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Research paper

Differential ecophysiological response of a major Mediterranean pine species across a climatic gradient

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The rate of migration and in situ genetic variation in forest trees may not be sufficient to compete with the current rapid rate of climate change. Ecophysiological adjustments of key traits, however, could complement these processes and allow sustained survival and growth across a wide range of climatic conditions. This was tested in *Pinus halepensis* Miller by examining seven physiological and phenological parameters in five provenances growing in three common garden plots along a climatic transect from meso-Mediterranean (MM) to thermo-Mediterranean (TM) and semi-arid (SA) climates. Differential responses to variations in ambient climatic conditions were observed in three key traits: (i) growing season length decreased with drying in all provenances examined (from 165 under TM climate to 100 days under SA climate, on average); (ii) water use efficiency (WUE) increased with drying, but to a different extent in different provenances, and on average from 80, to 95, to 110 μmol CO_2 mol^{-1} H_2O under MM, TM and SA climates, respectively; (iii) xylem native embolism was stable across climates, but varied markedly among different provenances (percent loss of conductivity, was below 5% in two provenances and above 35% in others). The results indicated that changes in growing season length and WUE were important contributors to tree growth across climates, whereas xylem native embolism negatively correlated with tree survival. The results indicated that irrespective of slow processes (e.g., migration, genetic adaptation), the capacity for ecophysiological adjustments combined with existing variations among provenances could help sustain *P. halepensis*, a major Mediterranean tree species, under relatively extreme warming and drying climatic trends.

Keywords: climate change, embolism, growth phenology, provenances, water use efficiency.

Introduction

Plants are sessile and hence cannot migrate during their ontogeny when local conditions become unfavorable for their growth or survival. This selected for a remarkable ability of plants to acclimate to a changing environment through physiological, molecular and genetic responses (Reyer et al. 2012, Shaw and Etterson 2012). Trees in particular are sensitive to climate change due to their longevity, extending over time-spans from decades to centuries. For example, extreme, rare climate events (e.g., a drought year once a decade) inevitably influence tree communities more than annuals and geophytes that can, for example, avoid germination under such events. Consistent, directional climate change taking place today on a global scale highlights the importance of tree acclimation (Allen et al. 2009).

Climate predictions indicate drying trends associated with reduced precipitation in the Mediterranean and other regions (Alpert et al. 2006, Burke et al. 2006, Christensen et al. 2007). Observed increases in global drought severity since 1952 and in evaporative demand in Israel since 1964 are expected to intensify during the 21st century (Cohen et al. 2002, Burke et al. 2006). Evidence for the impact of warming and drying on Mediterranean forests is already accumulating at an alarming pace and in 2008 alone at least five examples of distinct drought-induced forest stand dieback and decline events were observed. This includes *Abies cephalonica* Loudon forests in Greece (Raftoyannis et al. 2008); *Cedrus atlantica* Endl. in Algeria and Morroco; *Quercus*, *Pinus* and *Juniper* spp. in Turkey; and *Quercus suber* L. in France (Allen et al. 2009 and references therein). Impacts on Mediterranean *Pinus* spp. following the drought years of 1999–2000 are evident in Israel as well as in Greece (Koerner et al. 2005, Sarris et al. 2007) and Spain (Penuelas et al. 2001, Martinez-Vilalta and Pinol 2002). Additional implications of climate change on tree growth include reduced ability of forests to sequester carbon (Ciais et al. 2005) as well as shifting the timing of the growing season (Chmielewski and Rotzer 2001).

Common garden testing of tree populations of different origin, known as provenance trials, is a powerful tool for testing hypotheses of adaptation to climate in trees (Langlet 1971, Matyas 1996). Traditionally, provenance trials served in identifying provenances carrying the most desired phenotypes at the test location, and sometimes also as a basis for delineating seed planting and breeding zones (Raymond and Lindgren 1990). Intraspecific differences in physiological and hydraulic traits were studied in *Pinus sylvestris* L. (Martínez-Vilalta et al. 2009) and *Pinus pinaster* Ait. (Lamy et al. 2011). The 1975 UN FAO provenance trial program on Mediterranean conifers (www.fao.org/docrep/006/k1203e/K1203E08.htm) has a major section on Aleppo pine (*Pinus halepensis* Miller), a key forest tree across the Mediterranean region. Its distribution spans three climate types: meso-Mediterranean (MM) (Cfb, Koppen–Geiger climate classification), thermo-Mediterranean (TM) (Csa) and semi-arid (SA) (Bwh). The ability of *P. halepensis* to survive and grow in various environments indicates that it is a highly tolerant species. Field performance data of *P. halepensis* provenances collected routinely in numerous sites are being used in the selection of successful provenances for each location (Eccher et al. 1987, Fusaro et al. 2007). Ecophysiological parameters underlying *P. halepensis* provenance-level response to changes in environmental conditions were identified in seedlings (Tognetti et al. 1997, Calamassi et al. 2001, Baquedano et al. 2008), and also in field-grown trees (Schiller and Atzmon 2009). However, studies on the association between climate and adjustments in physiological traits are still scarce, and hence it is still not possible to predict provenance performance under varying climate conditions (Atzmon et al. 2004).

In this study we hypothesized that (i) ecophysiological traits should vary, both with climate and provenance; (ii) interactions of climate with provenance are expected and should result in differences in climate response thresholds and (iii) growth strategies rely on specific combinations of traits. To test these hypotheses we used five provenances originating from different environments, growing in three experimental forest plots in sites representing mesic to warm and very dry conditions. Seven eco-physiological parameters were selected to represent tree physiology (carbon assimilation rate, stomatal conductance and intrinsic water use efficiency) hydraulics (xylem conductivity and native embolism) phenology (trunk growing season length (GSL)) and integrated photosynthetic potential (leaf chlorophyll concentration). These parameters were identified as keys to *P. halepensis* functioning in previous studies (Klein et al. 2005, Maseyk et al. 2008, Klein et al. 2011). Observations were made along 2 years with focus on April–June, when plant physiological activities were intermediate between winter maxima and summer minima. Finally, we tested whether our findings could explain published data about the survival of provenances of this species under more extreme, arid conditions.

