As regards future improvement, a study on benefit of combination of breeding strategies in a stage-wise manner would be of value, e.g. phenotypic selection for growth rhythm traits followed by reselection by clonal testing (e.g. Cotterill 1984).

Acknowledgement

We gratefully acknowledge the "Kempestiftelserna" and the EU project FAIR5 PL97 3823 "Wood quality in Birch" for the financial support as well as Ola Rosvall for the valuable comments.

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Landscape Genetic Structure of *Pinus banksiana:* Seedling Traits

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(Received 15th December 2000)

Abstract

The extent and patterning of genetic diversity at a landscape scale (30 km x 30 km) was investigated using seedlings from 47 stands of *Pinus banksiana* Lamb. collected in a pine-oak barrens in west-central Wisconsin, USA. Seedlings grown for six months in a greenhouse were evaluated for the number of cotyledons, the length of the longest cotyledon, the number of early needle fascicles, seedling height, timing of bud set, and the dry weight of roots, foliage, stem and total seedling, shoot:root ratio and foliage:root ratio. A pronounced genetic structure exists for most traits, with stands showing significant differentiation at geographic distances up to 25 km. Seedlings originating from trees growing on sandy sites were larger than those from sandy-loam sites. The scale and pattern of differentiation for several traits parallels the scale and pattern of soil variation on the landscape, supporting the hypothesis that stand genetic differentiation corresponds to a gradient of environmental differences. The combined effect of soil texture, drainage and ground-water influence, apparently are the primary selective forces influencing among-stand genetic differentiation for the traits under study within this landscape. The results could be useful in a program of genetic resource management.

Key words: Landscape, genetic variation, genetic differentiation, quantitative traits, *Pinus banksiana*, spatial statistics, autocorrelation, kriging, forest gene conservation.

Introduction

Ecological factors help shape genetic architecture in plant populations, with correlations between environmental and

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genetic patterns of variation partly explained by adaptations to prevailing environmental conditions (YOUNG, 1995). Phenotypic differentiation can be observed in plants at many spatial scales, whenever localized selection forces are sufficiently intense (LINHART and GRANT, 1996). However, gene flow is a formidable "cohesive" force that reduces or prevents genetic differentiation among plant populations. Thus, the extent of population differentiation often viewed as the result of two opposing forces: natural selection and gene flow (HAMRICK *et al.*, 1992; LINHART and GRANT, 1996).

Evidence for the effects of environmental selection and genetic differentiation among plant populations even with intense gene flow in wind-pollinated species has been reviewed by YOUNG (1995). Well-studied examples include adaptation to different soil regimes, including heavy metal tolerance (e.g. ANTONOVICS *et al.*, 1971; WU *et al.*, 1975), or other fertility differences (DAVIES and SNAYDON, 1976). Cultural practices such as mowing regimes can give rise to differentiation in growth form (prostrate and erect) in adjacent populations (WARWICK and BRIGGS, 1978). Several conifer species also exhibit substantial genetic differentiation along altitudinal clines, including *Larix occidentalis* (REHFELDT, 1982), *Picea engelmannii* (REHFELDT, 1994), *Pinus contorta* (REHFELDT, 1988; XIE and YING 1995), *Pinus ponderosa* (REHFELDT, 1991, 1993; SORENSEN, 1994; SORENSEN and WEBER, 1994) and *Pseudotsuga menziezii* (CAMPBELL, 1979, 1986, 1991; REHFELDT, 1989).

Range-wide patterns of morphological variation in *Pinus banksiana* (jack pine) are linked to geographic clines, most of which are associated with climatic gradients (SCHOENIKE, 1976; RUDOLPH and YEATMAN, 1982). Phenological traits such as shoot elongation, timing of bud set and growth rate vary clinally and are correlated with photoperiod and length and temperature of the growing season (RUDOLPH, 1964; RUDOLPH and YEATMAN, 1982). Seedling traits also exhibit clinal patterns, with seed weight, number of cotyledons, seedling height, foliage and root dry weight all increasing with longer and warmer growing seasons at the seed origin (GIERTYCH and FARRAR, 1962; YEATMAN, 1965).

Resource managers need better information on the extent and patterning of genetic diversity at "landscape" scales on the order of 50 to 900 km^2. However, there are few studies of adaptive genetic variation of trees at such small geographical scales. One study documented genetic differentiation in root growth between adjacent jack pine stands growing in different moisture regimes (WRIGHT *et al.,* 1992). A second study identified a clinal pattern of genetic variation in jack pine along gradients of climatic differences in an area of approximately 90,000 km^2 (NIEJENHUIS and PARKER, 1996).

In the present study, we examine the patterning of genetic variation in quantitative traits for jack pine seedlings to determine whether apparently small environmental factors can lead to genetic differentiation on a local geographic scale. If genetic differentiation does exist among local stands, what is the spatial scale of such differentiation and what environmental factors might give rise to patterns of genetic variation? We approached these questions by studying the spatial patterning of genetic variability for quantitative traits of jack pine seedlings using seed collected from 47 stands representing a jack pine barrens landscape in west-central Wisconsin.

Material and Methods

Study area

A study area of approximately 30 km x 30 km was delineated in the Black River State Forest and Jackson County Forest in west-central Wisconsin, USA. We will refer to this study area as the "landscape", a region characterized by pine and oak barrens, with nearly level sand plains and sporadic sandstone buttes, and soils ranging from droughty infertile sands to more fertile outwash and dune sands (HOLE and GERMAIN, 1994). The vegetation is a mosaic dominated by woody plants including *Pinus banksiana* (jack pine), *P. resinosa* (red pine), *P. strobus* (white pine), *Acer rubrum* (red maple), *Quercus alba* (white oak), *Q. rubra* (northern red oak), *Q. palustris* (pin oak) and *Quercus velutina* (black oak) (CURTIS, 1959; KOTAR and BURGER, 1996), interspersed with extensive areas of marshlands and commercial cranberry bogs (*Figure 1*). The topography of the area is essentially flat, with elevations between 270 to 300 m above sea level (State of Wisconsin 1970), without important gradients in temperature (average annual temperature of 7.9 °C) or precipitation (average annual precipitation of 776 mm). Jack pine is a fire-maintained species (RUDOLPH and YEATMAN, 1982, and others), but stands are variable in composition and age due to frequent disturbance events, including past fires, pest outbreaks, timber harvesting and other land use. Most of the land is in public ownership today (either state or county), but agriculture was the dominant land use during the first half of the 20th century.

