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## Altitudinal genetic variation in *Pinus hartwegii* Lindl. I: Height growth, shoot phenology, and frost damage in seedlings

Héctor Viveros-Viveros<sup>a,1</sup>, Cuahtémoc Sáenz-Romero<sup>b,\*</sup>, J. Jesús Vargas-Hernández<sup>a,1</sup>, Javier López-Upton<sup>a,1</sup>, Gustavo Ramírez-Valverde<sup>c</sup>, Amalio Santacruz-Varela<sup>d</sup>

<sup>a</sup> Forestal, Campus Montecillo. Colegio de Postgraduados. Km 36.5 Carretera México-Texcoco. Montecillo, Estado de México 56230, Mexico

<sup>b</sup> Centre canadien sur la fibre de bois, Service canadien des forêts, Ressources naturelles, 1055 rue du P.E.P.S., C.P. 10380, succ. Sainte-Foy, Québec (Québec) G1V 4C7, Canada

<sup>c</sup> Estadística, Campus Montecillo. Colegio de Postgraduados. Km 36.5 Carretera México-Texcoco. Montecillo, Estado de México 56230, Mexico

<sup>d</sup> Genética, Campus Montecillo. Colegio de Postgraduados. Km 36.5 Carretera México-Texcoco. Montecillo, Estado de México 56230, Mexico

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## ABSTRACT

The altitudinal pattern of genetic variation in *Pinus hartwegii* Lindl. populations was explored for seedling height growth, frost damage, grass-stage, and phenological stage of the terminal shoot. A provenance test was conducted with open-pollinated seed from 13 populations collected along an altitudinal transect (3000–3600 m) at the National Park Pico de Tancitaro, Michoacán, center-west Mexico. Height growth of seedlings in a nursery was assessed at seven and 18 months of age. Frost damage at  $-15\text{ }^{\circ}\text{C}$  was evaluated in laboratory at 18 months of age; proportion of plants that had left grass-stage and stage of shoot development was assessed at the age of 22 months. Significant differences among provenances ( $P < 0.0001$ ) were detected for all of the evaluated characters. The variation among populations was structured as a moderate altitudinal cline, with populations from lower altitudes showing larger height growth in seedlings, larger proportion of frost damages, fewer seedlings in grass-stage and more seedlings with developed shoot, whereas in populations from higher altitudes, seedlings exhibiting shorter plant height, lower percentages of frost damage, more seedlings with unbroken grass-stage, and fewer seedlings with advanced shoot development were displayed. Options for seed and seedling movement along the altitudinal gradient are discussed under the scope of reforestation, aiming at ecological restoration, conservation of forest genetic resources, and assisted migration considering global warming. We suggest delineation of two altitudinal seed zones (Zone I: 3000–3350 m; Zone II: 3350–3700 m).

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### 1. Introduction

*Pinus hartwegii* Lindl. is a pine species of the temperate-cold zones of Mexico, confined to the highest peaks and mountains of Mexico and Central America, between altitudes of 3000 and 4000 m (Martínez, 1948; Lauer, 1973; Lauer and Klaus, 1975; Perry, 1991). Its natural distribution is discontinuous at the sites of

highest altitude, from the State of Nuevo León in the northwest of Mexico (approximately  $25^{\circ}\text{ LN}$ ) to sites near the border between Guatemala and El Salvador (approximately  $14^{\circ}\text{ LN}$ ) (Perry, 1991). The annual mean temperature of the sites, where this species is found, varies between  $7.5$  and  $10\text{ }^{\circ}\text{C}$ , with annual precipitation around 1000 mm (Lauer, 1973, 1978).

*Pinus hartwegii* is of great ecological importance for being probably the most cold-tolerant tree in Mexico (Lauer, 1973, 1978; Lauer and Klaus, 1975; Viveros-Viveros et al., 2007). This species constitutes the altitudinal limit of tree vegetation in high mountains and volcanoes which are also important National Parks of Mexico, such as the Volcán of Colima, Pico de Tancitaro, Nevado de Toluca, Ajusco, Popocatepetl, Iztaccihuatl, Malinche, and Citlaltépetl (Pico de Orizaba) on the Neovolcanic Axis (Lauer, 1973, 1978; Lauer and Klaus, 1975; Vera-Vilchis and Rodríguez-Trejo, 2007).

Its unique and extreme altitudinal distribution makes *P. hartwegii* a highly vulnerable species in view of global warming

\* Corresponding author. Present address: Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo (IIAF-UMSNH). Km 9.5 Carretera Morelia-Zinapécuaro. Tarímbaro, Michoacán 58880, Mexico. Tel.: +52 443 334 0475x118; fax: +52 443 334 0475x200.

E-mail addresses: [hectorvv@colpos.mx](mailto:hectorvv@colpos.mx) (H. Viveros-Viveros), [csaenz@umich.mx](mailto:csaenz@umich.mx) (C. Sáenz-Romero), [vargashj@colpos.mx](mailto:vargashj@colpos.mx) (J.J. Vargas-Hernández), [uptonj@colpos.mx](mailto:uptonj@colpos.mx) (J. López-Upton), [gramirez@colpos.mx](mailto:gramirez@colpos.mx) (G. Ramírez-Valverde), [asvarela@colpos.mx](mailto:asvarela@colpos.mx) (A. Santacruz-Varela).

<sup>1</sup> Tel.: +52 595 952 0246.

since its exclusive habitat could be reduced or even disappear. The climates existing at present will in future occur at higher altitudes and latitudes (Hughes, 2000; Rehfeldt et al., 2004, 2006a; Rehfeldt, 2006). For example, it has been estimated that the climate recently occurring at the sites where *Pinus oocarpa* Schiede grows, in the region of Uruapan, Michoacán, Mexico, will occur at an altitude 300 m higher by the year 2090 (Sáenz-Romero et al., 2006) or at sites farther north, as far away as the low parts of the Sierra Nevada of California (Rehfeldt et al., 2006b).

