Tree nursery environments and their effect on early trait variation

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Abstract

Despite the necessity of using nurseries to raise young plants and trees prior to transplantation into the landscape, not enough is known about the impact of their associated climate on the growth and development of seedlings from initial germination through to transplantation and beyond. Furthermore, the effect of nursery environment on trait interactions and age-age correlations and their subsequent relation to traits in mature trees is of significant importance and interest to forestry practitioners but requires long-term experimental trials measured for multiple traits in multiple years. A multisite progeny-provenance trial of the economically and ecologically important species, Scots pine, was established in three environmentally distinct nurseries in Scotland and measured for traits relating to survival, growth, form and phenology while in the nurseries. Temperature variation and photoperiod were the only uncontrolled environmental variables during this period, and their effect on measured traits was found to be significant among

nurseries from the first growing season onwards. Furthermore, interactions among traits were not always consistent among the different nurseries indicating that the use of proxy traits to enable selection of desirable traits may be more or less successful depending on the environment the trees are measured in. This study represents the first in a series examining trait variation in Scots pine from seedlings to mature trees, and highlights the importance of carefully considering the nursery environment when growing trees for transplantation.

Introduction

Globally, forests function as the major terrestrial carbon sink (Pan *et al.* 2011) and there is broad consensus that increasing the area of forest cover is vital to achieving net zero targets (Westaway *et al.* 2023). If it happens, a greater level of forest cover would also help tackle parallel environmental challenges like biodiversity loss, soil stabilisation and watershed management (Holl and Brancalion 2020). The degree of scaling up proposed is large, and has been exemplified in recent years by a proliferation of tree-planting initiatives such as the Trillion Trees Campaign: trilliontreecampaign.org, the Eden reforestation projects: edenprojects.org, and the Bonn Challenge www.bonnchallenge.org. This is creating an increased need for planting stock, which is most commonly supplied as nursery-grown saplings (Brancalion and Holl 2020) and places the forest nursery in a pivotal position, even as they continue to be required to supply the vast numbers of plants required for traditional forestry, amenity and conservation planting. In Great Britain alone, it is estimated that forest nurseries produced around 173M saplings for the year 2022-2023 (Forestry Commission 2023). Therefore, now and in the future, nurseries will

play a major role in the supply chain for forestry and tree planting of all kinds and will be key to the success of global tree planting efforts.

In their role as the primary location for raising young trees, choices made by nurseries have been shown to profoundly influence size and development of young trees, effects that may be observable long after transplantation. For example, the choice of fertiliser (Gruffman *et al.* 2012), growing medium (Heiskanen and Rikala 2000), container type, root management, and watering regime (Villar-Salvador *et al.* 1999) during early life stages can affect root growth, seedling morphology, and susceptibility to pests and pathogens (Selander and Immonen 1992). Even the timing of transplantation from the nursery to the field can impact performance (Luoranen 2018). Whilst soil, water and nutrients can be controlled quite precisely by nursery managers, the geographic location of a nursery and any protection measures used, such as glasshouse cover, determines other critical components of its environment such as climate and photoperiod . As variables like temperature and photoperiod can strongly affect the growth, development and biochemistry of seedlings (Downs and Borthwick 1956; Vapaavuori, Rikala and Ryyppö 1992; Nerg *et al.* 1994; Domisch, Finér and Lehto 2001) with effects lasting well beyond transplantation(Dhar *et al.* 2015), the nursery environment can be hugely influential.

The use of locally sourced seed is an explicit requirement when planting native species in the UK (Herbert, Samuel and Patterson 1999), but the location of the nursery in which those plants are raised is rarely specified. For example, the contemporary guiding document for UK forestry – The UK Forestry Standard (Forest Research 2023) - does not comment on nursery management, nor connect nursery practice to its recommendations on seed sourcing. Previous publications, such as

the Forestry Nursery Practice (Aldhous and Mason 1994), do consider site selection factors, but largely from the point of view of maximising plant performance, minimising risk and logistical considerations. In practice, choice of nursery is generally governed by what the market offers and by economics, consequently the nursery site may be environmentally distant from both the site of origin and planting site. If the effect of the nursery environment on plant phenotype is large, then the effect of local seed sourcing may be minimal in comparison. A quantitative evaluation of the impact of the nursery environment on trait variance is therefore potentially very important to seed sourcing policy, nursery practice and to the long-term success of planting initiatives.

The impact of the early growing environment is also a critical factor to consider in tree improvement and breeding programmes, particularly with regards to its effect on survival and on variation in desirable traits. Traditional breeding practice, which makes selections at around half rotation length (Zobel and Talbert 1984), is highly inefficient for trees with long rotation times and there is much interest in methods for screening trait variation at the nursery stage (Wu *et al.* 1997). However, trait values prior to transplantation (ca. two years) rarely predict values in later years (Burdon, Bannister and Low 1992; Hong, Fries and Wu 2015; Dong *et al.* 2018) (apart from species with very short rotation lengths, e.g. *Eucalyptus grandis* in Colombia, (Osorio, White and Huber 2003)). The use of ‘proxy’ traits to enable early selection relies on the stability of trait-trait relationships among environments, which may (Fukatsu *et al.* 2011) or may not be (Marron, Dillen and Ceulemans 2007) identifiable. Significant variation in age-age and trait-trait correlations among environments (Williams 1988; Osorio, White and Huber 2003) means that nursery effects may confound selection based on early traits. Similarly, the issue of

genotype-environment interactions may become increasingly important as larger numbers of trees are planted and on more challenging sites.