Field sampling

We selected stands dominated by jack pine for seed collection using the existing division of the landscape into 'management units', aggregates of relatively homogeneous forest stands designated by the Wisconsin Department of Natural Resources. Our goal was to cover as much of the landscape as possible, sampling at least one stand for each 'management unit' dominated by jack pine. Sampled stands were not in a regular grid, since jack pine has a patchy and irregular distribution within the landscape mosaic (*Figure 1*). The exact location of the sampled trees was influenced by our requirement that stands be separated by a minimum distance of 500 m to decrease the probability that sampled stands had a recent common origin from the same seed source.

During 1994 and 1995, we collected open-pollinated cones from ten to fourteen randomly-selected jack pine trees from each of 47 stands. We will refer to the progenies of these trees as "half-sib families" or simply "families". Most collections were made in August-September, when cone ripening occurs to minimize any bias toward trees bearing serotinous cones. Stands contained a mixture of serotinous and open-coned trees, and none were exclusively serotinous-coned individuals. We recorded on a map (scale = 1:50000) the position of each stand sampled, using coordinates in meters, and pooled the position of the trees from the same stand for an "average" location. Most sampled trees were separated by 30–50 m, with collections covering 2–3 ha.

Soil-Moisture classes

We defined 'soil-moisture classes' as a combination of characteristics of soil and habitat types. Stands were classified to major soil types based on soil survey maps at a scale 1:20000 obtained from the Wisconsin Department of Natural Resources. The 47 sampled stands corresponded to twelve recognized soil types. Soil types were then sorted by two criteria, texture and drainage, in that order (*Table 1*). Stands were also assigned to 'habitat types' following the methodology of KOTAR and BURGER (1996). All the sampled stands corresponded to either of two habitat types, a dry habitat (PVHa = *Pinus-Vaccinium-Hamamelis*) or a dry-mesic habitat (PVRh = *Pinus strobus-Vaccinium-Rubus hispidus*) (*Table 1*). Both habitat types are similar, but on the dry-mesic habitat, ground water influence is

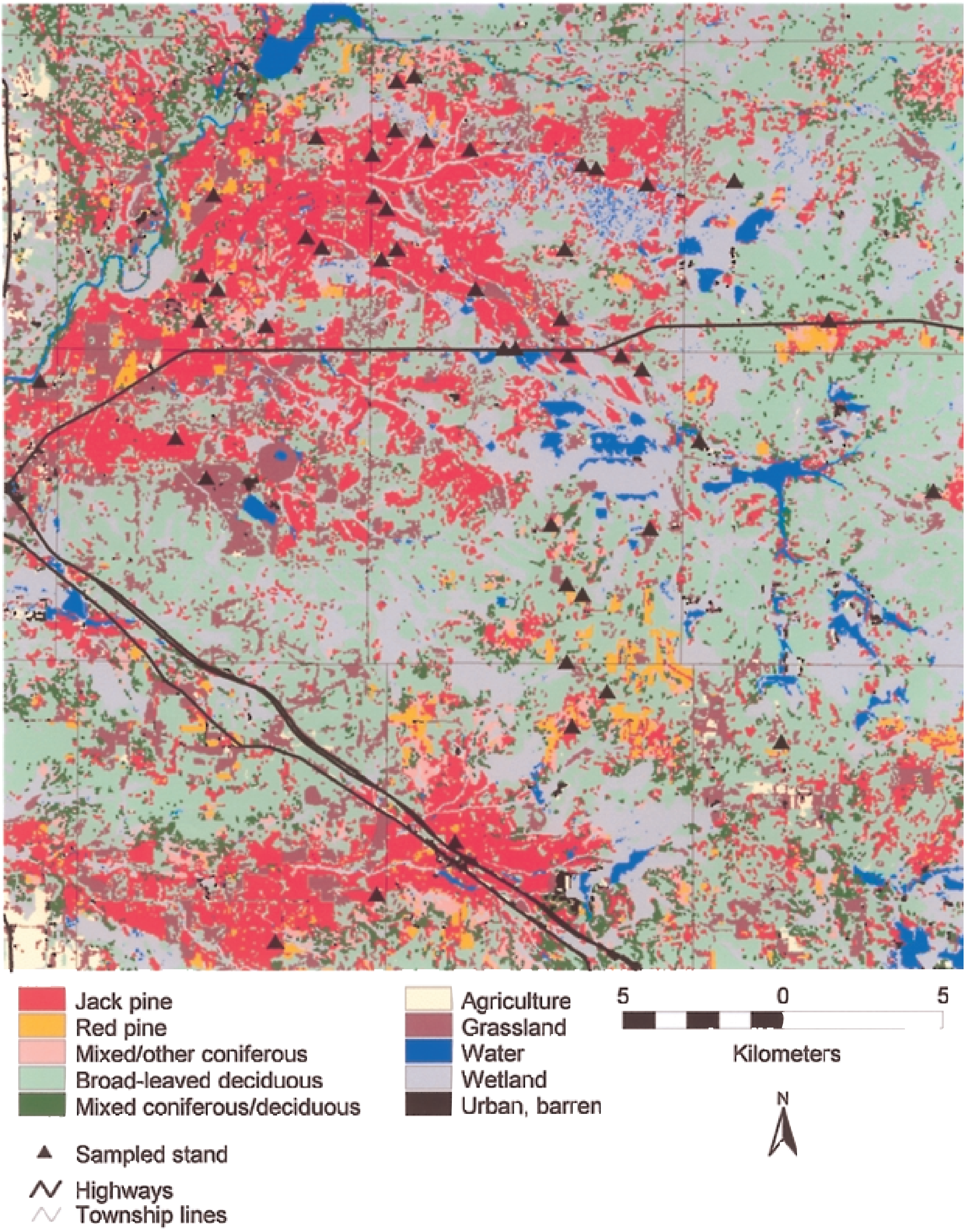

Figure 1. – Vegetation map of the study area indicating the 47 stands sampled in the Black River State Forest/Jackson County Forest, Wisconsin, USA. Source: Wisconsin Department of Natural Resources.

