

Seedling cold hardiness, bud set, and bud break in nine provenances of *Pinus greggii* Engelm.

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Abstract

Cold hardiness and timing of bud set and bud break are important processes that provide protection of nursery seedlings against low temperatures. Seedlings of 9 provenances of *Pinus greggii* from two different regions of Mexico were tested to determine cold hardiness, bud set, and bud break timing differences. Needle sections were exposed to freezing temperatures to determine an injury index of each provenance. In addition, bud set and bud break timing were recorded through the fall, winter and spring. There were significant differences in cold hardiness between seedlings from northern and southern provenances. At the maximum cold hardiness, the index of injury (LT_{50}) for northern provenances was $LT_{50} = -18^{\circ}\text{C}$, compared to -12°C for southern provenances. There was a considerable variation among the provenances in the proportion of seedlings that set terminal buds. Seedlings from northern provenances had greater proportions of seedlings that set a terminal bud than seedlings from southern provenances. There were also significant differences in the bud break timing in the following spring among the 9 provenances. Seedlings from northern provenances broke bud earlier than southern provenances. Cold hardiness, bud set, and bud break timing results may be useful to determine how far a specific seed source can be moved from its natural environment.

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1. Introduction

Differences in susceptibility to cold temperatures are often present in species that show a wide geographic distribution (Flint, 1972; Kuser and Ching, 1980; Alexander et al., 1984; McCamant and Black, 2000). Seedlings from northern provenances or higher elevation typically tolerate low temperatures better than seedlings from southern provenances or low elevation (Kuser and Ching, 1980; Thomas and Lester, 1992). Cold hardiness is an important factor related to the physiological condition of the seedlings (Johnson and Cline, 1991). Knowledge of seedling cold hardiness status can be valuable when protecting seedlings against low temperatures not only in the nursery but also after outplanting (Mexal et al., 1979; Glerum, 1985).

Cold hardiness also has been found to be related to bud set and bud break of some conifers (Kuser and Ching, 1980; Burr et al., 1989). Bud set and bud break are two precise phenological events in conifers (Hannerz, 1999) in response to the environmental conditions and this response is under strong genetic control (Ekberg et al., 1991). Both bud set and bud break can be used for screening clones and progenies in tree breeding programs (Hannerz, 1999).

Pinus greggii Engelm. occurs in natural stands in two widely separated regions of Mexico. These northern and southern populations of *P. greggii* are exposed to different environmental conditions. Trees from northern populations grow above 2200 m a.s.l., receive less than 800 mm in average annual precipitation, while the average annual temperature is about 14°C . On the other hand, the southern populations usually grow at elevations below 1900 m a.s.l., receive more than 800 mm/yr, and in some places greater than 1200 mm, with an average annual temperature near 17°C . Northern populations usually grow on sites with neutral to slightly alkaline pH while southern populations prosper in more acidic soils (Donahue,

