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A laboratory study of the effect of temperature on red pine seed germination

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Abstract

This study investigated the influence of temperature on the germination of red pine (*Pinus resinosa* Ait.) seed. Three aspects of the temperature–germination relationship were addressed. First, the influence of temperature on the rate of germination and percentage germination was studied to test the hypothesis that low ambient temperatures inhibit the northward migration of red pine. Second, a model relating degree days to accumulated germination was developed. Third, the role of prechilling red pine seed was explored.

Temperatures of 15°C or greater were required for germination of 80% or more of the seeds, which means that germination capacity is not limiting the expansion of the range of the red pine. Temperatures greater than 10°C were required for completion of the germination process of unchilled seeds. Approximately 75% of the variance in the germination data was explained using a simple stepwise linear regression using accumulated thermal time (degree days). Prechilling the seeds (2–5°C) resulted in increased germination at temperatures of 10 and 12.5°C compared with unchilled seeds (78% vs. 26%). Prechilling also reduced the mean germination time by at least 1 day, even at the optimum temperature (25°C).

Introduction

The range of red pine (*Pinus resinosa* Ait.) extends from southern Manitoba eastward to Newfoundland, and as far south as West Virginia (Fig. 1) (Burns and Honkala, 1990). This area covers the Great Lakes–St. Lawrence Forest Region and the southern sections of the Boreal Forest (Rowe, 1972). Red pine survived the most recent glaciation in refugia in the Appalachians (Wright, 1964) and reached a postglacial peak in abundance and northern extent between 5000 and 3000 years ago during a hypsithermal period. Haddow (1948) states that within 300 km of its northern limit, red pine is isolated and scattered. What hinders the migration of red pine to the north and west?

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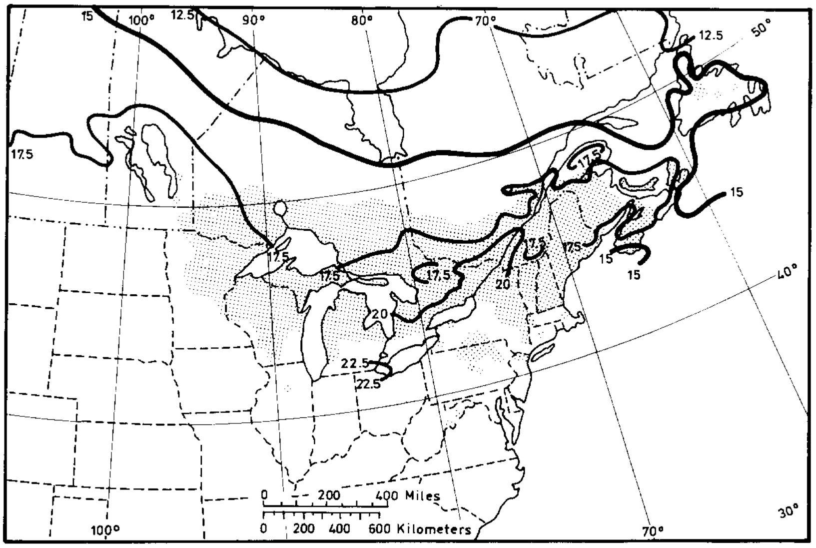

Fig. 1. The native range of red pine (Burns and Honkala, 1990) and mean monthly temperature (°C) for July 1951–1980 (Environment Canada, 1985).

Regeneration often limits the distribution of a species (Pigott and Huntley, 1978; Black and Bliss, 1980). Red pine produces viable seed even at the northern edge of its distribution and evidence suggests that populations are maintaining themselves by regeneration (Butson et al., 1987) at these northern locations where there has been no interference by human activity.

There are three possible explanations for the failure of red pine populations to migrate further north.

(1) Red pine is a fire-dependent species (Van Wagner, 1970; Heinselman, 1973; Bergeron and Brisson, 1990; Engstrom and Mann, 1991), i.e. fire initiates the regeneration process by opening up the canopy, preparing the seedbed, and removing competition; at the northern limit of red pine, the fire regime may be too severe for regeneration to occur except in sheltered areas.