near the surface, usually within 1 m (KOTAR and BURGER, 1996). Stands corresponding to the several combinations of texture, drainage and habitat-type characteristics were grouped into five 'soil-moisture classes', numbered from I to V to reflect an apparent gradient of moisture caused by the combined effect of texture, drainage and ground-water influence (*Table 1*). Some soil-moisture classes overlap with some soil types, a feature that we attribute to overlapping biophysical features such as soil moisture, soil texture and distance to underground water, probably at different scales, making it impossible to establish sharp boundaries among categories of our classification. The number of stands belonging to soil-moisture classes I, II, III, IV and V were 16, 6, 8, 6 and 11, respectively.

Greenhouse stand / progeny test

A randomly chosen sub-sample of three trees per each of the 47 collected stands was drawn from the larger collection of 82 stands. The full collection was used for another study of allo-

Table 1. – Soil-moisture classes (I to V) based on soil and habitat characteristics for 47 *Pinus banksiana* stands in west-central Wisconsin.

Soil type			Habitat type	Soil-moisture classes
Soil classes	Texture	Drainage		
TrB Tarr Sand, TrC Tarr sand, MaB Mahtomedi sand	Sandy	Extremely well	Dry*	I
TtA Tint sand, RkA Rockdam sand	Sandy	Moderate to poor	Dry*	II
RkA Rockdam sand, IxA Ironrun Ponycreek complex, Pv Ponycreek-Dawsil complex	Sandy	Moderate to very poor	Dry-Mesic†	III
LxB Ludington-Fairchild sands, MoB.Merit-Gardenvale silt loams, FeA Fairchild-Elm Lake	Sandy-loamy	Moderate to very poor	Dry*	IV
FeA. Fairchild-Elm Lake complex, FaA Fairchild sand, MrA Merrillan-Veedum complex	sandy-loamy to Sandy-loamy-clayey	Very poor	Dry-Mesic†	V

*Habitat PVHa: *Pinus-Vaccinium-Hamameli.*

† Habitat PVRh: *Pinus strobus-Vaccinium-Rubus hispidus* with ground water influence.

zyme variation reported elsewhere, and limited seed supply made it impossible to use all stands for both studies. Seeds were stratified 15 days and then germinated at room temperature in Petri dishes. Due to a combination of poor germination and limited seed, we had enough germinated seed from only three families from each of 13 stands, two families from each of 13 stands and one family from each of 21 stands. These provided a total of 86 half-sib families from 47 stands. Germinated seeds were transplanted to 15 x 4 x 4 cm 'root-trainers' in a greenhouse at the University of Wisconsin - Madison, Wisconsin. The growing medium was a steam-sterilized mix of 50:50 Plainfield sand and No.2 Farfard Growing Mix (peat, perlite and vermiculite, 1:1:1 by volume). The experimental design used randomized complete blocks with 6 replications, 86 half-sib-families and 4-seedling single-row-plots.

Seedling variables measured included number of cotyledons (one-month), length of the longest cotyledon (two-months), number of fascicles of secondary needles (three-months), timing of bud set (number of weeks from 16 May 1996 to the set of a resting bud) and total height (six-months). At the end of the growing season (October), one randomly chosen seedling per plot was harvested for estimation of dry weight of foliage, stem, root and total seedling (the sum of dry weight of root, stem and foliage), aerial shoot:root and foliage:root ratios.

Analysis of variance

We conducted an analysis of variance for each response variable and an estimation of the variance components using the General Linear Model Procedure (GLM) and the VARCOMP procedure of the Statistical Analysis System (SAS 1988). For the variables 'height', 'number of cotyledons', 'length of longest cotyledon', 'number of fascicles' and 'timing of bud set', all of which included 4 seedlings per plot, the statistical model was:

$$[1] \qquad Y_{ijkl} = \mu + \beta_i + \tau_j + \beta_i{*}\tau_j + \Psi_k(\tau)_j + \beta_i{*}\psi_k(\tau)_j + \varepsilon_{ijkl}$$

where: Y_{ijkl} = observation of the l^{th} seedling of the k^{th} family nested into the j^{th} stand in the i^{th} block, μ = general mean, β_i = effect of the i^{th} block, τ_j = effect of the j^{th} stand, $\beta_i{*}\tau_j$ = effect of the interaction between the i^{th} block and the j^{th} stand, $\psi_k(\tau)_j$ = effect of the k^{th} family nested into the j^{th} stand, $\beta_i{*}\psi_k(\tau)_j$ = effect of the interaction between the i^{th} block and the k^{th} family nested into the j^{th} stand, and ε_{ijkl} = error term.

For all the variables involving dry weight: 'foliage', 'stem', 'root', 'total', 'aerial shoot:root' ratio and 'foliage:root ratio' (only one seedling per plot), we used the following model:

$$[2] \qquad Y_{ijk} = \mu + \beta_i + \tau_j + \beta_i{*}\tau_j + \psi_k(\tau)_j + \varepsilon_{ijk}$$

All the effects were considered random.

We explored simple correlations among all the measured variables using Procedure CORR (SAS 1988).