At a warmer climate coming up in future, the species will face different scenarios: extinction, migration towards greater latitudes in the north, or towards higher altitudes, or the adjustment of their physiological processes to the new environmental conditions through mechanisms of phenotypical plasticity or adaptation (Ledig, 2004; Rehfeldt, 2004; Aiteken et al., 2008). Nevertheless, in the case of *P. hartwegii* the possibilities of migration are limited, since the high parts of the mountains are generally isolated among themselves (there is no natural connection among them), which might prevent the displacement of the populations. Furthermore, it has been estimated that the increase of temperature by 0.6 °C in the last 100 years has caused a diminution by 10.6% in its relative growth (Ricker et al., 2007).

In order to mitigate the potential negative effects of global warming on *P. hartwegii* it would be an alternative to carry out assisted migration, consistent in establishing reforestation using seeds of populations with better adaptation potential for future global warming than the local ones for a given site (St. Clair and Howe, 2007). This would be feasible if there were significant genetic variation among populations. Therefore, it is indispensable to know the genetic variation pattern among *P. hartwegii* populations.

It has been demonstrated that coniferous populations differentiate genetically in response to differential selection pressure occurring along the altitudinal gradient. The populations of lower altitudes have lower cold tolerance and higher growth potential (earlier shoot elongation, later growth cessation, and larger total shoot elongation) than those of higher altitude, in species like *Abies sachalinensis* (Schmidt) Mast. (Eiga and Sakai, 1984), *Pinus contorta* Carr. (Rehfeldt, 1985, 1988, 1989b), *Picea engelmannii* Carr. (Rehfeldt, 2004), *Pinus ponderosa* (Dougl.) Laws (Rehfeldt, 1991), *Pseudotsuga menziesii* (Mirb.) Franco (Rehfeldt, 1989a; St. Clair et al., 2005), *Tsuga heterophylla* (Raf.) Sarg. (Kuser and Ching, 1980), and *Podocarpus totara* (Benn. ex Don.) (Hawkins et al., 1991). Similar patterns have been described for Mexican coniferous populations distributed along the altitudinal gradients, such as *Pinus devoniana* Lindl. (also called *Pinus michoacana* Martínez) (Sáenz-Romero and Tapia-Olivares, 2008), and *P. oocarpa* (Sáenz-Romero et al., 2006).

The objectives of this study were: (a) to quantify the genetic variation existing among *P. hartwegii* populations along an altitudinal transect, with respect to plant growth potential, frost damage, plants that had left grass stage, and phenological stage of terminal shoot, (b) to delineate seed zones and seed transfer guidelines required for matching genotypes with the appropriate planting site environments, and (c) to suggest potential locations for establishing Forest Genetic Resources Conservation Units.

## 2. Materials and methods

### 2.1. Biological material and altitudinal transect

Seed samples were collected in the forests of the National Park Pico de Tancítaro, Michoacán, along a transect covering the natural altitudinal distribution of the species in the region, from 3000 to 3600 m of altitude. In the transect 13 sampling sites were

**Table 1**

Geographical and altitudinal localization of the *Pinus hartwegii* Lindl. populations in National Park Pico de Tancítaro, Michoacán, Mexico.

Population	Latitude (N)	Longitude (W)	Altitude (masl)
1	19° 25.120'	102° 18.750'	3600
2	19° 25.111'	102° 18.599'	3550
3	19° 25.179'	102° 18.589'	3500
4	19° 25.046'	102° 18.331'	3450
5	19° 25.128'	102° 18.399'	3400
6	19° 25.327'	102° 18.325'	3350
7	19° 25.416'	102° 18.187'	3300
8	19° 25.722'	102° 18.123'	3250
9	19° 26.036'	102° 17.998'	3200
10	19° 26.094'	102° 17.786'	3150
11	19° 26.221'	102° 17.454'	3100
12	19° 26.292'	102° 17.252'	3050
13	19° 25.967'	102° 16.972'	3000

established, one every 50 m of altitudinal difference (Table 1). At each site 11 healthy trees with cones were randomly selected, at a distance of at least 30 m from each other, to avoid possible effects of relatedness. Ten cones of each selected tree were taken and their altitudinal position was registered. Due to the very steep slopes at Pico de Tancítaro, the distances between sampled populations are short: between the highest and the lowest population there is about 1.85 km, and between the two most distant populations there is only 1.95 km.

Considering the discussion about the differences between *P. hartwegii* and *P. rudis* Endl. (Matos, 1995; Farjon and Styles, 1997), at the sites of lowest altitudes (3000 and 3050 m) it was avoided to include atypical individuals having longer cones and aciculae, than could reasonably be expected by the environmental effect of lower altitude. It was believed that such atypical individuals could probably be considered by many taxonomists as a taxon different from *P. hartwegii* (Martínez, 1948; Perry, 1991) or as a product of the hybridization between *P. hartwegii* and *P. montzuma* Lamb. (Matos and Schaal, 2000).

The seed was extracted from the cones manually, maintaining the identity per tree. In the month of July, 2005, the seed was put to germinate at environmental temperature. Germinated seeds were placed in rigid containers of 380 cm<sup>3</sup> (Broadway Plastics de Mexico<sup>®</sup>), with commercial Creci-root<sup>®</sup> substratum in a nursery of the Instituto de Investigaciones Agropecuarias y Forestales of the Universidad Michoacana de San Nicolás de Hidalgo (IIAF-UMSNH) in Morelia, Michoacán. In the nursery assay a randomized complete block design was used, with eight blocks, 13 populations, and nine plants per plot (experimental unit) in a row. In every plot, each of the nine plants originated from a different mother tree; in other words, the provenances were represented in the plots by nine half-sib families, and each half-sib family was represented by one single seedling per population per block. Additionally, a row of plants from families randomly taken was placed in the surroundings of the experiment in order to control the border effect.

Watering was provided as needed. Nitrogen fertilization (urea) was supplied twice (46-00-00, 0.2 g per seedling). Aphids *Pineus* sp. were controlled by two applications of systemic insecticide Confidor<sup>®</sup> (20 ml/l of water).