(2) Red pine populations could be relicts from a warmer period and are just maintaining existing populations (regeneration approximately equals senescence).

(3) Environmental conditions are marginal during some stages of the life cycle, e.g. temperatures for seed germination and establishment or winter survival.

In this study, three facets of the temperature–germination relationship were investigated. First, we examined whether low ambient temperatures during seed germination inhibit successful germination. Second, the relationship between cumulative percentage germination and cumulative thermal time (degree days) was investigated using a linear regression model. Third, the effect of prechilling seeds on the percentage rate of germination was examined.

Germination of red pine seed in relation to temperature has not been widely studied, unlike other conifer species (Allen, 1960; Adkins et al., 1984). Roth and Riker (1943) found that germination of red pine seed was poor when temperatures were lower than 15 °C. Another report states that the ideal temperatures for red pine germination are between 20 and 30°C (Anonymous, 1948). Similar values are listed by the International Seed Testing Association (1985) as being valid for seed testing, i.e. 20°C for 16 h in the dark, 30°C for 8 h in light.

Prechilling of seeds was included in this study for two reasons. First, prechilling the seed is representative of field conditions. Red pine seeds that fall to the ground in the autumn are usually covered by snow until spring. This period represents a natural prechill time with temperatures near 0 °C. Second, red pine seeds may exhibit conditional dormancy at low temperatures; prechilling may be needed to break this dormancy. Thus, prechilling may result in differences in the proportion of seeds germinating and/or in the rate of germination.

Materials and methods

Three provenances of red pine seed were obtained from the National Tree Seed Centre at the Petawawa National Forestry Institute (PNFI) in Chalk River, Ontario (Table 1). The seeds were stored in air-tight containers in a freezer maintained at −18 °C with moisture contents prior to the study from 4 to 8% (fresh weight basis).

Table 1
Red pine seed lot characteristics

Provenance	Year collected	Origin	Latitude (°)	Longitude (°)	Elevation above sea level (m)	Weight per 1000 seeds germinated (g)	Percentage germinated[1]
1	1983	Alexander Bay, Nfld.	49	54	120	7.49	95
2	1989	Chalk River, Ont.	46	77	180	9.48	85
3	1990	Upper Peninsula, MI	47	86	240	10.33	97

[1]Percentage germinated following a standard test conducted at the National Tree Seed Centre, PNFI. The standard test for red pine for all provenances includes four replications of 100 seeds with an 8 h photoperiod, day temperatures of 30°C (8 h), and night temperatures of 20°C. Seeds are reported as having germinated only if they have high vigour, i.e. the radicle must be at least four times as long as the seed after 3 weeks.

Conviron G-30 Seed Germinators (Conviron Canada, Winnipeg) at PNFI were used in this study during 1991 and 1992. These seed germinators allow control of temperature, humidity, and photoperiod. Seeds in groups of 50 from each provenance were placed on moistened Kimpak (cellulose paper wadding, Kimberley Clark, Green Bay, WI) in a germination box (Wang and Ackerman, 1983), each group in the germination box being positioned randomly. There were four replicates of each provenance for each temperature treatment. The relative humidity was maintained near 100% and the lighting set for a 16 h photoperiod, close to the actual photoperiod at the northern range (50°N) of red pine during the typical germination period in June. Photosynthetically active radiation was approximately 12 mmol m^{-2} s^{-1} from fluorescent lamps mounted in the side walls of the germinator, simulating field conditions, although Mirov (1967) reported that red pine does not require light for germination.

A variety of temperature treatments ranging from suboptimal to optimal were used in this experiment. This included temperatures of 10, 12.5, 15, 20 and 25°C for chilled and unchilled seeds, with additional treatments at 11 and 17.5°C for the latter. Optimum temperatures for red pine germination are a constant 25°C or a regime of 20°C at night (16 h) and 30°C during the day (8 h) (International Seed Testing Association, 1985). Constant temperatures were used throughout the experiment. Seeds for the prechilled seed treatments were placed on moistened Kimpak in a germination box and kept at 2–5°C in a standard upright refrigerator for 2 weeks prior to being placed in the germinator.