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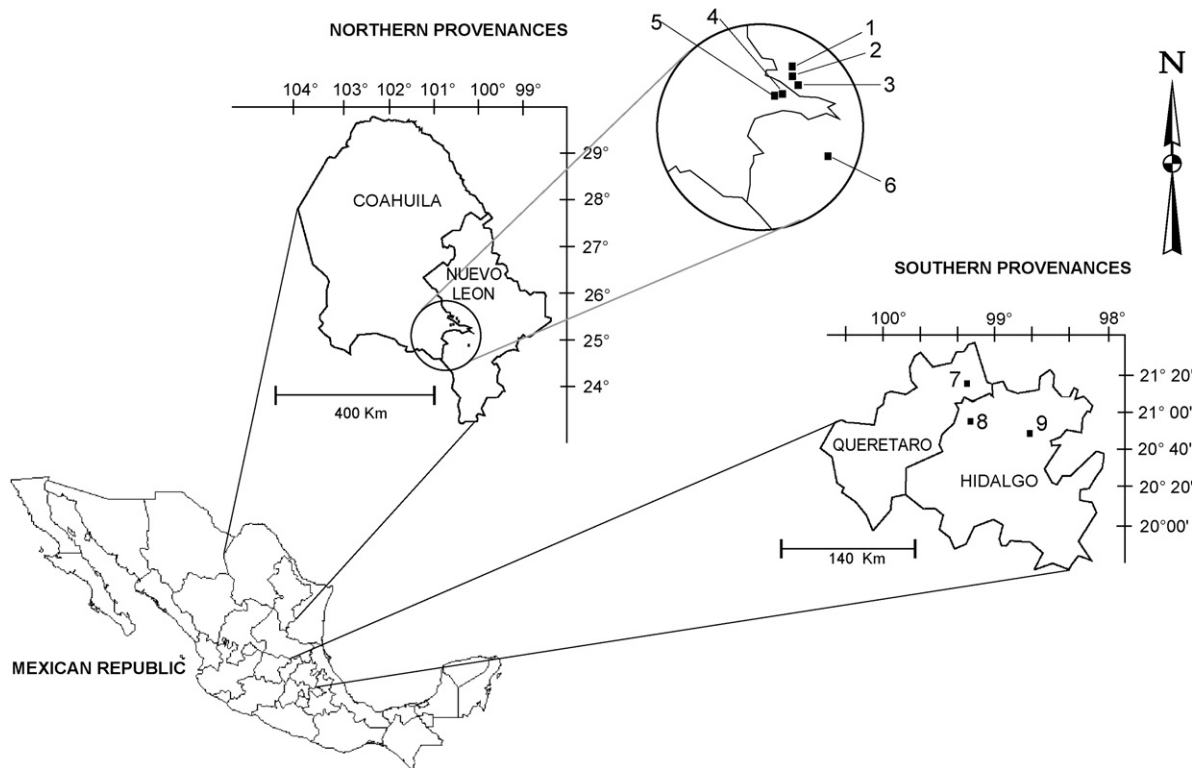


Fig. 1. Geographic location of the northern region (states of Coahuila and Nuevo Leon) provenances (1: Jamé, 2: Los Lirios, 3: San Juan, 4: Santa Anita, 5: El Conejo, and 6: Las Placetas) and southern region (states of Hidalgo and Querétaro) provenances (7: El Madroño, 8: El Piñon, and 9: Xochicoatlán).

1993). The great difference in environmental growing conditions between the two regions has influenced some morphological, physiological, and phenological traits in this species not only in adult trees but also in seedlings (Dvorak et al., 1996). The overall objectives of this study were to evaluate the variation of cold hardiness, bud set, and bud break in seedlings from 9 different provenances of *P. greggii*.

2. Materials and methods

2.1. Seed sources

P. greggii seed was collected in 1995 from two different regions of Mexico and stored for 3 years at 4 °C. The first region was located in the northern part of Mexico in the states of

Coahuila and Nuevo Leon, while the other is in the southern part in the states of Querétaro and Hidalgo (Fig. 1). The northern region included six provenances (Jamé, Los Lirios, San Juan, Santa Anita, El Conejo, and Las Placetas) while the southern region included three provenances (Xochicoatlán, El Piñon, and El Madroño) (Table 1).

2.2. Seedling production

Seedlings were produced at the Fabian Garcia Science Center at New Mexico State University (latitude 32°22', longitude 106°43'). The experiment was established in a greenhouse where 9 provenances were compared. Seedlings were grown in plastic propagation trays (Tray Master TM 060, The Lerio Corp, Mobile, Alabama), and each plastic tray

Table 1
Geographic location, elevation, and annual precipitation of the 9 provenances of *Pinus greggii* included in the study