Materials and methods

Plant material and site descriptions

The study included five provenances of Aleppo pine (*Pinus halepensis* Miller) grown from seeds collected from mature trees in natural stands of each provenance and sown in three sites of the UN FAO seed collection provenance program (SCM/CRFM/4 bis project, http://www.fao.org/docrep/006/k1203e/K1203E08.htm; see database in: http://147.100.66.194/ForSilvaMed). Out of 32 provenances 5 were selected to allow in-depth study while maintaining sufficient representation of the variety of habitats in which *P. halepensis* grows. Seed sources included contrasting sites from different areas around the Mediterranean (Figure 1) starting with mean annual precipitation (P) of 830 mm in Otricoli, Italy, to 310 mm in Senalba, Algeria (Table 1). All five provenances were represented by 10–12 actively growing trees in each of three experimental plots in three different climates: MM (in Castel Di Guido, Rome), TM (in Beit Dagan) and SA (in Yatir), see a summary of climate characteristics for the sites in Table 1.

The Castel Di Guido farm is located in the coastal plains 15 km west of Rome, Italy, 10 km east of the Tyrrhenian Sea shore, and its soil is mostly clay. The experimental plot was planted in 1975 in a randomized block design, with 9–25 replicates × 6–9 blocks at 3 × 3 m spacing. The Beit Dagan ARO Volcani Center farm is located in the coastal plains, 20 km south-east of Tel-Aviv, Israel, 20 km east of the Mediterranean Sea shore, and its soil is deep sandy to sandy-loam. The 1-ha experimental plot was planted in 1991 with 11 provenances in parallel rows with 12 replicates × 1 block at 2 × 4 m spacing. Yatir forest is a *P. halepensis*-dominated planted forest located at the northern edge of the Negev desert, Israel and covering

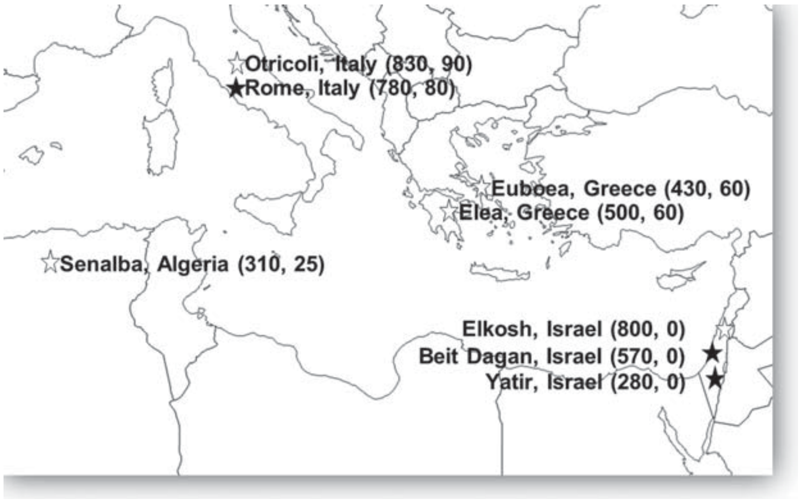

Figure 1. Geographic locations of the five *Pinus halepensis* provenances (open star symbols; Table 1) and the three experimental forest plots (closed star symbols; Table 1) included in this study and their mean annual and summer precipitation, respectively. Note that, in spite of proximity between the Beit Dagan and Yatir sites, climate types differ due to a sharp precipitation gradient.

Table 1. Summary of main geographical and climatic characteristics at the five provenances (North to South) from which seeds were collected and trees were included in this study and three experimental forest plots included in this study.

Provenance (FAO code) or site	Latitude	Longitude	*P* (mm)	PET (mm)	Aridity index (*P*/PET)	P_s (mm)
Otricoli, Italy (A 26)	42°24′N	12°38′E	830	~900	0.92	90
Euboea, Greece (A3)	38°58′N	23°18′E	430	~1250	0.34	60
Elea, Greece (A2)	37°46′N	21°32′E	500	~1350	0.37	60
Senalba, Algeria (A 30)	34°45′N	03°25′E	310	~1250	0.25	25
Elkosh, Israel (A7)	33°01′N	35°18′E	800	~1300	0.59	0
Rome, Italy	41°54′N	12°30′E	780	~1000	0.78	80
Beit Dagan, Israel	31°59′N	34°48′E	570	~1300	0.44	0
Yatir, Israel	31°20′N	35°04′E	280	~1600	0.18	0

P, mean annual precipitation; PET, mean annual potential evapo-transpiration; P_s, mean summer (May–September) precipitation. Data from: (i) FAO Forest Genetic Resources No. 5. Mediterranean Conifers, table 1; (ii) Palutikof et al. (1994); (iii) Mejstrik and Cudlin (1983); (iv) Meteorological measurements carried out by the authors in Rome and Yatir; (v) Klein Tank et al. (2002) (data and metadata available at http://eca.knmi.nl); (vi) Cohen et al. (2002).

an area of 2800 ha on predominantly light brown Rendzina, 25–100 cm deep. The Yatir experimental plot was planted in 1989–90 with 17 provenances in parallel rows with 12 replicates × 4 blocks with 4 × 4 m spacing. Previous reports about the performance of *P. halepensis* provenances in Yatir (Atzmon et al. 2004, Schiller and Atzmon 2009) refer to another experimental provenance plot, where micro-climatic conditions are drier than in the plot reported here. None of the plots were thinned, and with the exception of several tree mortalities, plot densities were kept as planted. To account for differences in trees' age among plots, field performance was measured in trees of similar age, but in different years. To avoid edge effects, sampling excluded trees growing in the margins of plots.

