

## **Seed Germination Variation among Crop Years from a *Pinus sylvestris* Clonal Seed Orchard**

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### **Author's contribution**

*The sole author designed, analyzed, interpreted and prepared the manuscript.*

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### **ABSTRACT**

Maternal effects were assessed by germinating seeds sourced over multiple years from the same cloned mother trees, comparing germination capacity and rate between crop years. The relationships between climatic variables, seed characteristics and germination capacity were determined, and thermal time parameters were used to predict seed dormancy release and germination under the climatic conditions in the year after seed collection. There were significant differences in seed weight ( $P < 0.05$ ), seed length and embryo occupancy (both  $P < 0.001$ ) among crop years. Temperature during the seed development period explained 70% of the variation in seed weight and 63% of the variation in embryo occupancy. Germination capacity was significantly ( $P < 0.001$ ) different among crop years, among temperatures and among chilling durations, and thermal time requirements for germination increased from older (2007) to younger (2012) seeds. The mean base temperature without chilling was  $7.1^{\circ}\text{C}$ , while after chilling it was  $4.6^{\circ}\text{C}$  and  $3.6^{\circ}\text{C}$  for four and eight weeks chilling respectively. The mean thermal time to 50% germination without chilling was  $135.1^{\circ}\text{Cd}$ , while after chilling it was  $118.3^{\circ}\text{Cd}$  and  $154.0^{\circ}\text{Cd}$  for four and eight weeks chilling respectively. This experiment demonstrates that year-to-year differences in the environment experienced by mother trees during seed maturation can affect seed germination characteristics.

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## 1. INTRODUCTION

Seeds are the main means by which trees and forests produce future generations; therefore, understanding their developmental and germination stages is vital to forest regeneration [1]. Seed traits, including seed size, level of dormancy and germination requirements often vary in parallel and are central components of life history strategies for many species [2]. The timing of germination has been shown to be under extremely strong and geographically-variable natural selection, but maternal effects can also impact seed characteristics [3]. Maternal effects are defined as the influence of the mother plant on the phenotype of her offspring via mechanisms other than the passing on of genetic information. In conifers, for example, the maternal environment is known to influence frost-hardiness of offspring [4]. The climatic conditions in which the mother plant grows may influence the size and morphology of seeds, which in turn may influence germination timing and success [5]. Environmental factors such as temperature during the seed maturation period have been found to influence seed germination of many species [6]. These factors may also influence the size of the seeds, which in turn may influence germination timing and success [5]. If conditions during seed maturation are altered, it is likely that germination behaviour

will also be altered [7]. However, there are only a few examples of significant maternal effects on germination characteristics [8]. It is therefore important to understand the cycle of seed production in *Pinus sylvestris* and to time management activities to take advantage of good crop years. The species has a three-year reproductive cycle typical of most species of *Pinus* [9]. These cycles include active periods during winter. There are three time periods that are particularly important in pine seed development: pollination, fertilisation and maturation. In *Pinus sylvestris* flowering starts in May to June of year 2 (Fig. 1), followed by pollen cone enlargement and shedding of pollen. *Pinus sylvestris* is wind pollinated; dry, sufficiently windy weather enhances both pollination and consequently the seed crop [10]. Pollination occurs over a short period in mid-late May of year 2, varying with season and altitude (it is delayed by 3-5 days for each 100 m rise in elevation) [10]. Fertilisation does not take place until the following spring (year 3) (Fig. 1), after which the seeds mature rapidly [9]. Seed cones are mature by late August of year three [11]. Seed dispersal is from December to March two years after pollination [12] (Fig. 1). Changes in temperature during winter in year 2/3 and early spring in year 3 may have important implications for the reproductive cycle [13]. Maturation

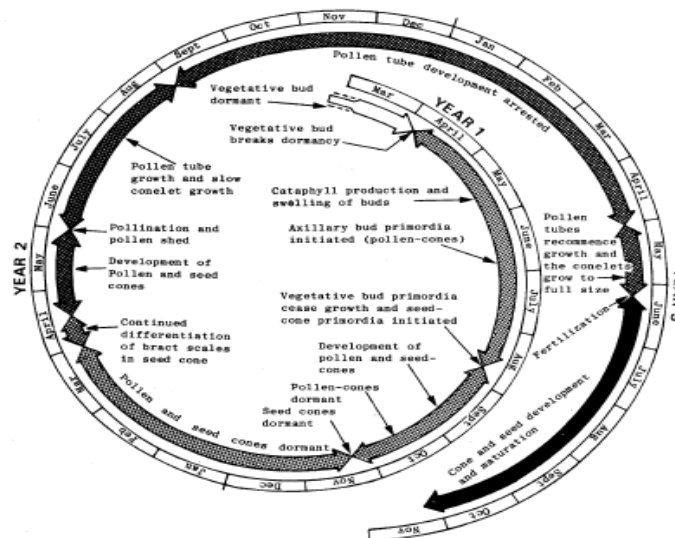


Fig. 1. Reproductive cycle of *Pinus sylvestris* [11]

conditions are known to influence the quality of conifer seed and can also influence the proportion of seeds that enter dormancy [8]. As a general rule, the lower the temperature during seed development, the higher the levels of dormancy [14] in seed dispersed from the parent plant after the seed maturation period. For *Pinus sylvestris* the best available planting material is produced from seed collected from seed orchards [12]. The size of seed crops from seed orchards varies from year to year, but there have been few investigations of the variation in germination characteristics between seed crops. Despite all the attention that climate change has attracted in the forestry sector, the effects of temperature changes on sensitive phases of the reproductive life cycle, including germination, have been virtually ignored [15]. This research provides the first estimates of maternal effects on germination characteristics of *Pinus sylvestris*.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The experiment was carried out from May to July 2014 at the Alice Holt Research Station of Forest Research, the research agency of the Forestry Commission of Great Britain.

### 2.2 Moisture Content

Seed moisture content was determined in accordance with ISTA rules [16]. Representative samples of 200 seeds per seed source were drawn after thoroughly mixing each seedlot to ensure homogeneity. Two replicates of 100 seeds of each seed source were placed in labelled and weighed moisture-proof tins (90 mm in diameter and 30 mm in height). Moisture content (MC, fresh weight basis) was calculated as a percentage to one decimal place as:  $MC = 100 \times (\text{fresh seed weight} - \text{dry seed weight}) / (\text{fresh seed weight})$ .

