Genetic Variation in Frost Damage and Seed Zone Delineation within an Altitudinal Transect of Pinus devoniana (P. michoacana) in Mexico

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Abstract
We explored the patterning of genetic variation among Pinus devoniana Lindl. (also known as P. michoacana Martín) populations to develop guidelines for seed and seedling movements, intended for improving the matching between genotypes and environments regarding frost tolerance, in reforestation programs. Open-pollinated seed from 16 populations along an altitudinal transect (1600 to 2450 m) were collected near Morelia, State of Michoacán, México. A common-garden provo- nance test, established with 2.5-year-old seedlings, was assessed for frost resistance conducting a laboratory frost damage test (–9°C). Results indicate that there were significant differences among provenances (P=0.0261) for frost damage. Variation among provenances was structured as an altitudinal cline, with populations from lower altitudes being the least tolerant to frost. Linear regression statistics suggest that for each increment of 100 m of provenance altitude, there will be a 5.2% decrease in frost damage. We suggest the use of two provisional altitudinal seed zones of 400 m breadth each (lower and upper limits for zone 1: 1600 m and 2000 m of altitude; for zone 2: 2000 and 2400 m, respectively), and for reforestation of a given site, the use of seedlings originated from seed of the same seed zone or within ±200 m of altitude from the elevation of the reforestation site.

Key words: Pinus devoniana, Pinus michoacana, altitudinal genetic variation, provenances, frost damage, frost hardiness, seed zoning, seed and seedling movement guidelines.

Introduction
Frost damage is one of the main causes of poor growth and mortality of seedlings in reforestations using pine species in México (BELLO-LARA and CIBRIÁN-TOVAR, 2000; SÁENZ-ROMERO et al., 2003). Frost damage accounts for the 14% of mortality in reforestations in the Mexican western State of Michoacán (SÁENZ-ROMERO and LINDIG-CEÑEROS, 2004). Frost damage also causes reduced growth and poor stem form (ANEKONDA and ADAMS, 2000). Occurrence of frost damage in provenance tests or field plantings is an indication of lack of compatibility between the genotype of the tree and the climate of the planting site (development is out of phase with the environ- ment). Thus, prevention of frost damage requires better matching between genotypes and environments.

Studies on conifer species in the Rocky Mountains have shown that populations are genetically differ-...
*P. devoniana* is widely used in Mexico for sawtimber, paper pulp and resin production. In the State of Michoacán in particular, the species is important for the local Native Indian handcraft industries, such as rustic furniture, marquetry and guitar parts (MUSÁLEM and SÁNCHEZ-CRUZ, 2003). Thus, success of *P. devoniana* plantations is very important not only for ecological restoration purposes, but also for the local economy, particularly that of the most impoverished segment of Mexican society, the Native Indians.

The goals of the present study are: a) to explore the patterning of genetic variation among *P. devoniana* populations along an altitudinal gradient, using provenance common garden tests and lab frost damage tests, and b) to delineate altitudinal seed zones as guidelines for controlling seed and seedling movement in reforestation.

Materials and Methods

Open pollinated seeds were collected from five to 10 trees (randomly selected among those trees bearing cones) from each of 16 natural *P. devoniana* populations. Collection was made along an altitudinal transect, from San Miguel del Monte (2450 m of altitude) to near Tumbisca village (1600 m of altitude), near Morelia, in the Mexican western State of Michoacán (Figure 1). The sampled populations were located approximately at every 50 m of altitudinal difference (Table 1). Sites at 1900 and 2000 m of elevation were not included because a low stand density made the sampling of at least 5 trees impossible. The trees represented by the samples are termed populations, whereas the location of a population is called provenance.

