Can native woodlands cope with climate change?

Measuring genetic variation & phenotypic plasticity in British populations of ash, rowan and silver birch

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Declaration of Authorship

I, Cristina ROSIQUE ESPLUGAS, declare that this thesis titled, 'can native woodlands cope with climate change? Measuring genetic diversity and phenotypic plasticity IN British populations of ash, rowan and silver birch' and the work presented in it are my own. I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at this University.
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- Where I have consulted the published work of others, this is always clearly attributed.
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
- I have acknowledged all main sources of help.
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Signed:

Date:

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J.M. de Sagarra

Lay Summary

Trees are very important organisms in all global ecosystems, and also they are crucial and bring many benefits to society. There is need for planting more trees, and the British government have targets of increasing the woodland cover in Great Britain (GB). The current system for sourcing seed for new tree planting in GB divides the country in four regions of provenance (ROPs), and trees have to be planted within those regions. However, there is no evidence on this system reflecting the genetic pattern of differentiation of the native tree populations of GB. In this study we want to examine this seed-sourcing system with the insight of our data. Moreover, under rapid climate change there is concern of whether and how trees will be able to adapt. There are two mechanisms with which trees can cope with the change of the environment. The first one is by genetic change; trees have long-generational changes for what selection of good genotypes will happen at the young seedlings which are growing currently. The other mechanism is with phenotypic plasticity, this allows the trees to change their phenotype without there any genetic change. Trees are long-lived organisms that have to endure the variable environment over their long lifetime, for what phenotypic plasticity is crucial for their survival. Common garden experiments are very useful to study genetic diversity and phenotypic plasticity, as they allow to separate the variation due to one and the other. In a common garden experiment trees grow under a common environment, for what the differences found across the experiment are due to genetic differences. Having replicated common garden experiments in distinct environments, allows to find the phenotypic plasticity when we compare trees from the same origin growing under different climates. Common garden experiments were planted in early 2000s with seed from over 30 native forests of ash, rowan and silver birch across all GB. This experiments are replicated between 2 and 4 times per species in contrasting locations around GB. We assessed the trees growing in these experiments, measuring several traits which are relevant to understand tree fitness and their capacity to adapt to climate change. In many studies tree height is used as a proxy for tree fitness, however we show that tree height is a multifactorial trait with limitations to understand the suitability of a tree to the environment where it is growing. We assessed our tree populations for growth (survival, tree height, DBH), stem form (number of forks), leaf phenology (leaf flushing and senescence) and leaf anatomical traits (leaf area, stomatal density and stomatal size). We analyse the levels of genetic diversity found for these traits and the phenotypic plasticity. GB has very distinct and heterogeneous environments and we have found that the patterns of differentiation of the trees for these traits has a geographical pattern following these environmental differences. There are differences along a latitudinal gradient and longitudinal, moreover, some traits are linked to specific water availability variables. Overall, we found that native populations of ash, rowan and silver birch are very diverse genetically and have high levels of phenotypic plasticity, which show that they are well adapted to the current diverse and oceanic climate of GB. The phenotypic plasticity they show will allow the current standing trees to survive the rapid climate change, and the high genetic diversity find across and within GB populations will allow natural selection to select the most suitable trees for the new climate.

UNIVERSITY OF EDINBURGH

Abstract

College of Science and Engineering School of Biological Sciences

Doctor of Philosophy

Can Native woodlands cope with climate change? Measuring genetic diversity and phenotypic plasticity in British populations of ash, rowan and silver birch.

by Cristina ROSIQUE ESPLUGAS

Rapid climate change is a significant threat to the long-term persistence of native tree populations. Concern has been expressed that tree populations might fail to adapt due to rate of change, insufficient adaptive variation in tree populations and limits to dispersal. In contrast, others have contended that most tree species have high phenotypic plasticity, maintain high levels of within-population genetic variation and exhibit effective gene dispersal capability, all characteristics which should enable an adaptive response. To assess the potential adaptability of tree populations we need to understand their genetic diversity and phenotypic plasticity to build on the currently limited evidence base and guide decisions about seed sourcing for establishment of new woodlands desired to meet ambitious planting targets. Currently the seed sourcing system divides the island in four regions of similar size although it is not based on any genetic or ecological information. We discuss the suitability of this system with the insight of the data collected from native tree populations growing in experimental trials. In this thesis we study genetic diversity and phenotypic plasticity patterns in over 30 native tree populations across all Great Britain for three broadleaved species: ash (*Fraxinus excelsior)*, rowan (*Sorbus aucuparia)*, and silver birch (*Betula pendula)*. To obtain these data we assessed the variation in multiple traits in several common garden experiments for each species, which were grown in contrasting environments. There is a tendency in provenance experiments to consider height as a proxy for fitness. We demonstrate that tree height is not enough to understand tree fitness and its adaptability capacity. We assessed our tree populations for growth (survival, tree height, DBH), stem form (number of forks), leaf phenology (leaf flushing and senescence) and leaf anatomical traits (leaf area, stomatal density and stomatal size).Great Britain has very distinct and heterogeneous environments likely to have given rise to adaptive differentiation. Knowing the geographical pattern of the genetic differences we can see the direction selective pressures have had on each of the traits studied, and we compare differences in patterns across the traits and species. Comparing populations growing in different environments we assessed the variation in phenotypic plasticity by trait and the direction of these plasticity. We found that tree populations across Great Britain are highly genetically variable and show genetic differences which have a geographical pattern, and that the patterns and size of the differences vary by species. Phenotypic plasticity varies across traits and interactions between genotype and environment make plasticity in some traits more unpredictable than others. We conclude that tree populations of ash, rowan and birch are well adapted to the diverse and oceanic climate of Great Britain, and that levels of genetic diversity and phenotypic plasticity provide a high capacity to respond to environmental change.

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Chapter 1: Introduction

1.1 Challenges in British forests

1.1.1 The value increasing forest cover, and having healthy resilient forests

With evidence of global warming and increasing observations of the biological responses to that warming (Parmesan, 2006; IPCC 2007), attention has turned to the fate of trees and forests. Forests in Europe will have to adapt not only to changes in mean climate variables but also to increased variability with greater risk of extreme weather events, such as prolonged drought, storms, winds and floods (Lindner, 2010). Tree species are central to many ecosystems and play key roles in providing habitat, food or mutualisms with many organisms, in addition to other ecosystem services and resources for humans. There are three possible fates for forest tree populations in a rapidly changing environment: persistence through migration to track ecological niches spatially, persistence through adaptation to new conditions in current locations or extinction (Aitken et al., 2008). Climate-based species distribution models predict the necessity of redistributions of tree species over the next century (Malcolm et al., 2002), although these are usually calculated without any intrinsic consideration of the biological capacity of populations for local adaptation. Trees live a long time and they might be adapted to the current conditions (or to past conditions or to the conditions in which they were initially selected in a younger age) but the trees that are growing now may not be adapted to the climate in the relatively near future.

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 & 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 & Shaw, 2001). There is evidence of local adaptation of introduced tree species within one or two generations of introduction (Bennuah, 1992; Saxe et al., 2001).

It is vital to ensure that the species and genotypes that are being proposed for planting today will be able to cope with the coming changes in the climate. The question that arises is whether native tree populations will be able to adapt or migrate sufficiently rapidly to keep pace with climate change, whether their levels of genetic diversity will be sufficient to allow a rapid adaptation despite long generation times. Some authors have suggested that the tree populations will fail to adapt because: the rate of climate change is likely to be too great compared to tree longevity, they do not maintain sufficient adaptive variation and the landscape is overly fragmented and impermeable to dispersal (Davis & Shaw, 2001; Jump & Peñuelas, 2005). In contrast, others take a different view and consider that most tree species will be able to adapt, as they have high phenotypic plasticity, high levels of within-population genetic variation and effective long distance gene dispersal capacity (Hamrick & Godt, 1996; Parmesan, 2006; Petit & Hampe, 2006).

In Great Britain (GB) forest land cover represents 11.6% of the total land area (Forestry Commission, 2003). Even though this figure is very low compared to the average forest cover for most other European countries (the average of Europe forest cover is 45% of land area), Britain's forests are of high importance for many environmental, social and economic reasons. There are governmental targets to increase this woodland cover during the coming decades, which means that new woodland planting will increase in the near future. The Scottish Forestry Strategy has set out a target of increasing forest cover from 17 per cent to 25 per cent of land area by 2050, Wales has a target to create 100,000ha new woodland over the next 20 years although England has not yet set a target for woodland creation (Atkinson & Townsend 2011). High costs are involved in establishing and maintaining new native forests in GB, therefore it is important to have as much evidence and information available as possible in order to maximise the likely success of planting by basing them on informed decisions from the outset. 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 survive for sufficient time for natural selection and evolutionary adaptation to take place (Clark, 2013; Cavers & Cottrell, 2014).

Management of broadleaved tree species has become increasingly important during recent decades and broadleaves now constitute 83 per cent of all new forest planting in the UK (Forestry Facts and Figures, Forestry Commission 2014). It has been shown that continental provenances planted in northern and western Britain have very low performance. Inspection of seed catalogues showed that seeds of many British native tree species have been imported into Britain from east and south-east Europe; which has been shown not to be well adapted to British conditions (Worrell, 1992; Hubert 2005). Many species which are frequently imported are widely planted for conservation purposes, but there is a risk that maladaptation could lead to problems in establishment (Whittet at al., 2016).

1.1.2 Seed sourcing guidelines in GB

In Britain, the UK Forestry Standard (Anon, 2004) encourages the use of local stock for planting of native tree species. These guidelines are based on the principle that locally sourced planting stock is likely to be the best adapted material available for a site (Hubert & Cottrell, 2007). The seed sourcing guidelines for native British trees, created by the Forestry Commission in 1999, are based on a system of four regions of provenance (NW, NE, SW and SE) which are subdivided into 24 seed zones. These regions of provenance (ROP) and seed zones (SZ), shown in Figure 1.1, were designed to encourage planting of local material, based on the assumption that local stock is more likely to be better adapted. Growers are encouraged to source their planting stock for planting schemes in ancient and semi-natural woodlands from within their local seed zone (UKWAS, 2013). Seed zone boundaries were delimited by major geomorphological landform boundaries and watersheds, but can include considerable environmental variation within them (Salmela et al., 2010).

This system has not been scientifically tested; therefore, it is not supported by a strong evidence base, which could show that the system is fit for its purpose, i.e. that the trees that are now being planted following these guidelines will be adapted to their new planting site. This is a key factor to have healthy forests which will have a better chance to cope with future climates. Concern has been raised regarding the use of the same seed-sourcing system for all the species and whether following the strategy of “local is best” is the most appropriate method of provenancing, especially when taking into account climate change (Ledig & Kitzmiller, 1992; Broadhurst et al., 2008; Aitken & Whitlock, 2013; Breed et al., 2013). Furthermore, it has been shown that there are high levels of climatic variation within some of the seed zones (Ennos et al., 1998; Salmela et al., 2010).

Elevation can vary within a seed zone, and there is adaptive variation due to elevation. An increase in elevation can be equated to an increase in latitude. Populations from high elevations tend to flush later than population from low elevations, but populations from lower elevations have high phenological plasticity. Seedlings from high elevation exhibited lower phenological plasticity to temperature than low-elevation provenances, which suggests they are under stronger selection pressures (Vitasse et al., 2009; 2013). For this reason, the seed sourcing guidelines also suggest to plant within similar altitude range, although it simplifies it to two categories (under and over 300 m) (Herbert, 1999).

Increasing concern about climate change, in particular, the suspicion that tree populations might struggle to cope with the changing conditions suggests that revision of guidelines might be necessary.

1.1.3 Climate change predictions in GB

The current climate in Great Britain is temperate oceanic with prevailing south-westerly winds from the Atlantic Ocean. These currents, known as the Gulf Stream, condition the climate and ensuring milder temperatures than would be expected for the latitude. In the western coastal region, where the moderating effects of the Gulf Stream are strongest, temperatures are milder and rainfall is greater than in the east. Therefore, the island has a climatic west – east gradient, with higher temperatures, precipitation and wind speeds in the west. The other stronger gradient in Great Britain is the north – south gradient, in the north of the country temperatures tend to be lower therefore there is a shorter growing season than in the south. The south east of the island can experience drought while in the north is uncommon. Another factor that affects the climate in GB is the topography. Areas in the west and north are more mountainous. The oceanic climate in GB has four distinct seasons. However, differences between these seasons are not as extreme and contrasting as those found in a continental climate (Barrow & Hulme, 1997).

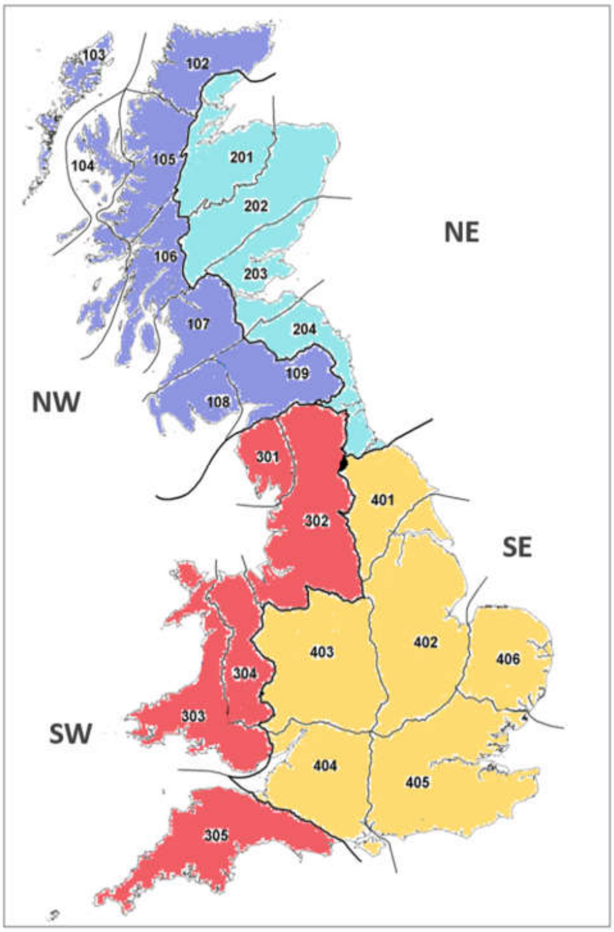


Figure 1.1. The four regions of provenance and 24 seed zones in Great Britain (modified from Herbert et al., 1999)

Temperature observations indicate that temperature in GB is increasing (Jones & Hulme, 1997). In the last 45 years, all parts of the UK have experienced increase in heavy precipitation events in winter; and most parts of the UK (except parts of the north of England and northern Scotland) have had dried summers, although annual precipitation sums have not shown clear change over time (Jenkins et al., 2008).

Climate projections for the UK (UKCP09) indicate an increase in mean summer temperatures of 3 – 4 °C by the 2080s under the medium emissions scenario, with the increase being greater in the south and east. Although little change in total annual rainfall is predicted, the distribution of this rainfall throughout the year will change. It is expected that summers will have a lower precipitation and winters will have higher precipitation totals, with a larger contribution coming from intense precipitation events in winter. As a result, summer droughts may become more frequent and severe. Furthermore, a larger proportion of the rainfall is projected to occur in shorter periods of time during extreme events. Cloud cover, particularly in summer, will decline. It is unlikely that an abrupt change to the Atlantic Ocean Circulation (Gulf Stream) will occur in the 21st century (Murphy et al., 2009).

Despite that higher temperatures and levels of CO_2 are expected to increase tree productivity and growth in areas which will not be limited by water availability (Saxe et al., 2001; Boisvenue & Running, 2006; Lindner et al., 2012); these projections of the climate changing in GB could have negative repercussions on forests. Warmer and drier summers will increase the risk of drought in some areas, mostly in the SE of England (Jerkins et al., 2010). Extreme drought events have been documented to lead to mortality in trees (McDowell et al., 2008), and examples of drought induced mortality events have been recorded in European forests (Allen et al., 2010). Furthermore, flooding might increase, due to the intense precipitation events which are expected to increase in winters. Trees can help mitigate flooding (Calder et al., 2003), however this is less effective when trees are dormant in winter.

1.2 Adaptive capacity of trees

To be able to understand how tree populations will react to climate change we first have to understand their past adaptive capacity and their current levels of adaptive genetic variation. Many species have wide geographical ranges, occupying very diverse environments which cause that selective pressures vary spatially (Kawecki & Ebert, 2004). 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 tuned to the seasonality of their environment. To respond to temporal environmental variation tree populations exhibit two mechanisms (Sultan, 1995): with phenotypic plasticity (which allows single genotypes to produce different phenotypes in different environments) or with adaptive genetic differentiation (differentiation due to different alleles; or different frequencies or allele combinations). Natural selection, genetic drift and mutations tend to increase genetic differentiation among populations, while phenotypic plasticity and gene flow tend to reduce it.

1.2.1 Genetic diversity in tree populations

Natural selection requires a source of heritable variation upon which to act. 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. In a population these sources of genetic diversity can come from mutation, from migration or gene flow. Genetic variation observed between populations maybe not be the results of adaptive divergence but the result of drift, migration or other characteristics of the populations' history (Kawecki & Ebert, 2004). When different populations in different locations experience different environmental conditions, natural selection causes populations to become adaptively different from one another (Kawecki & Ebert, 2004; Hereford, 2009), a process which can take place even in the absence of strong neutral genetic differentiation. This situation of local adaptation is common in trees (Savolainen et al., 2007) and has been demonstrated in many studies. Local adaptation is stronger in situations where selection pressures are intense, population sizes are large and where populations have been present in a given location for many generations (Savolainen et al., 2007).

Gene flow can be extensive in forest trees (Savolainen et al., 2007). Gene flow can counteract genetic differentiation and adaptation, allowing an influx of genetic variation from sites with different natural selection pressures. This has been suggested as one factor that might cause range limits in different species: if peripheral populations receive high levels of gene flow from other parts of the distribution, they might not be able to reach their optimum level of adaptation (García-Ramos & Kirkpatrick, 1997). When environments are diverse and gene flow limited, local adaptation may be constrained by small population sizes (Savolainen et al., 2007), in such cases gene flow enhances adaptive potential, avoiding inbreeding depression (Kremer et al., 2012). In GB genetic variation observed (mainly selectively neutral variation) in tree populations is also due to the relative recent history, as tree species mostly recolonized GB since 10,000 years ago, coming from refugia mainly in the south of Europe (Birks, 1989). 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). 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. Tree offspring can be very large and highly variable, and usually only a small proportion will survive to maturity. Natural selection is typically very strong at early life stages when trees are particularly sensitive to stress. (Persson & Stahl, 1990; Petit & Hampe, 2006).

Evolutionary change is usually expected to occur over long time scales and that the tolerance range of a species remains the same as it shifts its geographical range (Pearson & 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 poorly adapted to local conditions (Davis & Shaw, 2001). For example, rapid evolutionary change can and does occur as demonstrated by some plant species tolerance to heavy metals (Wu et al., 1975; Muller-Starck, 1985).

1.2.2 Phenotypic plasticity

Trees can respond to environmental change through acclimation within their own life time. The process by which individuals can alter their phenotype is known as phenotypic plasticity. Phenotypic plasticity involves non-heritable change in an individual's physiology in the absence of changes in their genetic constitution (Nicotra et al., 2010). Since trees cannot move and have long life-spans which include exposure to a wide range of conditions, plastic responses are highly developed in trees (Jump & Peñuelas, 2005; Alberto et al., 2013). Plasticity is under some genetic control and can therefore be acted on by selection to fit the demands of different environments (Bradshaw 2006; Lindner et al., 2010). Highly variable environments are likely to select for phenotypic plasticity across a range of traits, plastic responses will be crucial to cope with the current climate change (Jump & Peñuelas, 2005; Botero et al., 2015). However, plastic responses are, on the other hand, associated with ontogenic costs, or trade-offs among traits (DeWitt et al., 1998;

Valladares et al., 2007; Richter et al., 2012). Selection for plasticity may conceal adaptive genetic responses to directional change, although phenotypic plasticity has to be considered as an adaptive trait in itself (Jump & Peñuelas, 2005). There is plenty of evidence of phenotypic plasticity as a response mechanism to environmental change in temperate tree species (Barnett and Farmer, 1980 (*Prunus serotina*); Kramer, 1995 (seven tree species); Grantani et al., 2003 (*Quercus ilex*); Rehfeldt et al., 1999 (*Pinus contorta*)).

1.4. Measuring adaptive capacity of trees

1.4.1. Common garden experiments

To assess the potential for adaptive response in forest tree populations it is necessary to investigate current levels of phenotypic plasticity and patterns of adaptive genetic variation. To measure the sources of variation (genetic diversity or phenotypic plasticity) and the ways different populations have cope to adapt to environments we can use common garden experiments. These involve growing plants raised from seed together in a common environment. Because the environment is held constant, it is possible to observe the differences between tree populations which are determined by their genotype. Replicating the experiment in multiple environments allows comparison of trees from the same population growing in different environments; this enables assessment of phenotypic plasticity because responses of similar genotypes to different environments can be observed. Common garden experiments have revealed high among-population levels of genetic variation for quantitative traits related to adaptation, geographic structure of the variation along environmental gradients, and genotype-environment interactions, providing strong evidence of local adaptation of populations to climate (Howe et al., 2003; Savolainen et al., 2007).

Common garden trials of species such as Scots pine (Perks & MacKay, 1997), birch and oak (Worrell, 1992) have already demonstrated that British material is better adapted than provenances from elsewhere in Europe to conditions in Britain. However, to develop an understanding of the pattern of distribution of adaptive variation in our native tree species at a British, as opposed to a European scale, trials based on material collected from provenances throughout Britain are required. There is limited information on the distribution of adaptive variation and phenotypic plasticity for British tree populations. There are some results for *Betula pendula* (Blackburn & Brown, 1988; Billington & Pelham, 1991), *Pinus sylvestris* (Perks, 1994; Perks & McKay, 1997; Perks & Ennos 1999; Salmela et al., 2013), *Crataegus monogyna* (Jones et al., 2001) and *Fraxinus excelsior* (Cundall et al., 2003; Boshier & Stewart, 2005; Clark, 2013), although they were obtained from young provenance and progeny trials and based only on a restricted number of provenances. Also, the studies focus mainly on few characters such as tree height and bud flush.

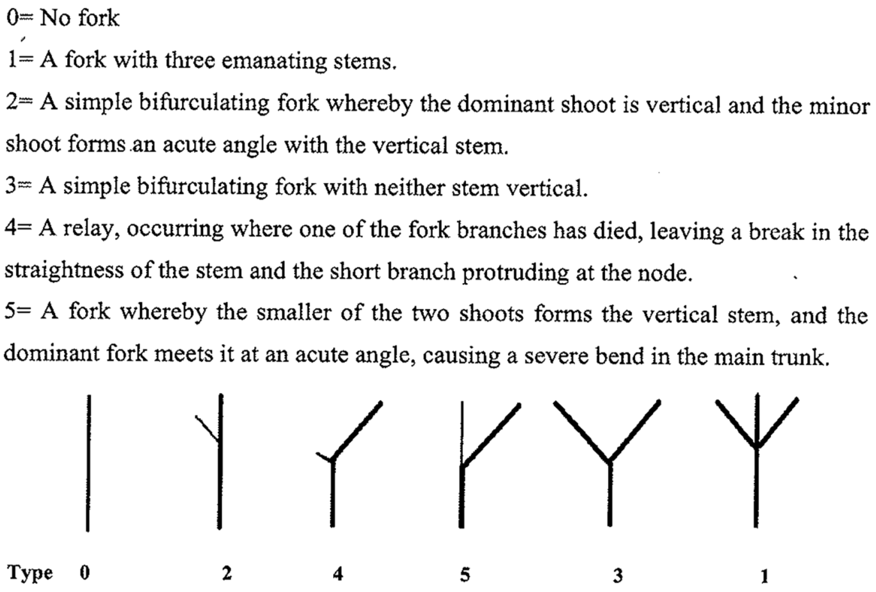
Relatively little information is available about the genetic variability in native tree populations for the whole range of Great Britain. There is a need for research to provide information on the pattern of adaptation, the extent of the phenotypic plasticity and the extent of variation for adaptive characters in British range. An understanding of these is necessary to create proper guidelines for seed transfer. Different species behave in different ways, for that reason, the adaptive differences may occur at differing spatial scales (Ennos et al,. 1998; Vitasse et al., 2009). This may be related to the reproductive strategies of the species or the complexity of environment they inhabit. In some cases significant differences may exist over short distances, while other species might be relatively similar across large areas. By studying variation in more than one tree species, different patterns may emerge, which would be of significance for management of genetic resources.

1.4.2. Measuring the relevant phenotypic traits

It is important to measure appropriate traits when assessing a tree; the characteristics which will allow assessment of tree fitness and its adaptive variation are those related to tree performance, and those traits responsive to environmental change. Such traits should include:

a) Tree performance: It is important that trees survive and grow well to achieve maturity and be able reproduce. The height of the trees and DBH are important traits as a measure of vegetative fitness. Also, tree height is related to the adaptation of the length of the growing season (Chuine et al., 2006). Stem diameter is an important measure to understand tree growth, it complements the height measurement analysing height:diameter ratios. They are also relatively simple and quick measurements, especially DBH. Survival, is essential for trees to survive to assure the viability of the population.

Stem form is also a very indicative trait. Excessive forking can indicate a maladaptation to the growing season (Park & Talbot, 2018) and stem inclination can be related with a weak root system (which can indicate lower tolerance to wind high intensity). Damage to terminal buds leads to outgrowth of other apical buds causing forking of trees. This can be considered a defect if the production of timber is an important objective. However, the cause of this forking gives interesting information. There is relation between early flushing and frost damage, which leads to poor stem form. Late spring frost causes damage to terminal buds, despite not being the only possible reason for forking (Morin & Chuine, 2014). For example in the case of ash trees in Britain, the main two causes of forking are frosts and ash bud moth (Kerr & Boswell, 2001). Seaman (2007) showed the relation between tree architecture and the timing of the bud burst in spring, using provenance trial of ash from different parts of Europe. Another important factor of stem form is apical dominance. A viable bud is needed in order for the tree to grow in upwardly direction. If a branch that is not the apical branch becomes dominant the tree will start to divide its resources into such new dominant branch(es), creating a bent trunk. There are different ways that stem form can be recorded. Some of the quantitative ways could be: number of forks, height to the first fork, number of branches, and distance of stem from the vertical at breast height. However, tree stems can be very variable and difficult to assess just with quantitative measures, for example, there are many types of forking, with loss or not of apical dominance (Figure 1.2). Counting the number of forks should theoretically be a wholly objective measure, although some trees can be difficult to assess due to other strange stem forms. To ensure consistency, the measurements should be done by one same person.



0= No fork

1= A fork with three emanating stems.

2= A simple bifurculating fork whereby the dominant shoot is vertical and the minor shoot forms an acute angle with the vertical stem.

3= A simple bifurculating fork with neither stem vertical.

4= A relay, occurring where one of the fork branches has died, leaving a break in the straightness of the stem and the short branch protruding at the node.

5= A fork whereby the smaller of the two shoots forms the vertical stem, and the dominant fork meets it at an acute angle, causing a severe bend in the main trunk.

Figure 1.2. Several types of tree forking. Extracted from Seaman 2007.

b) Adaptation to future climate change. The length of the growing season is expected to change with climate change due to higher average temperatures. Phenology measurements including the timing of bud flushing, leaf senescence and flowering reflect the adaptation to the environmental conditions where the tree grows. Furthermore, tree phenology can also be related with tree stress (e.g. Lindner et al., 2010). In addition to warmer temperatures, seasonal patterns of water availability are predicted to continue to change. Leaf anatomy and other functional traits, including leaf size and shape, stomata density, C-isotope discrimination analysis, hairiness, venation density; as an indication of adaptation to moisture availability and other environmental factors, competitive strength and water use efficiency (Pérez-Harguindeguy et al., 2013). In this thesis we will explain the analyses for the assessments of leaf phenology, leaf size and stomatal traits.

Leaf Phenology

For deciduous trees the timing of the bud burst (flushing) and leaf fall (senescence) define the duration of the canopy, determining the growing season of the tree and therefore the carbon assimilation (Baldocchi & Wilson, 2001); and also influence vegetative growth and fitness (Rathcke & Lacey, 1985; Lechowicz & Koike, 1995).

Leaf phenology has been mostly related to air temperatures, and it is expected that global warming will have an effect of extension of the growing season (Chmielewski & Rötzer, 2001). Especially for budburst, which is largely governed by temperature, is expected to occur earlier in the season (e.g. Cannell & Smith 1983; Murray et al., 1989; Hänninen 1991).

Leaf area

Leaf traits provide a link between various environmental factors and leaf functions (Xu et al., 2009). Leaf size has important consequences for the leaf energy and water balance. The size of a leaf has been related with nutrient availability and competitive strength, and the size and shape related to drought and light conditions. Intraspecific variation in leaf size has been connected with climatic variation, geology, altitude or latitude where heat stress, cold stress, drought stress and high radiation stress all tend to select for relatively small leaves (Parkhurst & Loucks, 1972; Orians & Solbrig, 1977; Box, 1996; Cornelissen, 2003). Phenotypic plasticity has been shown to be an important factor in many leaf traits that are environmentally affected (McLellan, 2000; Barkoulas et al., 2007; Xu et al., 2008). Specific leaf area (SLA) is the one-sided area of a fresh leaf, divided by its oven-dry mass. SLA is frequently used in growth analysis because it is often positively related to potential relative growth rate. SLA tends to scale positively with mass-based light-saturated photosynthetic rate and with leaf nitrogen concentration, and negatively with leaf longevity and carbon investment in quantitatively important secondary compound such as tannins and lignin (Pérez-Harguindeguy et al., 2013). Leaf area traits are important in plant ecology because they are associated with many critical aspects of plant growth and survival (Garnier et al., 2001b; Shipley & Vu, 2002) and also they play an important role in explaining variation in ecological behaviour in plants (Garnier et al., 2001a). Leaf area measurements are broadly used in the literature as a comparison between species, for functional traits ecology approaches or large-scale meta-analyses. However, within species variability for leaf traits has been shown to be high and is typically related to environmental variation. Plants adapted to drier environments tend to have smaller thicker leaves, with higher SLA, stomatal density and lower guard cell length than plants of the same species but from less dry areas (Carpenter &

Smith, 1975; Abrams, 1986; Abrams et al., 1990). This was shown well with green ash (*Fraxinus pennsylvanica*) in common garden experiments, where grown in the same conditions, trees from drier areas had higher SLA, lower stomata densities and smaller leaf sizes (Abrams *et al*., 1990).

Stomata density

Plant stomata are a vital gate between the plant and atmosphere, and they play a central role in plant responses to environmental conditions. Many researchers have reported stomatal density responses to various environmental factors, such as elevated CO2 concentration (Woodward, 1987), heat stress (Beerling & Chaloner, 1993), salt stress (Zhao et al., 2006), precipitation change (Yang et al., 2007), and plant density (Zhang et al., 2003) and water use and drought adaptation (Galmés et al., 2007; Xu & Zhou, 2008; Abrams 1990; Abrams et al., 1994).

1.4.3 Tree species chosen

The tree species which have been chosen for the current study are common ash (*Fraxinus excelsior* L.), rowan (*Sorbus aucuparia* L.) and silver birch (*Betula pendula* Roth). These three have been chosen for having distinct ecological requirements and life strategies (Table 1.1). Although they are native and distributed across all GB, as their distribution maps show (Figures 1.3, 1.4 and 1.5), their ecological niches are different. Rowan is the most similar in ecological distribution to birch, since both are relatively short-lived trees, most characteristic of acidic soil and extending to high altitudes. However, they differ very much in their reproductive biology, as birch is wind dispersed and vast number of seeds and colonize unshaded areas; a trait which ash and birch have in common. Rowan has a very distinct life history strategy compared with the other two species. Rowan is insect pollinated and produces fleshy fruits which are eaten by birds, the main seed dispersers (Snow & Snow, 1988). In many places (mainly at lower altitudes) it often behaves as a hardy pioneer or post-pioneer species (Kullman, 1986; Rameau et al., 1989), populations of which are later replaced by late-successional tree populations. At high altitude, however, it is one of the few species which can maintain the tree habit and its populations may be part of the late-successional vegetation.

Table 1.1. Comparison of ash, rowan & silver birch for their life strategy traits (Grime, 2007).

Traits	***Fraxinus excelcior***	***Sorbus aucuparia***	***Betula pendula***
Flowers	Wind-pollinated Flowers are variously male, female and hermaphrodite, and may occur in various combinations on each tree. The proportion of male and female flowers can vary from year to year	Insect-pollinated or selfed. <100 flowers in a compound corymb. Hermaphrodite.	Wind-pollinated, monoecious in catkins
Phenology	Flowers April to May before leaves and sets seed from September onwards. Leaves shed October to November.	Bud breaks in early spring. Flowers in May to June and sets seed in September to October. Leaves shed in autumn. Buds of montane populations show a considerable resistance to desiccation.	Flowers April and may before the leaves are expanded. Seeds shed from September onwards
Established strategy	Intermediate between competitor and stress-tolerant competitor. Wind dispersed.	Stress-tolerant competitor. Bird/mammal dispersed, also water.	Intermediate between competitor and stress-tolerant competitor. Wind dispersed.
Regenerative strategies	Regenerates by seeds, which germinate in spring usually in the second year after shedding. Immature embryo and germination chilling requirement. Seedlings and saplings, often in clumps, in shaded habit, frequently recorded from shaded slopes of both north and southern aspect, although only abundant on S-facing slopes.	Regenerates entirely by seed, which germinates in spring. Dormancy of seed broken by chilling. Sparse. No bias detected in unshaded sites but in shaded sites juveniles recorded more frequently on N-facing slopes. Seedlings very much shade tolerants.	Seed germinates in spring. Seedlings may occur at high densities. Germination may be inhibited by placement under leaf canopy. Seedlings very susceptible to drought. Root:shoot ratio never exceeds 1.
Distribution & Habitat	All British Isles, except for parts of the Scottish Highlands. A frequent constituent of hedgerows. Seedlings and saplings recorded or observed in all but aquatic habitats. Particularly frequent in woodlands and meadows, and on shaded river banks, lead-mine spoil and scree.	All the British Isles, although rare and possibly not native in some lowland C and E counties in England. Absent from wetlands. Seedlings and saplings mainly restricted to wooded sites, particularly on non-calcareous. Also recorded from skeletal habitats including lead mines and wasteland.	All through British Isles (90% vice-counties) but more common in the N and W. Seedlings and young saplings common in shaded habitats, particularly scrub and woodland on acidic strata. Also rocky open habitats. Is the second most common broad-leaved tree of British woodland and the most common in Scotland
pH	Juveniles widely distributed with slightly greater abundance in pH 6-8. Virtually absent from acidic soil.	Mainly restricted to soil of pH< 5.5, a few records up to 7.	Widely distributed, but mostly frequent and abundant below pH 5.
Altitude	Mostly 100-300m, observed to 400m (up to 585m).	Juveniles particularly frequent in gritstone (siliceous) woodlands over 200m, and observed up to 400m. Extends on rocky ground to over 900m.	Juveniles observed from 0-400, more abundant 200-300m, up to 760m.

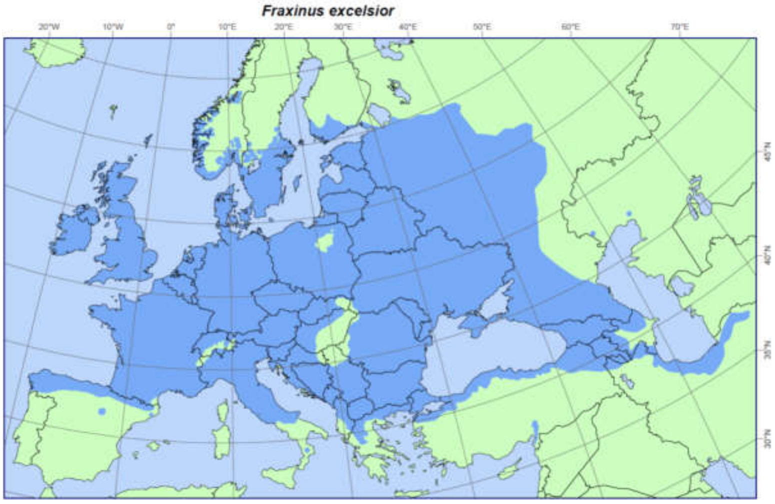


Figure 1.3. Common ash (*Fraxinus excelsior* L.) distribution in Europe (source: http://www.euforgen.org).

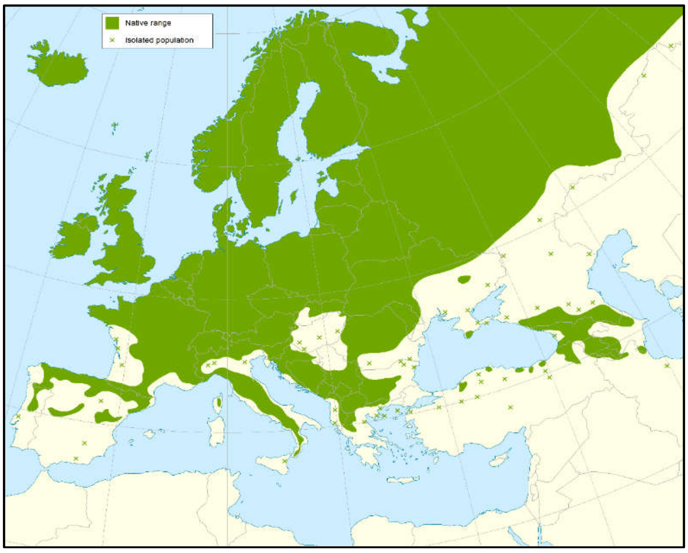


Figure 1.4. Rowan (*Sorbus aucuparia* L.) distribution in Europe (Caudullo, 2017).

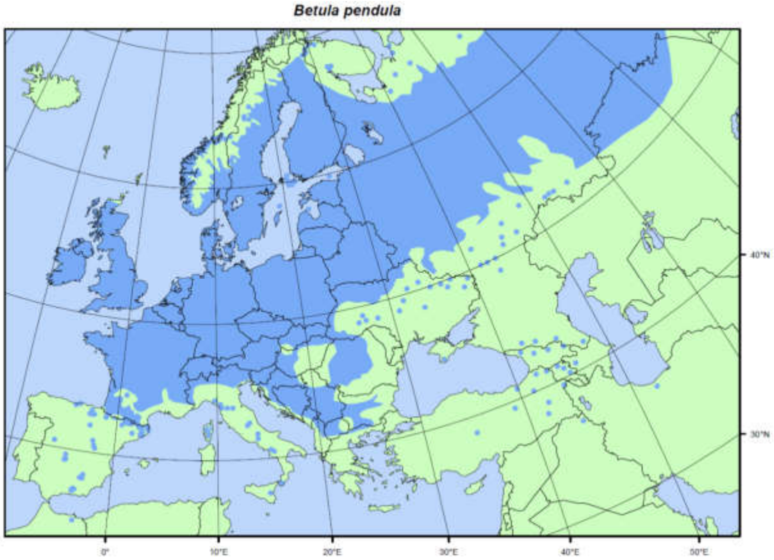


Figure 1.5. Silver birch (*Betula pendula* Roth) distribution in Europe (source: http://www.euforgen.org).

Fossil pollen records since the end of the last ice age indicate that most temperate forest tree species spent the cold period in southern Europe (Heuertz et al., 2004) and from there recolonized GB. The tree species survived the last glacial maximum (c. 25 000–16 000 year BP) in a range of geographically and ecologically different refugia and followed their own path during the postglacial recolonization of Europe. Thermophilous tree species were confined to refugia in southern Europe during the last glacial maciumym. Other species, such as birch, were able to survive at much higher latitudes (Willis et al., 2000).

For ash, Sutherland et al. (2010) found that neutral genetic differentiation among populations in GB is limited and that British ash belongs to a single large European meta-population which expanded from the Iberian Peninsula (Heuertz et al., 2004), with the exception of some rare chloroplast haplotypes in eastern Scotland. These rare haplotypes could have be derived from relict populations which survived in glacial refugia c. 12000 years BP, however, ash pollen is present in the south of England from 7000 BP, and it is thought to have become common in GB after 5000 years BP (Birks, 1989; Rackham, 2006). Studies investigating very small fragmented populations (n = 10-30) in southern Scotland showed that pollen flow among fragmented stands can be extensive, encouraging high levels of genetic diversity in seed crops, should conditions for natural regeneration exist (Bacles et al., 2005; Bacles & Ennos, 2008).

Fossil records show that birch was spread in central Europe during the ice age (Willis et al., 2000). Palmé (2003) found two major birch chloroplast haplotypes in Europe (one west one east) plus a few local ones, from which two one of the major haplotypes and a rare one are found in British birch populations. Populations in the Iberian Peninsula and in Italy did not take part in the birch postglacial recolonization of Europe. In contrast to thermophilous species that exhibit higher levels of variation in southern areas, the highest levels of variation in *Betula pendula* are found at high latitudes (Palmé, 2003). The two native birch species in GB (*Betula pubescens* Ehrh. and *B. pendula*) were present in the British Isles from about 13,500 BP (Pennington, 1981). Birks (1989) explains that before 10,000 BP birch was well established in much of central and northern England, southern Scotland, and parts of Wales. By 9750 BP birch spread (c. 250 m yr-1) and expanded in north Wales, Skye and the adjacent mainland, and by 9500 BP it expanded in parts of the Scottish Highlands, with the exception of the frozen areas.

Boyd & Dickson (1987) and Fossitt (1996) found rowan pollen in lake sediments dating from the early Holocene, in Scotland, which showed that around 6500 BP, rowan was present in the area. A study of a few rowan populations of central Europe (Raspé, 2001), found low genetic differentiation among rowan populations compared to the other tree species studied. This discrepancy might be accounted for by the contrasting life history traits of rowan, including bird dispersal of seeds and pioneer habit. The same low genetic differentiation was found in a previous study of isozyme variation (Raspé & Jacquemart 1998), suggesting high levels of gene flow. In a study which investigated small fragmented populations of rowan in southern Scotland (Bacles et al., 2004) found that despite being fragmented for many generations the populations retained substantial genetic diversity. However the populations were significantly genetically different amongst them, which could be due to reduced pollen flow.

1.5 Purpose of Thesis

The purpose of this thesis is to address the knowledge gap which exist regarding the understanding of the genetic diversity for adaptive traits in British tree populations. Information which is required to understand and address the challenges that tree populations in GB are facing and will continue to face in the future.

The value of the current study is that analyses of tree populations from all the three species distribution in GB, contemplating the wide range of environments found in GB, are provided. Moreover, we investigate a range of different traits which have not been studied before in British tree populations. Most studies which have looked at variation amongst British provenances of trees do it from the point of view of timber production and wood quality, and therefore they focus mostly on tree height, and many times conflate tree fitness with greater height, using one as a proxy for the other. In the current thesis we have not made that assumption, and we have tried to address trees as a whole with all their trait diversity, trying to understand the complexity of local adaptation from different points of view.

In each chapter we have addressed the following questions:

- Is there genetic diversity in the tree species provenances for that particular trait?
- Do these differences show a geographical pattern? Or are these associated more with a particular environmental variable?
- Are the patterns of genetic variation amongst provenances the same for the three species?
- Is there phenotypic plasticity for that particular trait? Is the site effect bigger than the provenance effect?
- Are there any genotype by environment interactions for that trait?

These questions are addressed at each of the data chapters (Chapter 3, 4 and 5) for several traits. Moreover, in Chapter 2 we address the diversity of environments found in GB, trying to understand the geographical patterns, as that diversity is the basis of the selective pressures which the tree populations are exposed to, and whether how our sampling has collected this diversity of environments in a representative way.

In the discussion (Chapter 6) we synthesise all the results from the traits to try to understand the adaptive variation as a whole, and its geographical distribution; also site effect and plasticity. We compare our results with the current seed system in GB. The general questions we want to answer with this thesis are:

1- Do British populations of ash, rowan and silver birch show high levels of genetic diversity within the British populations for relevant traits? Would this diversity be enough to allow an adaptive response to climate change?

2- Do these populations show high levels of phenotypic plasticity? Would this plasticity help trees cope with climate change?

3- Is the current seed sourcing guidelines for British tree species fit for purpose?

Chapter 2: Environmental setting of Great Britain

Abstract: The current seed sourcing system used in GB to plant new native woodlands divides the island into 4 large regions of provenance (NW, NE, SW, SE), and seed collected from within a region must be planted within its boundaries to receive grant funding. This system was set up in 1999, without any data on patterns of genetic diversity in actual tree populations to support the ROPs divisions. Given the substantial environmental variation across GB, it is important to gain an understanding of the patterns of climatic variation that are likely to have shaped the adaptive pressures across the island. The populations of ash, birch and rowan, used in all the data chapters of the current thesis were systematically sampled and distributed more or less evenly within the four ROPs. To analyse the pattern of climatic variation within and among the ROPs we used 13 climatic variables taken from each of our sampling locations and subjected this to PC analysis. The first two principal components accounted for 58% and 28% of the variation and were roughly associated with variation in mean temperature and continentality of climate respectively. Variation in PC1 value was largely accounted for by latitude of provenance ($R^2 = 0.52 – 0.72$), while PC2 was best explained by joint variation in latitude and longitude of provenance ($R^2 = 0.14 – 0.42$). By plotting provenance locations according to their PC1 and PC2 scores, we demonstrated that the climatic variation covered by the three species in our samples was largely overlapping, and that the trial sites had climates within the core climatic

envelope within GB for the three species. The trials should therefore allow meaningful comparisons of patterns of variation in adaptation across species. Additionally, we found extensive climatic diversity within each ROP. This was not evenly distributed across the 4 ROPs, and there was considerable overlap in the climatic envelopes of the four ROPs. We conclude that there is therefore no justification for including ROP as a nesting fixed factor in the analyses of morphological and phenological traits in our data chapters. However analysis of provenance variation with respect to latitude and longitude is justified and likely to be useful because of their relationships with climatic variation across GB.

2.1 Introduction

Many countries, including the UK, have seed sourcing guidelines for the planting of new trees which involve defining the geographical regions within which seed may be moved, ensuring that the risk of maladaptation is minimised (Ying & Yanchuk, 2006). The current UK seed sourcing system (Herbert et al., 1999) was defined in the absence of comprehensive data on the adaptive genetic variation of British trees species, and was based on major watersheds and geomorphological landforms, therefore making it a provisional system. Great Britain was divided into 24 seed zones (SZ) nested in four regions of provenance (ROP) (Figure 2.1), and planting locally seed collected from within each ROP is mandatory to obtain grants for planting new native woodland.

There are problems with this system because seed transfer guidelines should ideally be designed with information on genetic and phenotypic diversity of the tree populations across each species distribution. These data were not available but can be obtained from multi-site common garden experiments conducted in field conditions or fully reciprocal transplant experiments (Rehfeldt et al., 2002; Reich & Oleksyn, 2008; Wang et al., 2006b). Moreover, in contrast to other countries (Alía et al., 2009), in Great Britain (GB) the geographical division for seed sourcing is applied to all native trees and shrubs, with the only exception of *Pinus sylvestris* (Scots pine), which in GB is only considered native in Scotland.

Another shortcoming of this seed zone approach is that geographical proximity does not necessarily mean that environmental conditions are similar, as microclimate and topography can increase the environmental difference over a small spatial scale (Linhart & Grant, 1996; Bischoff et al., 2006; Jump et al., 2009; Loarie et al., 2009). This was demonstrated by Salmela et al. (2010), who showed that the current seed zones for Scots pine in Scotland include large climatic variation within them; and that most climatically similar native Scots pine sites were often not within the same seed zone.

In this thesis we bring evidence from replicated common garden experiments in three species to understand whether the current system of seed sourcing in GB based on large geographical regions is the appropriate one, and also whether it is fit for the three species studied. Before addressing the former question, we first analyse in this chapter: a) the seed sampling that composes our common garden experiments, to see the extent of environments in GB that these samples cover; and b) the diversity of environments within GB, which set the selective pressure for local adaptation of tree populations. In particular we aim to establish whether our sampling covers the full diversity of climatic conditions present in GB. In addition, we wish to determine whether our sampling covers similar range of climates for each species so that we can conduct species comparisons.

Another objective of this chapter is to establish a clear understanding of the climate pattern across GB to underpin the interpretation of the results presented in the later data chapters. An understanding of the climatic diversity and structure of our sampling is key to designing the appropriate data analysis approaches. A key question is whether we are justified in including ROP as a nesting fixed factor when analysing patterns of adaptive variation across GB. We also need to identify the most important climate variables on a GB scale so that we have some idea of the likely drivers of adaptation. We can then use our data from trials to test which climatic factors may be responsible for adaptive differentiation.

A final aspect covered by our analysis concerns the environments in which the trial sites are located. Trials for each species are situated in different locations, in a range of environments. We need to examine the climatic variables at these sites to see how different they are and to understand their relative suitability for the species being studied.

2.2 Material and Methods

2.2.1 Sampling provenances

The same sampling strategy was adopted for all three species; ash, *Fraxinus excelsior L.*, rowan, *Sorbus aucupari*a L. and silver birch, *Betula pendula* Roth .This was based on the Forestry Commission seed zone map of Great Britain (Forestry Commission, 1999; Figure 2.1). This divides the total area of Great Britain into 4 regions of provenance (North West NW, South West SW, North East NE and North West NW). These regions of provenance (ROPs) are further subdivided into a total of 24 seed zones based on natural topographical boundaries (Figure 2.1). Where possible two (three in a few cases for birch) self-sown semi-natural populations of each species (hereafter referred to as provenances) were sampled from each of the seed zones. Roughly equal numbers of seed were collected from a minimum of 20 (in birch a minimum of 30) maternal trees and combined together to constitute the provenance sample. Selection of mother trees was not deliberately biased towards superior phenotypes, and, where possible, mother trees were located at least 100m apart. For ash and rowan 42 provenances where sampled (Table 2.1 and 2.2, respectively) and for birch 32 provenances (Table 2.3).

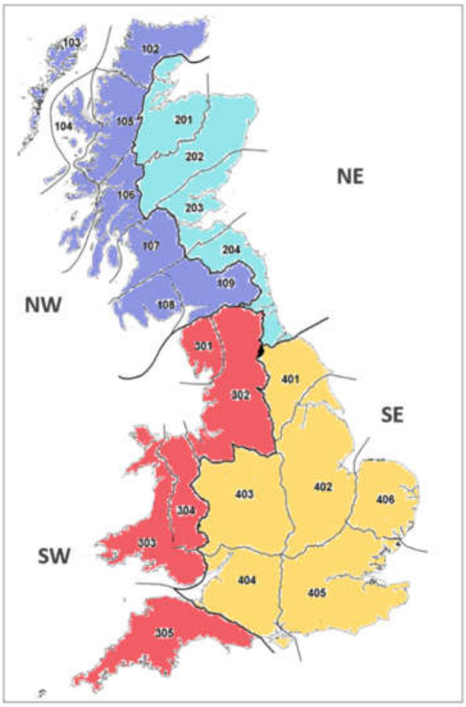


Figure 2.1. The four regions of provenance and 24 seed zones in Great Britain (modified from Herbert et al., 1999)

Table 2.1. Details of the ash provenance locations.

ROP	Ash provenances	Lat	Long	Alt (m)
NW	Duisdale, Skye	57.176	-5.751	18
	Kilninian, Mull	56.530	-6.208	71
	Rassal Wood, Kishorn	57.426	-5.591	78
	Ardtornish,Morvern	56.560	-5.741	20
	Glasdrum Wood, Loch Creran	56.574	-5.232	33
	Add Valley, Kilmichael Glassary	56.106	-5.420	30
	Clyde Valley	55.680	-3.913	159
	Shielhill Glen	55.911	-4.825	107
	Penpont	55.235	-3.853	90
	Nith Valley	55.320	-3.829	141
	Crawick Water	55.381	-3.929	162
	Warks Burn	55.088	-2.222	90
NE	Erchite Wood, Dores	57.368	-4.345	56
	Craigellachie	57.484	-3.170	102
	Fearnan Forest, Kenmore	56.579	-4.037	142
	Glen Lyon	56.602	-4.248	183
	Den of Alyth	56.623	-3.258	152
	Pitcairns Glen, Dunning	56.300	-3.573	119
	Tweed Valley North Glen	55.588	-2.662	68
	Castle Eden Dene, Peterlee	54.743	-1.352	102
SW	Witherslack	54.264	-2.870	79
	Park Wood&Hutton Roof	54.182	-2.689	170
	Via Gellia Woods	53.104	-1.619	239
	Upper Wharfedale	54.203	-2.104	202
	Greta Wood, Purbeck Ridge	50.637	-2.136	126
	Horner Wood, Porlock	51.189	-3.583	102
	Cardiff Area	51.546	-3.234	158
	Aberystwyth Area	52.430	-4.059	90
	Betws-y-Coed	53.079	-3.799	57
	Talgarth	51.986	-3.213	198
SE	Forge Valley	54.274	-0.490	52
	Ashberry Woods	54.262	-1.133	142
	Treswell Woods	53.308	-0.861	54
	Hayley Wood	52.158	-0.110	79
	Tick Wood, Ironbridge	52.621	-2.523	99
	Forest Bank, Marchington	52.852	-1.820	142
	Wyndcliff, Wye Valley	51.678	-2.679	208
	Midger Wood	51.606	-2.285	160
	Pheasant Copse, Petworth	51.011	-0.628	60
	Bignor Hill	50.908	-0.616	194
	Groton Wood	52.050	0.883	66
	Out Wood	52.166	0.415	96

Table 2.2. Details of the rowan provenance locations.

ROP	Rowan provenances	lat	long	Alt (m)
SW	Assynt	58.171	-5.261	27.48
	Inverpolly	58.093	-5.232	82.12
	Allt Volagir, South Uist	57.247	-7.310	68.76
	Salen, Isle of Mull	56.527	-5.961	5.80
	Tokavaig, Isle of Skye	57.139	-5.965	25.40
	Glen Loy	56.901	-5.071	61.96
	Add Valley	56.141	-5.486	33.72
	Strathlachlan	56.128	-5.152	70.32
	Falls of Clyde	55.653	-3.778	160.84
	Mugdock Country Park	55.971	-4.320	161.8
	Glenlee	55.088	-4.194	129.68
	Stroan Bridge	55.070	-4.545	66.72
	Lochwood	55.258	-3.443	180.2
	Ettrick Water	55.420	-3.133	234.16
SE	Bunchrew	57.471	-4.315	100.8
	Craigdarroch	57.574	-4.605	31.8
	Cleanhill Wood, Aberchirder	57.554	-2.636	158.76
	Birks of Aberfeldy	56.602	-3.872	285.96
	Pressmennan Wood	55.951	-2.589	149.84
	Castle Eden Dene	54.743	-1.351	107.64
NW	Horner Wood	51.194	-3.589	232.84
	Holford/Hodder's Combe	51.158	-3.218	164.88
	Duddon Valley	54.316	-3.230	61.84
	Naddle Forest	54.511	-2.805	291.24
	Brignall Banks	54.496	-1.913	222.36
	Gelt Wood	54.903	-2.733	139.16
	Brechfa	51.993	-4.063	146.28
	Beddgelert	53.025	-4.138	257.76
	Mynydd Du	51.954	-3.105	532.12
	Ugly House	53.106	-3.868	231.32
NE	Forge Valley& Raincliffe Woods	54.275	-0.484	89.64
	Ashberry and Reins Woods	54.253	-1.128	106.24
	St. He len's Wood, Coningsby	53.113	-0.123	6.24
	Moor Farm	53.156	-0.181	14.8
	Pepper Wood	52.368	-2.092	144.76
	The Ercall	52.687	-2.522	185.8
	King's Bottom, Longleat	51.191	-2.241	219
	Chestnuts Wood, Forest of Dean	51.829	-2.470	161.72
	Saxonbury Hill	51.076	0.251	183.12
	Seal Chart	51.278	0.237	129.44
	Culter's Wood, Freston, Ipswich	52.011	1.142	34.4
	Felbrigg great Wood, Cromer	52.913	1.264	83.24

Table 2.3. Details of the birch provenance locations.

ROP	Birch provenances	lat	long	Alt (m)
NW	Affric	57.31	-4.80	118
	Loch Creran	56.55	-5.28	26
	Dumfries	55.11	-3.58	29
NE	Elgin	57.42	-3.38	140
	Great Glen	57.21	-4.62	68
	Glen Garry	57.07	-4.83	54
	Spinningdale	57.89	-4.26	38
	Dunkeld	56.56	-3.56	143
	Alford	57.24	-2.67	142
SW	Bovey Tracey	50.59	-3.71	120
	Bovington Camp	50.71	-2.21	50
	Penrith	54.7	-2.96	280
	Ambleside	54.4	-2.98	60
	Bolton Abbey	54.04	-1.95	220
	Hamsterley Forest	54.7	-1.86	200
	Sheffield	53.28	-1.56	240
	Machynlleth	52.58	-3.85	90
	Taffs Well	51.55	-3.27	100
	Llanidloes	52.46	-3.53	180
	Persteigne	52.24	-3.05	200
	Llangollen	52.98	-3.19	170
SE	Castle Howard	54.12	-0.92	76
	Sand Hutton	54.02	-0.95	25
	Leicester	52.73	-1.23	160
	Clumber park	53.26	-1.06	55
	Cannock Chase1	52.72	-2.04	180
	Cannock Chase 2	52.74	-2.05	140
	Monmouth	51.79	-2.69	170
	Tollard Royal	50.96	-2.21	130
	Godalming	51.14	-0.6	170
	Basingstoke	51.38	-1.02	50
	Braintree	51.91	0.54	80
	Dunwich	52.26	-1.61	20

2.2.2 Trial sites locations

The provenances were planted in multiple trial sites across the country (Figure 2.2), two trials for ash (Llandovery and North York Moors), four for rowan (Alice Holt, Llandovery, North York Moors, Dornoch) and four for birch (Llandovery, Thetford, Drummond and Dornoch). Most of these trial sites (Llandovery trial sites for the three species, Alice Holt, Thetford, North York Moors for ash) are established on fertile soils previously used as arable land where trees might not have been growing naturally. The other trial sites are in more demanding locations: steep hills, damp soils, locations exposed to high winds, where some of the species will experience challenging growing conditions (North York Moors rowan: Drummond and Dornoch, rowan and birch).