Field performance and growth phenology

Periodic measurements of tree height and diameter at breast height (DBH, 1.3 m) were performed every 1–3 years from the time of planting on 10 trees of each provenance and site. Height was measured using a PM-5 clinometer (Suunto, Sylvan Lake, MI, USA) and DBH was estimated from tree circumference. Since planting density differed among sites, individual measurements of DBH were normalized by the standard stand density of 1343 trees ha^{-1} (3 × 3 m spacing). Dendrometers were prepared using stem circumference increment tape (EMS, Brno, Czech Republic) fitted with custom-made 50 mm steel tension coil springs (Hakfitz, Ramat Gan, Israel). During December 2008 dendrometers were installed on five trees of each provenance growing in Beit Dagan and Yatir. Trunk circumference increment was measured once in 2 weeks using a digital caliper (indication error 0.03 mm; Fuji, Japan). Observed growth rate in mm $month^{-1}$ was calculated from consecutive measurements divided by the time in between individual measurements. In order to estimate trunk GSL, increments in circumference were expressed as percent of the annual total for each individual tree, and GSL was defined as the sum (in days) of all 2-week periods where increment was ≥5% of the total annual increment.

Tree-ring $\delta^{13}C$

Tree cores were sampled between December 2008 and December 2009 using a 200 mm increment borer (core diameter 5.15 mm) equipped with starter (Haglof, Sweden). From each tree, two trunk cores were collected, at breast height at 0° (N) and 180° (S). The sampling design was 3 sites × 5 provenances × 8–10 trees × 2 sides = 270. Tree-rings of the growth years 1997–2000 were identified, cut into 7–12 equal intra-annual sub-sections and their $\delta^{13}C$ values were analyzed as described in Klein et al. (2005). To minimize analysis time and resources, isotopic analysis was performed on whole wood samples rather than cellulose, which is generally coherent (McCarroll and Loader 2004 and references therein). The $\delta^{13}C$ results were used to calculate the discrimination of the tree against ^{13}C (Δ) expressed in delta notation as parts per thousand (‰) deviations from the international carbon isotope standard (Coplen 1994):

$$\Delta = \frac{(\delta^{13}C_a - \delta^{13}C_t)}{(1 + \delta^{13}C_t)} \quad (1)$$

where $\delta^{13}C = (\delta^{13}C/\delta^{12}C)_{sample}/(\delta^{13}C/\delta^{12}C)_{reference} - 1$ and the reference is Pee Dee Belemnite carbonate, and subscripts a and t stand for atmospheric air and the tree-ring material, respectively. *Pinus halepensis* is a drought-avoiding species and therefore the deposition of carbon into tree-rings is typically restricted to October–May (Klein et al. 2005). This means that the annual $\delta^{13}C$ maxima occurred in March–May, when soil and atmospheric humidity were declining while still permitting leaf gas exchange. Δ values were calculated from annual $\delta^{13}C$ maxima values of trees from each provenance and climate and were further used in the derivation of the tree's intrinsic water use efficiency (WUE_i) using the following equation (adapted from Farquhar et al. 1982, Seibt et al. 2008):

$$WUE_i = \frac{C_a}{r\{[b - \Delta - f(\Gamma^*/C_a)] / [b - a + (b - a_m)(g_s / g_i r)]\}} \quad (2)$$

where C_a is the atmospheric CO_2 concentration in ppm (an annual global average; Robertson et al. 2001); r is the ratio of the diffusivities of CO_2 and water in air (1.6); a, a_m, b and f are the leaf-level discriminations against ^{13}C in the diffusion through the stomata (4.4‰), during dissolution and liquid phase diffusion (1.8‰), during biochemical CO_2 fixation (29‰) and in photo-respiratory CO_2 release (8‰), respectively; Γ^* is the temperature-dependent CO_2 compensation point of ca. 30–45 ppm (Maseyk et al. 2008); g_s/g_i is the ratio between stomatal and internal conductances to CO_2, respectively (0.5, according to Maseyk et al. 2011).

Leaf gas exchange

During March and May of 2009 and 2010, leaf stomatal conductance (g_s, in mol H_2O m^{-2} s^{-1}) and net carbon assimilation (A, in µmol CO_2 m^{-2} s^{-1}) were measured on three trees per provenance in the Yatir and Beit Dagan plots. On each field day, this was repeated 2–3 times from 9:00 to 15:00 on sunlit needles using an LI-6400 Photosynthesis System (Licor Inc., Lincoln, NE, USA) equipped with an LED lamp set on 1000 µmol m^{-2} s^{-1} PAR. These measurements were used in the calculation of intrinsic water use efficiency (WUE_i, in µmol CO_2 mol^{-1} H_2O) according to the equation:

$$WUE_i = \frac{A}{g_s} \quad (3)$$

Xylem hydraulic conductivity

Hydraulic conductivity was measured under low pressure (0.02 MPa) generated by a 2 m pressure head before and after perfusing the xylem tissue at a high pressure of 0.5 MPa. Light-exposed twig sections (30 cm long, 5 mm diameter) with 3–5 needle cohorts were cut in the field from the lower branches. From each provenance we sampled three twigs from three individual trees during morning hours (8:30–9:30) on 23 May at Yatir and on 8 June at Beit Dagan. Resin secretion from the cuts was eliminated by immediately placing both ends of the twig section (5 cm from cut tips) in a water bath at 95°C for 10 min (adapted from Waring and Silvester 1994) while still in the field. Measurements that we made in branches of *Olea europea* L. showed that K_s was not affected by this heat treatment. Next, the specific hydraulic conductivity K_s and maximum specific conductivity $K_{s\,max}$ (kg m^{-1} MPa^{-1} s^{-1}) were determined as described by Klein et al. (2011). Measurements of K_s and $K_{s\,max}$ were further used to identify loss of conductivity that can be attributed to xylem cavitation according to Eq. (4) (Tognetti et al. 1997):

$$PLC = 100\frac{(K_{s\,max} - K_s)}{K_{s\,max}} \quad (4)$$

where PLC is the percent loss of conductivity (%) due to cavitation.