Provenance	Code	State	Latitude	Longitude	Elevation (m a.s.l.)	Precipitation (mm)
Northern						
Jamé	1	Coahuila	25°21'N	100°36'W	2500–2600	<800
Los Lirios	2	Coahuila	25°22'N	100°32'W	2300–2600	<800
San Juan	3	Coahuila/N.L.	25°25'N	100°32'W	2450–2650	<800
Santa Anita	4	Coahuila/N.L.	25°27'N	100°34'W	2500–2600	<800
El Conejo	5	Coahuila/N.L.	25°28'N	100°34'W	2500–2600	<800
Las Placetas	6	Nuevo Leon	24°54'N	100°12'W	2400–2500	<800
Southern						
Xochicoatlán	7	Hidalgo	20°45'N	98°40'W	1800	1200
El Piñon	8	Hidalgo	20°54'N	99°11'W	1800–1900	800
El Madroño	9	Querétaro	21°14'N	99°13'W	1650–1840	1200

contained 60 cells with a volume of approximately 120 ml. Each cell had an average diameter of 4 cm and a depth of 15 cm. Griffin Corporation (Valdosta, GA) provided all the containers. Four trays (240 cells) of each provenance were grown.

On May 1, 1998, containers were filled with Metromix[®] 702 substrate (50–60% composted pine bark fines, 15–25% Canadian sphagnum peat moss, 10–15% medium grade horticultural vermiculite, and 5–15% horticultural perlite) was mixed with Osmocote[®], a granulated controlled release fertilizer using a cement mixer. The concentration of macronutrients for this experiment was 14–14–14 (N–P₂O₅–K₂O). The rate was 5 kg Osmocote/m³ growing media.

Seeds of *P. greggii* were soaked in water for 24 h, and sown singly in each cell to a depth of approximately 0.5 cm. Trays were hand-watered as needed during emergence. After emergence, irrigation was applied through micro-sprinklers as needed. Seedlings were grown in the greenhouse for 5 months. On October 4, 1998, seedlings were placed under a shadehouse for 2 weeks and then moved to the open to allow seedlings to cold-harden under natural conditions. Seedlings were placed outdoors in the same arrangement as in the greenhouse.

2.3. Cold hardiness measurement

On November 30, 1998, February 8, 1999, and April 6, 1999, a sample of 12 seedlings randomly selected from each provenance was shipped by overnight mail to the US Forest Service Coeur D'Alene Nursery's Quality Assurance Laboratory, Coeur D'Alene ID for cold hardiness determinations. Cold hardiness was measured using the freeze-induced electrolyte leakage (FIEL) technique (Burr et al., 1990), within 3 days for each shipping date.

The 12 trees were grouped into 4 replications of 3 trees each. A sample of needles from the middle section of each seedling stem was cut into 1 cm pieces, and 10 needle pieces were placed in each of 28 culture tubes containing 0.5 ml of distilled water. Four of the tubes were placed in a refrigerator at about 2 °C to serve as controls to measure the amount of electrolytes leakage at the end of the test.

The remaining 24 tubes were put in a low temperature alcohol bath at –2 °C. The temperature of the bath was lowered at a rate of 5 °C per hour. At each of six preselected temperatures, four tubes were removed and placed in a refrigerator to thaw. The temperatures were selected to span the expected range from no damage to complete kill. After thawing, 6 ml of distilled water was added to aid measurement, and all of the tubes were placed on a shaker to incubate 20 h overnight.

The next day, electrical conductivity of each tube was measured to determine the amount of electrolytes leaked from the tissue in response to damage from low temperature. The tubes were then boiled to completely kill the tissue and returned to the shaker overnight. On the third day, the electrical conductivity of the water was measured again to determine the total amount of electrolytes present.

An index of injury was calculated which represented the percentage of electrolytes that leaked out in response to low temperature damage. The temperature that caused a 50% index of injury (LT₅₀) and 95% confidence level was calculated.