Seedlings were grown in 380 cm³ *Broadway Plastics* de México® containers on commercial *Creciroot*® substrate. When the seedlings were 7 months old, they were transplanted to a rectangular wooden-structure raised bed, filled with a 40 cm – layer of 4:1 mix of *Creciroot*® substrate and local Andosol forest soil, placed over a 20 cm layer of extrusive volcanic small stones for improving drainage. The raised bed was built inside a shade house (35% shade) where a common garden test was established. The experimental design was randomized complete blocks, with five blocks, 16 provenances and six seedlings per plot in a row. Seedlings were spaced at 15 cm within plots and 15 cm among plots. The first and last plots were flanked by a protection row from randomly chosen seedlings. Both nursery and raised bed experiments were located at the Instituto de Investigaciones Agropecuarias y Forestales, Michoacan University, near Morelia, Michoacán, México (1830 m of altitude, 19°42’LN , 101°11’LW, annual mean temperature 17.5°C, annual mean precipitation 776 mm, GARCIA, 1988). Seedlings were watered as needed.

An artificially-induced frost damage test, using visual injury scoring on needle tissue, was conducted in the laboratory. The technique was developed based on REHFELDT (1980, 1985). The test was conducted at a seedling age of 2.5 years (almost two years on the raised bed) which meant that the grass stage had been passed. The freezing treatments were conducted in December and January while the seedlings were in the winter rest stage. Three healthy fascicles located 5 cm below the bud were removed from each seedling in the morning following a day of irrigation. The seedlings, therefore,
were free of draught stress. Fascicles were sealed in plastic bags and stored in a refrigerator at 7°C for 12 hours. Then, samples were placed inside a cardboard box in a freezer set at 1°C; a thermistor was placed inside the box. Space limitations required that treatments were run separately for each block. When the samples reached 1°C, the temperature was slowly decreased at a rate of 2°C/hour, until a target freezing temperature of –9°C was reached. The target temperature was previously determined from the results of pilot tests, selected with the goal of achieving 30 to 70% damage (ANEKODA and ADAMS, 2000), too low a temperature caused close to 100% damage on all samples, whereas too mild temperatures did not cause damage. After reaching –9°C, the temperature was slowly increased at approximately the same rate (2°C/hour), until reaching 0°C. The samples were then thawed slowly by allowing the freezer to return to room temperature. The fascicles were then set out on a lab bench for 72 hours at room temperature, to allow cold injury symptoms to develop. Damaged tissues turned from green to brown, depending on the damage intensity. Damage was scored using a 0 to 100% score, were 0% was no damaged fascicles (intense green color, turgent, flexible), 100% was seriously damaged fascicles (brown color, dehydrated needles, easily forming kinks when bending), and intermediate damage had intermediate values (10%, 20% ... to 90%). To minimize bias, all assessments were made by the same scorer.

Data were transformed to the arcsine of the square root of frost damage proportion to each seedling. Statistical analyses included analysis of variance to test significance among populations using PROC GLM of SAS (SAS, 1988). Percent of contribution of variance component to total variance was estimated using PROC VARCOMP METHOD = REML (SAS, 1988). These analyses used the following statistical model:

\[
Y_{ijk} = \mu + B_i + P_j + B \times P_i + \varepsilon_{ijk} \quad [1]
\]

where \(Y_{ijk}\) = observation on the \(k^{th}\) seedling of the \(j^{th}\) population in the \(i^{th}\) block, \(\mu\) = overall mean, \(B_i\) = effect of the \(i^{th}\) block, \(P_j\) = effect of \(j^{th}\) population, \(B \times P_i\) = interaction of block by population, and \(\varepsilon_{ijk}\) = error term, \(i = 1, \ldots, b, j = 1, \ldots, p, k = 1, \ldots, n\), where \(b = 5\), \(p = 16\), and \(n = 6\), which are the numbers of blocks, populations, and seedlings-per-plot, respectively, not considering mortality. Blocks and populations were considered as random effects, from which inferences were to be made about the species as a whole.

Table 1. – Location of sixteen Pinus devoniana populations sampled along an altitudinal transect in the region of Morelia, State of Michoacán, western México.