Analysis including soil-moisture classes

Relationships between seedling traits and environmental features were examined using analysis of variance models with soil-moisture classes as one classification variable. The models used are identical to model [1], and [2], but with 'soil-moisture classes' added as a source of variation. Stands were nested within soil-moisture classes and corresponding interaction terms were added. The model for 'number of cotyledons', 'length of longest cotyledon', 'number of fascicles', 'timing of bud set' and 'height' was:

$$[3] \qquad Y_{hijkl} = \mu + \alpha_h + \beta_i + \tau_j(\alpha)_h + \alpha_h{*}\beta_i + \beta_{i*}\tau_j\,(\alpha)_h \\ + \Psi_k(\tau(\alpha))_{hj} + \beta_i{*}\psi_k(\tau(\alpha))_{hj} + \varepsilon_{hijkl}$$

where α_h = effect of the h^{th} soil-moisture class; $h = 1, ..5$; $\tau_j(\alpha)_h$ = effect of the j^{th} stand nested into the h^{th} soil-moisture class; $\alpha_h{*}\beta_i$ = effect of the interaction h^{th} soil-moisture class with i^{th} block; $\beta_i{*}\tau_j(\alpha)_h$ = effect of the interaction i^{th} block with the j^{th} stand nested into h^{th} soil-moisture class; $\psi_k(\tau(\alpha))_{hj}$ = effect of the k^{th} family nested into the j^{th} stand nested into the h^{th} soil-moisture class; $\beta_i{*}\Psi_k(\tau(\alpha))_{hj}$ = effect of the interaction between the i^{th} block, and the k^{th} family nested into the j^{th} stand nested into the h^{th} soil-moisture class. Soil-moisture class was considered a random effect. The model for dry weight variables was:

$$[4] \qquad Y_{hijk} = \mu + \alpha_h + \beta_i + \tau_j(\alpha)_h + \alpha_h{*}\beta_i + \beta_{i*}\tau_j\,(\alpha)_h \\ + \Psi_k(\tau(\alpha))_{hj} + \varepsilon_{hijk}$$

We obtained least square means for each soil moisture class, using models [3] and [4], for those traits that were significantly different among soil-moisture classes. We estimated Pearson product moment correlation coefficients (r) between least square means for seedling traits per soil moisture class and the soil-moisture classes.

Spatial variation

Spatial patterning across the landscape was explored by estimating Moran's I values for several distance intervals (Sokal and Oden, 1978), where:

[5] $$I = n \Sigma w_{ij} z_i z_j / W \Sigma z_i^2$$

where n is the number of stands under comparison, w_{ij} is the weight given to the join between localities *i* and *j* (the values are 1 for a pair of localities within the distance class for which the autocorrelation coefficient is being calculated and 0 if it is not), $z_i = X_i - X$ where X_i is the value of the variable for stand *i* and X is the mean of the variable for all stands, $z_j = X_j - X$ where X_j is the value of the variable for stand *j* , and $W = \Sigma w_{ij}$

Estimates of Moran's I were made for all traits by using as X_j and X_j the least square means for each stand obtained from ANOVA models [1] and [2] for the respective variables. An additional analysis of variance was used to estimate the least square means for 10 stands which had only a single family representing the stand (using averages per stand per block).

We calculated Moran's I values for 8 distance intervals: 2, 4, 6, 8, 10, 15, 20 and 25 km using Gamma Design Software (1995). Previously, we screened many combinations of 'step distances' but this set provided the best balance between the minimum and maximum distances sampled among stands, and the requirement that a sufficient number of pairs of joins per distance class exist for statistical testing purposes. The number of joins per interval of distance was: 70, 194, 226, 266, 246, 572, 374 and 184, respectively.

Test of significance for Moran's I values ($I_{observed}$) were conducted by contrasting estimated values of t against tabled values:

[6] $$t = (I_{expected} - I_{observed}) / (\text{square root of } \mu_2)$$

[7] $$I_{expected} = -1/(n-1)$$

and μ_2 is defined under the randomization assumption, as (Sokal and Oden, 1978):

[8] $$\mu_2 = \frac{n[(n^2-3n+3)S_1-nS_2+3W^2]-b_2\,[(n^2-n)S_1-2nS_2+6W^2]}{(n-1)(n-2)(n-3)W^2} - (n-1)^{-2}$$

where $S_1 = (1/2)\Sigma\,(w_{ij} + w_{ji})^2$, $S_2 = \Sigma\,(w_{i.} + w_{.j})^2$, and $w_{i.}$ and $w_{.j}$ are sums of the *i*th row and *j*th column of the weight matrix, respectively, and $b^2 = \Sigma\, z^4 / (\Sigma\, z^2)^2$, a sample coefficient of kurtosis.

Landscape maps were developed for soil-moisture classes and total dry weight values of seedlings, using 'kriging' techniques. A kriging map represents interpolated values of points not physically sampled and is made after fitting a regression model with semivariance values from the points physically sampled (Legendre and Fortin, 1989). A semivariance (γ) for a given distance (*h*) is defined as (Rossi *et al.*, 1992):

[9] $$\gamma_h = [1/(2N_h)]\,[\Sigma(x_i + x_i + {}_h)^2]$$

where N_h = number of pairs of points separated by distance *h*; x_i and x_{i+h} = measured sampled value at points *i* and *i+h*, respectively. We estimated semivariances by entering the least square means by stand as x_i and x_{i+h} using the same interval distances used for estimation of Moran's I. We fitted semivariance values to a set of isotropic and anisotropic regression models: linear, linear/sill, spherical, exponential and Gaussian and selected the "best" model as the one producing the largest r^2 and smallest sum of squares of the error (Rossi *et al.*, 1992). The selected model was used for interpolation by blocks representing 750 m^2 portions of the actual landscape, excluding large polygons that had no sampled stands. Interpolated values where used for generating maps. Estimation of semivariances, model fitting, kriging and mapping were conducted using GS+ (Gamma Design Software 1995).

Results

Among Stand Variation

Analyses of variance revealed significant differences among stands for number of cotyledons, length of the longest cotyledon, number of fascicles, seedling height, timing of budset (*Table 2*), dry weight of root, foliage, stem and total seedling weight (*Table 3*). No significant differences were detected for shoot:root and foliage:root ratios. Significant differences existed among families-within-stands for the number of cotyledons, length of longest cotyledon, number of fascicles and timing of bud set (*Table 2*), but not for seedling height nor for any of the dry weight traits (*Table 3*).

We attribute the significant differences among blocks in most of the traits to temperature differences within the greenhouse, where blocks near the greenhouse walls received more sun irradiation and drier earlier than blocks located in the middle of the greenhouse.