Pinus sylvestris L. (Scots pine) is a globally important tree species with a natural range that extends from southern Spain to northern Finland, and from western Scotland to far-eastern Russia. A foundation species with high conservation value, it has been shown to exhibit high adaptive trait variation across the landscape it inhabits at both regional and international scales (Salmela *et al.* 2011; Perry *et al.* 2016; Donnelly *et al.* 2018; Benavides *et al.* 2021; Ramírez-Valiente *et al.* 2021). *Pinus sylvestris* is also an economically valuable timber production species and is widely planted both within and beyond its natural range: it is estimated that it occupies more than 20 percent of the productive forest area in the EU (Mason and Alía 2000) and, within Great Britain, it is the second most abundantly grown species in tree nurseries after Sitka spruce (Forestry Commission 2023). *Pinus sylvestris* grows (both naturally and planted) across a vast and heterogeneous landscape. It may therefore need to survive and thrive in environments with huge temperature variance, or in others with relatively stable temperature profiles across seasons and years.

Common garden trials are widely used in forestry to disentangle the genetic component of phenotypic variation from the effects of environmental variation. Extending this approach, but using the common garden design to control the genetic composition of test populations, it is possible to use multiple common gardens in different environments to examine the environmental component of phenotypic variation. Nurseries, in their efforts to minimise environmental variation within a single site and to standardise conditions for each plant, are effectively functional

common gardens. Here we compare multiple common garden nurseries, each encapsulating the same genetic variation, to identify the contribution of the nursery environment to phenotypic variation and estimate genotype by environment effects. By doing so, we evaluate the importance of the nursery environment to longer term development of the plant, and establish a baseline for interpreting field trial results in the years to come. In addition, these results will be important in enabling age-age correlations to be explored as trees mature in field trials.

For this study, we compared the performance of *P. sylvestris* seedlings in three nursery environments. The plants had a common genetic background, collected systematically from across the natural range in Scotland, and were grown under standard soil, nutrient and watering regimes. We quantified the effect of the nursery environment on early growth and development and survival and examined relationships within and among traits.

Methods

Source of material

Seedling source and nursery environments are detailed in Beaton *et al.* (2022). Briefly, seed was collected from 10 mother trees from each of 21 populations of *P. sylvestris* from across the native range in Scotland in March 2007. Following stratification, seeds were germinated at the James Hutton Institute, Aberdeen (latitude 57.133214, longitude -2.158764) in June 2007 and transplanted into pots in an unheated glasshouse. The full final collection consisted of 210 families (10 families from each of 21 populations) each consisting of 24 half-sibling progeny (total 5,040 individuals).

Nursery environment

After transfer into individual pots (the same compost and pots were used for all plants) and with the intention of eventually using these plants to establish field based provenance trials, 8 seedlings per family were moved to one of three nurseries (total 1,680 seedlings per nursery) in July 2007: an unheated nursery glasshouse (NG) at the James Hutton Institute, Aberdeen; an outdoor nursery in the west of Scotland (NW) at Inverewe Gardens, (latitude 57.775714, longitude -5.597181); an outdoor nursery in the east of Scotland (NE) at the James Hutton Institute (location as above). In each nursery, trees were arranged in 40 randomised blocks, where each block contained two trees per family (total 42 trees). Watering was automatic in NG, and manually, as required, for NE and NW. The watering regime ensured that water was never limiting and waterlogging of seedlings was avoided. No artificial light was used in any of the nurseries. In May 2010, the pots containing the seedlings from NG were moved outdoors to Glensaugh in Aberdeenshire (latitude 56.893567, longitude -2.535736).

Hourly temperature was recorded at each of the nursery sites using data loggers from July 2007 until December 2010. Data loggers were suspended 50 cm above the ground under an aluminium foil-covered funnel. Daily minimum, maximum and average temperatures (between 0000-2300 each day) and the daily temperature variances were calculated for each nursery over this period. Daily variance was also partitioned into separate values for the day and night period, where day variance was estimated between the hours of sunrise and sunset between 0000-2300 and night variance was estimated between the hours of sunset and sunrise between

0000-2300. Sunset and sunrise for each day at each nursery were obtained from www.timeanddate.com.

On the few occasions on which more than one hourly record per 24 hours was missing, the daily temperatures were replaced with average daily temperature data derived from the nearest weather stations to the nurseries (obtained from the Met Office National Meteorological Archive for the period July 2007 to December 2010). Weather stations were: Poolewe (0.7 miles from NW); Craibstone (4.3 miles from NE and NG) and Glensaugh No 2 (0.1 miles from NG when moved outdoors to Glensaugh). Where weather station data were missing (only relevant for Poolewe over this period) they were replaced with measurements from the next closest weather station: Aultbea No 2 (4.2 miles from NW). Due to the differences in the location between the weather stations and the nurseries and the different methods of recording temperature, data from weather stations were adjusted prior to inclusion in the 'nursery temperatures' dataset by comparing daily temperatures across the whole period. Missing logger temperatures were directly replaced, where possible, by checking the weather station temperature at the missing time point and calculating the mean logger temperature recorded at all other instances that the same weather station temperature was recorded. In the few instances where there were no direct replacements, replacement logger values were obtained by taking the mean value for the three logger records either side of the missing logger data point in a dataset which was ordered by the weather station temperatures. For missing data in NG (prior to May 2010), replacement temperatures derived from NE were used instead of weather station data. Where hourly data were missing, temperature variance could not be recorded.

The growing season length in each year at each nursery was estimated as the period bounded by a daily mean temperature >5 °C for >5 consecutive days and daily mean temperature <5 °C for >5 consecutive days (after 1st July). During the defined growing season period trees were assumed to be ‘growing’ whereas outwith the defined growing season trees were assumed to be ‘not growing’. The previously described temperature variances were compared between these two different periods. The growing degrees were estimated for each day in the period July 2007 to December 2010 as the number of degrees above 5 °C. The cumulative growing degree days (GDD) were estimated by summing the growing degrees for each period leading up to phenology assessments at each nursery from 1 January in 2008. Chilling days were defined as the days in which the average maximum temperature did not exceed 5 °C.

Phenotype assessments

Seedling phenotypes were assessed for a range of traits reflecting different life history characteristics (Table 1): growth (annual increment in stem diameter and height; absolute stem diameter and height; relative growth rate); form (current year needle length, total number of buds; ratio of absolute height to absolute stem diameter); phenology (timing of budburst and growth cessation); and survival.