<table>
<thead>
<tr>
<th>Num.</th>
<th>Altitude (m)</th>
<th>Locality</th>
<th>Site</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2450</td>
<td>SMM</td>
<td>Peña de San Pedro</td>
<td>19°35'52.10&quot;</td>
<td>101°07'55.5&quot;</td>
</tr>
<tr>
<td>2</td>
<td>2400</td>
<td>SMM</td>
<td>Peñas de San Pedro</td>
<td>19°35'55.70&quot;</td>
<td>101°07'52.10&quot;</td>
</tr>
<tr>
<td>3</td>
<td>2350</td>
<td>SMM</td>
<td>La Lobeca</td>
<td>19°35'50.40&quot;</td>
<td>101°07'41.60&quot;</td>
</tr>
<tr>
<td>4</td>
<td>2300</td>
<td>SMM</td>
<td>La Virgeneta</td>
<td>19°35'29.00&quot;</td>
<td>101°07'18.70&quot;</td>
</tr>
<tr>
<td>5</td>
<td>2250</td>
<td>RTS</td>
<td>Banco de balastre</td>
<td>19°34'57.40&quot;</td>
<td>101°07'17.80&quot;</td>
</tr>
<tr>
<td>6</td>
<td>2200</td>
<td>RTS</td>
<td>Banco de balastre</td>
<td>19°35'04.70&quot;</td>
<td>101°07'03.90&quot;</td>
</tr>
<tr>
<td>7</td>
<td>2150</td>
<td>RTS</td>
<td>Loma del Patio</td>
<td>19°35'17.30&quot;</td>
<td>101°06'48.10&quot;</td>
</tr>
<tr>
<td>8</td>
<td>2100</td>
<td>RTS</td>
<td>Loma del Patio</td>
<td>19°35'30.30&quot;</td>
<td>101°06'42.70&quot;</td>
</tr>
<tr>
<td>9</td>
<td>2050</td>
<td>RTS</td>
<td>La Cruz</td>
<td>19°35'34.80&quot;</td>
<td>101°06'38.10&quot;</td>
</tr>
<tr>
<td>11</td>
<td>1950</td>
<td>RTS</td>
<td>El Aserradero</td>
<td>19°35'12.30&quot;</td>
<td>101°06'17.70&quot;</td>
</tr>
<tr>
<td>13</td>
<td>1850</td>
<td>Tumbisca</td>
<td>El Cuitito</td>
<td>19°35'00.90&quot;</td>
<td>101°05'26.80&quot;</td>
</tr>
<tr>
<td>14</td>
<td>1800</td>
<td>Tumbisca</td>
<td>El Puertecito</td>
<td>19°35'05.90&quot;</td>
<td>101°05'02.60&quot;</td>
</tr>
<tr>
<td>15</td>
<td>1750</td>
<td>Tumbisca</td>
<td>Cerro Márquez</td>
<td>19°35'05.70&quot;</td>
<td>101°04'48.20&quot;</td>
</tr>
<tr>
<td>16</td>
<td>1700</td>
<td>Tumbisca</td>
<td>El Reparo</td>
<td>19°34'48.30&quot;</td>
<td>101°04'24.30&quot;</td>
</tr>
<tr>
<td>17</td>
<td>1650</td>
<td>Tumbisca</td>
<td>El Tularcillo</td>
<td>19°34'58.70&quot;</td>
<td>101°04'06.20&quot;</td>
</tr>
<tr>
<td>18</td>
<td>1600</td>
<td>Tumbisca</td>
<td>El Tularcillo</td>
<td>19°34'40.00&quot;</td>
<td>101°03'55.20&quot;</td>
</tr>
</tbody>
</table>

SMM = San Miguel del Monte, RTS = Road Tumbisca – San Miguel del Monte.
The relationship of genetic variation among populations to the altitude of the provenance was assessed with a linear model, using PROC REG (SAS, 1988). The model was:

\[ Y_{ij} = \beta_0 + \beta_1 X_i + \epsilon_{ij} \]  

where \( Y_{ij} \) = population mean of frost damage, \( \beta_0 \) = intercept, \( \beta_1 \) = regression parameter, \( X_i \) = altitude (m) of \( i \)th population origin, \( \epsilon_{ij} \) = error.