### 2.2. Characteristics evaluated in the plants

At seven (February, 2006) and 18 months of age (December, 2006), beginning with seed germination, total plant height was measured (mm). In the months of December 2006 and January 2007, frost damage in plants was assessed through artificial induction of low temperatures under laboratory conditions, based on the technique used by Rehfeldt (1980, 1985) and modified by

Sáenz-Romero and Tapia-Olivares (2008). Due to the limited space of the freezer, the evaluations were conducted separately for each of the test blocks (in other words, the test was conducted in eight runs, one run by each block); thus, in each frost damage run all the populations were represented (117 seedlings per run, from 13 populations and 9 families per population, when no previous mortality occurred), and the variation among the runs remained assigned to the blocks. In order to avoid effects of water stress, the tests were initiated one day after watering the plants. Five sound fascicles from the upper part of the shoot were taken, 5 cm below the terminal bud. These were put into hermetically sealed plastic bags and kept in refrigeration (4 °C) for 12 h. Afterwards, the bags were placed in cardboard boxes, in a freezer at 1 °C during 12 h, and immediately, the temperature was reduced at a rate of 2 °C per hour, until reaching –15 °C. In previous assays, at this temperature, damages varying from 30 to 70% appeared in plants of different *P. hartwegii* populations; within these damage levels, it is more feasible to distinguish differences among populations or genotypes (Anekonda and Adams, 2000). After reaching –15 °C, the temperature gradually increased at a rate of 2 °C per hour until reaching 0 °C. Then the samples were removed from the freezer and taken out of the bags, keeping them at environmental temperature (approximately 20 °C) during 72 h, until the damage symptoms became evident. The damages were evaluated visually, establishing the damage percentage in foliage according to its severity in categories at intervals of 10%. Zero percent corresponded to fascicles without damage (intense green color, turgent, and flexible), whereas 100% corresponded to the completely damaged fascicles (brown color, dehydrated, and rigid) (Sáenz-Romero and Tapia-Olivares, 2008).

On April 3rd, 2007 (at 22 months of age), the percentage of plants per population that had left grass stage was assessed assigning the values of 1 to plants having already left the grass stage (that is, with elongated buds or finishing this process, and with >6 cm stem height), and values of 0 to plants that had not yet left grass stage (without elongated buds, and with ≤6 cm stem height). The phenological stage of the terminal shoot was assessed only with those seedlings having already left grass stage, using a variable denominated stage of shoot development, assigning values of 1 to the plants at advanced stage of terminal shoot development (stem height ≥10 cm and elongated needles on the shoot), or values of 0 to the plants at an early stage of shoot development (6 cm > stem height < 10 cm, and without needles on the shoot). With these data, the proportion of plants with advanced shoot development per population was estimated for each experimental unit.

2.3. Statistical analysis

For total plant height at seven and 18 months of age as well as for the frost damage, an analysis of variance was conducted by

means of the following complete model:

$$Y_{ijk} = \mu + \beta_i + \tau_j + \beta_i \times \tau_j + F_k(\tau_j) + E_{ijk} \tag{1}$$

where:  $Y_{ijk}$  = value of the  $ijk$ -th observation,  $\mu$  = general mean,  $\beta_i$  = effect of the  $i$ -th block,  $\tau_j$  = effect of the  $j$ -th population (altitude above sea level),  $\beta_i \times \tau_j$  = interaction of the  $i$ -th block with the  $j$ -th population,  $F_k(\tau_j)$  = effect of the  $k$ -th family nested in the  $j$ -th population, and  $E_{ijk}$  = experimental error. All effects were assumed to be random.

For the percentage of plants that had left grass stage and percentage of plants at advanced stage of shoot development, a variance analysis was carried out, utilizing the mean values per experimental unit by means of GLM of SAS procedure (SAS Institute, 1988), based on the following incomplete statistical model:

$$Y_{ij} = \mu + \beta_i + \tau_j + E_{ij} \tag{2}$$

where:  $Y_{ij}$ , value of the  $ij$ -th experimental unit;  $\mu$ , general mean;  $\beta_i$ , effect of the  $i$ -th block;  $\tau_j$ , effect of the  $j$ -th population (altitude); and  $E_{ij}$ , experimental error.

In order to determine the altitudinal pattern of genetic variation, the relationship existing among the mean values per population of the assessed characteristics with altitude above sea level of the sites was modeled, using the procedure REG of SAS (SAS Institute, 1988) with the following statistic model:

$$Y_{ij} = \beta_0 + \beta_1 X_i + \varepsilon_{ij} \tag{3}$$

where:  $Y_i$ , population mean;  $\beta_0$ , intercept;  $\beta_1$ , regression parameter;  $X_i$ , altitude ( $m$ ) of  $i$ -th provenance; and  $\varepsilon_{ij}$ , error.

Least significant difference (LSD) of population means was used to estimate the altitudinal intervals that must separate populations before one can be reasonably certain of genetic differentiation (Rehfeldt, 1991, 1994). LSD ( $\alpha = 0.20$ ) of those variables with high correlation against altitude, were translated to an altitudinal interval, which was used as limit for delineating altitudinal seed zones for seed guideline and seedling transfers (Rehfeldt, 1983, 1991; Sáenz-Romero and Tapia-Olivares, 2008).

The degree of association among the variables was evaluated by analysis of correlation among the means per population of all the variables using CORR of SAS procedure (SAS Institute, 1988).

3. Results and discussion

3.1. Genetic differences among populations

Significant differences ( $P < 0.0001$ ) were found among *P. hartwegii* populations in all the evaluated characteristics (Table 2). Despite the short geographical distances between populations (only up to 1.95 km), the differential selective forces apparently are strong enough to promote genetic differentiation

**Table 2**  
Mean square, percentage of contribution to total variance (%) and significance level ( $P$ ) of plant characteristics of 13 *Pinus hartwegii* Lindl. populations.