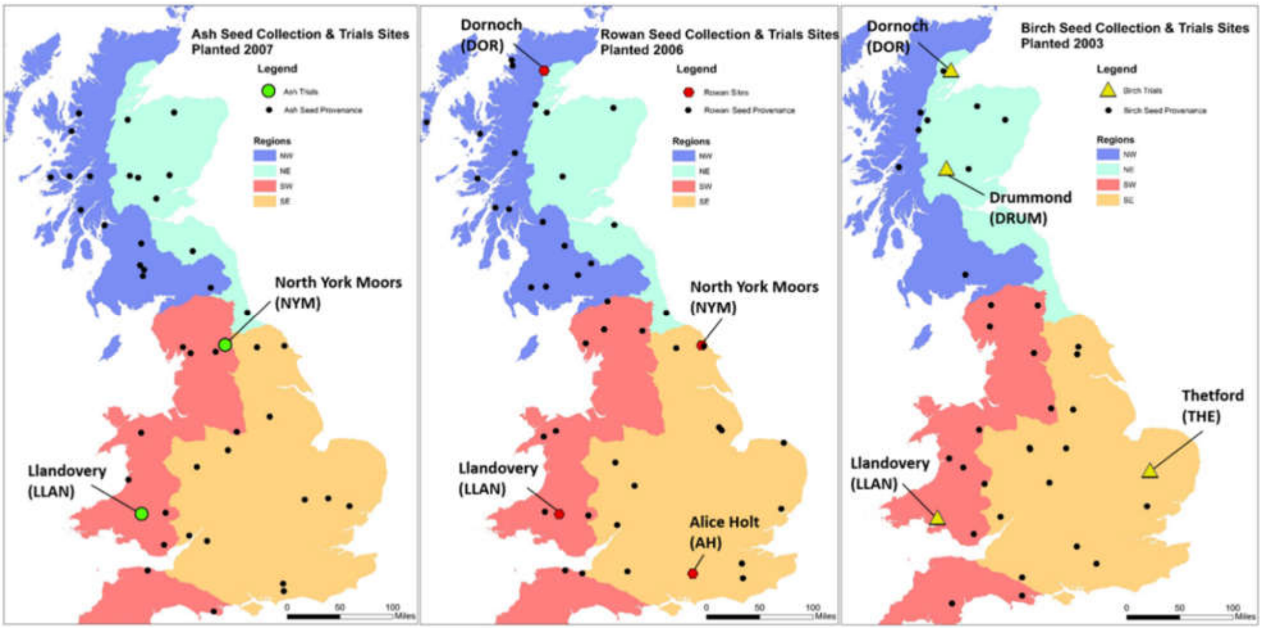


Figure 2.2. Seed collection (black small dots) and trial sites locations for ash (green dots, left), rowan (red dots, centre) and birch (yellow triangles, right)

Table 2.4. Trial site locations and altitude, for the three species.

Spp	Trial Site	Planting Year	County	Lat	Long	Alt (m)
Ash						
	NYM (North York Moors)	2007	East Yorkshire	54.3	-1.9	113
	LLAN (Llandovery)	2007	Carmarthenshire	51.9	-3.8	215
Rowan						
	DOR (Dornoch)	2006	Sutherland	58.1	-4.4	130
	NYM (North York Moors)	2006	East Yorkshire	54.3	-0.5	197
	LLAN (Llandovery)	2006	Carmarthenshire	51.9	-3.8	240
	AH (Alice Holt)	2006	Surrey	51.2	-0.8	118
Birch						
	DOR (Dornoch)	2003	Sutherland	57.9	-4.1	135
	DRUM (Drummond)	2003	Perthshire	56.6	-4.1	218
	THET (Thetford)	2003	Norfolk	52.4	0.6	54
	LLAN (Llandovery)	2003	Carmarthenshire	52.6	-4.1	114

2.2.3 Environmental covariates

Climatic data for each of the provenance and trial site locations were obtained from the Met Office. A total of 13 climatic variables (Table 2.5) were chosen that were considered relevant for plant growth. We acknowledge that there will be differences at the microclimatic level, which will not be reflected in the large scale extrapolated climatic data provided by the Met Office, in 5 by 5 km polygons (Perry & Hollis, 2005). However, as the differences in these variables are very large across GB, we expect them to provide a good signal of climatic variation.

Table 2.5. Environmental variables used in the PCA analysis.

Variable	Variable explained MetOffice (1970-2011), 5x5km
AP	Total precipitation (monthly mean)
ConsDryDays	Maximum number of consecutive dry days (annual mean)
DaysSnowLying	Days of snow lying (monthly mean)
EXT	Extreme temperature range (annual mean)
GDD	Growing degree days (annual mean)
GFD	Days of ground frost (monthly mean)
GSL	Growing season length (annual mean)
MeanCC	Mean cloud cover (monthly mean)
MFT	Mean air temperature February (coldest month)
MJT	Mean air temperature July (warmest month)
MWSpeed	Mean wind speed at 10 m (monthly mean)
SunHours	Sunshine duration (monthly mean)
VAPPressure	Mean vapour pressure (monthly mean)

2.2.4 Analyses of principal components

A principal component analysis (PCA) was carried out with the 13 climatic variables (Table 2.4) derived from the 116 provenance locations of all three species, which comprise 42 provenances of ash, 42 of rowan and 32 of birch planted in at least one of the trials (Tables 2.1 to 2.3). In order to determine where the trial sites fit climatically with respect to the provenances, a separate PCA was carried out which additionally included the climatic data from the trial sites for each of the three species (Table 2.4). Correlations were calculated amongst climatic variables, and with PC components and coordinates (latitude and longitude), using Pearson's correlation coefficient. For the first two PCA components, linear regressions (single and multiple) were applied to test the relationship with latitude and longitude of the home site. Linear regression plots were drawn. Data analysis and visualisation were performed in the R statistical environment (R version 3.2.3, Core Team, 2015).

2.3 Results

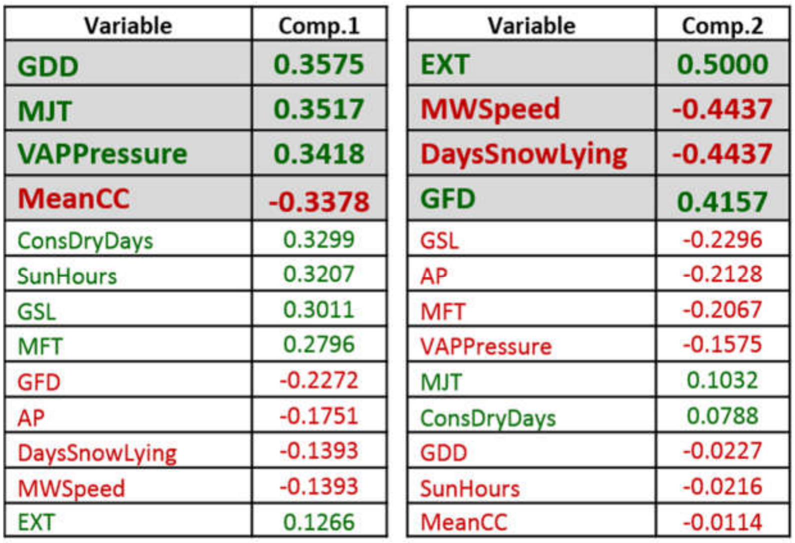
2.3.1 PCA all species -Provenance locations climatic data vs. geographical pattern

The first two components together account for 80% of the variation, 58% PC1 and 22% PC2. The last 4.4% of the variance is explained between the 5th and 13th component (Table 2.6).

Table 2.6. Proportion of the variance in the first 5 components of the PCA analysis.

	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5
Proportion of variance	0.583	0.216	0.123	0.034	0.015
Cumulative proportion	0.583	0.799	0.922	0.956	0.971

Table 2.7. Loadings of each variable to PC1 and PC2.



Variable	Comp.1
GDD	**0.3575**
MJT	**0.3517**
VAPPressure	**0.3418**
MeanCC	**-0.3378**
ConsDryDays	0.3299
SunHours	0.3207
GSL	0.3011
MFT	0.2796
GFD	-0.2272
AP	-0.1751
DaysSnowLying	-0.1393
MWSpeed	-0.1393
EXT	0.1266

Variable	Comp.2
EXT	**0.5000**
MWSpeed	**-0.4437**
DaysSnowLying	**-0.4437**
GFD	**0.4157**
GSL	-0.2296
AP	-0.2128
MFT	-0.2067
VAPPressure	-0.1575
MJT	0.1032
ConsDryDays	0.0788
GDD	-0.0227
SunHours	-0.0216
MeanCC	-0.0114

The climatic variables which contribute over 30% of their loadings and over 0.8 on Pearson's correlation coefficient to the first principal components (PC1) are the following: growing degree days (+36%, cor= 0.98), mean July temperature (+35%, cor= 0.97), vapour pressure (+34%, cor= 0.94), consecutive dry days (+33%, cor= 0.90), sun hours (+32%, cor= 0.88), and growing season length (+0.30, cor= 0.83) are positively correlated; cloud cover (-34%, cor= -0.93) is negatively correlated with PC1.

The climatic variables which contribute over 20% of their loadings and over 0.3 on Pearson's correlation coefficient to the second principal components (PC2) are the following: extreme temperature range (+50%, cor= -0.84) and ground frost days (+0.42, cor= 0.70) are positively correlated with PC2; wind speed (-44%, cor= -0.74), days of snow lying (-44%, cor= -0.74), growing season length (-23%, cor= -0.38), annual precipitation (-21%, cor= -0.36), and February mean temperature (-21%, cor= -0.35) are negatively correlated with PC2.

PC1 is positively correlated with parameters associated with to warmer and drier environments, and negatively correlated with cloud cover. In contrast, PC2 is positively correlated with extreme temperature range (which would be related to stronger continentality) and ground frost days; and it is negatively correlated with temperature, precipitation and wind. In the PCA plot (Figure 2.3) PC1 is on the x axis, and the provenances located towards the right of the plot will tend to have a warmer climate, with a longer growing season, higher vapour pressure and less cloud cover. PC2 provides the y axis; provenances in the lower part of the plot tend to be from sites which experience more precipitation, more wind and more days of snow lying; and provenances on the top part of the y axis will be more continental and have more frost days.

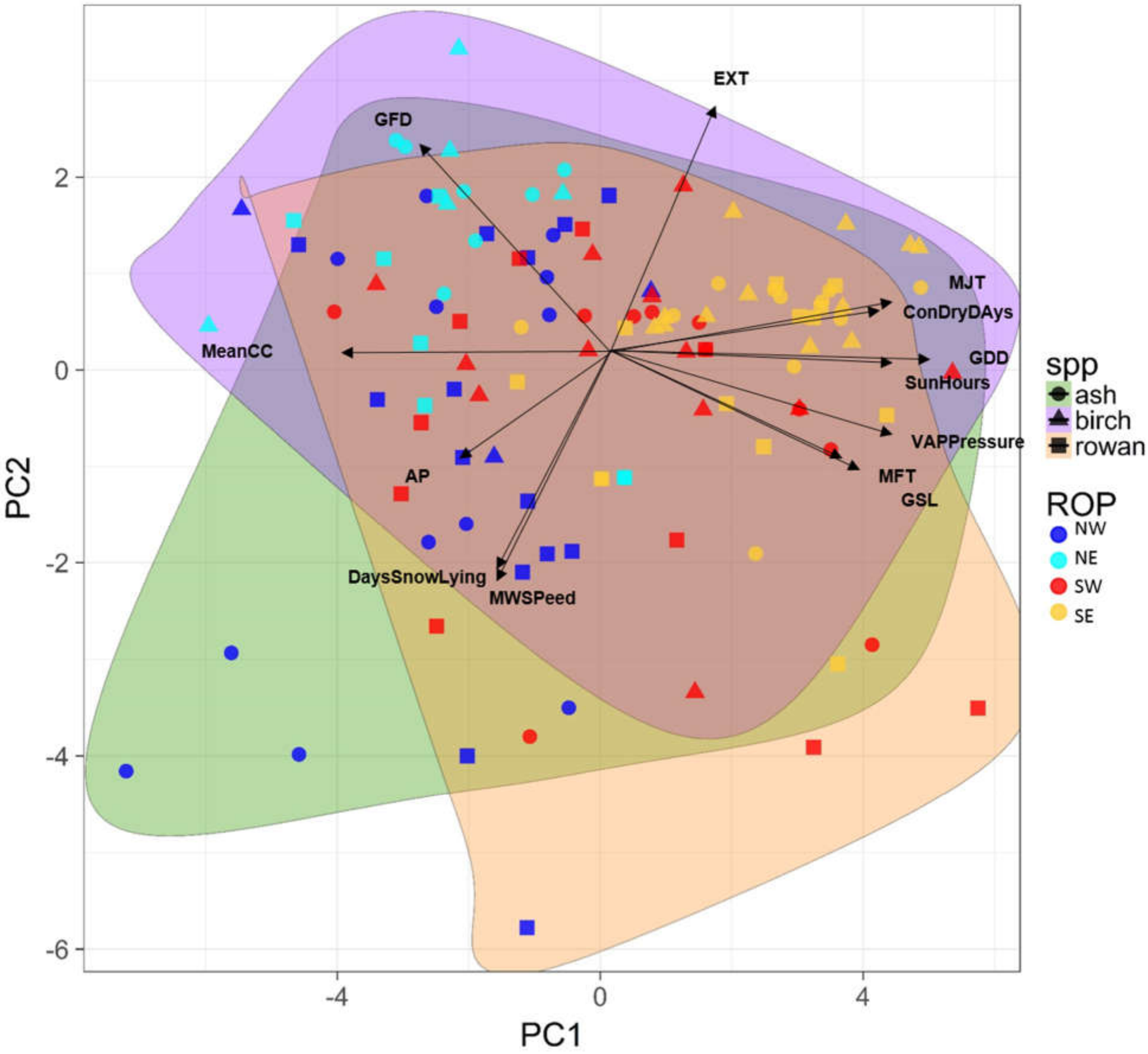


Figure 2.3. Scatterplot of the provenances’ PC1 and PC2 values. The three species: ash (round points and green polygon), rowan (square points and orange polygon), and birch (triangle points and purple polygon). The colour of the points corresponds to the ROP of the provenance. The arrows correspond to the direction and contribution in the plot of the 13 climatic variables.

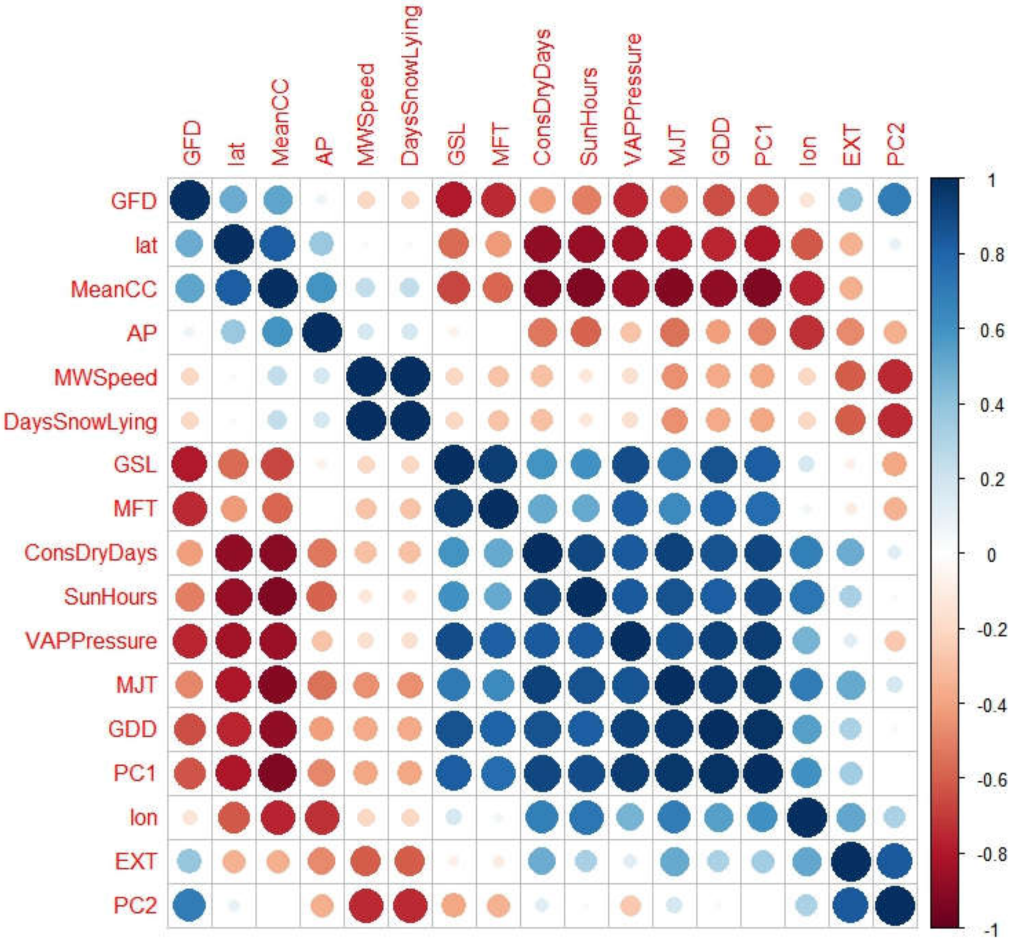


Figure 2.4. Correlation amongst all the 13 environmental variables, blue indicates a positive correlation and red indicates a negative correlation.

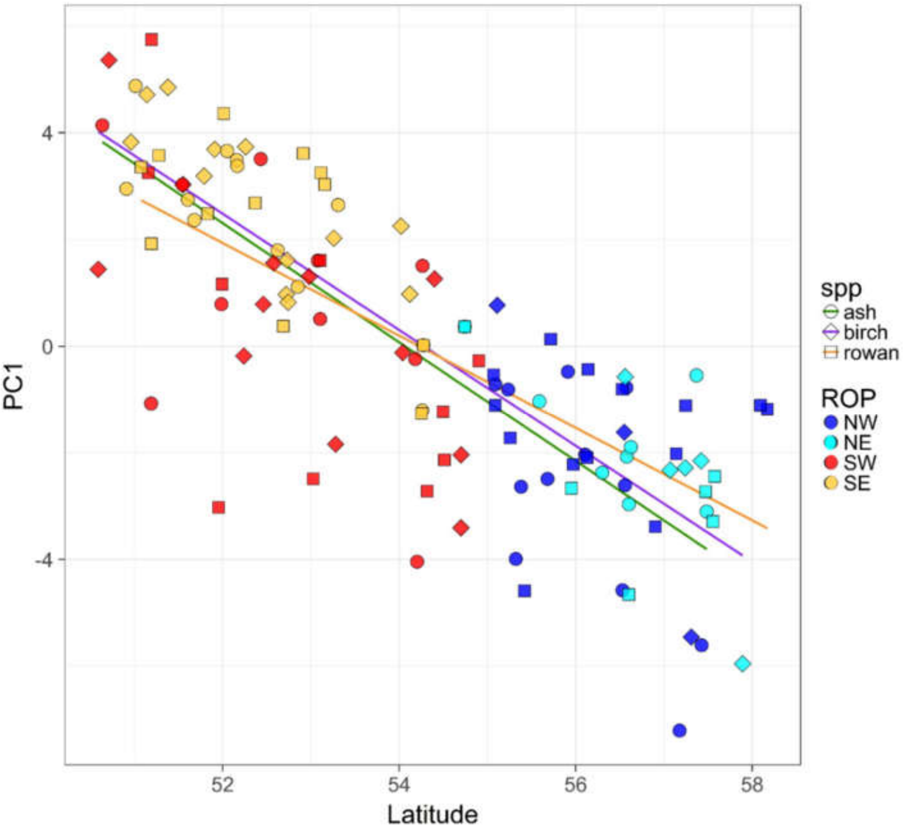
Table 2.8. Pearson's correlation coefficients between each variable and PC1 and PC2.

Variable	**Pearson's corr. coeff**	
	PC1	**PC2**
MeanCC	-0.9298	-0.0191
GFD	-0.6254	0.6958
AP	-0.4819	-0.3561
MWSpeed	-0.3834	-0.7428
DaysSnowLying	-0.3834	-0.7428
EXT	0.3483	0.8370
MFT	0.7696	-0.3461
GSL	0.8288	-0.3844
SunHours	0.8828	-0.0361
ConsDryDays	0.9080	0.1319
VAPPressure	0.9408	-0.2637
MJT	0.9680	0.1727
GDD	0.9841	-0.0381

The individual climatic variables used to calculate PC1 and PC2 are correlated with latitude and longitude. It is important therefore to determine the geographical patterns of variation for the more complex PCs. Within GB the latitude and longitude of sites are naturally correlated because of the orientation of GB on the globe (corr= -0.62); towards the north the island also goes towards the west. This means that longitudinal clines need to be understood in the context of latitude. Thus it is important to look not only at simple regression of PCs on latitude and longitude, but on multiple regression on latitude and longitude together. For PC1 latitude alone explains most of the variation, with a negative regression on latitude for all three species (ash R^2: 68%, $p<0.001$; rowan R^2: 52%, $p<0.001$; and birch R^2: 70%, $p<0.001$) (Table 2.10). For PC1 the three species show a very similar slope and intercept with PC1 values increasing to the south (Figure 2.5). For PC2 a multiple regression on latitude and longitude explains most of the variation, whereas simple linear regressions with either latitude or longitude, are not significant or explain very much less of the variation (Table 2.10). Multiple regression explains 23% ($p<0.01$) of variation in ash, 14% ($p<0.05$) in rowan and 42% ($p<0.001$) in birch.

Table 2.9. Pearson's correlation coefficients for each variable with latitude and longitude.

Variable	**Pearson's corr. coeff**	
	Lat	**Long**
Latitude	1	-0.6168
Longitude	-0.6168	1
PC1	**-0.8010**	**0.6027**
PC2	**0.0912**	**0.3116**
MeanCC	0.8265	-0.7607
GFD	0.4965	-0.1388
AP	0.3731	-0.7263
DaysSnowLying	0.0313	-0.2071
MWSpeed	0.0313	-0.2071
EXT	-0.3407	0.5145
MFT	-0.4300	0.0518
GSL	-0.5649	0.1695
GDD	-0.7552	0.5448
MJT	-0.8008	0.6951
VAPPressure	-0.8337	0.4601
SunHours	-0.8626	0.7290
ConsDryDays	-0.8856	0.6818



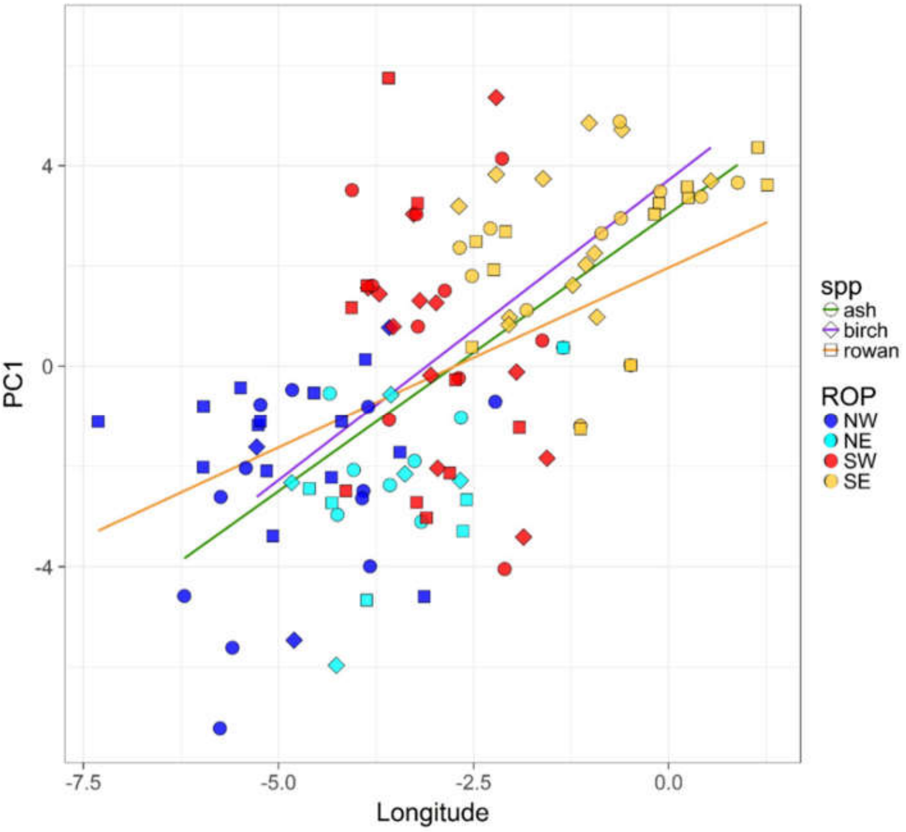
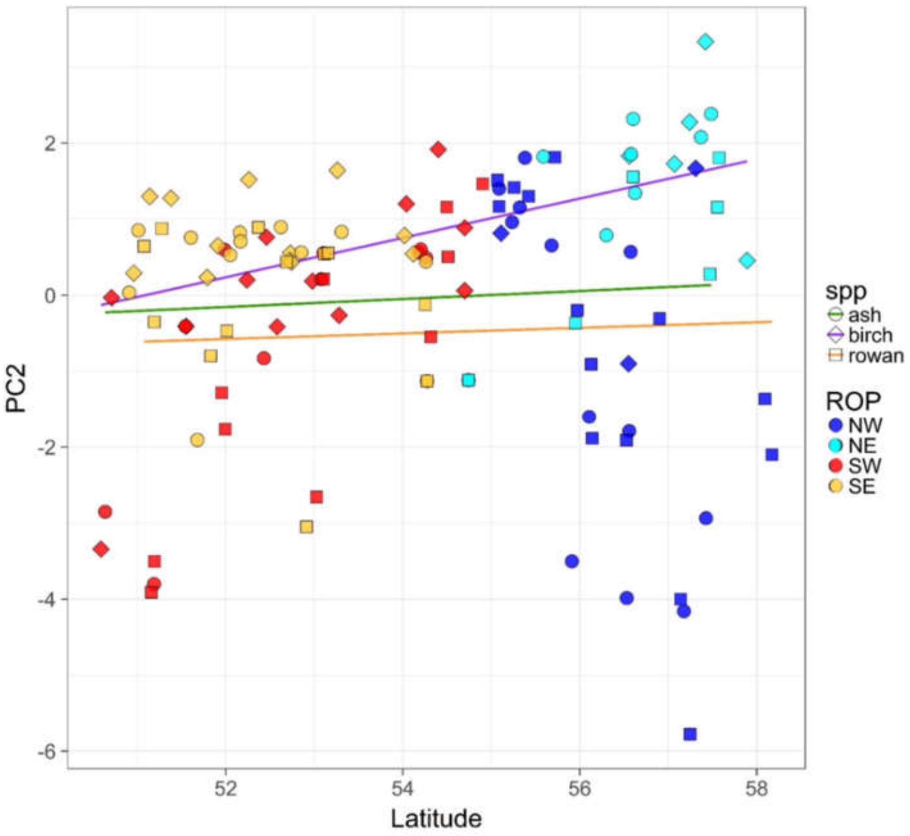


Figure 2.5. Regression of PC1 with latitude (top) and longitude (below). Green line ash, orange rowan and purple birch.



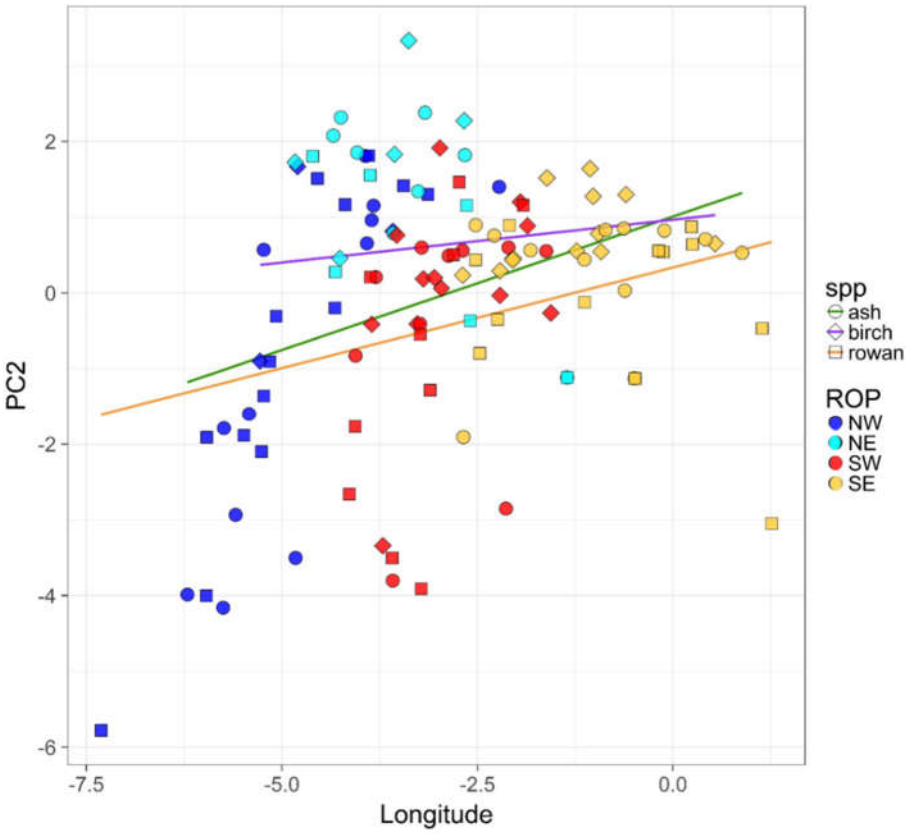


Figure 2.6. Regression PC2 with latitude (top) and longitude (below). Green line ash, orange rowan and purple birch.

Table 2.10. Linear regressions (single & multiple) of PC1 and PC2 with latitude and longitude.

		Ash	**Rowan**	**Birch**
PC1	**Latitude**	-Lat *** R^2: 0.6794 p-value: 1.204e-11	**-Lat ***** **R^2: 0.5202** p-value: 4.306e-08	**-Lat ***** **R^2: 0.7018** p-value: 1.355e-09
	Longitude	+Long *** R^2: 0.4486 p-value: 7.406e-07	+Long *** R^2: 0.2898 p-value: 0.0001401	+Long *** R^2: 0.3071 p-value: 0.0005929
	Multiple regression	**-Lat *** + Long *** **R^2: 0.7056** p-value: 1.661e-11	-Lat *** + Long ns R^2: 0.523 p-value: 2.029e-07	-Lat *** + Long ns R^2: 0.7168 p-value: 4.315e-09
PC2	**Latitude**	ns	ns	+Lat * R^2: 0.2189 p-value: 0.004056
	Longitude	+Long * R^2: 0.1001 p-value: 0.02333	R^2: 0.06616 p-value: 0.05507 (ns)	ns
	Multiple regression	**+Lat ** + Long ***** **R^2: 0.2337** p-value: 0.002098	**+Lat * + Long**** **R^2: 0.1377** p-value: 0.021	**+Lat *** + Long **** **R^2: 0.4199** p-value: 0.0001417

2.3.2 PCA all species - Provenance locations and differences across the four ROPs

In the PC1 versus PC2 scatterplot we drew a polygon around the provenances for each ROP (Figure 2.7). The largest overlap of the ROPs' areas is between the NW and SW ROPs, which have also the largest polygons, while the NE ROP has the smallest polygon.

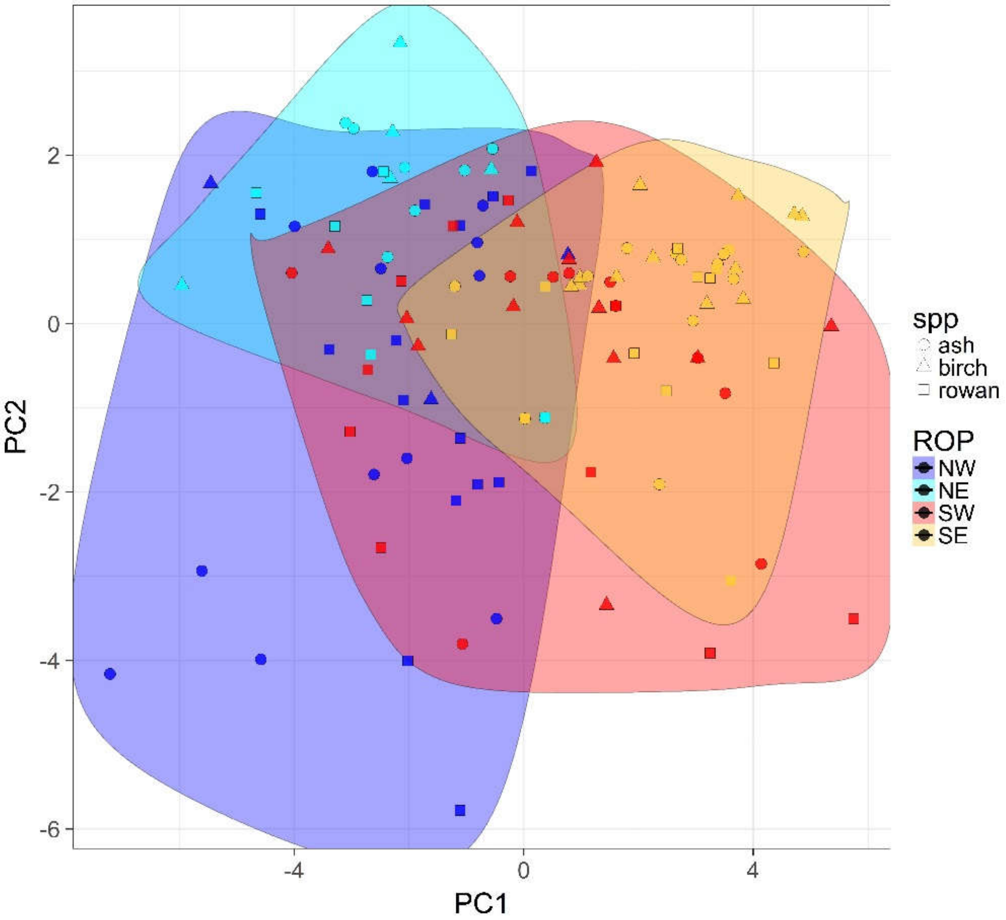


Figure 2.7. Scatterplot of the provenances' PC1 and PC2 values. The three species: ash (round points), rowan (square points), and birch (triangle points). The colour of the points corresponds to the ROP where the provenance is from. The polygons correspond to the 4 ROPs.

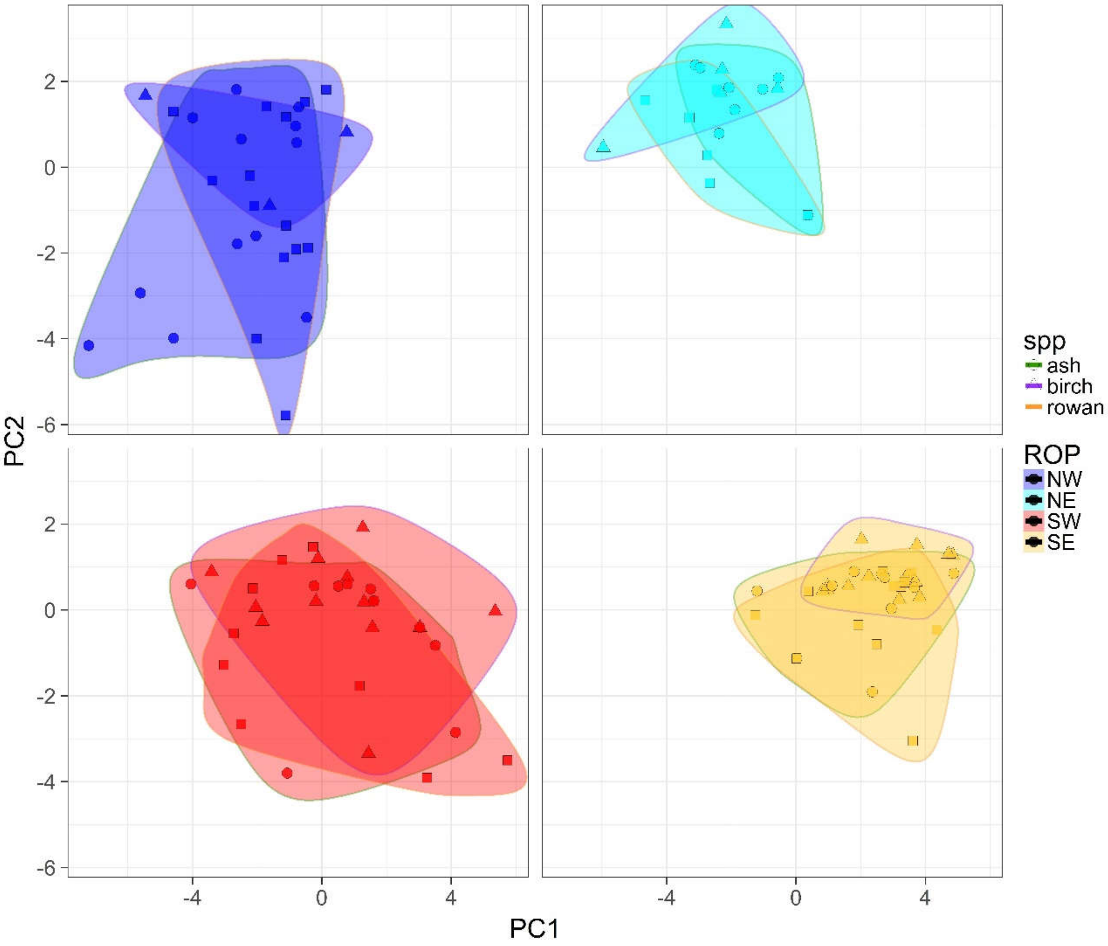


Figure 2.8. Scatterplot of the provenances' PC1 and PC2 values, separated by ROP and polygons by species. Provenances separated by the four ROPs: NW (dark blue, top left), NE (pale blue, top right), SW (red, bottom left), and SE (yellow, bottom right). Each polygon is one species: ash (round points, green polygon-outline), rowan (square points, orange polygon-outline), and birch (triangle points, purple polygon-outline).

We drew PC1 versus PC2 scatterplots for each ROP, to display the differences in the distribution of each species for each ROP (Figure 2.8). For the NW ROP, we see that the polygons for each of the three species only overlap over a small area, the ash provenances' polygon being the largest. For the SW ROP, we find that the polygons are larger than for the other ROPs, but quite similar among tree species. For the eastern ROPs, the polygons are smaller than the ones of the western ROPs, but quite similar in size amongst species, except for birch in SE which is much smaller. Overall, we see that the birch provenances occupy a smaller climatic range than the other species for the NW, NE and SE ROPs; while rowan and ash polygons overlap much more, except in the NW ROP.

2.3.3. PCA all species -Provenance and trial site locations

In the PCA which incorporated the trial sites locations in the scatterplot of PC1 and PC2 (Figure 2.9) the trial sites lie within the core overlapping area of all three species, with the rowan Dornoch and the Alice Holt sites being the most outlying. In the more detailed scatterplot (Figure 2.10) it is clear that the provenances closest, in terms of climatic environment, to each trial site, are in many cases from a different ROP. In the Dornoch birch trial site, which is in ROP NE, the closest birch provenances in the scatterplot are from ROP SW and NW. In the rowan trial site located in the North York Moors (ROP SE) the closest provenance is from ROP NE. In the ash site of North York Moors (ROP SW) the closest ash provenances are from ROP NW and NE.

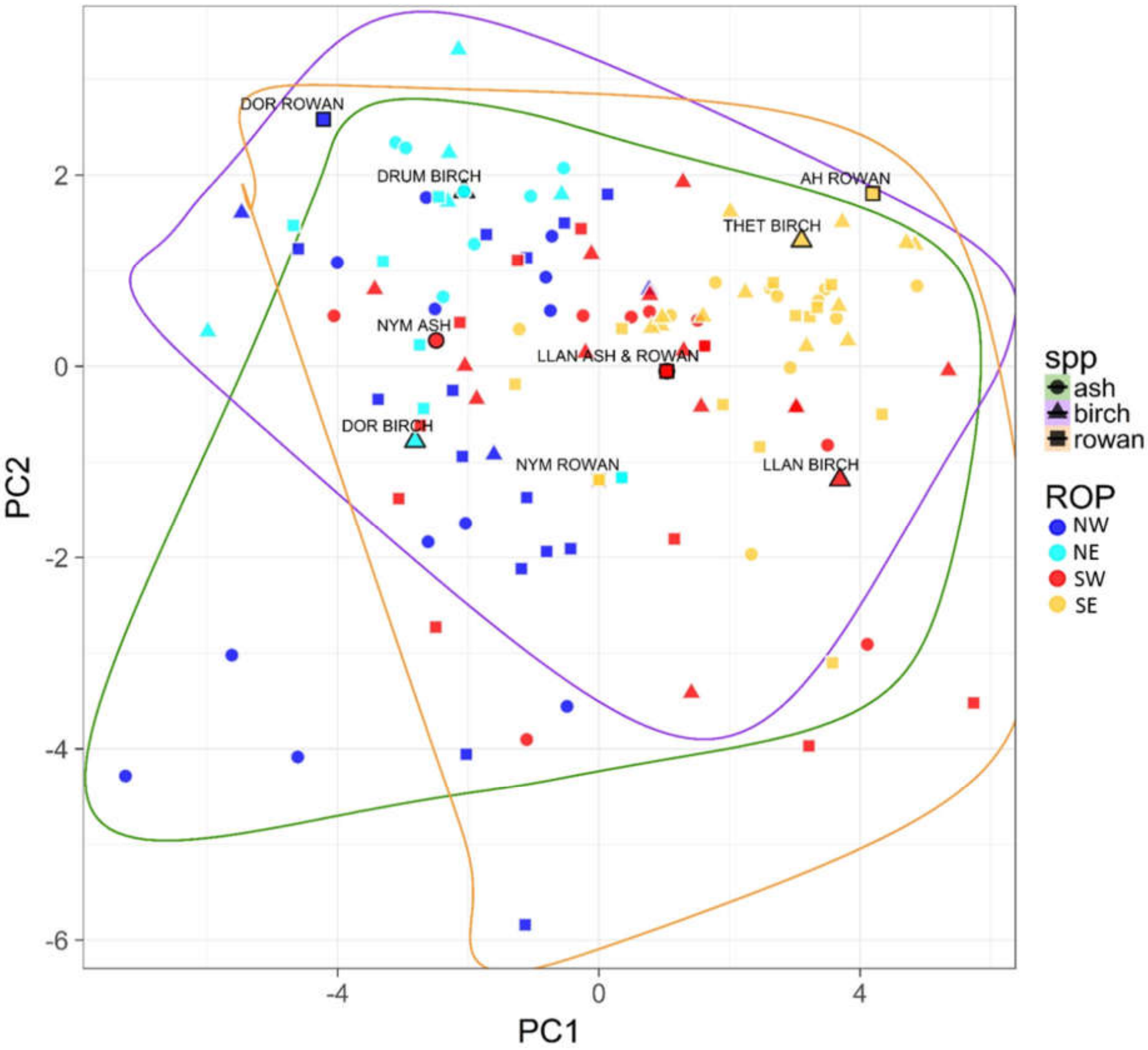


Figure 2.9. Scatterplot of provenance and trial sites PC1 and PC2 values. The three species: ash (round points and green polygon), rowan (square points and orange polygon), and birch (triangle points and purple polygon). The colour of the symbol corresponds to the ROP where the provenance or trial site is located. The symbols for the trial sites have a black outline and are labelled with the name of the trial site.

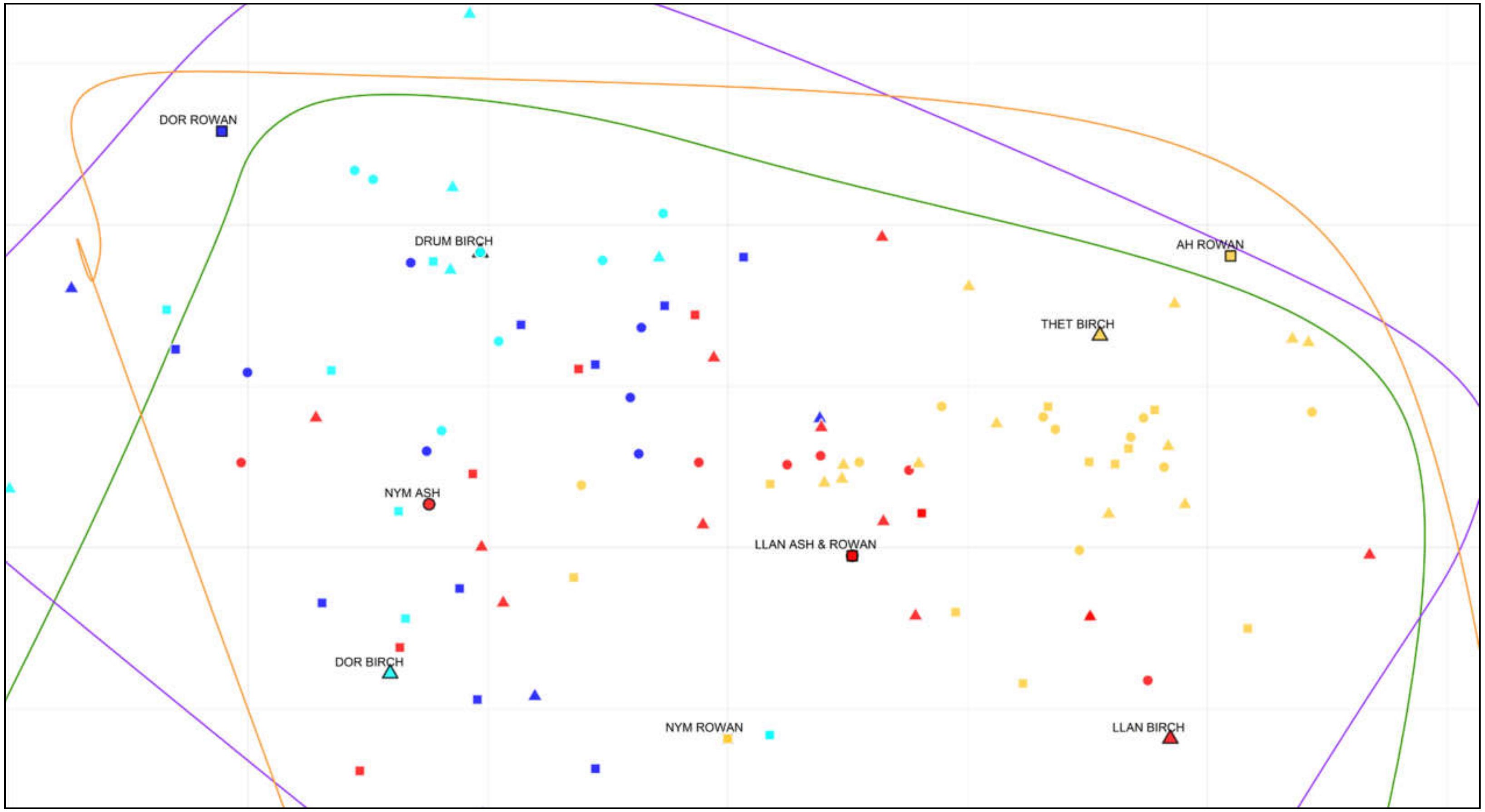


Figure 2.10. Detailed zoom into the trial site location, for the relative environmental distance between the trial sites and the most similar provenances. The legend id the following: circles ash, squares rowan and triangles birch. The colours represent the ROPs: dark blue NW, pale blue NE, red SW and yellow SE.

2.4 Discussion

This chapter explores the sampling locations to understand the variation of the climatic and geographic factors we start with and which we will use in the later chapter to explain the variation found in the results. Another reason that justifies the incorporation of this chapter is to explain why we had not included the seed zones or regions of provenance in the data analyses in the following chapters. It had been shown before (Salmela et al., 2010) how British seed zones, while they are geographically close by, it does not mean that they are climatically uniform and that provenances from different seed zones can be more climatically similar than others form the same seed zone. Also, topography is another factor that affects climate in a way that complicated defining seed zones (Campbell, 1978).

We have seen that there is a large amount of climatic variation in Great Britain and that the current seed zones might not be good enough to help us understand the variation. We have found that latitude is a major factor which encompasses most important variation, however we see that longitude is also important as it incorporated other aspects that latitude cannot. For example, in Finland or other Nordic countries latitude is the key factor to explain the variation north to south of tree variation (Viherä-Aarnio and Velling, 2008), however in GB we have an east west gradient as well due to continentality and topography.

Another thing to take into account is that latitude incorporates a very important non-climatic variable: photoperiod. The photoperiod is a key variable for tree growth (Downs & Borthwick, 1956), and its gradient, which is very large in GB, is incorporated in latitude, as they are negatively correlated.

With the analyses in this chapter we try to observe if our sampling is equilibrated within and between species, so that there we can interpret properly the results late on. The trial sites of the three species, we have found that the most different amongst them within a species are the rowan trial sites, they are the most extreme climatologically within the British climate. For this reason, we can expect that the phenotypic plasticity we might encounter could be larger in rowan because of the trial sites where trees are growing.

We have found that the provenances sampled come from a large range of environments, and that these are distributed across the three species in a similar pattern. The sampling locations for ash, rowan and birch share a core climatic envelope. There are also a few outlier provenances located out of the main core, 3 ash from NW provenances, 3 outliers of rowan from SW and NW, and 3 birch outliers of ROP NE and NW. Overall however the sampled provenances of the 3 species fall in the same range of environments, and this justifies across species comparison of patterns of adaptive variation with respect to environment in the following chapters.

The main climatic variables which contributed to the first principal component of the analysis were related with temperature, PC1 being positively correlated with growing degree days, growing season length, mean temperature in July, vapour pressure and consecutive dry days. The second principal component was more related with extreme and cold temperature (aspects of continentality), being correlated with extreme temperature range, windiness, snow and frost.

We found that latitude and longitude are highly correlated with PC1 and PC2. This justifies our use of longitude and latitude as a proxy for a combination of climate variables in the next chapters. In PC1 latitude explains a large component of the variation, between 50% and 70%, in the three species. In PC2 the variability is best explained by a multiple regression of latitude and longitude together. This geographical climatic structure of the sampling is very similar between the three species. Latitude explains the greatest variation for PC1 in rowan and birch for PC1, while in ash it is latitude together with longitude. For PC2 multiple regression of latitude and longitude best explains the variation for the three species.

The ROPs do not form four discrete climatic clusters separated from one another. Sampling locations from the four ROPs overlap in the PC1-PC2 scatterplot, the NW and the SW ROPs overlapping the most. Moreover, the four ROPs show a great degree of variation within them climatologically, with the western NW and SW ROPs exhibiting the greatest climatic variation across the locations where the three species grow. For this reason, we will not use ROP as a fixed nesting factor for the analyses of the adaptive differentiation data in the subsequent chapters, and will instead restrict ourselves to providing the colour coding for ROPs on figures to facilitate ease of results interpretation.

We found that most of the trial sites locations fall in the core area of the provenances, and at least the trials from each species fall within the polygon of each species. The sites located more outside of the core area are the rowan trials of Dornoch and Alice Holt. Thus, the conditions under which the trials have been grown are appropriate for studying patterns of adaptive variation of the three species, since they do not fall outside the normal range of conditions that would be encountered by the species in GB.

Finally, we found that in many cases the provenances more closely climatologically to the trial sites are not the most geographically proximate, and in some cases are from a different ROP altogether. These results therefore call into question the use of a seed zone systems with fixed boundaries such as that presently used in GB, because there is often a poor correlation between geographic and environmental differences between sites.

For these reasons exposed we find we justify why the best option is to use latitude and longitude for our analyses and other individual variables if they are important in the hypothesis. To not constraint our data with artificial boundaries, we do not incorporate the seed zones or the regions of provenance in our analyses on the following chapters as we have shown they do not fit well the variability of climates found in GB.

Chapter 3: Variation in growth traits among British populations of Ash, Rowan and Birch.

Abstract: In this chapter we study the variation in tree growth amongst British provenances of three broadleaved trees, the plasticity they show when growing in different environments, and correlations of growth traits with other relevant traits. We analysed tree height, DBH, survival and stem forking in over 30 native populations for 3 different broadleaved species: ash (*Fraxinus excelsior* L.), rowan (*Sorbus aucuparia* L), and silver birch (*Betula pendula* Roth.). These were sampled throughout Great Britain and grown in common garden experiments, in contrasting environments. The results showed that tree height and DBH decreases with increasing latitude of the provenance origin site. Tree height however is positively correlated with stem forking, which can be due to frost damage. We found different geographic patterns amongst the three species in their genetic variation for these traits. We also found large site effects, which in the case of birch included change in height:DBH ratio. These results show that it is important to analyse tree height together with other traits to understand whether trees are well adapted to the conditions where they are growing. This is of special relevance for planting trees in locations further north than their sites of origin.

3.1 Introduction

Understanding the distribution of genetic variation among tree populations is a crucial component of managing forest resources, in particular for the development of evidence-based policies for seed sourcing (Thomas et al. 2015; Whittet et al 2016). Knowledge of the genetic diversity, together with the variation in phenotypic plasticity, is needed to understand the capacity to adapt of tree populations. There are targets to increase the woodland cover in Great Britain (Scottish Executive, 2006; Forestry Commission, 2007; Welsh Assembly Government, 2009), which, will require planting of a large number of trees. Genetic resources in British native species are poorly characterised: most work has been done on non-native conifers, as they are the predominant timber production species (Lines, 1966, 1987, 1996 and 1967; Lee, 2002 and 2003; Samuel et al., 2007; Lee & Connolly, 2010). In particular, despite recent efforts (Hubert et al, 2010), little is known about the genetic diversity of native broadleaved species (Boshier & Stewart, 2005; Cavers & Cottrell, 2015).

To better understand how adaptive genetic variation is arranged in native broadleaved trees we can conduct and interpret results from long term common garden experiments. This type of experiment allows us to compare genetic origins within a trial site to measure the genetic component of variation (individual variation in genotype). It also allows comparison of the same genetic origin amongst trial sites to measure the environment component of variation (phenotypic plasticity). Assessments from well replicated common garden experiments established in multiple locations are of great practical value for management of genetic resources because they can be used both to determine patterns of genetic variation amongst populations of a focal species to guide seed transfer, and to gain an understanding of variation in phenotypic plasticity, which may be important in acclimatation to rapidly changing environments (Valladares et al., 2007).

In this study, we describe results from analysis of growth traits in a comprehensive series of field provenance tests of three British native broadleaf species, common ash (*Fraxinus excelsior* L.), rowan (*Sorbus aucuparia* L.) and silver birch (*Betula pendula* Roth). Since ash and silver birch have been used as sources of timber in Great Britain, a limited evidence base on provenance variation already exists (Worrell et al., 2000;

Cundall et al., 2003; Lee et al., 2015). In contrast, since rowan has been of lower silvicultural interest, knowledge of its genetic resources are extremely limited. Nevertheless these data are valuable because rowan is very frequently supplied in mixtures for new native woodland creation (Russell & Evans, 2003). Moreover its contrasting reproductive biology (animal pollination and dispersal) compared with ash and birch (which are wind pollinated and dispersed), may provide broader insights into patterns of variation across native GB tree species.

Height growth is particularly frequently assessed in provenance trials, because it ought to reflect an individual's ability to compete for resources, survive and grow (until the age of assessment) and, ultimately, height may confer an advantage in terms of pollen and seed dispersal (Ying & Yanchuk, 2006; Savolainen et al., 2007). Because it can be measured relatively easily and is of strong selective and silvicultural importance, it is a commonly measured trait in provenance trials. Tree height, in common with many other traits, is influenced by genetic factors, environmental factors and their interaction (Callaham, 1962; Morgenstern, 1996). Tree populations are likely to be subject to soft (i.e. non-lethal) directional selection on variation in height growth from generation to generation (Aitken et al., 2008), due to competition among neighbouring trees.

If tree growth reflects the adaptation of that tree to the length of the growing season in its site of origin, deployment of non-local planting stock based on seed collected from a warmer environment than that of the planting site provides a way to make gains in productivity in the absence of improved planting stock (Zobel & Talbert; 1984, White et al., 2007). Because such gains are based on historical adaptation to longer growing season than that of the planting site, it is possible that non-local trees which grow faster will also be exposed to temperatures during their growing period much lower than those to which they are adapted. If this causes damage there may undesirable side effects of exploiting longer growing seasons to increase productivity.

For this reason, differentiation among population means for height can be strong (Alberto et al., 2008; Aitken & Bemmels, 2016). However, considering variation in height as a proxy for fitness on its own can be problematic, for instance if height is only investigated at an early age, prior to the breakdown of age-age correlations (Mwase et al., 2008), or in the absence of severe climatic episodes (Worrell et al., 2000; Benito-Garzon et al., 2013). For these reasons, it is worthwhile considering covariance among other traits to add information about the trees' performance. Height is considered in many studies a proxy for good adaptation. Being taller will confer a competitive advantage for light availability, but it is worthwhile to investigate which trade-offs come with greater growth.

For this reason, measuring other traits such as stem form and survival can bring a better understanding of the tree response to the environment. Maladaptation to climate change can be measured by observing the overall shape and form of the tree. Tree shape variability can be due to different causes, but stem forking (as the loss of apical dominance in the main trunk or main branches) is due to the damage of the apical bud, which could be due to a poorly timed growth initiation in spring which can lead to frost damage of the bud (Kerr & Boswell, 2001). There are cases of tree mortality sue to frost damage (Park & Talbot, 2018).

In this chapter, we will present results from a series of comprehensive multi-site provenance trials of ash, birch and rowan in Great Britain. The knowledge that is currently available is limited both in terms of the number of provenances and the geographical scope of the studies, or is mainly focused on tree height alone. There is need of more evidence of adaptive variation in native tree species. The objective of this study is to discover whether (a) native British populations show genetic diversity for growth and in the geographical pattern of this variation. This can be captured with tree growth measurements amongst British provenances growing in the same environment; (b) there is evidence of phenotypic plasticity for tree growth.This can be shown by comparing the same provenances across sites; (c) there are genotype by environment interactions, due to difference in the ranking of provenances between trials, or other reasons; and (d) there are correlations with other traits which will extend our understanding of the adaptedness of the trees to the environment in which they are growing.

We expect that northern provenances will be shorter, as they adapted to shorter growing seasons, compared with southern provenances. We also expect that the environment of the planting site will affect the growth of the trees; growth will be overall lower in the most northern trial sites, due to the colder climatic conditions, but greater height of southern provenances will be retained, though this may be accompanied by more damage by frost. We hypothesise that trees from northern provenances will have been under greater selective pressures due to harsher environment conditions, for what they will be more constrained in their phenotypic plasticity, compared with the southern provenances. Regarding tree form, our expectation is that taller trees will have higher number of forks, and provenances from more oceanic origins, which have over the long term experienced higher incidence of late frosts, will fork less. We also propose that low survival will be an indication of mal-adaptation.

We expect to find differences amongst the three species, as they each have different ecological requirements. Ash grows primarily in fertile and more calcareous soils and prefers more benign climatic conditions; silver birch and rowan are much short lived trees which grow well in harsher environments. The use of three tree species allows us to see whether the predicted patterns of genetic variation and their response to being moved across different environments, are the same amongst the three species.

3.2 Materials and methods

3.2.1 Sampling strategy

The same sampling strategy was adopted for all three species; ash, *Fraxinus excelsior L.*, rowan, *Sorbus aucupari*a L. and silver birch, *Betula pendula* Roth .This was based on the Forestry Commission seed zone map of Great Britain (Forestry Commission, 1999; Figure 3.1). This divides the total area of Great Britain into 4 regions of provenance (North West NW, South West SW, North East NE and North West NW). These regions of provenance are further subdivided into a total of 24 seed zones based on natural topographical boundaries (Figure 3.1). Where possible two (three in a few cases for birch) self-sown semi-natural populations of each species (hereafter referred to as provenances) were sampled from each of the seed zones. Roughly equal numbers of seed were collected from a minimum of 20 (in birch a minimum of 30) maternal trees and combined together to constitute the provenance sample. Selection of mother trees was not deliberately biased towards superior phenotypes, and, where possible, mother trees were located at least 100m apart.

3.2.2 Trials and provenances by tree species

After extraction, the seeds collected were sown in nurseries and the resulting seedlings were planted in common garden experiments, 2 for ash, 4 for rowan and 4 for birch (location in Figure 3.2). The sites represent a range of environmental conditions (as illustrated in Table 3.1).