Leaf chlorophyll concentration

During May 2009 and April 2010, healthy 1-year-old needles were collected in three replications from three trees per provenance in Yatir and Beit Dagan. Needles (50 mg leaf tissue per sample) were cut into 5 mm segments and immersed in 1 ml 80% aqueous acetone solution together with two bearing balls (diameter = 3 mm). Leaf specimens were ground using a ball mill (Retsch, Hann, Germany) at a frequency of 25 s^{-1} until full extraction was achieved (~5 min), followed by centrifugation at 14,000 rpm for 10 min. The supernatant of each specimen was transferred into a micro-well in a 96-well plate, where its absorbance was measured at 663.6 and 646.6 nm using a tunable micro-plate reader (Molecular Devices, Sunnyvale, CA, USA). Concentrations of chlorophylls *a* and *b* were determined following subtraction of the absorbance of a blank sample (containing solution but not leaf sample) according to Porra et al. (1989). Results were expressed on a dry weight basis following the drying of remaining needles in the sample at 60°C for 48 h.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using JMP software (Cary, NC, USA). Measurements of individual trees were used as observations, where climate type (three levels, nominal variable), provenance (five levels, nominal variable) and their interaction were defined as factors (Table 2). The relatively high number of combinations (3 × 5) together with resource limitations meant a limited sample size ($n = 3$–10). Differences between responses were considered significant when type 3 sum of squares met the *F*-test criterion at probability <0.05. In addition to the ANOVA, the effect of climate in individual provenances and the effect of provenance in the three climate types were also analyzed (Table 2). Means were compared using Tukey's HSD test, reported as letters indicating significant differences in Figures 2, 3 and 5. To test the relationship between variance in field performance (increment growth) and variance in physiological traits, data were analyzed by partial least-square regression (PLS) and the relative percent of variation in performance explained by each of the six parameters is reported in Table 2.

Results

Field performance and tree physiology

At 19 years after planting at each site, average normalized DBH was 24.1, 21.3 and 10.5 cm in the Rome, Beit Dagan and Yatir sites, respectively (Figure 2a). Trees growing in Rome reached a mean height of 12.2–15.9 m compared to 8.6–12.0 m at Beit Dagan and Yatir (data not shown), consistent with the precipitation gradient (Table 1), and especially in the Greek provenances Elea and Euboea. The reduction in annual stand-level basal area increment (BAI; $m^2\ ha^{-1}\ year^{-1}$) as a function of 100 mm reduction in precipitation, $d(BAI)/d(P_{100})$, was estimated at 0.82, 0.81, 0.31, 0.29 and 0.17 for the provenances Elea, Euboea, Elkosh, Senalba and Otricoli, respectively. Correlation coefficients between BAI and precipitation (r^2, with * indicating significant at $\alpha = 0.05$) were 0.73*, 0.68*, 0.49*, 0.44* and 0.11, respectively.

To distinguish between provenance-level physiological responses to dry conditions we used the seasonal changes in carbon isotopic composition and specifically the $\delta^{13}C$ maxima in the annual tree-rings. Such maxima can be expected to reflect the lowest seasonal discrimination against ^{13}C, which is associated with assimilation under the drier part of the growing season. While in Rome $\delta^{13}C$ values ranged between −23 and −30‰, in Beit Dagan they were between −21 and −27‰ and in Yatir between −19 and −26‰, as expected along a drying gradient. The annual maximum $\delta^{13}C$ values were then used in estimating intrinsic water use efficiency (WUE_i; $\mu mol\ CO_2\ mol^{-1}\ H_2O$) using Eq. (2) (Seibt et al. 2008). WUE_i was inversely related to precipitation, but with no significant differences among provenances, except for the Beit-Dagan site (Figure 2b; $P = 0.0161$, Table 2). Across provenances, the WUE_i increase per 100 mm decrease in *P*, $d(WUE_i)/d(P_{100})$, ranged between 2.7 and 4.1 (with r^2 ranging between 0.53 and 0.87, significant at the 0.05 level in all provenances).

Leaf gas exchange measurements were used in an attempt to distinguish between the effects of *A* or g_s on WUE_i (calculated from instantaneous gas exchange measurements using Eq. 3 and derived from tree-rings). Note, however, that because of the

Table 2. *P* values (probability > *F*, an effect is significant if $P < 0.05$, in bold) from ANOVA for (i) the effects of climate, provenance and their interaction (C × P) on main response parameters, (ii) the effect of climate in individual provenances and (iii) the effect of provenance in the three climate types. Results of PLS are reported as the relative percent of variation in field performance (DBH) explained by each of the six parameters.

Variable	DBH (cm)	WUE_i ($\mu mol\ CO_2\ mol^{-1}\ H_2O$)	*A* ($\mu mol\ m^{-2}\ s^{-1}$)	g_s ($mol\ m^{-2}\ s^{-1}$)	Growing season length (days)	PLC (%)	Chlorophyll conc. ($mg\ gDW^{-1}$)
Climate	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	<0.1	<0.1
Provenance	**<0.001**	n.s.	**<0.05**	**<0.05**	**<0.001**	**<0.05**	**<0.001**
C × P	**<0.001**	**<0.05**	**<0.05**	n.s.	n.s.	n.s.	**<0.05**
Otricoli	**<0.001**	**<0.001**	**<0.05**	<0.1	**<0.05**	n.s.	n.s.
Euboea	**<0.001**	**<0.05**	n.s.	n.s.	**<0.05**	n.s.	n.s.
Elea	**<0.001**	**<0.05**	n.s.	<0.1	**<0.001**	n.s.	**<0.05**
Senalba	**<0.001**	**<0.001**	n.s.	<0.1	**<0.001**	n.s.	n.s.
Elkosh	**<0.001**	**<0.001**	n.s.	**<0.05**	n.s.	n.s.	n.s.
SA	n.s.	n.s.	n.s.	n.s.	<0.1	n.s.	**<0.001**
TM	n.s.	**<0.05**	**<0.05**	<0.1	**<0.001**	**<0.05**	**<0.05**
MM	**<0.001**	n.s.					
% variation explained		39.05	16.07	7.98	18.70	4.37	13.84