Growth traits were assessed annually from 2007-2010. Annual increments in height and base stem diameter were measured as the increase from the end of one growing season to the end of the next (for comparisons of height increment across multiple years, height in the first year of growth in 2007 is considered as the height increment for this year), whilst absolute growth was measured at the end of the

growing season in 2010. Relative growth rate was estimated as the percentage increase in height (increment) relative to the absolute height in the previous year.

Mortality was recorded each year from 2007 to 2010 and survival was recorded as the percentage of surviving trees by 2010. Relative growth rate was estimated as the percentage increase in height (increment) relative to the absolute height in the previous year and was recorded for 2008 to 2010.

Form was measured annually from 2007-2009. Needle length was measured for three needles per tree and a mean value was obtained. The total number of buds on each seedling was counted in 2008 and 2009. The ratios of absolute height to absolute stem diameter were recorded in 2010.

Phenology was assessed weekly during the spring and autumn of 2008. Budburst was defined as the number of days from 31 March 2008 to the time when newly emerged green needles were observed. The estimated proportion of trees which had burst bud in each nursery was calculated at each assessment. In order to compare budburst progression across the three sites, the point at which an estimated 50 % of trees had undergone budburst (BB_{50}) was calculated as follows:

$$BB_{50} = (50 - B_{i<50}) - \frac{B_{i>50} - B_{i<50}}{A_{i>50} - A_{i<50}} + A_{i<50}$$

where *i<50* is the *i*th assessment performed at each nursery where < 50 % of trees had undergone budburst (*i<50*: NE = 2; NG = 3; NW = 2); *i>50* is the *i*th assessment where > 50 % of trees had undergone budburst (*i>50*: NE = 3; NG = 4; NW = 3); *A* is the variable associated with each assessment performed at each nursery (*A*: number of days after 31 March; or cumulative growing degree days since 1 January); and *B*

is the percentage of trees observed with budburst at each nursery at each assessment.

Growth cessation was defined as the number of days from 10 September 2008 to the time when no further height growth was observed. In order to compare the growing season length at each nursery with the duration of growth observed in the seedlings, ‘growth duration’ was calculated for each seedling as the number of days between budburst and growth cessation.

Table 1. Traits measured in individual seedlings, grouped into ‘Growth’, ‘Form’, ‘Phenology’ and ‘Survival’. Units for each trait are indicated, along with the years in which data were collected.

Trait	Code	Unit	Years
Growth			
Stem diameter - absolute	DA	mm	2010
Stem diameter - increment	DI	mm	2009-2010
Height - absolute	HA	mm	2010
Height - increment	HI	mm	2007-2010
Relative growth rate	HR	%	2008-2010
Form			
Number of buds	Bu	Count	2008-2009
Height to stem diameter ratio	HD	Ratio	2010
Mean needle length	NL	mm	2007-2009
Phenology			
Budburst	BB	Days	2008
Growth cessation	GC	Days	2008
Growth duration	GD	Days	2008
Survival			
Survival	Su	%	2010
Mortality	M	0/1	2007-2010

Statistical analysis

Paired t tests were performed using R (R Core Team 2024) to compare nursery means for annual climatic variables (mean daily temperature, daily variance, growing degree days) and climatic variables measured within and outside the growing season (day temperature variance and night temperature variance).

Traits and climatic variables were normalised using the minimum and maximum value recorded for that trait/climatic variable for each nursery as follows:

$$x_{normalised} = \frac{x - \min(x)}{\max(x) - \min(x)}$$

Coefficient of variation, calculated as standard deviation divided by the mean, was estimated for each trait in each nursery. Where traits were measured in multiple years only the most recent year was used.

To evaluate relationships among traits, Pearson's correlation coefficients and significance values were estimated for trees growing in each nursery using the 'Hmisc' package (Harrell Jr 2024) in R (R Core Team 2024).

Nested analyses of variance (ANOVA) were performed in Minitab version 21 (Minitab 2024) for all trees with nursery as a fixed effect and block nested within nursery as a random effect. Nested ANOVA was also used to analyse the distribution of variance among these traits. Tukey's post-hoc tests were performed to determine whether mean values among nurseries were significantly different from one another for all traits and for mean temperature, temperature variance (daily, day and night) and growing degree days for each year. ANOVAs were repeated with all terms included as random effects to identify their contribution to the total variance.

Narrow sense heritability (h^2), which is the proportion of total phenotypic variance (V_P) explained by additive genetic effects (V_A) (Falconer and Mackay 1996), was estimated using among family, block and residual variance (V_{fam}, V_{block} and V_{res} respectively) from data pooled across populations and analysed separately for each nursery:

$$h^2 = \frac{V_A}{V_P} = \frac{RV_{fam}}{V_{fam} + V_{block} + V_{res}}$$

where R is the relatedness of individuals within families (assumed to be half-siblings, R = 4). Standard errors for heritability estimates were calculated as follows (Visscher 1998):

$$SE_{h^2} = R\sqrt{\frac{2(1-\frac{h^2}{R})^2\left[1+(s-1)\frac{h^2}{R}\right]^2}{s(s-1)(f-1)}}$$

where *R* is the relatedness of trees within families, *s* is the mean number of offspring per family and *f* is the mean number of families.

Results

Climatic variation among nurseries

Trees growing at each of the three nurseries experienced broadly similar mean daily temperatures for each year between 2007 and 2010 which were not significantly different (Figure 1) with the exception of NE and NG in both 2008 and 2009, where mean daily temperature at NG was significantly warmer than at NE in both years and for NG and NW in 2009. Trees at NG also experienced the fewest chilling days (total 119 between 2007 and 2010, compared with NE = 157 and NW = 123) and had higher daily GDD throughout the majority of the year (Figure 1) compared to the

other two nurseries. In contrast, the daily temperature variance that trees were exposed to was consistently and significantly lower for trees growing at NW than for trees growing at either NG or NE (Figure 1). The daily temperature variance that trees experienced in NG and NE was also significantly different, although the magnitude of the difference was less than when comparing NW with either NE or NG (Figure 1).