Trait	Block			Population			Block × Population			Family (Population)			Error	
	MS	%	$P$	MS	%	$P$	MS	%	$P$	MS	%	$P$	MS	%
Height at 7 months	0.7	6.0	0.0001	1.9	3.0	0.0001	0.7	7.9	0.0001	0.5	3.0	0.0311	0.4	80.1
Height at 18 months	61.9	3.7	0.0001	47.3	3.0	0.0001	12.0	3.5	0.0121	21.7	14.9	0.0001	8.5	74.9
Frost damage	15590.0	13.6	0.0001	3024.1	2.4	0.0001	1346.2	7.8	0.0001	1152.8	6.2	0.0001	688.6	70.0
Plants that had left grass stage	801.4	12.1	0.0012	1183.5	32.2	0.0001							210.5	55.7
Stage of shoot development	334.7	3.5	0.0966	1275.3	40.9	0.0001							185.3	55.6

Error terms used for  $F$  test for seedling height and frost damage were for block:  $MS_{Block \times Population}$ ; for populations:  $MS_{Block \times Population} + MS_{Family (population)} - MS_{Error}$ ; for interaction block × population and for family (population):  $MS_{Error}$ . For plants that had left grass stage and for the stage of shoot development, the error term for block and populations was:  $MS_{Error}$ .

among populations, even under the likely presence of an active gene flow (mainly thought pollen) among populations (Bradshaw, 1972).

The percentage of contribution of the populations to total variance for total seedling height at seven and 18 months of age was 3%; in frost damage the populations contributed with 2.4% (Table 2). These values are close to those reported on plant height at 30 months of age (3.3%) in *P. oocarpa* (Sáenz-Romero et al., 2006). The contribution of populations to total variance for the percentage of plants that had left grass stage and for the percentage of plants with advanced stage of shoot development was very high: 32.2% and 40.9%, respectively. This indicates important genetic differentiation among populations for shoot elongation dynamics. However, the comparison among traits for population contribution to total variance needs to be taken with caution, because for percentage of plants with breakage of the grass stage and stage of shoot development, there was no estimation of the variation among families within populations, as there was for seedling height and frost damage.

### 3.2. Altitudinal pattern of genetic variation

The correlation between the population mean and the altitude of their origin was negative for all the variables, but the importance of correlation was variable for the different characters, and in the case of plant height it was different according to plant age.

Plant height showed negative association with the altitude of the site of origin; the plants of populations from lower altitudes grew taller than those of higher altitude (Fig. 1). This pattern was more pronounced at 7 months of age ( $r = -0.71$ ,  $P = 0.0065$ , Fig. 1a) than at 18 months ( $r = -0.53$ ,  $P = 0.0630$ , Fig. 1b). This pattern of altitudinal genetic differentiation is common in several conifer species, but the magnitude of negative association between plant height growth and altitude of the population can be variable in each species. For example, a highly pronounced altitudinal pattern has been found in *Pseudotsuga menziesii* [Mirb.] Franco var. *glauca* Beissn. at 3 years of age ( $r = -0.81$ ), when populations from lower altitude grow taller (Rehfeldt, 1989a), but the correlation is less marked in *Larix occidentalis* Nutt. ( $r = -0.47$ ) (Rehfeldt, 1982). Also, the patterning can vary among species. In some cases, populations from middle elevation grow taller than those from lower or higher elevation (Ohsawa and Ide, 2007), as for example, in the case of *Pinus brutia* (Isik and Kara, 1997), and for basal diameter of 6-month-old *P. oocarpa* seedlings (Sáenz-Romero et al., 2004).

Maternal effects like seed size could have accentuated altitudinal differentiation in plant height at 7 month of age in the present study. Since seed size was not evaluated, we cannot estimate the magnitude of the possible maternal effect nor rule it out. In *Pinus taeda* L. and *Pseudotsuga menziesii* [Mirb.] Franco var. *menziesii*, for example, it has been found that seed size influences total plant height during the first months of age and generates confusion with the growth potential associated to genetic factors (Dunlap and Barnett, 1983; Sorensen and Campbell, 1985; St. Clair and Adams, 1993). However, there is also evidence that it is not recommended to adjust nursery results to seed weight for two-year-old *Pseudotsuga menziesii* seedlings, because maternal effects seem to have also a genetic component (Sorensen and Campbell, 1993).

The damages caused by low temperatures showed a marked pattern of genetic altitudinal differentiation. The plants with provenance from populations of lower altitude presented more frost damage than those from higher altitude ( $r = -0.71$ ,  $P = 0.0061$ , Fig. 2). Based on the statistical model (3), the regression equation ( $r^2 = 0.5098$ ,  $P = 0.0061$ ) was obtained (Fig. 2), which indicates that for every 100 m less altitude of population, the frost

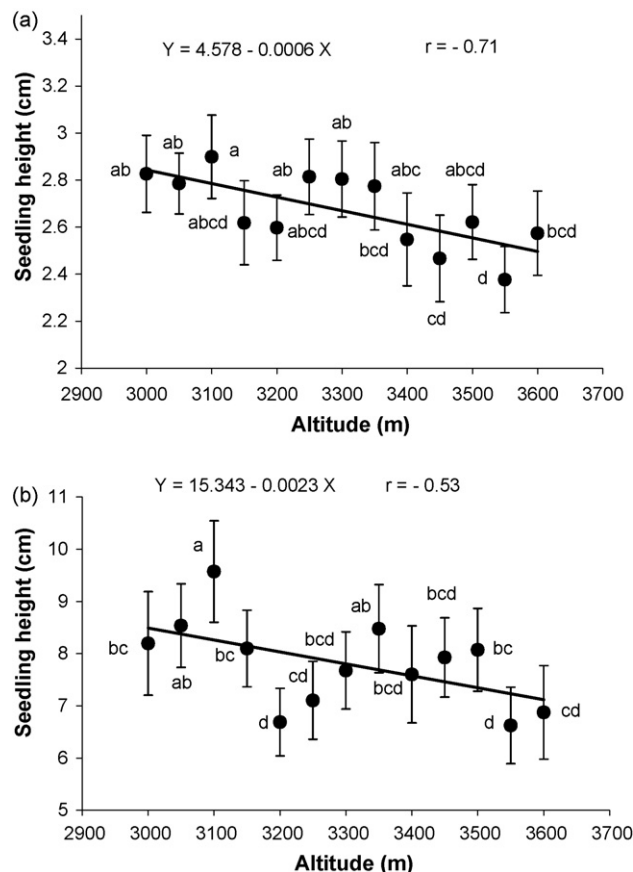


Fig. 1. Relationship among means of plant height per population at 7 (a) and 18 (b) months of age, against altitude of origin of 13 *Pinus hartwegii* Lindl. populations. The vertical bars represent standard deviation and different letters indicate different mean grouping according to Duncan ( $\alpha = 0.05$ ) test.

damages increase by approximately 2.6%. For example, it is expected that the plants from sites at an altitude of 3600 m (highest altitudinal limit of their distribution) may present mean damage of 53%, while the plants from an altitude of 3000 m (lowest limit) would have damages of 69%. A similar pattern was found in *P. devoniana*, where for every 100 m of altitudinal difference there was a differential of 5.2% of frost damage (Sáenz-Romero and Tapia-Olivares, 2008).