The least significant difference (LSD, \( \alpha = 0.20 \)) multiple mean comparison was conducted to estimate the altitudinal interval that must separate populations before one can be reasonably certain of genetic differentiation (Rehfelt, 1991).

Analysis of original and transformed data (arc sine of square root) gave essentially the same results; thus, results from regression analysis are presented using untransformed data, which is clearer for interpretation.

Results and Discussion

Genetic differences among provenances

There were significant differences among provenances for frost damage (\( P = 0.0261 \)) that accounted for 8% of the total variance (Table 2). Differences among provenances are structured as an altitudinal cline along which populations from lower altitude had more frost damage than populations from higher altitudes (\( r^2 = 0.8046, P = 0.0001 \), Figure 2).

The altitudinal cline of genetic variation among populations suggests that incidence of temperatures below zero along the P. devoniana altitudinal distribution has acted as a selective force resulting in genetic differentiation of populations; populations from lower altitudes are less tolerant to freezing temperatures than populations from higher altitudes. That trend is similar to what has been demonstrated for Pinus contorta (Rehfelt, 1985, 1988), for Pseudotsuga menziesii var. glauca (Rehfelt, 1989) in the Rocky Mountains and for Pseudotsuga menziesii var. glauca in western Oregon and Washington (StClaire, 2006). For the latest, genetic differentiation among populations is more pronounced for cold hardness than for other traits, such as bud burst, bud set and 2-year-old biomass; also fall cold hardness is strongly correlated to cold season temperatures of the source environment (StClaire, 2006).

The slope of the regression line (\( \beta_1 = -0.05161 \)) estimates the change in freezing damage along the altitudinal gradient. Thus, for our test, a freezing temperature of \(-9^\circ C\) would cause a decrease in injury of 5.2% for each increase of 100 m of provenance altitude, and for each decrease of 100 m of provenance altitude, there should be an increase of 5.2% of frost damage. Consequently, a population originated at 2000 m of altitude should have 20.8% more frost damage than a population originated at 2400 m.

The relationship between altitude and amount of frost damage also illustrates the amount of damage that can be avoided if the right provenance is chosen for a given reforestation site, and conversely, the amount of damage that will possibly occur when a wrong provenance is chosen. For example, if a site at the upper altitudinal limit (2450 m) of the P. devoniana natural distribution in the area is reforested using seed from the lowest altitudinal Table 2. – Analysis of variance for frost damage of sixteen P. devoniana provenances.

<table>
<thead>
<tr>
<th>S.V.</th>
<th>d.f.</th>
<th>%*</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>4</td>
<td>8.77</td>
<td>5.69</td>
<td>0.0006</td>
</tr>
<tr>
<td>Provenance</td>
<td>15</td>
<td>7.92</td>
<td>2.65</td>
<td>0.0261</td>
</tr>
<tr>
<td>Blo * Prov</td>
<td>56</td>
<td>13.16</td>
<td>1.84</td>
<td>0.0009</td>
</tr>
<tr>
<td>Error</td>
<td>254</td>
<td>70.16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Percentage of contribution of variance component to total variance.

Figure 2. – Linear regression of population average percentage of frost damage with respect to elevation of origin of provenance.
limit (1600 m) of the distribution, there will be an unnecessary 44.2% greater frost damage than that on seedlings originated from local seed would be, if a temperature of about −9 was reached during the winter dormant period.