Four sets of traits appeared to be correlated (Pearson correlation > |0.5| and P-value < 0.001) including: (a) height, dry weight of root, foliage, stem and total seedling, (b)

Table 2. – Percentage contribution to total variance, test of significance and averages of seedling traits for 47 *Pinus banksiana* stands in west-central Wisconsin.

Source of Variation	df	Number of cotyledons	Length of longest cotyledon	Number of fascicles	Height	Timing of bud set
Stand	46	5 **	14 **	5 **	4 **	6 **
Block	5	0 ns	1 ***	9 ***	7 ***	5 ***
Stand x Block	230	2 **	2 *	0 ns	0 ns	0 ns
Family (Stand)	39	11 ***	33 ***	7 ***	2 ns	3 **
Block x Family (Stand)	194	0 ns	6 ***	15 ***	26 ***	9 ***
Error	1/	81	44	63	60	77
Average		4.4 units	21.0 mm	4.5 units	50.7 mm	22.1weeks

Significance: * = P < 0.1; ** = P < 0.05; *** = P < 0.01; ns = no significant. 1/ Error's degrees of freedom for each trait are 1402, 1369, 1364, 1355 and 1133, respectively.

Table 3. – Percentage contribution to total variance, test of significance and averages of seedling traits for 47 *Pinus banksiana* stands in west-central Wisconsin.

Source of Variation	df	Root	Foliage	Stem	Total	Shoot:Root ratio	Foliage:Root ratio
Stand	46	6 *	8 *	8 **	8.5 **	1.4 ns	0.23 ns
Block	5	12 ***	9 ***	9 ***	10.8 ***	5.3 ***	1.08 ***
Stand x Block	215	0 ns	1 ns	0 ns	0.0 ns	0.0 ns	0.25 ns
Family (Stand)	39	0 ns	4 ns	0 ns	0.3 ns	0.4 ns	0.30 ns
Error	167	82	78	83	80.4	92.8	0.30
Average		188 mg	290 mg	43 mg	521 mg	1.85 ratio	1.62 ratio

Significance: * = P < 0.1; ** = P < 0.05; *** = P < 0.01; ns = no significant.

shoot:root ratios and foliage:root ratios, (c) the number of fascicles and timing of bud set (negatively correlated), and (d) the number and the length of cotyledons. This last set was not highly correlated (Pearson correlation < |0.36|) with other variables.

A principal component analysis (SAS 1988) yielded four principal components which we interpreted as: (a) a 'biomass' component including height and all dry weight traits, (b) an 'architecture' component including the shoot:root and folaige:root ratios, (c) a 'phenology' component (d) a 'cotyledons' component. Analyses using these four principal components yielded essentially the same results as for the sets of individual variables and are not presented.

Variation among soil-moisture classes

When 'soil-moisture class' was included as a source of variation, several traits differed among 'soil-moisture classes' but stand-level variation was reduced in significance (*Tables 4* and *5*). 'Soil-moisture class' as a variance component, contributes in average to 5.7% of total variance (averaging across those traits that were significantly different for 'soil moisture classes'), which approximately is twice as much as the variance explained by 'stand' (2.6% in average). Within stand variation contributes with the largest proportion of the total variance: 77.7% (a result from adding 7.3% of family average and 70.4% of the average error term). Remaining traits behaved in a different fashion: timing of bud set (*Table 4*), aerial shoot:root ratios and foliage:root ratios (*Table 5*) were not significantly different among soil-moisture classes.

Negative correlations were observed between least square means for several seedling traits and the 'soil-moisture classes', including the number of cotyledons (–0.98), length of the longest cotyledon (–0.80), dry weight of roots (–0.84), foliage (–0.81), stem (–0.73) and total seedling (–0.82) with P-values of 0.002, 0.053, 0.039, 0.047, 0.078 and 0.046, respectively. The relationship between 'soil-moisture classes' and the number of cotyledons or dry weight of seedlings illustrate typical situations (*Figure 2*).

Spatial variation

Among-stand differentiation for seedling traits is indicated by significant and negative autocorrelations at 25 km for length of the longest cotyledon (*Figure 3.A*), dry weights of stems (*Figure 3.B*), roots (*Figure 3.C*), and total seedling (*Figure 3.D*). Correlograms without significant autocorrelations are not shown.

Table 4. – Percentage contribution to total variance, test of significance and averages of seedling traits for 47 *Pinus banksiana* stands in west-central Wisconsin. Soil-moisture classes (Soil) are included as a source of variation.

Source of Variation	df	Number of cotyledons	Length of longest cotyledon	Number of fascicles	Height	Timing of bud set
Soil	4	4.9 ***	14.1 ***	1.70 *	0.9 *	0.6 ns
Stand (Soil)	42	1.3 ns	2.1 ns	3.98 *	3.6 *	5.3 **
Block	5	0.0 ns	0.7 ***	9.35 ***	6.9 ***	5.3 ***
Soil x Block	20	0.0 ns	0.0 ns	0.09 ns	1.1 ns	0.4 ns
Block x Stand (Soil)	210	2.8 **	2.5 *	0.00 ns	0.0 ns	0.0 ns
Family [Stand (Soil)]	39	10.6 ***	32.1 ***	6.85 ***	2.1 ns	2.9 **
Block x Family [Stand (Soil)]	194	0.0 ns	6.0 ***	15.18 ***	26.0 ***	8.6 ***
Error	1/	80.3	42.5	62.85	59.5	76.8
Average		4.4 units	21 mm	4.5 units	50.7 mm	22.1 weeks

Significance: * = P < 0.1; ** = P < 0.05; *** = P < 0.01; ns = no significant. 1/Error's degrees of freedom for each trait are 1402, 1369, 1364, 1355 and 1133, respectively.

Table 5. – Percentage contribution to total variance, test of significance and averages of seedling traits for 47 *Pinus banksiana* stands in west-central Wisconsin. Soil-moisture classes (Soil) are included as a source of variation.