Ash (*Fraxinus excelsior* L.)

Seed from a total of 42 provenances of ash was grown in nurseries located near the trial sites. Nurseries were located at Whixley (Yorkshire) and Carmarthen (South Wales).

In 2007, one-year-old seedlings were planted in two common garden experiments (hereafter referred to as trials). The two trials are in South Wales (Llandovery - LLAN) and Yorkshire (North York Moors - NYM). The Llandovery trial contains trees from the 42 provenances, the one in the North York Moors has 40 provenances (Figure 3.2 and Table 3.2).

At each trial site the provenances were grown in a randomised block experiment. Each provenance was present as a single plot in each block, and there were three blocks. The plots consisted of 36 (6 x 6) trees in Llandovery and 30 (6 x 5) trees in North York Moors. Trees were planted at a distance of 2 metres apart.

All measurements in the Llandovery trial were made before the trial was infected by ash dieback (Jo Clark, *pers. comm.*). In North York Moors nearly all the measurements were collected before the trees were infected with the exception of DBH and forking, which was done the same year when a few of the trees had started showing some signs of infection. Since then, both trial sites have shown high level of infection.

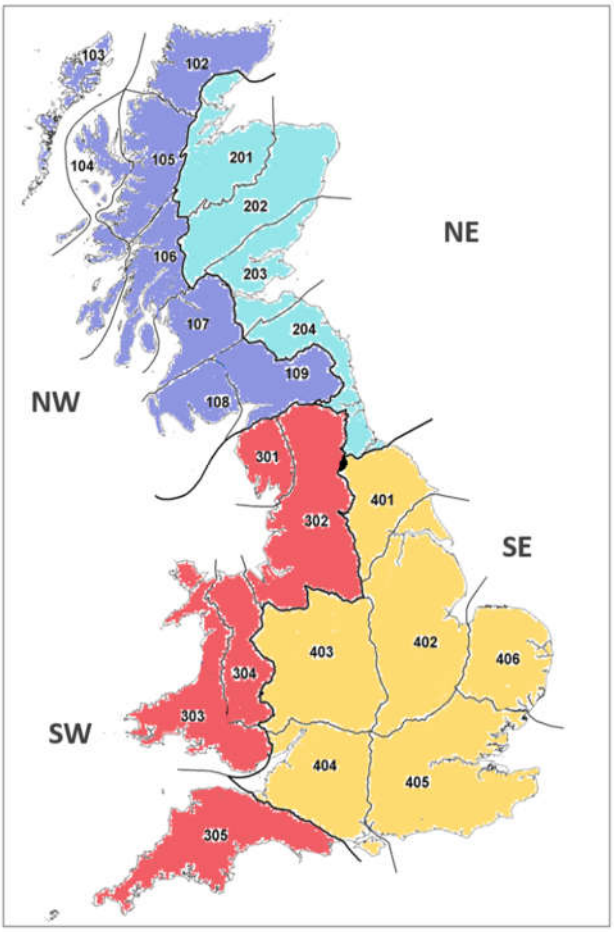


Figure 3.1. The four regions of provenance and 24 seed zones in Great Britain (modified from Herbert et al., 1999)

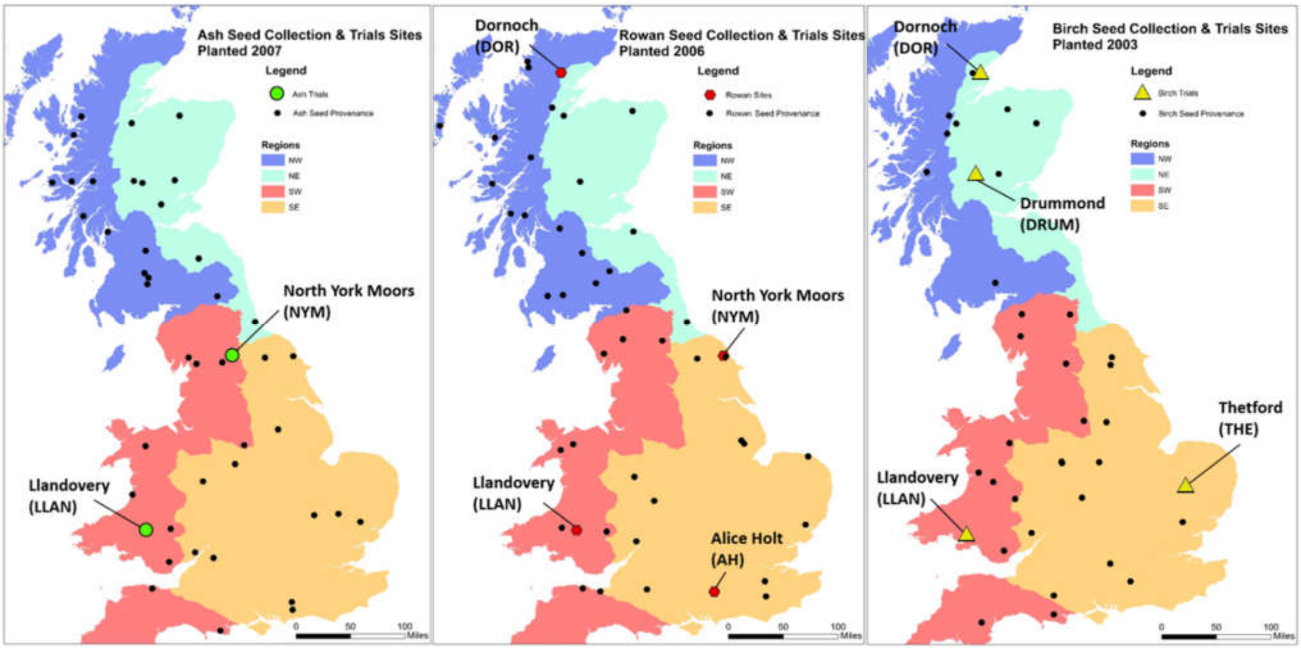


Figure 3.2. Seed collection (black small dots) and trial sites locations for ash (green dots, left), rowan (red dots, centre) and birch (yellow triangles, right).

Rowan (*Sorbus aucuparia* L.)

The rowan berries, from a total of 42 provenances, were sent to Forest Research's Newton field station near Elgin for manual extraction of seed from the fruits. The seeds were sown and raised into seedlings at Forest Research's nursery (Northern Research Station, south of Edinburgh). In 2006, when the seedlings were one year old they were planted in four trials around Great Britain. The trials sites (Figure 3.2) were in South Wales (Llandovery - LLAN), the South of England (Alice Holt - AH), East Yorkshire (North York Moors - NYM) and the North of Scotland (Dornoch - DOR). However, the number of provenances represented at each of the trial sites is not consistent due to shortage of plants raised from some provenances. Consequently, some provenances are absent from some of the trials (Table 3.3). From the 42 total provenances, only 29 of them were planted in all four trials.

The experiment is based on a randomized block design, and consists of three replicated blocks. Trees were planted at a distance of 2 metres. Each plot contains 9 (3 x 3) trees from the same provenance.

Silver Birch (*Betula pendula* Roth)

The silver birch seeds, from a total of 33 provenances, were grown in the Forest Research's nursery (Northern Research Station, south of Edinburgh). Four trial sites were planted in 2003, two in Scotland at Drummond (DRUMM) and Dornoch (DOR), one in South Wales at Llandovery (LLAN) and one in Norfolk at Thetford(THET). The majority of the provenances are represented at Drummond, Llandovery and Thetford, however the Dornoch trial contains only 12 provenances (Table 3.4).

A randomized block design was used based on three replicated blocks. Each plot consisted of 25 (5 x 5) to 36 (6 x 6) trees per provenance, depending on the site. Trees were planted at 2 m spacing.

Table 3.1. Details of the all the trial sites.

Spp	Trial Site	Planting Year	County	Lat	Lon	CT	MD	Alt (m)	AP (mm)	GDD	DAMS	Soil Type
Ash												
	NYM (North York Moors)	2007	East Yorkshire	54.3	-1.9	9.0	153	113	960	1475	10.0	Riverine Floodplain. Clay to Sandy Loam. Freely draining floodplain soils.
	LLAN (Llandovery)	2007	Carmarthenshire	51.9	-3.8	8.1	108	215	1329	1470	15.0	Sandstone and Mudstone. Sand to Loam. Freely draining acid loamy soils over rock
Rowan												
	DOR (Dornoch)	2006	Sutherland	58.1	-4.4	4.2	91	130	1115	1070	15.2	Podzol. Clayey loam to sandy loam. Glacial till.
	NYM (North York Moors)	2006	East Yorkshire	54.3	-0.5	8	149	197	759	1328	14.8	Very acid loamy upland soils with a wet peaty surface. Silty loam to sandy loam. Peaty.
	LLAN (Llandovery)	2006	Carmarthenshire	51.9	-3.8	8.1	109	240	1329	1474	15.1	Sandstone and Mudstone. Sand to Loam. Freely draining acid loamy soils over rock.
	AH (Alice Holt)	2006	Surrey	51.2	-0.8	10.2	186	118	785	1772	13.4	Slowly permeable seasonally wet slightly acid but base-rich loamy and clayey. Claystone/mudstone. Loam to clay.
Birch												
	DOR (Dornoch)	2003	Sutherland	57.9	-4.1	4.1	99	135	745	1097	15.2	Umbrisol. Sand to loam. Peaty Gley.
	DRUM (Drummond)	2003	Perthshire	56.6	-4.1	6.1	89	218	1262	1143	11.0	Umbrisol. Clayey loam to sandy loam. Glacial till.
	THET (Thetford)	2003	Norfolk	52.4	0.6	10.8	214	54	608	1751	12.4	Chalk. Chalky, sandy loam. Freely draining sandy breckland soils.
	LLAN (Llandovery)	2003	Carmarthenshire	52.6	-4.1	7.1	139.	114	1503	1754	13.8	Glacial till. Loam to clayey. Slowly permeable seasonally wet acid loamy and clayey soils.

The information relating to the climatic conditions at each of the trial sites was obtained from ESC-Ecological Site Classification (Clare and Ray 2001). We focused on five parameters because we considered them likely to reflect elements of the climate that are key to tree growth: temperature, water availability, and other variables which are dependent on the geographical location of the site. The indices and their definitions are:

CT: Continentality, corresponds to the Conrad Index (Conrad 1946). $CT = 1.7 [A/\sin(\varphi + 10)] - 14$. Where A is the difference between the mean temperature of the warmest and coldest month in degrees Celsius and φ is latitude in degrees. Lower values indicate more oceanic climates.

MD: Moisture deficit (mm).To consider the effects of the dryness of the growing season moisture deficit is calculated by considering the potential evaporation and precipitation. Higher values indicate drier sites.

AP: Annual precipitation. Average annual rainfall (mm) between 1961 and 1990.

GDD: growing degree days, the cumulative sum of the number of degrees Celsius above 5 ^{o}C on each day of the year in which mean temperature exceeds 5 ^{o}C. Values expressed here are long term mean annual values for the years spanning from 1961 to 1990.

DAMS: Wind exposure measured by **Detailed Aspect Method of Scoring**: This provides an indication of wind exposure, the higher the values the more exposed the site. Sites with values under 12 can be considered to be sheltered.

Soil Type: the soil information data were obtained from different inventories, using the UK Soil Observatory (UKSO). The European Soil Bureau description (The European soil database, 2006) was used to assign a soil type for all British trial sites. The soil texture and soil group data were obtained from Soilscapes (Cranfield University, 2017) for Welsh and English locations, and from the national soil map of Scotland (Soil Survey of Scotland Staff, 1981) for the Scottish trials.

Table 3.2. Details of the ash provenances locations and the trial site they are planted in. The “X” indicates presence of a provenance at a given site.

ROP	Country	Ash provenances	Lat	Long	Alt (m)	Site	
						LLAN	NYM
NW	Scotland	Duisdale, Skye	57.176	-5.751	18	x	x
		Kilninian, Mull	56.530	-6.208	71	x	x
		Rassal Wood, Kishorn	57.426	-5.591	78	x	x
		Ardtornish,Morvern	56.560	-5.741	20	x	x
		Glasdrum Wood, Loch Creran	56.574	-5.232	33	x	x
		Add Valley, Kilmichael Glassary	56.106	-5.420	30	x	x
		Clyde Valley	55.680	-3.913	159	x	x
		Shielhill Glen	55.911	-4.825	107	x	x
		Penpont	55.235	-3.853	90	x	x
		Nith Valley	55.320	-3.829	141	x	x
		Crawick Water	55.381	-3.929	162	x	x
	England	Warks Burn	55.088	-2.222	90	x	x
NE	Scotland	Erchite Wood, Dores	57.368	-4.345	56	x	x
		Craigellachie	57.484	-3.170	102	x	x
		Fearnan Forest, Kenmore	56.579	-4.037	142	x	x
		Glen Lyon	56.602	-4.248	183	x	x
		Den of Alyth	56.623	-3.258	152	x	
		Pitcairns Glen, Dunning	56.300	-3.573	119	x	x
		Tweed Valley North Glen	55.588	-2.662	68	x	x
	England	Castle Eden Dene, Peterlee	54.743	-1.352	102	x	x
SW	England	Witherslack	54.264	-2.870	79	x	x
		Park Wood&Hutton Roof	54.182	-2.689	170	x	x
		Via Gellia Woods	53.104	-1.619	239	x	x
		Upper Wharfedale	54.203	-2.104	202	x	x
		Greta Wood, Purbeck Ridge	50.637	-2.136	126	x	x
		Horner Wood, Porlock	51.189	-3.583	102	x	x
	Wales	Cardiff Area	51.546	-3.234	158	x	x
		Aberystwyth Area	52.430	-4.059	90	x	x
		Betws-y-Coed	53.079	-3.799	57	x	x
		Talgarth	51.986	-3.213	198	x	x
SE	England	Forge Valley	54.274	-0.490	52	x	x
		Ashberry Woods	54.262	-1.133	142	x	x
		Treswell Woods	53.308	-0.861	54	x	
		Hayley Wood	52.158	-0.110	79	x	x
		Tick Wood, Ironbridge	52.621	-2.523	99	x	x
		Forest Bank, Marchington	52.852	-1.820	142	x	x
		Wyndcliff, Wye Valley	51.678	-2.679	208	x	x
		Midger Wood	51.606	-2.285	160	x	x
		Pheasant Copse, Petworth	51.011	-0.628	60	x	x
		Bignor Hill	50.908	-0.616	194	x	x
		Groton Wood	52.050	0.883	66	x	x
		Out Wood	52.166	0.415	96	x	x

Table 3.3. Details of the rowan provenances locations and the trial sites in which they are planted. The “X” indicates presence of a provenance at a given trial site.

ROP	Country	Rowan provenances	lat	long	Alt (m)	Site			
						AH	DOR	LLAN	NYM
SW	Scotland	Assynt	58.171	-5.261	27.48		x		x
		Inverpolly	58.093	-5.232	82.12		x		
		Allt Volagir, South Uist	57.247	-7.310	68.76		x	x	x
		Salen, Isle of Mull	56.527	-5.961	5.80		x	x	x
		Tokavaig, Isle of Skye	57.139	-5.965	25.40		x	x	x
		Glen Loy	56.901	-5.071	61.96		x		
		Add Valley	56.141	-5.486	33.72	x	x	x	x
		Strathlachlan	56.128	-5.152	70.32	x	x	x	x
		Falls of Clyde	55.653	-3.778	160.84	x	x	x	x
		Mugdock Country Park	55.971	-4.320	161.8		x	x	x
		Glenlee	55.088	-4.194	129.68	x	x	x	x
		Stroan Bridge	55.070	-4.545	66.72		x		
		Lochwood	55.258	-3.443	180.2	x	x	x	x
		Ettrick Water	55.420	-3.133	234.16	x	x	x	x
SE	Scotland	Bunchrew	57.471	-4.315	100.8		x		x
		Craigdarroch	57.574	-4.605	31.8		x		x
		Cleanhill Wood, Aberchirder	57.554	-2.636	158.76		x		x
		Birks of Aberfeldy	56.602	-3.872	285.96	x	x		x
		Pressmennan Wood	55.951	-2.589	149.84	x	x	x	x
		Castle Eden Dene	54.743	-1.351	107.64	x	x	x	x
NW	England	Horner Wood	51.194	-3.589	232.84	x	x	x	x
		Holford/Hodder’s Combe	51.158	-3.218	164.88	x	x	x	x
		Duddon Valley	54.316	-3.230	61.84	x	x	x	x
		Naddle Forest	54.511	-2.805	291.24	x	x	x	x
		Brignall Banks	54.496	-1.913	222.36	x	x	x	x
		Gelt Wood	54.903	-2.733	139.16	x	x	x	x
	Wales	Brechfa	51.993	-4.063	146.28	x	x	x	x
		Beddgelert	53.025	-4.138	257.76	x	x	x	x
		Mynydd Du	51.954	-3.105	532.12	x	x	x	x
		Ugly House	53.106	-3.868	231.32	x	x	x	x
NE	England	Forge Valley& Raincliffe Woods	54.275	-0.484	89.64	x	x	x	x
		Ashberry and Reins Woods	54.253	-1.128	106.24	x	x	x	x
		St. He len’s Wood, Coningsby	53.113	-0.123	6.24	x	x	x	x
		Moor Farm	53.156	-0.181	14.8	x	x	x	x
		Pepper Wood	52.368	-2.092	144.76	x	x	x	x
		The Ercall	52.687	-2.522	185.8	x	x	x	x
		King’s Bottom, Longleat	51.191	-2.241	219	x	x	x	x
		Chestnuts Wood, Forest of Dean	51.829	-2.470	161.72	x	x		x
		Saxonbury Hill	51.076	0.251	183.12	x	x	x	x
		Seal Chart	51.278	0.237	129.44	x	x	x	x
		Culter’s Wood, Freston, Ipswich	52.011	1.142	34.4	x	x	x	x
		Felbrigg great Wood, Cromer	52.913	1.264	83.24	x	x	x	x

Table 3.4. Details of the birch provenances locations and the trial sites in which they are planted .The “x” indicates presence of a provenance at a given trial site.

ROP	Country	Birch provenances	lat	long	Alt (m)	Site			
						DOR	DRUMM	LLAN	THET
NW	Scotland	Affric	57.31	-4.80	118		x		x
		Loch Creran	56.55	-5.28	26	x	x	x	x
		Dumfried	55.11	-3.58	29	x	x	x	x
NE	Scotland	Elgin	57.42	-3.38	140	x	x	x	x
		Great Glen	57.21	-4.62	68				
		Glen Garry	57.07	-4.83	54		x	x	x
		Spinningdale	57.89	-4.26	38	x	x	x	x
		Dunkeld	56.56	-3.56	143	x	x	x	x
		Alford	57.24	-2.67	142		x	x	x
SW	England	Bovey Tracey	50.59	-3.71	120			x	x
		Bovington Camp	50.71	-2.21	50		x	x	x
		Penrith	54.7	-2.96	280		x	x	x
		Ambleside	54.4	-2.98	60		x	x	x
		Bolton Abbey	54.04	1.95	220		x	x	x
		Hamsterley Forest	54.7	-1.86	200	x	x	x	x
		Sheffield	53.28	-1.56	240			x	x
	Wales	Machynlleth	52.58	-3.85	90			x	x
		Taffs Well	51.55	-3.27	100		x	x	x
		Llanidloes	52.46	-3.53	180		x	x	x
		Persteigne	52.24	-3.05	200		x	x	x
		Llangollen	52.98	-3.19	170				x
SE	England	Castle Howard	54.12	-0.92	76		x	x	x
		Sand Hutton	54.02	-0.95	25	x	x	x	x
		Leicester	52.73	-1.23	160		x	x	x
		Clumber park	53.26	-1.06	55	x	x	x	x
		Cannock Chase1	52.72	-2.04	180		x	x	x
		Cannock Chase 2	52.74	-2.05	140	x	x	x	x
		Monmouth	51.79	-2.69	170		x	x	x
		Tollard Royal	50.96	-2.21	130	x	x	x	x
		Godalming	51.14	-0.6	170			x	x
		Baingstoke	51.38	-1.02	50		x	x	x
		Braintree	51.91	0.54	80	x	x	x	x
		Dunwich	52.26	-1.61	20	x	x	x	x

3.2.3 Measurements

Height

The trees were assessed for height at different times (between ages 1 and 10) the last measurement of which was made between 2010 and 2015 in different trials (detailed in Table 3.5). Measurements were made for each individual tree, all trees per plot. Height was measured to the nearest centimetre in one of two ways, either with a measuring tape while trees were short and with extendable measuring rod to reach the top when the trees were taller. Missing and dead trees were excluded to calculate mean values.

Diameter

Stem diameter at 1.35 m (breast height) was measured with a diameter tape to the nearest half centimetre (5mm). Stem diameter was not measured in rowan trees because most were multi-stemmed from the ground level, especially at Dornoch and Llandovery where many trees had over 10 stems. In the ash and birch trials tree diameters were measured in 2015 (when trees were 8 and 12 years old, respectively Only the trees in the centre of the plot were measured, the number depending on the trial. For plots of 25 trees the 9 (3x3) central trees were measured, for plots of 30 trees the 12 (3x4) central trees were measured and for plots of 36 the inner 16 (4x4) trees were measured. Missing and dead trees were excluded to calculate mean values.

Stem Forking

Ash and birch trees were assessed for stem forking in the two trials of ash (Llandovery and North York Moors) and three of birch (Llandovery, Drummond and Dornoch). The number of forks was counted as the number of times the main stem lost apical dominance. This was assessed following the main stem from the ground up, including the subsequent branches originated from each fork up to the crown, excluding the small twigs. Only the trees at the centre of the plot were assessed and the exact number per plot differed depending on the trial. For plots of 25 trees, the 9 (3x3) central trees were assessed, for plots of 30 trees, the 12 (3x4) central trees were assessed and for plots of 36, the inner 16 (4x4) trees were assessed. The average number of forks per plot was used in subsequent analyses. Rowan was not assessed for forking, once again because of its multi-stem habit and the difficulty of identifying which the main stem. Missing and dead trees were excluded to calculate mean values.

Survival

Survival was measured as a percentage of the remaining living trees compared to the number of trees planted in each plot.

Table 3.5. Data analysed by trial and species, the age of the trees and the date when each trait was measured are shown.

	Height			DBH			Forking			Survival		
Assessment	**Age**	**Year**	**Trial sites**	**Age**	**Year**	**Trial sites**	**Age**	**Year**	**Trial sites**	**Age**	**Year**	**Trial sites**
Ash	5	2012	All 2 trials	8	2015	All 2 trials	8	2015	All 2 trials	6	2013	All 2 trials
Rowan	9	2015	All 4 trials	n/a			n/a			9	2015	All 4 trials
Birch	8	2010	All 4 trials	12	2015	LLAN and DRUM	12	2015	LLAN and DRUM	8	2010	All 4 trials

3.2.4 Analysis

Data analysis and visualisation were performed in the R statistical environment (R version 3.2.3, Core Team, 2015).

Analysis of Sources of Variation

To determine the effects of provenance, site and their interaction on each trait (Table 3.5) the plot mean values were analysed in Analysis of variance (ANOVA) using a linear model. The different types of ANOVAs used were the following:

i. Analyses by species, by trait and by trial site. In the ANOVA provenance was a fixed factor and block was a random factor.
ii. Analysis by species and by trait across all trial sites. In the ANOVA site, provenance, and site by provenance interaction were fixed effects and block nested within site was a random factor.

Transformation was applied to the forking (logarithmic) and survival (arcsine) data to ensure that they fitted a normal distribution. For the analysis of variance across sites the provenances that were not present in all the trial sites were excluded. This gave 40 provenances for ash, 29 for rowan and 26 for birch (12 when including Doroch trial site). To visualize the site by trial interaction, interaction plots were undertaken.

Relationship with Environmental Factors

For each trait linear regressions were applied to test the relationship between provenance mean values and latitude and longitude of the home site. Linear regression plots were drawn.

Relationship among traits

To determine the relationships between provenance mean values for different traits, Pearson's correlation coefficients were calculated using data from individual sites.

3.3 Results

3.3.1 Ash

3.3.1.1 Tree growth Ash

i. Analysis of Variance

Differences amongst provenances: In ash there were significant differences in tree height among provenances in Llandovery ($p<0.001$) and in North York Moors ($p<0.05$). The differences amongst provenances were larger in Llandovery in absolute terms, however in both sites the tallest provenance was 45% taller than the shortest provenance.

For stem diameter, there were only significant differences amongst provenances in the Llandovery trial ($p<0.001$), no significant differences in North York Moors. The difference between the provenance with the biggest and the smallest DBH was higher in North York Moors (52.72%) than Llandovery (33.95%).

Site effect: There was a significant site effect ($p<0.001$) for ash height. There was also a significant site by provenance effect for height ($p<0.05$) (Figure 3.3). All the provenances were shorter in North York Moors than in Llandovery (Figure 3.3, Table 3.7). The site average height was 50.17% greater at Llandovery than at North York Moors. There was a greater range of height in Llandovery, from 1.707 m – 4.068 m (2.361 m), while in North York Moors was 0.585m – 1.920m (1.335m). The site by provenance effect could be due to this larger range of height amongst provenances in the southern trial.

For DBH there was a significant site effect ($p<0.001$), but no significant site by provenance interaction. DBH was on average 54.66% higher in Llandovery than in North York Moors.

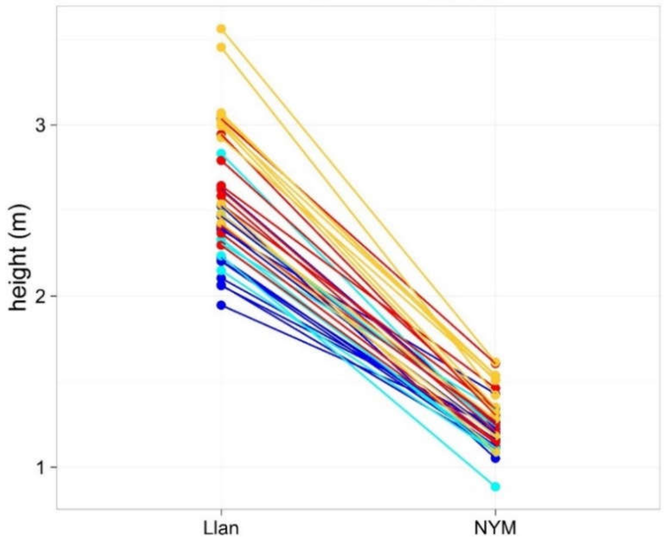


Figure 3.3. Ash height yr 5 interaction plot. Provenance means of each trial site. Colour legend: **yellow** (ROP SE), **red** (ROP SW), **dark blue** (ROP NW) and **pale blue** (ROP NE).

ii. Relationship of height with geographical location of seed origin

The latitude of the provenance explained around 40% of the variance in height (Figure 3.4) and in DBH in both trial sites ($p<0.001$) (Table 3.10), but there was no effect of longitude. The more northerly the provenance, the shorter were the trees and the smaller their stem diameters.

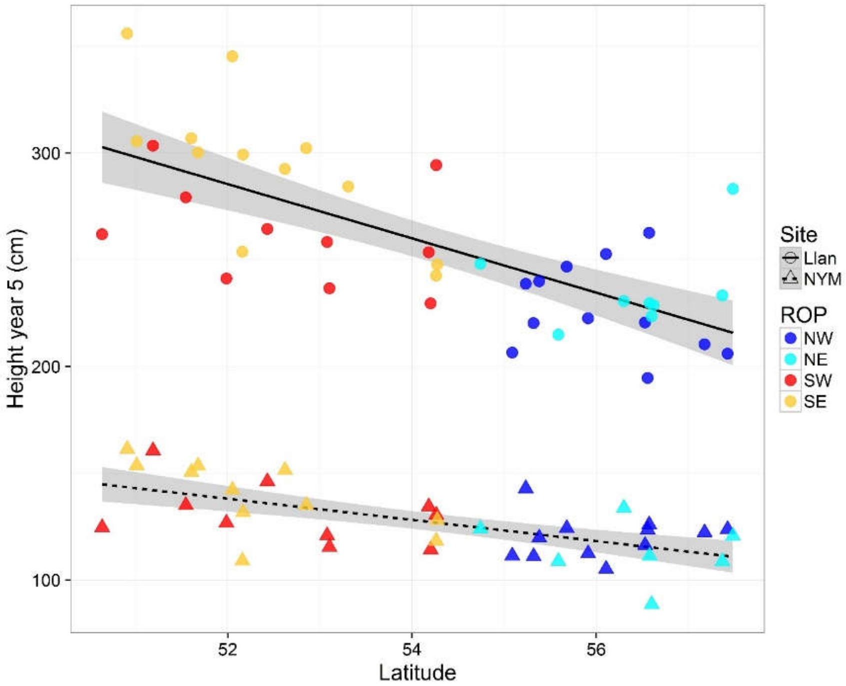


Figure 3.4. Ash trees height yr 5 in the two trial sites by latitude. Provenances means, arranged according to their latitude from more south (left on the x axis) to north (right). Regression Adj R^2 and p value in Table 3.10. Colour legend: **yellow** (ROP SE), **red** (ROP SW), **dark blue** (ROP NW) and **pale blue** (ROP NE). Latitude degrees of the LLAN trial 51.96 and NYM trial 54.3.

3.3.1.2 Stem forks Ash

i. Analysis of Variance

Differences amongst provenances: In ash, there were significant differences among provenances ($p<0.05$) in stem forking at North York Moors but not Llandovery. The difference between the most and least forked provenances were much higher in North York Moors (79.22%) than Llandovery (57.18%).

Site effect: There was a significant site effect ($p<0.01$) but no significant provenance by site interaction. Incidence of forking was 18.82% higher in Llandovery (with 2.4 forks on average) than at North York Moors (with 1.9 forks on average). All provenances forked less in North York Moors with the exception of six provenances (four from SE ROP, one from SW ROP and one from NW ROP) (Figure 3.3).

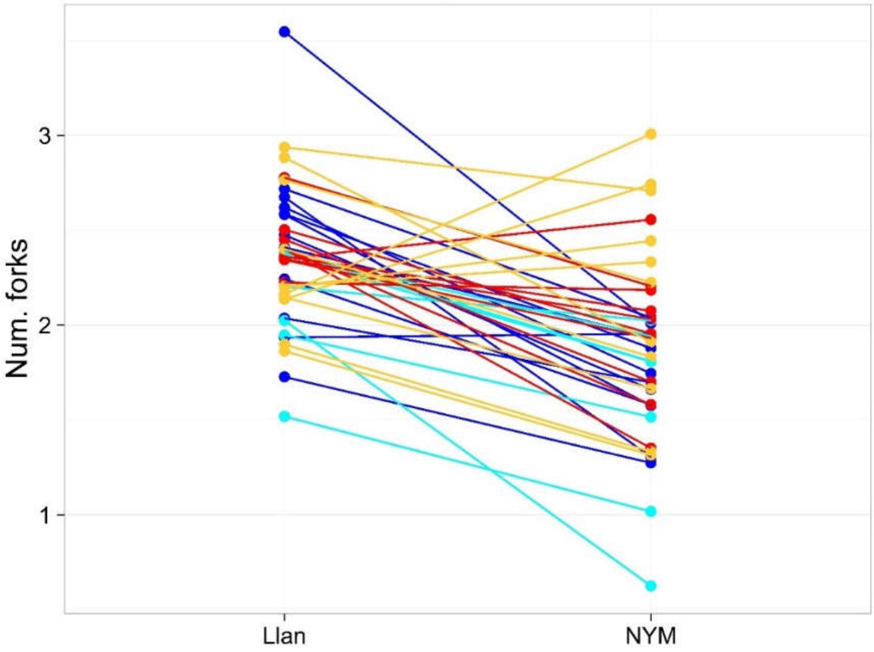


Figure 3.5. Interaction plot on provenance forking means in the two ash trials. Colour legend: yellow (ROP SE), red (ROP SW), **dark blue** (ROP NW) and pale blue (ROP NE).

ii. Relationship of forking with the geographical location of seed origin

There was a significant regression with latitude in North York Moors, where latitude explained 21% of the variation (p<0.001). Provenances from southern sites forked more than those from the north. In the case of Llandovery, latitude did not explain the variation. (Table 3.10 and Figure 3.6). Longitude had no effect.

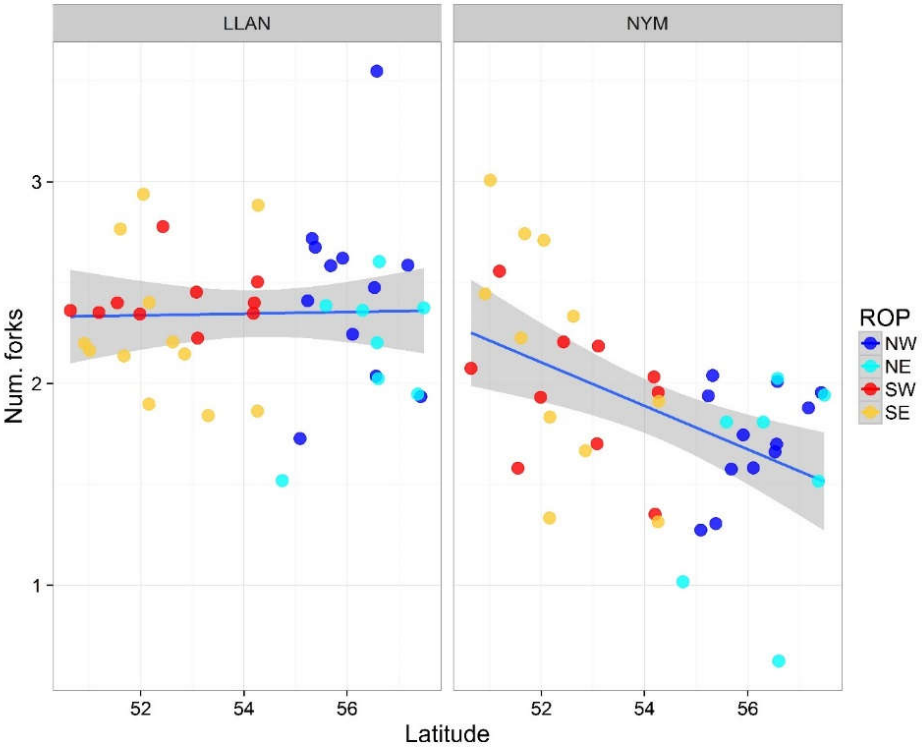


Figure 3.6. Regression of fork means by latitude, in both trial sites (LLAN left and NYM right). The regression in LLAN is not significant. Colour legend: **yellow** (ROP SE), **red** (ROP SW), **dark blue** (ROP NW) and **pale blue** (ROP NE).

3.3.1.3 Survival in Ash

i. Analysis of Variance

Differences amongst provenances: In ash there were no significant differences amongst provenances for survival in any of the sites.

Site effect: There was a significant site effect (p<0.001), survival was 7.75% higher on average in Llandovery. Most provenances had lower survival in North York Moors, with some exceptions from western provenances (Figure 3.7). However there was no significant interaction between site and provenance.

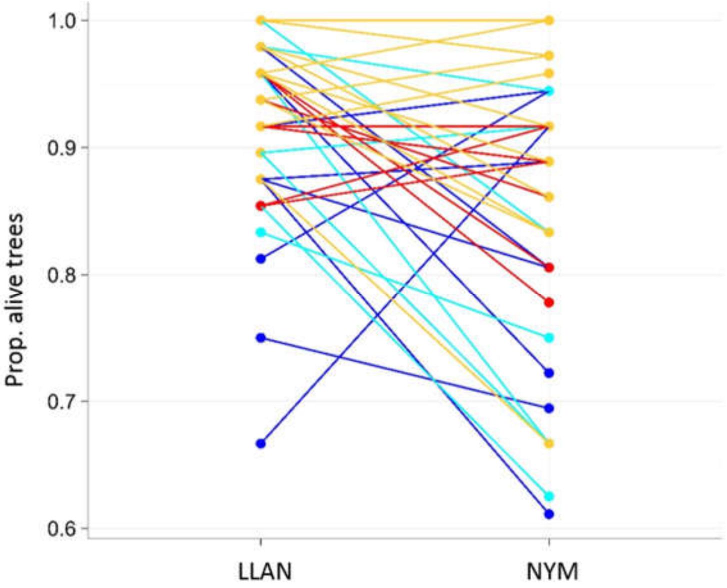


Figure 3.7. Interaction plot on provenance survival means in the two ash trials. Colour legend: **yellow** (ROP SE), **red** (ROP SW), **dark blue** (ROP NW) and **pale blue** (ROP NE).

ii. Relationship of survival with the geographical location of seed origin

There was no significant regression of survival with either latitude or longitude.

3.3.1.4 Relationships among traits in Ash

There was a significant (p<0.001) positive correlation between tree height and number of forks in both trials of ash. The Pearson's coefficient in Llandovery was 0.371 and North York Moors is 0.591. The tallest trees had greater number of forks, although this relationship was much clearer in North York Moors (Figure 3.8).

Height and DBH were strongly correlated at both sites, North York Moors (r = 0.89, p<0.001) and Llandovery (r= 0.81, p<0.001).

There were significant positive correlations between survival and height in both trials, Llandovery (r=0.402, p<0.001) and North York Moors (r=0.730, p<0.001).

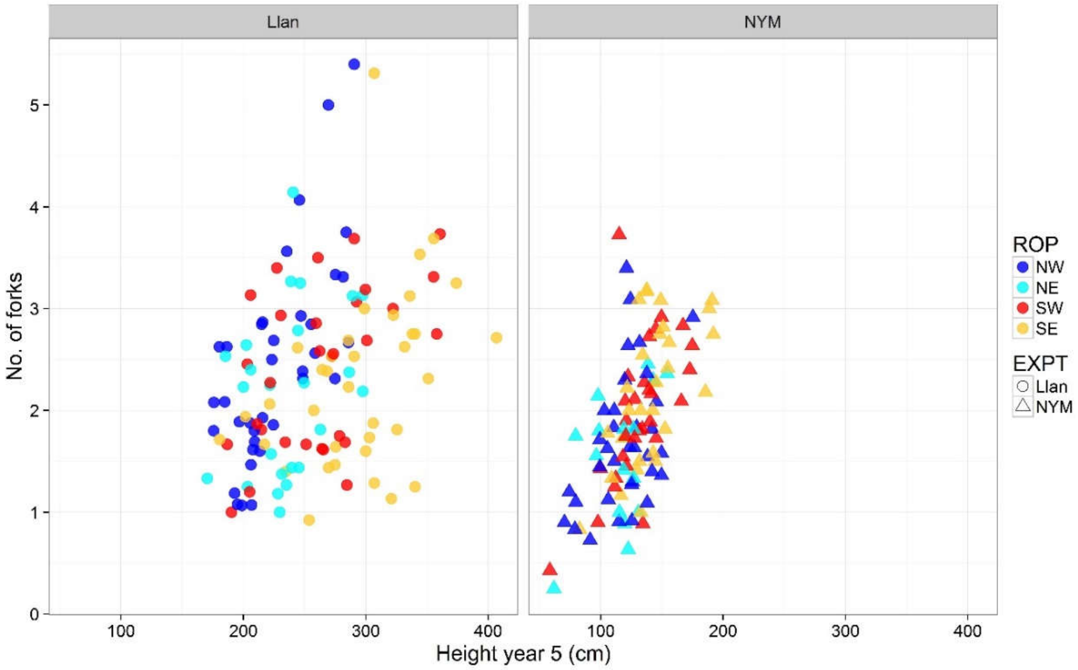


Figure 3.8. Number of forks versus height in the two ash trials, LLAN left and NYM right. Colour legend: **yellow** (ROP SE), **red** (ROP SW), **dark blue** (ROP NW) and **pale blue** (ROP NE).

3.3.2 Rowan

3.3.2.1 Tree height in Rowan

i. Analysis of Variance

Differences amongst provenances: In rowan there were significant differences amongst provenances at all trial sites ($p<0.001$ in Llandovery and Dornoch; $p<0.05$ in Alice Holt and North York Moors). The biggest difference between the tallest and shorts provenances were in Dornoch (65.94%) and the smallest difference in Alice Holt (24.03%). Despite the biggest difference in absolute terms being in Llandovery (2.8m of difference) compared with Dornoch (1.8m), in both cases this was because of one NW outlier provenance.

Site effect: There was a significant site effect ($p<0.001$) on height but no significant interaction between provenance and trial (Figure 3.9). The trees grew taller in Llandovery (Table 3.6, Figure 3.9) followed, in this order, by Alice Holt, Dornoch and North York Moors. Trees in Llandovery were on average 72.96% taller than in North York Moors.

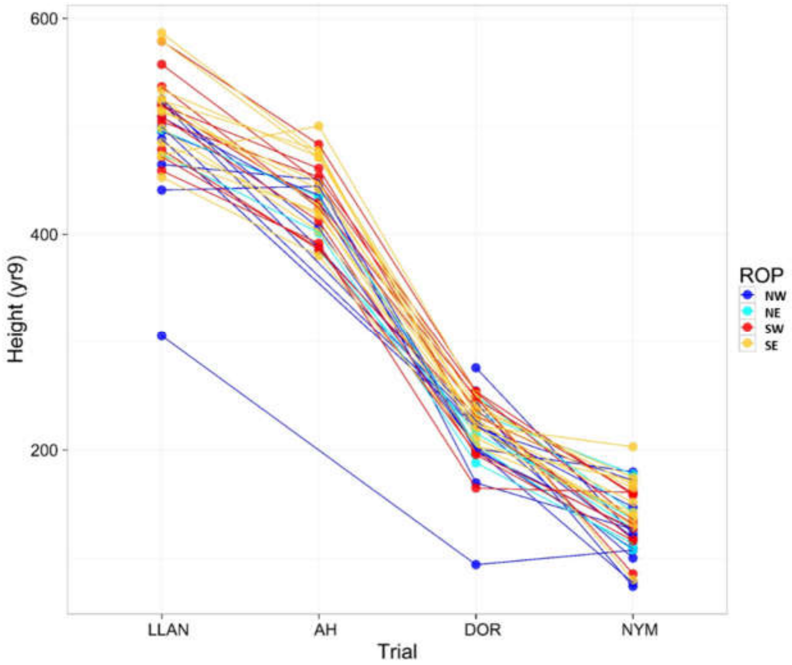


Figure 3.9. Interaction plot of the provenance means of the rowan tree height measured at age 9, in the four sites. Colour legend: yellow (ROP SE), red (ROP SW), dark blue (ROP NW) and pale blue (ROP NE).

ii. Relationship of tree growth with the geographical location of seed origin

In rowan, latitude explained height differences in the two southern trials (Llandovery and Alice Holt) where trees from northerly provenances were generally shorter (Table 3.10, Figure 3.10). The linear regression with latitude was not significant in the two most northern trials. Longitude was significant but explained only 4% of the variation among provenances in North York Moors.

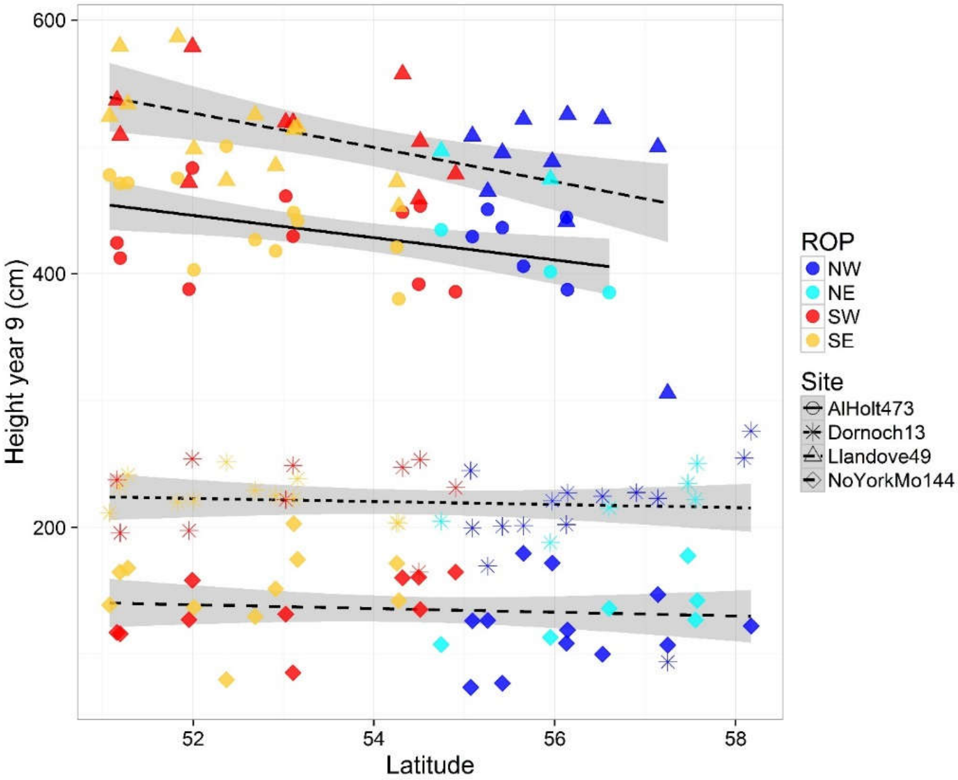


Figure 3.10. Rowan trees height yr 9 in the two sites versus latitude. Provenances means arranged according to their latitude from more south (left on the x axis) to north (right). Regression Adj R^2 and p value in Table 3.10. Colour legend: **yellow** (ROP SE), **red** (ROP SW), **dark blue** (ROP NW) and **pale blue** (ROP NE). Latitude of the AH 51.16, DOR 58.04, LLAN trial 51.96 and NYM trial 54.3.

3.3.2.2 Survival Rowan

i. Analysis of Variance

Differences amongst provenances: There were only significant differences on tree survival amongst provenances in the Llandovery ($p<0.05$) and North York Moors ($p<0.001$) trial sites. In these two trials the differences amongst survival provenance means were greater (22.22% in Llandovery and 83.33% in North York Moors).

Site effect: In rowan, the survival mean in all trials was over 90% live trees, except in North York Moors. The North York Moors site had a very cold, windy and wet environment (Table 3.1) which resulted in the death of many trees during some very cold winters. There was a significant site effect ($p<0.001$) for the tree survival and a significant interaction ($p<0.001$) between provenance and site. Llandovery has the highest survival mean, 35.51% higher than North York Moors.

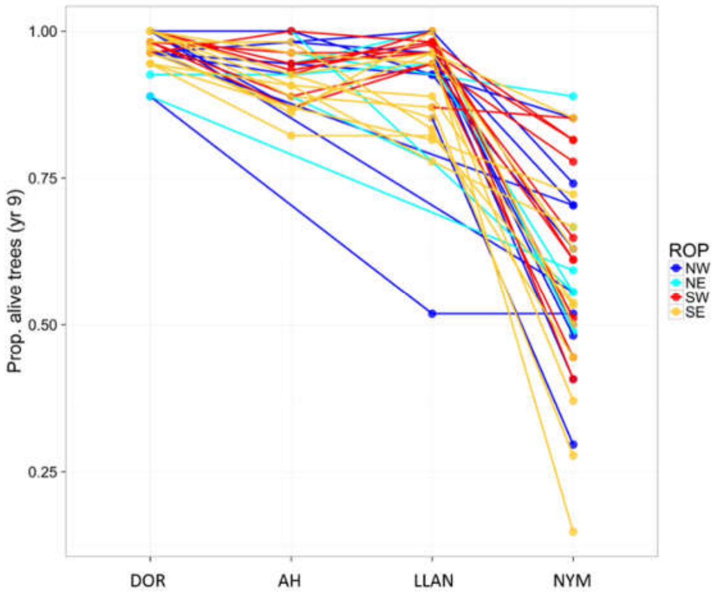


Figure 3.11. Interaction plot of the provenance means of the rowan tree survival measured at age 9, in the four sites. Colour legend: **yellow** (ROP SE), **red** (ROP SW), **dark blue** (ROP NW) and **pale blue** (ROP NE).

ii. Relationship with the geographical location of seed origin

There were no significant regressions with latitude or longitude in the trials where significant differences across provenances were found: Llandovery or North York

Moors. There was a significant regression with longitude in Alice Holt ($R^2 = 11\%$, $p<0.001$), where the eastern provenances survived less.

3.3.2.3 Relationships amongst traits

The only significant (positive) correlations found were between height and survival in Dornoch (0.191, $p<0.05$) and North York Moors (0.415, $p<0.001$). However, in Dornoch it is mainly due to one single provenance, with an outlier short height measurement; and in North York Moors the trial had a badly designed block arrangement and the height measurement differences were more due to the soil differences than the tree genetics.

3.3.3 Silver Birch

3.3.3.1 Tree growth Birch

i. Analysis of Variance

Differences amongst provenances: There were significant differences among provenances for height (all $p<0.001$) and DBH (all $p<0.001$ except Dornoch $p<0.05$) in all trial sites. The biggest difference between the tallest and shortest provenances was in Llandovery (40.89%), then Thetford and then Drummond. The biggest difference between provenance DBH was in Dornoch (76.42%), then Llandovery and then Drummond. Dornoch is a very harsh trial site, very wet and cold (more suitable for downy birch) and that is why trees have hardly grown in comparison to the other trial sites.

Site effect: Height was measured on the same year in Llandovery, Drummond and Thetford (not in Dornoch). There was a significant site effect ($p<0.001$) and a significant interaction between provenance and site ($p<0.01$) for birch height (Figure 3.12). The interaction is caused by greater differences in height amongst provenances in the southern trials. Trees were taller on average in Llandovery, 6% shorter in Thetford and 21.4% shorter in Drummond. DBH was measured in the same year in Llandovery, Drummond and Dornoch (not in Thetford). There was a significant site effect ($p<0.001$) and a significant interaction between provenance and site ($p<0.001$)

(Figure 3.12). The DBH was higher in Drummond, 10% lower in Llandovery and 89% lower in Dornoch.

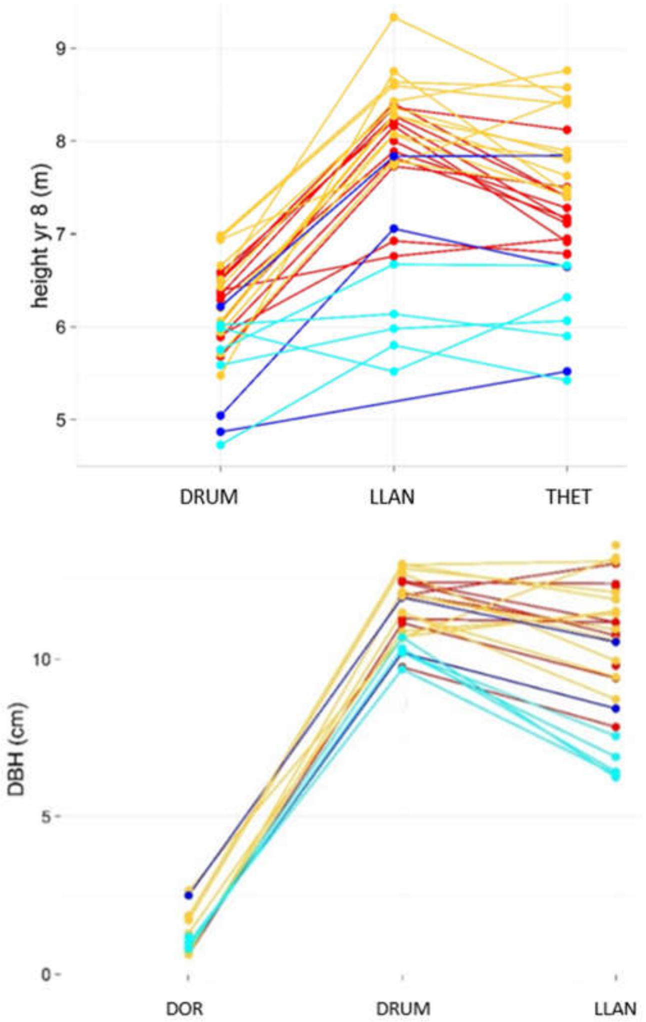


Figure 3.12. Interaction plots for height (top) (Drummond, Llandoveyr and Thetford) and DBH (bottom) (Dornoch, Drummond and Llandovery). Note that the trials are different. Colour legend: **yellow** (ROP SE), **red** (ROP SW), **dark blue** (ROP NW) and **pale blue** (ROP NE).

ii. Relationship of tree growth with the geographical location of seed origin

In birch latitude explained the differences in height in the Llandovery trial (51% of the variation); longitude explained the variation in Drummond (29%) and Dornoch (52%); and in Thetford both latitude and longitude were important predictors of variation (60%) (Table 3.10, Figure 3.13). This demonstrated a difference between the northern and southern trials: in the southern trials latitude accounts for a large proportion of the differences in height; and in the northern ones, variation in height is accounted by longitude.

DBH variation was explained by latitude in Drummond and by latitude and longitude in Llandovery (Table 3.10). There was no clear geographical pattern for Dornoch.

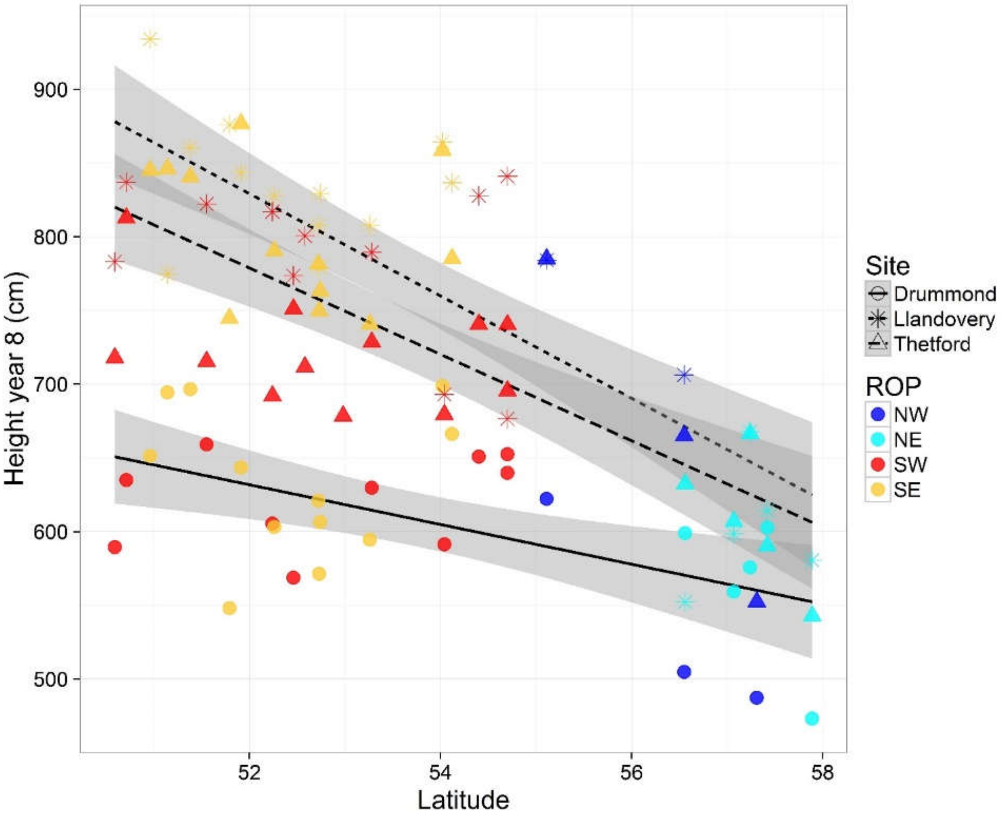


Figure 3.13. Birch height yr 8 in the three sites by latitude. Provenances means arranged according to their latitude from more south (left on the x axis) to north (right). Regression Adj R^2 and p value in Table 3.10. Colour legend: **yellow** (ROP SE), **red** (ROP SW), **dark blue** (ROP NW) and **pale blue** (ROP NE). Latitude degrees of the trials: LLAN 51.96, DRUM 56.57 and THET 52.41.

3.3.3.2 Stem forks Birch

i. Analysis of Variance

Differences amongst provenances: In birch, there were only significant differences amongst provenances in forking in Llandovery (p<0.01).

Site effect: There was a significant site effect (p<0.001) and a significant provenance by site interaction (p<0.05). Nearly all the provenances (except one from SE region) had fewer forks in Llandovery than in Drummond (Figure 3.14). Drummond had the most forks on average (4.2 forks), Dornoch 47% less (2.2 forks) and Llandovery 44% less forks (2.3 forks). Trees in Dornoch had fewer forks, but correcting by the tree height, forking could be seen as high; considering that the trees are on average around 6 meters shorter (over 75% shorter) than Llandovery and Drummond (Dornoch was measured 2 years earlier than the rest of trials).

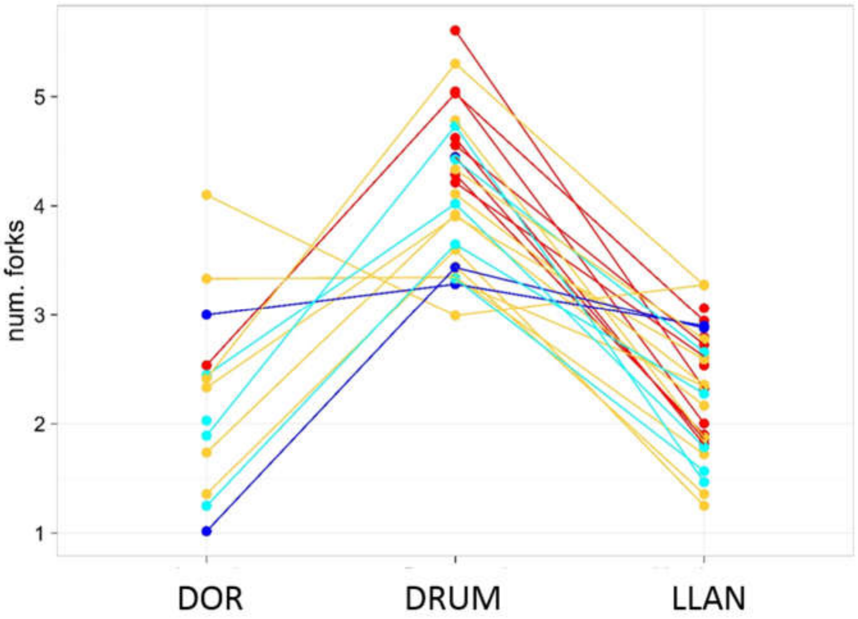


Figure 3.14. Interaction plot of number of forks for birch by trial, provenance means. Colour legend: **yellow** (ROP SE), **red** (ROP SW), **dark blue** (ROP NW) and **pale blue** (ROP NE).

ii. Relationship of forks with the geographical location of seed origin

In Dornoch forking variation was explained by longitude, the eastern provenances being the ones with higher number of forks (Table 3.10). There was no relationship with longitude in the other two sites. No association was found between forking and latitude.

3.3.3.3 Survival Birch

i. Analysis of Variance

Differences amongst provenances: In birch, there were only significant differences in survival amongst provenances ($p<0.05$) in the Llandovery trial, where the provenance with the highest survival had 52.17% more survival than the lowest.

Site effect: The average survival was the highest in Thetford (over 99%), followed by Drummond, then Dornoch and then Llandovery (30% less survival than Thetford). There is a significant site effect ($p<0.01$) but no significant interaction between provenance and site. (Figure 3.15). The interaction plot showed how most provenances had better survival in the Drummond trial (after Thetford), except for one provenance from SW ROP, which had the highest survival in Llandovery. For the common provenances between Llandovery and Dornoch, half have better survival in Llandovery and the other half in Dornoch.