For the purpose of this study, germination was defined as being complete when the radicle emerged from the seed. Germinated seeds were counted and removed every 24 h. The experiments were run for a minimum of 2–3 weeks and were continued as long as the seeds were still germinating (maximum 40 days). Degree days were calculated using Eq. (1)

$$DD = NDAY \cdot (\text{mean temperature} - \text{base temperature}) \qquad (1)$$

where DD is degree days and NDAY is the number of days. The base temperature is the temperature at which little or no germination takes place (10°C as derived from this study). Also, Kozlowski and Borger (1971) found that red pine growth was hindered by low temperatures, growth being minimal at 10°C. To discover a possible better fit, as described by the variance explained in the germination data, the base temperature was varied from 0 to 10°C. A base temperature of 10°C yielded the best fit, giving more credence to the selection of that temperature.

Two aspects of seed germination that might be influenced by temperature are the rate of germination and the percentage of seeds germinating. The rate of germination was used to contrast prechilled seeds with seeds not prechilled. The mean germination time (MGT) was calculated using Eq. (2) (Bewley and Black, 1985)

$$MGT = \sum(T \times N) / \sum N \qquad (2)$$

where T is the time in days and N is the number of seeds fully germinated on day T.

For the germination model, the data underwent an arcsine transformation (Steel and Torrie, 1980). The germination model is based on a forward stepwise linear regression, using Proc Stepwise of the Statistical Analysis Systems Institute (SAS, 1985). The model was designed to examine the influence of temperature, time to germination and provenance on germination. The transformed percentage germinated is the dependent variable and the degree days, temperature, number of days until germination and provenance are the independent variables. For regression analysis, the provenance was assigned a number between 1 and 3.

Results

Figure 2(a) shows the effect of temperature on the percentage of seed (not prechilled) germinated. As the temperature increases, the percentage of seed germinated also increases. There is some difference between provenances, but the trend for all three is similar. Below 15 °C, germination is hindered by the low temperature. At 10 °C, germination did not occur at all during the experiment. Figure 2(a) also shows that temperatures of 15 °C or warmer are required for significant germination of red pine seed. The relationships between temperature and germination for prechilled seeds (Fig. 2(b)) and those that were not prechilled (Fig. 2(a)) are similar except at temperatures below 15 °C, where the percentage of seed germinated is significantly higher for the prechilled seeds, indicating that prechilled seeds germinate at lower temperatures.

The relationship between degree days and the percentage of seed germinated is shown in Fig. 3, and is typical for all three provenances and for all temperatures of 15 °C and above. The rate of germination is linear over most of the plot and the germination model used this linear portion of the germination–rate relationship. The non-linear tails at both ends of the distribution were removed by a screening procedure. This eliminated all the data below 5% germination and points where the slope was less than 0.333, effectively expunged the region where the germination rate flattens out at the end of the germination period.

Table 2 shows the explained variance for a forward stepwise linear regression using the Stepwise procedure (SAS, 1985) by provenance and treatment (with and without prechilling). Terms were accepted only if they met the 0.05 significance level, which corresponds to an F value to enter of 4.0; terms were removed when they failed to meet the 0.05 significance level. The explained variance is approximately 75% with some variation between provenances and