### 2.3 Triphenyl Tetrazolium Chloride Test

Triphenyl tetrazolium chloride tests was conducted following procedures established by the International Seed Testing Association [17]. Representative samples of 200 seeds per seed source were drawn after thoroughly mixing each seedlot to ensure homogeneity. Four replicates of 50 seeds per seed source were fully imbibed in deionised water for  $17 \pm 1$  hours at room temperature. Imbibing allowed the seeds to be cleanly sliced longitudinally on either side of the

embryo without damaging the embryo itself. The sliced seeds were placed in petri dishes and soaked in a 0.5% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) for  $17 \pm 1$  hours at  $30^\circ\text{C}$  in the dark [18]. Soaking in the dark prevents non-embryonic material or dead embryos from absorbing the dye and creating false positives; the reaction that occurs within the tissue is light sensitive [19]. The seeds were removed from the TTC solution and washed with deionised water. The washed seeds were separated from the seed coat, opened to expose the embryos, and viewed under a light microscope to assess the staining patterns. Embryos and megagametophytes of viable seeds were stained bright red in colour, while non-viable ones were colourless or partially-stained (Fig.2). Only embryos and megagametophytes that were completely red-stained were considered viable, while partially-stained or unstained (colourless) embryos and megagametophytes were classified as non-viable. The percentage of viable seed (VS) was calculated as:  $VS = 100 \times (\text{number of red-stained embryos} / \text{total tested seeds})$ .

### 2.4 X - ray Test

Representative samples of 100 seeds per seed source were drawn after thoroughly mixing each seedlot to ensure homogeneity. Four replicates of 25 seeds per seed source were X-rayed using a Faxitron MX-20 machine. The seeds were exposed to the X-ray for 10-15 seconds. Full embryos appeared dark, while empty/ diseased/ damaged embryos appeared light (Fig. 3). Seeds in which the embryo filled  $\leq 20\%$  of the seed volume were classified as empty, while those with embryos occupying  $>20\%$  were classified as filled.

### 2.5 Seed Characteristics

Seed weight, seed length, embryo occupancy and seed coat colour were measured. Two samples of 25 seeds were randomly selected from each seed crop and weighed to the nearest 0.01 mg using an electronic microbalance. The graphics software Imagej, a Java-based image processing program, developed at the National Institute of Health [20] was used to measure seed length. Seed length was measured over the seed coat along the longest axis of the seed. Embryo length was measured on the X-rays of seeds. Embryo occupancy was then calculated as the length of the embryo as a percentage of the seed length. Seed coat colour was assessed

visually by comparing two random samples of 25 seeds from each crop year (Fig. 4). Seeds were categorised as black or brown.

## 2.6 Germination Characteristics

Three chilling durations (zero, four and eight weeks) at  $4 \pm 1^\circ\text{C}$  and four pseudo-replicates per treatment were incubated at ten constant temperatures (7, 10, 13, 15, 17, 20, 25, 30, 33, and  $35^\circ\text{C}$ ) for 21 days. The experiment was laid out as a randomized complete block design, together with four replicates. For each combination of chilling treatment and incubation temperature, four replications of 50 seeds were used. For each replicate, seeds were sown on a sheet of moist filter paper ( $90 \times 133$  mm) placed in a clear, transparent, rectangular plastic germination box ( $170 \times 110 \times 30$  mm). The filter paper was continuously moistened using deionized water through an absorbent filter wick. The lids of the germination boxes were securely closed to maintain the relative humidity. Seed sources were randomly arranged within the germination chambers and re-randomized after each germination count until the germination test ended. Germination was considered successful when the radicle protruded to three times the length of the seed (5 mm). Germination tests were carried out in germination chambers.

## 2.7 Data Analysis

Mean values of seed characteristics were calculated for each of the five crop years. Analyses of variance were used to determine the significance of differences in seed characteristics

among crop years. Seed germination data were used to calculate germination capacity and germination rate. Germination capacity (GC) was calculated as:  $GC = 100 \times S/T$ . Where S is the cumulative number of germinated seeds at the end of the experiment and T is the total number of sown seeds.

Statistical analyses were conducted at two levels. Firstly, differences in germination capacity and germination rate among seed sources and treatments (chilling temperatures and chilling durations) as factors were tested with a two-way ANOVA. None of the germination data from any of the experiments required transformation. Where differences among seed sources and / or treatments were significant ( $P < 0.05$ ) a multiple comparison *post hoc* test was performed (Tukey test) to determine the significance of pairwise differences between means. Data analyses were conducted using Genstat (14<sup>th</sup> Edition, VSN International Ltd) and SPSS (20<sup>th</sup> Edition).

## 2.8 Thermal Time Model and Parameters

Thermal time parameters were estimated using methods described by [21] and a Genstat programme [22]. For each seed source, cumulative germination data for each day of germination tests at sub-optimal and optimal temperatures ( $\leq 20^\circ\text{C}$ ) were used to estimate base temperature ( $T_b$ ) and thermal time to 50% germination ( $\theta_{50}$ ) using the GLM procedures of Genstat. The maximum number of germinated seeds recorded in each treatment combination



Scale: 1 mm

**Fig. 2. Results from tetrazolium testing for seed viability. Live tissue stained bright red and dead tissue colourless or partially-stained**

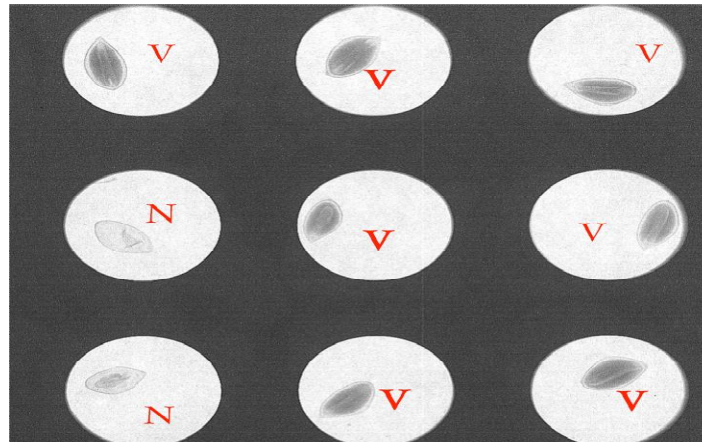


Fig. 3. Results from X-ray testing for seed quality. Seeds labelled V are filled, while seeds labelled N are empty