Source of Variation	df	Root	Foliage	Stem	Total	Shoot:Root ratio	Foliage:Root ratio
Soil	4	5 **	6 ***	6 ***	7.0 ***	0.0 ns	0.0 ns
Stand (Soil)	42	2 ns	2 ns	3 ns	2.9 ns	1.6 ns	1.6 ns
Block	5	12 ***	9 ***	9 ***	10.9 ***	5.7 ***	4.6 ***
Soil x Block	20	0 ns	0 ns	0 ns	0.0 ns	0.0 ns	0.0 ns
Block x Stand (Soil)	195	0 ns	2 ns	0 ns	0.0 ns	0.0 ns	0.0 ns
Family [Stand (Soil)]	39	0 ns	4 ns	0 ns	0.3 ns	0.4 ns	0.1 ns
Error	167	81	76	82	79.0	92.3	93.7
Average		188 mg	290 mg	43 mg	521 mg	1.85 ratio	1.62 ratio

Significance: * = P < 0.1; ** = P < 0.05; *** = P < 0.01; ns = no significant.

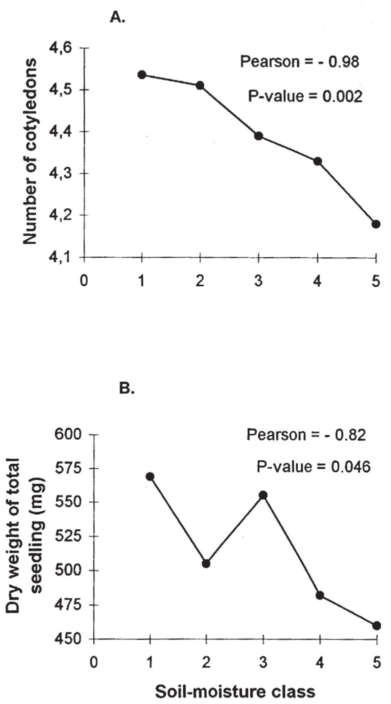

Figure 2. – Correlations between soil-moisture classes and least square means of *Pinus baksiana* seedling traits: Number of cotyledons (A) and Dry weight of total seedling (mg) (B).

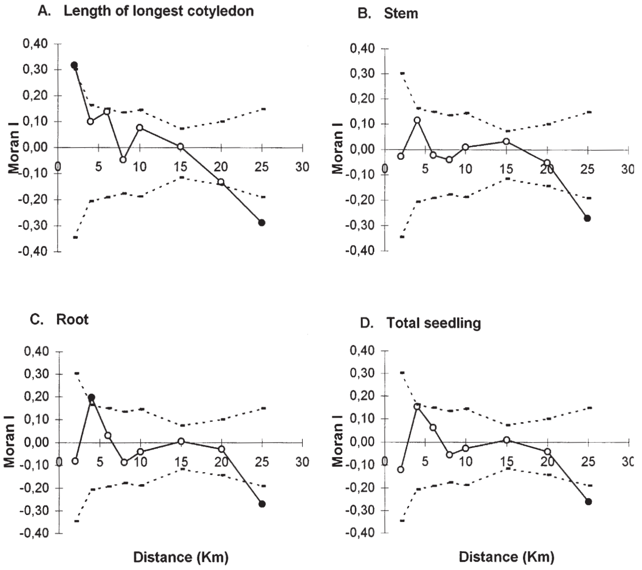

Figure 3. – Autocorrelograms (solid lines) of Moran's I values for *Pinus banksiana* seedling traits: length of longest cotyledon (A), dry weight of stem (B), dry weight of root (C) and dry weight of total seedling (D). Filled circles are significant autocorrelations. Dotted lines indicate 95% null hypothesis confidence regions.

A 'kriging' map (*Figure 4*) demonstrates the pattern of variation observed over soil-moisture classes across the landscape, with sandy-loamy sites in the north having the highest values, while the droughty sites in the south have the smallest values. A kriging map of total seedling dry weight (*Figure 5*) reveals a spatial pattern for a seedling size gradient with the largest seedlings from stands in the south and progressively smaller seedlings distributed on more mesic soil-moisture classes in the north.

Discussion

Small but significant differences exist among *Pinus banksiana* stands for almost all early growth traits. Stands appear to become slightly genetically differentiated as an adaptive response to a gradient of soil-moisture classes on this barrens landscape. Larger seedlings originated from sites with droughty soils, with seedling size declining as site quality improves.

Among stand variation

Several traits including number of cotyledons, length of longest cotyledon, number of fascicles, seedling height, timing of bud set, dry weight of root, stem, foliage and total seedling all were significantly different among stands. This finding supports the idea that significant genetic differentiation for seedling traits can occur among stands of forest trees separated by relatively short distances, consistent with results from WRIGHT *et al.* (1992).

Among-stand differences for timing of bud set were unexpected because flushing and bud set are governed mostly by photoperiod and the number of days with temperatures above 10 °C (RUDOLPH, 1964; RUDOLPH and YEATMAN, 1982; MAGNUSSEN and