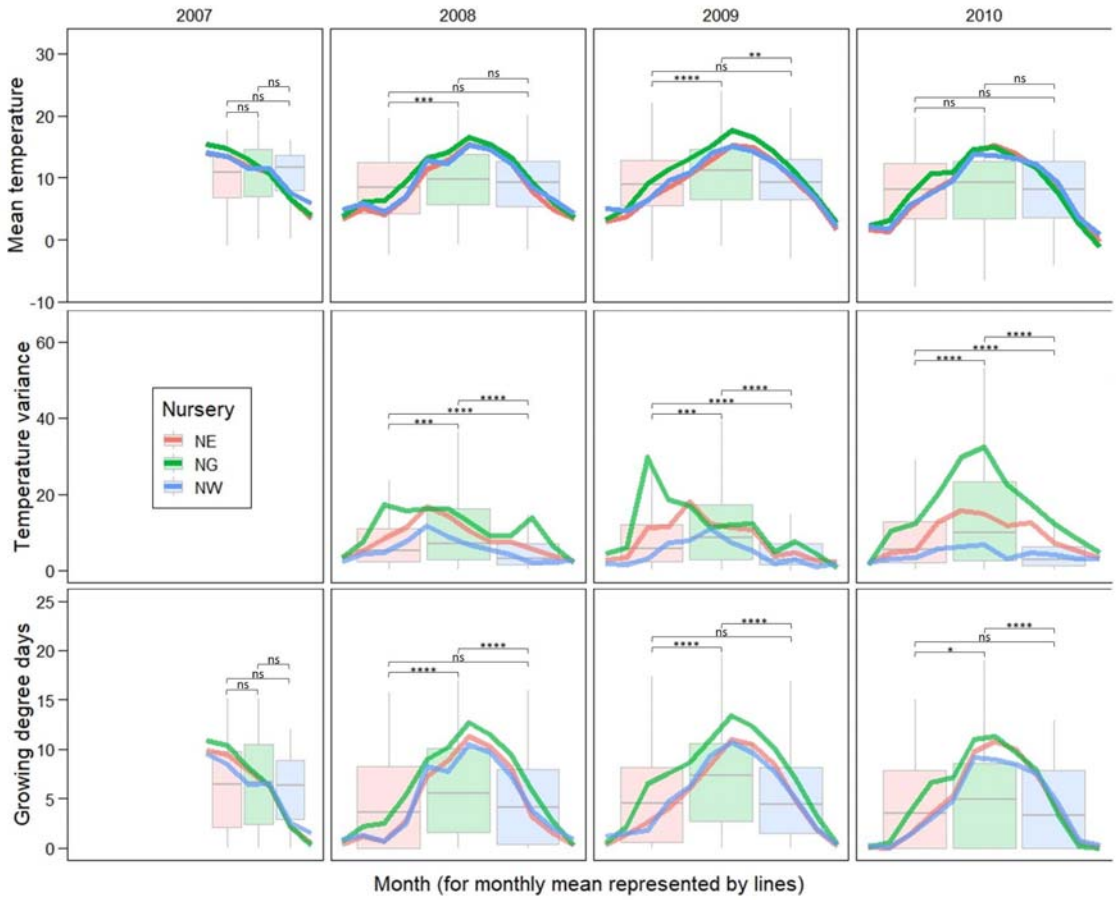

Figure 1. Climatic variation at the three nurseries for the period July 2007 to December 2010 when P. sylvestris seedlings were growing in pots at the sites. Lines indicate smoothed monthly means for each year. Boxplots indicate values for the entire year: solid grey lines indicate the median value; the bottom and top of the boxes indicate the first and third quartile; and the upper and lower whiskers extend to

the highest and lowest values within 1.5 times for interquartile range. No data are included for daily temperature variance in 2007 due to the extent of missing hourly data for this year. Significant differences among nurseries within each year are indicated with asterisks (: p = 0.01-0.05; **: p = 0.001-0.01; ***: p = 0.0001-0.001; ****: p < 0.0001; ns: not significant. Units: mean temperature, °C; daily temperature variance, °C^2, growing degree days, number of degrees above 5 °C for each day.*

A climatic profile was generated for each of the nurseries based on differences in normalised values for mean daily temperature, temperature variance during the day and night, GDD and chilling days (Figure 2a), highlighting differences among the three nurseries summarised in the text above.

Interestingly, the differences in temperature variance were most pronounced among nurseries and consistent among years during the daytime within the growing season period (Figure S1). During this period, daytime variance was significantly higher at NG than at NE or NW, and higher at NE than at NW in all years. In contrast, variance during the night-time was lowest at NG compared to the outdoor nurseries in all years outside the growing season (mean night temperature variance 2008-2010: NE = 2.40 °C^2; NG = 1.35 °C^2; NW = 2.17 °C^2).

Trait variation among nurseries

Trees growing in NG had higher mean values for traits relating to growth than those growing outdoors in NE and NW by 2010 (Figure 2b): they had thicker stems and grew taller and faster, on average. They also had larger increments for both height and stem diameter in 2010. Trees grown in NE were the smallest and thinnest with the slowest growth rate and lowest increments on average in 2010. In contrast, mean values for traits relating to form, and survival were lower, and phenology was