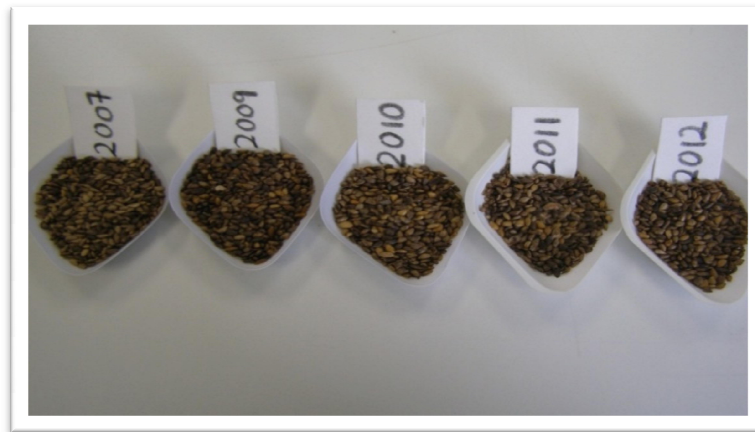


Fig. 4. Seed coat colour of five *Pinus sylvestris* seed crops from a clonal seed orchard (2007, 2009, 2010, 2011 and 2012). Picture taken June 2014

were used as the binomial totals for fitting the models [23]. Base temperature is the temperature below which there is no germination and is assumed to be constant for a particular seedlot. Thermal time to 50% germination has units of degree-days ( $^{\circ}\text{Cd}$ ) or degree-weeks ( $^{\circ}\text{Cw}$ ). According to [21] the values of base temperature and thermal time to 50% germination can be estimated iteratively using repeat probit regression, varying base temperature until the best fit is obtained. The distribution of thermal time requirements is given by the following:  $\text{probit}(g) = k + \frac{(T - T_b)t_g}{\sigma}$ .

Where,

probit (g) is probit units of germination; T is temperature;  $T_b$  is base temperature;  $t_g$  is time to

germination percentage g;  $(T - T_b)t_g$  is thermal time for a given percentage of germination g in degree-days;  $\sigma$  is the standard deviation of thermal time for germination, and k is a constant. Probit can be generalised and reparameterised<sup>22</sup> as:

$$\text{logit}(g) = \beta_1 + \beta_2(T \times t_g) - \beta_3 t_g;$$

Where,

probit is replaced by the logit function for ease of fitting

$\beta_1 = k$ ;  $\beta_2 = 1/\sigma$  and  $\beta_3 = \beta_2 T_b$ ; after fitting this model,  $T_b$  can then be directly estimated as:

$T_b = \frac{\beta_3}{\beta_2}$ ; while thermal time to 50 % germination is directly estimated as:  $\theta_{50} = \frac{\beta_1}{\beta_2}$ .

### 3. RESULTS

There were significant ( $P < 0.001$ ) differences among crop years in viability and percentage of filled seeds, but no significant differences among crop years in moisture content (Table 1). There were signs of a decrease in both viability and percentage of filled seed with increasing time since seed collection (Table 1). Results of analyses of variance showed that there were significant differences in seed weight ( $P < 0.01$ ), seed length and embryo occupancy (both  $P < 0.001$ ) among crop years (Table. 1). However, there were no statistically-significant differences ( $P = 0.878$ ) in seed coat colour among crop years (Table 1).

#### 3.1 Germination Capacity

Germination capacity was significantly ( $P < 0.001$ ) different among crop years, among chilling durations and among temperatures. There were significant ( $P < 0.001$ ) interactions between crop year and chilling, between crop year and temperature, and between chilling and temperature (Table 2). There was also a significant ( $P < 0.001$ ) three-way interaction between crop year, chilling and temperature (Table 2). Crop years are confounded with seedlot age; the effect of seedlot age was therefore tested by replacing crop year with seedlot age and re-running the analysis of variance. The main effect of seedlot age was not significant ( $P = 0.594$ ), however there were significant interactions between seedlot age and chilling and between seedlot age and temperature. Germination capacity varied among crop years, temperatures and chilling treatments (Fig. 2). Germination capacity increased with

chilling duration. Without chilling, germination capacity ranged from 0% to 79% and values increased with increasing temperature; no seeds germinated at lowest temperature (7°C) used. After four weeks chilling germination capacity ranged from 0% to 95% and after eight weeks chilling germination capacity was between 5.5% and 96%. Chilling treatment not only increased germination capacity but also widened the range of temperatures at which germination occurred (Fig. 2). Germination capacity increased with increasing temperature up to an optimum of 20°C. Above 20°C germination capacity decreased as temperature increased (Fig. 2). Seeds from the 2010 crop year had the lowest germination capacity at the optimum temperature of 20°C in all chilling treatments, while seeds from the 2012 and 2011 crop year showed the highest germination capacity at 20°C after four and eight weeks respectively (Fig. 2).

#### 3.2 Base Temperature

Base temperature decreased with increasing chilling duration in all crop years except 2007 which showed an increase from four weeks chilling to eight weeks chilling. Base temperature increased with increasing time since crop harvest; for example, the 2007 crop year had a higher base temperature than the 2009, 2010, 2011 and 2012 crop years. After all chilling treatments the 2007 crop year had the highest base temperature and the 2012 crop year had the lowest (Fig. 6). Over the crop years used in this experiment, the average base temperature without chilling was 7.1°C, while after four and eight weeks chilling it averaged 4.6°C and 3.6°C respectively.