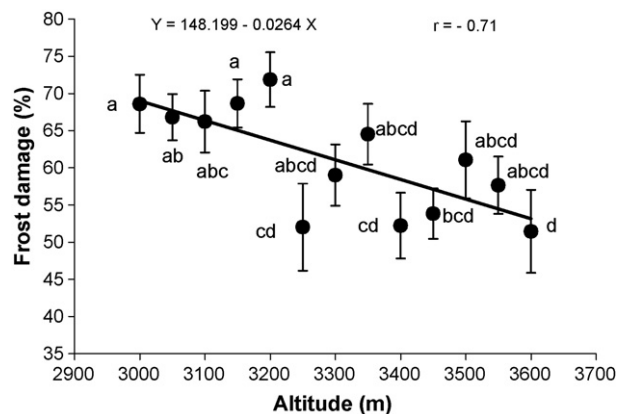
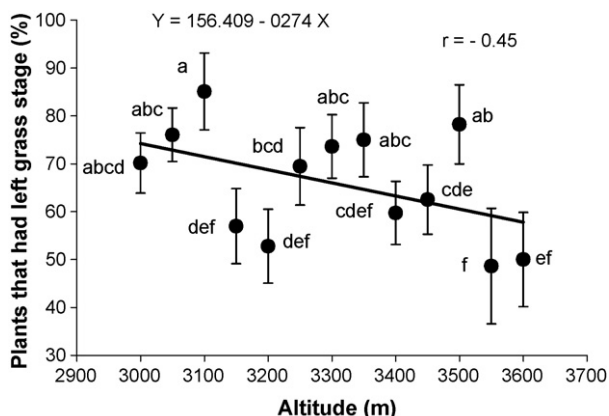


Fig. 2. Relationship between the percentage of frost damage per population and altitude of origin of 13 *Pinus hartwegii* Lindl. populations. The vertical bars represent standard deviation and different letters indicate different mean grouping after Duncan ( $\alpha = 0.05$ ) test.

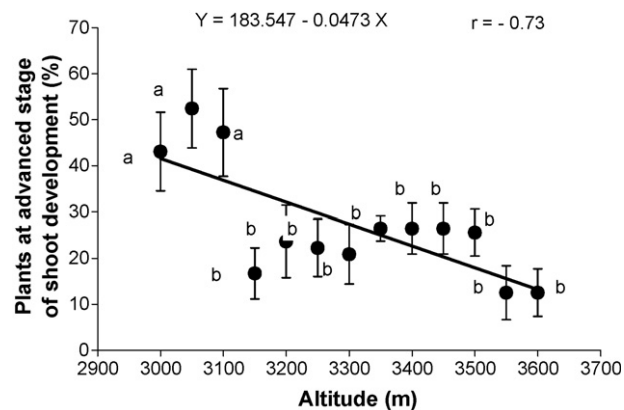


**Fig. 3.** Relationship between the percentage of plants having left grass stage at 22 months of age per population and altitude of origin of 13 *Pinus hartwegii* Lindl. populations. The vertical bars represent standard deviation and different letters indicate different mean grouping after Duncan ( $\alpha = 0.05$ ) test.

A negative relationship between frost damage and altitude has also been found for *Pseudotsuga menziesii* var. *glauca* ( $r = -0.66$ ; Rehfeldt, 1989a), *Pseudotsuga menziesii* var. *menziesii* ( $r = -0.75$ ; Martínez-Mier et al., 2005), and *Pinus contorta* Dougl. spp. *latifolia* Engelm. ex Watson ( $r = -0.46$ , Wei et al., 2001 and  $r = -0.65$ , Rehfeldt, 1985). However, it is notorious that within the populations there is an important source of variation in this characteristic, which is reflected in standard deviation of the population mean as well as in the contribution of the families to total variance (6.2%,  $P = 0.0011$ , Table 2). This genetic variability among and within populations can be important for plant adaptation facing the new conditions generated by the climatic change.

The percentage of plants which at the age of 22 months had already left grass stage, presented negative correlation with the altitude of the site of origin (Fig. 3), though relatively weak ( $r = -0.45$ ) and not significant ( $P = 0.1181$ ). Nevertheless, the results show important variability among populations with respect to the proportion of individuals having broken grass stage at this age. There are no previously published studies that show the dynamics of grass stage with respect to population altitude in this species, as far as we know.

The stage of terminal shoot development at the age of 22 months (by April 3rd, 2007, taking into account only those plants that had left grass stage), also showed a marked pattern of genetic differentiation among the populations negatively associated to altitude ( $r = -0.73$ ,  $P = 0.0046$ ), where the populations of lower elevation had shoots with higher degree of development compared to populations of higher altitude (Fig. 4). Globally, this indicates that the plants of lower altitude initiate shoot development earlier or at more accelerated growth rate than the plants originally coming from higher altitudes. Analyzing the data in detail, such pattern actually is differentiated in two population groups: one group made up of three populations originated from an altitude between 3000 and 3100 m, with earlier or quicker development,



**Fig. 4.** Relationship between percentage of plants at an advanced stage of shoot development per population and altitude of 13 *Pinus hartwegii* Lindl. populations. The vertical bars represent standard deviation and different letters indicate different mean grouping after Duncan ( $\alpha = 0.05$ ) test.

and a second larger group composed by the remaining populations (from an altitude of 3150–3600 m) with later or slower development. Similarly, in *Pinus contorta* var. *latifolia* populations, a negative association has been demonstrated, between the degree of shoot development and the altitude of the populations ( $r = -0.69$ , Rehfeldt, 1985). The same relationship becomes evident in the moment when the period of seasonal growth finishes, in the plants of *Pseudotsuga menziesii* var. *menziesii* ( $r = -0.4$ , Griffin and Ching, 1977).