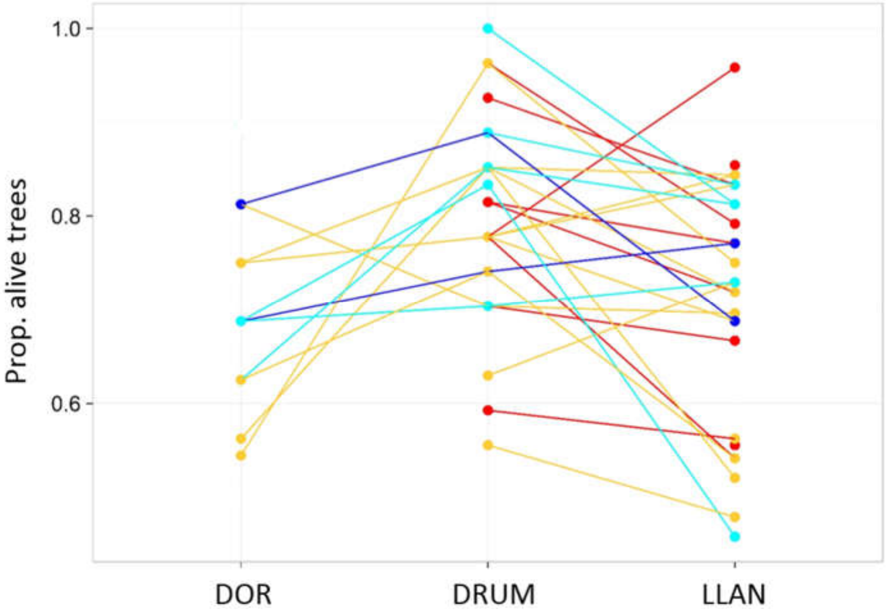


Figure 3.15. Interaction plot of survival data for birch by trial, provenance means. Thetford excluded because all provenances nearly zero mortality. Colour legend: **yellow** (ROP SE), **red** (ROP SW), **dark blue** (ROP NW) and **pale blue** (ROP NE).

ii. Relationship of survival with the geographical location of seed origin

In birch, there was a significant regression of survival on longitude at the Drummond and Llandovery trials, in both cases the eastern provenances survived less (Figure 3.16). In Llandovery longitude explains 10% of the variation and in Drummond explains 26% (Table 3.10). Overall the provenances from ROP SE had lower survival in the three trial sites, as the most eastern coincide with the most southern provenances.

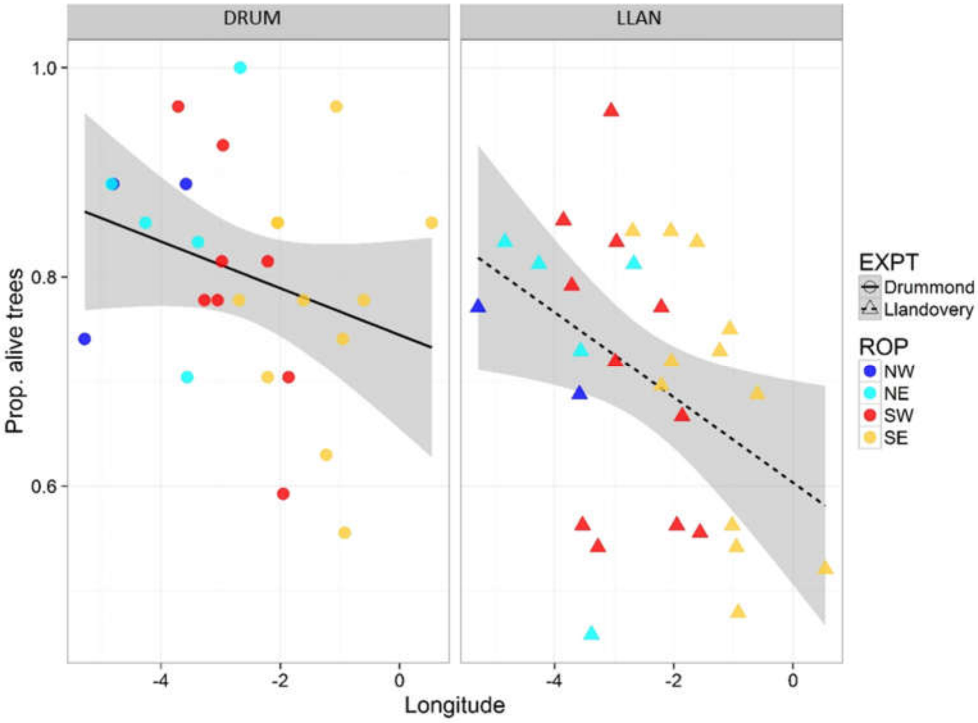


Figure 3.16. Birch survival means for Drummond (left) and Llandovery (right) by longitude. Provenances means arranged according to their latitude from more west (left on the x axis) to east (right). Regression Adj R^2 and p value in Table 3.10. Colour legend: **yellow** (ROP SE), **red** (ROP SW), **dark blue** (ROP NW) and **pale blue** (ROP NE). Longitude degrees for DRUM -4.11 and LLAN -4.08.

3.3.3.4 Relationship amongst traits

There was a positive correlation between height and DBH in Llandovery ($r=0.705$ $p<0.001$), Drummond ($r=0.633$, $p<0.001$) and Dornoch ($r=0.602$, $p<0.001$). There is a positive correlation between number of forks and height in Dornoch ($r=0.409$, $p<0.05$), but not in the other sites. There were no significant correlations between height and survival in any trial site.

Table 3.6. Height means by species, trial sites and ROPs (the values inside a square are significantly different amongst ROPs)

HEIGHT *Species*	*Region*	*Llandovery*	*North York Moors*	*Alice Holt*	*Dornoch*	*Drummond*	*Thetford*
Ash	**trial mean**	**256.475**	**127.798**				
5 yr	**NW**	226.796	119.779				
	NE	236.488	115.866				
	SW	262.246	130.704				
	SE	294.669	141.107				
Rowan	**trial mean**	**506.903**	**137.064**	**433.044**	**220.955**		
9 yr	**NW**	480.091	122.008	422.422	209.589		
	NE	486.566	129.489	412.927	214.596		
	SW	519.856	132.11	431.675	228.555		
	SE	514.014	153.218	442.16	225.385		
Birch	**trial mean**	**773.536**			**177.538**	**608.026**	**728.978**
8 yr	**NW**	745.218			173.319	538.06	667.522
	NE	602.645			156.381	561.93	607.743
	SW	787.385			163.571	622.191	721.986
	SE	836.766			205.955	632.919	801.849

Table 3.7. DBH means by species, trial sites and ROPs (the values inside a square are significantly different amongst ROPs)

DBH *Species*	*Region*	*Llandovery*	*North York Moors*	*Dornoch*	*Drummond*	*Thetford*
Ash	**trial mean**	**7.247**	**3.286**			
	NW	6.801	2.962			
	NE	6.814	2.961			
	SW	7.410	3.481			
	SE	7.846	3.659			
Birch	**trial mean**	**10.251**		**1.249**	**11.374**	
	NW	9.485		1.667	10.257	
	NE	6.694		1.012	10.190	
	SW	10.867		0.625	11.706	
	SE	11.446		1.470	11.902	

Table 3.8. Forks means by species, trial sites and ROPs (the values inside a square are significantly different amongst ROPs)

FORKS *Species*	*Region*	*Llandovery*	*North York Moors*	*Drummond*	*Dornoch*
Ash	**trial mean**	**2.364**	**1.919**		
8 yr	**NW**	2.506	1.744		
	NE	2.183	1.577		
	SW	2.418	2.024		
	SE	2.304	2.179		
Birch	**trial mean**	**2.341**		**4.158**	**2.213**
yr	**NW**	2.889		3.721	2.008
	NE	1.951		4.032	1.939
	SW	2.429		4.726	2.536
	SE	2.332		3.913	2.546

Table 3.9. Survival means (proportion of alive trees) by species, trial sites and ROPs (the values inside a square are significantly different amongst ROPs)

SURVIVAL *Species*	*Region*	*Llandovery*	*North York Moors*	*Alice Holt*	*Dornoch*	*Drummond*	*Thetford*
Ash	**trial mean**	**0.916**	**0.845**				
2015	**NW**	0.882	0.821				
	NE	0.917	0.794				
	SW	0.917	0.833				
	SE	0.950	0.914				
Rowan	**trial mean**	**0.918**	**0.592**	**0.923**	**0.978**		
2015	**NW**	0.906	0.595	0.932	0.974		
	NE	0.970	0.604	0.949	0.961		
	SW	0.954	0.665	0.933	0.988		
	SE	0.887	0.527	0.905	0.978		
Birch	**trial mean**	**0.701**			**0.733**	**0.799**	**0.993**
2015	**NW**	0.729			0.750	0.840	0.996
	NE	0.729			0.768	0.846	0.995
	SW	0.710			0.813	0.796	0.993
	SE	0.673			0.674	0.771	0.988

Table 3.10. List of traits measured by species and sites, number of provenances, presence (y)/absence (n) of provenance effect and site effect, and linear regression variables and Adjusted R^2 values. All coefficients were significant at $P<0.01$ (except the R2 values with (*) at $P<0.05$)

Spp	*Trial site*	*Trait*	*No. of provenances*	*Prov. effect*	*Site effect*	*Geographical variable*	*R^2*
Ash	Llan	Height	41	y	y	Latitude	0.46
		DBH	41	y	y	Latitude	0.36
		Forks	41	n	y	ns	-
		Survival	41	n	y	ns	-
	NYM	Height	39	y	y	Latitude	0.40
		DBH	39	n	y	Latitude	0.41
		Forks	39	y	y	Latitude	0.21
		Survival	39	n	y	ns	-
Rowan	Llan	Height	33	y	y	Latitude	0.17
		Survival	33	y	y	ns	-
	NYM	Height	38	y	y	Longitude	0.04
		Survival	38	y	y	ns	-
	Dor	Height	41	y	y	ns	-
		Survival	41	n	y	Latitude	0.02*
	AH	Height	30	y	y	Latitude	0.54
		Survival	30	n	y	Longitude	0.11
Birch	Llan	Height	29	y	y	Latitude	0.51
		DBH	29	y	y	Lat +Long	0.51
		Forks	29	y	y	ns	-
		Survival	29	y	y	Longitude	0.10
	Drum	Height	29	y	y	Longitude	0.29
		DBH	26	y	y	Latitude	0.48
		Forks	26	n	y	ns	-
		Survival	29	n	y	Longitude	0.26
	Thet	Height	31	y	y	Lat +Long	0.60
		Survival	31	n	y	ns	-
	Dor	Height (y6)	13	y	-	Longitude	0.52
		DBH	13	y	y	ns	-
		Forks	13	n	y	Longitude	0.22*
		Survival	13	n	y		

3.4 Discussion

We have investigated variation in tree growth, stem forking and survival for three tree species. Although there is much variability among trials and provenances, the results of assessments in common garden experiments have enabled us to differentiate between the genetic and the environment components of the variation found. Our results have shown that there is: 1) phenotypic plasticity (growth is very dependent on the site where trees are growing), 2) genetic differentiation (there are clear differences in growth trait variation among British provenances), and 3) interaction between these two. Furthermore, we have shown that differences in height growth tend to be related to latitude of origin, although in the northern trial sites longitude also was important. Height variation was positively correlated with DBH and forking, but with differences amongst species.

3.4.1 Phenotypic plasticity in tree growth

It has been well studied how a single genotype can produce different phenotypes when grown in different environments (Bradshaw, 1965). For all three species, the largest proportion of the variation in height and DBH was explained by the environment (trial site).

In general, our results showed that all provenances attained greater height when grown in southern locations and lower growth in northern locations. In all three species the tallest trees are found in the Llandovery site, which is the warmest and with lowest moisture deficit. Similar results were obtained by Cundall et al. (2003) who found that on average, tree height was greatest at the two Welsh trials sites.

The ash provenances grew 50% taller in Llandovery than in North York Moors. The rowan provenances grew taller in Llandovery, on average 56% taller than in Dornoch and 73% taller than in North York Moors. Birch provenances also grew taller in the Llandovery site, 21% taller than in Drummond and 77% taller than in Dornoch. Moreover, we have found that the degree of plasticity is different. Not all provenances showed the same amount of plasticity when moved to a different environment (this is explained in section below 3.4.3).

For ash, DBH was smaller in northern trials. In contrast, DBH of birch was greater in a northern trial. In other studies of British ash and silver birch (Worrell, 2000; Cundall et al., 2003; Lee et al., 2015) these differences in growth due to the site effect have also been shown. For all species, the greatest height was observed at the Llandovery trials, which are situated in a warm and wet area.

3.4.2 Genetic variation in tree growth

There are clear differences in growth trait variation (height and DBH) among British provenances for all three species. Despite the large site effect, there were differences among provenances, which were significant for all species in all trial sites. As the environment at each trial site was uniform, these differences have to be accounted for by genetic differences, which suggests a degree of local adaptation has taken place in British tree populations of these three species. The difference between the tallest and the shortest provenance within each trial site varied across sites. In ash both trial sites showed the same differences amongst provenances, for both sites the tallest provenance was 45% tallest than the shortest. In rowan, the tallest provenance was 24% taller than the shortest in Alice Holt, 48% taller in Llandovery, 64% in North York Moors and 66% in Dornoch. In birch, the tallest provenance within a trial was 32% taller than the shortest in Drummond, 33% taller in Dornoch, 29% in Thetford and 41% in Llandovery. Overall, we see that the differences due to genetic variation within a trial are of the same magnitude or greater than the differences across trials. However, the differences between provenances are better shown in some trials than others, usually differences show better in southern trials.

Other studies for ash and birch studied variation between British populations, but across a smaller geographical range or based on many less provenances; this may explain the inability of previous studies of British ash to detect clear provenance differences (Savill et. al, 1999; Cundall et al., 2003). In rowan, generic variation in British populations has not been studied before

Out of our three study species, rowan showed the least differences in height among provenances. There are examples in the literature where genetic variance can be observed in some environments but not others (Thompson, 1991), and that is what we can see in our height results for rowan, where the same provenances show very little differences amongst them in the most northern site while they show great differences amongst provenances in the two most southern sites.

3.4.3 Genotype by environment interaction

We found significant genotype by environment interactions for tree height in all species. The magnitude of differences among provenances varied by trial site and in other cases the ranking changed. Differences among provenances at southern trial sites were larger than at northern trials, both for height and DBH. Although the ranking of provenances for height was stable across sites (with the exception of a few provenances), there was a larger range of height means among provenances in the southern trials, which gave rise to significant provenance by site interactions. In some cases the proportional difference between the tallest and shortest provenance was greater in northern trials, although this was mainly due to one or a few outlier provenances for which growth was very limited at the northern sites. This trend is clear in all three species.

Moreover, we have found differences in the amount of phenotypic plasticity between provenances. Genetic variation for phenotypic plasticity has been well documented (Schlichting, 1986). We hypothesised that the provenances from northern (harsher) environments would show less plasticity, due to having stronger selective pressures, a trend which has been recognised in deciduous trees for leaf phenology (Vitasse et al., 2013), where provenances from higher altitude showed less phenotypic plasticity than provenances from lower altitude. Southern provenances have the ability to fully exploit the growing season when planted in the southern trials and will therefore show high plasticity among sites. Northern provenances are genetically adapted to grow only within a shorter growing season, so they cannot exploit the longer growing season in the southern trials, showing less plasticity. We, therefore, expect that northern provenances will show smaller differences in height when growing in different environments, compared with southern provenances. We have found differences in the relative height increase between trial sites, comparing the most northern trials with the trial which shows the greatest growth for all three species (Llandovery). In ash, relative height growth of northern provenances is 49% greater when growing in Llandovery compared to North York Moors, while relative height growth of southern provenances is 51% (ROP SE the most with 52%). In rowan, the relative height growth at Llandovery compared to Dornoch is the same for northern and southern provenances (56.1% in both cases). In birch, the southern provenances change more between Drummond and Llandovery (southern provenances are 23% taller at Llandovery compared to Drummond, while the northern ones are 18% taller). The difference in absolute height increase between the northern provenances and the southern provenances was significant for both ash and birch, however the relative increase (Figure 3.17) was only significant in birch. These differences in relative height growth suggest that northern provenances have less phenotypic plasticity for height on average in birch. A degree of differences between northern and southern provenances were found in ash, but no differences in rowan.

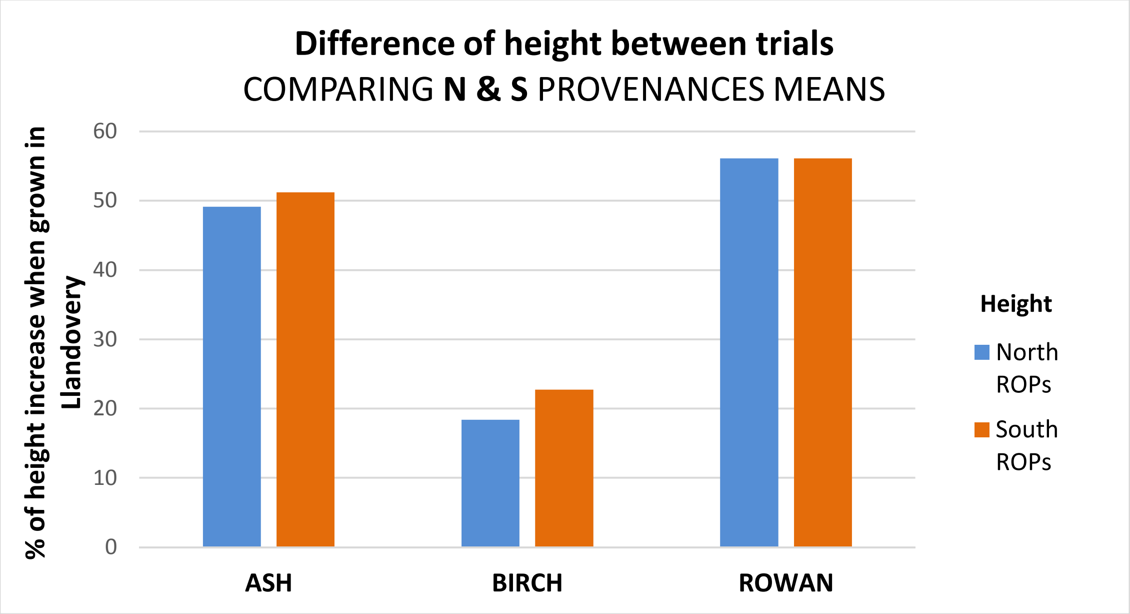


Figure 3.17. Percentage of increase of tree height in provenances grown at Llandovery compared to performance at northern sites (NYM in ash, DRUMM in birch and DOR in rowan). The bars represent the average increase for all the northern provenances in **blue** (ROPs NW and NE) and for all the southern provenances in **orange** (ROPs SE and SW).

3.4.4 Geographical patterns of adaptive variation in tree growth

It has been well studied that trees from warmer climates have a greater stem height than trees from colder climates, and these differences show when growing under common conditions (Aitken & Bemmels, 2016). Indeed, it is a well-established strategy in plantation forestry to plant trees from provenances south of the planting site to gain faster growth by exploiting the adaptation of these non-local trees to longer growing seasons (Zobel & Talbert, 1984). Although this clinal latitudinal change is generally observed in common garden trials other climatic factors at the source site can also be very important, and, in some cases, longitude can be more important than latitude (Langhammer, 1982; Hubert, 2005). We found that in those trials in which there were clear differences among provenances for height growth, this tended to be related to latitude. However, in the northern trials, variations amongst provenances tended to be accounted for by longitude.

Our results, based on a large number of provenances sourced from across Britain, show that this latitudinal cline in height and DBH is particularly clear among British ash populations, with southern provenances achieving greater growth. Other studies which included British ash provenances did not find a geographical pattern of variation in height growth within ash provenances in Britain, perhaps due to the inclusion of only a limited number of British provenances (Cundall et al., 2003; Clark, 2013), or provenances from a very restricted geographic area (Boshier & Stewart, 2005). Boshier & Stewart (2005) investigated variation in growth among 20 provenances from a small geographical range in England and Wales but found no clear geographical pattern. Other studies which included non-British provenances alongside British provenances, found that the non-British most southern provenances were the tallest (Cundall et al. 2003, Clark 2013) and northern British provenances in general were shorter that the non-British.

Variation in growth traits in rowan has been far less studied, perhaps due to its lower economic value. The only study based on British rowan populations (Barclay & Crawford, 1984) studied the effect of altitude on the first few weeks of seedling growth and found that seedlings from a higher altitude origin had a higher relative growth. This is contrary to expectation, as the general trend is that temperature declines with higher altitude, in a similar way that it declines with increasing latitude (Jump et al., 2009). However this study only compared seed of 15 trees in the same site growing at different altitude (from 0 to 600 m) and only looked at early seedling growth. In contrast, an experiment comparing populations of rowan from throughout its range in Sweden (Baliuckas et al., 2005) found that the populations from the highest altitude had the poorest performance, which they described with the vague concept "health status" and height. Popov (1990) studied six Western-Russian populations along a latitudinal gradient and found that crown density was greater in provenances from the south. Our results, based on a large number of provenances from across the whole of Britain, show that significant height differences amongst British rowan provenances are only shown in the southern trials, where height declines with increasing latitude of origin. However, this was not observed at the northern trial sites, where differences among provenances were much smaller. Another consideration to take into account is that rowan displays different growth habits, from a single stemmed tree to a multi-stemmed form. For this reason, the height measured as the tallest stem, might not be the best way to measure growth in rowan, as the same biomass can be in the form of one tall stem, or many shorter ones. These differences in habit might vary across environments and provenances. The number of stems should be a trait to taken into account when measuring rowan growth. Tanentzap et al. (2012) consider that the multi-stemmed architecture in temperate woodland trees species is an advantageous trait for shade tolerance and herbivory damage. However, the phenotypic plasticity as a response to different environments has not been studied.

Geographic patterns of variation found in the growth of birch existed but were complex, different by site and different for height and DBH. Height differences in the southern trials were explained by latitude (decreasing with increasing latitude), but in the two northern trials variation was better explained by longitude. However in either case, the most southern provenances were the tallest - it has to be noted that latitude and longitude are themselves correlated in Britain, as the most eastern part of the country is also the most southern, and the most western parts are also the most northern. Previous work on silver birch in Great Britain largely supports our results for height in birch, where trees from southern provenances transferred to northern sites had greater relative height growth, compared to local or more northern provenances. Trials based on a more restricted geographical range of provenances which included only Scottish and northern-English provenances, showed a clear relationship with latitude, whereby provenances from the north were shorter than those from further south (Lee et al. 2015). Another study found that height was correlated with both latitude and longitude, where provenances from the south-west were the tallest

(Worrell 2000). In Finland, latitudinal transfer functions of growth (based on a larger geographical range) were curvilinear, where good height growth was found in local stock and in provenances originating from up to 2 degrees further south; bur provenances transferred from further south showed poor height growth (Vihera Aarnio & Velling, 2008; Vihera Aarnio et al., 2013).

Overall, in ash and birch latitude has a great effect in tree growth, and in a lesser extent for rowan; while longitude is also important for tree growth in silver birch. Across all trial sites, the northern provenances in ash and birch are 15% shorter than the southern provenances for these two species, however, overall in rowan the northern provenances are 6% shorter than the northern ones.

3.4.5 Correlations of Height with other traits

Our results show that height is an informative trait, which reveals differences among provenances effectively. In general, we expect that height can provide a relatively good indication of tree fitness. However in the sections below we provide clear examples of situations in which consideration of trait covariance can result in different sets of conclusions, thereby improving our understanding of suitability for planting sites.

Forking

The tallest trees observed (in both trial sites for ash and in the most northern birch site) were those which also had the highest number of forks. This positive correlation between height and forking has also been found before in ash in Britain (Mwase et al., 2008). However in another study the opposite trend was found (Foggo, 1996), which related forking with producing shorter shoots. Also, Kerr & Boswell (2001) consider that younger or shorter trees have greater risk of forking because damaging frosts occur near to the ground. The trend we have found which shows that the tallest, southern provenances have a higher rate of forking has also been shown in silver birch experiments in Finland (Viherä-Aarnio & Velling, 2008; Viherä-Aarnio et al., 2013), where forking represents the trade-off against height growth. A probable explanation for this relationship is that past adaptation to longer growing seasons in trees from southern provenances provides strong vegetative vigour but also results in the timing of growth initiation being inappropriately phased to avoid late spring or early autumn frosts when these provenances are grown away from their home site; and thus, instances of frost damage (measured as number of forks) are more frequent (Wardle, 1961; Ningre et al., 1992; and Kerr & Cahalan, 2004). Although frost events seem not to have been sufficiently severe to result in mortality in standing live trees (survival was generally high at all sites), overall stem form is compromised and the presence of forks is indicative of maladaptation to the length of the growing season at the trial site. Wind can also be a cause of forking by bud damage (Kerr and Boswell, 2001), and windiness increases in a northerly and westerly cline in Great Britain (Clare & Ray, 2001). This possible maladaptation to growing season length and wind intensity, would have been concealed if height alone was taken as a single proxy for fitness. In contrast to our results Malcolm & Worrell (2001) found, in their silver birch progeny trial that the differences in forking amongst families was negatively rather than positively related to height, however this relationship was only just significant (p=0.048).

We have also found a large site effect for forking. In ash, the frequency of forking was uniformly high at Llandovery, and lower overall in North York Moors but with differences amongst provenances. In the Llandovery ash trial we do not find significant differences amongst provenances for forking, while in North York Moors the southern provenances fork more. In birch, the number of stem forks also varied by trial, in an opposite trend to ash: forking being higher in the north (Drummond) rather than the south (Llandovery). In the most northern and western birch trial (Dornoch) the variation was explained by longitude, where the eastern provenances forked more. We see that the oceanic climate to which the western provenances will probably be better adapted, can have an effect as it has more late spring and early autumn frost. In the ash trials, Llandovery is more oceanic than North York Moors; in birch, Drummond is more oceanic that Llandovery (both have the same longitude, but Drummond is more northerly).

DBH

Our results show a high correlation between height and DBH in ash and birch (DBH was not assessed in rowan), which agrees with findings in other studies (Malcolm & Worrel, 2001; and Mwase et al., 2008). However, when DBH is considered together with height measurements, the site effect on the height:DBH ratio revealed a physiological trade-off in birch trees, whereby trees in the southern trial were thinner and taller, while in the northern trial they were thicker and shorter. This shows a different resource allocation strategy when the same provenances grow in different environments. Height-DBH relationships have been recognized as one of the ways in which trees respond to changes in the environment (Körner, 1998). These allometric relationships in tree growth have been studied broadly in many tree species, and have been shown to change with planting site quality, temperature and water supply (Aiba & Kitayama, 1999; Martinez & López-Portillo, 2003). Height and diameter growth have been found to have different temperature sensitivities in conifers (Li et al, 2003; Li & Yan, 2004) and this may be an underlying reason for the difference in height;DBH ratio between trial sites. Wang et al (2006) found that a lower proportion of biomass is allocated to height growth than to DBH under conditions of intensified winter cold in *Betula ermanii* Cham.. Another study (Wang et al, 2017) on the same birch species found that height-DBH ratios were modulated by temperature and other environmental factors such as soil depth, water availability, wind speed and snow cover. In previous studies, other birch species (*Betula nana* L., *Betula populifolia* Marsh., *Betula papyrifera* Marsh., and *Betula alleghaniensis* Britt.) have been found to show a higher degree of plasticity than species from other genera co-existing in the same environment (Bretharte et al., 2001; Ellum et al., 2004). Neighbour-competition effects have also been found on height-DBH relationships, including in silver birch (Hanry & Aarseen, 1999; Ilomäki, 2003). At the time of planting, spacing between trees at both of our birch sites was the same, which ought to have minimised differences in the level of neighbour-competition between the sites and so we expect that the difference in height-DBH ratio we found in our birch trial sites could be due to the difference in site conditions. Drummond (north) was the colder, drier and windier of the two sites while Llandovery (south) had a much more oceanic climate.

Additionally, prevalence of competing vegetation was higher at Llandovery, which had a very high density of bramble bushes (*Rubus fruticosus*)).

We have found this difference in height:DBH ratio due to environment of the planting site in birch but not in ash. In the northern site of ash (North York Moors) trees are much shorter and thinner than in the southern trial site. It is clear that this variability in height:DBH ration in birch is driven by the environment; why this resources allocation strategy gives survival advantage, has to be further studied. Wind damage could be a possible explanation, but greater stem diameter is discussed as being correlated with being more susceptible to wind damage, as it reduces the flexibility of the tree (King, 1986; Rich et al., 2007) and root system is more related to wind damage resistance (Nicoll & Ray, 1996).

Survival

In ash and, to a lesser extent, rowan, survival is positively correlated with height. A possible explanation is that fast early height growth confers an advantage in terms of access to light availability (King, 1990). Considering trials were planted with a uniform spacing of 1.8-2 m, it is unlikely that trees would experience much competition for light, at least for the first years. Ash seedlings have been shown to have a lower survival rate under low light and higher growth rate at high light exposure (Petritan et al, 2007). On the other hand, rowan seedlings are considered exceptionally shade-tolerant (Pigott, 1983; Zebre, 2001). Therefore, light competition in the ash trial could contribute to the higher mortality in shorter trees, but this is less likely to be in the case for rowan due to its greater shade tolerance. Another explanation for the higher mortality in shorter trees, could be severe damage by ground frosts (Kerr and Boswell, 2001), but our results in forking do not suggest that would be a general trend. Yet another possible explanation for lower survival in ash and rowan northern provenances, could be explained by inbreeding in the most isolated populations in harsher environments. If the slower growing northern provenances of rowan have some level of historical inbreeding, amongst other things (such as harsh climatic conditions, which can affect pollinators as well) would influence a lower quality of seed in the populations from harsher environments, as it has been shown in other studies in rowan (Barclay & Crawford, 1984; Sperens, 1996; Sæbø & Johnsen, 2000).

However, studies carried out on isolated rowan and ash populations in the south of Scotland did not find inbreeding, despite being in a fragmented landscape (Bacles et al., 2004; Bacles et al., 2005); the high levels of genetic diversity could have been maintained by bird seed-dispersal in rowan, and by wind-dispersed pollen in ash. Lower seed quantity and quality (low germination) has also been found in higher altitude populations of silver birch in Sweden (Holm, 1994). However, in birch, despite the absence of a clear correlation between tree height and survival, we see an opposite trend: the trees from the SE ROP were the tallest in all trials but also showed the highest level of mortality, except when planted in the south-east. In birch we have shown a clear relationship between survival and longitude, the oceanic-continental cline in Great Britain is important for the survival of birch trees, showing an East-West split. In comparison, in silver birch provenance trials in Finland (Viherä-Aarnio et al., 2013), there was a relationship between latitude and survival: the local and the provenances from locations further north than the planting site, had a higher survival rate than the southern provenances. Provenances transferred west also showed very poor survival in Finland (Viherä-Aarnio & Velling, 2008).

3.4.6 Conclusion

Our results confirm the existence of genetic differentiation between British populations of ash, rowan and birch for height, as well as high level of phenotypic plasticity. This local adaptation present in British populations and the effect of the planting site, have to be taken into account when planting new trees.

In the literature, height alone is frequently used as a proxy for fitness on its own. However, it is important to consider other traits when assessing suitability for tree planting, both if the interests are commercial (where both tree height and form are valued quantity and quality of timber) or for conservation purposes (where tree survival and fitness is more important than yield). We have shown that trees with higher survival might be the tallest in ash, but not in birch for example Also, achieving greater height if it is as a result of being in leaf for longer can very often come with the associated risk of frost damage. We consider that the trees which grow taller are not necessarily the best adapted to a given site, and the concept of tallest trees being best adapted is sometimes confused in the literature because of the desirability of tall trees for timber production objectives.

In this chapter, we have shown that with easy and quick measurements of tree stem (height, DBH and forking) and tree survival we can find clear differences amongst British provenances, as well as the large effects of the planting site. We have clearly demonstrated the importance of considering height in relation to other traits in order to improve our understanding of how this trait impacts on adaptedness of provenances. With this and the subsequent two chapters, we will provide more insight into tree local adaptation, by moving forward from the basic tree height measure on to traits that are more demanding to assess.

Our results show a large amount of variation (genetic differences and phenotypic variation) on tree height amongst British populations. We have shown that the genetic differences are mostly explained by latitude, which negatively correlates with the growing season length. Therefore, this genetic differentiation we have found in British populations of ash and birch on their growth would explain an adaptation of the tree populations to the different gradient of growing season length across GB. Tree height is not so differentiated in rowan populations as it is in ash and birch, we want to find out if rowan populations are genetically differentiated looking at other traits. We want to find out if the genetic differences amongst tree populations (of ash, rowan and birch) in GB are only due to different adaptation to growing season length, or whether there are other adaptive pressures which can explain genetic differences amongst populations. In the next chapters we address this issue, examining other traits a part form tree stem growth.

Chapter 4: Variation in spring and autumn leaf phenology in British Ash and Rowan populations.

I. Variation in leaf phenology in ash populations across GB

II. Comparison of leaf phenology in ash and rowan

Abstract: In this chapter we address two main questions, a) are there genetic differences in leaf phenology amongst provenances of British ash and rowan trees? and b) are the patterns of adaptive variation in leaf phenology the same in different species? Our study focuses on native populations of ash (*Fraxinus excelsior* L.) (n=42) and rowan (*Sorbus aucuparia* L.) (n=40), sampled throughout Great Britain and grown in common garden experiments. Trees were assessed in the spring for leaf flushing and autumn for leaf senescence. We found that ash provenances differed in timing of leaf flushing following a latitudinal cline, while rowan provenances showed a longitudinal cline. Differences amongst provenances were smaller for leaf senescence, with a clear latitudinal cline in ash but no obvious geographic pattern in rowan. We also found that the differences amongst provenances were much larger than the plasticity shown in the differences between the trial sites. In ash, we also found a correlation between stem forking and timing of flushing, with early flushing provenances tending to be more forked indicating repeated frost damage. Our results suggest that, as their leaf phenology is strongly genetically determined, moving trees to locations with different growing season lengths could have consequences for fitness in the long term.

4.1 Introduction

Phenology is the study of the relationship between timing of recurrent life-history events and seasonal and annual climatic changes. In deciduous trees, the predominant non-reproductive phenological cycle is the active growth period, which is defined by the timing of bud development and leaf expansion (flushing) in spring and leaf colour change and shed (senescence) in autumn. It is key because it determines the amount of time that the trees are actively photosynthesising, which in turn affects the annual amount of biomass produced (Häkkinen et al., 1998; Mezel & Fabian, 1999; Linkosalo et al., 2006). If the timing of leaf emergence or senescence is not in synchrony with key seasonal weather patterns the maladaptation can have serious impacts on tree health and fitness (Perry, 1971; Mezel & Fabian, 1999; Doi & Katano, 2008; Vitasse et al., 2018). For example, young buds and leaves are highly sensitive and can be completely destroyed by extreme frost during early development and emergence (Häkkinen et al., 1998; Inouye, 2000; Rodrigo, 2000). This can result in stem forking (Kerr & Boswell, 2001), which compromises timber quality and potentially reproductive output if flower development is also affected (Gardner, 1977; Augspurger, 2009). The timing of leaf flush and shed is thought to be determined by a trade-off between maximum use of resources for growth and avoidance of damage by frosts (Heide et al., 1985, Saxe et al., 2001; Aitken et al., 2008).

It has also been shown that phenology plays a crucial role in the carbon balance of terrestrial ecosystems (Keeling et al., 1996), in plant competition (Rathcke & Lacey, 1985), in pest and disease control (Penfound et al., 1945) and can have impacts across trophic levels (Crawley & Akhteruzzaman, 1988; Koenig et al., 2015; Sinclair et al., 2015; Bucharova et al., 2016). Since phenological events often respond to temperature cues, there is also concern about the impact that climate change will have on their timing; and there are widespread reports of advances in spring phenology, not only in trees (Fu et al.,2015) but in many other groups (Thackeray et al., 2016). Trees can adapt to change in the environment in two ways: responding with acclimation, reversible plastic changes on the phenotype; or with genetic change. Phenotypic plasticity is an essential component of plants' response to an ever-changing environment (Jump & Peñuelas, 2005, Nicotra et al., 2010), however it might drive a mismatch with fitness consequences (DeWitt et al., 1998). Genetic variation for phenology would allow an adaptive response over time (Aitken et al., 2008).

For these reason, is important to separate the two components of variability found in tree populations. It is very important to understand the relative extents of genetic and environmental control over the phenological variation of native tree populations to provide evidence of whether and how standing trees can acclimate to different environments, and whether populations have the capacity for evolutionary adaptation to the changing climate.

Phenology in the wild has been observed and very well recorded *in situ* in the natural environment for many centuries. In Great Britain, where there is a very good tradition of phenological observation, some records go as far as 1736 (Sparks & Carey, 1995). These show that timing of spring events vary substantially across the country, typically being earlier in the warmer southern parts. In temperate regions, spring phenology is negatively correlated with temperature (Roy & Sparks, 2000; Fitter & Fitter, 2002, Schwartz et al., 2006). There are many examples that show large differences in timing of key phenological events across Great Britain as a whole (such as Phillimore et al, 2010), however differences can also be found at smaller spatial scales (pollen shed in Scots pine in the Scottish Highlands, Whittet et al., 2017), and there may be also some levels of variation within single populations (Rousi et al., 2011). The *in situ* timing of the leaf flush has been well recorded in British trees, all over the country (Murray et al., 1989; Abernethy et al., 2017).

However, *in situ* observations do not provide insights into the relative extents of genetic and environmental (plastic) contributions to phenological variability. Common garden experiments are used to estimate these two components of the variability (and their interaction), where trees from different provenances are grown in a common environment and phenotypic differences can be inferred to be due to genetic differences.

Generally, there is a rather limited knowledge of forest genetic resources in Great Britain (Boshier & Stewart, 2005; Cavers & Cottrell, 2015). There has been little assessment of genetic variation in the leaf phenology of native tree populations. The few common garden experiments on tree phenology in British provenances include ash (Clark, 2013), hawthorn (Jones et al. 2001), Scots pine (Perks & Ennos, 1998; Salmela et al., 2011), oak (Wilkinson et al, 2016) and silver birch (Pelham et al., 1988; Billington & Pelham, 1991; Worrell et al, 2000). However, these studies tended to involve a relatively small number of provenances from a limited region of the country, sometimes as part of larger scale experiments involving continental provenances. Moreover, there are no studies which look at autumn leaf phenology (except for Blackburn and Brown, 1998, which looked at a few progenies of silver birch assessed for evidence of autumn frost damage). This work has shown that climate in Britain is characterized by a variable but high incidence of frosts between the months of April and May (Blackburn and Brown, 1988; Pelham et al., 1988; Billington and Pelham, 1991, Worrell et al., 2000).

Spring phenology is advancing under climate change (Roberts et al., 2015; Fu et al., 2015; Thackeray et al., 2016). However, Vitasse et al. (2017; 2018) have shown that, in Switzerland, advances in the timing of key spring phenological events are greater than the advance in the date of the latest spring frost. It is known that there is a genetic component to leaf phenology the timing of which is also modulated by the environment so that it exhibits inter-annual variation (Wilson & Baldocchi, 2000). Both temperature and photoperiod have a role in controlling the timing of bud burst, although there are differences between species and different hypotheses on the effect of these factors (Vitasse & Basler, 2013; Tansey et al., 2017).

In this chapter, we describe results of assessments of spring and autumn leaf phenology in multi-site common garden field experiments of ash (*Fraxinus excelsior* L.) and rowan (*Sorbus aucuparia* L.), containing a comprehensive sampling of the British distributions of each species. Our overall objective was to evaluate the relative extent of genetic variation and phenotypic plasticity in leaf phenology in native British populations of both species. We studied two species, as differences between species in the timing of bud burst have been shown previously (Murray et al., 1989; Abernethy et al., 2017), and aim to discover whether patterns of variation are the same in the two species.

In this chapter we addressed the following questions:

-Do native populations of ash and rowan show genetic differentiation for leaf phenology? i.e. do they show differences amongst provenances when grown in the same location?

- Do native populations of ash and rowan show phenotypic plasticity for leaf phenology? i.e. do provenances perform differently at different locations?

- To what extent does the site of origin of the provenance determine timing of leaf phenological events? Are the patterns of variation for leaf phenology different between ash and rowan?

4.2 Materials and methods

The study was based on measurements of the timing of bud burst and senescence carried out in multi-site common-garden provenance experiments (trials) of ash and rowan established by Forest Research.

4.2.1 Sampling strategy for trials.

To establish the trials, a common seed sampling strategy was adopted for both species based on the Forestry Commission seed zone map of Great Britain (Forestry Commission, 1999; Figure 4.1). The system of seed zones divides the total area of Great Britain into 4 regions of provenance (North West NW, South West SW, North East NE and North West NW). These regions of provenance are further subdivided into 24 seed zones based on natural topographical boundaries (Figure 4.1). Where possible, two self-sown semi-natural populations of each species (hereafter referred to as provenances) were sampled from each seed zone. Approximately equal numbers of seed were collected from a minimum of 20 maternal trees and mixed to constitute the provenance sample. Selection of mother trees was not biased towards individuals with superior phenotypic characteristics (e.g. size, form), and where possible, sampled mother trees were located at least 100 m apart.

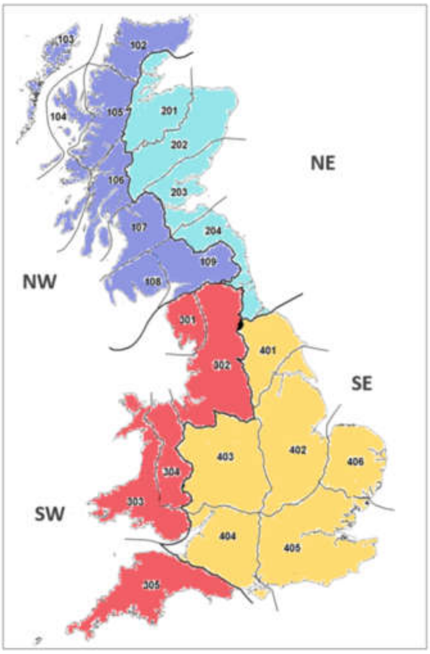


Figure 4.1. The four regions of provenance (ROP, coloured areas, heavy black boundary, labelled as northwest - NW, northeast - NE, southwest - SW, southeast - SE) and 24 seed zones (fine black boundary, numbered using FC seed zone codes) in Great Britain (modified from Herbert et al., 1999)

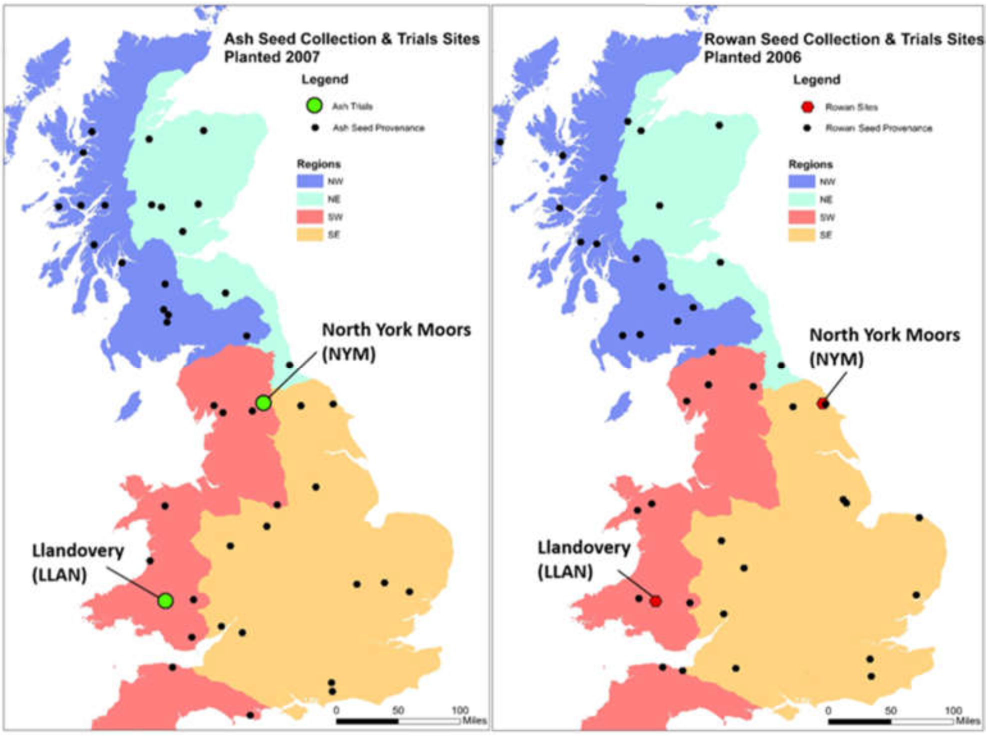


Figure 4.2. Seed collection sites (small black dots) and trial site locations for ash (green dots, left) and rowan (red dots, centre). Coloured areas show regions of provenance (Herbert et al., 1999).

4.2.2 Experimental design by tree species

Trials were established in environmentally contrasting field sites, with two sites per species (Table 4.1, Figure 4.2). The trial sites named ‘Llandovery’ are in the same location for both species, and are adjacent to one another. The trial sites named ‘North York Moors’ are located 112 km apart (Figure 4.2) in different parts of East Yorkshire: the ash trial site is west of the rowan site, at lower elevation and in more continental conditions; the rowan trial is located at higher altitude but is near the coast (Table 4.1, Figure 4.2).

Ash (*Fraxinus excelsior* L.)

Seed from 42 provenances of ash was germinated and grown in nurseries located near the trial sites, at Whixley (Yorkshire) and Carmarthen (South Wales).

In 2007, one-year-old seedlings were planted in two common garden experiments. The two trials are located in South Wales (Llandovery - LLAN) and East Yorkshire (North York Moors - NYM). The Llandovery trial contains trees from the 42 provenances, the one in the North York Moors has 40 provenances (Figure 4.2 and Table 4.2). There are 40 provenances common to both trials.

At each site, the experiment followed a randomized block design, consisting of three replicated blocks with each provenance represented by a single plot in each block. The plots consisted of 36 (6 x 6) trees from the same provenance in Llandovery and 30 (6 x 5) trees in North York Moors and were planted at 2 m spacing.

Careful inspection of plants for symptoms of disease at the time of assessment confirmed that measurements from the Llandovery trial were completed before the trial was infected by ash dieback, *Hymenoscyphus fraxineus*. In North York Moors nearly all the measurements were collected before the trees were infected with the exception of DBH and forking, which were assessed in the year when trees began showing signs of infection. Since then, both trial sites have developed high levels of infection (J. Clark, pers. comm).

Table 4.1. Location and climate details for the ash and rowan trial sites.

Spp	Trial Site	Planting Year	County	Lat	Long	CT	MD	Alt (m)	AP (mm)	GDD	DAMS	Soil Type
Ash												
	NYM (North York Moors)	2007	East Yorkshire	54.3	-1.9	9.0	153	113	959.8	1475	10.0	Riverine Floodplain. Clay to Sandy Loam. Freely draining floodplain soils.
	LLAN (Llandovery)	2007	Carmarthenshire	51.9	-3.8	8.1	108.2	240	1329	1474	15.1	Sandstone and Mudstone. Sand to Loam. Freely draining acid loamy soils over rock
Rowan												
	NYM (North York Moors)	2006	East Yorkshire	54.3	-0.5	8	149	197	759.2	1328	14.8	Very acid loamy upland soils with a wet peaty surface. Silty loam to sandy loam. Peaty.
	LLAN (Llandovery)	2006	Carmarthenshire	51.9	-3.8	8.1	108	240	1329	1474	15.1	Sandstone and Mudstone. Sand to Loam. Freely draining acid loamy soils over rock.

The information relating to the climatic conditions at each of the trial sites was obtained from ESC-Ecological Site Classification (Clare and Ray 2001). We focused on five parameters because we considered them likely to reflect elements of the climate that are key to tree growth: temperature, water availability, and other variables which are dependent on the geographical location of the site. The indices and their definitions are:

CT: Continentality, corresponds to the Conrad Index (Conrad 1946). $CT = 1.7 [A/\sin(\phi + 10)] - 14$. Where A is the difference between the mean temperature of the warmest and coldest month in degrees Celsius and ϕ is latitude in degrees. Lower values indicate more oceanic climates.

MD: Moisture deficit (mm).To consider the effects of the dryness of the growing season moisture deficit is calculated by considering the potential evaporation and precipitation. Higher values indicate drier sites.

AP: Annual precipitation. Average annual rainfall (mm) between 1961 and 1990.

GDD: growing degree days, the cumulative sum of the number of degrees Celsius above 5 °C on each day of the year in which mean temperature exceeds 5 °C. Values are long term mean annual values for the years spanning from 1961 to 1990.

DAMS: Wind exposure measured by **Detailed Aspect Method of Scoring**: This provides an indication of wind exposure, the higher the values the more exposed the site. Sites with values under 12 can be considered to be sheltered.

Soil Type: the soil information data were obtained from different inventories, using the UK Soil Observatory (UKSO). The European Soil Bureau description (The European soil database, 2006) was used to assign a soil type for all British trial sites. The soil texture and soil group data were obtained from Soilscapes (Cranfield University, 2017) for Welsh and English locations, and from the national soil map of Scotland (Soil Survey of Scotland Staff, 1981) for the Scottish trials.

Rowan (*Sorbus aucuparia* L.)

Fruits from a total of 42 provenances were sent to Forest Research's Newton field station near Elgin for manual extraction of seed, then sown and raised into seedlings at Forest Research's nursery (Northern Research Station, Edinburgh, NT248639). In 2006, when the seedlings were one year old they were planted in two field based common garden experiments. Due to a shortage of plants from some provenances, only 33 were planted in both trials (Table 4.3).

The trial sites (Figure 4.2) were in South Wales (Llandovery - LLAN) and East Yorkshire (North York Moors - NYM). At each site, the experiment followed a randomized block design consisting of three replicated blocks with each provenance represented by a single plot in each block. The plots consisted of 9 (3 x 3) trees from the same provenance, planted at 2 m spacing.

Table 4.2. Details of the ash provenance source locations and the trial site in which they were planted. X indicates presence of a provenance at a given site.

ROP	Country	Ash provenances	Lat	Long	Alt (m)	Site	
						LLAN	NYM
NW	Scotland	Duisdale, Skye	57.17551	-5.75117	18	x	x
		Kilninian, Mull	56.52959	-6.20799	71	x	x
		Rassal Wood, Kishorn	57.42572	-5.59116	78	x	x
		Ardtornish, Morvern	56.55802	-5.74080	20	x	x
		Glasdrum Wood, Loch Creran	56.57409	-5.23204	33	x	x
		Add Valley, Kilmichael Glassary	56.10608	-5.42003	30	x	x
		Clyde Valley	55.67987	-3.91378	159	x	x
		Shielhill Glen	55.91098	-4.82460	107	x	x
		Penpont	55.23484	-3.85285	90	x	x
		Nith Valley	55.32013	-3.82903	141	x	x
		Crawick Water	55.38070	-3.92901	162	x	x
	England	Warks Burn	55.08810	-2.22184	90	x	x
NE	Scotland	Erchite Wood, Dores	57.36812	-4.34520	56	x	x
		Craigellachie	57.48392	-3.17014	102	x	x
		Fearnan Forest, Kenmore	56.57883	-4.03655	142	x	x
		Glen Lyon	56.60154	-4.24476	183	x	x
		Den of Alyth	56.62334	-3.25773	152	x	
		Pitcairns Glen, Dunning	56.29959	-3.57373	119	x	x
		Tweed Valley North Glen	55.58841	-2.66224	68	x	x
	England	Castle Eden Dene, Peterlee	54.74302	-1.35233	102	x	x
SW	England	Witherslack	54.26362	-2.87015	79	x	x
		Park Wood&Hutton Roof	54.18157	-2.68983	170	x	x
		Via Gellia Woods	53.10412	-1.61940	239	x	x
		Upper Wharfedale	54.20254	-2.10359	202	x	x
		Greta Wood, Purbeck Ridge	50.63671	-2.13570	126	x	x
		Horner Wood, Porlock	51.18901	-3.58257	102	x	x
	Wales	Cardiff Area	51.54568	-3.23397	158	x	x
		Aberystwyth Area	52.43015	-4.05854	90	x	x
		Betws-y-Coed	53.07863	-3.79924	57	x	x
		Talgarth	51.98553	-3.21297	198	x	x
SE	England	Forge Valley	54.27437	-0.49037	52	x	x
		Ashberry Woods	54.26218	-1.13339	142	x	x
		Treswell Woods	53.30786	-0.86124	54	x	
		Hayley Wood	52.15840	-0.10991	79	x	x
		Tick Wood, Ironbridge	52.62181	-2.52287	99	x	x
		Forest Bank, Marchington	52.85206	-1.82028	142	x	x
		Wyndcliff, Wye Valley	51.67790	-2.67969	208	x	x
		Midger Wood	51.60551	-2.28572	160	x	x
		Pheasant Copse, Petworth	51.01113	-0.62770	60	x	x
		Bignor Hill	50.90848	-0.61611	194	x	x
		Groton Wood	52.04999	0.88313	66	x	x
		Out Wood	52.16578	0.41539	96	x	x

Table 4.3. Details of the rowan provenance source locations and the trial sites in which they were planted. X indicates presence of a provenance at a given trial site.

ROP	Country	Rowan provenances	lat	long	Alt (m)	Site	
						LLAN	NYM
SW	Scotland	Assynt	58.1707	-5.2616	27.48		X
		Allt Volagir, South Uist	57.2473	-7.3102	68.76	X	X
		Salen, Isle of Mull	56.5270	-5.9606	5.80	X	X
		Tokavaig, Isle of Skye	57.1387	-5.9650	25.40	X	X
		Add Valley	56.1406	-5.4861	33.72	X	X
		Strathlachlan	56.1284	-5.1529	70.32	X	X
		Falls of Clyde	55.6528	-3.7777	160.84	X	X
		Mugdock Country Park	55.9707	-4.3205	161.80	X	X
		Glenlee	55.0884	-4.1935	129.68	X	X
		Lochwood	55.2577	-3.4428	180.20	X	X
		Ettrick Water	55.4201	-3.1327	234.16	X	X
		Bunchrew	57.4713	-4.3153	100.80		X
		Craigdarroch	57.5742	-4.6051	31.80		X
		Cleanhill Wood, Aberchirder	57.5543	-2.6364	158.76		X
SE	Scotland	Birks of Aberfeldy	56.6024	-3.8716	285.96		X
		Pressmennan Wood	55.9513	-2.5885	149.84	X	X
		Castle Eden Dene	54.7433	-1.3507	107.64	X	X
		Horner Wood	51.1943	-3.5885	232.84	X	X
		Holford/Hodder’s Combe	51.1583	-3.2183	164.88	X	X
		Duddon Valley	54.3164	-3.2298	61.84	X	X
NW	England	Naddle Forest	54.5114	-2.8047	291.24	X	X
		Brignall Banks	54.4959	-1.9129	222.36	X	X
		Gelt Wood	54.9028	-2.7329	139.16	X	X
		Brechfa	51.9930	-4.0633	146.28	X	X
		Beddgelert	53.0247	-4.1380	257.76	X	X
		Mynydd Du	51.9544	-3.1046	532.12	X	X
	Wales	Ugly House	53.1057	-3.8683	231.32	X	X
		Forge Valley& Raincliffe Woods	54.2748	-0.4837	89.64	X	X
		Ashberry and Reins Woods	54.2533	-1.1284	106.24	X	X
		St. He len’s Wood, Coningsby	53.1132	-0.1226	6.24	X	X
NE	England	Moor Farm	53.1562	-0.1814	14.80	X	X
		Pepper Wood	52.3682	-2.0916	144.76	X	X
		The Ercall	52.6865	-2.5223	185.80	X	X
		King’s Bottom, Longleat	51.1909	-2.2406	219.00	X	X
		Chestnuts Wood, Forest of Dean	51.8293	-2.4703	161.72		X
		Saxonbury Hill	51.0763	0.2509	183.12	X	X
		Seal Chart	51.2778	0.2374	129.44	X	X
		Culter’s Wood, Freston, Ipswich	52.0105	1.1419	34.40	X	X
		Felbrigg great Wood, Cromer	52.9134	1.2639	83.24	X	X

4.2.3. Measurements

4.2.3.1 Phenology measurements

The ash and rowan trials were assessed for leaf phenology, in spring and autumn. The different methods of assessment are explained below and the list of assessments are provided in Table 4.6.

4.2.3.1.1 Spring phenology:

Spring phenology was assessed using a six-stage ordinal morphological scale for both species. The top bud of each tree was visually assessed to determine which of the six stages best described the development stage of the leaf. In this scoring system stage 1 corresponds to a dormant fully closed bud while stage 6 corresponds to a fully expanded bud (Table 4.4, Figure 4.3).

The terminal bud of each tree was assessed and scored on each visit. If the terminal bud was damaged, missing or dead then the next highest bud on the main stem was assessed. If the tree had more than one leading stem (i.e., was forked), then the bud on the stem with the largest diameter was assessed. Where the two stems were found to be equal in diameter, the highest bud was assessed. If forks were equal in height and diameter then the bud giving the highest score was recorded.

The ash trials were visited in late spring 2013, when the trees were 7 years old. They were assessed on 13 occasions in Llandovery and on 15 occasions in North York Moors, between April and June. Rowan sites were visited on three occasions in late March to early April of 2008, when the trees were 2 years old (Table 4.6).

Table 4.4. Stages of bud flushing in both species

species	Flushing stages (scores)
ASH	1. Bud closed, black, fully dormant winter state. 2. Bud swollen but still closed, green-black in colour. 3. Bud scales partially separated, some leaves visible. 4. Bud scales completely separated, leaves visible but still furled and extending <1cm beyond scales. 5. Leaves elongated >1cm from scales and spreading but leaflets still furled. 6. All leaflets separated and shoot expanding.

ROWAN	1. Bud closed fully dormant winter state. 2. Bud swollen and the bud scales just started to open, however the bud is still vertical. 3. Bud scales separated and the tightly furled leaves visible. The bud is bent sideways and can appear “hooded”. 4. Bud scales completely separated, leaves starting to unfurl and separate but the leaflets (pinnae) on each leaf still furled. The leaves appear brownish in colour since the underside is predominantly visible. 5. Leaves elongated and leaflets starting to separate as well. The appearance is now much more green since the top side of the leaves is visible. 6. All leaflets separated on lowest two leaves and shoot expanding.