**Table 1. Seed quality (moisture content (%), viability (%) (TTC) and percentage of filled seeds (X-ray)), characteristics (weight, mean length and embryo (Occupancy) of five seed crops of *Pinus sylvestris* from a clonal seed orchard**

Crop year	Moisture content (%)	Viability (%) TTC test	Filled seed (%)X-ray	Seed weight (mg)	Mean seed (mm)	Embryo occupancy (%)	Black seed coat colour (%)
2007	7.3	90	90	7.5	4.2	66	53
2009	7.3	91	93	7.5	4.3	69	52
2010	7.4	93	93	7.2	4.3	65	53
2011	7.2	93	94	7.7	4.8	68	50
2012	7.3	95	95	8.3	4.8	73	49
P-value	NS	***	***	***	***	***	NS

NS: not significant; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

### 3.3 Thermal Time to 50% Germination

Thermal time to 50% germination increased with increasing time since harvest; after all chilling durations the 2007 crop year had the lowest thermal time to 50% germination, while the 2012 crop year had the highest (Fig. 7). Crop years with higher base temperatures had lower thermal times to 50% germination (Figs. 3 and 4). Over all the crop years in this experiment, the mean thermal time to 50 % germination without chilling was 135.1°Cd, while after four and eight weeks it averaged 118.3°Cd and 154.0°Cd respectively.

### 3.4 Relationships between Weather Conditions, Seed Characteristics and Germination Capacity

Weather data showed that the 2012 crop year had the highest mean monthly temperature (14.8°C) during the pollen development and pollination period (May, June and July of the second year of the reproductive cycle) while the 2010 crop year had the lowest temperature (13.3°C) during the same period (Fig. 8 (a)). The 2012 crop year had the highest (104.2 mm) and 2010 had the lowest (27.3 mm) mean monthly precipitation during the pollen development and pollination period (Fig. 8 (b)). The results of correlation and linear regression analysis of seed characteristics against temperature and precipitation during the pollen development and pollination period are shown in Table 5. None of the correlations was significant, and temperature and precipitation explained very little of the variation (2.7% to 37.9%) in seed characteristics. The 2012 crop year had the highest mean monthly temperature (15.2°C) during the period of pollen tube growth and fertilization (May, June and July of the third year of the reproductive cycle) while the 2010 crop year had the lowest temperature (14.0°C)

during the same period (Fig. 8 (a)). The 2012 crop year had the highest mean monthly precipitation (109.5 mm) during the period of pollen tube growth and fertilization and 2009 had the lowest (51.5 mm) (Fig. 8 (b)). The results of correlation and linear regression analysis of seed characteristics against temperature and precipitation during the pollen tube growth and fertilisation period are shown in Table 3. There was a significant correlation between precipitation and seed length ( $r = 0.921$ ,  $P < 0.05$ ). Temperature explained 40.9% of the variation in seed weight and 38.0% of the variation in embryo ratio; precipitation explained much more of the variation (39.9% to 84.4%) in seed characteristics (Table 4).

The 2012 crop year had the highest mean monthly temperature (17.3°C) during the seed development and maturation period (June, July and August of the third year of the reproductive cycle) while the 2010 crop year had the lowest temperature (15.1°C) during the same period (Fig. 8 (a)). The 2012 and 2010 crop years also had the highest (123.8 mm) and lowest (52.1 mm) mean monthly rainfall during the seed development and maturation period (Fig. 8 (b)).

There were significant correlations between temperature and embryo ratio ( $r = 0.958$ ,  $P < 0.05$ ), between precipitation and seed weight ( $r = 0.882$ ,  $P < 0.05$ ) and between precipitation and seed length ( $r = 0.950$ ,  $P < 0.05$ ). Temperature explained 91.8% of the variation in embryo ratio; precipitation explained 77.8% of the variation in seed weight and 95.0% of the variation in seed length (Table 5). None of the correlations was significant. Seed characteristics explained 42.0% to 79.7% of the variation in germination capacity (Table 6).

**Table 2. Results of analysis of variance of germination capacity of five seed crops of *Pinus sylvestris* from a clonal seed orchard after three chilling durations and at ten temperatures**

Source of variation	d.f	F-value	P-value
Crop year	4	20.340	***
Chilling	2	1648.258	***
Temperature	9	1294.146	***
Crop year × chilling	8	10.763	***
Crop year × temperature	36	4.444	***
Chilling × temperature	18	53.317	***
Crop year × chilling × temperature	72	2.174	***
Error	450		
Total	599		

\*\*\*  $P < 0.001$

**Table 3. Relationships (correlation coefficient,  $r$ , and coefficient of determination,  $R^2$ ) between seed characteristics and mean monthly temperature and mean monthly precipitation during the pollen development and pollination period of *Pinus sylvestris***

		Temperature	Precipitation
Seed weight	$r$	0.165 <sup>NS</sup>	0.161 <sup>NS</sup>
	$R^2$	0.027	0.379
Seed length	$r$	-0.255 <sup>NS</sup>	0.076 <sup>NS</sup>
	$R^2$	0.065	0.006
Embryo ratio	$r$	0.241 <sup>NS</sup>	0.441 <sup>NS</sup>
	$R^2$	0.058	0.194

**Table 4. Relationships (correlation coefficient,  $r$ , and coefficient of determination,  $R^2$ ) between seed characteristics and mean monthly temperature and mean monthly precipitation during the pollen tube growth recommencement and fertilisation period of *Pinus sylvestris***

		Temperature	Precipitation
Seed weight	$r$	0.640 <sup>NS</sup>	0.831 <sup>NS</sup>
	$R^2$	0.409	0.691
Seed length	$r$	0.016 <sup>NS</sup>	0.921*
	$R^2$	0.002	0.848
Embryo ratio	$r$	0.616 <sup>NS</sup>	0.631 <sup>NS</sup>
	$R^2$	0.380	0.399

**Table 5. Relationships (correlation coefficient,  $r$ , and coefficient of determination,  $R^2$ ) between seed characteristics and mean monthly temperature and mean monthly precipitation during the seed development and maturation period of *Pinus sylvestris***

		Temperature	precipitation
Seed weight	$r$	0.870 <sup>NS</sup>	0.882*
	$R^2$	0.757	0.778
Seed length	$r$	0.459 <sup>NS</sup>	0.950*
	$R^2$	0.211	0.903
Embryo ratio	$r$	0.958*	0.726 <sup>NS</sup>
	$R^2$	0.918	0.528