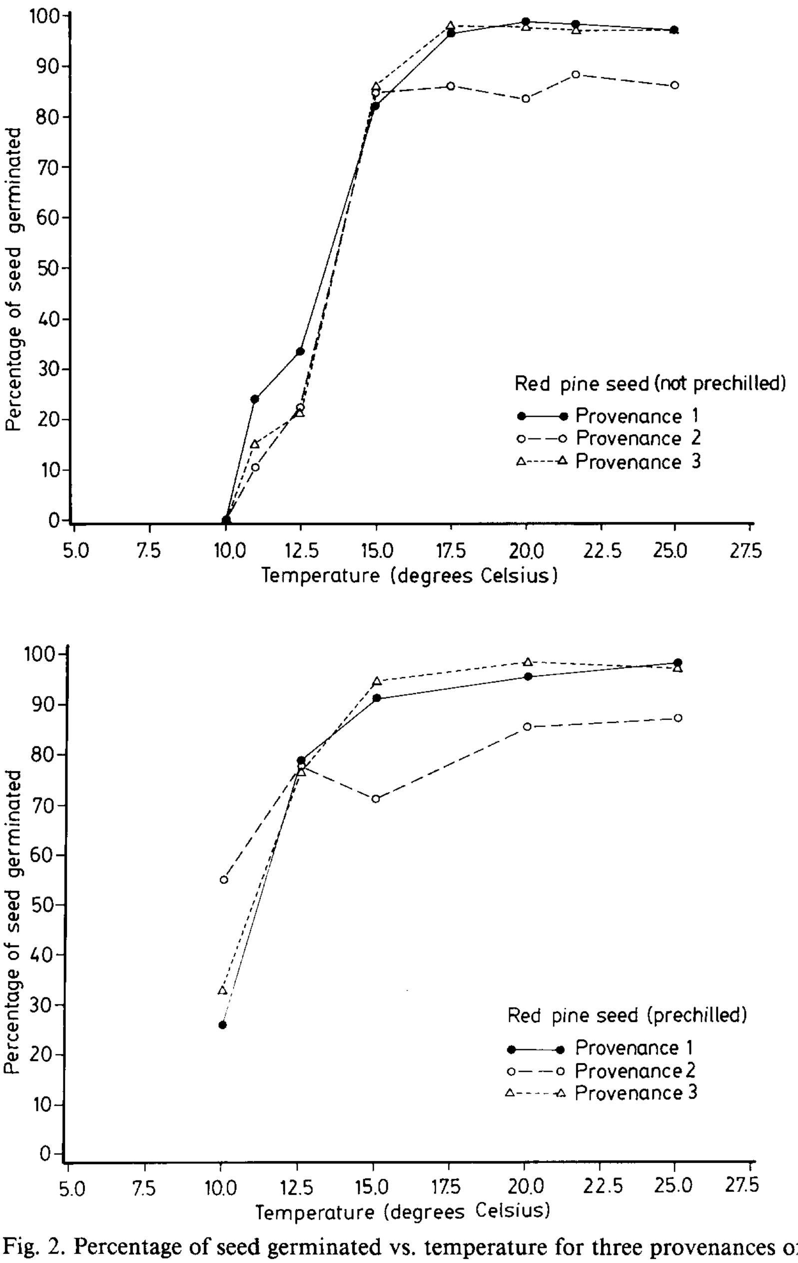

Fig. 2. Percentage of seed germinated vs. temperature for three provenances of red pine seed: (a) not prechilled; (b) prechilled.

treatments. These regression equations are valid only for the temperature range over which they were developed, namely 15–25°C.

Figure 4 illustrates mean germination time in relation to temperature. Pre-

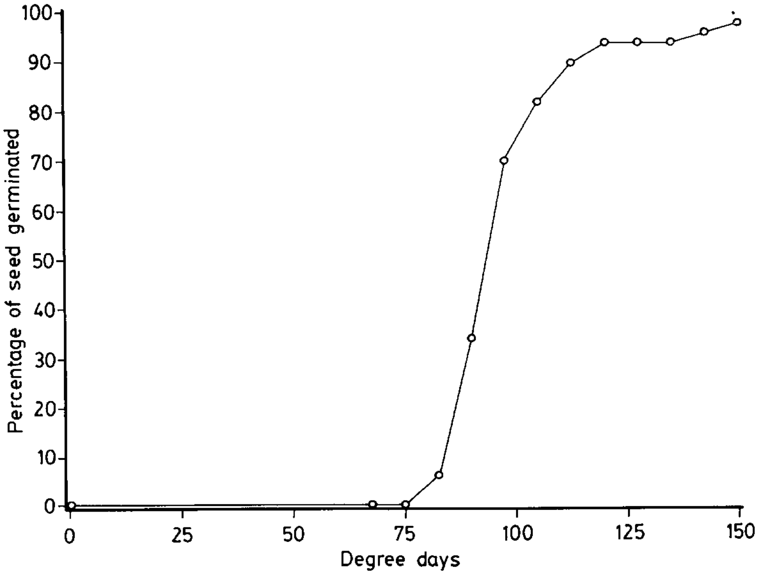

Fig. 3. Typical cumulative germination (%) of red pine seed vs. degree days (base 10°C). (Data from 17.5°C for Provenance 1.)