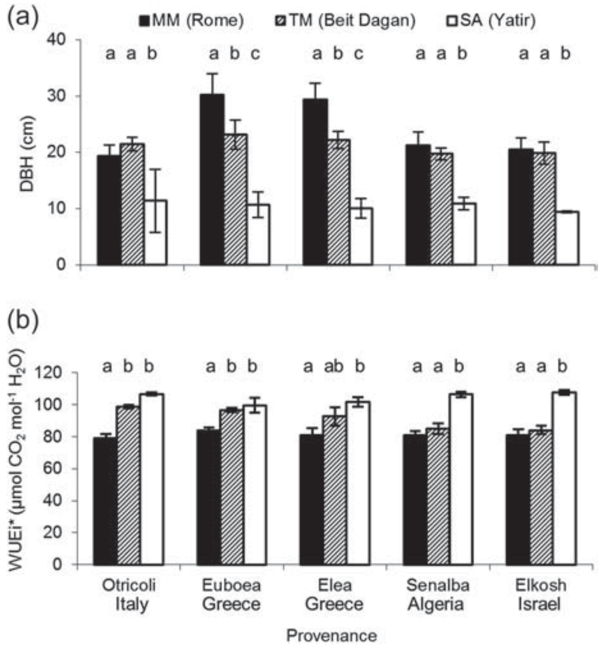

Figure 2. Mean DBH (a) and δ^{13}C-derived WUE$_i$ (b) of trees from each provenance and climate (SA, semi-arid; TM, thermo-Mediterranean; MM, meso-Mediterranean) 19 years after planting (2008–2009 in Yatir, 2010 in Beit Dagan and 1994 in Rome). WUE$_i$ values were calculated from δ^{13}C measurements on tree-rings formed during years with precipitation within 0.5 SD of the multiannual average; 1997 in Yatir and Beit Dagan, 1999 in Rome. Similar letters indicate a non-significant difference ($\alpha > 0.05$) between trees of a provenance growing under different climate types. Error bars represent the standard error of the mean ($n = 8$–10 and 3–5, respectively).

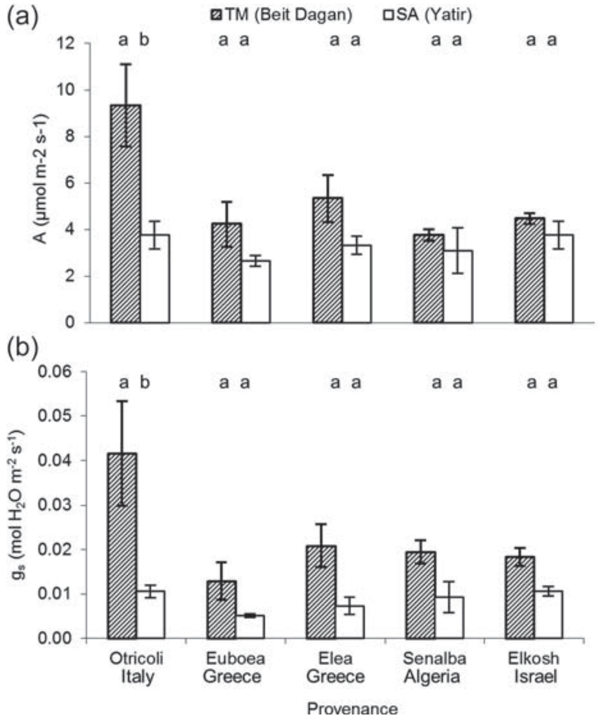

Figure 3. Mean net assimilation rates (a) and stomatal conductance (b) measured during May in leaves of trees from each provenance and two climate types (SA, semi-arid; TM, thermo-Mediterranean). Similar letters indicate a non-significant difference ($\alpha > 0.05$) between trees of a provenance growing under different climate types. Error bars represent the standard error of the mean ($n = 3$).

different time scales involved, comparing instantaneous gas exchange results with long-term integrated tree ring data only serves as an indication for the possible underlying processes. In general, values of A and g_s were comparable across provenances, with the exception of higher assimilation rates in the Italian provenance Otricoli under TM climate (Figure 3, Table 2). Along the drying climate trend across sites, A values decreased by ~40% and g_s values decreased by ~60%. The decrease in A with drying is consistent with the lower growth observed along the climatic trend (Figure 2a) and may indicate an agreement between the short- and long-term results. The A/g_s ratio (Eq. (3)) increased by ~100% with drying, compared to 15% increase in δ^{13}C-derived WUE$_i$. In turn, the results indicate that the higher WUE$_i$ under SA climate may have been the result of a greater decrease in stomatal conductance, as compared with A. Note that the decrease in A values in response to the climate gradient was not significant in Senalba and Elkosh (Table 2). This was also reflected by lower WUE$_i$ in Senalba and Elkosh under TM climate than in other provenances, as shown above (Figure 2b).