The remaining trees were monitored for the number that set bud. In addition, these trees were observed during and after the winter in order to determine the timing for the terminal bud break for each provenance. Starting on March 1, 1999 (date when the first bud break was detected), bud break was recorded weekly as the cumulative number of trees that broke bud and started new growth.

2.4. Statistical analyses

Chi square analysis was used to compare the percentage of bud set for each provenance. Percentage data were transformed prior to analyses. Bud break data were analyzed to determine the average date when each provenance reached 10%, 50%, and 90% bud break. These means were recorded as the cumulative numbers of days since the first visible bud break started until reaching 10%, 50%, and 90% bud break for each provenance. These data were subjected to analysis of variance.

3. Results

3.1. Cold hardiness

Seedlings from northern provenances were more cold hardy throughout the test period than southern provenances (Fig. 2). During the first evaluation date (November 30, 1998) northern provenances cold hardiness averaged –17 °C (LT₅₀) while southern provenances only averaged –11 °C (LT₅₀). For the second evaluation date (February 8, 1999) LT₅₀ of northern provenances almost reached –18 °C, but southern provenances averaged only –12 °C. Finally, cold hardiness decreased for the third date (April 6, 1999) with northern provenances averaging –11 °C (LT₅₀) compared to only –9 °C for southern provenances.

Among the six northern provenances, there were two distinct groups. Four of the six provenances (Jamé, San Juan, Santa

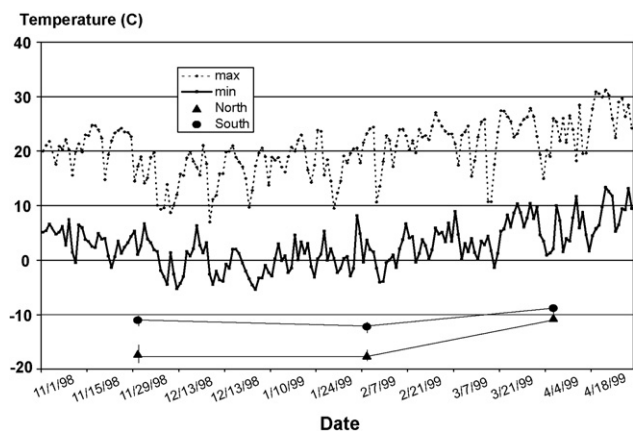


Fig. 2. Cold hardiness (LT₅₀) of northern and southern provenances of *Pinus greggii* evaluated on three dates. Vertical bars represent one S.E. of the mean.

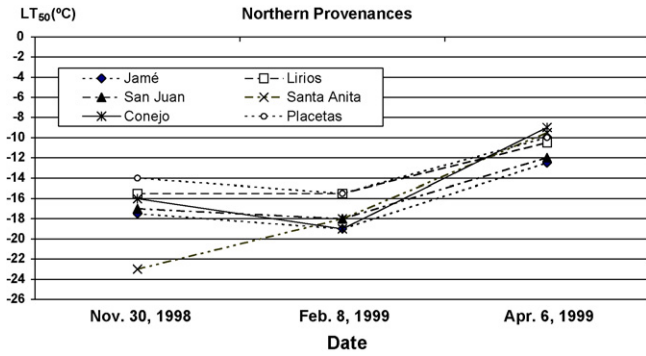


Fig. 3. Cold hardiness (LT_{50}) variation among six northern provenances of *Pinus greggii* evaluated on three dates.

Anita, and El Conejo) had similar cold hardiness patterns but the provenances Los Lirios and Las Placetás did not cold-harden as much as the others (Fig. 3). At the maximum cold hardiness registered during the second evaluation date (February 8, 1999), Los Lirios and Las Placetás only reached $-15.5\text{ }^{\circ}\text{C}$ (LT_{50}) in comparison to -18 or $-19\text{ }^{\circ}\text{C}$ (LT_{50}) for the other four northern provenances (Fig. 3). Furthermore, among all provenances, Santa Anita was the most cold hardy during the first evaluation date ($LT_{50} = -23\text{ }^{\circ}\text{C}$). In contrast, the provenance Las Placetás only reached $-14\text{ }^{\circ}\text{C}$ (Fig. 3).