Table 2

Variance explained and variable selected by forward stepwise linear regression

Provenance	N[1]	Variance explained	Variables selected
Not prechilled			
1	66	0.76	DD(0.76)
2	61	0.83	DD(0.74) Temp.(0.09)
3	71	0.80	DD(0.66) Temp.(0.14)
All	198	0.76	DD(0.69) Temp.(0.04) or Prov.(0.03)
Prechilled			
1	49	0.69	DD(0.61) Temp.(0.08)
2	37	0.78	DD(0.63) Temp.(0.14)
3	48	0.67	DD(0.53) NDAY(0.14)
All	134	0.69	DD(0.61) Temp.(0.08)

[1]Number of data points used in the regression.

DD, degree day; temp., temperature; prov., provenance; NDAY, number of days.

Values in parentheses are the amounts of variance explained by that variable.

chilling decreases mean germination time. The difference between treatments became less pronounced as the temperature increased. Even at the optimum temperature of 25°C, however, the difference was still more than 1 day, which is about 16% of the total germination time.

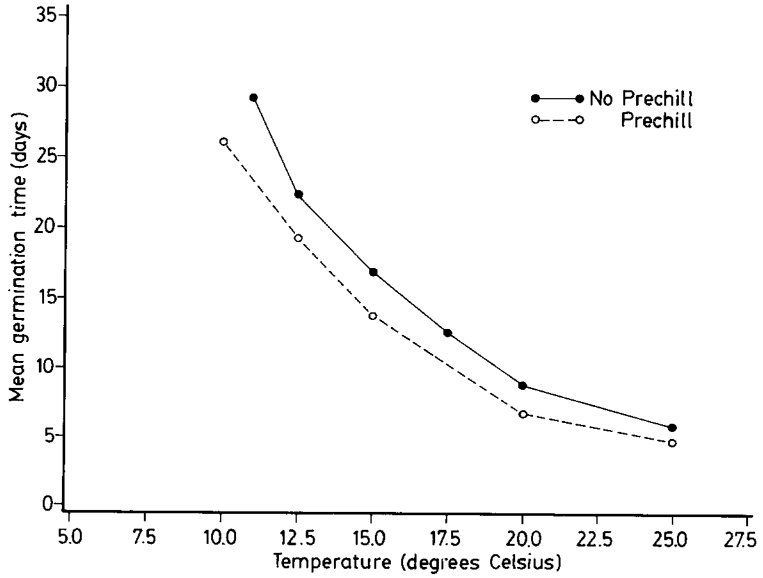

Fig. 4. Mean germination time (MGT) vs. temperature for red pine seed (all provenances combined) with and without moist prechilling.

Discussion

A mean temperature of around 15 °C for a period of 2–3 weeks (mean germination time of 16.9 days) is required for significant red pine seed germination unless the seed is prechilled. This assumes that all of the other stringent requirements for red pine regeneration are met (Van Wagner, 1970). Figure 1 (Environment Canada, 1985) shows the 15 °C isotherm for July in Canada, calculated in sheltered screens located 1.5 m above the ground, is well north of the red pine limit. Toy et al. (1978) provide an equation to predict the mean monthly soil temperature based on the mean monthly air temperature. According to this equation, a mean monthly soil temperature of 15 °C is obtained from a mean monthly air temperature of 10 °C. In shaded forest soils, however, the equation would not be appropriate. Because red pine is intolerant to shade, all successful regeneration will take place on exposed soil. This study has shown that some germination is possible at temperatures below 15 °C, although the time required for germination is greatly increased. It is possible that if the trial had run for 2 months or longer, a small percentage of seed might have germinated even at 10°C. Roth and Riker (1943) observed a trend similar to that revealed in this study, although their results were much lower in terms of the percentage of seeds germinated (85% vs. 45%), especially at temperatures of 15 °C or below, probably due to differences in seed quality and the environment. They found that seedlings from seeds germinated at temperatures below 15 °C were not as healthy as those germinated at higher temperatures (between 15 and 30 °C).