Trunk growth phenology and leaf chlorophyll concentration

Differences in trunk growth phenology between provenances were found under identical climatic conditions in each site. Each provenance had a unique growth phenology (Figure 4) in terms of start date, end date and the GSL, defined as the sum (in days) of all 2-week periods when the increment was 5% of the annual total or more. Under TM climate, trunk growth initiation followed the beginning of the rainy season in October. At that time Elea growth rate was 2.6 ± 0.7 mm month^{-1}, and other provenances between 1.8 ± 0.6 and 2.3 ± 0.4 mm month^{-1}. Otricoli reached that rate in early December, while other provenances caught up only in early February of the following year. Growth continued in all provenances until early (Elkosh) to mid- (all others) April, with rates of up to 6.7 ± 1.0 mm month^{-1} (Euboea; Figure 4). GSL was significantly lower under SA climate ($P < 0.0001$, Table 2), predominantly due to a delayed starting date: Elea trees were again the first to resume growth, but only in mid-January, and they had the longest growing season (128 days, Figure 5a) and reached rates of 5.3 ± 0.4 mm month^{-1}, whereas other provenances' growth rates were between 2.4 ± 0.8 and 4.2 ± 0.2 mm month^{-1}. Euboea had a very short growing season (80 days). In TM climate GSL was also high for Otricoli, while Senalba and Elkosh had intermediate GSLs. Elkosh was the only provenance whose GSL did not significantly change with climate ($P = 0.1308$, Table 2).

Leaf chlorophyll concentrations were not sensitive to climatic conditions, with comparable values across sites in each

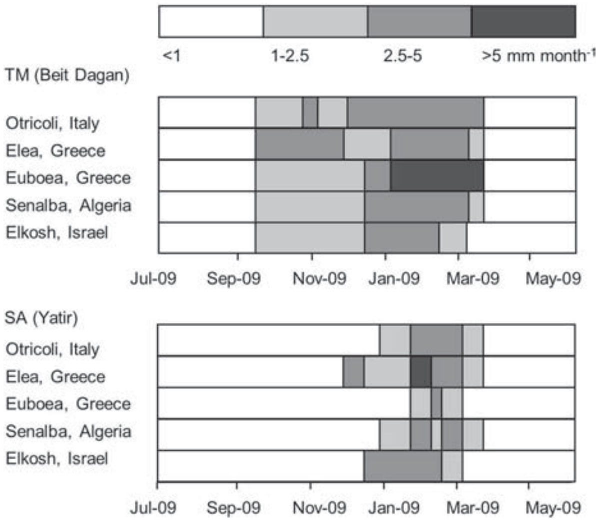

Figure 4. Quantitative trunk growth phenograms averaged across trees from each provenance and two climate types (SA, semi-arid; TM, thermo-Mediterranean).

provenance (Figure 5c; $P = 0.086$, Table 2). However, chlorophyll concentrations were 30% higher in the leaves of Euboea (Figure 5c), yielding a significant provenance effect on chlorophyll concentration ($P < 0.0001$, Table 2). The higher chlorophyll concentration in Euboea was consistent with its higher trunk growth rate, i.e., up to 6.7 mm month^{-1}, as compared with other provenances 3–5 mm month^{-1} (Figure 4), and may reflect higher integrated photosynthesis in this provenance.

Xylem hydraulic conductivity

All provenances had lower hydraulic conductivities (K_s) in SA than in TM climate: ~27 and ~120 g m^{-1} MPa^{-1} s^{-1}, respectively (Table 3). Elkosh and Elea had the highest K_s, whereas Senalba had the lowest K_s (and $K_{s\ max}$) values at both sites. Measurements of K_s and $K_{s\ max}$ were further used to identify PLC, which can be attributed to xylem cavitation, according to Eq. (4). Elkosh and Elea PLC was low (0–12%), Otricoli and Euboea was moderate (26–44%) and in Senalba PLC was high, i.e., 70% or more (Figure 5b). Provenance PLC values did not change significantly with site ($0.1083 < P < 0.8139$, Table 2).

Discussion

Ecophysiological response of Pinus halepensis to drying and warming

This study was motivated by the growing realization that the current rate of climate change may exceed the rate of evolutionary changes, genetic adaptation or migration in Mediterranean forest trees. Alternatively, the potential for ecophysiological adjustments in key traits may be sufficient to ensure the success of a genotype across a wide range of climatic conditions, as described by Bradshaw (1965). The trees included in this study showed a remarkable ability to survive and grow under a range of climate conditions (Figure 2a), sometimes contrasting strongly with those prevailing at their seed source sites (Table 1).

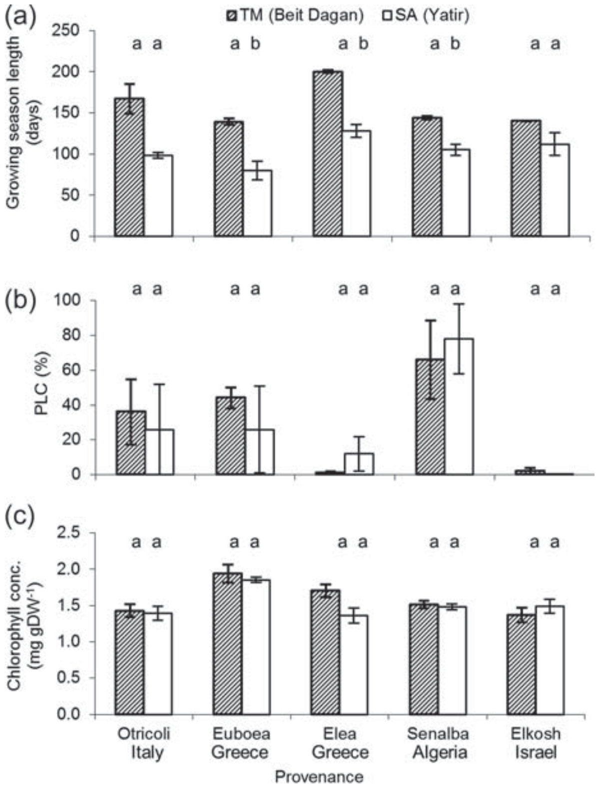

Figure 5. Mean growing season length (a), percent loss of xylem conductivity (b; measured during May–June) and leaf chlorophyll concentration (c; measured during April–May) of trees from each provenance and two climate types (SA, semi-arid; TM, thermo-Mediterranean). Similar letters indicate a non-significant difference ($\alpha > 0.05$) between trees of a provenance growing under different climate types. Error bars represent the standard error of the mean ($n = 5$, 3 and 9 respectively).