earlier, for trees growing in NG than those growing in one or both outdoor nurseries (Figure 2b) with the exception of needle length. Trees growing at each nursery had distinct profiles for mean trait values across the trait groups: trees growing at NE generally had higher values for traits relating to form and survival, trees growing at NG had higher values for traits relating to growth and trees growing at NW had moderate values for traits relating to growth and form but also had the highest values for phenology traits (indicating both late budburst initiation but also late growth cessation). Coefficient of variation (CV) for each trait at each nursery were generally the inverse of observed mean values (Figure 2c), for example: whereas trees growing at NG had the highest mean values for all traits relating to growth, they had the lowest CVs for growth traits, with the exception of relative growth rate. As a result, the profiles of each nursery for CVs was quite different to their profiles for mean trait values (Figures 2b and 2c): trees growing at NG showed greater variation than the other nurseries for traits relating to phenology and, to a lesser extent, form; trees growing at NW had moderate to high variation in growth traits compared to the other nurseries, as well as for two of the three traits relating for form; and trees growing at NE showed low overall levels of variation compared to trees growing at the other two nurseries, but had distinctively high values for specific traits within each group, namely needle length (form), growth cessation (phenology) and stem diameter increment (growth). Comparing climatic and trait profiles for each nursery allows simple relationships to be identified, for example: trees growing at NG and experiencing higher values for climatic variables during the growing season than trees at NE also have higher mean trait values and lower CVs for those traits, whereas trees growing at NE which experience higher values for climatic variables outside the growing season (more chilling days and higher nighttime temperature

variance) than NW have higher values for traits relating to form (with the exception of needle length). Interestingly, differences among nurseries in daytime temperature variance correspond inversely to differences in coefficient of variation (so, trees growing in nurseries with less temperature variance have higher coefficients of variation) for most growth traits.

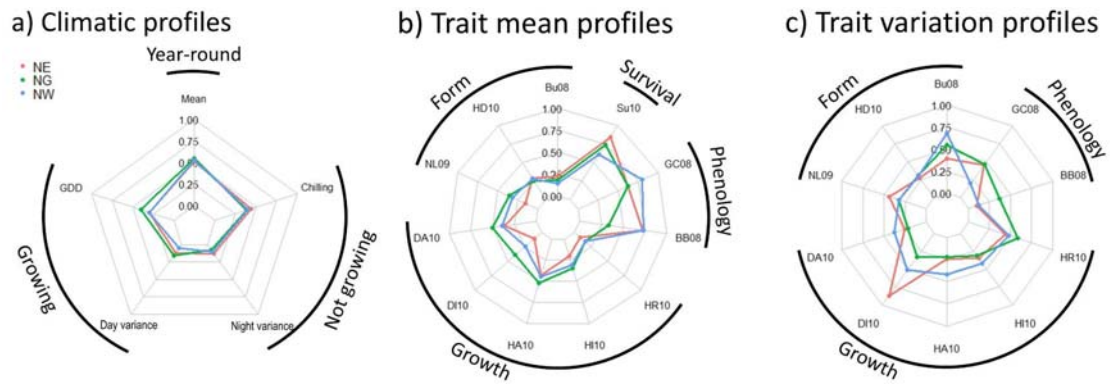

Figure 2. Differences among nurseries (NE, NG and NW) based on normalised variables, represented as profiles for a) climatic variables; b) mean trait values; and c) coefficient of variation for trait values. Traits relating to form, growth, phenology (b and c) and survival (b only) are indicated. For climatic profiles: 'Growing' refers to variables recorded within the growing season; 'Not growing' refers to variables recorded outside the growing season; 'Mean' is the mean average daily temperature; 'Chilling' is the mean number of days over the period 2007-2010 outside the growing seasons where the mean maximum temperature <5 °C; 'Night variance' is the mean variance in temperature during the night outside of the growing season; 'Day variance' is the mean variance in temperature during the day within the growing season; 'GDD' is the mean growing degree days within the growing season. For climatic profiles, data from 2007 to 2010 are used with the exception of temperature

variances when data from 2007 are not included. For traits measured in multiple years, only the most recent year is shown.

All trait means were significantly different among the three nurseries for each trait and each year with the exception of height increment in 2009 and relative growth rate in 2010, both among NG and NW, and growth cessation among NE and NG (Figure S2). It is notable that the length of needles was considerably longer in all nurseries in 2008 than in either 2007 or 2009 (Figure 3; Figure S3). Needles which grew in 2008 were, on average, 3.93 (NE) to 4.59 (NW) times longer than those which grew in 2007, and 2.11 (NW) to 3.21 (NE) times longer than needles which grew in 2009.

Overall survival was much lower for trees in NW compared to the other two nurseries (Figure S3) although this was due to a large spike in mortality in 2008 (Figure S4). Mortality was highest/joint-highest at NW in three of the four assessment years and was also extremely high in the other year. Mortality at the other outdoor site (NE) was the lowest of all nurseries across all four years. By 2010, there were significant differences among nurseries in seedling mortality. The highest levels of mortality in a single year were observed in 2008 at NW (25.03 % annual mortality) which accounted for over half of the seedling deaths across the period for this nursery. In contrast, mortality in 2010 was low for all nurseries.

The association between trait variation and mortality was examined by comparing the relative difference in trait means between trees that were alive and those that died in the year after a trait was measured (Figure S5). Trait values were nearly always lower (for traits relating to growth and form) or later (for traits relating to phenology) for trees that died in the following year. Therefore, trees that died in the

following year were more likely to be shorter, thinner, grow less rapidly, form fewer buds and shorter needles, and burst bud and stop growing later than trees which remained alive (Figure S5). Exceptions to this were growth cessation in 2008 for trees growing in NG, some growth traits (absolute and increment height and relative growth rate) for trees growing in NW in 2008 and needle length in 2009 for trees growing in NW and NE.

The relative difference in trait values between trees that were alive and those that died increased as trees got older (although differences over more than one year can only be compared for growth traits, as traits relating to phenology and form were not measured in multiple years, with the exception of needle length, Figure S5). However, the numbers of trees in the 'dead' group also reduced substantially over this period, from an average of 221 trees in 2007 to 64 trees in 2008 and 10 trees in 2009 compared to those in the 'alive' group (average numbers of trees counted for each trait: 1,313 in 2007; 1,198 in 2008; 1,191 in 2009).