There are various approaches to modelling cumulative germination (Roberts, 1988; Brown and Mayer, 1988a,b) including polynomial models, exponential models, and gamma and Weibull distributions. We used a linear regression because it was easy to use and our data were linear for most of the cumulative germination curve (Fig. 3). We should note that if we were interested in the entire cumulative germination curve, more sophisticated techniques would have been required. The linear model explained about 75% of the variance, with the degree day variable being responsible for most of that.

The explained variance for the prechilled seeds is similar to that for seeds not prechilled. These regression equations suggest that the germination process is highly dependent on accumulated thermal time (degree days). This analysis was performed on a dependent data set and was to ascertain what, if any, variables were important for germination. What is responsible for the remaining unexplained variance? Perhaps it is due to germination processes that are not highly temperature dependent. Was there measurement error, which would include the practice of counting the seeds only once a day? In such a situation, seeds germinating within a few hours of each other might have significantly different degree day values. Also, a subjective visual examination of the seeds was performed to determine whether germination had occurred. Furthermore, some variance was no doubt due to the inherent variability among seeds of the same seedlot, even though each replicate had 50 seeds chosen at random. The varying lengths of cold storage of the seed did not add any uncertainty to the data; red pine seeds remain viable for a long time when properly stored (Eliason and Heit, 1973). Considering the sources of uncertainty, an explained variance of 75% appears to be a good fit of the data.

Prechilling of red pine seed speeded up the germination process. Even under optimum conditions, the prechilled seeds germinated, on average, 1 day faster than those seeds that were not prechilled (Fig. 4). Wang et al. (1993) found similar results when working with black spruce (*Picea mariana* (Mill.) B.S.P.) seeds. The reason for the increased rate of germination may be that there are processes occurring during germination that may not be highly temperature dependent. For example, imbibition of water and protein hydration are already underway during the prechilling stage. Other studies with conifer seeds have shown the importance of moisture content and prechill duration on germination (Gosling and Rigg, 1990; Downie and Bergsten, 1991).

Significantly more prechilled seeds germinated at temperatures less than 15 °C compared with those seeds that were not prechilled. These results indicate that red pine seeds exhibit a conditional dormancy; prechilling may be necessary to break this dormancy at low temperatures (below 15 °C). In the natural environment, red pine will undergo prechilling as the seed is covered by snow during winter. This would set up conditions in the spring to allow for significant germination to take place at temperatures below 15°C.

The duration of the prechilling period was not varied in this study. It is probable that a period longer than 2 weeks will reduce the mean germination time even further (Gosling and Rigg, 1990).

Although not an objective of this study, it is obvious that the difference in response among the provenances was minimal. The provenances covered a large geographical area and a large climatological gradient, yet the responses to temperature and prechilling were nearly identical. This is not surprising given the uniform genetics of the species (Fowler and Lester, 1970; Fowler and Morris, 1977).

Based on the present study, it appears that germination is not limited by temperature at the present-day northern range of red pine. The time required for germination of red pine seed at the present northern limit would be approximately 9 days based on average weather conditions in July. If red pine migration was hindered only by germination temperature, the expected northern limit would follow the 10°C. This would mean that red pine could germinate in any forest existing in Canada at present. However, field experiments of red pine seed germination would be required to verify the scenario based on these laboratory experiments. The lack of red pine migration to the north could be due to the fire regime. If forest fires were less frequent and not quite as intense, red pine could spread inland away from the lakes and northward as well. Finally, the possibility exists that present populations might be relicts of an earlier expansion that are simply maintaining themselves in sheltered environments.

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