However, there is a significant association between the fact that plants had left grass stage or not, and their height growth and the stage of shoot development. This is reflected in the magnitude of correlation between the percentage of individuals having left grass stage, and the variables plant height at 18 months of age ( $r = 0.821$ ,  $P = 0.001$ ) and stage of shoot development ( $r = 0.704$ ,  $P = 0.007$ ) (Table 3). In other words, the populations which had the largest proportion of individuals having left grass stage at 22 months, had the greatest mean values of height at 18 months, and the largest percentage of individuals with further advanced stage of shoot development at the age of 22 months. It is not possible to completely separate the effect of continuance or not from grass stage and growth potential, due to the fact that shoots of the plants, having not yet left grass stage, could not have developed more than 6 cm by the month of April (22 months of age). Based on the aforesaid, it is convenient to carry out studies similar to the present one in a longer term, which allows assessing the growth potential once all the plants surpass the grass stage.

The differentiation among populations associated to the altitudinal gradient found in all the analyzed variables, indicates that along the altitudinal gradient where *P. hartwegii* is distributed, on the Pico of Tancítaro, Michoacán, there is a differential selection, probably due to differences of temperature and precipitation, associated to altitude, strong enough to promote genetic differentiation among the populations, as an adaptive mechanism (Endler, 1977; Rehfeldt, 1988, 1989a, 1989b).

**Table 3**

Pearson's correlation coefficient ( $r$ ) and significance ( $P$ , in parenthesis) of variables evaluated in plants of 13 *Pinus hartwegii* Lindl. populations.

Traits	Height at 18 months	Frost damage	Plants that had left grass stage	Stage of shoot development
Height at 7 months	0.619 (0.024)	0.372 (0.211)	0.769 (0.002)	0.632 (0.020)
Height at 18 months		0.407 (0.167)	0.821 (0.001)	0.750 (0.003)
Frost damage			0.245 (0.421)	0.465 (0.109)
Plants that had left grass stage				0.704 (0.007)

### 3.3. Seed zoning and implications of the study

Due to the fact that there are significant differences among populations in total plant height at seven and 18 months of age, in frost damage, and in proportion of plants that have left grass stage and reached an advanced stage of shoot development at 22 months of age, it is important to delineate altitudinal seed zones and to establish criteria for moving seed according to altitude, with the purpose of conducting ecological restoration, commercial plantations, and defining strategies of genetic conservation.

For frost damages, the least significant difference ( $\alpha = 0.2$ ) indicates that two populations are significantly different if they differ by 9.12% or more in average frost damage. Therefore, the LSD ratio with respect to the regression coefficient suggests that populations separated by 346 m are likely to be genetically different. The breadth of a seed zone was defined rounding up such altitudinal difference to 350 m. A similar result was found for 7-month-old seedling height (another variable with large association to altitude).

For practical purposes, we suggest the delimitation of two seed zones, the first zone delimited between 3000 and 3350 m of altitude, and the second zone between 3350 and 3700 m (Table 4). The first zone has its lower limit at the lowest altitudinal distribution of the populations in the studied area, and also aims at capturing the variability of a set of lower altitude populations that seems to behave distinctively: they have the largest values of frost damage (populations between 3000 and 3200 m, Fig. 2) and also the largest and most distinctive shoot development (between 3000 and 3100 m, Fig. 4). The second seed zone aims at capturing the variability of most of the remaining populations, from 3350 m to the upper altitudinal limit.

For ecological restoration, the seeds collected in a given seed zone must be used within the same zone, or within an interval of  $\pm 175$  m from the place where it was collected, which corresponds to half of the breadth of an altitudinal seed zone (Table 4). This way, it would be expected that the frost damage of the plant at the planted site will not be significantly different from the expected frost damage if the plants were from local origin. If plants of the populations from lower elevation are utilized to reforest the highest seed zones, the risk of high mortality because of frost damages has been run. On the contrary, if seed of populations from higher altitude is used to reforest areas of lower altitude, inferior mean growth will be obtained than at utilizing local seeds.

However, for commercial plantations (purposes at present not made in the studied area, but that could eventually be done in future, or in other *P. hartwegii* natural distribution areas), it might be worth moving seeds from the first to the second seed zone, and to run the risk of having some frost damage if such loss is surpassed by the gains in growth potential.

Nevertheless, the aforementioned will be modified because of global warming, since it is foreseen that the risk of frost damages will diminish in the near future. It is also reasonable to assume that if only local seed is used for reforestation, in future the populations will be gradually more stressed by the higher temperature without increase of precipitation (Sáenz-Romero et al., 2006). Therefore, to mitigate the potential negative effects of global warming, it may be

preferable to move the seed upwards according to altitude than to use local seed. To what extent would altitudinal displacement upwards have to be made? The response to such question requires estimates of the global warming for the region at different altitudes.

For genetic conservation of the natural populations, one desirable first step is to establish Forest Genetic Resources Conservation Units (FGRCUs) (Sáenz-Romero and Tapia-Olivares, 2003; Sáenz-Romero et al., 2003). A FGRCU is a representative natural stand of any species with a management priority to maintain genetic diversity, as well as to allow natural evolutionary forces to mold the genetic structure of the population (Ledig, 1988, 1992; Millar and Libby, 1991; Sáenz-Romero et al., 2003). We propose to establish at least one FGRCU in each seed zone. The FGRCU also can be used as seed source for restoring natural populations. Guidelines for using seed originated from a FGRCU would be basically the same as for ecological restoration.

Seed zone delimitation might be needed for other areas of México where *P. hartwegii* occurs at even higher altitudes than at Pico de Tancitaro; for example, on the volcanoes Volcán de Colima, Nevado de Toluca, Popocatepetl, Iztaccíhuatl, and Citlaltépetl, where populations distribute between altitudes of 3000 and 4000 m (Perry, 1991). Lacking results of provenance tests of populations collected on those volcanoes, perhaps an acceptable management decision could be to use temporarily seed zones with basically the same criteria as in this report, delineating for those volcanoes one additional seed zone for the upper part of *P. hartwegii* distribution. Thus, seed zones could have limits at: 3000, 3350, 3700, and 4050 m.