Associations between nursery climate and trait variation

The timing of budburst in 2008 was significantly different among nurseries (Figure S2) with budburst occurring, on average, 23 days earlier at NG than at either NE or NW. The estimated number of days (since 31 March 2008) by which 50 % of the trees had burst bud was 38.7 and 38.8 days in NE and NW, respectively, but only 16 days in NG (Figure S6). In contrast, over the same period, the differences among nurseries in GDD was much lower (Figure S6, NE: 207.3; NG: 237.7; NW: 238.1). Trees at NE required fewer cumulative GDD, and those at NW and NG required more cumulative GDD, to complete budburst throughout the assessment period but only by around 30 degree days. Trees at NW were exposed to two chilling days

during the budburst period (5th and 6th April 2008) but the environments were otherwise similar among the two outdoor nurseries (NE and NW, Figure 1).

Despite significantly earlier initiation of budburst in NG, trees ceased growing at around the same time as those in NE (Figure 2 and Figure S2) which was on average about 13 days earlier than in NW. The duration of growth (period between budburst and growth cessation) therefore varied on average between 166 and 189 days (NE and NG, respectively) with trees in NW actively growing for an average of 178 days each year. Mean growth duration was significantly positively associated with growing season length at each site (Figure 5) although the amount of variation in this trait at each site was substantial.

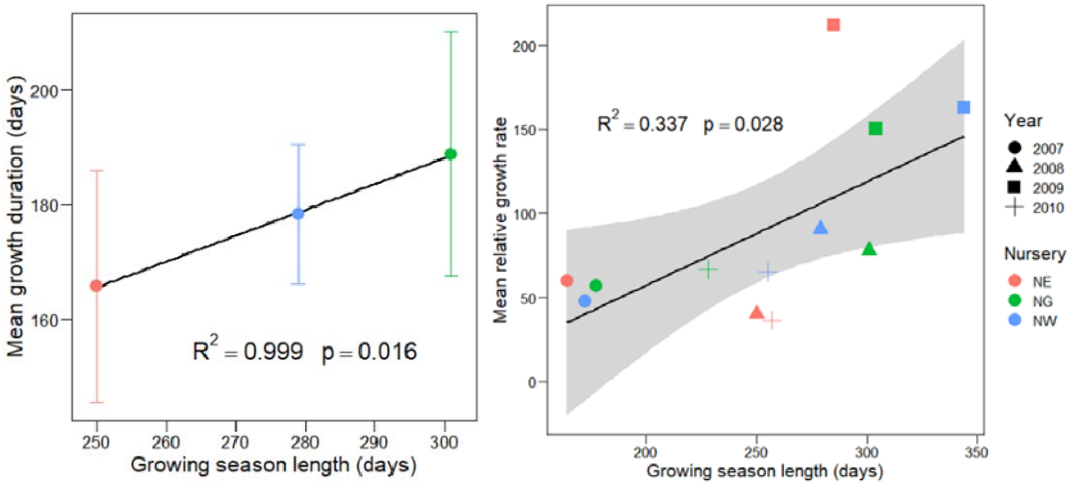

Figure 5. Linear regressions between growing season length (the period in days bounded by a daily mean temperature >5 °C for >5 consecutive days and daily mean temperature <5 °C for >5 consecutive days, after 1st July) and a) mean growth duration in 2008; and b) mean relative growth rate. Mean growth duration: number of days between budburst and growth cessation among trees at each site in 2008. Error bars are one standard deviation either side of the mean. Mean relative growth

rate: mm per year as a proportion of its size at the end of the previous growing season from 2007 to 2010.

There was a positive relationship between mean relative growth rate and the growing season length at each site in each year (between 2007 and 2010, Figure 5), with a possible threshold for growing season length (270 days) below which the growth rate appeared to be limited. In-season performance might also be strongly associated with the length of growing season in the previous year, due to provisioning of the bud during the growing season, but these comparisons will be more powerful using field rather than nursery data, due to the comparatively small number of datapoints available for the latter.

Relationships within and among traits

Age-age correlations were performed for measurements of a given trait obtained in pairs of years for all trees in each nursery site separately (Figure S7). The majority of age-age correlations were significant across each pair of years in each of the nurseries, with the exception of needle length (between years 2007 and 2008 in NG; between years 2008 and 2009 in NE), diameter increment (between years 2009 and 2010 in NG), and height increment (between years 2007 and 2009, and 2007 and 2010 both in NG). Traits were consistently either positively or negatively correlated among years, with the exception of height increment and needle length. Correlations among height increments in different years for individuals in NG were negative when 2010 is included (in contrast to the same years in other nurseries for this trait) – this is the year during which the pots were moved outdoors from the glasshouse. The patterns of pairwise comparisons among the sites were more similar for the outdoor sites (NE and NW) than NG. Correlations generally increased in significance as the

age of the trees increased and as the number of years between comparisons decreased (Figure S7).

The number of traits measured across multiple years that were consistently significantly associated (in the same direction) among years were reduced prior to subsequent analyses, either to a single (most recent) assessment year (for traits for which successive measurements were not independent: DA, HA) or to a mean value among years (HR, DI). Traits which were not consistent among years (HI, NL) were considered separately for each year.

Pairwise comparisons among growth, form and phenology traits both within and among years (Figure S8) show that pairs of traits were more highly significantly correlated in NW than in either NG or NE, particularly among growth traits and among budburst and other traits. Relationships were often inconsistent among sites: of the 105 pairwise comparisons among traits, 21 were not consistent (i.e. not always either positive or negative) among nurseries. Of the inconsistent correlations, nearly all (N = 17) involved needle length or height increment (Figure S8). Significantly associated growth traits were always positive, with the exception of height increment in 2007 in NE and NW and in all years at NG (Figures S7 and S8). Phenology traits were highly significantly positively correlated with one another and were generally negatively correlated with traits related to form (with the exception of height to diameter ratio and needle length in 2009). Considering only traits that were measured in the same year (i.e. 2008 for phenology traits), days to budburst were negatively correlated with height increment in NW, positively correlated in NE and not associated in NG. Days to budburst were highly significantly negatively correlated with all growth traits at NW, whereas correlations were either not

significant or positively significant for growth traits in NG and NE. In contrast, days to cessation of growth were highly positively correlated with most growth traits in NG (with the exception of height increment in 2007) but associations were much weaker and/or negatively correlated in the other nurseries. Thus, in general, a long growing season between budburst and growth cessation resulted in larger plants.