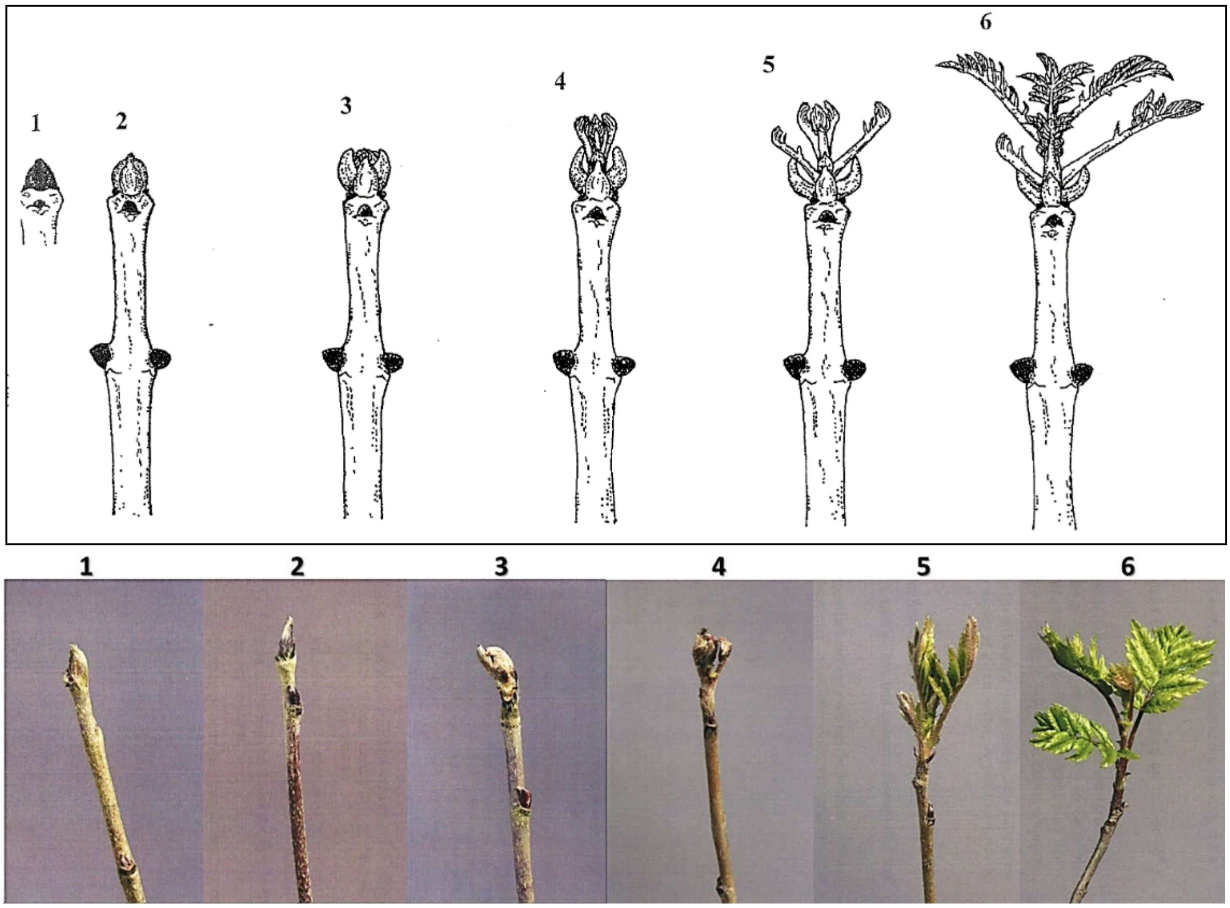


Figure 4.3. Stages of bud flushing in both species, ash above and rowan below.

4.2.3.1.2 Autumn phenology:

Assessments of autumn phenology were based on foliage throughout the entire crown rather than a single leaf or a sub-set of leaves. The crown of the tree was visually inspected and assessment was based on the proportion of the crown that was visible to the assessor standing on the south-facing side of each tree. In this case, different visual scales were used for each species.

For ash, senescence was measured as the percentage of the canopy which had shed its leaves (Table 4.5). This ranged from stage 1 (0% leaf loss) to stage 6 (100% leaf loss).

For rowan, the scoring system was based on the proportion of crown that was still green rather than yellow, brown or absent due to leaf fall. Trees were ranked from 5 (81-100% green) to 1 (0-20% green) (Table 4.5). All lost leaves were counted as "not green", identifying the gaps in regular patterns of the leaves plus the leaf scars on the shoots. Where loss of colour had led to a patchy distribution of green on the leaves, the assessor estimated the proportion of total green colour in the crown, rather than by individual leaves.

Both ash trials were assessed in autumn 2014, one year later than spring phenology measurements were made. In Llandovery trees were scored on 20 occasions and in North York Moors, trees were scored on 23 occasions (Table 4.6). For rowan, assessments were conducted in 2008, during the same year as spring phenology assessments were made. The trees were scored on 3 dates in early autumn, at different dates on each trial. Scoring schedules had to be planned in advance and prior knowledge of the most appropriate timing of assessments was lacking. In consequence the timing of assessments was rather too early in the season and scoring was done before most trees were in an advanced stage of leaf senescence.

Table 4.5. Leaf senescence scale for ash and rowan.

Ash Leaf senescence scale		**Rowan Leaf senescence scale**	
1	No leaf loss	**1**	0-20% green
2	1-25% leaf loss	**2**	21-40% green
3	26-50% leaf loss	**3**	41-60% green
4	51-75% leaf loss	**4**	61-80% green
5	76-99% leaf loss	**5**	81-100% green
6	100% leaf loss		

Table 4.6. Range and frequency of dates on which ash and rowan trials were assessed for spring and autumn leaf phenology.

Species	Trial Site	Spring phenology	Autumn phenology
Ash	**Llandovery**	*13 visits* 23rd Apr - 19th June 2013	*20 visits* 2nd of Sept - 11th of Nov 2014
	North York Moors	*15 visits* 22nd Apr - 18th June 2013	*23 visits* 26th of Aug - 10th of Nov 2014
Rowan	**Llandovery**	*3 visits* 18th Mar, 3rd & 10th Apr 2008	*3 visits* 8th, 18th Sept & 2nd Oct 2008
	North York Moors	*3 visits* 19th Mar, 3rd & 10th Apr 2008	*3 visits* 10th, 18th & 22nd Sept 2008

4.2.4.2 Stem form

Ash trees were assessed for stem form in both trial sites by counting the number of forks on the main stem. This metric represents the number of occasions upon which the main stem had lost apical dominance due to bud damage, resulting in the subsequent branches coming from these forks up to the crown. The average number of forks per plot was used as the measure of stem form. Data were log transformed prior to analysis to meet assumptions of normality.

4.2.4 Climatic data

Temperature is known to affect tree leaf phenology (Wielgolaski, 1999). Long-term average temperature data were obtained from the Met Office to compare the three years in which phenology data were collected. For the trial sites in Llandovery, data were obtained from the nearest Met Office meteorological station (Saron, N 52.01 W4.31), which is approximately 32 km west of the trial site. For the two North York Moors trials, temperature data were based on regional averages obtained from the Met Office for North East England. The data obtained were data for those two areas between 1995 and 2015 as seasonal averages summaries: Winter (WIN, from the start of December to the end of February), Spring (SPR, start of March until the end of May), Summer (SUM, from the start of June until the end of August), and Autumn (AUT, from the start of September to the end of November). We compared the season average temperature of the years when phenological data was collected (2008 for rowan spring and autumn phenology; 2013 for ash spring phenology; and 2015 for ash autumn phenology) with the long-term average temperatures over the past 25 years in the same location (Figure 4.5 for Llandovery and Figure 4.6 for North York Moors).

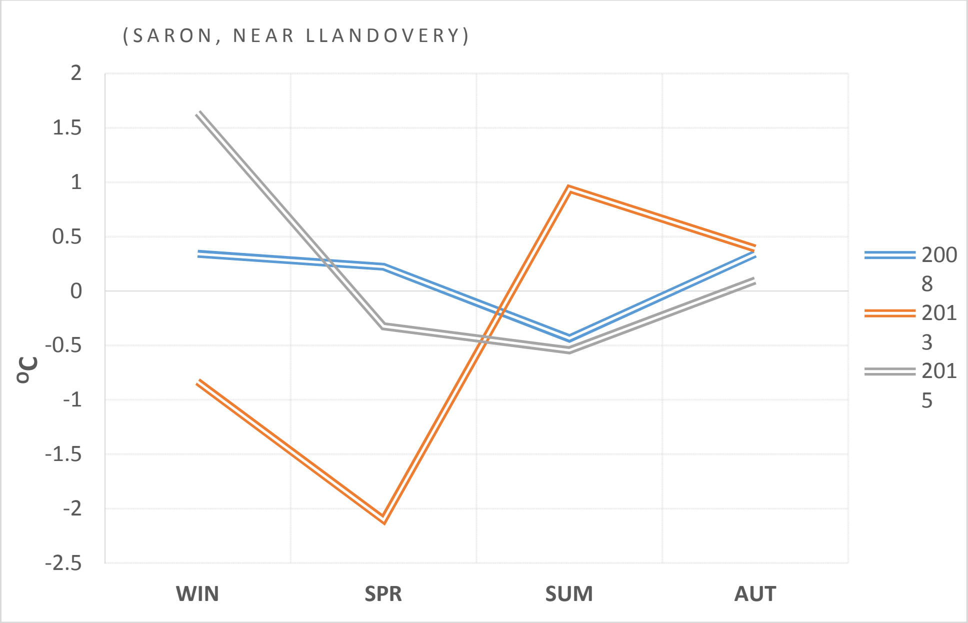


Figure 4.5. Difference between the average temperature in years 2008, 2013 and 2015, and the average of the last 25 years, at the Saron climatic station. Temperature is the seasonal average (WIN=winter, SPR=spring, SUM=summer and AUT=autumn. Data from Met Office).

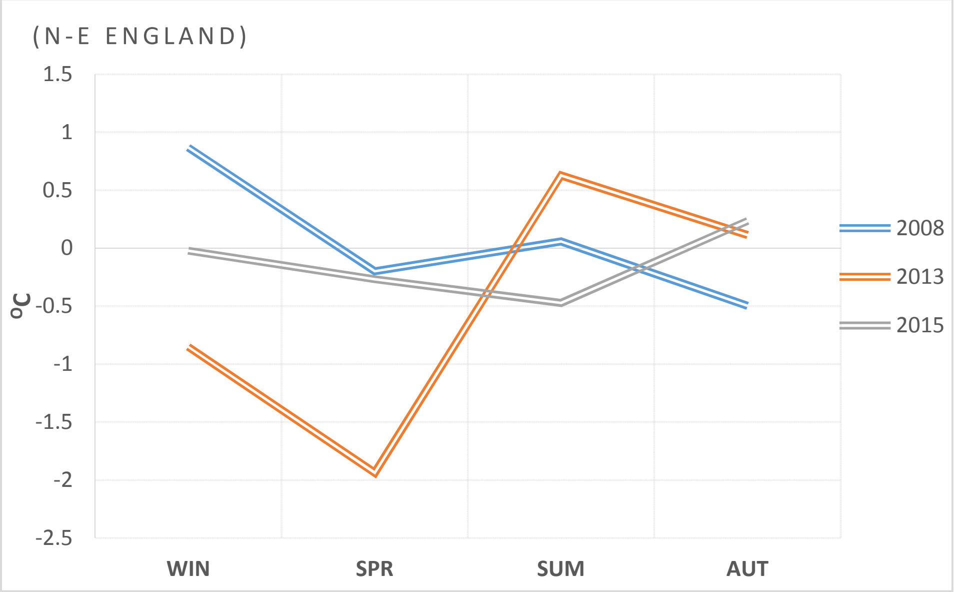


Figure 4.6. Difference between the average temperature in years 2008, 2013 and 2015, and the average of the last 25 years in the North of England Region. Temperature is the seasonal average (WIN=winter, SPR=spring, SUM=summer and AUT=autumn. Data from Met Office).

4.2.5 Data analysis

Estimating means of leaf flushing and senescence.

For analysis, dates were transformed into Julian dates (JD), commencing from January 1.

For ash, due to the large numbers of observations of spring and autumn phenology it was possible to construct an accurate model of leaf phenology of the trees. To predict the timing of transition between ordinal stages, ordinal logistic regression was applied using 'cumulative link models'. Using these models, estimates of the dates (expressed in Julian days) on which 50% of trees per plot were at the fully flushed stage (stage 6) ('JD50_F'), and on which 50% of the trees in the plot reached full defoliation (100% leaf loss) ('JD50_S'). For each plot, curves were modelled with a common slope, which mirrored the raw data distribution (Figure 4.7). For each trait, the mean JD50 per provenance was the average of modelled JD50 from three plots per provenance. Confidence intervals (at 95% level) associated with mean provenance values were calculated (as the mean trait value ± 1.96 times the standard deviation).

For rowan, as frequency of observations was lower, it was not possible to model phenology and the response variable used was a simple mean score per plot on the date when differences among provenances were greatest at each site.

To enable direct comparison between ash and rowan, mean flushing score on the date on which there was the greatest variation amongst provenances was also calculated for both species.

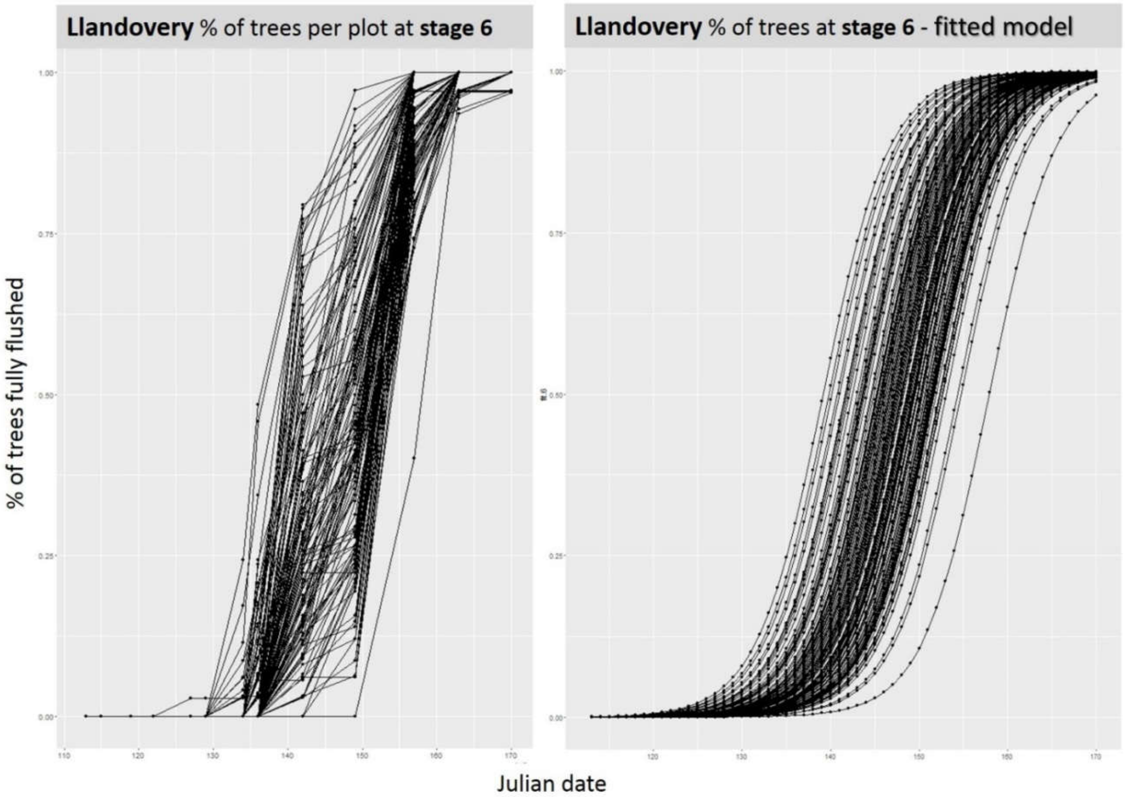


Figure 4.7. Mean per plot of trees in fully flushed (staged 6) in Llandovery (left) versus the fitted model. Each plot is represented by a line in the ordinal logistic regression (right).

Analysis of Sources of Variation

Analysis of variance (ANOVA) based on linear models was used to determine the effects of provenance and site on the plot means for each trait. The different models of ANOVA used were as follows:

i. By species, trait and trial site; provenance was a fixed factor and block was a random factor.
ii. By species and trait across all trial sites; site, provenance, and site by provenance interaction were fixed effects, block nested within site was a random factor.

Relationship with site of origin

For each trait, linear regressions were used to test the relationship between provenance means and latitude and longitude of the home site. Multiple linear regressions including both longitude and latitude were carried out; if the relationship with only one of the coordinates was significant then a single linear regression was done. Linear regression plots were drawn.

Phenological variation in leaf flushing and senescence has been shown elsewhere to be driven by multiple environmental factors (White et al., 1997; Vitasse et al., 2009) which vary with location. In GB, the predominant axes of environmental variation are captured well by latitude and longitude, which summarise climatic variables (as shown in Chapter 2). Here we use latitude and longitude as proxies for local environmental variation.

Trait covariation.

In ash, to test for covariation between phenology and form, Pearson's correlation coefficients were calculated using plot means for flushing and forking from individual sites.

Statistical analyses were performed in R version 3.2.3 (R Core Team, 2015). The data packages for data management, analysis and visualisation used were "dplyr" (Wickham and Francois 2015) and "ggplot" (Wickham, 2009). Cumulative link models were processed using the 'clm' command in the 'ordinal' package in R (Christensen, 2015)

4.3 Results

4.3.1 Leaf phenology in Ash

4.3.1.1 Ash spring leaf phenology

Significant differences were found among provenances for the timing of spring leaf flush (JD50_F) at both sites (Table 4.7; Figure 4.8). The mean JD50_F was earlier by three days in the northern trial site (NYM) (24th of May) than it was in southern trial site (Llandovery) (27th of May) (Table 4.8; Figure 4.9). In both trials, the means across provenances from each ROP showed a similar geographical pattern (Table 4.8). In general, provenances from the south east ROP flushed earliest, and provenances from the north-west ROP flushed latest, although there was variation among provenances from the same region (Figure 4.8). Latitude explained around 40% of the variation at both trial sites (Figure 4.10). The difference between JD50_F of the earliest and latest provenances was 18 days in Llandovery (from Julian date 129 to 157) and 11 days in North York Moors (140 to 151). No significant interaction was found between provenance and trial site for JD50_F. However there was a significant site effect (Table 4.9). The interaction plot (Figure 4.9) shows how nearly all provenances flush earlier at the North York Moors site. The trees growing in the more northerly trial site were fully flushed slightly earlier.

Table 4.7. Analysis of variance of JD50_F in ash at the two trial sites.

		Df	Sum Sq	Mean Sq	F value	Pr(>F)
Llandovery	**PROV**	41	1267.69	30.9193	11.2493	<2e-16 ***
	BLOCK	2	5.29	2.6429	0.9615	0.3866
	Residuals	82	225.38	2.7485		
North York Moors	**PROV**	39	631.46	16.1914	4.5148	2.496e-08 ***
	BLOCK	2	35.04	17.5223	4.8859	0.01036 *
	Residuals	69	247.46	3.5863		

Table 4.8. Mean values of JD50_F by ROP and trial site

Trial Site	**Llandovery (JD50_F)**		**North York Moors (JD50_F)**		**Difference LLAN - NYM**
	Mean trial	**147.26**	**Mean trial**	**144.70**	**2.55**
ROP	**NW**	150.03	**NW**	146.94	3.00
	NE	148.79	**NE**	145.94	2.84
	SW	145.70	**SW**	143.42	2.32
	SE	144.78	**SE**	142.57	2.09

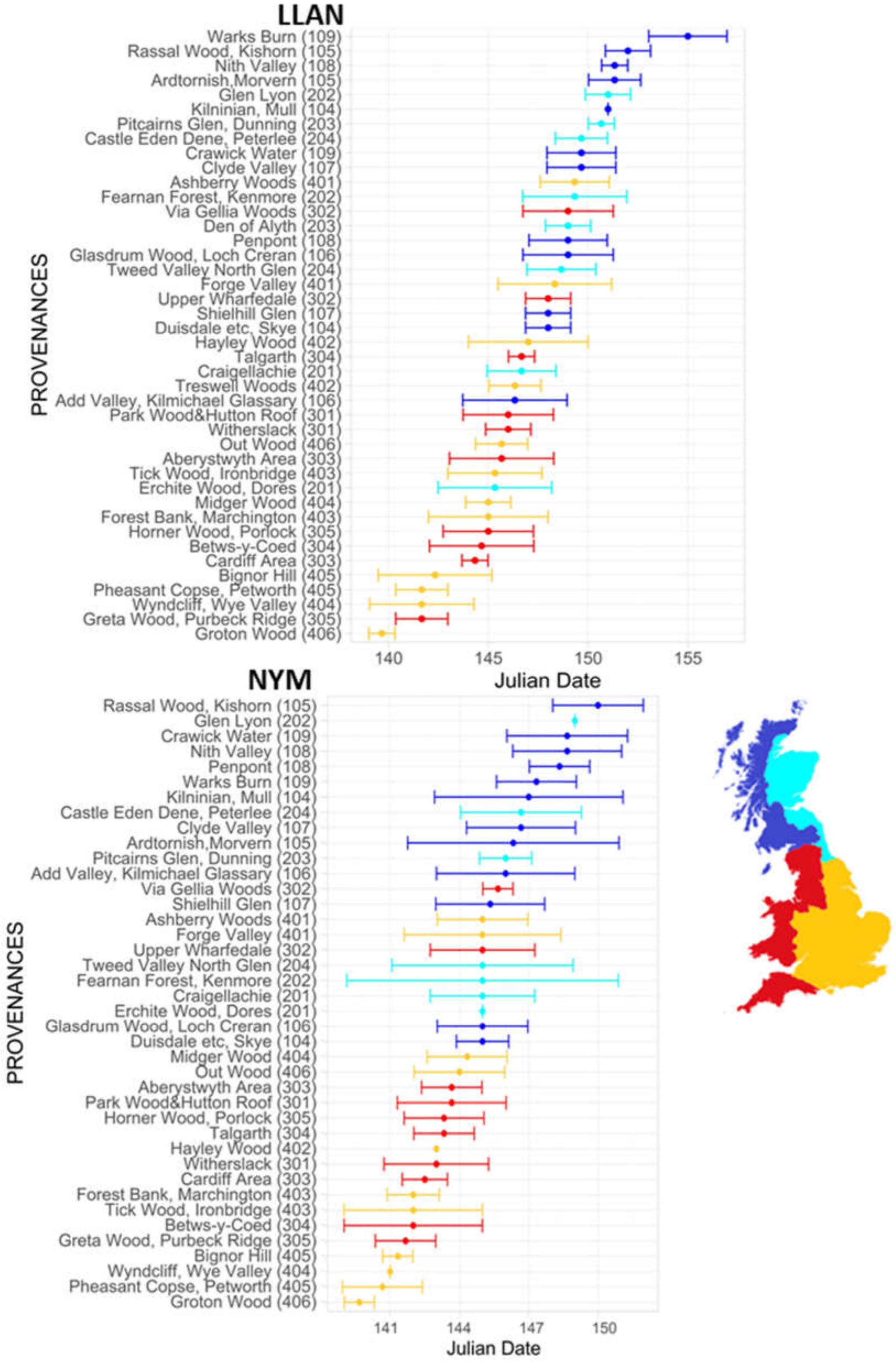


Figure 4.8. Mean provenance JD50_F, with 95% confidence intervals, with colours corresponding to ROP (following inset map).

Table 4.9. Analysis of variance table with interaction between provenance and site.

		Df	Sum Sq	Mean Sq	F value	Pr(>F)
JD50_F JD 50% trees Fully flushed	**PROV**	41	1779.32	43.40	13.86	<2e-16 ***
	SITE	1	369.42	369.42	117.97	<2e-16 ***
	Prov x Site	39	142.38	3.63	1.16	0.2542 ns
	BLOCK	4	40.33	10.08	3.22	0.0144 *
	Residuals	151	472.84	3.13		

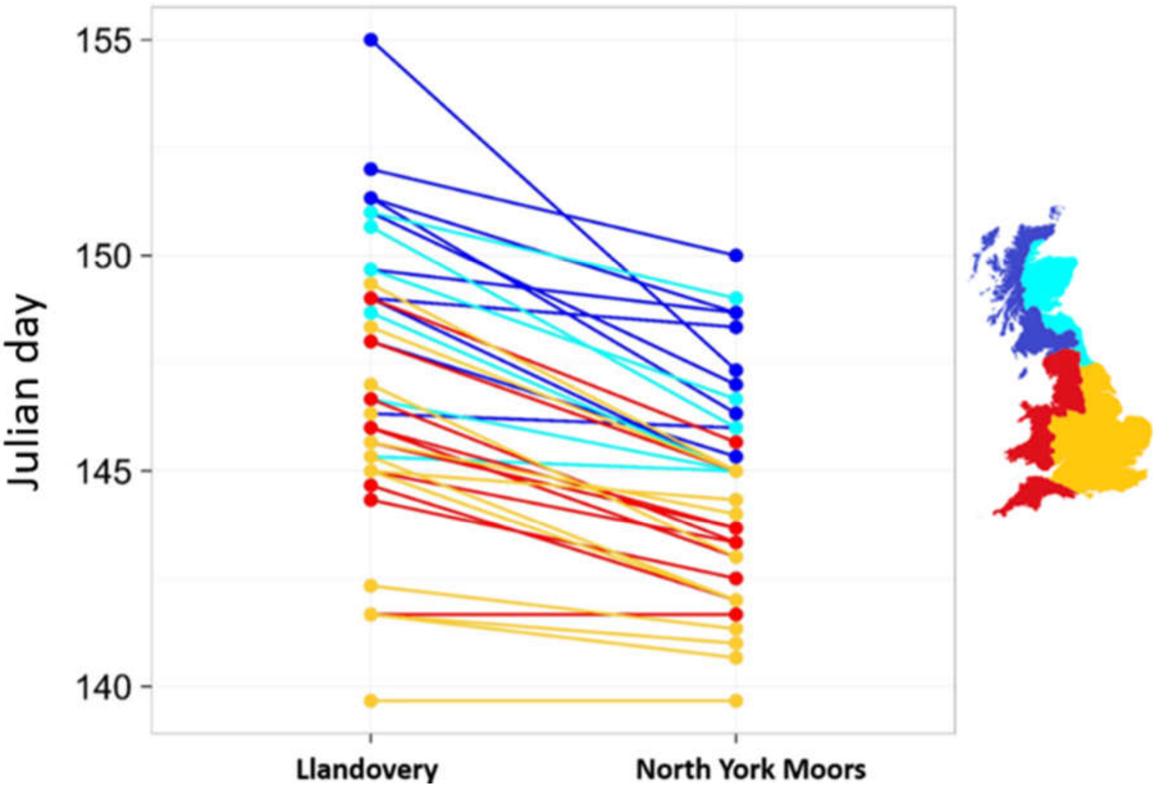


Figure 4.9. Plot of interaction between JD50_F and trial site per provenance. Colour represents the ROP (following inset map).

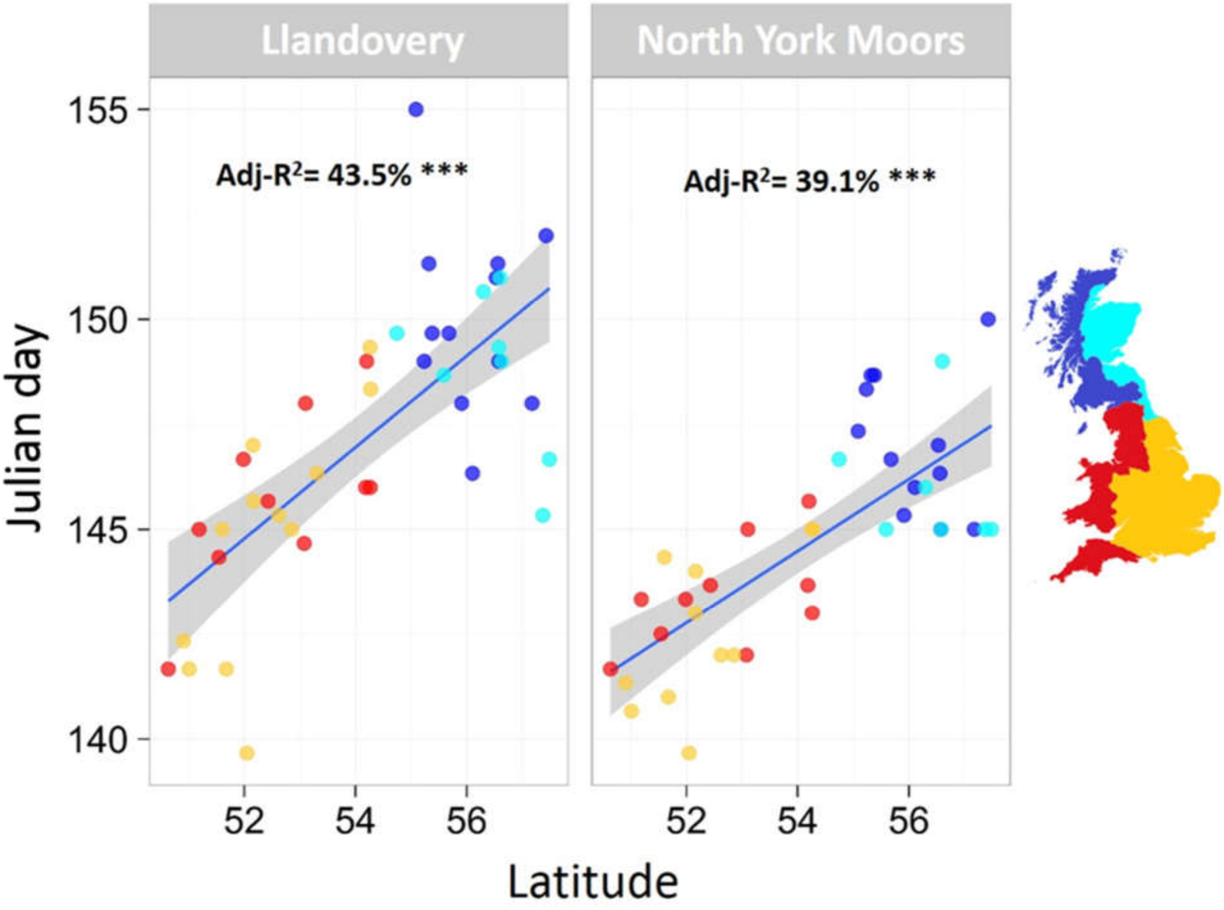


Figure 4.10. Linear regression of JD50_F with Latitude per provenance at the Llandovery and North York Moors trial sites. Colour represents ROP (following inset map).

4.3.1.2 Ash stem forking and leaf flushing

At each site, a significant negative correlation was found between number of forks and JD50_F (Llandovery, $R = -0.18$, $p < 0.05$; North York Moors, $R = -0.23$, $p < 0.05$), whereby early flushing was related to higher number of stem forks (Figure 4.11). The effect was most pronounced at North York Moors, which exhibited greater variation in the number of stem forks, as forking at Llandovery was generally high for all provenances.

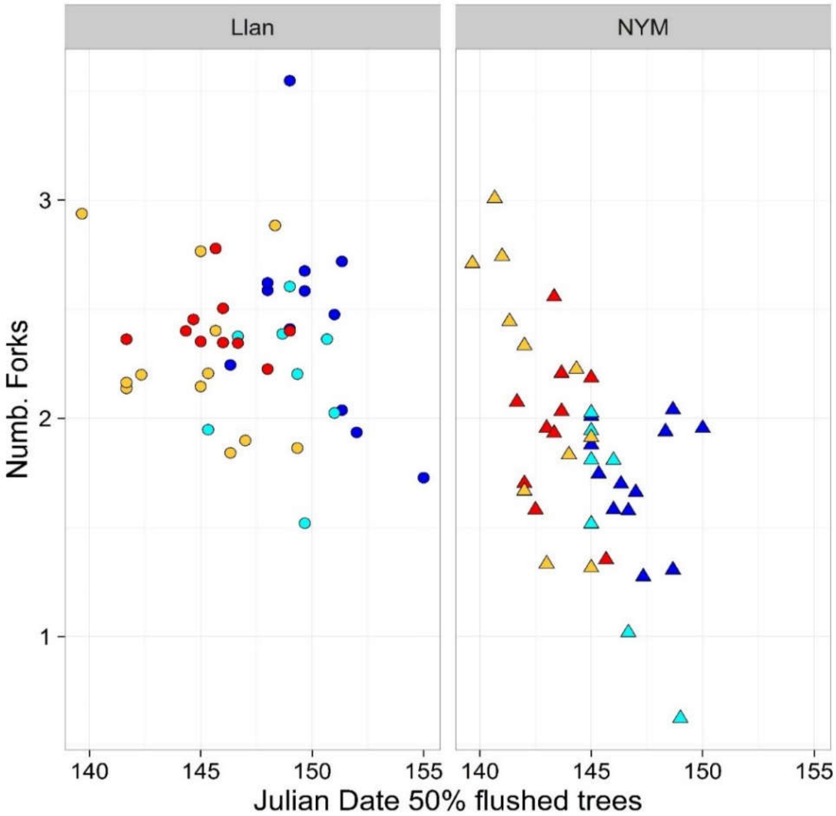


Figure 4.11. Scatterplot of the mean number of forks and JD50_F by provenance. The colour corresponds to ROP.

4.3.1.3 Ash autumn leaf phenology

For leaf senescence, there were significant differences amongst provenances in the North York Moors trial only (Table 4.10), which can be observed in the aerial photograph (Figure 4.12), taken at the time of investigation. The trial average JD50_S was 8 days earlier in North York Moors than in Llandovery, meaning that the trees lost all their leaves earlier in the North York Moors trial (Table 4.11). As with spring phenology, the order of provenances in both trials show a similar geographical pattern. Provenances from the north-west ROP lost their leaves earliest, whilst those from the south eastern ROP lost their leaves latest.

The difference between the earliest and latest provenances was larger at Llandovery (37 days, from JD 278 to 315) than at North York Moors (24 days, from JD 279 to 303). There was a large site effect (Table 4.12): in the interaction plot (Figure 4.14) all provenances except one (from north east region) lose their leaves earlier in the North York Moors trial than in Llandovery. There was no significant interaction between provenance and trial site, although the ranking of provenances from the earliest to the latest to lose their leaves differed between sites (Figure 4.9). At Llandovery, the provenances show a clearer latitudinal cline than at North York Moors, when the provenances are ranked according to their JD50_S. Latitude explained 44% of the variation in North York Moors compared to only 32% in Llandovery (Figure 4.15).

Figure 4.12. Aerial photograph of part of the ash trial at the North York Moors site taken from a drone during leaf senescence, October 2015.

Table 4.10. Analysis of variance of JD50_S for ash at the two trial sites.

		Df	Sum Sq	Mean Sq	F value	Pr(>F)
Llandovery	**PROV**	41	1718.10	41.90	1.4986	0.06074 NS
	BLOCK	2	748.43	374.21	13.3828	9.337e-06 ***
	Residuals	82	2292.90	27.96		
North York Moors	**PROV**	39	1185.77	30.40	2.1599	0.002579 **
	BLOCK	2	1153.86	576.93	40.9841	1.857e-12 ***
	Residuals	69	971.31	14.08		

Table 4.11. Mean values of JD50_S by trial site and ROP.

Trial Site	**Llandovery (JD50_S)**		**North York Moors (JD50_S)**		**Difference LLAN - NYM**
	Mean trial	**299.52**	**Mean trial**	**291.33**	**8.34**
ROP	**NW**	296.78	**NW**	289.65	7.16
	NE	296.63	**NE**	291.12	5.79
	SW	301.87	**SW**	292.97	8.90
	SE	302.25	**SE**	291.80	10.77

Table 4.12. Analysis of variance of JD50_S.

		Df	Sum Sq	Mean Sq	F value	Pr(>F)
JD50_S JD 50% leafless trees	**PROV**	41	1835.7	44.8	1.7188	0.009971**
	SITE	1	3865.3	3865.3	148.3859	< 2.2e-16 ***
	Prov x Site	39	1083.7	27.8	1.0668	0.379841 ns
	BLOCK	2	1181.0	590.5	22.6698	2.383e-09 ***
	Residuals	153	3985.5	26.0		

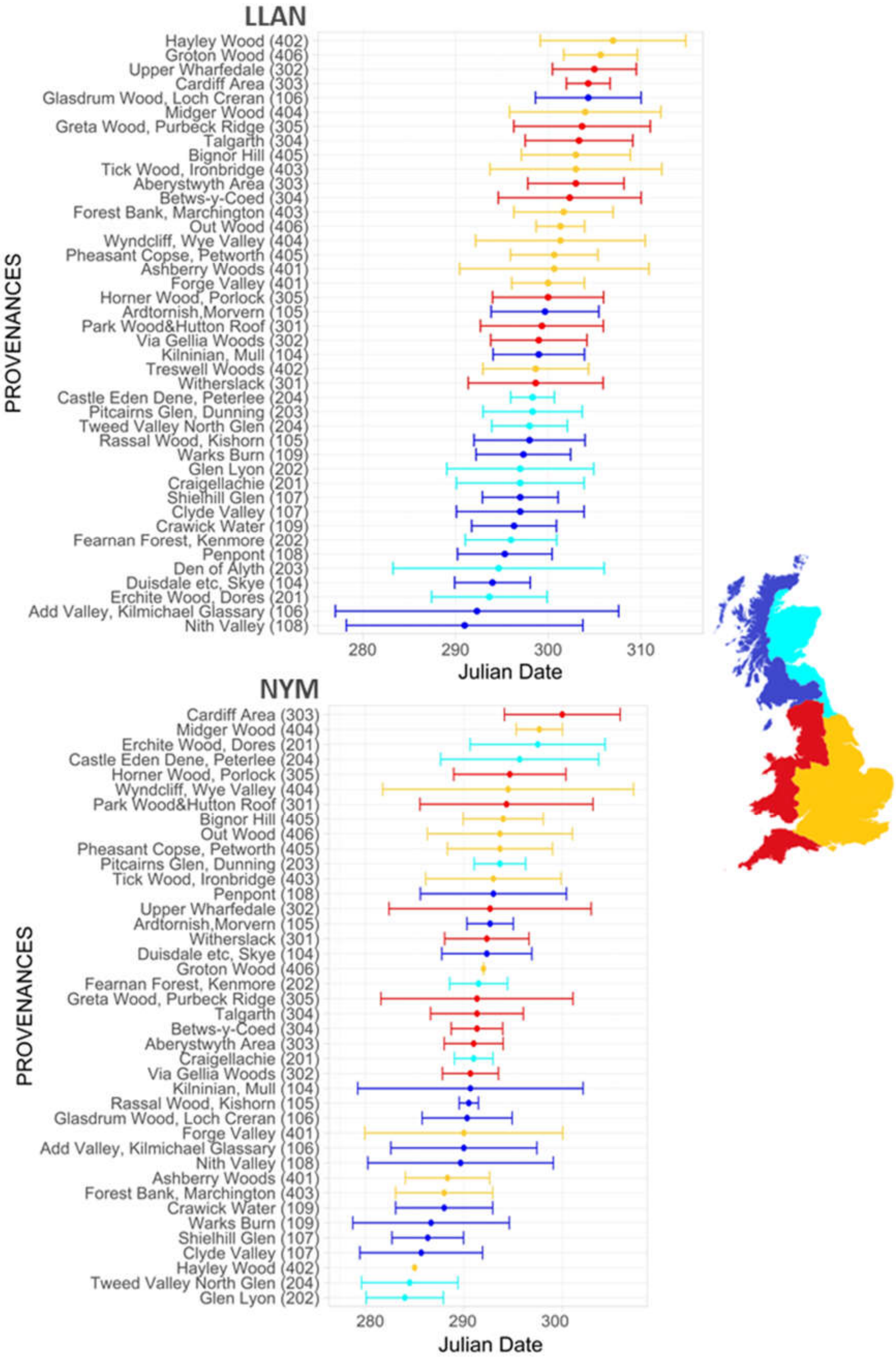


Figure 4.13. Mean JD50_S, with 95% confidence intervals for each provenance at each trial site. Colours correspond to ROP (following inset map).

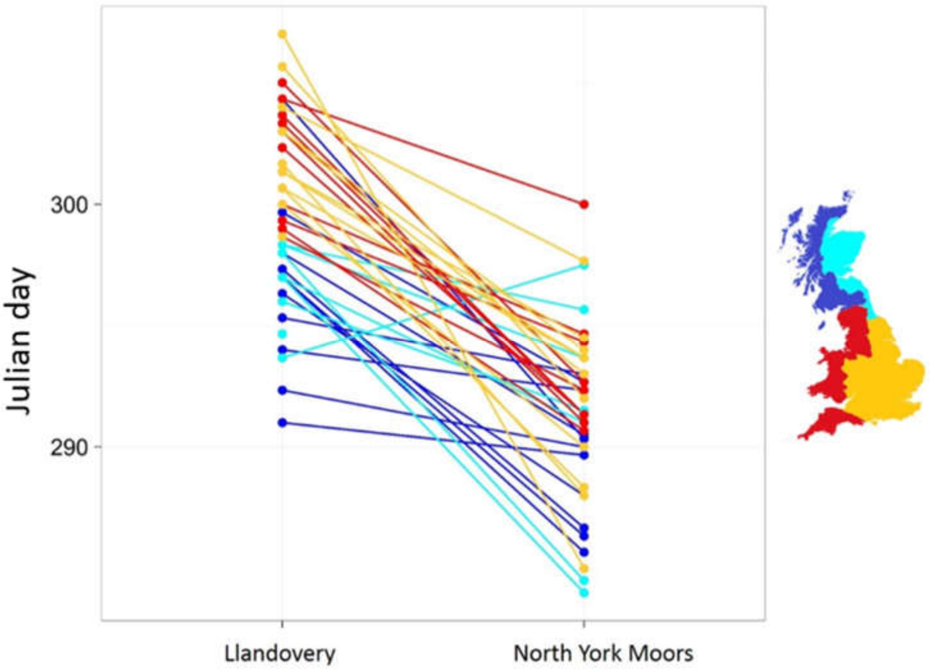


Figure 4.14. Plot of the interaction between JD50_S and trial site per provenance. Colours correspond to ROP (following inset map).

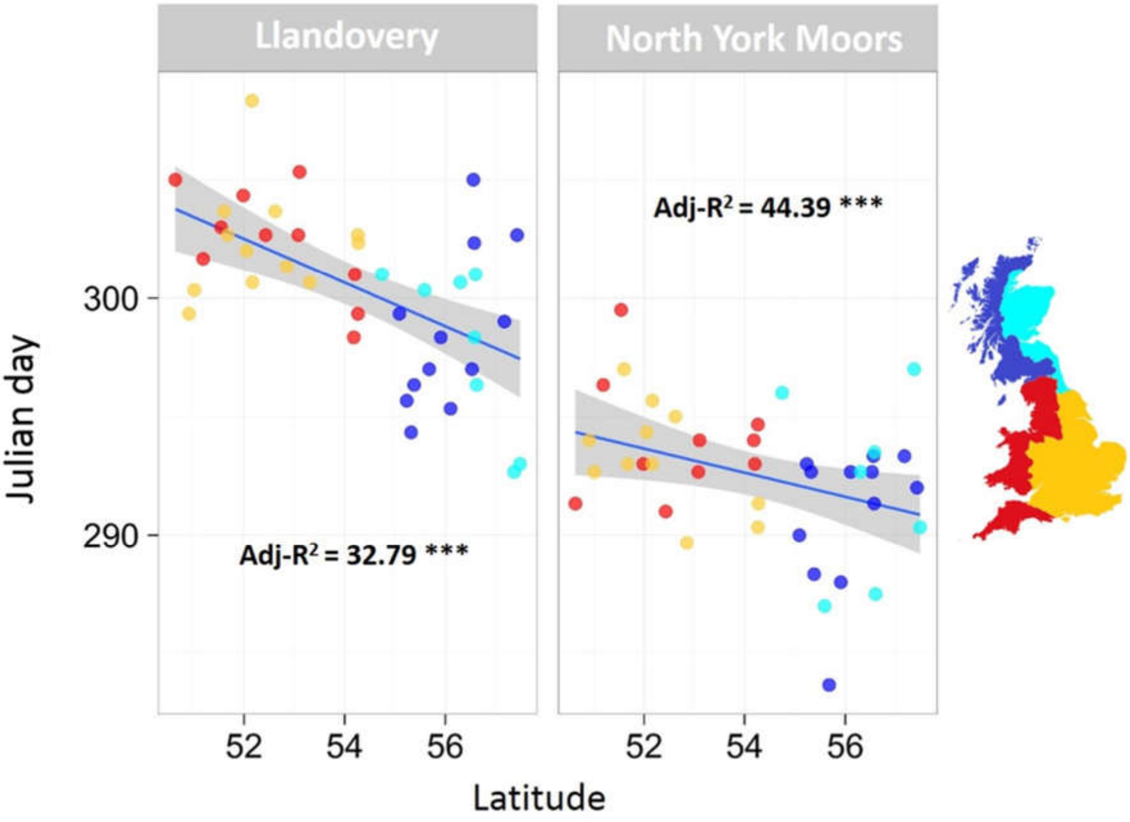


Figure 4.15. Linear regression of JD50_S versus latitude. Each point represents a provenance mean. Colours correspond to ROP (following inset map).

4.3.2 Comparison of Leaf phenology in Ash and Rowan

4.3.2.1 Spring leaf phenology

4.3.2.1.1 Genetic diversity and plasticity in both species

Ash: There were significant differences amongst ash provenances at most of the scoring dates (apart from the earliest and latest dates, there were significant differences in Llandovery between JD113 and 163, and in North York Moors between JD119 and 162). The dates when the differences amongst provenances where the greatest were JD136 for Llandovery and JD33 for North York Moors (Table 4.13). There was no significant interaction between provenance and site. In ash the differences amongst provenances were greater in Llandovery than in North York Moors (Figure 4.17). The trees from NW were the least flushed (Table 4.14).

Rowan: For rowan, there were significant ($p<0.001$) differences amongst provenances at the three dates when trees were scored in both trial sites, the date when the differences were greater was JD101. There was also a significant interaction between provenance and site ($p<0.001$). The differences between provenances were much greater in Llandovery (Figure 4.16) than North York Moors. All provenances except a few from SW and NW ROPs were more advanced in Llandovery than in North York Moors. In both trial sites the eastern provenances were the most advanced in their flushing stage.

Table 4.13. Analysis of variance of the flushing mean score on the dates when the differences amongst provenances were the greatest, for rowan and ash.

Species	Mean Flushing Score		**ANOVA TABLE**				
			Df	**Sum Sq**	**Mean Sq**	**F-value**	**Pr(>F)**
Ash	Llandovery JD 136	**PROV**	41	43.554	1.06229	8.2897	4.065e-16 ***
		BLOCK	2	0.131	0.06528	0.5095	0.6027 NS
		Residuals	82	10.508	0.12815		
	North York Moors JD 133	**PROV**	39	19.9163	0.51067	4.5461	2.176e-08 ***
		BLOCK	2	1.6489	0.82445	7.3393	0.001288 **
		Residuals	69	7.7509	0.11233		
Rowan	Llandovery JD 101	**PROV**	33	199.894	6.0574	33.7205	< 2.2e-16 ***
		BLOCK	2	2.830	1.4150	7.8772	0.0006061 ***
		Residuals	122	21.916	0.1796		
	North York Moors JD 101	**PROV**	38	77.927	2.05071	10.5783	< 2.2e-16 ***
		BLOCK	2	2.924	1.46212	7.5421	0.0007851 ***
		Residuals	135	26.171	0.19386		

Table 4.14. Mean flushing score by region of provenance (ROP), trial site and species for the date (expressed in Julian days, JD) on which there was most difference amongst provenances for the mean leaf flushing score.

		Mean Flushing score			
	TRIAL SITE	LLANDOVERY		NORTH YORK MOORS	
	Species	**Ash**	**Rowan**	**Ash**	**Rowan**
	Date of assessment (Julian days)	**136**	**101**	**133**	**101**
	Trial mean	**3.13**	**3.47**	**3.34**	**3.10**
ROP	**NW**	2.63	2.63	2.95	2.68
	NE	2.86	4.14	3.11	3.18
	SW	3.36	2.66	3.53	2.75
	SE	3.61	4.44	3.72	3.81

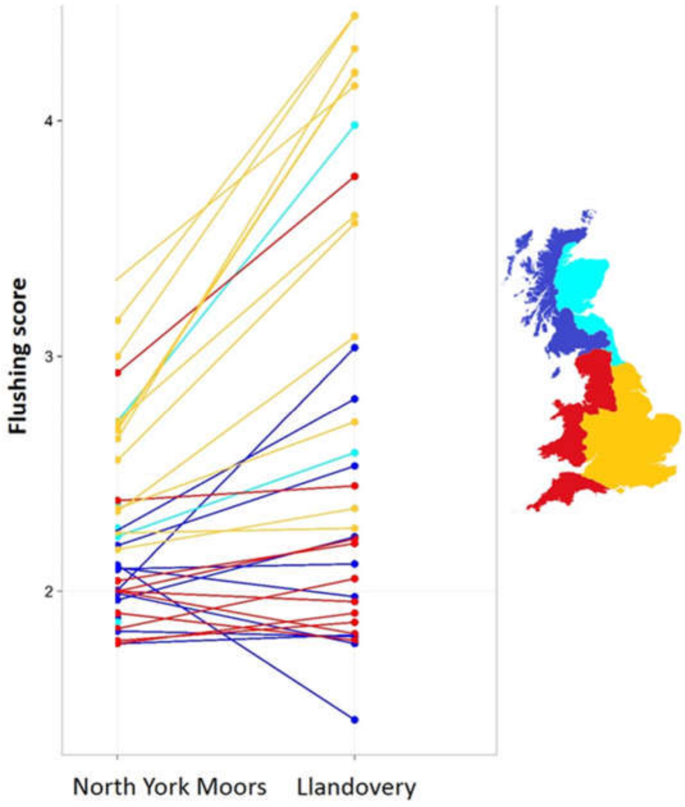


Figure 4.16. Interaction plot for rowan, mean flushing score by provenance in each trial site on JD 101. Colours correspond to ROP (as indicated in the map).

4.3.2.1.2 Comparison of spring leaf phenology between species

Spring leaf phenology was recorded at high frequency throughout the flushing period for ash, but at only three time points for rowan. In 2008, rowan trees started flushing in both trial sites over a month before the ash trees recorded in 2013 (Figure 4.16). In Llandovery where the two species grow adjacent to one another, at the last scoring date for rowan (Julian date 101) the average flushing score for the whole trial was 3.5. This score was not reached in the ash Llandovery trial until over a month later (the average score at JD 136 is 3.1). In North York Moors, the ash trees also took over a month longer than the rowan trees to reach the same mean flushing score (rowan mean score is 3.2 in JD101, while ash mean score is 3.34 in JD133).

On the date on which there was most difference amongst provenances for the mean leaf flushing score (Table 4.13), there were strong relationships between flushing scores and the locations of origin of the provenances. In ash there was a significant negative regression with latitude (for both trial sites, explaining over 35% of the variation), while in rowan there was a significant positive regression with longitude (for both trials, explaining over 45% of the variation) (Table 4.15, Figure 4.18). In ash the southern provenances flushed earlier, while in rowan the eastern provenances grew earlier.

Table 4.15. Adjusted R^2 values of the linear regressions of leaf phenology scores against latitude and longitude, by species and trials.

Spp	*Trial site*	*Trait*	*Julian Days*	*No. of provenances*	*Geographical variable*	*R^2*	*p-value*
Ash	LLAN	Bud flush	136	41	Latitude	0.41	P<0.001
		Leaf loss	294	41	Latitude	0.27	P<0.001
	NYM	Bud flush	133	39	Latitude	0.35	P<0.001
		Leaf loss	293	39	Latitude	0.20	P<0.001
Rowan	LLAN	Bud flush	101	33	Longitude	0.48	P<0.001
		Leaf loss	276	33	Latitude	0.08	P<0.01
	NYM	Bud flush	101	38	Longitude	0.45	P<0.001
		Leaf loss	262	38	Longitude	0.16	P<0.001

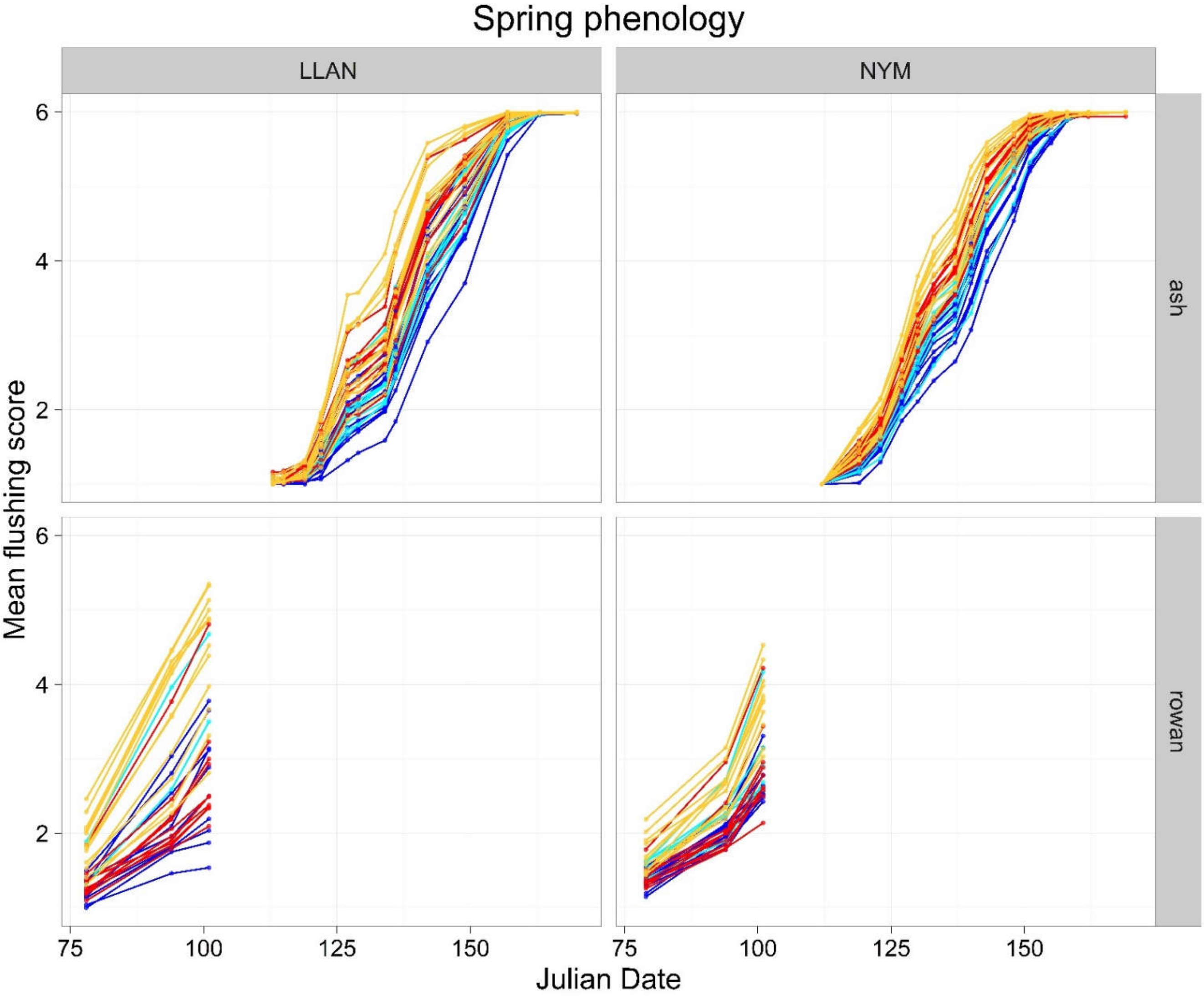


Figure 4.17. Mean flushing score value per provenance in each trial site (Llandovery left, North York Moors right) and both species (ash above, rowan below), on all the dates when the trees were scored. The x axis is time in Julian days, from 76 (16th March) to 170 (19th June). Very important, the ash data was recorded in 2013 and rowan data on 2008. The y axis is the flushing score (when 1 is dormant bud to 6 which is fully flushed). Colours correspond to ROP.

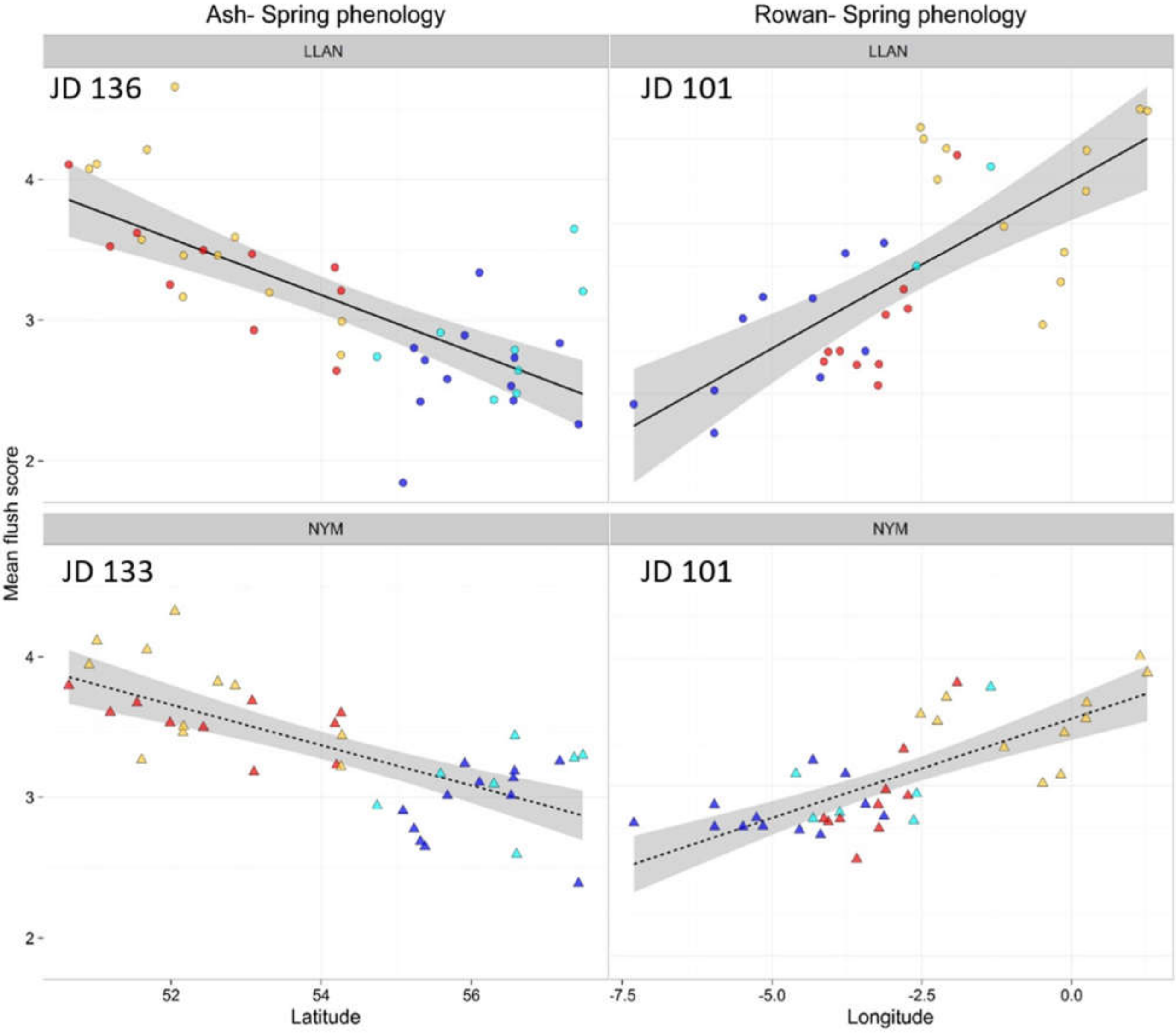


Figure 4.18. Linear regression of the mean flushing score per provenance with latitude in ash (left) and longitude in rowan (right), by trial site (North York Moors below and Llandovery above). The mean flushing score corresponds to the assessment date when differences amongst provenances were greatest (see Table 4.13). Colours correspond to ROP.