On the other hand, given that the variation within populations was higher than that among populations (Table 2), the possibility arises that plants of each population naturally achieve certain adaptation to the new environmental conditions, generated in view of the climatic change, thanks to survival of certain families within every population.

## 4. Conclusion

Significant differences among populations were found for total plant height at 7 and 18 months of age, damages due to low temperatures, breakage of the grass stage, and percentages of plants with well-developed shoots in *P. hartwegii*. The differentiation pattern found was moderately clinal, associated to altitude above sea level of the site of origin of each population. The population from lower elevation had taller plants, larger percentages of frost damage, higher proportion of plants that had left grass stage, and higher percentage of plants with advanced shoot development.

We suggest to delineate two seed zones within the altitudinal distribution of *P. hartwegii*, each zone of 350 m of altitudinal breadth. The genetic differentiation shown among populations suggests, for management purposes, to transfer seeds and plants in intervals of  $\pm 175$  m, but preferably to use local seed. In order to lessen the potential effects of global warming, it is suggested to transfer seeds or plants from lower to higher altitude, though it still must be determined to what extent altitudinal transfer should be carried out.

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**Table 4**  
Limits and ranges of two *Pinus hartwegii* Lindl. altitudinal seed zones.

Seed zone	Altitudinal limits (m)		Range	Interval ( $\pm$ )
	Lower	Upper		
1	3000	3350	350	175
2	3350	3700	350	175