For further analyses, traits within groups which were consistently highly significantly positively correlated with one another were reduced to a single representative trait (height increment 2008 to 2010, relative growth rate and absolute and increment stem diameter were all represented by absolute height in 2010; needle length in 2007 and 2008 were represented by number of buds; growth cessation was represented by budburst)

Partitioning trait variance

There were highly significant differences among nurseries (i.e. environmental variation over a large scale) as well among blocks within nurseries (environmental variation over a small scale) for every trait (Table S1). Nursery accounted for a greater proportion of variation than block for needle length, number of buds, height increment and budburst (Figure S9), whereas the contribution of block to the total variance was greater than nursery for height to diameter ratio and absolute height.

Narrow sense heritability of growth traits was highest for trees growing in NG (Figure 6). For traits relating to form (specifically, bud counts and height to diameter ratio), heritability values were lower for trees growing in NG compared to those growing in NE and NG. Narrow sense heritability was highest overall for height increment at all three nurseries (Figure 6) but was very low for the other growth trait (absolute height) in all nurseries. Standard errors were extremely large, particularly in NW.

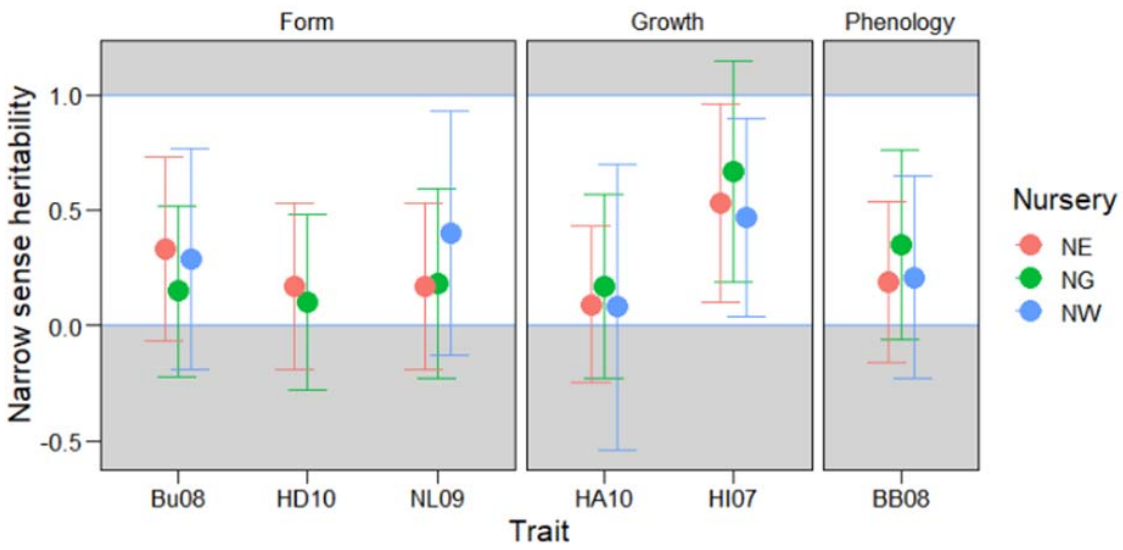

Figure 6. Estimated narrow sense heritability for each trait at each nursery (NG, NE, NW) with associated standard error indicated by error bars. The acceptable range of narrow sense heritability values is indicated by a white background (h^2 = 0 to 1) and the area outside of this range is indicated by a grey background ($h^2 > 1$; $h^2 < 0$). There is no value for narrow sense heritability for HD10 in NW due to zero contribution of family to the total variance.

Discussion

This study highlights the profound impact that relatively small differences in the temperature profiles of nurseries can have on trait variation in tree seedlings. The two outdoor nurseries, only ca. 200 km apart, were exposed to the same nutrient, pot and watering regimes and experienced similar average temperatures and monthly growing degree days. The major difference between the sites occurred during the daytime within the growing season, when daily temperature variance was much higher for trees growing in the nursery in the east of Scotland compared to the nursery in the west. Seedlings in the glasshouse nursery, in contrast, experienced

high daily temperature variance (which was also higher than in the neighbouring outdoor nursery), but temperatures were also higher overall. The effects of warmer mean temperatures (Morison and Morecroft 2006) and extremes of temperature (Niu *et al.* 2014) on plant adaptive traits are increasingly well recognised, especially in the light of an anticipated increase in extreme weather events due to climate change (Herring *et al.* 2022). However, the contrasting effects of temperature variance and mean temperatures and their comparative impact on the growth and development of plants and trees, is less well understood.

In our study, all traits were strongly and significantly affected by the nursery environment. The effect was particularly pronounced between the glasshouse and neighbouring outdoor nursery, where the main difference was mean temperature, compared to the two outdoor nurseries, where the main difference was daytime temperature variance during the growing season. The distinct climatic profiles generated for each nursery demonstrated the comparative importance of differences in temperature mean and variance on adaptive traits.

Growing the seedlings under high temperature variance (resulting in more days at the extreme ends of the temperature range) but with the same mean temperature led to lower rates of growth: a finding supported by Chiang, Bånkestad and Hoch (2020), who report significantly taller seedlings when grown in fixed or sinusoidal temperature regimes compared to the same species grown in pots outdoors with a naturally fluctuating and high temperature variance: but only within in spring. When grown in the summer, the same seedlings grown in a fixed environment were reported to be significantly shorter than those grown in the outdoor environment, possibly because the lower temperatures that seedlings were exposed to in the

outdoor environment in the spring did not occur during summer (Chiang, Bånkestad and Hoch 2020). The extent of the difference between day time and night time temperatures is also known to directly influence key plant traits relating to growth and form, such as internode length and height (Myster and Moe 1995). Similarly, the allocation of resources to traits relating to tree form in this study appeared to be more important in the nursery with both high variance and low mean temperatures, indicating that a more conservative growth strategy was potentially balanced by investment in traits relating to tree form (Banin *et al.* 2012), such as number of buds.