The sources of phenotypic variance in the seven parameters were examined according to three categories: (i) environmental effects (E), represented by the three climate types (C); (ii) genetic effects (G), represented by the five provenances (P); and (iii) the interaction between the two (E × G), here (C × P). (i) The climate effect was significant in five out of the seven parameters examined (Table 2), with some traits showing marked variations in all provenances. This included the expected reductions not only in GSL (Figure 5a, ~40% decrease from TM to SA climate) and in mean stomatal conductance, g_s (Figure 2b, ~60% decrease from TM to SA climate) but also in xylem conductivity, which requires structural changes (Table 3, ~77% decrease from TM to SA climate). (ii) Differences between provenances were significant in all but one trait, WUE$_i$. Notably, such variations imparted some compensation for the low climate effect in traits, such as in leaf chlorophyll content and in PLC (Table 2). These two traits did

Table 3. Mean (SE)-specific xylem conductivity values (in g m^{-1} MPa^{-1} s^{-1}) before (K_s) and after ($K_{s\ max}$) perfusion at high pressure measured in twigs of trees from the five provenances in Beit Dagan and Yatir.

Climate (site)	Thermo-Mediterranean (Beit Dagan)		Semi-arid (Yatir)	
Provenance	K_s	$K_{s\ max}$	K_s	$K_{s\ max}$
Otricoli	84.5 (6.8)	146.3 (43.7)	20.4 (3.0)	27.6 (2.8)
Euboea	56.6 (4.5)	102.5 (4.0)	24.1 (9.2)	37.6 (15.4)
Elea	291.8 (76.3)	294.5 (74.0)	24.9 (0.6)	33.8 (0.2)
Senalba	18.8 (6.8)	90.5 (44.6)	7.6 (0.2)	23.5 (9.4)
Elkosh	156.2 (20.1)	159.6 (17.3)	60.0 (14.3)	61.3 (28.8)

not vary with climate but varied significantly among provenances (ranges of ~30 and ~70%, for chlorophyll and PLC, respectively). The stable PLC across climates is in agreement with the poor correlation of embolism resistance with climate reported for other pine species (Martínez-Vilalta et al. 2009, Lamy et al. 2011). The use of provenances (as opposed to identical genotypes, e.g., from clones) means that the observed variations might include some intra-provenance genetic variance, but valuable quantification of provenance-level plasticity can nevertheless be gained (e.g., Tognetti et al. 1997; Calamassi et al. 2001; Atzmon et al. 2004; Baquedano et al. 2008; Schiller and Atzmon 2009). (iii) C × P interactions include situations where a parameter changes across climates (sites), yet differences in the extent of the response are also observed among provenances. These included changes across climates in the mean rate of leaf assimilation, A (Table 2; Figure 3b), in WUE$_i$ (Figure 2b) and in the maximum growth rate (Figure 4).

Nicotra et al. (2010) asked whether the incidence of adaptive plasticity may vary among types of traits (e.g., those related to anatomy versus those related to allocation or physiology). Our results show that traits related to xylem anatomy can be both plastic (e.g., K_s) and rigid (e.g., PLC). Similarly, physiological leaf parameters included the plastic assimilation rate and the rigid chlorophyll level.

Interactions between specific traits

In addition to the three main categories of variations above (climate, provenances and their interactions), combinations of traits are also important: a limitation by a rigid, non-plastic, trait can be compensated for by association with another trait that has a high level of plasticity (Bradshaw 1965; Bonaparte and Brawn 1975; Sadras et al. 2009). For example, the stable PLC across climates could be compensated for by decreasing K_s in some provenances (Table 3 and Figure 5b). Combinations of traits also include GSL and PLC: a low GSL value helps avoiding unfavorable conditions, and therefore the need for high xylem resistance to embolism (low PLC). In contrast, maintaining high GSL critically depends on low PLC. Such a relationship was exemplified by the two Greek provenances: Euboea had the lowest GSL (Figure 5a) and ~35% PLC (Figure 5b), while Elea had the highest GSL and a PLC of only ~10%. The strategy of Euboea seems to rely on synchronization of cambial activity with relatively high water availability during the wet season, as previously reported for other sites (Breitsprecher and Bethel 1990; Brochert 1994), and specifically for *P. halepensis* in Yatir (Klein et al. 2005). At the other extreme, the early onset of the growing season in Elea exposes it to high water potential gradients, increasing the selective advantage of resistance to embolism (Klein et al. 2011).

Another combination of traits may exist between GSL and chlorophyll level. Assuming that the 30% higher chlorophyll content in Euboea (Figure 5c) is translated into higher integrated photosynthetic capacity, it potentially contributed to its higher trunk growth rate (up to 6.7 ± 1.0 mm month^{-1} compared with 3.8 ± 0.7 to 5.0 ± 0.2 in other provenances, Figure 4). But we note that the correspondence between high chlorophyll and a high growth rate was not found in the short-term, late season, leaf gas exchange measurements (Figure 3a). Leaf chlorophyll may therefore provide a better indicator of long-term integrated photosynthetic capacity (e.g., Brougham 1960; Gratani et al. 1998). Across all five provenances, the correlation coefficient between GSL and chlorophyll content was 0.66, which was significant at $\alpha = 0.1$. Elevated chlorophyll content can be a key element in a growth strategy where trunk growth occurs in a short yet strong growth pulse, without reducing field performance (for example, Euboea DBH was similar to or higher than that of other provenances, Figure 2a).