4.3.2.2 Autumn leaf phenology

4.3.2.2.1 Genetic diversity and plasticity in both species

Ash: For ash there were significant differences in timing of leaf senescence (mean provenance score) amongst provenances in both trials on most of the dates scored (the dates scored when differences among provenances are significant are in Llandovery between JD 294 and 315, and in North York Moors between JD 241 and 297). The dates on which there was the greatest range of mean leaf loss scores amongst provenances (and these were significant) were JD 294 in Llandovery and JD 293 in North York Moors (Table 4.16). There were no significant interaction between provenance and site. The greatest difference between provenances was found in Llandovery. In Llandovery SE provenances were the less senesced, while in North York Moors it was the SW provenances (Table 4.17).

Rowan: There were significant differences amongst provenances at the three dates scored in Llandovery and on the first two dates in North York Moors. The date when the range of senescence across provenances was greater was JD 276 in Llandovery and JD 262 in North York Moors (Table 4.16). There was a significant (p<0.001) interaction between provenance and site. There was stronger differentiation between provenances for leaf senescence in the North York Moors than in Llandovery, and trees senesced earlier in the former. All provenances senesced earlier in North York Moors, with the exception of one provenance from the NW ROP (Figure 4.19).

Table 4.16. Analysis of variance of the flushing mean score on the dates when the differences amongst provenances were the greatest, for rowan and ash.

Spp	Mean Senescence Score		**ANOVA TABLE**				
			Df	**Sum Sq**	**Mean Sq**	**F-value**	**Pr(>F)**
Ash	Llandovery JD 294	**PROV**	41	26.9521	0.65737	1.9257	0.006007 **
		BLOCK	2	4.2136	2.10681	6.1716	0.003186 **
		Residuals	82	27.9924	0.34137		
	North York Moors JD 293	**PROV**	39	8.9092	0.22844	2.5304	0.0003716 ***
		BLOCK	2	2.5493	1.27466	14.1194	7.242e-06 ***
		Residuals	69	6.2291	0.09028		
Rowan	Llandovery JD 276	**PROV**	33	14.3486	0.43481	3.7619	5.178e-08 ***
		BLOCK	2	0.9819	0.49097	4.2478	0.01647 *
		Residuals	122	14.1009	0.11558		
	North York Moors JD 262	**PROV**	38	29.911	0.7871	1.7325	0.0118957 *
		BLOCK	2	6.704	3.3518	7.3775	0.0009105 ***
		Residuals	135	61.335	0.4543		

Table 4.17. Mean senescence score by region of provenance (ROP), trial site and species. On a given date (expressed in Julian days, JD), which is the date when there are more differences amongst provenances for the mean leaf senescence score.

		Mean senescence score			
	TRIAL SITE	LLANDOVERY		NORTH YORK MOORS	
	Species	**Ash**	**Rowan**	**Ash**	**Rowan**
	Date of assessment Julian days	**294**	**276**	**293**	**262**
	Trial mean	**4.37**	**2.58**	**5.40**	**2.40**
ROP	**NW**	4.37	2.53	5.53	2.09
	NE	4.90	2.47	5.50	2.28
	SW	4.08	2.56	5.27	2.51
	SE	3.97	2.66	5.31	2.72

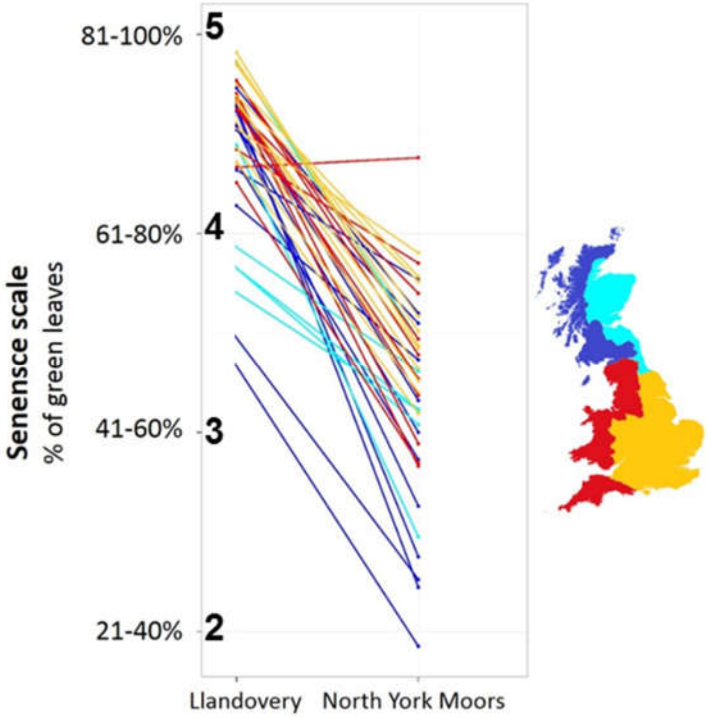


Figure 4.19. Interaction plot for rowan, mean senescence score by provenance in each trial site on JD 101. Colours correspond to ROP (as indicated in the map).

4.3.2.2.2 Comparison of spring leaf phenology between species

There was a much smaller time difference between species for leaf senescence than for leaf flushing, taking into consideration that they were assessed in different years (although 2008 and 2015 had similar average weather, Figure 4.5 and 4.6) and different locations for North York Moors. For ash, the measured variable was leaf loss in ash, whilst for rowan the scale incorporates leaf colour change and leaf loss together. Although the scales were not equivalent and the senescence process of leaf colour change and leaf loss is different across species, both track leaf loss and can be compared. Leaf loss is the last stage in autumn leaf senescence, following a gradual leaf colour change. When rowan trees were changing colour, ash trees had already started to lose leaves for the years sampled (Figure 4.20).

There were greater differences between provenances in Llandovery for ash but in North York Moors for rowan. On average, trees were more senesced in Llandovery in ash but more advanced in North York Moors in rowan.

There were strong relationships between leaf senescence and the sites of origin of the provenances for the means at the dates when the greatest differences between provenances were observed (Table 4.17). In ash there was a positive significant ($p<0.001$) regression of leaf loss score on latitude (for both trial sites, explaining less than 10% of the variation, Table 4.15). In the Llandovery rowan trial there was a significant ($p<0.01$) negative regression of green crown score on latitude (8% explained variation), while in North York Moors there was a significant ($p<0.001$) positive regression of green crown score on longitude (16% explained variation). In ash in both trials the southern provenances were the fastest to senesce. In rowan there were differences between the two trials, in Llandovery the southern provenances lost their leaves later, while in North York Moors the eastern provenances lost their leaves the latest (Figure 4.21).

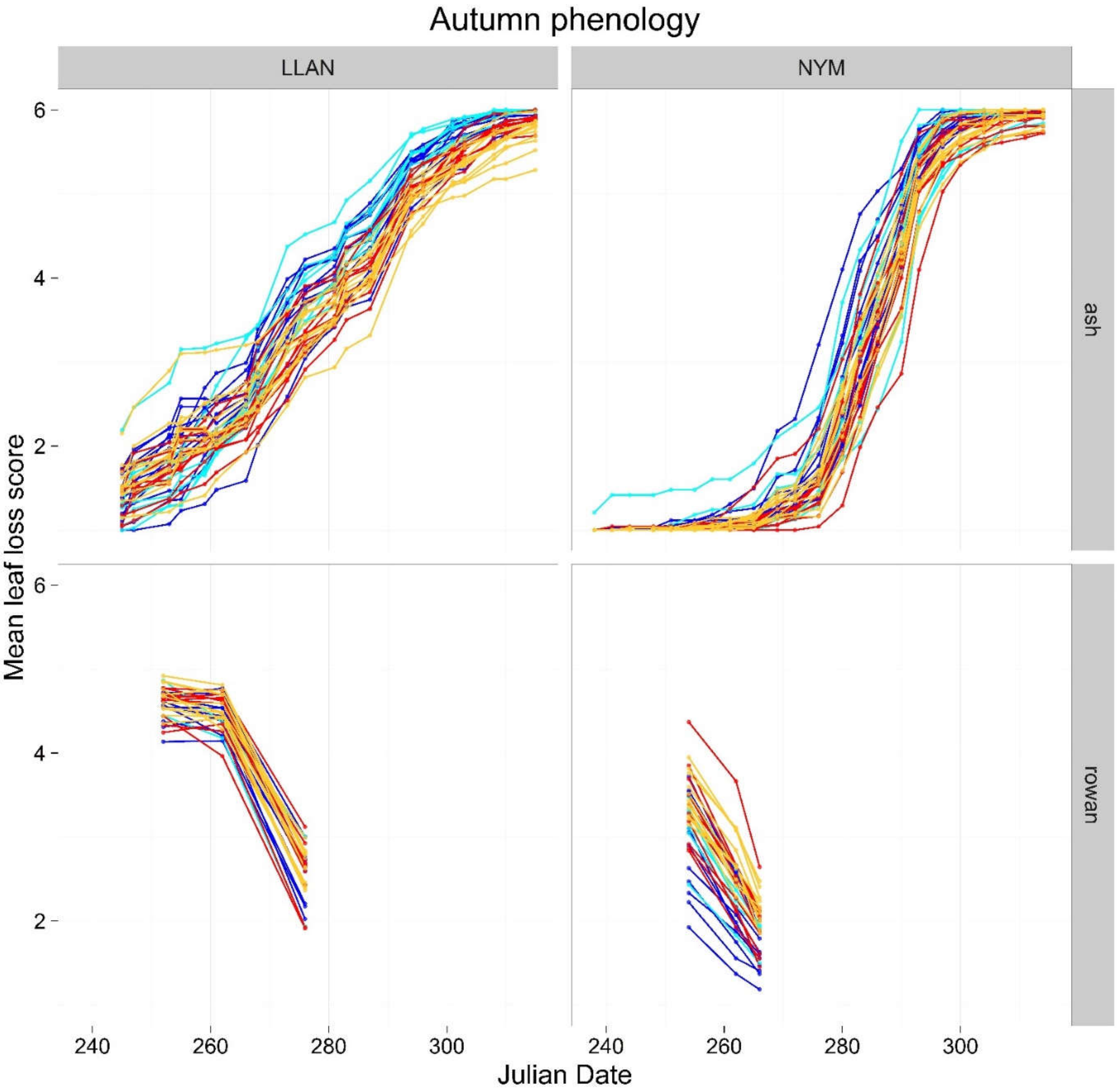


Figure 4.20. Mean leaf loss score per provenance in each trial site (Llandovery left, North York Moors right) and for both species (ash above, rowan below), on all the dates when the trees were scored. The x axis is time in Julian days, from 238 (26th August) to 315 (11th November). Note that the ash data was recorded in 2015 and rowan data in 2008. The y axis is the senescence score, note that the leaf loss scales are different between ash and rowan (table 2). The ash score increases as leaf loss progresses towards total leaf loss (stage 6), while rowan score decreases towards no green leaves left (stage 1).

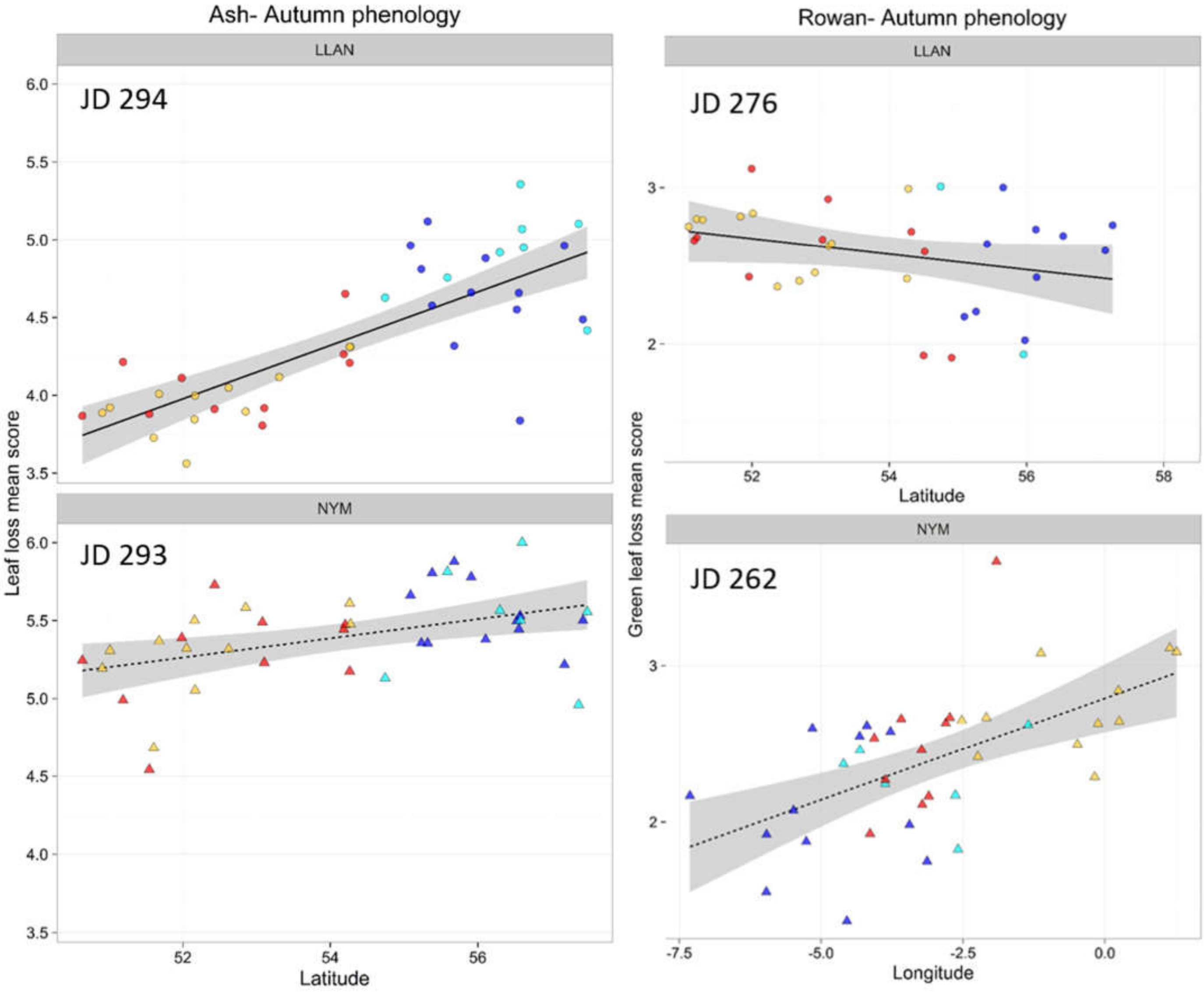


Figure 4.21. Linear regression of the mean senescence score per provenance with latitude in ash in both trials and rowan in Llandovery, and with longitude in rowan in North York Moors, by trial site (North York Moors below and Llandovery above). R-sq values in Table 15. The mean flushing score was taken on the date when the difference amongst provenances was greatest (see Table 4.17).

4.3.2.3 Summary differences amongst species for leaf phenology

We found big differences between ash and rowan for timing of leaf flushing, but smaller differences for leaf senescence. We have to take into account the climatic differences experienced during the years in which the trees were assessed. However the same patterns are found in the 3 locations, which have distinct climates. Spring 2013 was much colder (2 °C less on average) than spring 2008, when ash and rowan were respectively assessed for leaf flushing. Autumn 2008 and 2015 were much more similar in terms of average temperature (Figures 4.5 and 4.6).

Southern provenances of ash tended to flush earlier and senesce later than those from more northern latitudes. In the case of rowan, the eastern provenances were inclined to flush earlier than those from the west. The pattern of leaf senescence was less clear for rowan, although site had an effect: in North York Moors the provenances from the east senesced later than those from the west, whilst in Llandovery the populations from the south senesced earlier than those from the north.

For both species the provenance effect was much greater for leaf flushing than for senescence. Also the provenance effect was greater than the trial effect for both species, especially for leaf flushing. There was more variation within trials than across trials.

4.4 Discussion

We have found differences: 1) between species, 2) across trial sites, and 3) across provenances for leaf phenology in the two species investigated. Our results showed that differences between provenances in the timing of leafing and leaf shed persist when the trees are grown under the same conditions in a common garden trial; and this indicates that these phenological traits have a genetic basis. Within each of the trial sites analysed and for both species, we found genetic differentiation amongst native provenances for leaf flushing and senescence. Moreover, these differences amongst provenances were related to the location of the origin of the seed, which showed the geographical pattern of differentiation for leaf phenology across GB. Despite the large site effect, variation amongst provenances was greater than the plasticity individual provenances showed across the different trial sites. The patterns of geographical variation between ash and rowan were different. We also found that stem forking is correlated with date of leaf flushing in ash. These results show that trees are adapted to a determinate growing season and transfer of material from its site of origin to another location can result in damage to the trees.

4.4.1 Spring phenology

In ash there was a clear latitudinal cline for spring phenology, where the provenances originating from the south flushed earlier than those from the north. Air temperature is the most important factor in regulating budburst and leaf-out in temperate and boreal woody plants (Linkosalo et al., 2006). This latitudinal cline effect is typical in *in situ* studies, where in temperate regions it has been observed that flushing begins in southern locations and that spring phenology of many tree species correlates negatively with temperature at the growing site (Polgar & Primack, 2011; Roberts et al., 2015). This pattern is also shown in common garden trials in which leaf phenology has been studied which have shown a latitudinal cline in the origin of provenances in several deciduous species including: oak (Kleinschmit, 1993; Liepe, 1993; Deans & Harvey, 1995; Ducousso et al., 1996; Vitasse et al., 2009a; Wilkinson et al., 2017) and many other temperate broadleaf species (Kramer, 1995; Karlsson et al 2003), including British ash (Clark, 2013). In comparison to Clark (2013) our study extends the geographical area to the whole of GB with a larger number of provenances, which has allowed us to capture a greater scope of variation for leaf flushing phenology within British ash populations.

Although this latitudinal cline was relatively clear at a British scale it became less so when the range of provenances was extended to include provenances from mainland Europe. Smith (2011) and Clark (2013) established common garden experiments in England with a range of both British and continental provenances and found that Eastern European provenances of ash flushed earlier than those of British origin from similar latitudes. Hence, whilst latitude may capture the primary sources of environmental variation, other factors are clearly important at larger geographic scales. For example, in this case it is likely that provenances from Eastern Europe are adapted to a continental climate where winters are cold, and once spring temperatures start to rise cold spells are not common and late frosts are less likely to occur. In provenance trials of oak, spring leaf phenology was found to be genetically determined and was strongly correlated with adaptive characters such as spring frost tolerance (Ducousso, 1996). Provenances from northern locations and those which originated from locations close to sea level were the latest to flush and were more tolerant of exposure to late spring frost (Ducousso, 1996). Hence we would expect that trees from oceanic climates will have a higher chilling requirement before starting to flush to avoid frost damage. Chill temperatures in winter and spring release bud dormancy by lowering the thermal time required for the buds to develop to budburst (Cannell, 1997). This can explain why in our results for spring phenology in rowan, the eastern provenances flushed earlier than those from the west, so that we detected a longitudinal cline. Rowan flushes much earlier than ash (over a month before in our provenance trials and also in *in situ* observations of others (Abernethy et al., 2017) on average 20 days before), and for this reason rowan is more likely to be at risk of exposure to late spring frosts at the time of flushing than ash. Therefore, in response to the western climate in Great Britain being more oceanic than that in the east on average, the western provenances of rowan may have adapted to have a higher chilling requirement before flushing to avoid the more frequent late spring frosts.

It is well recognised that for traits such as bud burst, temperate perennial species respond differently to particular environmental cues (Murray et al., 1989), and the factors which trigger bud flush vary among species. Differences in factors such as; levels of chilling, thermal time after chilling or photoperiod affect species in different ways (Cannell & Smith, 1983; Cannell & Smith, 1986; Murray et al., 1989; Heide, 1993). The timing of bud burst in rowan and ash has been found to be unaffected by the photoperiod (Heide, 1993; Basler & Körner, 2012). Laube et al. (2014) found that chilling was an important factor in budburst in ash, and that the lack of chilling can delay budburst. Leaf spring phenology is a trade-off between minimizing the risk of freezing damage and maximizing the length of the growing season (Cannell, 1997).

Ash is the latest native tree species to flush in Great Britain in the wild (Abernethy et al., 2017). This may be due to its lack of frost tolerance, so that its late flushing in the spring represents an avoidance strategy. Dormant trees are very cold hardy, but young shoots are frost sensitive (Hemery et al., 2009). However, late frosts can cause forking by damaging the flushed buds (Kerr and Boswell, 2001). In our ash experiments, there was a positive correlation between flushing earlier and higher number of forked stems, demonstrating the consequences of mismatch between local climate and spring leaf phenology.

Our results in ash flushing showed greater differences amongst provenances than amongst trial sites. However, these differences amongst provenances varied across trial sites and genetic differences were more expressed in one trial than the other. We found greater differentiation for leaf flushing across provenances in Llandovery than in North York Moors. In Llandovery there were 15 days between the earliest and the latest provenance to flush (JD50-F), but only 10 days in North York Moors.

4.4.2 Autumn phenology

For ash, we showed that the southern provenances senesced later than those from more northern latitudes, suggesting they are adapted to a longer growing season. We found greater and more significant differences amongst provenances in leaf flushing than in leaf senescence, in both species, consistent with other studies (Vitasse, 2009c). It has been shown that leaf phenology (Chmielewski and Rotzer 2001; Sparks and Menzel 2002; Vitasse et al., 2009b), and for ash in particular, no relationship between temperature and time of senescence has been found (Vitasse et al., 2009b).

We also found less marked differences amongst ash and rowan in the timing of senescence; again consistent with other studies (Menzel, 2000). In ash, latitude explained a very large part of the variation amongst provenances; however the relationship with rowan was less clear. In the rowan trial in North York Moors there was a clear longitudinal cline for rowan (where the eastern provenances senesced later) but, unexpectedly, in Llandovery northern provenances senesced later. Although autumn phenology and the climatic drivers that regulate this have been less studied (Estrella and Menzel, 2006), it has been documented that in many (but not all, (Heide and Prestrud, 2005) woody species, growth cessation and dormancy are induced by decreasing day length during late summer and autumn (Downs and Borthwick, 1956; Nitsch, 1957; Heide, 1974; Håbjørg, 1978). If, in rowan, day length is a more important driver of senescence, it could explain why most northern provenances in the southern trial (Llandovery) senesced later, as they experienced the biggest shift in day length. Autumn frosts are also a problem when planting trees adapted to a longer growing season than the planting site. Deans & Harvey (1995) found that oak provenances which senesced later than others were more badly damaged by autumnal frosts. Autumn frost damage increases the risk of incomplete nutrient remobilisations due to damage to functional leaves (Keskitalo et al., 2005).

4.4.3 Phenotypic plasticity

Leaf phenology has genetic and environmental components, as we have shown in our results; the replication of our trials allows assessing the plastic component of the variation. To look at plasticity we can compare results from the two different trial sites for each species. Temperate trees species possess considerable plasticity in their leaf phenology that allows them to respond to inter-annual changes in the climate (Kramer, 1995; Vitasse, 2010 & 2013). Our results show greater variation within than amongst trial sites in ash for both spring and autumn phenology, which indicates that the genetic effect is greater than the site or environmental effect when considering phenological traits. Kramer (1995) found differences amongst species in the levels of plasticity; some species exhibited plasticity in the timing of key events during both spring and autumn, whereas other species did not show any plasticity in autumn phenology.

All provenances (except one from the southwest ROP) flushed earlier in the North York Moors trial site than in Llandovery, although in most cases this difference was very small, the average being 2.5 days and the largest being 7 days. The North York Moors site was more continental and colder than the Llandovery site, which may allow the chilling requirement for initiation of bud flush to be achieved earlier. However, despite a difference in the extent of variation among provenances at the two trial sites, the ranking of provenances was very similar and there was no significant interaction between provenance and site.

For ash, the site effect was bigger for senescence than for flushing. The average difference in JD50_S between the trial sites was over 8 days, and the biggest difference of provenances between two sites is 22 days. The provenances with the largest differences in JD50_S between sites were from the southeast ROP. On the other hand, the difference amongst provenances was greater than for leaf flushing. The difference between the earliest and the latest provenance to reach JD50_S was 16 days at both North York Moors and Llandovery. All provenances (except one from the northeast ROP) senesced earlier in North York Moors than in Llandovery. For this trait, the ranking of provenances changed slightly more than for flushing across the trial sites, although there was still no significant interaction between provenance and site. At North York Moors the ranking of the provenances by their JD50_S shows a much less clear division between northern and southern provenances than it showed at Llandovery. We hypothesise that the differences between spring and autumn phenology could be explained because leaf flushing might be under stronger selective pressures; perhaps because it is much more damaging for a tree to have damage on the newly flushed leaves, which would compromise the growing season length, than the getting frost damage in the end of the growing season, when most of the photosynthesis would have already occurred. Some studies have shown that genetic control for autumn leaf phenology is not as strongly associated with climatic factors as spring leaf phenology (Chmielewski and Rotzer 2001; Sparks and Menzel 2002; Vitasse et al., 2009b). However, other studies have found the opposite trend, that autumn phenology was under stronger genetic control and showed less phenotypic plasticity than spring phenology (Howe et al. 2004; Savolainen et al. 2007; Alberto et al. 2013). Alberto et al. (2013) found higher levels of quantitative genetic differentiation (Q_{ST}) among populations in a range of studies bud set (n=16) than bud flush (n = 36). Aitken & Bemmels (2016) also found that clinal variation for autumn phenology was more consistent than for spring phenology. We found more plasticity in autumn phenology than spring phenology for both rowan and ash, while other studies found the opposite.

In rowan, spring phenology was more advanced, and differences amongst provenances were greater, at Llandovery than at North York Moors. The biggest difference between sites were found in provenances from eastern ROPs, which were more advanced in Llandovery. At North York Moors, which is a much colder site than Llandovery, flushing started later and senescence started earlier on average so that trees had a shorter growing season in the North York Moors. In leaf senescence however, we found greater differences amongst provenances in North York Moors than Llandovery.

We found that some provenances seemed to have more plasticity than others; i.e. their phenology changed more across sites. Although no general pattern was very clear, provenances from the southeast ROP had more plasticity in leaf senescence. It has been found that provenances from higher altitudes have less plasticity for leaf flushing (Vitasse, 2013), which maybe could be comparable with the highest latitude provenances. The northern provenances will be under more selective pressure due to the harsher climatic conditions (colder springs and autumns) which can cause greater damage to the leaves than the climatic conditions in the most southern areas.

Availability of phenological data collected over more than a single season would help to improve our understanding of temporal, rather than spatial variation in phenotypic plasticity in our study organisms. Another weakness in our data is that flushing and senescence are recorded in different years, with very different average temperatures.

4.4.4 Practical implications

Many studies show that leaf phenology is advancing with climate change (Morin et al., 2009; Lebourgeois et al., 2010), however frost damage in spring will continue to be a risk for bud burst (Vitasse et al., 2014). Studies suggest that with climate change in Britain frequency of spring frosts is not going to decrease, as the average of spring frosts has not decreased in the last 40 years (Figure 4.20), despite springs on average having been warmer in the last decade. The earlier spring phenology that has already been observed accompanied by the lack of change predicted in frequency of spring frosts (Morison and Mathews, 2016) is likely to mean that late frosts will continue to cause damage to trees whose leaf emergence coincides with the occurrence of late frosts.

It is assumed that it is beneficial for perennial deciduous plants to maximise leaf life span. As leaf life span increases, the potential carbon gain (Kikuzawa 1994) and efficiency of nutrient use increases (Eckstein et al. 1999). However, the risk of frost damage must be taken into account (Bennie et al., 2010). Vitra et al. (2017) shows that extreme cold events in winter are not critical for trees, as freezing resistance is enhanced in winter, but that the timing of budburst is a critical component for tree fitness.

This has implications when planting trees in Great Britain from southern provenances which are adapted to warmer climates with longer growing seasons or more continental climates. Another thing to take into consideration when considering provenance movement for planting is the degree of unpredictability of how the trees will react to the new environment. This is especially of interest when there is a genotype by environment interaction. For spring and autumn phenology of rowan, we found significant provenance by site interactions, which show some provenances having an opposing response for timing of leaf flushing and leaf senescence at the two sites. This shows that acclimation does not always happen in the same direction and therefore it can be positive or negative, adding complexity to our capacity to predict change, and to predict the impacts of environmental change. Together, these considerations add to possible risks of northward transfer of provenances, as it could have damaging effects of the trees fitness due to asynchrony with the weather.

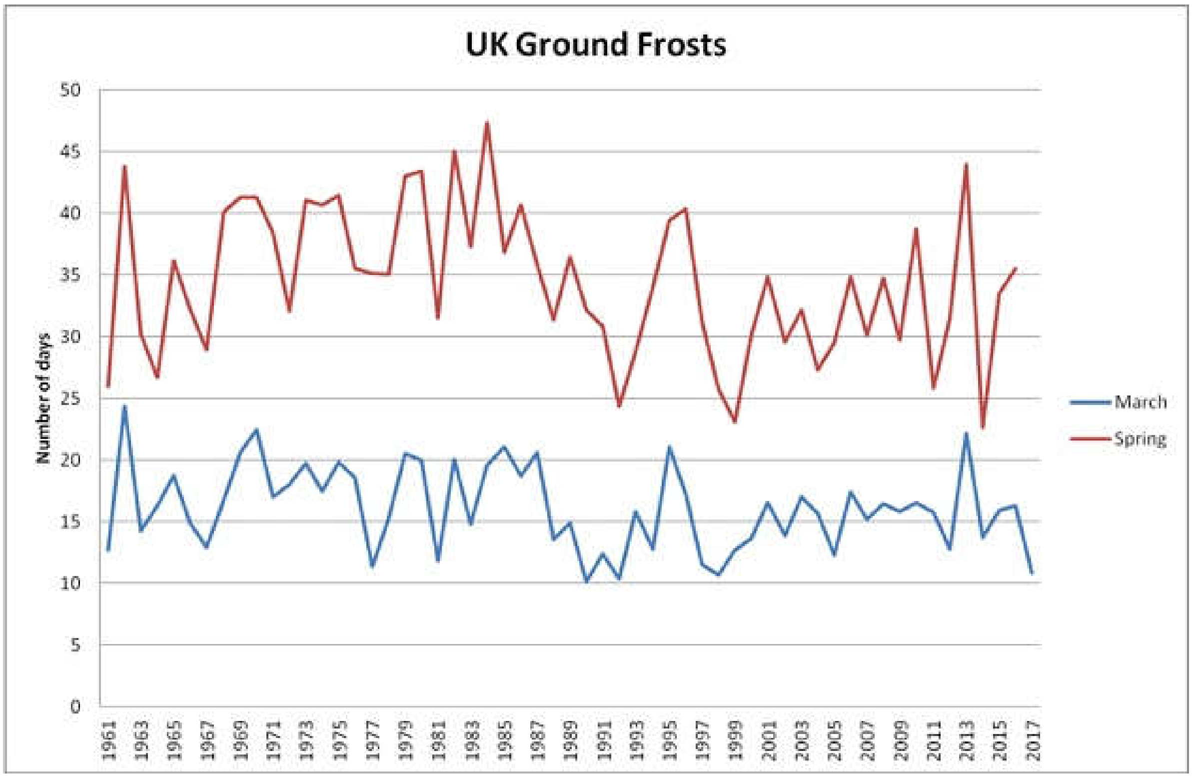


Figure 4.22. Number of days with ground frost in UK since 1961 in the month of March (blue line) and in Spring (red line), i.e. March, April and May. Met Office data.

Chapter 5: Do British tree populations show genetic differentiation in leaf traits?

Abstract: Plant functional traits have been shown to determine how plants respond to environmental factors. These traits, however, are usually compared between rather than within species. Here, we present results on leaf anatomy traits (leaf area, stomatal density and stomatal length) measured across native provenances of ash, rowan and silver birch from all over Great Britain grown in several replicated common garden experiments. We show distinct genetic diversity for these traits across natural populations, which are related with environmental covariates. Trees from drier provenances have greater leaf area, lower stomatal density and greater stomatal length; while trees from origins with greater annual precipitation show lower leaf area, greater stomatal density and lower stomatal length. Moreover, these traits show extensive phenotypic plasticity across the different environments. In the case of leaf area, there is a strong environment by genotype effect, which makes leaf area unpredictable when provenances are grown in novel environments. Stomatal density does not show interaction between site and provenance, and plasticity is in the same direction as the genetic pattern of variation. These results provide an understanding of the patterns of adaptation for these species in Great Britain, identifying key environmental drivers; they can also help to predict how these tree populations might react to climate change.

5.1 Introduction

Plant functional traits have been described as the plant features (morphological, physiological, phenological) that represent ecological strategies and determine how plants respond to environmental factors, affect other trophic levels and influence ecosystem properties (Pérez-Harguindeguy et al., 2013). However, this is a description which is applied mainly at the taxon level: the main concept of plant functional type proposes that species can be grouped according to common responses to the environment (Lavorel & Garnier, 2002). The variation found in plant functional traits has been mainly used to study broad differences amongst species to tackle ecological questions, and as a tool for comparing distant ecosystems with very little taxonomic overlap (Reich et al. 1997; Díaz et al. 2004). The main goal is to build a big database with a predictive set of local, regional and global relationships between plants and environment, and represents a step towards understanding and predicting the distribution of species in present and future environments (Grime et al., 1988; Keddy, 1992; Westoby, 1998, Grime et al., 2014).

Leaf functional traits have been proposed to be of primary importance as leaves are the plant organs most relevant to the acquisition and use of resources (Westoby, 1998; Weiher et al., 1999). There are many functional leaf traits, such as leaf area which has been related to climatic variation, geology, altitude and latitude, where heat stress, cold stress, drought stress, nutrient stress and high-radiation stress all tend to select for relatively small leaves (Pérez-Harguindeguy et al., 2013).

Leaves are the principal photosynthetic organs of plants, providing the supply of carbohydrates (Wright et al., 2004; Pallardy, 2008), and their growth is a compromise between maximal photosynthetic output and the constraints imposed by the environment (such as water availability). Leaf size has been found to reduce towards drier environments when water is a limiting factor (Battaglia et al., 1998, Bruschi et al., 2003; Gratani et al., 2003) and also has been found to decrease towards higher altitudes (Zhag & Marshall, 1995; Fonseca et al., 2000; Scheepens et al., 2010; Bresson et al., 2011; Milla & Reich, 2011). Species from colder sites display leaves which grow slower and have lower cell numbers. Having similar cell size to species growing in warmer habitat, this results in a tendency for species which are cold-adapted to have small leaves. This is observed across both altitudinal and latitudinal gradients (Körneret al., 1989; Joelet al., 1994; Körner, 1999; Farrellet al., 2006; Sunet al., 2006).

Stomata are the pores on the plant photosynthetic tissues which allow gas exchange and transpiration. They are composed of two guard cells which are responsible for the opening and closing of the stomata, the mechanism used to regulate the plant's water loss. Stomatal traits have been shown to respond to many different environmental factors. However, clear patterns in these relationships have not been found as they vary depending on plant genus, species, families and genotypes (Dillen et al., 2008). It is considered that plants are able to control stomata development in new organs to adapt to environmental conditions (Casson & Gray, 2008; Hamanishi et al., 2012). Stomatal density is one of these functional traits which has usually been found to increase as an adaptation to drier environments (Carpenter and Smith, 1975; Abrams, 1994; Hogan et al., 1994). Water stress has been found to increase stomatal density, which is frequently accompanied by a decrease in stomatal size (Dunlap & Stettler, 2001; Pearce et al., 2005; Xu & Zhou, 2008; Fraser et al., 2009; Laajimi et al., 2011). However, the opposite trend has been found as well, where water reduction increased stomatal density (Salisbury, 1927; Quan&Jones, 1977). Another common trend which has been shown to alter stomatal density is altitude whereby stomatal densities have been reported to increase with altitude (Kouwenberg et al., 2007; Premoli & Brewerm 2007; Bresson et al., 2011) but also to decrease (Hultine and Marshall 2000; Schoettle and Rochelle 2000).

Leaf functional traits in plants have been mostly studied *in situ*, relating the measurements of those traits to the environment they grow, and, as mentioned earlier, studied at a species level. It is considered that the plasticity of these traits does not affect the ranking of species amongst different environments (Garnier et al., 2001). We, on the other hand, are interested in studying diversity within species. Within the range of a given species environments can vary substantially producing differential selection pressures across a species' distribution. The importance of genetic variation in traits of ecological significance has been raised before (Geber & Griffen, 2003). There are many examples which show that there are both genetic differences (Warren et al., 2005; Dillen et al., 2008; Marchin et al., 2008; Stojinic et al., 2015) and phenotypic plasticity (Abrams, 1994; Bresson et al., 2011; Thomas, 2011) related to environmental factors.

Variation in leaf traits may be attributed to plasticity, genetic variation, or both. Many of the studies on leaf functional traits look at plants *in situ* where variation due to genetic differences and plasticity cannot be separated. Also, in many cases plant species are studied in controlled environment conditions in which extreme treatments are imposed, such as water reduction levels which might not occur in normal circumstances in the natural distribution of the species. Moreover, most studies that have looked at differences within species have focused more on extreme climates such as very xeric, or drought tolerant species, or looking at a species across a steep altitudinal range. In contrast there are not many examples of studies performed in a temperate climate. Common garden experiments help to measure and differentiate the sources of variation observed. Differences among populations within a common garden will be due to genetic differences and differences across the replicated common garden experiments will be due to phenotypic plasticity, with the genotype by environment interaction also being estimated. This information is key to being able to understand how the studied populations will react to climate change.

Here we use field based common garden experiments in trial sites located within the natural range of each species, and in different contrasting environments, to study genetic differences between provenances, phenotypic plasticity and genotype x environment interaction for leaf functional traits. Two leaf anatomy traits have been measured, leaf size and stomata leaf structure (stomatal density and length). We studied the variation of these two traits within three species with distinct ecological niches (ash, *Fraxinus excelsior* L.; rowan, *Sorbus acuparia* L.; and silver birch *Betula pendula* Roth), from which seed was collected in a systematic way across Great Britain and planted in multiple and contrasting sites. This enabled us to estimate; the genetic component of the variation, the plastic environmental response component and the interaction between them. With this information we can have a better understanding of how future climate change will affect the British populations of these tree species. The populations we study are part of the most north-western part of the distribution of these three tree species, as all three grow across Europe in different environments.

Both leaf size and stomata anatomy have been related with water availability. What we are interested to know is whether in GB these parameters also explain the variation in these traits. The particular questions we are going to address are: a) Is there any genetic diversity among provenances for these leaf traits?; b) Are these differences related to environmental factors related to water availability at the seed origin site?; c) Is there a geographical pattern across GB for these two traits?; d) Is there phenotypic plasticity?; e) Is the direction of the phenotypic plasticity likely to lead to tree acclimation? i.e. is the direction of plastic change the same as the direction of adaptation found in the pattern of genetic diversity?; and f) Are there any significant provenance by site (genotype by environment) interactions?

5.2 Materials and Methods

5.2.1 Sampling strategy

The same sampling strategy was adopted for all three species; ash, *Fraxinus excelsior L.*, rowan, *Sorbus aucupari*a L. and silver birch, *Betula pendula* Roth .This was based on the Forestry Commission seed zone map of Great Britain (Forestry Commission, 1999; Figure 5.1), which divides Great Britain into 4 regions of provenance (ROP: North West NW, South West SW, North East NE and North West NW). These ROPs are further subdivided into a total of 24 seed zones based on natural topographical boundaries (Figure 5.1). Where possible, two (three in a few cases for birch) self-sown, semi-natural populations of each species (hereafter referred to as provenances) were sampled from each of the seed zones. Roughly equal numbers of seed were collected from a minimum of 20 (in birch a minimum of 30) maternal trees and combined together to constitute the provenance sample. Selection of mother trees was not deliberately biased towards superior phenotypes, and, where possible, mother trees were located at least 100m apart.

5.2.2 Trials and provenances by tree species

After extraction, the collected seeds were sown in nurseries and the resulting seedlings were planted in a series of common garden experiments, 2 for ash, 4 for rowan and 4 for birch (locations in Figure 5.2). The trial sites represent a range of environmental conditions (as illustrated in Table 5.1).

Ash (*Fraxinus excelsior* L.)

Seed from a total of 42 provenances of ash was grown in nurseries located near the trial sites. Nurseries were located at Whixley (Yorkshire) and Carmarthen (South Wales).

In 2007, one-year-old seedlings were planted in two common garden experiments (hereafter referred to as trials). The two trials are in South Wales (Llandovery - LLAN) and Yorkshire (North York Moors - NYM). The Llandovery trial contains trees from 42 provenances; the one in the North York Moors has 40 of these (Figure 5.2 and Table 5.2).

At each trial site the provenances were grown in a randomised block experiment. Each provenance was present as a single plot in each block, and there were three blocks. The plots consisted of 36 (6 x 6) trees in Llandovery and 30 (6 x 5) trees in North York Moors. Trees were planted at a distance of 2 metres apart.

All measurements in the Llandovery trial were carried out before there were any visible signs of infection by ash dieback. In North York Moors nearly all the measurements were collected before the trees showed any signs of the disease with the exception of DBH and forking assessments. For these characters trees started to show signs of infection during the year of assessment. Since then, both trial sites have shown high levels of infection (Jo Clark, pers. comm.).

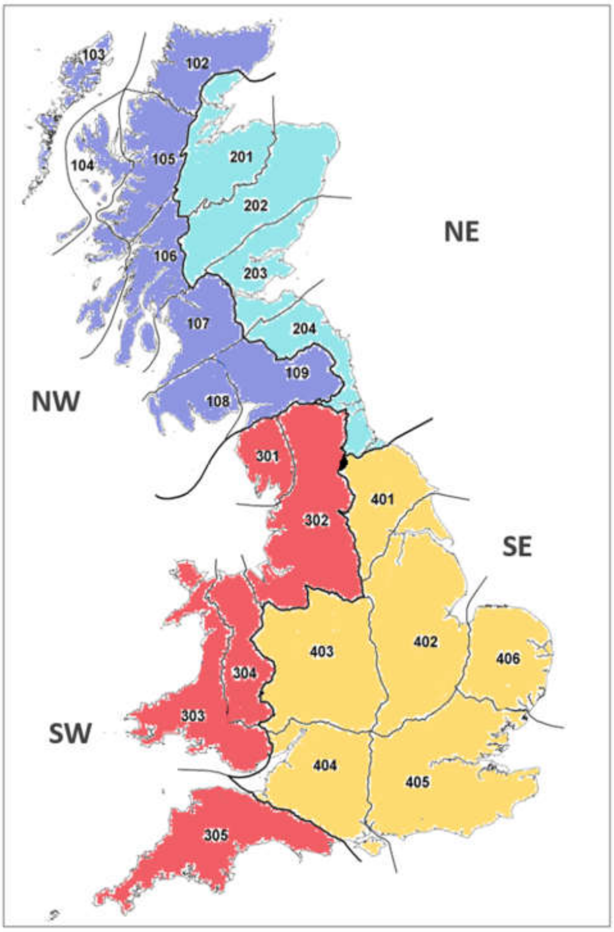


Figure 5.1. The four regions of provenance and 24 seed zones in Great Britain (modified from Herbert et al., 1999)

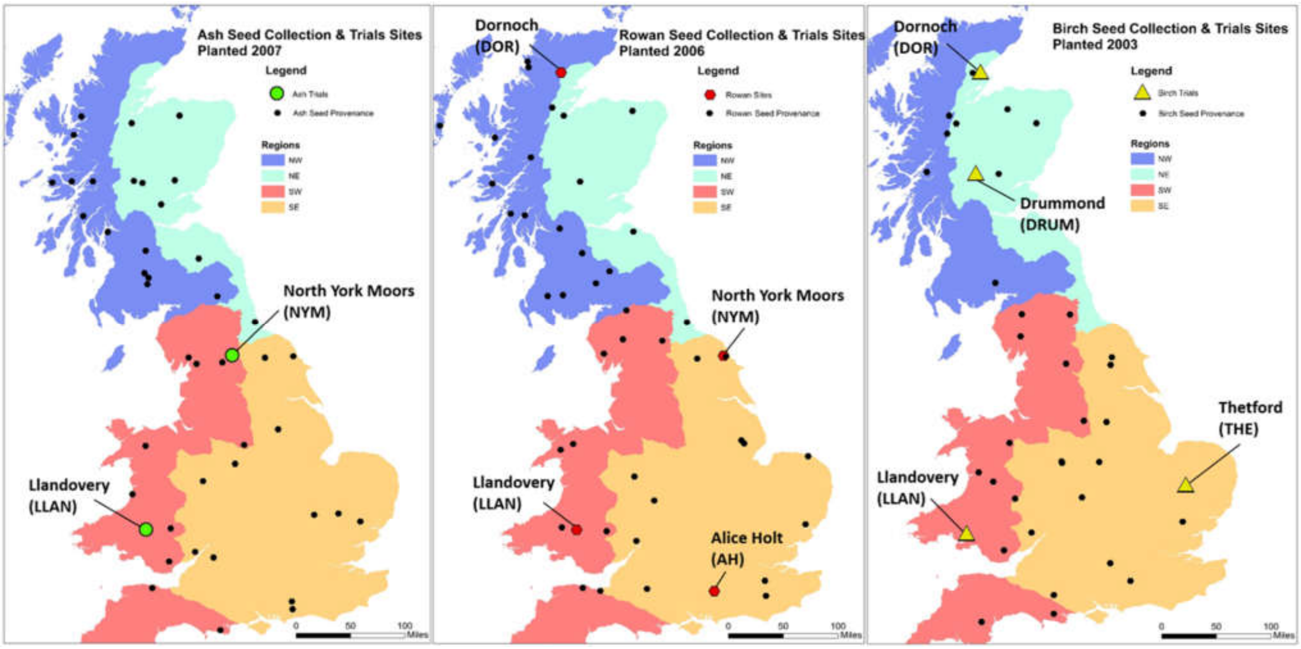


Figure 5.2. Seed collection (black small dots) and trial sites locations for ash (green dots, left), rowan (red dots, centre) and birch (yellow triangles, right).

Rowan (*Sorbus aucuparia* L.)

The rowan berries, from a total of 42 provenances, were sent to Forest Research's Newton field station near Elgin in northeast Scotland for manual extraction of seed from the fruits. The seeds were sown and raised into seedlings at Forest Research's nursery (Northern Research Station, south of Edinburgh). In 2006, when the seedlings were one year old they were planted out in trials in four locations across Great Britain. The trial sites (Figure 5.2) were located in; South Wales (Llandovery - LLAN), the South of England (Alice Holt - AH), East Yorkshire (North York Moors - NYM) and the North of Scotland (Dornoch - DOR). However, the number of provenances represented at each of the trial sites is not consistent due to shortage of plants raised from some of the provenances. Consequently, some provenances are absent from some of the trials (Table 5.3). From the 42 total provenances for which seed was collected, only 29 were planted in all four trials.

The experiment is based on a randomized block design, and consists of three replicated blocks. Trees were planted at a distance of 2 metres. Each plot contains 9 (3 x 3) trees from the same provenance.

Table 5.1. Details of the three species trial sites.

Spp	Trial Site	Planting Year	County	Lat	Long	CT	MD	Alt (m)	AP (mm)	GDD	DAMS	Soil Type
Ash												
	NYM (North York Moors)	2007	East Yorkshire	54.3	-1.9	9.0	153	113	960	1475	10	Riverine Floodplain. Clay to Sandy Loam. Freely draining floodplain soils.
	LLAN (Llandovery)	2007	Carmarthenshire	51.9	-3.8	8.1	108	215	1329	1470	15	Sandstone and Mudstone. Sand to Loam. Freely draining acid loamy soils over rock
Rowan												
	DOR (Dornoch)	2006	Sutherland	58.1	-4.4	4.2	91	130	1115	1070	15	Podzol. Clayey loam to sandy loam. Glacial till.
	NYM (North York Moors)	2006	East Yorkshire	54.3	-0.5	8	149	197	759	1328	15	Very acid loamy upland soils with a wet peaty surface. Silty loam to sandy loam. Peaty.
	LLAN (Llandovery)	2006	Carmarthenshire	51.9	-3.8	8.1	109	240	1329	1474	15	Sandstone and Mudstone. Sand to Loam. Freely draining acid loamy soils over rock.
	AH (Alice Holt)	2006	Surrey	51.2	-0.8	10.2	186	118	785	1772	13	Slowly permeable seasonally wet slightly acid but base-rich loamy and clayey. Claystone/mudstone. Loam to clay.
Birch												
	DRUM (Drummond)	2003	Perthshire	56.6	-4.1	6.1	89	218	1262	1143	11	Umbrisol. Clayey loam to sandy loam. Glacial till.
	THET (Thetford)	2003	Norfolk	52.4	0.6	10.8	214	54	608	1751	12	Chalk. Chalky, sandy loam. Freely draining sandy breckland soils.
	LLAN (Llandovery)	2003	Carmarthenshire	52.6	-4.1	7.1	139	114	1503	1754	14	Glacial till. Loam to clayey. Slowly permeable seasonally wet acid loamy and clayey soils.

CT: Continentality, MD: Moisture deficit, AP: Annual precipitation, GDD: growing degree days, DAMS: Wind speed score (Details of variables in section 5.2.3)

Silver Birch (*Betula pendula* Roth)

The silver birch seeds, collected from a total of 33 provenances, were grown in the Forest Research's nursery (Northern Research Station, south of Edinburgh). Three trial sites were planted in 2003, one in Scotland at Drummond (DRUMM), one in South Wales at Llandovery (LLAN) and one in Norfolk at Thetford (THET) (Table 5.4).

A randomized block design was used based on three replicated blocks. Each plot consisted of 25 (5 x 5) to 36 (6 x 6) trees per provenance, depending on the site. Trees were planted at 2 m spacing.

Table 5.2. Details of the ash provenances locations and the trial site they are planted in. The "X" indicates presence of a provenance at a given site.

ROP	Country	Ash provenances	Lat	Long	Alt (m)	Site	
						LLAN	NYM
NW	Scotland	Duisdale, Skye	57.176	-5.751	18	x	x
		Kilninian, Mull	56.530	-6.208	71	x	x
		Rassal Wood, Kishorn	57.426	-5.591	78	x	x
		Ardtornish,Morvern	56.560	-5.741	20	x	x
		Glasdrum Wood, Loch Creran	56.574	-5.232	33	x	x
		Add Valley, Kilmichael Glassary	56.106	-5.420	30	x	x
		Clyde Valley	55.680	-3.913	159	x	x
		Shielhill Glen	55.911	-4.825	107	x	x
		Penpont	55.235	-3.853	90	x	x
		Nith Valley	55.320	-3.829	141	x	x
		Crawick Water	55.381	-3.929	162	x	x
	England	Warks Burn	55.088	-2.222	90	x	x
NE	Scotland	Erchite Wood, Dores	57.368	-4.345	56	x	x
		Craigellachie	57.484	-3.170	102	x	x
		Fearnan Forest, Kenmore	56.579	-4.037	142	x	x
		Glen Lyon	56.602	-4.248	183	x	x
		Den of Alyth	56.623	-3.258	152	x	
		Pitcairns Glen, Dunning	56.300	-3.573	119	x	x
		Tweed Valley North Glen	55.588	-2.662	68	x	x
	England	Castle Eden Dene, Peterlee	54.743	-1.352	102	x	x
SW	England	Witherslack	54.264	-2.870	79	x	x
		Park Wood&Hutton Roof	54.182	-2.689	170	x	x
		Via Gellia Woods	53.104	-1.619	239	x	x
		Upper Wharfedale	54.203	-2.104	202	x	x
		Greta Wood, Purbeck Ridge	50.637	-2.136	126	x	x
		Horner Wood, Porlock	51.189	-3.583	102	x	x
	Wales	Cardiff Area	51.546	-3.234	158	x	x
		Aberystwyth Area	52.430	-4.059	90	x	x
		Betws-y-Coed	53.079	-3.799	57	x	x
		Talgarth	51.986	-3.213	198	x	x
SE	England	Forge Valley	54.274	-0.490	52	x	x
		Ashberry Woods	54.262	-1.133	142	x	x
		Treswell Woods	53.308	-0.861	54	x	
		Hayley Wood	52.158	-0.110	79	x	x
		Tick Wood, Ironbridge	52.621	-2.523	99	x	x
		Forest Bank, Marchington	52.852	-1.820	142	x	x
		Wyndcliff, Wye Valley	51.678	-2.679	208	x	x
		Midger Wood	51.606	-2.285	160	x	x
		Pheasant Copse, Petworth	51.011	-0.628	60	x	x
		Bignor Hill	50.908	-0.616	194	x	x
		Groton Wood	52.050	0.883	66	x	x
		Out Wood	52.166	0.415	96	x	x

Table 5.3. Details of the rowan provenance locations from which seed was collected and the trial sites in which the seedlings were planted. The "X" indicates presence of a provenance at a given trial site.

ROP	Country	Rowan provenances	Lat	Long	Alt (m)	Site			
						AH	DOR	LLAN	NYM
NW	Scotland	Assynt	58.171	-5.261	27.48		x		x
		Inverpolly	58.093	-5.232	82.12		x		
		Allt Volagir, South Uist	57.247	-7.310	68.76		x	x	x
		Salen, Isle of Mull	56.527	-5.961	5.80		x	x	x
		Tokavaig, Isle of Skye	57.139	-5.965	25.40		x	x	x
		Glen Loy	56.901	-5.071	61.96		x		
		Add Valley	56.141	-5.486	33.72	x	x	x	x
		Strathlachlan	56.128	-5.152	70.32	x	x	x	x
		Falls of Clyde	55.653	-3.778	160.84	x	x	x	x
		Mugdock Country Park	55.971	-4.320	161.8		x	x	x
		Glenlee	55.088	-4.194	129.68	x	x	x	x
		Stroan Bridge	55.070	-4.545	66.72		x		
		Lochwood	55.258	-3.443	180.2	x	x	x	x
		Ettrick Water	55.420	-3.133	234.16	x	x	x	x
NE	Scotland	Bunchrew	57.471	-4.315	100.8		x		x
		Craigdarroch	57.574	-4.605	31.8		x		x
		Cleanhill Wood, Aberchirder	57.554	-2.636	158.76		x		x
		Birks of Aberfeldy	56.602	-3.872	285.96	x	x		x
		Pressmennan Wood	55.951	-2.589	149.84	x	x	x	x
		Castle Eden Dene	54.743	-1.351	107.64	x	x	x	x
SW	England	Horner Wood	51.194	-3.589	232.84	x	x	x	x
		Holford/Hodder's Combe	51.158	-3.218	164.88	x	x	x	x
		Duddon Valley	54.316	-3.230	61.84	x	x	x	x
		Naddle Forest	54.511	-2.805	291.24	x	x	x	x
		Brignall Banks	54.496	-1.913	222.36	x	x	x	x
		Gelt Wood	54.903	-2.733	139.16	x	x	x	x
	Wales	Brechfa	51.993	-4.063	146.28	x	x	x	x
		Beddgelert	53.025	-4.138	257.76	x	x	x	x
		Mynydd Du	51.954	-3.105	532.12	x	x	x	x
		Ugly House	53.106	-3.868	231.32	x	x	x	x
SE	England	Forge Valley& Raincliffe Woods	54.275	-0.484	89.64	x	x	x	x
		Ashberry and Reins Woods	54.253	-1.128	106.24	x	x	x	x
		St. Helen's Wood, Coningsby	53.113	-0.123	6.24	x	x	x	x
		Moor Farm	53.156	-0.181	14.8	x	x	x	x
		Pepper Wood	52.368	-2.092	144.76	x	x	x	x
		The Ercall	52.687	-2.522	185.8	x	x	x	x
		King's Bottom, Longleat	51.191	-2.241	219	x	x	x	x
		Chestnuts Wood, Forest of Dean	51.829	-2.470	161.72	x	x		x
		Saxonbury Hill	51.076	0.251	183.12	x	x	x	x
		Seal Chart	51.278	0.237	129.44	x	x	x	x
		Culter's Wood, Freston, Ipswich	52.011	1.142	34.4	x	x	x	x
		Felbrigg Great Wood, Cromer	52.913	1.264	83.24	x	x	x	x

Table 5.4. Details of the birch provenance locations from which seed was collected and the trial sites in which the seedlings were planted .The “x” indicates presence of a provenance at a given trial site.

ROP	Country	Birch provenances	Lat	Long	Alt (m)	Site		
						DRUMM	LLAN	THET
NW	Scotland	Affric	57.31	-4.80	118	x		x
		Loch Creran	56.55	-5.28	26	x	x	x
		Dumfries	55.11	-3.58	29	x	x	x
NE	Scotland	Elgin	57.42	-3.38	140	x	x	x
		Great Glen	57.21	-4.62	68			
		Glen Garry	57.07	-4.83	54	x	x	x
		Spinningdale	57.89	-4.26	38	x	x	x
		Dunkeld	56.56	-3.56	143	x	x	x
		Alford	57.24	-2.67	142	x	x	x
SW	England	Bovey Tracey	50.59	-3.71	120		x	x
		Bovington Camp	50.71	-2.21	50	x	x	x
		Penrith	54.7	-2.96	280	x	x	x
		Ambleside	54.4	-2.98	60	x	x	x
		Bolton Abbey	54.04	-1.95	220	x	x	x
		Hamsterley Forest	54.7	-1.86	200	x	x	x
		Sheffield	53.28	-1.56	240		x	x
	Wales	Machynlleth	52.58	-3.85	90		x	x
		Taffs Well	51.55	-3.27	100	x	x	x
		Llanidloes	52.46	-3.53	180	x	x	x
		Persteigne	52.24	-3.05	200	x	x	x
		Llangollen	52.98	-3.19	170			x
SE	England	Castle Howard	54.12	-0.92	76	x	x	x
		Sand Hutton	54.02	-0.95	25	x	x	x
		Leicester	52.73	-1.23	160	x	x	x
		Clumber park	53.26	-1.06	55	x	x	x
		Cannock Chase1	52.72	-2.04	180	x	x	x
		Cannock Chase 2	52.74	-2.05	140	x	x	x
		Monmouth	51.79	-2.69	170	x	x	x
		Tollard Royal	50.96	-2.21	130	x	x	x
		Godalming	51.14	-0.6	170		x	x
		Basingstoke	51.38	-1.02	50	x	x	x
		Braintree	51.91	0.54	80	x	x	x
		Dunwich	52.26	-1.61	20	x	x	x

5.2.3. Environmental covariates

Latitude, longitude, altitude and climatic data were obtained for each trial site and provenance origin site locations from which seed was collected. The interpolated long-term average data relating to the climatic conditions at each of the trial and provenance sites were obtained from two sources. Continentality, Annual Precipitation, Moisture Deficit, Mean Wind speed were obtained from ESC-Ecological Site Classification (Clare and Ray 2001; Growing degree days and Consecutive Dry days were obtained from the Met Office (methods of the interpolation used from the Met Office historic data: Perry & Hollis, 2005). We focused on five parameters for the trial sites (continentality, moisture deficit, annual precipitation, growing degree days and wind speed) because we considered them likely to reflect elements of the climate that are key to tree growth: temperature, water availability, and other variables which are dependent on the geographical location of the site. For the analyses of the provenance sites, we used environmental factors which are related to water use availability (annual precipitation and consecutive dry days), as the leaf area and stomatal traits are functional traits which have been found to be influential (Pérez-Harguindeguy et al., 2013). The indices and their definitions of all the climatic parameters are the following:

i. AP – Average annual rainfall (mm) between 1961 and 1990.

ii. CDD – Consecutive Dry Days (days): Maximum number of consecutive dry days, meaning the longest spell of consecutive days with precipitation ≤ 0.2 mm (annual mean).

iii. CT – Continentality: corresponds to the Conrad Index (Conrad 1946). This is calculated as follows:

$$CT = 1.7\,[A/\sin(\phi + 10)] - 14$$

Where A is the difference between the mean temperature of the warmest and coldest month in degrees Celsius and φ is latitude in degrees. Lower values indicate more oceanic climates.

iv. GDD - Growing degree days: This is quantified as the cumulative sum of the number of degrees Celsius above 5 °C on each day of the year in which mean temperature exceeds 5 °C. Values expressed here are long term mean annual values for the years spanning from 1961 to 1990.

v. MD – Moisture deficit (mm): To consider the effects of the dryness of the growing season moisture deficit is calculated by considering the potential evaporation and precipitation. Higher values indicate drier sites.

vi. Wind exposure measured by DAMS – Detailed Aspect Method of Scoring: This provides an indication of wind exposure, the higher the values the more exposed the site. Sites with values under 12 can be considered to be sheltered.