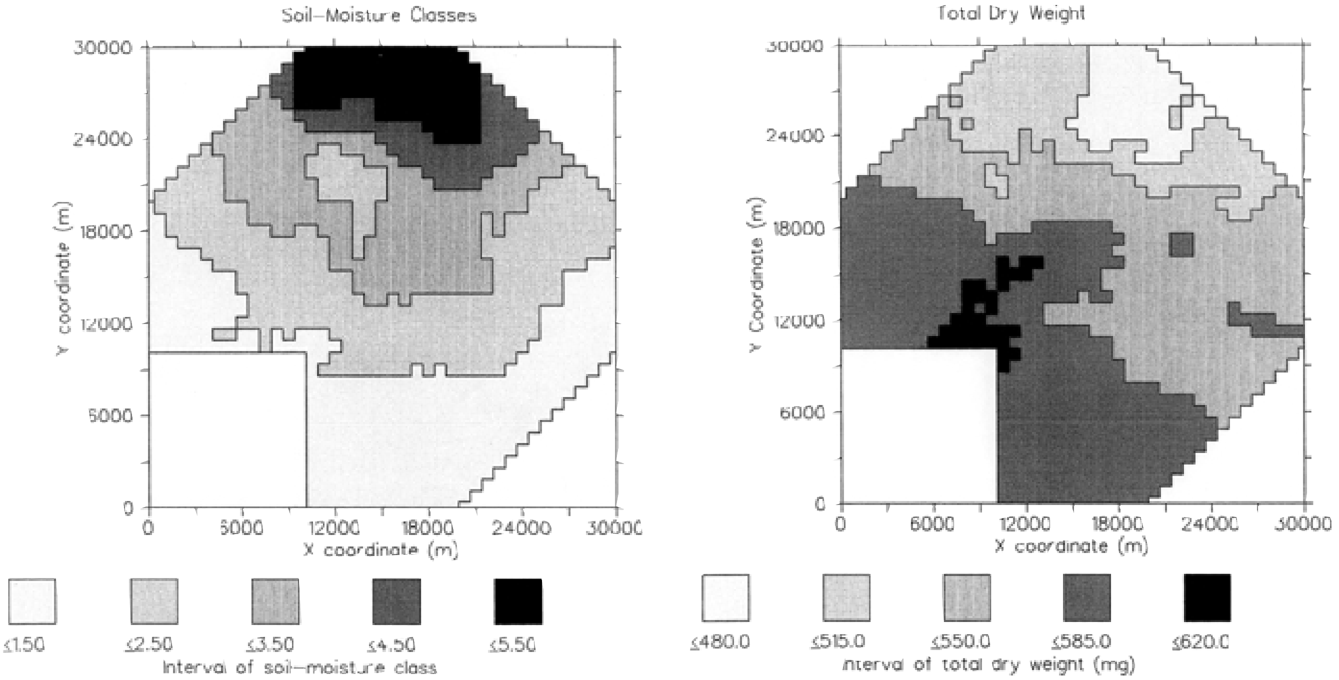

Figure 4 and 5. – Kriging maps of the studied area (Black River State Forest/Jackson County Forest, Wisconsin) for soil-moisture classes (*Fig. 4*) and dry weight of 6-month-old *P. banksiana* seedlings (*Fig. 5*).

YEATMAN, 1989). These variables should have negligible differences on our small (30 km x 30 km) landscape. However, KREMER and LARSON (1982) found bud morphology polymorphism (including early vs. late bud-set types in the first growing season) among and within jack pine families. Our finding of among-stand differences in bud-set can be interpreted as further evidence for the existence of high bud-set polymorphism that may have adaptive advantage.

Shoot:root and foliage:root ratios were not significantly different among stands. The lack of significant differences in shoot:root ratios suggests that some architecture characteristics are 'fixed', and differences might only be apparent on a scale larger that the landscape studied. Our finding is consistent with results from STRAUSS and LEDIG (1985), who found a relatively constant allometric relationship between different seedling architectures across groups of pines but not within species.

The lack of significant variation among families within-stands for all the dry weight traits might be due to small sample sizes, because we harvested only one seedling per plot and there were 21 stands with only one family. Our finding is contrary to expectations, since jack pine progeny tests commonly reveal differences among families (RIEMENSCHNEIDER, 1981; RUDOLPH and YEATMAN, 1982; MAGNUSSEN and YEATMAN, 1989).

Soil-moisture classes

Most seedling traits vary across 'soil-moisture classes'. When 'soil-moisture classes' are included in the ANOVA, variation among stands becomes non-significant in several traits, suggesting that most of the variation among stands is actually variation among 'soil-moisture classes'. We believe that the combined effect of soil texture, drainage and ground-water influence are the primary selective forces influencing among-stand differentiation within this landscape for the traits under study. The lack of significance for shoot:root and foliage:root ratios among 'soil-moisture classes' suggests that these seedling architecture traits remain relatively constant across this landscape.

Negative correlations between several seedling traits and 'soil-moisture classes' may be the result of a gradient in seedling size across 'soil-moisture classes', as stands from droughty sites (soil-moisture classes I and III) produced larger seedlings than stands from the more mesic sites (soil-moisture classes IV and V). These results might be due to seed-size effects, wherein trees growing on drier sites produce larger seeds (and therefore seedlings) as an adaptive response to drought stress. WRIGHT *et al.* (1992) reported larger seed coming from drier sites in their study of jack pine, a finding consistent with reports of maternal effects in seed-size variation for jack pine seedling growth (YEATMAN, 1965; RADSLIFF, 1981). There is a marked tendency for many plants, including trees, to show increases in seed weight across broad gradients of soil moisture apparently as an evolutionary response to drought stress in xeric habitats (BAKER, 1972).

Spatial analysis

Significant negative autocorrelations of Moran's I suggest an among-stand differentiation observed at a scale of approximately 25 km, a spatial scale at which environmental features impact the among-stand patterning of variation. However, the large number of non-significant autocorrelations and the erratic pattern of some correlograms for traits such as the number of cotyledons or seedling height, indicates that spatial differentiation for most traits is only moderate to weak. This makes sense if the environmental differences are small and/or gene flow among stands is extensive due to long-distance pollen dispersal, both factors that counteract population differentiation (LINHART and GRANT, 1996). NIEJENHUIS and PARKER (1996) also found significant spatial differences in adaptive traits among jack pine stands governed by a climatic cline, although their study area is approximately 10-fold larger than ours.

Applications for forest gene conservation

Many forest managers in Wisconsin prefer to plant *Pinus resinosa* (red pine) on sites where natural stands of *Pinus banksiana* have been harvested. Red pine is considered more productive and less prone to insect defoliation, so is preferred for many commodity production situations. However, the large-scale conversion of natural stands of jack pine to red pine can pose the risk of fragmenting the genetic structure across a landscape, disrupting a long evolutionary history of adaptation to environmental selection forces (Ledig, 1992). The fact that a large number of natural jack pine stands in the Black River State Forest/Jackson County Forest occur on public land represents a unique opportunity to manage forest genetic diversity.

The information generated by this study could be used to incorporate genetic conservation practices into ongoing reforestation efforts. Given the landscape scale at which genetic structure appears to occur in jack pine, only modest numbers of stands need be managed explicitly to conserve genetic diversity. This could be accomplished by promoting natural regeneration (Millar and Libby, 1991) for a sub-set of stands established at for each 'soil-moisture class' in the Black River State Forest/Jackson County Forest, to ensure continuity of the spatial diversity at which differentiation occurs.