However, it remains to be seen, what the compromise between frost damage and growth potential might be. It is known that populations from lower altitudes have more growth potential than populations from higher altitudes (REHFELDT, 1988, 1991; SÁENZ-ROMERO et al., 2006), and trees with more growth potential appeared to be more susceptible to cold injury (AITEKEN et al., 1996). Consequently, there tends to be a negative relationship between growth potential and freezing tolerance.

**Altitudinal seed zoning**

The least significant difference (LSD, $\alpha = 0.20$) between two populations was 19.99% of frost damage. The LSD ratio with respect to the regression coefficient suggests that populations separated by as little as 387 m are likely to be genetically different. Considering that the maximum altitudinal interval of *P. devoniana* natural distribution in the studied region is of 850 m of altitudinal difference (from 1600 to 2450 m), two seed zones would cover almost all the interval of the natural distribution. Rounding up the LSD to 400 m of altitudinal difference, the altitudinal seed zones for *P. devoniana* in the Morelia region can be simplified and defined as in Table 3, delimitation starting at 1600 m of altitude. Notice that an alternative criterion is to use altitudinal intervals of ± 200 m.

Suitable guidelines for ecological restoration would be:

(a) For reforestation of a given seed zone, using seedlings originated from the same seed zone, and alternatively (b) for reforestation of a site at a given altitude, using seedlings originated from seed collected from ± 200 m of altitude regarding the elevation of the reforestation site.

These practical guidelines should be viewed as provisional. Future analyses will consider population growth potential and will assess the importance of compromises between frost resistance and growth potential in limiting seed transfer. Field data from tests located at different altitudes and on different regions of the species natural distribution range would be of particular value.

Extrapolation of the use of suggested altitudinal intervals of ± 200 m for moving seeds and seedlings in other regions, where *P. devoniana* is distributed, is suggested with caution (although altitudinal value of upper and lower boundaries might shift). This is a reasonable management suggestion for the time while new data from provenance tests, eventually placed and evaluated in different regions, are available. Considering that altitude is a surrogate variable for temperature gradients (ROSENBERG, 1974) not only in Michoacán but anywhere in the species range, it is expected that limiting the altitudinal transfer of seed and seedling movement of *P. devoniana* to ± 200 m from the location of the seed collection to the reforestation site, will improve the matching between genotypes and environments.

Ideally, in the future, altitude limits of suggested seed zones need to be translated to climate variables, such as temperature, precipitation, degree days and annual moisture index (relationship between degree days and precipitation; see SÁENZ-ROMERO et al., 2006). However, that requires availability of enough weather data, something difficult to obtain in México and Guatemala. In any case, although provisional, the practical use of the current results is highly recommended, largely because no guidelines currently exist for this species in México.

**Conclusions**

Significant differences among provenances were detected for freezing injury. Genetic differences formed an altitudinal cline of decreasing injury with increasing altitude of the provenance. At a given freezing temperature (−9°C), for each increase or decrease of 100 m of altitude of the provenance, there should be a 5.2% decrease or increase in frost damage, respectively.

We suggest to split the altitudinal distribution of *P. devoniana* into two altitudinal seed zones of 400 m breadth (starting at 1600 m of altitude), and for reforestation of a given site to use seedlings originated from the same seed zone or within ± 200 m of altitude from the elevation of the reforestation site.

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**References**


Evaluation of Provenances of Eucalyptus camaldulensis and Clones of E. camaldulensis and E. tereticornis at Contrasting Sites in Southern India

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Abstract

A total of 188 open-pollinated families of Eucalyptus camaldulensis Dehnh. from 18 Australian natural provenances and 15 selected Indian families of the “Mysore Gum” land race were evaluated in three provenance-family trials at contrasting sites in southern India. At two years of age, the fastest growth was recorded at the driest site in Tamil Nadu, where E. camaldulensis provenances from Queensland were superior to those from Northern Territory and Western Australia, and the Indian land race. Provenance differ-