**Table 6. Relationships (correlation coefficient,  $r$ , and coefficient of determination,  $R^2$ ) between seed characteristics and germination capacity of *Pinus sylvestris***

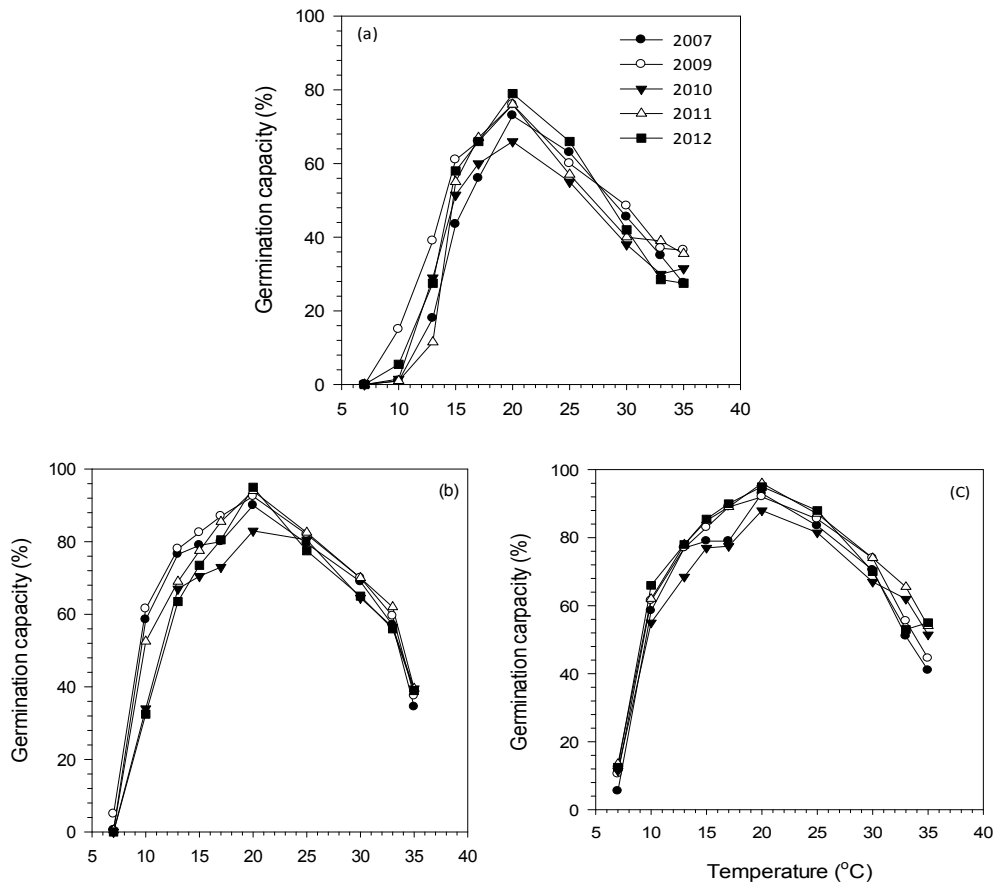
		Germination capacity
Seed weight	$r$	0.797 <sup>NS</sup>
	$R^2$	0.640
Seed length	$r$	0.651 <sup>NS</sup>
	$R^2$	0.420
Embryo ratio	$r$	0.796 <sup>NS</sup>
	$R^2$	0.630

#### 4. DISCUSSION

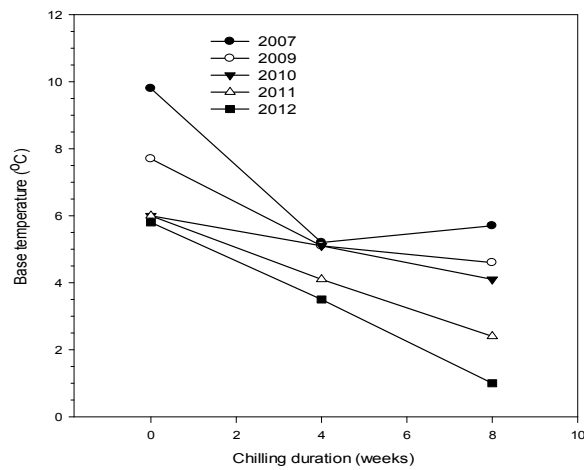
According to Gosling [24], dry conifer seed kept at 6-8% moisture content (fresh weight basis) can be stored with little deterioration for up to 10 years. For example, *Pinus ponderosa*, *Pinus elliotii* and *Pinus taeda* seeds maintained their germination capabilities after storage for six or seven years [25], although [26] reported a 32% reduction in germination of *Pinus echinata* seeds

stored for ten years. Biochemical and physiological changes during storage include oxidative damage, alterations in reserve substances, chromosomal dislocations and leakage of substances from seed [27]. However, germination information provided by the seed supplier (Forestry Commission) suggests that age had not affected germination capacity of the seedlots used in this experiment, as levels reached were comparable to the germination