Contribution of ecophysiological traits to growth

In contrast to the differential response in specific traits among provenances, the resulting overall tree performance was similar across provenances (Figure 2a), in agreement with an earlier *P. halepensis* study showing that trait plasticity allows different ecotypes to assume similar phenotypes (Baquedano et al. 2008). Differences in growth among provenances were usually small, and only under the wettest conditions, in the Rome site, large variations in growth rate were observed: Elea and Euboea performed better than Senalba and Elkosh. An association of growth rate with climate conditions at the seed source has been described by Fusaro et al. (2007), who found growth inhibition of Elkosh grown in Pescara, Italy ($P = 1000$ mm), and Otricoli grown in Cagliari on the Sardinia coast ($P = 500$ mm). Note that selection of provenances under dry conditions according to growth data alone would fail in this case: high performance under MM climate (Elea, Euboea) did not carry over to drier conditions.

To quantify the relative contribution of each physiological parameter to a trait related to the overall fitness of the tree (increment growth, measured as BAI), PLS was performed. Among six parameters, 39 and 19% of the fit to BAI was explained by WUE$_i$ and GSL, respectively (Table 2). The contribution of other parameters was 14% or lower. The ability of a genotype to succeed under climate change may depend on its overall fitness, expressed in growth and even more importantly, its survival. The survival aspects could be explored by extending the results obtained in the present study to consider two previous, consecutive studies (Atzmon et al. 2004, Schiller and Atzmon 2009). These studies examined the survival rates of the same provenances used here, planted in 1985 in an experimental plot in the Eastern Yatir area, where conditions are considerably harsher: lower elevation with strong southern aspect, and poor and drier soil. In contrast to the result reported above, high mortality ratios were observed in the Eastern Yatir site, but with large differences between provenances. These differences in mortality across a short geographical gradient are consistent with the notion that Yatir represents the 'dry timberline' for Aleppo pine and possibly for forests in general (Maseyk et al. 2008). In the Eastern Yatir site, mortality ratios in Elkosh and Elea were 18 and 26%, respectively, in 1997, increasing to 42 and 55% in the following survey in 2007. In contrast, Senalba and Otricoli showed mortality ratios as high as 55 and 59%, respectively, in 1997, and 82% mortality for both provenances in 2007 (Euboea was not included in those studies). Notably, among the seven parameters we examined, PLC levels were always lower (Figure 5b), and K_s levels were always higher (Table 3) in the surviving provenances compared to the high mortality provenances. Differential responses in other parameters, including WUE$_i$, did not align with the observed differences in survival. The correlation coefficient between PLC and mortality ratio was 0.78, which was significant at $\alpha = 0.1$. This finding also points to hydraulic failure as the tree mortality mechanism, rather than carbon starvation (Martinez-Vilalta and Pinol 2002, Brodribb and Cochard 2009, Sala et al. 2010, Klein et al. 2011).

Plotting PLC against WUE$_i$ and GSL, the two traits contributing most to growth, in a 3D representation of a response space (Figure 6), demonstrates the idea of an optimal combination of traits for fitness. This optimum, corresponding to the deepest corner in the plot, is represented by low GSL (i.e., growth retention), low PLC (i.e., low embolism) and high WUE$_i$. The observed superiority of Elkosh and Elea over Otricoli and, to a larger extent, over Senalba, is consistent with the analysis presented in Figure 6. Furthermore, while across the climatic gradient in our study a range of combinations and tradeoffs allowed moderate growth of all provenances under SA conditions (below 300 mm annual precipitation), challenging the same provenances with yet harsher conditions (Atzmon et al. 2004, Schiller and Atzmon 2009) indicated that ultimately embolism-resistant xylem is the critical element for tree survival under dry conditions (Brodribb and Cochard 2009, Klein et al. 2011).

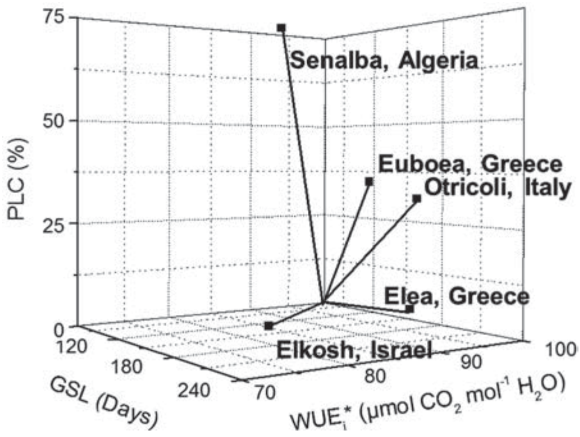

Figure 6. Mean responses of trees from the five provenances with respect to major traits: GSL in Beit Dagan and Yatir ($n = 10$), PLC in Beit Dagan and Yatir ($n = 6$) and WUE$_i$ in Beit Dagan ($n = 6$).

Implications for forestry under climate change

Today forests around the world are challenged by an increasingly faster rate of changes in environmental conditions, already severely affecting vulnerable populations (Allen et al. 2009 and references therein). In many cases, these include trees that grow near the limit of their natural distribution, such as the dry timberline case of the Yatir forest. Our analysis suggests several implications for forestry. (i) Considering the adjustments in key traits both in any given *P. halepensis* provenance and across a range of available provenances and climates could provide a powerful management tool to address the dangers of reduced forest distribution with warming and drying climate trends, such as predicted for the entire Mediterranean and other regions (Cohen et al. 2002, Christensen et al. 2007). (ii) A single advantageous trait may be insufficient for sustainability. For example, selection of the provenance Otricoli based on high WUE$_i$ or Senalba based on low water use in currently favorable conditions would fail under extreme conditions. (iii) Ultimately, selection for low PLC must be an important component in the face of predicted drying and warming. Our findings indicate that at least for *P. halepensis*, the existing gap between plant adaptability and the changing environment could be closed by ecophysiological response at the species and provenance level, as well as by exploiting the range of performance offered by provenances of different seed sources. Such an approach, in turn, will potentially permit sustained forest productivity and distribution even under relatively severe climate change scenarios across the Mediterranean region. Future forest planning should consider testing for ecophysiological adjustment in key traits in candidate tree species and genotypes selected for planting today, whose growth should be ensured in the future.

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Conflict of interest

None declared.

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