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## References

- Aiteken, S.N., Yeaman, S., Holliday, J.A., Wang, T., Curtis-McLane, S., 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evol. Appl.* 1, 95–111.
- Anekonda, T.S., Adams, W.T., 2000. Cold hardiness testing for Douglas-fir tree improvement programs: guidelines for a simple, robust and inexpensive screening method. *West. J. Appl. For.* 15, 129–136.
- Bradshaw, A.D., 1972. Some of the evolutionary consequences of being a plant. *Evol. Biol.* 5, 25–47.
- Dunlap, J.R., Barnett, J.P., 1983. Influence of size on germination and early development of loblolly pine (*Pinus taeda* L.) germinants. *Can. J. Forest Res.* 13, 40–44.
- Eiga, S., Sakai, A., 1984. Altitudinal variation in freezing resistance of Saghalien fir (*Abies sachalinensis*). *Can. J. Bot.* 62, 156–160.
- Endler, J.A., 1977. *Geographic Variation, Speciation and Clines*. Princeton University Press, Princeton, N. J., USA.
- Farjon, A., Styles, B.T., 1997. *Pinus* (Pinaceae). *Flora Neotrópica Monograph* 75. New York Botanical Garden, N.Y.
- Griffin, A.R., Ching, K.K., 1977. Geographic variation in Douglas-fir from the coastal ranges of California. I. Seed, seedling growth and hardiness characteristics. *Silvae Genetica* 26, 149–157.
- Hawkins, B.J., Sweet, G.B., Greer, D.H., Bergin, D.O., 1991. Genetic variation in the frost hardiness of *Podocarpus totara*. *N.Z. J. Bot.* 29, 455–458.
- Hughes, L., 2000. Biological consequences of global warming: is the signal already apparent? *Trends Ecol. Evol.* 15, 56–61.
- Isik, K., Kara, N., 1997. Altitudinal variation in *Pinus brutia* Ten. and its implication in genetic conservation and seed transfers in southern Turkey. *Silvae Genetica* 46 (2–3), 113–120.
- Kuser, J.E., Ching, K.K., 1980. Provenance variation in phenology and cold hardiness of Western Hemlock seedlings. *Forest Sci.* 26, 463–470.
- Lauer, W., 1973. The altitudinal belts of the vegetation in the central Mexican highlands and their climatic conditions. *Artic Alpine Res.* 5, 99–113.
- Lauer, W., 1978. Timberline studies in central Mexico. *Artic Alpine Res.* 10, 383–396.
- Lauer, W., Klaus, D., 1975. Geocological investigations on the timberline of Pico de Orizaba, Mexico. *Artic Alpine Res.* 7, 315–330.
- Ledig, F.T., 1988. The conservation of diversity in forest trees. *Bioscience* 38, 471–478.
- Ledig, F.T., 1992. A comprehensive strategy for the conservation of forest genetic resources. In: *Proceeding of the First International Symposium on the Development of Natural Resources and Environmental Protection*, Institute of Natural Resources and Environment, Korea University, Seoul, Korea, October 13–18, pp. 325–344.
- Ledig, F.T., 2004. Conservación y manejo de recursos genéticos forestales. In: Vargas-Hernández, J.J., Bermejo-Velázquez, B., Ledig, F.T. (Eds.), *Manejo de Recursos Genéticos Forestales*. CONAFOR-Comisión Forestal de América del Norte, México, pp. 3–18.
- Martínez, M., 1948. *Los Pinos Mexicanos*. Ediciones Botas, México D.F.
- Martínez-Mier, A., Mondino, V., Gallo, L., 2005. Evaluación de daños por heladas tardías en ensayos de procedencias de pino oregón introducidos en el norte de la región Andino Patagónica Argentina. *Bosque* 26, 113–120.
- Matos, J.A., 1995. *Pinus hartwegii* and *P. rudis*: a critical assessment. *Syst. Bot.* 20, 6–21.
- Matos, J.A., Schaal, B.A., 2000. Chloroplast evolution in the *Pinus montezumae* complex: a coalescent approach to hybridization. *Evolution* 54, 1218–1233.
- Millar, C.L., Libby, W.J., 1991. Strategies for conserving clinal, ecotypic, and disjunct population diversity in widespread species. In: Falk, D.A., Holtsinger, K.E. (Eds.), *Genetic and Conservation of Rare Plants*. Oxford University Press, New York, pp. 149–172.
- Ohsawa, T., Ide, Y., 2007. Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. *Global Ecol. Biogeography* 17, 152–163.
- Perry, J.P., 1991. *The Pines of Mexico and Central America*. Timber Press, Portland, Oregon, USA.
- Rehfeldt, G.E., 1980. Cold acclimation in populations of *Pinus contorta* from the Northern Rocky Mountains. *Botanical Gazette* 141, 458–463.
- Rehfeldt, G.E., 1982. Differentiation of *Larix occidentalis* populations from the Northern Rocky Mountains. *Silvae Genetica* 31, 13–19.
- Rehfeldt, G.E., 1983. Seed transfer guidelines for Douglas-fir in central Idaho. USDA For. Serv. Res. Note INT-337.
- Rehfeldt, G.E., 1985. Ecological genetics of *Pinus contorta* in the Lower Snake River Basin of Central Idaho. USDA For. Serv. Res. Note INT-354.
- Rehfeldt, G.E., 1988. Ecological genetics of *Pinus contorta* from the Rocky Mountains (USA): a synthesis. *Silvae Genetica* 37, 131–135.
- Rehfeldt, G.E., 1989a. Ecological adaptations in Douglas-fir (*Pseudotsuga menziesii* var. *glauca*): a synthesis. *Forest Ecol. Manag.* 28, 203–215.
- Rehfeldt, G.E., 1989b. Genetic variances and covariances in freezing tolerance of lodgepole pine during early winter acclimation. *Silvae Genetica* 38, 133–137.
- Rehfeldt, G.E., 1991. A model of genetic variation for *Pinus ponderosa* in the Inland Northwest (USA): applications in gene resource management. *Can. J. Forest Res.* 21, 1491–1500.
- Rehfeldt, G.E., 1994. Evolutionary genetics, the biological species, and the ecology of the Interior cedar-hemlock forest. In: *Proceedings of Interior cedar-hemlock-white-pine forest: ecology and management*, Pullman, Washington State University, pp. 91–100.
- Rehfeldt, G.E., 2004. Interspecific and intraspecific variation in *Picea engelmannii* and its congeneric cohorts: biosystematics, geneecology, and climate change. *Gen. Tech. Rep. RMRS-GTR-134*. USDA For. Serv.
- Rehfeldt, G.E., 2006. A spline climate model for western United States. *Gen. Tech. Rep. RMRS-GTR-165*. USDA For. Serv.
- Rehfeldt, G.E., Tchebakova, N.M., Parfenova, E., 2004. Genetic responses to climate and climate-change in conifers of the temperate and boreal forests. *Recent Res. Dev. Genetics Breeding* 1, 113–130.
- Rehfeldt, G.E., Crookston, N.L., Warwell, M.V., Evans, J.S., 2006a. Empirical analyses of plant-climate relationships for Western United States. *Int. J. Plant Sci.* 167, 1123–1150.
- Rehfeldt, G.E., Warwell, M.V., Crookston, N.L., Evans, J.S., 2006b. Using biotic community and species-specific bioclimatic models to invasive species and impacted ecosystems. Poster presented in: *Invasive species research; strengths, needs and future plans*. Albuquerque, New Mexico. March 22–24, 2006.
- Ricker, M., Gutiérrez-García, G., Daly, D.C., 2007. Modeling long-term tree growth curves in response to warming climate: test cases from a subtropical mountain forest and a tropical rainforest in México. *Can. J. Forest Res.* 37, 977–989.
- SAS Institute, 1988. *SAS/STAT User's Guide*, Release 6.03 Edition. SAS Institute, Cary, NC, 1028 pp.
- Sáenz-Romero, C., Snively, A., Lindig-Cisneros, R., 2003. Conservation and restoration of pine forest genetic resources in Mexico. *Silvae Genetica* 52, 233–237.
- Sáenz-Romero, C., Tapia-Olivares, B.L., 2003. *Pinus oocarpa* isoenzymatic variation along an altitudinal gradient in Michoacán. *México Silvae Genetica* 52, 237–240.
- Sáenz-Romero, C., Viveros-Viveros, H., Guzmán-Reyna, R., 2004. Altitudinal genetic variation among *Pinus oocarpa* populations on Michoacán, western México. Preliminary results from a nursery test. *Forest Genetics* 11, 343–349.
- Sáenz-Romero, C., Guzmán-Reyna, R., Rehfeldt, G.E., 2006. Altitudinal genetic variation among *Pinus oocarpa* populations in Michoacán, México; implications for seed zoning, conservation of forest genetic resources, tree breeding and global warming. *Forest Ecol. Manag.* 229, 340–350.
- Sáenz-Romero, C., Tapia-Olivares, B.L., 2008. Genetic variation in frost damage and seed zone delineation within an altitudinal transect of *Pinus devoniana* (*P. michoacana*) in México. *Silvae Genetica* 57 (3), 165–170.
- Sorensen, F.C., Campbell, R.K., 1985. Effect of seed weight on height growth of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var. *menziesii*) seedlings in a nursery. *Can. J. Forest Res.* 15, 1109–1115.
- Sorensen, F.C., Campbell, R.K., 1993. Seed weight–seedling size correlation in coastal Douglas-fir: genetic and environmental components. *Can. J. For. Res.* 23, 275–285.
- St. Clair, J.D., Adams, W.T., 1993. Family composition of Douglas-fir nursery stock as influenced by seed characters, mortality, and culling practices. *New Forests* 7, 319–329.
- St. Clair, J.D., Mandel, N.L., Vance-Borland, K.W., 2005. Geneecology of Douglas-fir in western Oregon and Washington. *Ann. Bot.* 96, 1199–1214.
- St. Clair, J.D., Howe, G.T., 2007. Genetic maladaptation of coastal Douglas-fir seedlings to future climates. *Global Change Biol.* 13, 1441–1454.
- Vera-Vilchis, V.y., Rodríguez-Trejo, D.A., 2007. Survival and height increment of *Pinus hartwegii* two years after prescribed burns and experimental forest fires. *Agrociencia* 41, 219–230.
- Viveros-Viveros, H., Sáenz-Romero, C., López-Upton, J., Vargas-Hernández, J.J., 2007. Growth and frost damage variation among *Pinus pseudostrobus*, *P. montezumae* and *P. hartwegii* tested in Michoacán, México. *Forest Ecol. Manag.* 253, 81–88.
- Wei, R.P., Lindgren, K., Lindgren, D., 2001. Parental environment effects on cold acclimation and height growth in lodgepole pine seedlings. *Silvae Genetica* 50, 252–257.