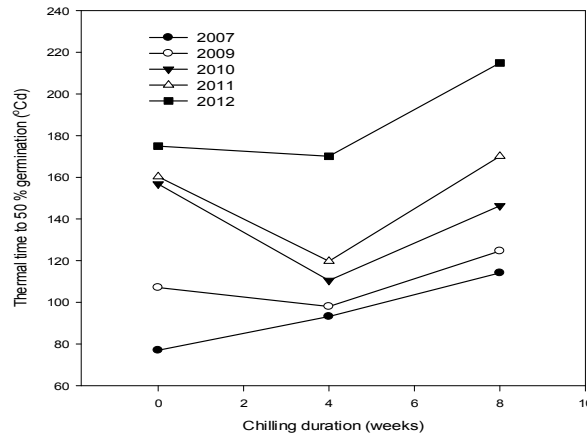




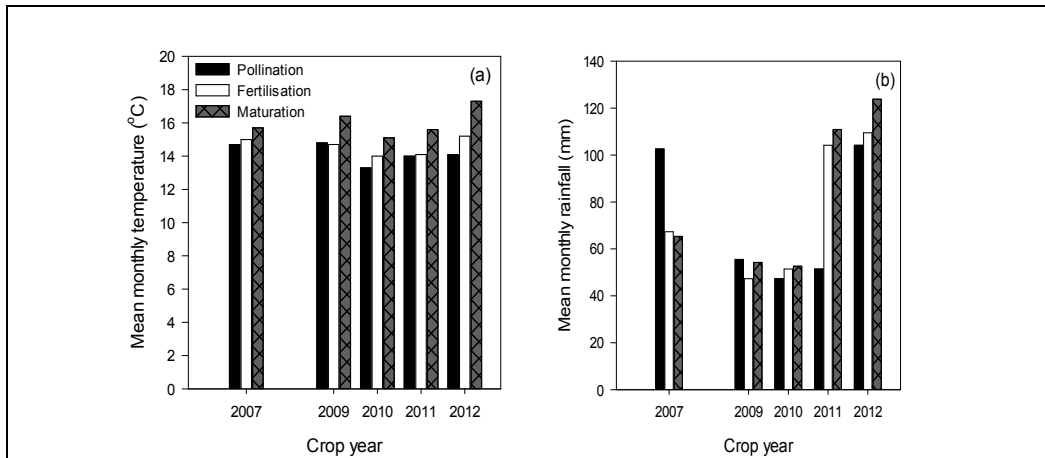
**Fig. 5. Germination capacity of five seed crops of *Pinus sylvestris* from a clonal seed orchard as a function of temperature**  
 ((a) zero weeks chilling; b) four weeks chilling and c) eight weeks chilling. Each curve corresponds to different crop year)



**Fig. 6. Base temperature (°C) for five seed crops of *Pinus sylvestris* from a clonal seed orchard after three chilling durations (zero, four, and eight weeks) based on germination at sub-optimum temperatures (7°C to 20°C)**



**Fig. 7. Thermal time to 50 % germination (degree-days) for five seed crops of *Pinus sylvestris* from a clonal seed orchard after three chilling durations (zero, four, and eight weeks) based on germination at sub-optimum temperatures (7°C to 20°C)**



**Fig. 8. Mean monthly (a) temperature (°C) and (b) precipitation (mm) at a seed orchard of *Pinus sylvestris* during periods of pollen development and pollination, pollen tube growth and fertilisation, cone and seed development and maturation in five seed crops. climatic research unit of the University of East Anglia (2014)**

values given on the certificates provided with the seeds. Genetic traits and environmental factors are the major determinants of seed size and shape [28]. The size and quality of *Pinus sylvestris* seed vary greatly between years, stands and individual trees [10]. Since the seeds were collected from same location, from trees of approximately the same age and the same clonal mixture, observed differences in seed variables may be attributed to maternal differences arising from the diverse climatic conditions prevailing during seed development. *Pinus sylvestris* seed development has been reported to be delayed by

decreasing mean temperatures during seed development [29].

It is generally accepted that heavier seeds germinate better than lighter seeds [30]. The results of this experiment are consistent with this general trend, suggesting that germination characteristics depend partly on the resources allocated to the seed by the mother plant. The differences in seed weight between the five crop years may explain their different behaviours during the germination test, and their differing sensitivity to low and high temperatures. Seeds

from crop years 2011 and 2012 had the highest weights and were least sensitive to high temperatures. Larger seeds have higher levels of starch and other foods, and this may be one factor which influences the germination of seed and the growth of seedlings [31]. [32] concurs that seed weight indicates the presence of food reserves in the megagametophyte which can support higher levels of germination. However, [33] observed no relationship between seed weight and germination capacity and rate in *Pseudotsuga menziesii* seeds from 19 seed orchard trees. The inclusion of seed weight in the delineation and understanding of geographical variation has been advocated because of the low plasticity of this character. However, according to Udval and Batkhuu, [34] the weight of seed is closely related to local climatic and site conditions. According to Leikola et al. [35] variation between years is mainly an effect of temperature conditions during the reproductive cycle. [36] suggest that variation in seed quality occurs as a result of climatic conditions and is often reflected in early seedling variation. It is possible that variation in morphological characters among crop years is due to variation in resource availability during development. Data on seed weight and embryo occupancy of seeds from the 2010 and 2012 crop years suggest that the low germination capacity of the 2010 crop year and high germination capacity of the 2012 crop year are due primarily to maternal effects resulting from weather conditions during the seed development and maturation period, rather than genetic factors. Mean monthly temperature and precipitation during this period were both low in 2010 and high in 2012.

Weather-driven maternal effects are common in plant species inhabiting harsh environments [37] and can result in increased seed dormancy. Pine trees growing in infertile and dry habitats have lighter-coloured seeds, while those from fertile and wet habitats have darker seeds [34]. Seeds attain their final colour at physiological maturity and colour is therefore related to seed dormancy and germination [38]. Seed colour affects various aspects of germination, such as water uptake by the seed, and is sometimes related to seed weight [39]. However, results of the experiment described here suggest that seed coat colour did not differ significantly among crop years and that seed colour is not a dependable determinant of seed dormancy, germination behaviour or seed weight in *Pinus sylvestris*. It is possible that the conditions for seed maturation and development were rather favourable in the seed orchard from

which seeds were collected and that differences in seed coat colour did not develop in the same way as in many earlier studies on seed coat colour. The seedlots were processed before storage to eliminate dubious seeds and this processing might have selectively removed seeds of a particular colour. The effect of crop year is confounded with seed age, and increasing seed age is associated with lower germination vigour; with increasing age germination capacity, germination rate and absolute seed weight decrease [40]. This pattern is partly reflected in the results of this experiment as older (2007) seeds had lower germination capacity and rate than younger (2012) seeds; however, the seed certificates suggest that seeds from crop years 2007 and 2010 had a relatively low germination capacity of 63-64% when tested soon after they were collected. This suggests that climatic conditions during seed development and maturation had an effect on germination.