For each trial site, the soil information data were obtained from different inventories, using the UK Soil Observatory (UKSO). The European Soil Bureau description (The European soil database, 2006) was used to assign a soil type for all British trial sites. The soil texture and soil group data were obtained from Soilscapes (Cranfield University, 2017) for Welsh and English locations, and from the national soil map of Scotland (Soil Survey of Scotland Staff, 1981) for the Scottish trials.

5.2.4 Measurements

Leaf sampling

Leaves were collected in June and July 2015 in the two ash (LLAN and NYM), four rowan (LLAN, AH, NYM and DORN), and three birch trials (LLAN, DRUM and THET). For each provenance 9 leaves were analysed: the leaves were collected from the three middle trees per plot, three plots per site, one leaf per tree. The leaves were collected from the south facing side of the tree. When possible, leaves were collected from a height of 1.5-2.0m from the ground in the canopy, although there were unavoidable exceptions. For example, nearly all trees in the birch trials and some ash trees were very tall and extendable loppers had to be used to reach even the lowest branches, and conversely, in the case of the northern rowan trials, some trees were shorter than 1.5m. The leaves sampled were on the basis of being in the largest size category, mature, and with a healthy appearance (i.e. no leaf discolouration, no visible infections or any other damage). These leaves were removed from the tree by cutting the petiole, and kept in a zip-sealed plastic bag inside a portable cool box and then stored in a fridge to stop them from drying. Processing was done as soon as possible (within the next 2-3 days) before any visible damage occurred to the leaves.

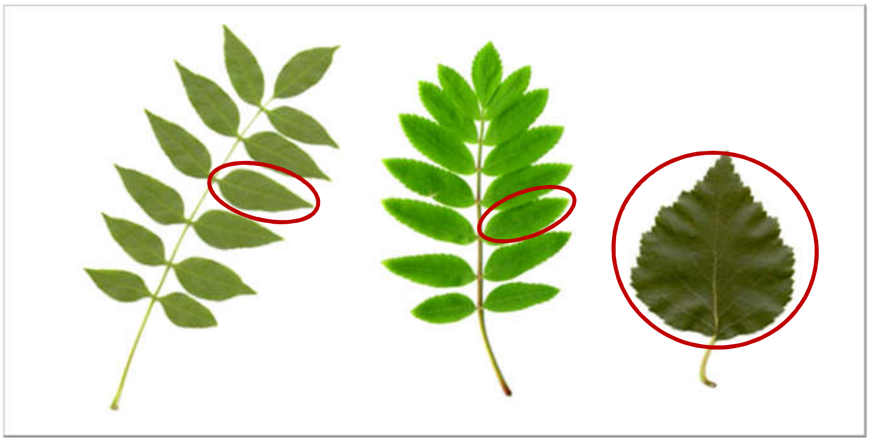


Figure 5.3. Leaves of ash, rowan and birch (left to right). The red circles indicate the part of the leaf that was measured.

Sample processing

To measure the stomatal traits, leaf surface imprints were obtained from fresh leaves as soon as possible following field collection (following the procedure described by Stojnić, 2015). Clear nail varnish was applied to a small area (approx. 5mm x 10mm) on the abaxial leaf epidermis and left to dry for 3 to 5 minutes. A small square of transparent adhesive tape (approx. 2 x 3 cm) was then pressed onto the varnished surface, gently peeled off along with the layer of nail varnish and attached to a microscope slide. This process was carried out for all the samples. For those species with compound leaves (ash and rowan) the procedure was applied to an area of a single leaflet from the middle part of the leaf (Fig. 3). Samples used to measure leaf shape were mounted in a plant press and dried in an oven at 90 °C for a minimum of 48hr.

Leaf area

To measure their area, pressed and dried leaves were scanned using a CanoScan LiDE 220 Flatbed Scanner at its highest maximal resolution (4800 x 4800 dpi). The resulting images were analysed using the software Image J (version 1.49v, as downloaded in October 2015; Schneider et al., 2012) using the Bio-Formats package plugins (© 2005-2015 University of Dundee & Open Microscopy Environment; Linkert et al, 2010). For birch, entire leaves were measured; whereas for ash and rowan, the largest central leaflet was measured (Figure 5.3). The software Image J was used to quantify leaf area (LA, cm^2), described as the total surface area of the lamina.

Measurement of Stomata

The leaf surface imprints were observed under a microscope (Nikon Phase Contrast 1.25 Microscope Alphaphot-2 Ys2, Japan) to count all the stomata in the field of view (a circle of radius 225 μm). Usually around 30 stomata were found, but this could rise to more than 70 stomata in ash samples. The area observed was selected randomly, avoiding regions intersected by leaf nerves. Stomatal density (SD) was calculated as the number of stomata found per square millimetre of abaxial leaf surface. Stomata length (SL, μm) was measured on a single stoma in the centre of the microscope field of view. A microscope scale eye graticule was used at a magnification of x40 with a x10 objective (Figure 5.4). This procedure was conducted three times per sample for ash and once per sample for rowan and birch, giving a total of 9 observations per plot for ash and 3 per plot for rowan and birch. Mean values per plot were used for statistical analyses.

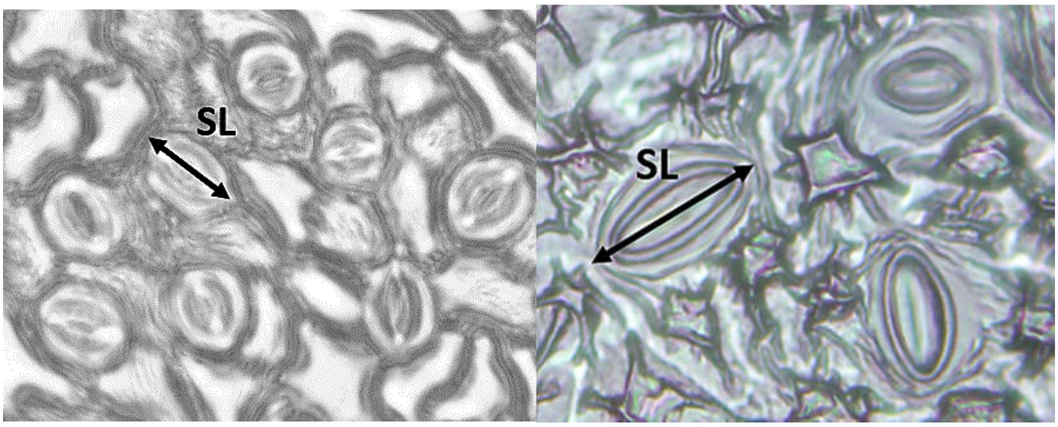


Figure 5.4. Two microscope stomatal observations at x40 magnification. Rowan (left) has smaller and shorter stomata than ash (right).

5.2.5 Data analysis

Statistical analyses were performed in R version 3.2.3 (R Core Team, 2015). The data packages for data management, analysis and visualisation used were "dplyr" (Wickham and Francois 2015) and "ggplot2" (Wickham, 2009).

Analysis of sources of variation

To determine the effects of provenance and site, the plot mean values were analysed in analysis of variance (ANOVA) using a linear model. The different models of ANOVA used were as follows:

i. Separate analysis for each species, trait and trial site combination. In the ANOVA provenance was a fixed factor and block was a random factor. All the provenances were used.
ii. Analysis for each species and trait combination across all trial sites. In the ANOVA trial site, provenance, and trial site by provenance interaction were fixed effects and block nested within site was a random factor. Only provenances common to all trials for each species were used.

Relationship with environmental factors

For each trait, linear regressions were used to test the relationship between provenance mean values and latitude (LAT), longitude (LON) together with the individual environmental variables annual precipitation (AP) and number of consecutive dry days at the home sire. These two individual variables were chosen because they are most closely related to water relations at the home site. Multiple linear regressions including both longitude and latitude were also carried out. If only one of these factors was significant then a single linear regression was reported.

Relationship among traits

To determine the existence and strength of covariance of LA, SD and SL over provenances, Pearson's correlation coefficients were calculated using provenance means from individual sites.

5.3 Results

5.3.1 Leaf Area

- *Ash*

Differences amongst provenances: There were significant differences in LA amongst provenances in both trial sites (LLAN $p<0.05$, NYM $p<0.05$). The ranges of LA provenance means were very similar between the two sites (LLAN 10.1-17.2 cm^2 and NYM 10.0-17.2 cm^2) (Figure 5.5).

Differences amongst trial sites: There were significant differences amongst the ash trials (Figure 5.5) for LA ($p<0.05$). On average, the leaves in Llandovery had a larger area (13.6 cm^2) than in North York Moors (12.6 cm^2).

Provenance by site interaction: There was a significant interaction between provenance and trial site for LA ($p<0.05$). There were numerous crossing interactions, the ranking of provenances changing across the two sites (Figure 5.6).

Relationship with environment at site of origin: At Llandovery there was a significant multiple regression of LA with latitude and longitude (R^2: 26%, $p<0.001$), with LA of provenances increasing towards the south and east. At this site LA also had a significant negative regression on AP (R^2: 24%, $p<0.001$), leaf area decreasing with increase in precipitation. There was no significant regression on CDD. In North York Moors there were no significant regressions of LA provenance means on any environmental variables (Table 5.5, Figure 5.7).

- *Rowan*

Differences amongst provenances: In the two southern trial sites there were significant differences in LA among provenances (Llandovery ($p<0.001$) and Alice Holt ($p<0.05$), but no significant provenance differences at the two northern trials. The range of provenance means for LA was much larger in Llandovery (5.5-10.0 cm^2) than at Alice Holt (4.7-6.5 cm^2).

Differences amongst trial sites: There were significant differences amongst the trials for LA ($p<0.001$). The largest rowan leaves were in Llandovery (7.6 cm^2), then Alice Holt (5.6 cm^2), Dornoch (5.0 cm^2) and North York Moors (3.7 cm^2) (Figure 5.5).

Provenance by site interaction: There was a significant interaction between provenance and trial site ($p<0.001$). There were crossing interactions, the ranking of provenances changing across the four sites (Figure 5.6).

Relationship with environment at site of origin: In the two southern sites provenance variation in LA was explained by Longitude (LLAN R^2: 6% $p<0.05$, AH R^2: 11% $p<0.001$), where LA declined towards the west. In contrast in the northern trial sites there were significant regressions of provenance mean LA on latitude with LA declining to the north, though these explained very little of the variation (NYM R^2: 3% $p<0.05$, DOR R^2: 10% $p<0.001$). The climatic variables AP and CDD also explained part of the provenance variation in LA. All the trial sites also showed a significant positive regression of LA on CDD. In all case LA was greater in provenances from drier sites, though again the amount of variation explained was small (Llandovery R^2: 4%, $p<0.05$; Alice Holt R^2: 12%, $p<0.001$; Dornoch R^2: 6%, $p<0.05$; North York Moors R^2: 5%, $p<0.05$ (Table 5.5, Figure 5.7).

-Birch

Differences amongst provenances: At Thetford, but not at other trial sites, there were significant differences amongst provenances for LA ($p<0.001$). The range of provenance means in Thetford was 9.4-19.8 cm^2.

Differences amongst trial sites: There were significant differences amongst the birch trials for LA ($p<0.001$). On average, leaves were smaller in the most northerly trial, Drummond (11.1 cm^2), while mean LA values at Llandovery (13.3 cm^2) and Thetford (13.5 cm^2) were more similar (Figure 5.5).

Interaction between provenance and site: There was a significant provenance by trial interaction for LA ($p<0.05$). There were crossing interactions, the ranking of provenances changing across the three sites (Figure 5.6).

Relationship with environment at site of origin: At Thetford provenance LA variation was explained by longitude (R^2: 6%, $p<0.05$) and CDD (R^2: 9%, $p<0.01$). LA increased in provenances from eastern and drier sites. There were no significant regressions of provenance LA on environmental variables at Llandovery or Drummond (Table 5.5, Figure 5.7).

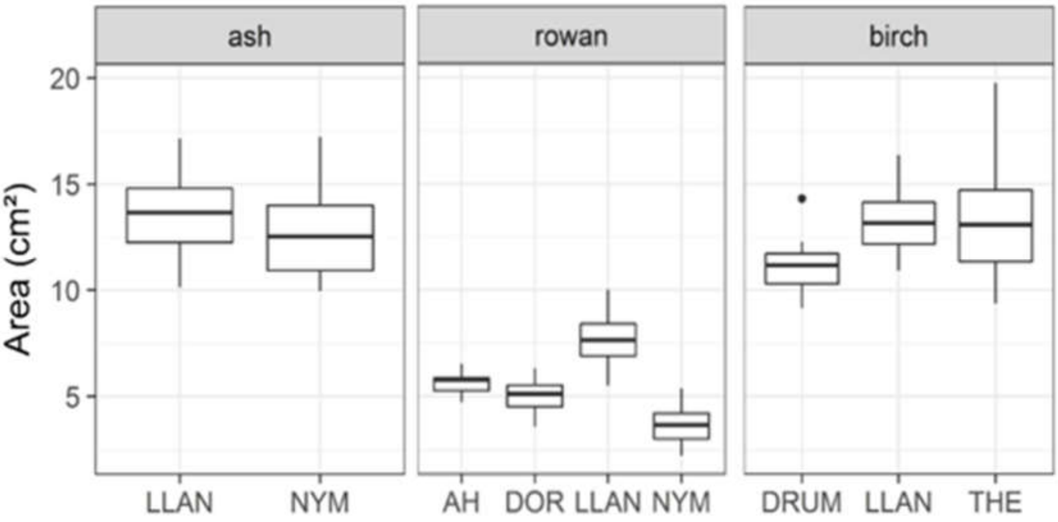


Figure 5.5. Among site variation in mean leaf area for three species. All three species show significant differences amongst trials (p<0.001).

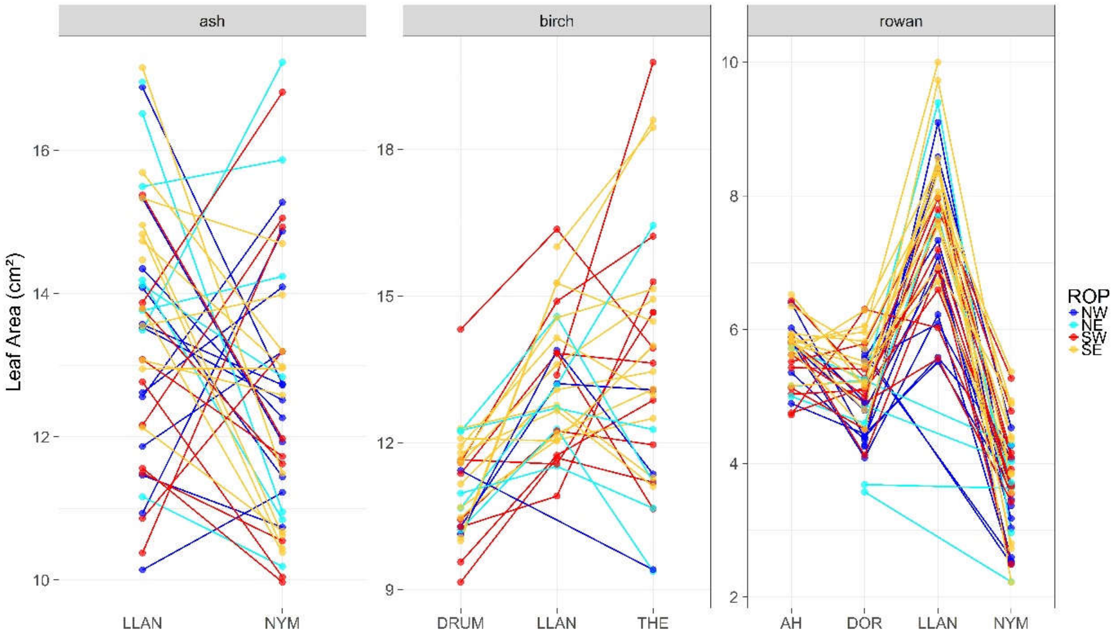


Figure 5.6. Interaction plot for changes in mean provenance leaf area over sites for three tree species. Note that the y axes are different for each species. The colours correspond to the ROPs.

Table 5.5. Significance of differences among provenance for leaf area, and the relationship with geographic and climatic variables for each species and trial site. All regressions were significant at $p<0.01$, except those indicated by '*' where significance was $p<0.05$. (+) and (-) indicate slope of regression.

Spp	*Trial site*	*No. of prov.*	*Prov. effect*	*Geogr/Environmental variable Regression sign and R^2*			
				Lat	Long	AP	CDD
Ash	Llan	42	*	**(-) Lat (+) Long 0.26**		**(-) 0.24**	ns
	NYM	40	*	ns	ns	ns	ns
Rowan	Llan	34	***	ns	**(+) 0.06***	**(-) 0.22**	**(+) 0.04***
	NYM	39	ns	**(-) 0.03***	ns	ns	**(+) 0.05***
	Dor	42	ns	**(-) 0.10**	ns	ns	**(+) 0.06**
	AH	31	*	ns	**(+) 0.11**	ns	**(+) 0.12**
Birch	Llan	30	ns	ns	ns	ns	ns
	Drum	28	ns	ns	ns	ns	ns
	Thet	32	***	ns	**(+) 0.06***	ns	**(+) 0.08**

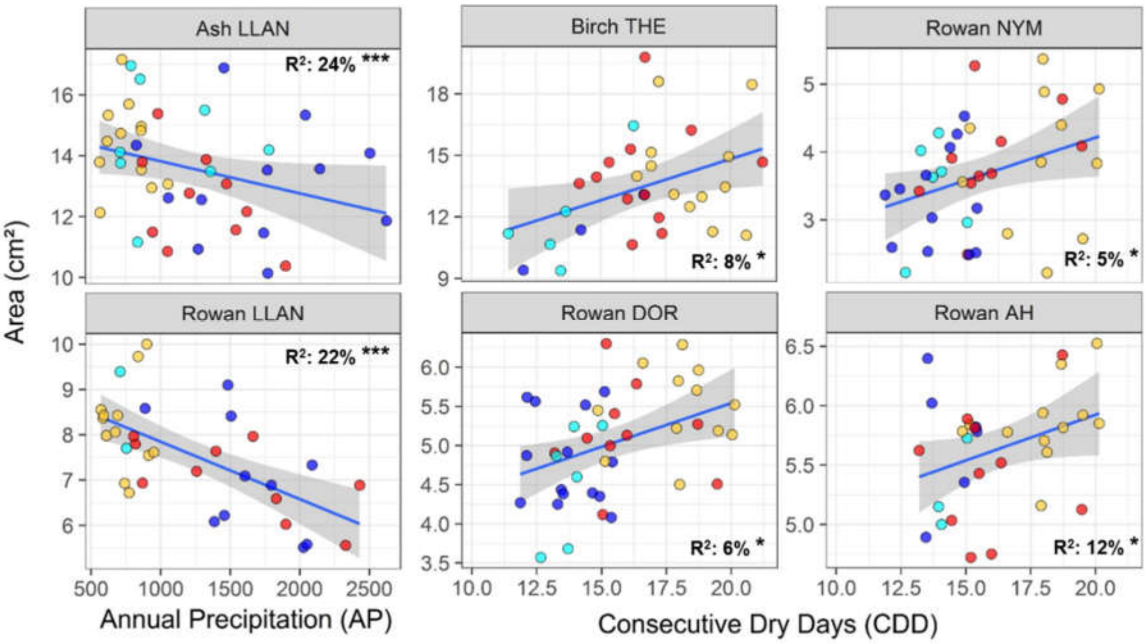


Figure 5.7. Linear regressions for trial sites where there was a significant relationship of provenance LA with Annual precipitation (AP) or Consecutive dry days (CDD) at site of origin. Each dot is a provenance mean. Colour corresponds to the ROP: dark blue NW, pale blue NE, red SW and yellow SE. Significance of R^2 ***$p<0.001$, **$0.001>p<0.01$, and *$0.01>p<0.05$.

5.3.2 Stomatal results

There were differences in stomata density (SD) and stomata length (SL) amongst species (Figure 5.5). Ash had the highest mean values for SD (315.8 stomata/mm^2) and lowest mean value for SL (23.6 µm); rowan and birch had similar mean values of SD (rowan 160.0 and birch 161.5 stomata/mm^2) and SL (rowan 27.5 and birch 28.2 µm) (Figure 5.8).

4.3.2.1 Stomatal Density (SD)

- Ash

Differences amongst provenances: In Llandovery there were significant differences in SD amongst provenances ($F_{(41,81)}$=1.6554, p=0.027). No significant differences were found in North York Moors. The provenance means range for SD was greater in Llandovery (range 259-354 stomata/mm^2) than in North York Moors (range 276-364 stomata/mm^2), but the coefficient of variation for SD was the same (13%) at both sites (Table 5.6).

Differences amongst trial sites: There were significant ($p<0.001$) differences amongst trials for SD (Figure 5.8). The most northerly trial, North York Moors, had higher stomata density (mean SD: 326 stomata/mm^2) than the Llandovery trial site (mean SD: 306 stomata/mm^2).

Interaction between provenance and site: There was no significant interaction between provenance and trial site for SD.

Relationship with environment at site of origin: Provenance SD showed significant regressions on environmental factors at the site of origin at the Llandovery trial site, but not at the North York Moors site. In Llandovery there was a significant positive regression of SD on Latitude (R^2: 16%, $p<0.001$), and significant negative regressions of SD on Consecutive Dry Days (CDD) (R^2: 12%, $p<0.01$). SD was greater in northern provenances, and those from wetter sites (Figure 5.9).

- Rowan

Differences amongst provenances: In all the rowan trials sites except North York Moors there were significant differences in SD amongst provenances (Llandovery $F_{(33,62)}$=2.08, p=0.0066; Alice Holt $F_{(30,115)}$=2.20, p-value=0.0016; Dornoch

$F_{(41,82)}$=1.89, p-value=0.0083). The range mean and coefficient of variation for provenance mean SD was similar among these sites (range 137-217 stomata/mm^2, CV=15% Dornoch; range 130-202 stomata/mm^2, CV=14% Llandovery; range 123-183 stomata/mm^2, CV = 17% Alice Holt).

Differences amongst trial sites: There were significant ($p<0.001$) differences amongst the rowan trials (Figure 5.8), for provenance mean SD. The Llandovery trial had the highest mean SD (171.1 stomata/mm^2), followed by Dornoch (SD 165.4 stomata/mm^2), Alice Holt (SD 153.9 stomata/mm^2), and North York Moors (SD 153.2 stomata/mm^2). The western trial sites had greater SD than those in the east.

Interaction between provenance and site: There was no significant interaction between provenance and trial site for provenance mean SD.

Relationship with environment at site of origin: SD had significant negative regressions with longitude in Llandovery (R^2: 17%, $p<0.001$), Alice Holt (R^2: 16%, $p<0.01$), and Dornoch (R^2: 4%, $p<0.001$); the eastern provenances have lower SD than those from the west. SD also showed a significant positive regression on AP in all the four trials (LLAN R^2: 9 %, $p<0.001$; AH R^2: 23%, $p<0.001$; DOR R^2: 5%, $p<0.001$; NYM R^2: 3%, $p<0.001$), the provenances with greater annual precipitation had greater SD. SD had a significant negative regression on CDD in Llandovery (R^2: 12%, $p<0.01$) and Alice Holt (R^2: 10%, $p<0.01$), where the provenances from origins with more CDD had lower SD (Table 5.7, Figure 5.9).

- *Birch*

Differences amongst provenances: There were no significant differences amongst provenances for SD at any of the birch trial sites. The coefficient of variation for SD in birch was similar to than in rowan and ash (Llandovery 12%, Thetford 12%, and Drummond 15%).

Differences amongst trial sites: There were significant differences ($p<0.05$) amongst the birch trial sites (Figure 5.8). The trial with higher SD was the most northerly one, Drummond (SD 166.1 stomata/mm^2); Llandovery and Thetford were more similar to each other (Llandovery SD 156.6 stomata/mm^2; Thetford SD 158.2 stomata/mm^2).

Interaction between provenance and site: There was no significant interaction between provenance and trial site for either SD.

Relationship with environment at site of origin: The variation in SD showed a significant positive regression on Latitude (R^2: 4%, $p<0.05$) in Llandovery, a negative regression on CDD (R^2: 5%, $p<0.05$) in Llandovery, and a negative regression on AP (R^2: 7%, $p<0.001$) in the Thetford trial site (Table 5.7, Figure 5.9). However these relationships explained very little of the variation in SD.

Table 5.6. Coefficient of variation, standard variation and mean of SD, by trial site.

spp	*trial*	*SD sd*	*SD mean*	*Coeff. variation*
ash	LLAN	39.03632	305.6506	**12.77 %**
	NYM	42.34146	325.6097	**13.00 %**
rowan	LLAN	23.13802	171.0859	**13.52 %**
	AH	25.78971	153.9118	**16.76 %**
	DOR	25.04954	165.4158	**15.14 %**
	NYM	22.47358	153.1943	**14.67 %**
birch	LLAN	19.35205	156.5828	**12.36 %**
	DRUM	25.0827	166.0662	**15.10 %**
	THET	19.41451	158.2439	**12.27 %**

4.3.2.2 Stomata Length (SL)

- Ash

Differences amongst provenances: There were no significant differences amongst provenances for SL at any of the trial sites.

Differences amongst trial sites: There were significant differences amongst trials for SL ($p<0.001$, Figure 5.8). The most northerly trial, North York Moors, had larger stomata (mean SL: 25 µm) than the Llandovery trial site (SL: 23 µm).

Interaction between provenance and site: There was no significant interaction between provenance and trial site for SL.

Relationship with environment at site of origin: SL showed significant regressions on environmental factors at the site of origin at the Llandovery trial site, but not at the North York Moors site. In Llandovery SL showed significant positive regressions on CDD (R^2: 3%, $p<0.05$) (Table 5.7), SL increased towards drier provenances.

- Rowan

Differences amongst provenances: There were significant differences for SL amongst provenances in the two southern trial sites (Llandovery $F_{(33,62)}$= 1.65, p= 0.0439; Alice Holt $F_{(30,115)}$= 1.65, p= 0.0323), but not in the two northern ones. The range of SL provenance means was 10 µm in Llandovery (25-35 µm), and 4 µm in both Alice Holt (24-28 µm) and Dornoch (30-26 µm).

Differences amongst trial sites: There were significant differences amongst the rowan trials (Figure 5.8) for SL. The Llandovery trial had the largest mean SL (28.0 µm) together with North York Moors (28.0 µm), followed by Dornoch (SL 27.5 µm) and Alice Holt (SL 26.3 µm).

Interaction between provenance and site: There was no significant interaction between provenance and trial site for SL.

Relationship with environment at site of origin: SL in rowan showed significant regressions on environmental factors only at Alice Holt: a positive regression on CDD (R^2: 12%, p<0.01) and on Longitude (R^2: 7%, p<0.001), and a negative regression on AP (R^2: 7%, p<0.001) (Table 5.7). SL increased in eastern and drier provenances.

- Birch

Differences amongst provenances: There were no significant differences amongst provenances for SL at any of the birch sites.

Differences amongst trial sites: There were significant differences amongst the birch trial sites for SL (p<0.001, Figure 5.8). The trial with largest SL was the most northerly one, Drummond (SL 29.2 µm), Llandovery and Thetford were more similar (Llandovery SL 27.6 µm; Thetford SL 27.5 µm).

Interaction between provenance and site: There was no significant interaction between provenance and trial site for SL.

Relationship with environment at site of origin: There were no significant regressions of provenance mean SL on environmental variables in any trial site (Table 5.7).

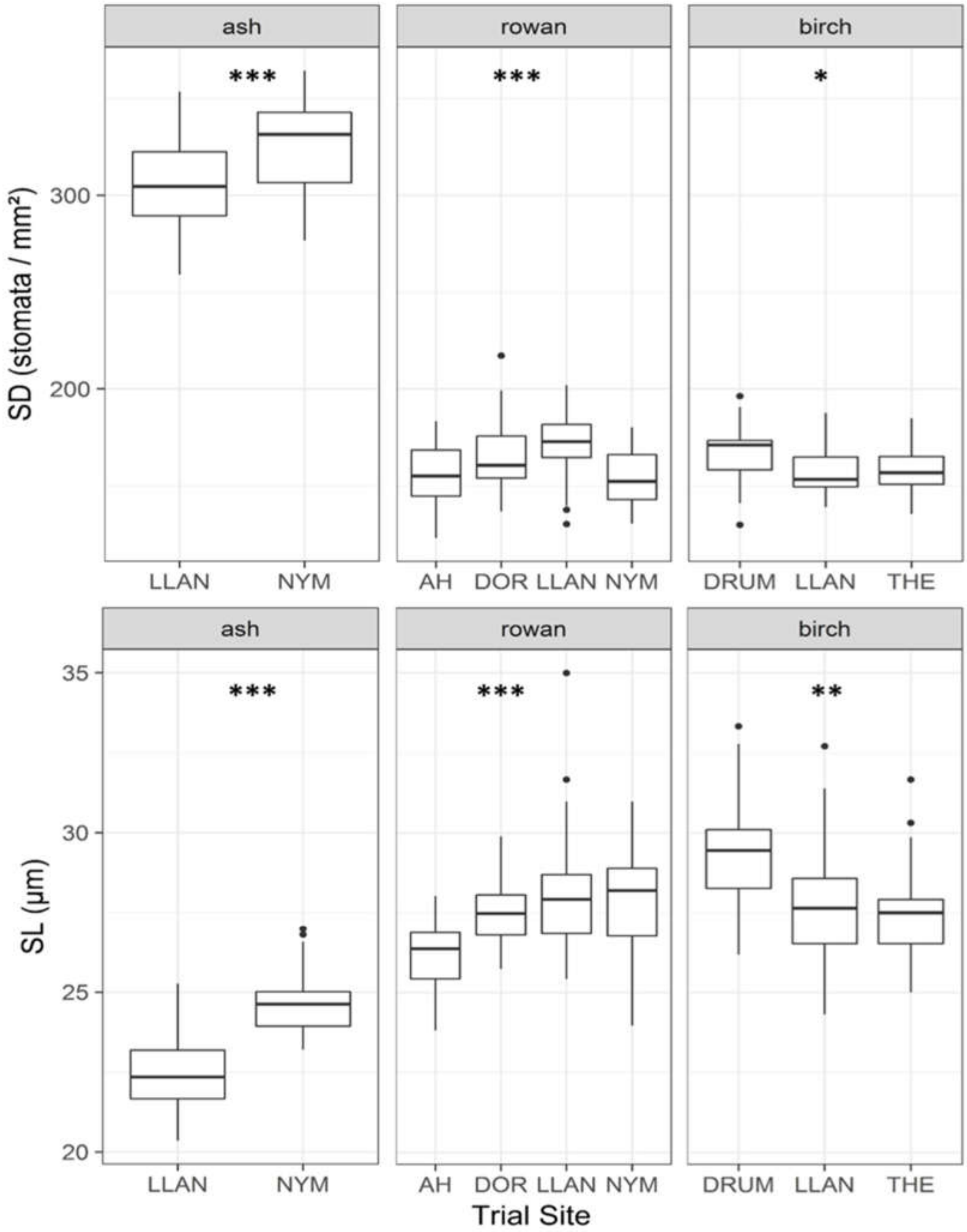


Figure 5.8. Among site variation in mean stomatal density (SD) (above) and stomatal length (SL) (below) per species. Asterisks indicate the level of significance of the effect of trial in one-way ANOVAS for SD and SL within species *** p<0.001, ** 0.001>p<0.01, and *0.01>p<0.05).

Table 5.7. Significance of provenance effect and relationship with geographic and climatic variables for each species and trial site, for stomata density (SL) and stomata length (SL). All regressions were significant at $p<0.01$ except those with the R^2 values indicated by ‘*’, where significance was $p<0.05$. (+) and (-) indicate slope of regression.

Trait	*Spp*	*Trial site*	*No. of prov.*	*Prov. effect*	*Geogr/Environmental variable Regression sign and R^2*			
					Lat	Long	AP	CDD
SD	Ash	Llan	42	*	**(+) 0.16**	ns	ns	**(-) 0.12**
		NYM	40	ns	ns	ns	ns	ns
	Rowan	Llan	34	**	ns	**(-) 0.17**	**(+) 0.09**	**(-) 0.12**
		NYM	39	ns	ns	ns	**(+) 0.03***	ns
		Dor	42	**	ns	**(-) 0.04***	**(+) 0.05**	ns
		AH	31	**	ns	**(-) 0.16**	**(+) 0.23**	**(-) 0.10**
	Birch	Llan	30	ns	**(+) 0.04***	ns	ns	**(-) 0.05***
		Drum	28	ns	ns	ns	ns	ns
		Thet	32	ns	ns	ns	**(-) 0.07**	ns
SL	Ash	Lland	42	ns	ns	ns	ns	**(+) 0.03***
		NYM	40	ns	ns	ns	ns	ns
	Rowan	Llan	34	*	ns	ns	ns	ns
		NYM	39	ns	ns	ns	ns	ns
		Dor	42	ns	ns	ns	ns	ns
		AH	31	*	ns	**(+) 0.07**	**(-) 0.07**	**(+) 0.12**
	Birch	Llan	30	ns	ns	ns	ns	ns
		Drum	28	ns	ns	ns	ns	ns
		Thet	32	ns	ns	ns	ns	ns

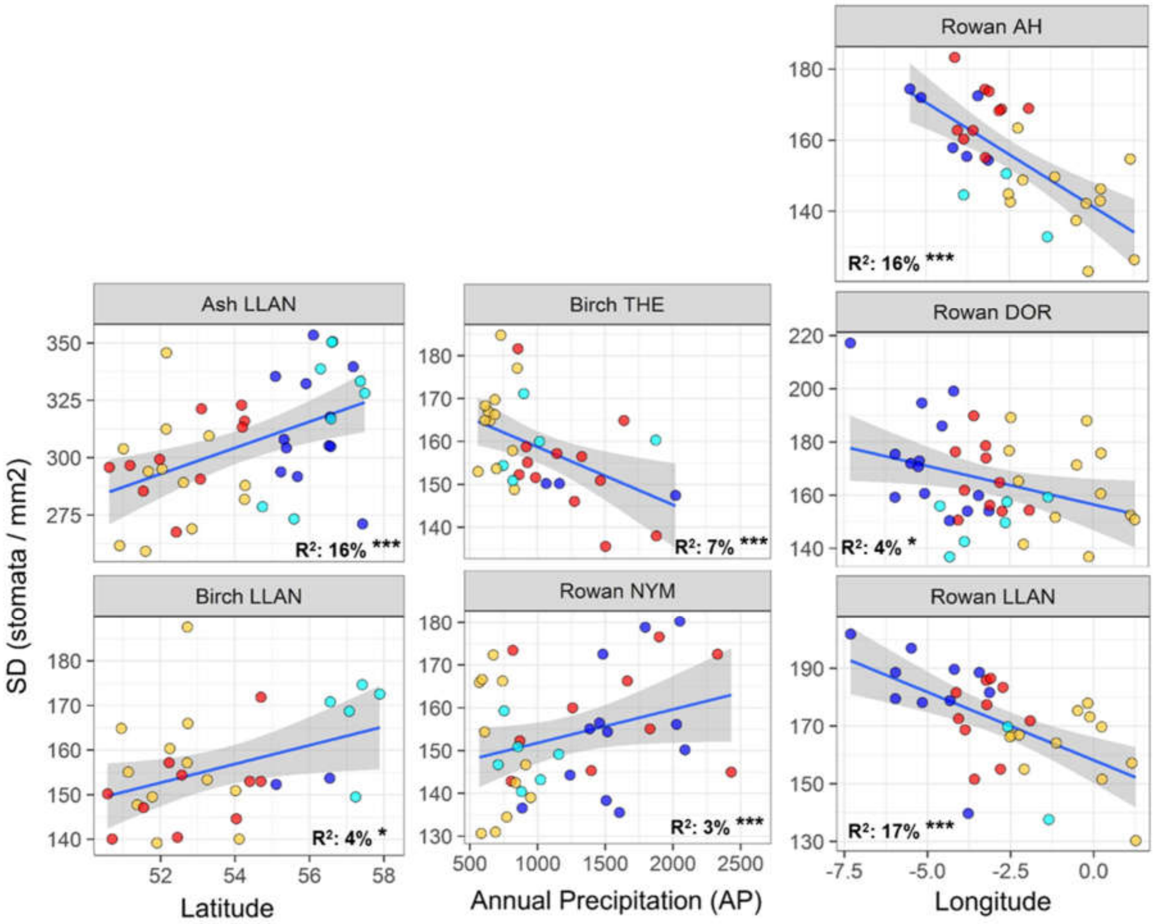


Figure 5.9. Regression of SD (number of stomata / mm^2) with latitude, longitude and annual precipitation by species and trial site. Points correspond to provenance means and the colour corresponds to ROP.

5.3.2 Correlations between Provenance Means for Leaf Characters

Correlation between SD and SL

There were significant negative correlations between the provenance means of SD and SL (higher SD corresponds to lower SL) in the following trial sites: ash Llandovery (-0.5, $p<0.001$), rowan Llandovery (-0.5, $p<0.01$), rowan Alice Holt (-0.7, $p<0.001$), rowan Dornoch (-0.4, $p<0.05$), and birch Thetford (-0.4, $p<0.05$). There were no significant correlations in the other trial sites (Table 5.8).

Despite there being a negative correlation between SD and SL within many trial sites, when looking across different trials on average the trials which had the greatest SD also had the greatest SL, for all three species (Figure 5.8).

Table 5.8. Correlation between SD and SL by species and trials.

Spp	trial	SD vs SL	Correlation stats	Pearson's coefficient
ASH	LLAN	***	t = -3.7858, df = 40, p-value = 0.0005039	-0.514
	NYM	ns		
ROWAN	LLAN	**	t = -3.1507, df = 32, p-value = 0.003522	-0.487
	AH	***	t = -5.0461, df = 29, p-value = 2.231e-05	-0.684
	NYM	ns		
	DOR	*	t = -2.5738, df = 40, p-value = 0.01387	-0.377
BIRCH	THET	*	t = -2.5324, df = 30, p-value = 0.0168	-0.420
	DRUM	ns		
	LLAN	ns		

Correlation between SD and leaf Area

Correlations between provenance means for LA and SD in each site showed only one significant correlation In the rowan Llandovery trial site a significant positive correlation was found, 0.39 was the Pearson's coefficient ($t = -2.4055$, $df = 32$, $p = 0.02$).

Correlation between SL and leaf Area

Correlations between provenance means for LA and SL in each site showed positive significant correlations in tow trials of rowan (Dornoch 0.4, p<0.05; North York Moors 0.3, p<0.05) and one of birch (Thetford 0.5, p<0.001) (Table 5.9).

Table 5.9. Correlation between LA and SL by species and trials.

Spp	**trial**	**LA vs SL**	**Correlation stats**	**Pearson's coefficient**
ASH	LLAN	ns		
	NYM	ns		
ROWAN	LLAN	ns		
	AH	ns		
	NYM	*	t = 2.4229, df = 37, p-value = 0.02041	0.370
	DOR	*	t = 2.0422, df = 40, p-value = 0.04777	0.307
BIRCH	THET	**	t = 3.041, df = 27, p-value = 0.005195	0.505
	DRUM	ns		
	LLAN	ns		

5.4 Discussion

Leaf functional traits such as leaf area, stomata density and stomatal length show great variability among populations of trees when measured *in situ*. Until now it has proved very difficult to understand either the causes of this variation or the patterns of variation that are found, and there is a lack of consensus across the literature (Dillen et al., 2008; Pallardy, 2008; Bresson et al, 2011). In order to analyse the underlying causes of the variation observed *in situ* (genetic, environmental, and an interaction between genotype and environment) and the environmental pressures that have affected these traits, it is essential to make measurements on these traits in common garden experiments at multiple sites. Here we have undertaken such analysis in common gardens for three native tree species sampled from across their natural ranges in Britain.

5.4.1 Main findings

1. All three species show significant provenance variation affecting leaf size, and this character is also affected by planting site environment. However, the most striking characteristic of the genetic variation in leaf size for all three species is that its expression is dependent on the environment in which the trees are grown. So differences in leaf size may be found in some planting sites and not in others, and the ranking of provenances may vary between sites in very unpredictable ways. In this situation it may not be easy to analyse in detail the relationship between leaf size and environmental variables because the pattern will not be consistent over experimental sites.

2. Density of stomata is a character which shows variation among provenances in ash and rowan but not birch, and it is affected by environment in all three species. However, in contrast to leaf size there are no significant genotype x environment interactions in any species. In this situation it is therefore worth exploring the patterns of variation among provenances in ash and rowan which are more likely to be consistent over experimental sites. In ash SD increases with latitude of provenance

origin (greater SD towards the north), while in rowan SD decreases with longitude of provenance origin (greater SD towards the west).

3. For SL there is evidence of provenance variation only in rowan, but of environmental variation in all three species. As for SD there is no G x E interaction. For rowan SL decreases with latitude (in opposite direction to SD).

4. In all three species there is a negative correlation between provenances means for SD and SL.

5. Differences across species were found for SD and SL. Ash has a much greater SD and lower SL than rowan and birch (which are more similar between them). The SD counts we reported are of very similar magnitude to other studies in ash (Hölscher et al. 2002) and silver birch (Kostina et al., 2001).

5.4.2 Pattern of variation for leaf size

For ash and rowan, leaves were smaller in some trials in provenances from the north-west (towards colder and wetter sites of origin), and were larger towards the south-east (drier sites of origin). This same trend is found in Warret et al (2005), where in common garden experiments the eucalyptus provenances from greater rainfall areas had smaller leaves. Also, studies of an altitude gradient have found a reduction in leaf size reduced towards higher altitudes provenances in *in situ* observations (Bresson et al., 2011) and in common garden experiments (Stojnic et al, 2015).

In other studies carried on in drier climates, leaves tend to be smaller *in situ* towards the equator as water is the limiting factor and leaf reduction is considered an adaptation to drought (Adams, 1994; Castro-Díez et al. 1997, Groom and Lamont 1997, Bussotti et al. 2000, Wright et al. 2001, Rowland, 2001; Lamont et al. 2002; Bruschi et al. 2003; Gratani et al. 2003). Some of these studies conducted in common gardens found genetic diversity in leaf size (Bussotti et al, 2000; Rowland, 2001), but patterns of variation were in the opposite direction to our results. In Bussotti et al. (2000), when leaf area was measured in common gardens of beech provenances from throughout

Italy, leaf area decreased towards the south. This was explained as an adaptation to the extreme drought from Mediterranean summers, which will be more acute in the most southern provenances.

However these explanations for patterns of leaf size variation developed in xeric systems are not likely to be applicable to ash, rowan and birch in GB. This is because these populations are not at the southern edges of their species’ distributions, but towards the north and western edges where they have had to adapt to grow in the temperate and oceanic climate in GB. Despite there being some relative drought in summer in some areas and over long time cycles, this is not the major limiting factor for tree growth in GB. The limited or conservative leaf size found in northern and western provenances must be due to selection by an environmental factor other than drought. We hypothesis that the limiting factor which is driving genetic diversity towards having smaller leaves in northern and more oceanic provenances is the colder temperatures in those areas in GB, which reduce the growing season length towards the north with increasing risk of frost damage towards the north and west. As shown in Figure 4.10, there is a positive correlation between wet and cold areas in GB. That could be why we find significant relationships between leaf size and annual precipitation, as the wettest sites in GB are also the coldest.

Although there is some signal from our common garden experiment that provenances from northern and western sites having reduced leaf size, the most striking result is that the provenances react differently in different environments. On average leaf size is greater in the western trial sites of Llandovery, for all three species, which are the wettest and warmest sites for all three species. We see an opposite pattern between genetic diversity and phenotypic plasticity: in relation to all trial sites the leaves grow bigger in the wettest site (Llandovery), but within trial sites the provenances from wettest origins have smaller leaves.

There are significant genotype by environment interaction in all three species for leaf area. These include crossing interactions, where the ranking of provenances changes across sites. This shows that it is unpredictable how leaf size will react if provenances are moved between different environments. There is genetic diversity within the species, but this expresses differently depending on the environment where the trees are grown. The southern trials show a greater range of leaf area and show more differences across provenances. The genetic diversity is expressed more in these southern trial sites in terms of phenotypic variation

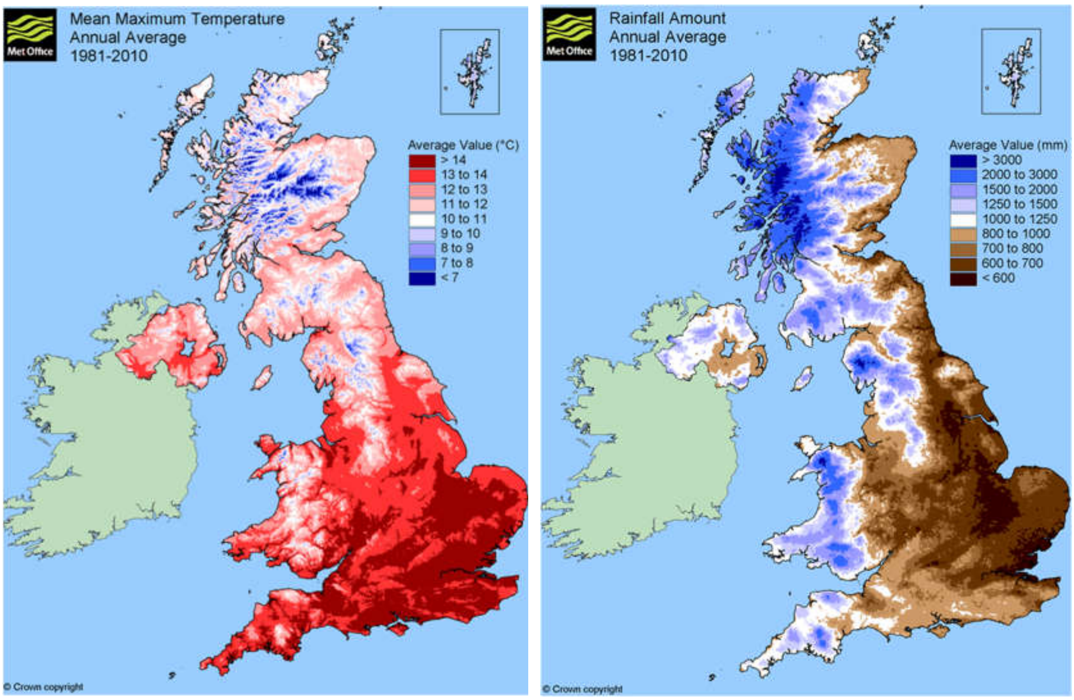


Figure 4.10. MetOffice average climatic data, mean maximum temperature and mean rainfall (1961-2010).

5.4.3 Patterns of variation for stomatal density

For the character of stomatal density, significant provenance differences were found in ash and rowan, and there were no genotype x environment interactions. The consistent pattern of variation for ash was that SD increased with latitude of origin. For rowan, in contrast, SD decreased with longitude of the provenance. We find again this same geographical pattern as previously found for growth and phenology traits, where the provenance differences across sites for rowan are explained by longitude, while for ash these are explained by latitude.

Another very interesting result was that the plastic response in SD found across trial sites, was in the same direction as the genetic differences across provenances. When looking at the differences across sites within species we found that in ash all provenances had greater SD when planted in the more northerly trial sites; while in rowan SD was greater in the western than in the eastern trial sites. For this trait phenotypic plasticity appears to operate in the same direction as adaptation, a phenomenon known as acclimation. This same direction of genetic diversity and phenotypic plasticity has been found in common garden experiments on riparian North-American poplars (Dunlap and Stettler 2001; Pearce et al. 2005). Here diverse poplar genotypes show genetic differences whereby individuals from drier locations have higher SD and smaller stomata, and respond to drier environments by producing leaves with a higher density of smaller stomata.

No consensus has been reached on the factors determining patterns of SD among provenances and the reasons why SD changes under different environmental conditions (Bresson et al., 2011). Depending on the study opposite trends have also been found. Usually, high stomata density has been considered a drought adaptation; provenances from drier sites, and also modifications on water availability stress (increased drought), results in an increase in stomatal density (Gindel, 1969; Dunlap and Stettler 2001; Abrams, 1994; Pearce et al. 2005; Xu and Zhou 2008; Fraser et al. 2009; Laajimi et al. 2011). This is the opposite of what we found in our results, where (for both genetic diversity and plasticity) SD decreased as site of origin or growth became drier. However, the studies in which SD increases with drier conditions tend to be conducted in semi-arid environments and on species which have adapted to cope with severe drought. These conditions do not hold for our study of temperate forest trees in an oceanic British climate. The selective pressures will be different in different climates.

There are other studies (Salisbury, 1927; Quane & Jones, 1977; Bussotti et al., 2005) where the opposite trend has been found, parallel to our own results. A study of beech trees across Italy (Bussotti et al., 2005) found that stomata density declined towards the south of Italy. They hypothesised that increased SD in the northern stands was an adaptation to the risk of physiological water stress (Flückinger et al., 1986), or an induction of increased SD by high ozone concentrations due to pollution (Pääkkönen et al., 1998). However, this is not an explanation that would be consistent with our findings, as the north-west of GB where we find the highest SD, is less polluted than the south of GB and moisture deficit is the lowest in GB. An alternative explanation is that higher SD can be an advantage when there is excess water availability but lower temperature, and flooding is a risk (Gomes and Kozlowski, 1980; Bradford and Hsiao, 1982; Kozlowski, 1997; Yordanova et al., 2005). This explanation would be consistent with the results found in Scottish populations of *Pinus sylvestris* (Donnelly, 2015), where the number of stomata increased towards the west of Scotland where precipitation and waterlogging is higher.

For our study based in GB the climate cline is more comparable to altitudinal changes than semiarid environments clines, as the latitude and altitude change in environmental clines are usually parallel (Randin et al., 2013). There are many studies which have documented the effect of altitude on stomata density. Both trends between altitude and SD have been found, the majority finding that SD increases towards higher altitude in *in situ* observations (Körner & Mayr 1981, Hovenden & Brodribb 2000, Hovenden & Vander Schoor 2006, Kouwenberg et al. 2007, Premoli & Brewer 2007, Bresson et al.,2011). However when common garden experiments were conducted, these did not find genetic differences among provenances across the altitude range. An exception was the work of Hover & Brodribb (2000), where population differentiation for stomata density was found. A possible explanation of why only phenotypic and not genetic differences were found for SD, is that the provenance sampling was done across a relative small geographic area, despite being in a very steep altitudinal and environmental gradient. Similar discrepancies have been found between the results from studies of comparable latitudinal and altitudinal gradients, due to the former involving sampling over a much greater geographic distance than the latter (Randin et al., 2013). For example, in Bresson et al. (2011), a study on sessile oak and beech progenies in the French Pyrenees, did not find differences in the common gardens but did find differences across the same progenies *in situ*. The progenies sampled in this study were quite close together in distance (most provenances collected within a 25 km radius, with only one beech provenance 100km further than the rest), which could mean that the populations could maintain high levels of gene flow between them.

Attempts have been made to explain changes in SD with altitude in terms of many factors including change in CO_2 air concentration, change in light, or decrease of air humidity (Bresson et al., 2011). There are also many factors which have been shown to influence SD: water availability, temperature, CO_2 concentration, and light amongst others (Beerling & Chaloner, 1993; Kouwenberg, 2007; Casson & Gray, 2008). In some cases the change in SD shows a quadratic response curve. For example Xu & ZhoU (2008) found that moderate water deficits increased SD, but more severe deficits led to a reduction. However, it has not yet been possible to find a consistent explanation for the pattern of variation in SD across species and environments. In our results, however, we have found clear patterns of provenance variation for SD in both ash and rowan associated with latitude and longitude respectively.

5.4.4 Patterns of variation for SL

In this study we used SL as a proxy for stomatal size. For SL we found provenance differences in rowan, where longer stomata were found in provenances from the east. There were also significant differences across trial sites for SL for all three species. Again, as with SD, there is no consensus in the literature on the pattern variation in stomatal size to be expected across environments. Usually smaller stomata have been related to higher drought adaptation (Dunlap & Stettlet, 2001; Pearce et al., 2005), which is the opposite to what we have found but in accordance with other studies such as Bussotti et al., 2005).

5.4.5 Relationship between leaf characters

When looking at the relationship between leaf and stomatal characters we found a strong significant negative correlation between SD and SL for the mean provenance values, and this was maintained across the tree species and in most trial sites. Provenances with higher stomatal density tend to have shorter stomata and vice versa. This negative correlation between SD and SL is a well-known pattern across species

(Gindel 1969; Franks & Farquhar 2001; Uprety et al. 2002; Franks et al., 2009) and it is thought it allows for quick changes in the stomata conductance. Hetherington & Woodward (2003) proposed that small stomata have shorter response times, and that this, in combination with their usually high densities, allows the leaf to attain high stomatal conductance rapidly under favourable conditions, but then to rapidly reduce conductance when evaporative conditions are unfavourable. While this would be an advantage over larger, slower stomata, it is unclear why a plant should revert back to larger, slower stomata under less demanding conditions.

It has been shown that SD is set in very early stage of leaf development (Casson & Gray, 2008). For this reason, it could be that leaf size and leaf development differences would affect SD variation, and the differences we have recorded on SD could be determined more by leaf development differences than genetically. However, we have rejected this hypothesis. We have not found significant correlations between SD and LA, except for one trial site of rowan. We found significant correlations between LA and SL in two sites for rowan ($p=0.048$ and $p=0.021$) and one in birch ($p<0.01$). Furthermore, the patterns of variation across sites were very different for leaf size and SD; while SD does not show significant site by provenance interactions and has a very predictable plasticity trend, leaf area shows a very strong interaction between site and provenance.

5.4.6 Conclusion

Our findings have revealed patterns which were previously unrecognised among populations of these 3 species in GB. The genetic variation and high levels of phenotypic plasticity indicate that leaf traits respond adaptively to environmental variation. Our results demonstrate that the leaf size, stomata density and stomata size are related to water availability environmental variables from their site of origin, and also the planting site. This is important as with climate change water availability is predicted to change in GB (IPPC, 2013).

Northern provenances have smaller leaves as an adaptation possibly to colder and windier conditions. Currently the southern provenances have the largest leaves, which are the provenances that are predicted to experience drought with climate change. This could be a problem as larger leaves have more evapotranspiration and therefore lose more water. However, we have shown that phenotypic plasticity in leaf size makes the same provenances have smaller leaves in the driest trial sites and larger leaves in the wettest trials. This plasticity could compensate and help the tree populations from the south east of GB cope with the future predicted drought.

For the stomata density we have seen this trait is positively correlated with precipitation, and we hypothesis it is an adaptation to help increase evapotranspiration when dealing with waterlogging. It is predicted that waterlogging and flooding will increase in parts of GB with climate change. Phenotypic plasticity in stomata density has the same trend, it increases the density with wetter sites, this means that plasticity can help cope it. However, we have not tested how drought will affect stomata density in GB tree populations, so we do not know if the current adaptation on stomata traits will be suitable for dealing with climate change.

The important thing we have found is that within British populations of ash, rowan and birch there is a large amount of variability for leaf size and stomata traits, both genetically and phenotypic plasticity. This means tree populations have an adaptive capacity for when the climate conditions change, and the selective pressures act upon the tree populations.

Chapter 6: Discussion

(Including multi-trait analyses as summary of all the results)

6.1Summary of key findings

6.1.1 Summary by chapter

The exploration of the climatic factors that was performed in Chapter 2 set the foundation of the analyses that were carried out in the subsequent chapters. We carried out a principal components analysis using 13 climate variables to characterize the environments of each of the provenances of the three broadleaved species which provided seed for the establishment of the common garden trials. By plotting provenance locations according to their PC1 and PC2 scores, we demonstrated that the climatic variation covered by the three species in our samples largely overlapped, which confirmed that it was valid to compare our results across species using the sampled provenances present in the trials. In addition, most of the trial sites had climates which lay within the core climatic envelope for the three species within GB. Therefore, the trial sites were likely to present conditions that were within the climatic range that British populations would normally experience and we could be confident that we were not exposing the material to extreme conditions that they were unlikely to experience naturally. Additionally, we found extensive climatic diversity within each region of provenance (ROP), with considerable overlap in the climatic envelopes of the four ROPs, for this reason we did not include ROP as a fixed factor in the subsequent analyses. We found that latitude and longitude explained and summarized the environmental diversity of GB effectively, justifying our use of these in the analyses.

Chapter 3 explored the variation in growth traits, stem forking and survival in British provenances of ash, rowan and silver birch in the replicated common garden trials. Comparison of results within trials revealed genetic differentiation (there are clear differences in growth trait variation among British provenances), and comparison of results between replicated trials showed that these traits exhibit phenotypic plasticity (growth is very dependent on the site where trees are growing), There was also an interaction between the genetic and environmental components of variation. Differences in height growth tended to be related to latitude, although in the northern trial sites, longitude also was important. Rowan was the species which showed smaller differences amongst provenances for tree height. Height variation was positively correlated with DBH and forking.

Chapter 4 examined phenological traits in the common garden trials. There was differentiation amongst native provenances for leaf flushing and senescence in ash and rowan. The variation amongst provenances was greater than the phenotypic plasticity individual provenances showed across different environments. Stem forking was correlated with date of leaf flushing in ash. These results show that trees are adapted to a determinate growing season at their site of origin and transfer of material from its site of origin to another location can result in frost damage to the apical buds which results in forking and poor form in trees. We also found very significant differences between ash and rowan for their geographical patterns of both leaf flushing and leaf senescence: in ash, the southern provenances flushed earlier and senesced later, in rowan the eastern provenances flushed earlier and senesced later.

Chapter 5 presented results on leaf anatomy traits (leaf area, stomatal density and stomatal length) measured across native provenances of ash, rowan and silver birch. We showed distinct genetic diversity for these traits, which are related with environmental covariates (consecutive dry days and annual precipitation). Trees from drier provenances had greater leaf area, lower stomatal density and greater stomatal length; while trees from origins with greater annual precipitation showed lower leaf area, greater stomatal density and lower stomatal length. Moreover, these traits showed extensive phenotypic plasticity across the different environments. In the case of leaf area, there was a strong environment by genotype effect, which makes leaf area unpredictable when provenances are grown in novel environments. Stomatal density did not show interaction between site and provenance.