The role of mean temperature on plant growth is better understood, with higher mean temperatures generally positively associated with higher rates of growth (measured as specific leaf area (Poorter *et al.* 2010). Our study supported this finding, with seedlings grown in a nursery with warmer mean temperatures showing higher rates of growth. Although the temperatures of our nurseries were not controlled, and only a few nursery environments were studied, the environments we studied are analogous to those used in the forestry industry (i.e. outdoor nurseries and glasshouses). We only recorded air temperature, but soil temperature mean and variance may also have been important factors for the growth and development of the seedlings. Soil temperature is unlikely to have been consistent among the glasshouse and outdoor environments: it has been reported that soil temperature inside pots tracks but lags behind the air temperature until solar radiation falls directly onto pots, when the temperature of the soil within the pots can rise to more than 20 °C higher than the air temperature (Poorter *et al.* 2016) and that increased soil temperature is associated with increased growth rate (Weih and Karlsson 1999) in saplings.

Growing Scots pine seedlings in nursery environments with different temperature means, variances and photoperiods also had strong effects on phenological variation. Budburst timing was strongly associated with growing degree days at all sites, but was not fully explained by differences among sites. This suggests a complex relationship between warm temperatures (i.e. growing degree days) and cold temperatures (often referred to as the chilling requirement), although the number of nurseries/years assessed in this study is too low to enable clear conclusions to be reached. Plant species generally cease growing in response to decreasing photoperiod (Nitsch 1957; Singh *et al.* 2017) although some may respond to decreasing temperature instead (Heide and Prestrud 2005). In our study, trees growing in neighbouring nurseries, outside or within a glasshouse, experienced the same photoperiod but different mean temperatures. Whereas budburst occurred much earlier in the glasshouse than in the neighbouring outdoor nursery (reflecting the different cumulative growing degree days over the preceding period), growth cessation occurred on average at a similar time.

The accuracy of using young seedlings to predict economic traits at harvest is of major interest and importance in forestry (Lee 2002; Hong, Fries and Wu 2015), but detailed early assessments of tree seedlings are rarely available. This study and previous work indicate that we cannot make predictions based on trait correlations at a very early age, but that age-age correlations strengthen with age. Within these experimental trials, which will be transplanted to field sites, the same traits will continue to be measured into maturity enabling age-age correlations from seedling to mature trees to be estimated with high resolution (many traits will be measured annually). This study highlights the relative consistency of age-age correlations among the different nursery environments, with the exception of height increment in

NG in the years before and after trees were moved outside. The latter showed that plants experienced a shock as a result of the move outdoors which impacted their subsequent growth. The well-documented phenomenon of ‘transplantation shock’ is known to induce changes to growth and development (Close, Beadle and Brown 2005) whereby growth is impaired for a period following transfer. The accuracy of age-age correlations should also, therefore, be reviewed in the context of the general environment the trees are measured and the specific environment the trees have been exposed to in the recent past.

The interactions among different traits were also affected by the nursery that plants were grown in. For example, while timing of growth cessation was positively correlated with most growth traits in the protected glasshouse environment, these relationships were weaker in outdoor environments, possibly because these traits were not responding to a single climatic variable but to a combination of many. Similarly, traits relating to form were inconsistent in their relationship with growth traits among nurseries, with more positive associations observed in the nurseries with higher temperature variance compared to the one with low temperature variance. These findings will be investigated and tested further once trees are transplanted to their field locations, as measurements can be made over many more years.

The observation that narrow sense heritability values vary not only among traits but also among different environments has been recognised in many systems (reviewed by Hoffmann and Merilä (1999)). These findings have led to the proposal of various hypotheses that aim to explain why the favourability of the conditions in which organisms grow should lead to higher or lower heritability of quantitative traits. In our

study, it appears that growing seedlings in the warmer (and, presumably, more favourable) environment of the glasshouse resulted in higher narrow sense heritability values for growth and phenology traits compared to seedlings grown in cooler conditions, although the errors in all estimates were large. This could be due to the genetic potential being reached in the more favourable environment (Gebhardt-Henrich and Van Noordwijk 1991; Hoffmann and Merilä 1999). In contrast, narrow sense heritability for traits relating to form had higher values for seedlings grown in the cooler (i.e. putatively less favourable) conditions in the outdoor nurseries, possibly because traits relating to form are more important for long-term survival in unfavourable conditions than those relating to rapid growth (Banin *et al.* 2012).

Conclusions

Despite the relatively low levels of environmental variation among the three nurseries, there were significant effects on seedling mortality, variation in traits relating to growth, form and phenology, interactions among traits, and on narrow sense heritability. These findings have implications for the choice of nursery in which plants are raised given that effects may persist and affect performance in the field. Growers may wish to assess firstly whether raising plants in relatively benign nursery environments is necessarily appropriate given the shock they receive when moved to less benign conditions, and secondly whether the phenotypes produced by nursery conditions benefit or hamper the plants on transfer to the field. In any case, we have demonstrated the importance of measuring and collecting data at an early stage in order to compare trait variation throughout the lifetime of trees, from seedling through to maturity. The trees in this study have now been transplanted to three field

environments (Beaton *et al.* 2022) and subsequent analyses will explore ontogenetic effects on intraspecific genetic variation in a wide range of phenotypic traits.

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Conflict of interest statement

None declared

Data availability statement

The data underlying this article are available from the Environmental Information

Data Centre (EIDC, https://eidc.ac.uk), at https://doi.org/10.5285/29ced467-8e03-4132-83b9-dc2aa50537cd

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