Studies by [41] showed that *Picea glauca* seeds from mother trees grown in colder conditions germinate earlier and reach higher germination percentages than seeds from trees grown in warmer conditions. These results contrast with the findings reported in this experiment, in which seeds from mother trees maturing in colder seasons germinated later and had lower germination capacity (Table 1 and Fig. 3). This discrepancy may arise because the environmental factors that critically limit germination differed between the two experiments. Results of this experiment do agree with several others in boreal conifers that have reported that maternal effects are mechanisms for adaptation [42] and suggest that the maternal influence on seed differs between years. In the experiment described here, differences in maternal effects between crop years were evident in germination capacity and rate, which were higher in crop year 2012, when conditions were warm during seed maturation. [43] found that meteorological conditions accounted for 74 % of the inter-annual variability in viability and germination capacity of viable seeds of *Pinus banksiana*. Other studies have identified maternal effects as important in explaining the variation in both germination capacity and dormancy in plant species other than trees [44]. For example, [44] found that in resource-limiting environments, seeds of semi-arid Mediterranean plant species have higher levels of seed dormancy. The proportion of unchilled seeds that failed to germinate differed between crop years,

suggesting different degrees of dormancy. These differences were reflected by the variation in thermal time parameters (base temperature and thermal time). This kind of variation among crop years is normally a result of maternal genotype and maternal environment during the time of seed development and maturation [8], and allows seeds to respond to their future environment long before germination. According to Houle and Filion [43] conifer trees may respond faster to change in temperature than expected. Variation in paternal genotype might have also played a role in offspring variation. The mother trees were open-pollinated, meaning the pollen cloud responsible for fertilisation could have come from a variety of clones in the seed orchard and is likely to have differed from year to year. Germination differences among seeds harvested in different years can also be expected because seed germination is affected by environmental conditions during processing at least until seed has dried [45].

The results were characterized by variation in both base temperature and thermal time to 50% germination among the five crop years, with more variation in the zero chilling treatment (Figs. 8 and 6). This variation within the same species may reflect different environmental conditions during seed development [46] resulting in different dormancy levels. There was a trade-off between base temperature and thermal time to 50% germination, with crop years having a higher base temperature also having lower thermal time to 50% germination. The results are in agreement with the findings by [47] who found that the higher the base temperature, the shorter the cumulative thermal time required to reach 50% germination. Species with high base temperature values often grow in locations with high annual temperatures, such as tropical regions [47], but in this experiment seeds developing under high temperatures had a lower base temperature (e.g. 2012 crop year) and those experiencing low temperatures during maturation had a higher base temperature. Crop year variation clearly influences the sensitivity of the seed germination response to temperature [46]. Significant correlations between weather conditions and seed characteristics were found in this experiment. Although correlations between seed characteristics and germination capacity were not significant, seed weight and embryo ratio explained 63-64% of variation in germination capacity (Table 6). This suggests that some characteristics of *Pinus sylvestris* seeds from seed orchard, to some extent, could

be estimated from climate change predictions of future temperature and precipitation.

## 5. CONCLUSION

Classification of seed dormancy offers a structured approach to collecting basic information on seed characteristics and can help identify likely factors required for dormancy alleviation. and warm seed maturation temperatures induce high and low levels of dormancy respectively in *Pinus sylvestris*. The results presented in this study suggest that a reduction in temperature during seed maturation lead to an increase in dormancy levels. Increases in dormancy levels delay germination, thus shifting the time-frame of regeneration due to differences in environmental conditions. In natural regeneration it is important that correct dormancy levels are induced, to ensure germination occurs at the right time. These findings suggest that there may be a simple but effective mechanism allowing seeds to predict their future success by utilizing information about the environment of their mother.

## COMPETING INTERESTS

Author has declared that no competing interests exist.

## REFERENCES

1. Tomášková J, Vítámvás JK. Testing of germination of spruce, pine and larch seed after 10 years from collection. Journal of Forest Science. 2014;60:540-543.
2. Evans AS, Cabin RJ. Can dormancy affect the evolution of post-germination traits? The case of *Lesquerella fendleri*. Ecology.1995;76:344-356.
3. Donohue K. Completing the cycle: Maternal effects as the missing link in plant life histories. Philosophical Transactions Royal Society of the Biological Sciences. 2009;364:1059-1074.
4. Johnsen O, Skroppa T, Junttila O, Daehlen OG. Influence of the female flowering environment on autumn frost-hardiness of *Picea abies* progenies. Theoretical and Applied Genetics.1996;92:797-802.
5. Castro J. Short delay in timing of emergence determines establishment success in *Pinus sylvestris* across microhabitats. Annals of Botany. 2006;98: 1233-1240.