6.1.2. Patterns of variation

6.1.2.1 Patterns of genetic diversity across traits

In the results chapters we have presented the analyses for nine traits of adaptive significance measured in the three species; all nine traits were measured in ash but only seven were assessed in the other two study species. A different complement of seven traits was measured in rowan and birch (Table 6.1). We found genetic differences for most of these traits in most trial sites (Table 6.2). Height and DBH had clear differences across provenances, whereas for survival the difference between provenances was not so clear. The stem forking had a very clear provenance effect for ash but less so for birch. Flushing had very strong differences across provenances, which were greater than the differences found for senescence. The leaf anatomy traits showed fewer differences across provenances for birch, and the stomatal genetic differences were most clear in rowan. We carried out two different PCA analyses to look at the patterns of variation that arise with all the traits together. We carried out PCA, which had one value for PC1 and PC2 per provenance. Secondly, we carried out PCAb, which had the provenances scores replicated for each trial site, to address phenotypic plasticity.

PCA of all traits by species

We have combined these traits measured in the different trial sites into a principal component analysis by species, to understand the patterns of variation across provenances that arise when the data for traits are considered together. For the PCA we did not include all the trial sites but used data from: Llandovery and North York Moors for ash; Alice Holt, Llandovery and Dornoch for rowan; and Llandovery and Drummond for birch. This was because for the analysis there could not be more columns (traits) than row (provenances).

Table 6.1. List of traits by species, ‘x’ indicates assessed and ‘-’ indicates not assessed.

Trait	Ash	Rowan	Birch
Survival	x	x	x
Height	x	x	x
DBH	x	-	x
Forking	x	-	x
Flushing	x	x	-
Senescence	x	x	-
Leaf area	x	x	x
Stomata density	x	x	x
Stomata length	x	x	x

Ash: The two first components account for 50% of the variation (38% PC1 and 12% PC2). PC1 corresponds mainly to growth (Height, DBH and survival) and leaf senescence, and separates provenances by taller and early flushers versus shorter and late flushers (Figure 6.1). PC1 is correlated positively with latitude (Pearson’s correlation coefficient r=+0.75) and negatively with longitude (r=-0.48) (Figure 6.2). PC2 loadings are the greatest for leaf traits SD, SL and LA, separating between provenances with large SD versus provenances with small leaves and small SL (Figure 6.1). PC2 is correlated negatively with latitude (r=-0.13) and positively with longitude (r=+0.18) (Figure 6.2).

Rowan: The first two components of the PCA for rowan account for the 40% of the variation (PC1 26%, PC2 14%). PC1 separates provenances between the late flusher provenances with large SD from the provenances which flush earlier and have smaller SDs (Figure 6.1). PC1 is correlated positively with latitude (r=+0.47) and negatively with longitude (r=-0.76) (Figure 6.2). PC2 separates the tallest and latest to senesce from the other provenances (Figure 6.1). PC2 is positively correlated with both latitude (r=+0.45) and longitude (r=+0.24) (Figure 6.2).

Birch: The first two components of the birch PCA explain 43% of the variation (28% PC1, 15% PC2). PC1 separates the provenances according to their height and DBH (Figure 6.1). PC1 is correlated positively with latitude (r=+0.71) and negatively with longitude (r=-0.38) (Figure 6.2). PC2 separated the provenances between trees with large leaves from provenances with more forks (Figure 6.1). PC2 is negatively correlated with latitude (r=-21) and positively with longitude (r=+0.15) (Figure 6.2).

Differences across species in height vs other traits

We find differences across species, different traits having more weight in determining the variation across provenances. For both ash and birch we find that most of the variation is explained by differences across provenances in growth (Height, DBH); while in rowan, most of the variation is explained by flushing time and SD. In the second PC2 (which in three species accounts for up to 15% of the variation) for rowan more weight is given to height and leaf senescence while in birch and ash leaf traits are more important. Overall, we see that for ash and birch, tree growth is what separated most the provenances and in secondary terms leaf traits; while in rowan SD and leaf phenology (both spring and autumn) are most important to explain the variation, with height being secondary. This could be explained by differences in the biology and life strategy of the different tree species. Rowan is a shorter tree usually located in the understory and shade tolerant (Grime, 2007) while ash and birch are much taller trees which compete more for light. The importance of leaf phenology in rowan may be explained by the fact that it flushes very early in the season and senesces later (compared with ash and birch, Grime 2007), and is therefore under greater selective pressures to adapt to avoid early and late frosts.

Geographical pattern of the trait variation

When looking at how the traits are correlated with latitude and longitude we find differences across species and traits (Tables 6.2). Ash has mainly a latitudinal pattern, rowan a longitudinal pattern, and in birch latitude and longitude are both important depending on the trial site. We can see that ash has a very large latitudinal effect for all traits with the only exception in leaf area. As we have shown in chapter 5, leaf traits are more affected by climatic variables; in this case of leaf area for ash, annual precipitation explains the same amount of variation as latitude and longitude together, and precipitation in GB towards the North and West of the country. In rowan we find that leaf traits, phenology and stomata traits are mostly explained by longitude. However, variation in height in the southern trials (and leaf area in the northern trials, although very small part of the variation) is explained by latitude. In birch we find a mixture of latitude and longitude effects. Height is explained by latitude in the southern, and by longitude in the northern ones; DBH is always by latitude; forking by longitude in the most northern trial; and survival by longitude (Table 6.2).

ROPs as a grouping system of trait variation

This complex pattern that differs geographically depending on the trait (Table 6.2) and between the three species is well summarized by the PCA and visualized in Figure 6.2. Looking at the ROPs of each provenance in the scatterplots of PC1 vs. PC2 (Figure 6.1), we see that in ash the PCA separates provenances by latitude, a group of provenances from SW and SE together, and then a group with the NW and NE provenances which include two SW and two SE provenances. Rowan, on the other hand, in the PCA the NW and SW provenances come together in one group, separated from the SE provenances; the two NE provenances are one in each group. And finally in birch, we find that SE and SW provenances are together in one big group that occupies most of the scatterplot, which means there is a lot of variation; variation which does not get separated by the two ROPs SW and SE. Then, we find a very distinct group of NE provenances, and the two NW provenances are one in each group.

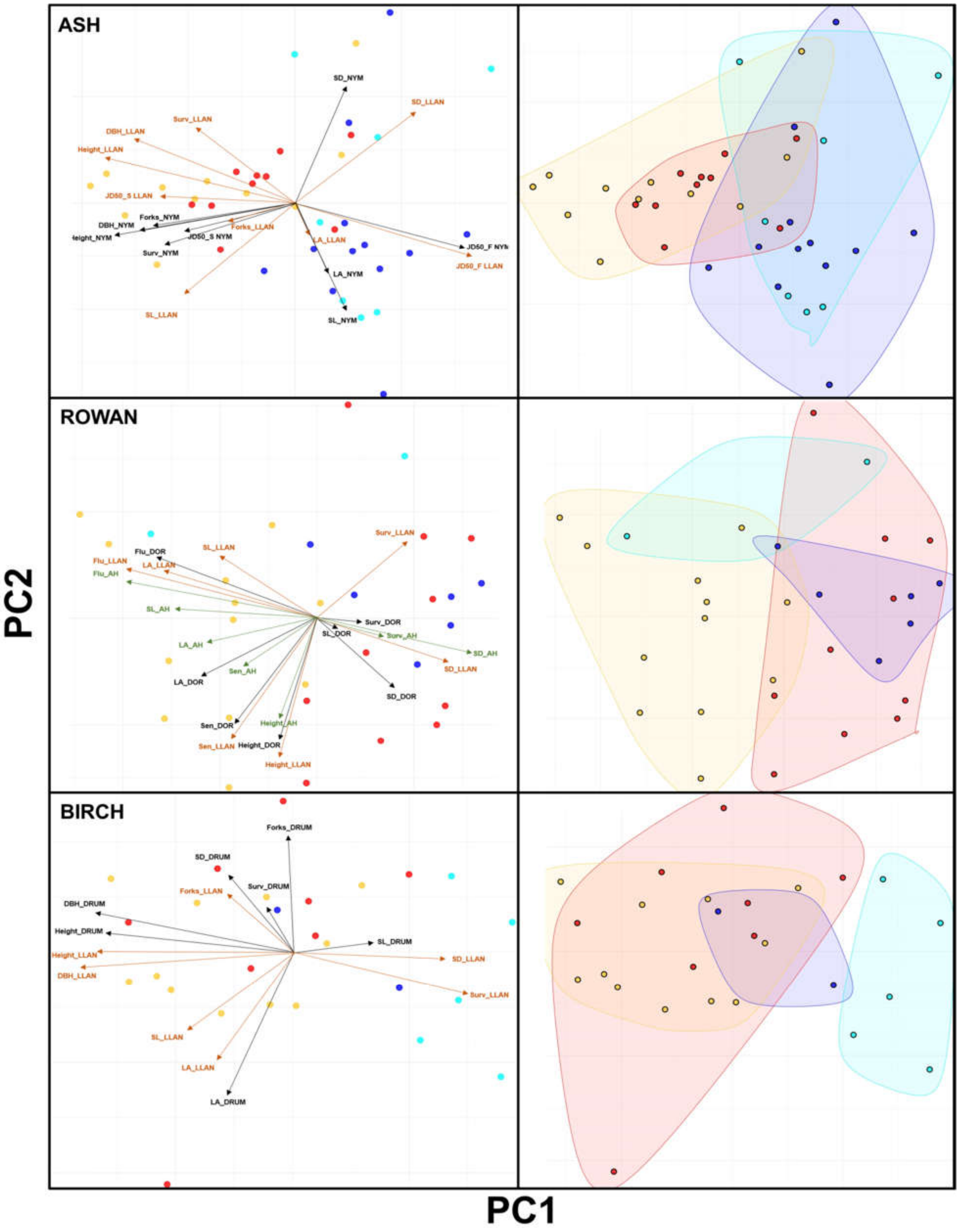


Figure 6.1. Scatterplot of PC1 vs. PC2 for the three species. Each dot is a provenance score for PC1 and PC2. On the left we have the plots with the arrows of each trait in each trial, and on the right we have the polygons with the ROP of the provenances. The colour represents the ROPs (**dark blue** NW, **pale blue** NE, **red** SW and **yellow** SE).

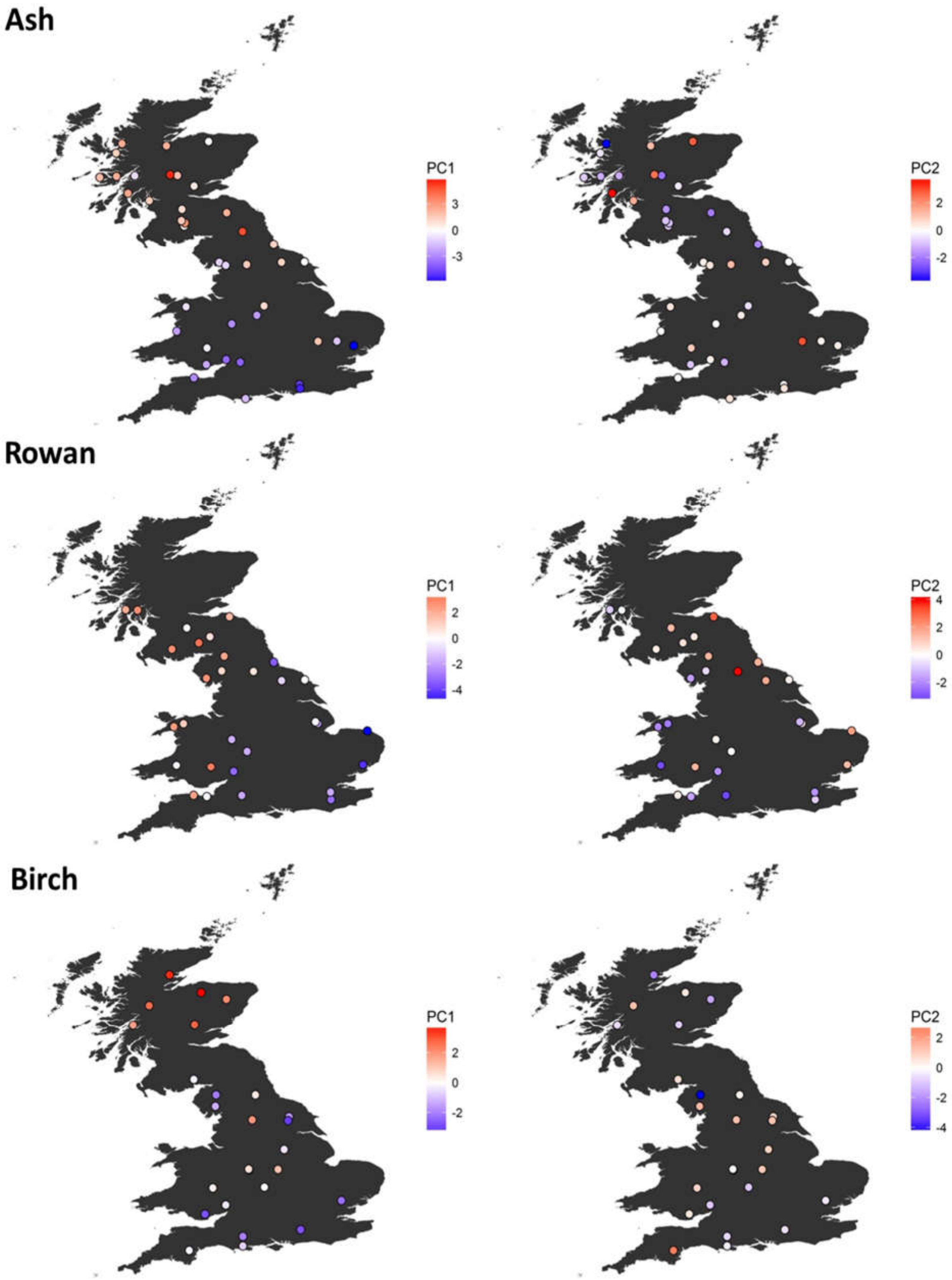


Figure 6.2. Values from the PC analysis by species, the provenance values of the PC1 (left) and PC2 (right) are mapped in the provenance locations.

Table 6.2. Summary table of all the traits analysed in the thesis, by species and trial site. It includes if there are significant provenances for that trait in that trial site, and whether these differences are explained by latitude, longitude or both. The significance coding is the following of the p values is: '***' 0-0.001, '**' 0.001- 0.01, '*' 0.01-0.05, 'ns' >0.05. Not all the traits were sampled in all the trial sites, when that is the case it is identified with a hyphen (-). The R-squared values which represent less than 10% of the variation are in italics font.

Spp	Site	Var.	Trait								
			GROWTH				PHENOLOGY		LEAF ANATOMY		
			Surv	Height	DBH	Forks	Flush	Senes	LA	SD	SL
Ash	**LLAN**	**~prov**	ns	***	***	ns	***	***	*	*	ns
		R^2	ns	-Lat 46%***	-Lat 36%***	ns	Lat 41%***	Lat 27%***	+Lon -Lat 27%***	+Lat 16%***	ns
	NYM	**~prov**	ns	*	ns	***	***	**	*	ns	ns
		R^2	ns	-Lat 40%***	-Lat 39%***	-Lat 21%***	Lat 35%***	Lat 20%***	ns	ns	ns
Rowan	**AH**	**~prov**	ns	*	-	-	***	ns	*	**	*
		R^2	-Lon 11%***	-Lat 54%***	-	-	Lon 41%***	ns	+Lon 11%**	-Lon 16%**	*+Lon 7%***
	LLAN	**~prov**	*	***	-	-	***	***	***	**	*
		R^2	ns	-Lat 17%***	-	-	Lon 48%***	*Lat 8%***	*+Lon 6%**	-Lon 17%**	ns
	NYM	**~prov**	***	*	-	-	***	***	ns	ns	ns
		R^2	ns	*+Lon 4%****	-	-	Lon 45%***	Lon 16%***	*-Lat 3%**	ns	ns
	DOR	**~prov**	ns	***	-	-	***	*	ns	**	ns
		R^2	*-Lat 2%**	ns	-	-	Lon 33%***	ns	-Lat 10%***	*-Lon 4%**	ns
Birch	**LLAN**	**~prov**	***	***	***	***	-	-	ns	ns	ns
		R^2	+Lon 10%**	-Lat 51%***	-Lat +Lon 51%***	ns	-	-	ns	*+Lat 4%**	ns
	THET	**~prov**	ns	***	-	-	-	-	***	ns	ns
		R^2	ns	-Lat 51%***	-	-	-	-	*+Lon 6%**	ns	ns
	DRUM	**~prov**	ns	***	***	***	-	-	ns	ns	ns
		R^2	+Lon 27%***	+Lon 29%***	-Lat 48%***	*-Lat 7%**	-	-	ns	ns	ns
	DOR	**~prov**	ns	***	*	ns	-	-	-	-	-
		R^2	ns	+Lon 51%***	ns	+Lon 22%*	-	-	-	-	-

6.1.2.2 Phenotypic plasticity

Having more than one common garden experiment by species has allowed us to see that the provenances behave very differently when growing in different environments, and that these differences vary across traits as well. Plasticity is a very important trait in itself which is determined genetically (Donohue et al., 2001), and that allows trees, being sessile organisms, to respond rapidly to changes to environmental changes in a shorter timescale than the life of a plant (Bradshaw, 1965).

We have shown in the results chapters that some provenances (especially the southern ones) vary more across sites than other provenances, meaning they have more plasticity, especially in some traits such as height and leaf phenology. Other cases of provenance effect on the levels of plasticity have been shown (Donohue et al., 2001; Vitasse et al., 2009). In our case, it could mean that the northern provenances are more conservative as they have adapted to stronger adaptive pressures. This could be parallel to the case explained in Vitasse et al (2009) of provenances from higher altitude showing less phenotypic plasticity.

Phenotypic plasticity can be positive or negative, which can cause fitness problems. It is important for a species to be well adapted to the environment in which it grows to increase efficiency and therefore increase competitiveness. However, trees as being long lived sessile organisms have developed high levels of phenotypic plasticity to cope with changes in the environment (Jump & Peñuelas, 2005). Plastic responses for plants have been reported as adaptive (Poorter & Lambers, 1986; Dudley, 2004), but also examples of maladaptive plasticity have been shown (van Kleunen & Fischer, 2005; Ghalambor et al., 2007).

In our results we find cases where the direction of plasticity can be in the same direction as adaptive variation (such as SD, SL or tree height) and others which plasticity goes on the opposite direction to the genetic variation found across provenances (the case of leaf area).

In our results we have shown that GB provenances for ash, rowan and silver birch have high levels of phenotypic plasticity, and that this degree of plasticity is different across provenances and across traits. For most traits however, we have found that there was more differentiation within a trial (due to genetic differences) than across trials, despite there being big differences.

PCAb for site effect

We now will look at the direction of plasticity by species and traits. We carried out a second PCA (which we referred to as PCAb) which included the values for each trait, with all the provenance means for each trial site. This allowed us to understand the difference between trial sites for each trait and species, and the direction of plasticity between the sites.

Ash: The first two components of PCAb (Figure 6.3) account for 64% of the variation (PC1b 48%, PC2b 16%). PC1b (which corresponds mainly to growth, survival, forking and senescence) separates the two trials clearly; there is only some overlap between the provenances of SE ROP from North York Moors with the provenances of NW from Llandovery. We see that all provenances are taller and thicker, fork more, plus senesce later in Llandovery than in North York Moors. PC2b separates the provenances by flushing time and leaf area, which splits within the two trials the provenances by north and south.

Rowan: The first two components for rowan explain 63% of the variation (PC1b 38% and PC2b 25%). The first one separates the provenances growing in North York Moors from the rest as being with the lowest survival, height, LA and SD compared with the other trials. PC2b separates the trials the provenances by flushing and senescence time, leaf area and SL. The provenances growing in Llandovery are the ones flushing earlier and with greater SL. We find the most diversification of the provenances within the Llandovery trial site (Figure 6.3).

Birch: In the birch PCAb the two first components explain 63% of the variation (PC1b 38% and PC2b 25%). PC1b separates the two trial sites by provenances growing taller and with bigger leaves in Llandovery than Drummond, and with greater SD and forking in Drummond. PC2b separates the provenances within their trial cluster by their DBH and SL (Figure 6.3).

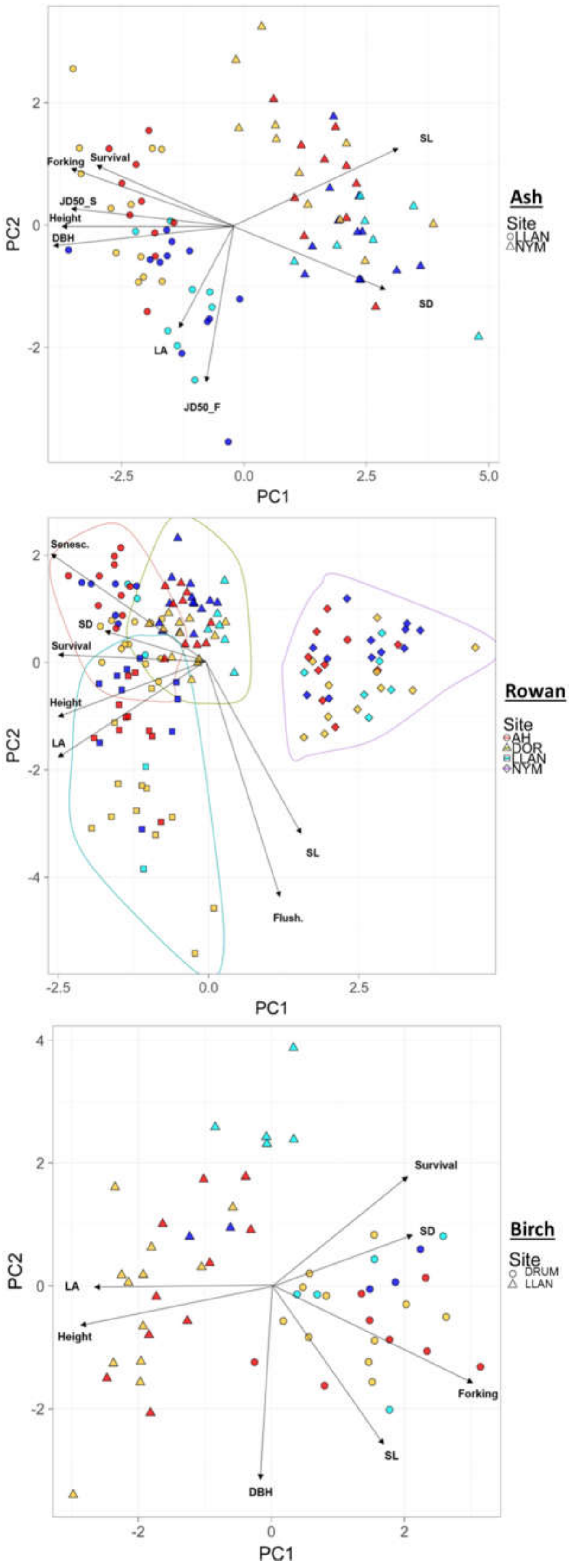


Figure 6.3. Scatterplot of PCAb for the three species. Each dot is the score for PC1b and PC2b in the provenances at the different sites. The colour represents the ROPs (**dark blue** NW, **pale blue** NE, **red** SW and **yellow** SE).

6.1.2.3 Genotype by environment

Genotype by environment interactions (GxE) exist when differences between genotypes are not consistent from one environment to another (Baker, 1988). GxE interactions have been well studied in plants using multi-environment trial as a methodology, especially by tree and crop breeders to test for their improved genotypes across different environments (Cooper & DeLacy, 1994). GxE interactions can express in many forms (Allard & Bradshaw, 1964), the two main ones being: a) interactions due to the heterogeneity of genotype variance amongst environments, and b) those due to the lack of correlation of the genotypic performance amongst the different environments (Cooper& DeLacy, 1994). The latter form of GxE interaction results in the re-ranking of the genotypes across the environments (which results in crossing provenance means in the interaction plots) (Baker, 1988) and these type of interactions are the most rejected by breeders as they impede good predictions of genotypes' performance (Gregorius & Namkoong, 1986).

In our results chapters we shown the two types of interactions, which in some cases were significant in the analyses of variance and others not. We found GxE interactions of the first type in the three species. In tree height for ash and birch due mainly to the greater differences in height amongst provenances in the southern trials. However in birch some change of ranking occurred across provenances from the same ROP. In rowan we found a significant GxE interaction due to the big range of survival differences across provenances in the North York Moors trial site, while in the other trial sites survival was over 90% for most provenances.

We found crossing GxE interactions with great change of provenance ranking on leaf area for all three species, then for leaf phenology in rowan, and for forking in birch. We also found the provenance means ranking changed between the two sites for ash in forking, survival and leaf phenology, and for birch in survival, but the interactions proved not to be significant. Height in rowan also had ranking change of provenances across sites, but the interaction was not significant in the ANOVA.

Using the PCA and PCAb biplots to examine GxE

GxE interactions vary across traits in our results, moreover the interactions we found of the first type (due to increased variance of genotypes due to the environment) have shown us the importance of having several trial sites. We have shown in the results chapters, how important is to have studied the provenances growing in different environments, as some of the genetic variation across provenances for some traits have arisen in some trial sites but not in others. This is especially true for the leaf traits, or for example in height for rowan. In PCAb we have shown that provenances are more different between each other (appear more scattered in the biplot) within some trials than in others. In the PCAb biplots (figure 6.3) we can see than in ash the provenances spread similarly within the two trial sites. In rowan, on the other hand, we find the most differentiation in Llandovery between sites, then in North York Moors, while in Alice Holt and Dornoch provenances overlap very much one cluster. In birch we see that the provenances in Llandovery are more spread across the PC2b and form a larger cluster than the provenances in Drummond.

On the other hand, we can also learn from GxE interactions looking at the first PCA (Figure 6.1). The interesting result from the first PCA is that allows us to compare the direction of each trait in separating the provenances across the different trial sites, and that way observe the genotype by environment interactions.

Ash: in ash we found significant interactions between provenance and site in height, and leaf area. There was no significant interaction for DBH, fork or survival. This is reflected in the PC2 of the first PCA, where height, DBH and survival for the two trials have opposite sign (Table 6.3). In PC1 SL pulls the provenances in opposite directions for the two trials, indicating the direction of plasticity. However, for most traits in ash, the two trials sites pull the provenances toward the same direction. In PC2 for height, DBH and survival the two trial sites separate the provenances in different directions.

Table 6.3. Loadings for PCA (figure 6.1), per trait and trial for PC1 and PC2 in ash.

Trait	Comp.1		Comp.2	
	LLAN	**NYM**	**LLAN**	**NYM**
Height	-0.3414	-0.3363	**0.1496**	**-0.1427**
DBH	-0.3006	-0.3121	**0.2325**	**-0.1318**
Forks	-0.1183	-0.2932	-0.0727	-0.1199
Survival	-0.1651	-0.2561	**0.2795**	**-0.1763**
JD50_F	0.3263	0.3148	-0.2195	-0.1930
JD50_S	-0.2342	-0.1990	**0.0265**	**-0.1247**
SL	**-0.1863**	**0.0854**	-0.3469	-0.4196
SD	0.1926	0.0805	0.3162	0.4022
LA	0.0197	0.0584	-0.1072	-0.2920

Rowan: we found significant interactions between provenance and site in survival, leaf flushing, leaf senescence, and leaf area. In the PCA we find that SL, survival, and leaf area are the traits which pull provenances in different directions depending on the trial site where they grow (Table 6.4).

Table 6.4. Loadings for PCA (figure 6.1) per trait and trial for PC1 and PC2 in rowan.

Trait	Comp.1			Comp.2		
	AH	**LLAN**	**DOR**	**AH**	**LLAN**	**DOR**
Height	-0.1182	-0.1112	-0.109	-0.3666	-0.4284	-0.3975
Survival	0.1220	0.1680	0.1012	**-0.0481**	**0.2281**	**-0.0244**
Flush	-0.3661	-0.3570	-0.3038	0.1456	0.1663	0.1606
Senesce	-0.1452	-0.1545	-0.1454	-0.1707	-0.3401	-0.3022
SL	**-0.2527**	**-0.2122**	**0.0235**	**0.0189**	**0.1710**	**-0.0094**
SD	0.3261	0.2962	0.1333	-0.1009	-0.1302	-0.2011
LA	-0.2048	-0.2836	-0.2111	**-0.0729**	**0.1393**	**-0.1901**

Birch: we found a significant interaction between provenance and site in tree height, DBH, forking and leaf area. However, in the PCA we see that survival SL and SD are the traits that separate provenances by trial site the most (Table 6.5).

Table 6.5. Loadings for PCA (figure 6.1), per trait and trial for PC1 and PC2 in birch.

Trait	Comp.1		Comp.2	
	LLAN	**DRUM**	**LLAN**	**DRUM**
Height	-0.4225	-0.3923	0.0293	0.1026
DBH	-0.4740	-0.3948	**-0.0325**	**0.1539**
Forks	-0.1085	-0.0071	0.2247	0.3873
Survival	**0.2966**	**-0.046**	**-0.1622**	**0.1640**
SL	**-0.2136**	**0.1171**	**-0.3368**	**0.0258**
SD	**-0.1027**	**0.2725**	**-0.0219**	**0.2868**
LA	-0.1488	-0.1365	-0.4105	-0.5870

6.2 Practical implications

6.2.1 Is the current seed zone system supported by our data?

We have shown that British populations of ash, rowan and silver birch have great genetic differentiation for the nine traits studied. They show genetic differences from each other for tree height, stem diameter, leaf phenology, leaf size, stomata size and density; and that such differences have consequences for survival and stem form. The observed differences tend to show a geographical pattern which varies among species. Variation in ash follows a clear latitudinal cline, in rowan a clear longitudinal cline and in birch tends to vary with both latitude and longitude.

These phenotypic differences ought to be taken into account when considering strategies for seed sourcing for establishment of new native woodland in GB. However, provenances from within a single ROP show high levels of variability (Figure 6.1), which means that planting within ROPs does not mean necessarily than provenances will be well adapted to the planting site. Maps in the appendix (A.1, A.2 and A.3), show that the provenances geographical difference does not agree with the similarities they show in the traits we have assessed. This is one clear shortcoming of the existing system of seed sourcing for native trees and shrubs.

Based on the broad geographic patterns of variation identified, we suggest that ash trees should be planted within a similar latitude to their home site to ensure adaptation to the current conditions of their planting site, as latitudinal transfer has been shown to compromise stem form and the greatest differences for all traits are between northern and southern provenances. For birch we see that SE and SW provenances show very similar variation which overlap; however, within the northern provenances there is a clear longitudinal differentiation.

Rowan shows a very different pattern from ash and birch and we have observed a clear longitudinal pattern in the genetic diversity. Rowan provenances from the SW and SE ROPs are very much differentiated, and so we consider that rowan should be planted within the same longitude as much as possible, advising against transfer from east to west, or west to east. We see also that multivariate trait values for the provenances from the NW overlap completely with the SW provenances (Figure 6.1) from which we conclude that latitude is less important than longitude in determining the variation among rowan populations.

Taken together, our results from three ecologically contrasting species demonstrate that the standardised series of seed zones currently applied to all tree species can oversimplify patterns of variation within and among tree species. Further research may help to determine whether a more sophisticated approach, involving separate sets of seed zones for different species, or groups of species is desirable. Such systems are in place elsewhere (e.g. Alìa et al., 2009; Pierangelo et al., 2017) and, given the complexity observed among the three species studied in this thesis, it would provide clear advantages in GB as well. Since the spatial scales at which traits have been found to vary are rather large, these do not need to be over-complicated, and indeed, may simplify current difficulties associated with sourcing planting stock for new woodland creation (O' Neill et al., 2014; Whittet et al., 2016).

6.2.2. Can the native populations of ash, rowan and birch adapt to climate change?

We have shown that provenances for ash, rowan and silver birch possess great levels of genetic differentiation within their British distribution, which can be presumed to be due to past selection and genetic adaptation since they recolonized the British Isles, due to the diversity of environments found in GB (as shown in Chapter 2). It is expected that the past and current selective pressures which have formed the current genetic pattern in tree populations will change with global warming and the effects it will have on many environmental variables. It is unclear whether adaptive genetic change will be quick enough to allow native populations of trees to regenerate and survive among increasingly changing conditions. As trees are long lived organisms (especially ash, which lives longer than rowan and birch), changes in the genomes of the populations might take longer to achieve the best adapted genotype for the new and changing environments. However, in addition to high levels of genetic variation, we have shown that British populations of these tree species exhibit high levels of phenotypic plasticity, which would allow existing generations of trees, and those which are newly grown to cope with a degree of environmental change. We consider that if levels of gene flow (which might be greater for ash and birch as they are wind pollinated) are maintained between the southern provenances towards the northern ones; and natural regeneration is allowed, which would permit that adaptive pressures select the seedlings with the best genotypes to the new climate, native populations of these trees should thrive under the new environmental change. Since all of the species studies also occupy large ranges throughout other parts of Eurasia, and have demonstrated adaptation to the environment in regions which are considerably warmer than GB, a lagged adaptive response is a realistic expectation. Phenotypic plasticity as it is a much quicker mechanism, could buffer the needed adaptive change during the generations required for genetic adaptation to take place.

Moreover, it has been shown that genetic variation that is selectively neutral in one environment can become adaptively significant in another (Hall et al., 2010; Anderson et al., 2013). There is probably more genetic variation within British tree provenances which in our study has not appeared as the environmental pressures were not there to express it. In the absence of a particular selective pressure (for example extreme drought which is predicted to increase in frequency in parts of GB), genetic variation for these traits present in the tree populations is carried as neutral variation. Exposure to such extremes may expose cryptic genetic variation, which would enable an adaptive response (Donnelly, 2015).

Leaf functional traits and phenology

Leaf phenology is one trait which has awaken a great deal of concern in the context of climate change (e.g. Cleland et al., 2007; Körner & Basler, 2010), as it is a trait which is very sensitive to climate and one which shows high levels of plasticity, as it varies inter-annually according to climatic variability (Delpierre et al., 2009). We have shown that (ash and rowan) provenances have high genetic differentiation for this trait, the pattern of which corresponds to the current climate in GB. We have seen that for rowan the longitudinal cline is very important, showing a cline that is probably driven by the difference between the continental (east) and oceanic (west) climate, an adaptation which is particularly important in order to avoid the higher rate of frost on the western part of the country. We have seen different directions in plasticity between species and between spring and autumn phenology. Moreover, with the GxE interactions and because it is such a plastic trait, it is difficult to predict how the different provenances would react in a change of climate, as we have seen that the rankings change across sites. This unpredictability increases the concern of the already shown effects of tree phenology changes with climate change and its impacts in the trophic levels of the ecosystems (Both et al., 2009). However, we have shown that the British provenances for ash and rowan show very strong genetic differences which are related to the climatic pattern in GB; so potentially tree populations could eventually adapt to the new climatic conditions. In the meantime, phenotypic plasticity will help trees cope, but as we see the unpredictability factor might create big consequences for the ecosystems, for tree herbivores and the rest of the trophic levels.

We have seen in our results for ash that early flushing provenances have more forking incidence. There is concern that with the changes in leaf phenology will increase frost damage in temperate zones and therefore increase stem forking. Morin & Chuine (2014) modeled 22 species and found that risk of frost injury changed for with climate change for all tree species (mostly decreasing). However other views (Vitasse, 2017), suggest that the risk the spring damage might be maintained, especially with the incorporation of provenances adapted to warmer climes.

Our results demonstrated that the leaf traits measured in our study are related to water availability environment variables at the provenance site of origin. This is an important finding as water availability is predicted to change with climate change in GB (IPCC, 2013; Morison and Mathews, 2016). Variation in leaf size exhibits a strong interaction between genotype and environment, and several climatic factors seem to play an important role in determining expressed variation in this trait. It is likely that adaptive change has occurred to allow provenances to cope with colder conditions towards the north and west, as low temperature represent a major source of physiological stress trees have experienced since recolonising GB. This could be a problem if severe droughts increase in GB with climate change, as trees have rarely been exposed to extreme drought pressure in the recent past, and with their current genetic pattern leaves are bigger in the areas where drought is expected to increase most (IPCC, 2013). Having bigger leaves means that there is more evapotranspiration, and therefore lower water use efficiency, which would be a disadvantage to cope with drought.

Phenotypic plasticity for leaf size exhibits trends which are opposed to the pattern of genetic variation. Leaf sizes in our results increases towards the wettest-warmest trial sites. This could indicate with the overall increase of temperature with climate change, leaf size might increase in trees growing in locations where there is no shortage of water but where cold temperatures currently limit leaf size. Such an increase in leaf area in response to warmer future conditions would help increase photosynthesis and growth in these trees (Saxe et al., 2001; Boisvenue and Running, 2006). However, we have not been able to directly observe whether plasticity in leaf size reacts in drier trial sites, to know how drought increase in GB might affect tree leaf size.

The provenances which had lower SD were from areas of GB where drought is predicted to increase with climate change. Higher SD is recognised as an adaptation to severe drought, as it helps trees to have a quick response, and more efficient gas exchange, in the periods when conditions are optimum (Hetherington & Woodward, 2003). The genetic diversity we found has not been exposed to drought pressure, a feature that is reflected in the different pattern we observe in GB, where high SD seems to represent an adaptation to the high precipitation and lower temperatures experienced in the NW of GB. These two factors which are likely to lead to waterlogging plus drought might occur in different seasons in the same locations in GB in the future (IPCC, 2013).

SD acclimation (phenotypic plasticity) happened in the same direction as genetic adaptation in our trial sites. For SD plasticity currently helps the individual tree move towards the required phenotype for the selective pressures found in different locations in GB. However we do not know how SD would react if trees were growing in a much drier conditions predicted for the future, as we have not exposed the trees to that water stress.

If currently we move provenances within GB, the leaf size change is very unpredictable, while SD acclimation to the new climate will change following the same geographical pattern found in the genetic diversity geographical pattern. For SD, the phenotypic plasticity found in British provenances shows acclimation in the right direction, towards increasing fitness. However, with the future climate changing the outcomes both for leaf area and stomata traits are unpredictable.

Our results for leaf functional traits show that genetic diversity of ash, rowan and birch in GB for these two functional leaf traits, seem to show the opposite trend to other environments where drought is already a major limiting factors. It has been suggested (Jump et al., 2006 & 2010; Cavin & Jump, 2017) that populations which have not yet been under drought selection might be harder hit by climate change where drought will be a selective pressure. If summer droughts increase in GB, especially towards the SE and our data do not inform this contention as British populations have not yet been subjected to severe droughts. To determine how the natural populations of GB trees will react to drought, they would have to be studied under drought conditions, so that the genetic diversity and the plasticity for drought tolerance are expressed.

6.2.3 Summary of practical implications

In this thesis we have found new results that help us understand tree populations variation and their capacity to future adaptability. The most important finding in the thesis are the following:

- The British populations of ash, rowan and birch show high levels of variability in genetic differences for growth, phenology and functional leaf traits. They also show high levels of phenotypic plasticity for these traits. This degree of variation found within species shows that these species have adaptive capacity and enough plasticity to cope with climate change.

- We have found that the variation between tree populations tends to show some geographical structure but that it does not correspond to the configuration of existing seed zones and ROPs. Furthermore, some provenances show differentiation over environmental or climatic distances, rather than simply geographic distance and this is something that could be considered in the development of alternative systems for seed sourcing.

- The three species do not show the same geographical pattern of genetic variation and so the common seed zones for all species are not fit for purpose. Levels of variation among populations of ash show a clear latitudinal cline, where the southern provenances are more similar amongst them and the northern ones are more differentiated between them. Variation in rowan shows a very clear longitudinal differentiation, where the NW and the SW provenances cluster together. Variation in birch shows a latitudinal cline, where the SE provenances are very similar amongst them and form a cluster within the SW cluster; while among the northern provenances there is a clear differentiation by longitude.

To finalise we will address the question in the title of the thesis: “Can native woodlands cope with climate change?”. Our answer is: mostly, yes. With the current climate change predictions for GB, we consider that British tree populations will be able to adapt and cope with the change of environment. In most of GB the increase of temperatures will increase the growing season length and in many cases this will result in a climate which is more benign. We have shown that all provenances and species trees grow taller when planted in a warmer trial site. Plasticity will help trees use the longer growing season and there will probably be a natural succession process whereby the southern provenances will move north as they show higher growth and could out-compete the local ones. However, because it is uncertain whether the frequency or severity of late spring and early autumn frosts rate will change, conservative growth of the northern provenances might prove to have an advantage to cope with the oceanic frosts events and the extreme weather events (such as winter flooding) that are also predicted to occur. The only cases when we cannot answer whether tree populations might be able to adapt and cope with the climate changing is in the cases where severe drought is predicted to occur. This is predicted to happened mostly in the south east of GB. However, as we have not put the pressure of drought to our trial sites we do not know how much variation is already present (dormant) for this trait, whether there is any at all or whether there is some phenotypic plasticity mechanism that could help the trees that are growing currently there buffer the drought stress.

6.3. Opportunities for further research

6.3.1 Ideas to improve the research

To study local adaptation in the strictest sense and gain a greater understanding, the best methodology would be to use reciprocal transplant experiments (Kawecki & Ebert, 2004) in which seed from multiple environments is raised reciprocally in each source environment. Reciprocal transplant experiments allow to determine more precisely the environmental factors associated with the diversity found. Another methodology which would enable stronger inferences to be made about the levels of genetic diversity found within provenances is to conduct progeny tests, which maintain family structure, in which the identity of parent trees is retained throughout the course of the experiment. Provenance-progeny tests enable calculation of quantitative genetic parameters (e.g. heritability, selection gradients), and enable variation among families within populations to be calculated. Such an experimental configuration, although harder to scale than the provenance tests we have assessed would provide the best evidence of the ability of trees to adapt to specific changes in the environment.

In the view of climate change there are new climatic pressures predicted to appear onto which will affect GB tree populations. The effects of these should be examined to see the amount of variation for these particular traits that exists already in GB, and to be able to assess the possible damage that these pressures could cause to tree fitness.

We have seen that forking is often positively associated with height growth, presumably as a consequence of frost damage on early flushing genotypes. Whilst this is indicative of maladaptation, we do not see higher levels of mortality in early flushing genotypes. On the contrary, provenances which show higher levels of mortality tend to be those from harsher home environments (e.g. cold, wet and windy sites). More detailed study on the effect of leaf spring phenology, height and stem form may help to determine which levels of stem forking truly compromise a trees' potential to survive and reproduce.

To understand better the plasticity in leaf phenology and the climatic variables which affect it, assessments over multiple years in the same sites would be a key data to try to understand how climate change might affect leaf phenology.

6.3.2 What we need to know next: New questions which have arisen from this thesis

a) *Are extreme events likely to exert stronger selection than directionally changing climate? Do trees show genetic variation in their tolerance to, or recovery from extreme events?*

Alongside directional change in the values of average climatic variables, extreme events such as flooding, droughts and heatwaves are expected to increase in frequency and severity in the future (IPCC, 2013). While exposure of tree populations rapid directional climate change will incur an adaptive lag, the selective pressure upon trees from extremes are stronger and more immediate. To explore whether tree populations show variation in resistance to extremes, and whether this can be used in deployment of seed for new planting sites, experiments could be conducted as pot-based studies to look at the variation existing in the resistance to drought/flooding/heatwaves/outbreaks; or alternatively some treatments could be done in the trials sites that already exist.

b) *Has the environment during nursery production a significant effect later in tree performance? Are these effects reversible? Is there potential in treating early tree seedlings growth conditions to enhance phenotypic plasticity due to epigenentics? How much of the phenotypic plasticity could be due to epigenetics, could some of it be hereditable?*

Epigenetics is still a topic not well understood, many questions remain about the mechanisms and roles of epigenetic processes in enabling rapid adaptation of plants to their environment, especially in forest trees (Bräutigam et al., 2013). However, it can be the reason for some of the variation we cannot explain and also something that it should be understood to be able to use in forestry and maybe help adaptation of trees to a changing environment. Forest trees offer excellent opportunities to examine some of the most compelling questions of ecological epigenetics (Bossdorf et al. 2008), particularly those related to the interplay between epigenetic variation and phenotypic plasticity in natural populations, and the role of epigenetic variation in evolutionary processes. There is still a lot to understand about the maternal effects, and the effect of environmental conditions during embryogenesis. For example, Dewan et al. (2018) have found that the maternal temperature during seed maturation affects seed germination and timing of bud set in seedlings of European black poplar. It is a field which needs more research and it has great potential. Also, it has to be taken into account the importance of how the nursery practises and the way new forests are created (sometimes seeds in orchards far from the planting site and seedlings grown in mainland Europe or artificial climatic conditions (Whittet et al., 2017)) affect later on the performance or suitability of trees to the environment they are planted. Natural regeneration could possibly be the best way to enhance acclimation to the new climate?

c) *With climate change how will tree populations be affected forest pests and diseases? Are there genetic differences regarding resistance?*

Physiological stress imposed by changes in climate is likely to weaken the defences of trees to herbivores, pests and pathogens (Telford et al., 2015), many of which are exotic and are expanding their ranges due to climate change (e.g. Battisti et al., 2005) or increased levels of international trade in live plants (Brasier, 2008).

Since the interaction between climate change and exposure to new pests and pathogens will be critical in the ongoing resilience of forests, provenance trials offer a unique opportunity to study genetic variation in resistance to these challenges.. For ash dieback, differences among provenances for resistance have been shown (Pliura et al., 2012; Stocks et al., 2017). Central and eastern Scottish provenances were found to be more resistant than those from further south, maybe it could be explained by leaf phenology. However, another theory is that resistance is greater in these populations due to some rare chloroplast haplotypes from refugial populations in the Forth and Clyde valley (Sutherland et al., 2010). Emerald ash borer LATIN NAME is a possible emerging threat to ash in the future and there is some weak evidence that there is a negative genetic correlation between susceptibility to the two systems (Sollars et al., 2017). Birch dieback, associated with *Anisogramma virgultorum* and *Marssonnina betulae* found to show differences among provenances at Drummond trial (De Silva, 2008).

6.4 Concluding remarks

Climate change is a challenge for ecosystems around the world. With this thesis we provided data that adds evidence towards the understanding of this topic and which may lead to improvements in policy and woodland management decision making. Large knowledge gaps exist in many aspects of this important issue. We have particularly focused on the measurement of the variation in genetic diversity and phenotypic plasticity in British populations of three native tree species. We have broadened the range of traits which are usually measured, and taken steps to release the restrictions of the timber quality approach. We have compared the behavior of traits such as height with that of more functional traits, in the end we have seen that for ash and birch (not so much for rowan) height remains an appropriate trait for demonstrating large genetic differences between provenances and between trial sites. However, we have shown that height is not necessarily an indicator of good adaptation to the planting site environment, as mismatch in the leaf phenology with the local conditions can cause damage to the tree, which can be reflected in the stem form. Moreover, leaf functional traits link to water availability have proven to show genetic differentiation and phenotypic plasticity. We have seen that seed sourcing rules need to be modified, tailored to individual species. These new rules need not be complex if we understand the most important environmental variables to which each native tree species is adapted across GB. We have found than British native populations of ash, rowan and silver birch show large genetic differences amongst them in several traits and also with high levels of phenotypic plasticity; both things which we consider that will help these populations cope with climate change.

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Appendix

Provenance maps by species. Provenances located in the GB map and in the biplot according to their PC1 and PC2 scores, from the PCA (Figure 6.1). Outliers from the core group of provenances in the biplot have been pointed in the maps as well.

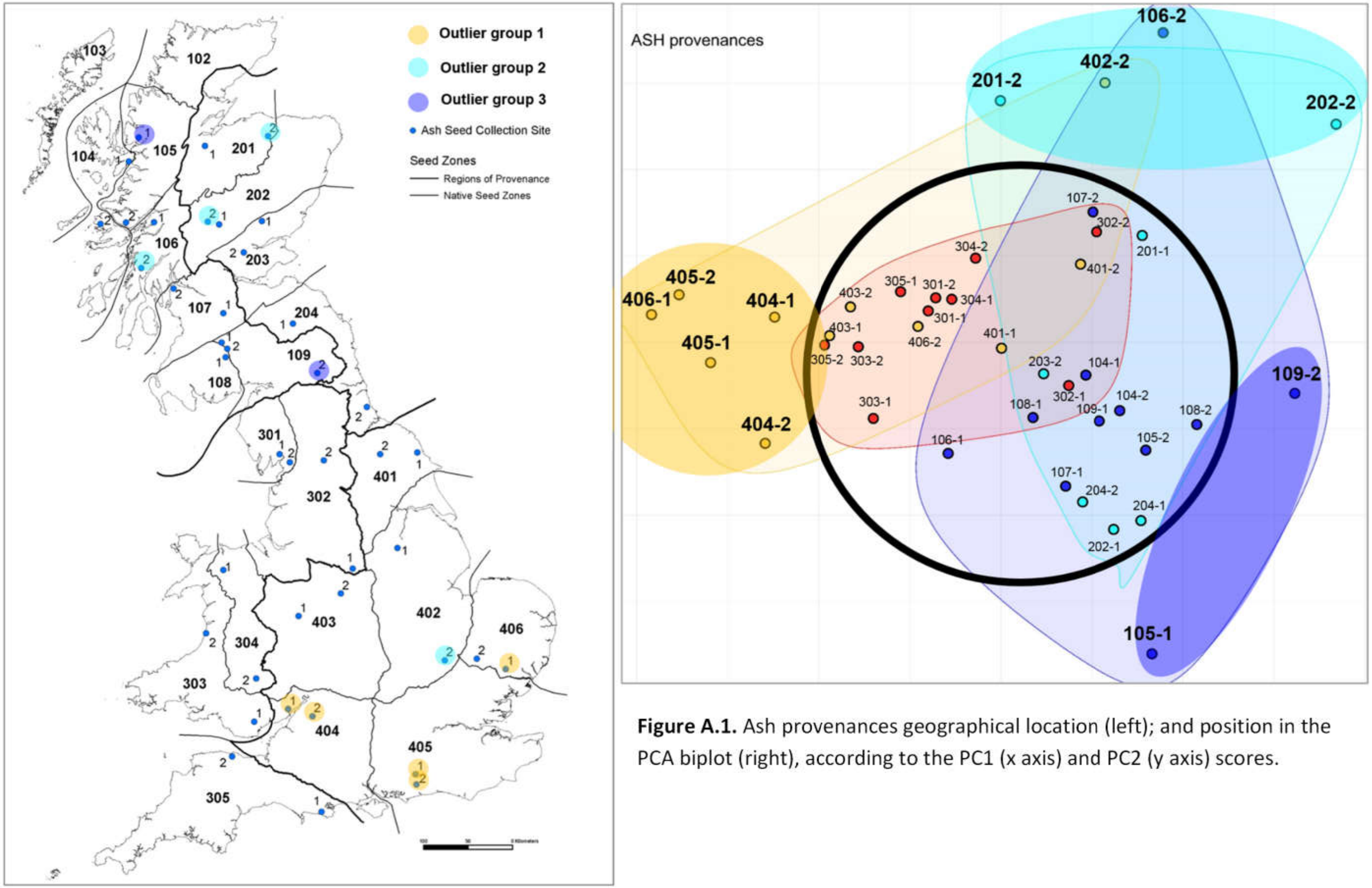

Figure A.1. Ash provenances geographical location (left); and position in the PCA biplot (right), according to the PC1 (x axis) and PC2 (y axis) scores.

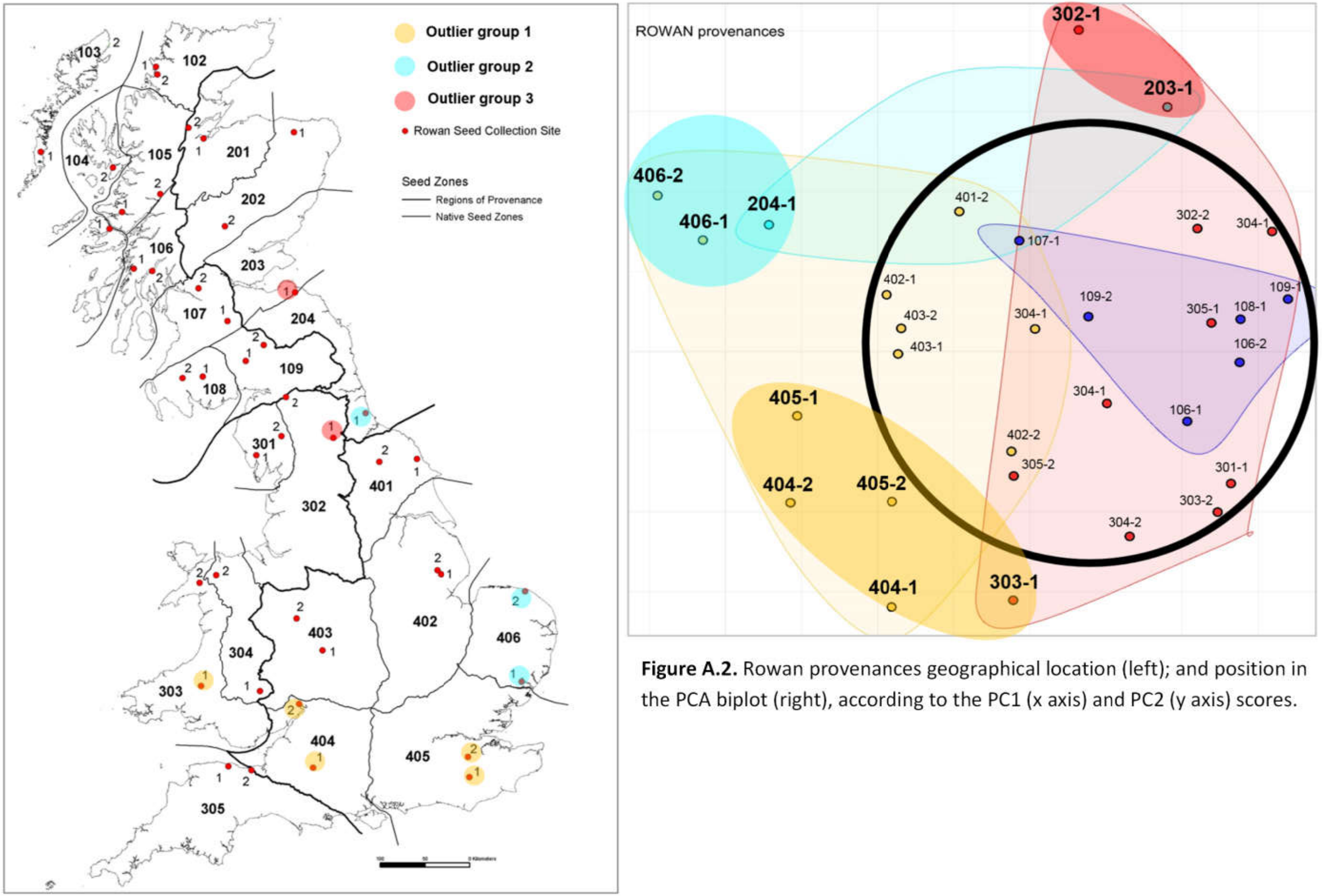

Figure A.2. Rowan provenances geographical location (left); and position in the PCA biplot (right), according to the PC1 (x axis) and PC2 (y axis) scores.

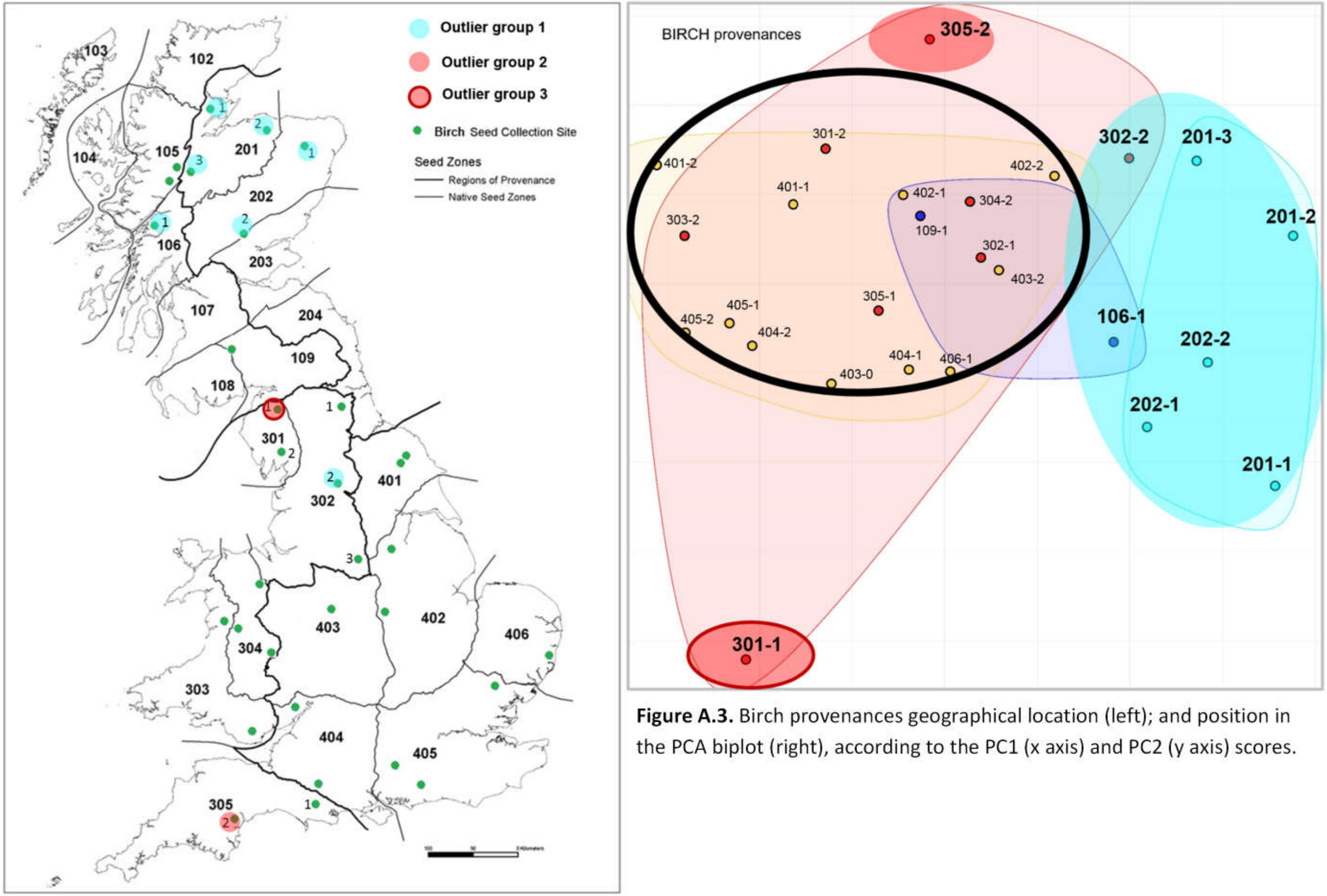

Figure A.3. Birch provenances geographical location (left); and position in the PCA biplot (right), according to the PC1 (x axis) and PC2 (y axis) scores.