3.2. Bud set

There was considerable variation among the provenances in the proportion of seedlings that set terminal buds (Fig. 4). In general, seedlings from northern provenances had a greater proportion of seedlings that set terminal bud than seedlings from southern provenances. However, northern provenances also showed greater variation among provenances (Fig. 4). The provenance El Conejo had the highest percent bud set among northern sources (44%), while the provenance Jamé had the lowest value (13%). On the other hand, bud set percentages for southern provenances ranged from 5% for El Piñón to 6% for Xochicoatlán, and 8% for El Madroño.

3.3. Bud break

There were significant differences in the bud break timing among the 9 provenances (Fig. 5). Seedlings from northern

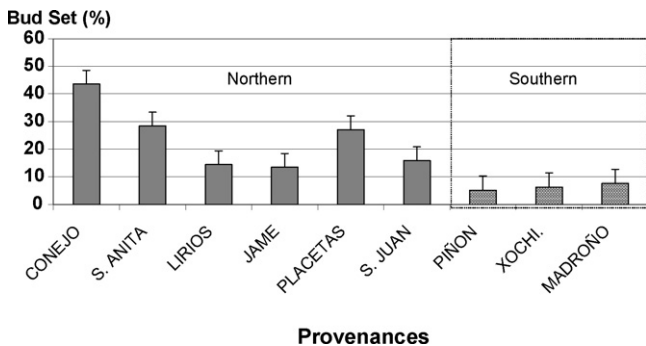


Fig. 4. Percentage of bud set for seedlings from 9 provenances of *Pinus greggii* evaluated 10 months from sowing.

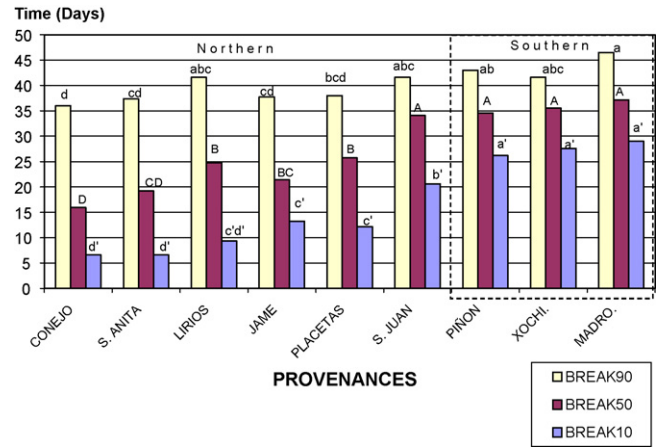


Fig. 5. Days to 10%, 50%, and 90% bud break for 9 provenances of *Pinus greggii*. Values followed by the same letter within the same bar series are not significantly different based on Duncan’s Multiple Range test, $P < 0.05$.

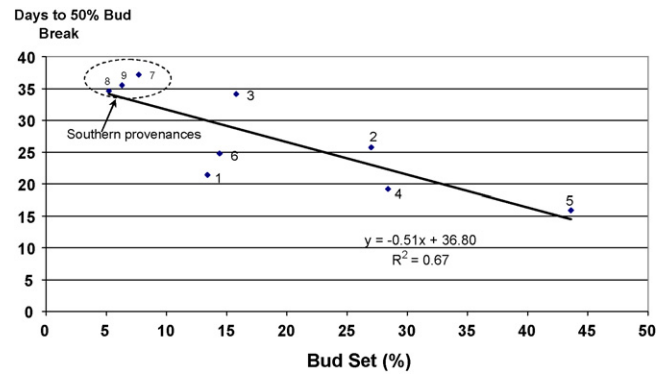


Fig. 6. Relationship between bud set and timing for bud break among provenances. Northern region included 6 provenances (1: Jamé, 2: Los Lirios, 3: San Juan, 4: Santa Anita, 5: El Conejo, and 6: Las Placetás) and southern region included 3 provenances (7: El Madroño, 8: El Piñón, and 9: Xochicoatlán) of *Pinus greggii*.