Conclusions

Spatial patterning of environmental features, especially soil-moisture classes, appears important in molding the spatial patterning of genetic differentiation among jack pine stands in a landscape without apparent strong climatic and biophysical clines. The differentiation among stands occurs at a scale corresponding to distances of 25 km, and it is negatively correlated with 'soil-moisture classes'. The information generated by this study could be used to establish genetic conservation practices to preserve part of the landscape genetic structure.

Acknowledgements

This study was supported in part by funds from the Bureau of Forestry, Wisconsin Department of Natural Resources (WDNR) to RPG, and by a graduate studies fellowship to CSR from the Mexican Council of Science and Technology (CONACYT). CSR gratefully acknowledges the support from the Centro de Investigación en Genética y Ambiente, Universidad Autonoma de Tlaxcala, Tlaxcala, México, during his stay in USA. We thank Andrew Monk for assistance with seed collections; Trent Marty (WDNR) for providing access to seed collections and logistic support; Jim Adams, Lynn Hummel and Laura Van Slyke of the University of Wisconsin-Madison Walnut Greenhouses for seedling maintenance; Ted Sickley for preparing the vegetation map. We also thank Erik Nordheim and Alejandro Munoz-delRio for help with statistical analyses; Jaya Iyer, John Kotar and Tim Burger provided insightful discussions about soil-moisture classes; Michael Casler, Eric Kruger, Erik Nordheim, Don Waller and one anonymous reviewer for valuable suggestions to improve the manuscript.

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Diversity Among Circum-Mediterranean Populations of Aleppo Pine and Differentiation from Brutia Pine in their Isoenzymes: Additional Results[*]

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(Received 13th June 2001)

Summary

Seed material from 20 circum-Mediterranean *Pinus halepensis* Mill (Aleppo pine) populations and 32 *Pinus brutia* Ten. Subsp. brutia populations of range-wide distribution in Turkey was used to analyze the genetic diversity and structure of the species, by applying the starch gel electrophoresis technique to isoenzymes extracted from the seed megagametophytes.

The results show that not all the enzyme systems were resolved equally in the two species, therefore, data for several loci are not available for one or the other species. Significant differences between the two species in the overall average of allele frequencies in many loci can be observed. The largest differences occurred in the Aco, Acp, Adh$_2$, Gdh, Got$_1$, Mdh$_1$, Pgm$_2$, Pgi$_2$, 6Pgd$_2$ and 6Pgd$_3$ enzyme systems and loci. In these enzyme systems allele frequencies alternated between the first and second locus in the two species.

In Aleppo pine, statistical analysis of allele frequency data revealed that the mean number of alleles per locus ranged between 1.3 and 2.0; the percentages of polymorphic loci at the 0.95 criterion ranged between 16.0 and 44.0; the direct count mean heterozygosity ranged between 0.025 and 0.144; and Hardy-Weinberg expected heterozygosity ranged between 0.052 and 0.188. A UPGMA dendrogram based on the genetic distance-Wagner procedure aggregated the 20 Aleppo pine populations mainly into three geographically distinct groups, namely: a western Mediterranean group that includes populations from Morocco, Spain and France; an eastern European group comprising Greek and Italian populations; and an eastern Mediterranean group comprising populations from Turkey, Jordan and Israel. The four Tunisian Aleppo pine populations were divided, according to allele frequencies in several enzyme systems, between the eastern and the western Mediterranean groups. Significant differences among the groups were found also in several genotype frequencies but especially in those of the Aap 1-1, Lap 0-1 and 2-2 genotypes.

In *Pinus brutia* Ten. The results revealed that the number of alleles per locus ranged from 1.95 to 2.40 with an average of 2.16; the percentage of polymorphic loci, by the 95% criterion, ranged from 65% to 75% averaging 68%; the effective number of alleles per locus ranged from 1.41 to 1.60, with an average of 1.49. Consequently, the observed and expected heterozygosities were 0.191 (ranging from 0.144 to 0.267) and 0.271 (ranging from 0.233 to 0.312), respectively.

Key words: Pinus halepensis, *Pinus brutia*, Allozymes, Genetic diversity and structure.

Introduction

The availability of Aleppo pine (*Pinus halepensis* Mill.) seed material, collected within the framework of the IUFRO-FAO project on Mediterranean pine species (Morandini, 1976), was the basis of the genetic diversity analysis, done by means of the isoenzyme starch gel electrophoresis technique (Conkle *et al.*, 1982), among 19 circum-Mediterranean populations of Aleppo pine and 10 *Pinus brutia* Ten. populations (Schiller *et al.*, 1986; Conkle *et al.*, 1988). Subsequent studies that utilized the same technique concerned themselves only with the regional distribution of the genetic diversity in *Aleppo and Brutia pines* (e.g., Grunwald *et al.*, 1986; Teisseire *et al.*, 1995; Agundez *et al.*, 1997; Korol and Schiller, 1996; Agundez *et al.*, 1999; Puglisi *et al.*, 1999; Kara, *et al.*, 1997; Panetsos *et al.*, 1998; Gani-Gulbaba and Ozkurt, 2000). Several other studies used biochemical traits as genetic markers, such as resin monoterpene composition analyzed with the gas chromatography technique, to evaluate the genetic diversity among populations of Aleppo pine (e.g., Schiller and Grunwald, 1986, 1987; Baradat *et al.*, 1989, 1995). Recently high levels of genetic diversity among circum-Mediterranean populations of Aleppo pine was revealed by means of the chloroplast microsatellites technique (e.g., Bucci *et al.* 1998; Morgante *et al.*, 1998; Vendramin *et al.*, 1998).

The aim of the present study was to extend our knowledge of the intra- and intergenetic diversity and the phylogenetic relationships among circum-Mediterranean occurrences (provenances) of Aleppo and Brutia pines by means of the isoenzyme technique. To compare the results echived on *P. halepensis*

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