6. Figueroa R, Herms DA, Cardina J, Doohan D. Maternal environment effects on common groundsel (*Senecio vulgaris*) seed dormancy. *Weed Science*. 2010;58: 160-166.
7. Donohue K, Rubio de Casas R, Burghardt L, Kovach K, Willis CG. Germination, Post-germination Adaptation, and Species Ecological Ranges. *Annual Review of Ecology, Evolution, and Systematics*. 2010;41:293-319.
8. Gutterman Y. Maternal effects on seeds during development. In: Fenner, M. (Editor). *Seeds: The ecology of regeneration in plant communities*. Cambridge: Centre for Biosciences and Agriculture International; 2000.
9. Nygren M. Germination characteristics of autumn collected *Pinus sylvestris* seeds. *Acta Forestalia Fennica*; 1987.
10. Mason WL, Hampson A, Edwards C. *Managing the Pinewoods of Scotland*. Forestry Commission, Edinburgh; 2004.
11. Fletcher AM. Flower, fruit and seed development and morphology. In: Gordon, A.G. (editor). *Seed Manual for Forest Trees*. Forestry Commission Bulletin No. 83. Her Majesty's Stationery Office (HMSO), London; 1992.
12. Forestry Commission. *National Inventory of Woodland and Trees, Great Britain*. Forestry Commission, Edinburgh. UK; 2003.
13. Cannell MGR. Analysis of risks of frost damage to forest trees in Britain. In: Tigerstedt, P.M.A., Puttonen, P. and Koski, V. (Editors). *Crop physiology of forest trees*. Helsinki University Press, Helsinki; 1985.
14. Probert RJ. The role of temperature in the regulation of seed dormancy and germination. In: Fenner, M. (Editor). *Seeds: The ecology of regeneration in plant communities, Centre for Biosciences and Agriculture International (CABI)*. Wallingford; 2000.
15. Gosling PG, McCartan SA, Peace AJ. Seed dormancy and germination characteristics of common alder (*Alnus glutinosa*) indicate some potential to adapt to climate change in Britain. *Journal of Forestry*. 2009;82:573-582.
16. ISTA (International Seed Testing Association). *International Rules for Seed Testing*. Seed Science Technology, 21 (supplement); 1999.
17. ISTA (International Seed Testing Association). *International rules for seed testing*. Seed Science Technology. 1985; 13:299-355.
18. ISTA (International Seed Testing Association). *International rules of seed testing*. International Seed Testing Association. Bassersdorf, Switzerland; 2009.
19. AOSA (Association of Official Seed Analysts). *Tetrazolium Testing Handbook*. Contribution No. 29, Las Cruces, New Mexico; 2000.
20. Schneider CA, Rasband WS, Eliceiri KW. National Institutes of Health (NIH) Image to Image J: 25 years of image analysis. *Nature Methods*. 2012;9:671-675.
21. Ellis RH, Covell S, Roberts EH, Summerfield RJ. The influence of temperature on seed germination rate in grain legumes. II. Intraspecific variation in chickpea (*Cicer arietinum*) at constant temperatures. *Journal of Experimental Botany*. 1986;37:1503-1515.
22. Jinks RL, Willoughby I, Baker C. Direct seeding of ash (*Fraxinus excelsior*) and sycamore (*Acer pseudoplatanus*): The effects of sowing date, pre-emergent herbicides, cultivation, and protection on seedling emergence and survival. *Forest Ecology and Management*. 2006;237:373-386.
23. McCartan SA, Jinks RL, Barsoum N. Using thermal time models to predict the impact of assisted migration on the synchronization of germination and shoot emergence of oak (*Quercus robur*). *Annals of Forest Science*. 2015;72:479-487.
24. Gosling PG. *Raising trees and shrubs from seed*. Forestry Commission Practice Guide. Forestry Commission, Edinburgh, UK; 2007.
25. Donald DGM, Jacobs CB. The effect of storage time, temperature and container on the viability of the seed of four pine species. *South African Journal of Botany*. 1990;154:41-46.
26. Barnett JP. Long-term storage of longleaf pine seeds. *Tree Planter's Notes*. 1969; 20:22-25.
27. Tommasi F, Paciolla C, de Pinto MC, Gara LD. Effects of storage temperature on viability, germination and antioxidant metabolism in *Ginkgo biloba* seeds. *Plant Physiology and Biochemistry*. 2006;44: 359-368.

28. Schmidt L. Guide to handling of tropical and subtropical forest seed. Danida Forest Seed Centre, Denmark; 2000.
29. Simak M. The influence of low temperature on embryogenesis in Scots pine. Royal College of Forestry, Department of Reforestation; 1972.
30. Blate GM, Peart DR, Leighton M. Post-dispersal predation on isolated seeds: A comparative study of 40 tree species in a Southeast Asian rainforest. *Oikos*. 1998; 82:522-538.
31. Wood DW, Longden PC, Scott RK. Seed size variation, its extent, source and significance in field crops. *Seed Science and Technology*. 1977;2:337-352.
32. Sarvas R. Investigations on the flowering and seed crop of *Pinus sylvestris*. *Communications Instituti Forestalis Fenniae*. 1962;53:1-19.
33. El-Kassaby YA, Edward DGW, Taylor DW. Genetic control of germination parameters in Douglas-fir and its importance for domestication. *Silvae Genetica*. 1992;41: 48-54.
34. Udval B, Batkhuu N. Seed and Cone Characteristics of Scots pine (*Pinus sylvestris*) from diverse seed sources in northern Mongolia. *Eurasian Journal of Forest Research*. 2013;16:57-62.
35. Leikola M, Raulo J, Pukkala T. Prediction of the variations of the seed crop of Scots pine and Norway spruce. *Folia Forestalia*. 1982;537:1-43.
36. Basada RM. Effect of seed size on germination, seedling survival and height growth of white lauan (*Shorea contorta*). *Silvae Tropical*. 1979;4:77-80.
37. Moles AT, Westoby M. Latitude, seed predation and seed mass. *Journal of Biogeography*. 2003;30:105-128.
38. Powell AA. The importance of genetically determined seed coat characteristics to seed quality in grain legumes. *Annals Botany*. 1989;63:169-175.
39. Atis I, Atak M, Can E, Mavi K. Seed coat colour effects on seed quality and salt tolerance of red clover (*Trifolium pratense*). *International Journal of Agriculture and Biology*. 2011;13:363-368.
40. Bewley JD, Black M, Halmer P. The encyclopaedia of seeds: Science, Technology and Uses. The Centre for Biosciences and Agriculture International (CABI). Wallingford, United Kingdom; 2006.
41. Stoehr MU, L'Hirondelle SJ, Binder WD, Webber JE. Parental environment aftereffects on germination, growth, and adaptive traits in selected white spruce families. *Canadian Journal of Forest Research*. 1998;28:418-426.
42. Johnson O, Fossdal CG, Nagy N, Molmann J, Daehlen OG, Skroppa T. Climatic adaptation in *Picea abies* progenies is affected by the temperature during zygotic embryogenesis and seed maturation. *Plant Cell Environment*. 2005; 28:1090-1102.
43. Houle G, Filion L. Interannual variations in the seed production of *Pinus banksiana* at the limit of the species distribution in Northern Québec, Canada. *American Journal of Botany*. 1993;80:1242-1250.
44. Tielborger K, Petru M. An experimental test for effects of the maternal environment on delayed germination. *Journal of Ecology*. 2010;98:1216-1223.
45. Tekrony DM. Precision is an essential component in seed vigour testing. *Seed Science and Technology*. 2003;31:435-447.
46. Daws MI, Lydall E, Chmielarz P. Developmental heat sum influences recalcitrant seed traits in *Aesculus hippocastanum* across Europe. *New Phytologist*. 2004;162:157-166.
47. Dürr C, Dickie JB, Yang XY, Pritchard HW. Ranges of critical temperature and water potential values for the germination of species worldwide: Contribution to a seed trait database. *Agricultural and Forest Meteorology*. 2015;200:222-232.

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