provenances showed earlier bud break than southern provenances. Seedlings from the provenance El Conejo reached 10% bud break at 6 days, 50% bud break at 16 days, and 90% bud break at 36 days counting since the very first seedling started the bud break process (March 1). Most of the northern provenances reached 50% bud break at 25 days compared to 35 days for southern provenances (Fig. 5). In addition, seedlings from the San Juan provenance were delayed in breaking bud compared to the other northern provenances. San Juan seemed more similar in behavior to the southern provenances. In addition, bud break in the spring was negatively correlated to the bud set from the previous fall among northern and southern provenances (Fig. 6). Provenances that had a high percentage of bud set in the fall seemed to initiate bud break in the spring sooner than those that had a lower percentage of bud set.

4. Discussion

P. greggii cold hardiness, bud set, and bud break characteristics varied among the provenances included in the

study. There was a general latitudinal relationship between location and cold hardiness. Seedlings from northern provenances had greater cold hardiness, greater percent bud set, and initiated bud break earlier than southern provenances. These results agree with previous studies with other conifers (Kuser and Ching, 1980; Beuker, 1994).

Bud break of seedlings from northern provenances may respond only to temperature while southern provenances appeared to respond to both photoperiod and temperature. All northern provenances initiated bud break in early March when the photoperiod was less than 12 h. In contrast, southern provenances started bud break only after the beginning of the spring (March 21). Northern provenances may be adapted to cooler temperatures. Furthermore, northern Mexico is more xeric and seedlings may be adapted to initiate growth before the onset of summer drought.

Among the northern provenances, seedlings from Los Lirios and Las Placetas do not cold-harden as much as the other 4 provenances. The natural distribution for these two provenances, while similar to the others, is lower in elevation. Thus, they may be adapted to warmer environments. This agrees with other studies with *Populus* (McCamant and Black, 2000). Northern provenances were more cold hardy than southern provenances, especially during the fall and winter. However, these values may not represent the maximum cold hardiness of *P. greggii* seedlings. The provenances were not tested in January when maximum cold hardiness might be expected. Furthermore, the winter of 1998/1999 in New Mexico was not particularly cold as the minimum temperature was about -5°C , but it was a good test to demonstrate that southern provenances could be moved into the northern range with some caution.

These provenance variations can be useful for selection and improvement of this species as suggested by others (Kariuki, 1998). However, it is important to maintain seed source identity since *P. greggii* consists of two varieties (Donahue and López-Upton, 1999). Different provenances of this species can be used in selection or breeding programs to improve the cold resistance and increase the growth of selections for reforestation programs. Regardless, seed source must be an important consideration for this species during nursery production and reforestation programs. Northern species grow slower than southern species (López-Upton et al., 2004; Aldrete et al., 2005) which places northern species at a disadvantage if moved south. However, southern species should be moved into the northern range with caution because of the risk of freeze injury.

5. Conclusions

Southern provenances of *P. greggii* differed in the ability to acclimate to cold temperature by as much as 6°C compared to northern sources. Northern sources became cold hardy to about -18°C , while southern sources could harden to only -12°C . Furthermore, there was more variation in cold hardiness among northern sources, apparently due to elevational differences among seed sources. Northern sources tended to have a higher

percentage of seedlings setting bud in the fall, and initiated growth earlier in the spring in spite of having a well-developed overwintering bud. However, the northern sources did not grow as much as southern sources, even with earlier bud break. This data may help in developing seed source movement guidelines for Mexican conifer species.

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