SILVA FENNICA

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Germination differences between natural and afforested populations of *Pinus brutia* and *Cupressus sempervirens*

Highlights

- Silvicultural practices of raising and outplanting seedlings yielded contrasting outcomes in our species.
- Afforested *Pinus brutia* populations acquired ability to tolerate drought stress at intermediate and hot temperatures compared to natural populations, which may indicate local adaptation.
- Natural *Cupressus sempervirens* populations showed higher salt-tolerance than afforested populations.
- Seed germination was optimal under intermediate temperatures and deionized water for both species.

Abstract

In afforestation, silvicultural processes of raising and planting seedlings under certain conditions can yield contrasting outcomes in tree stock performance. Moderate nursery conditions may select against stress tolerance whereas planting seedlings in stressful environments at afforestation sites may select for higher stress tolerance compared to natural populations. We compared germination performance between natural and afforested populations of *Pinus brutia* Ten. subsp. *brutia* and *Cupressus sempervirens* L. var. *horizontalis* (Mill.) under differing stress treatments. Seeds were collected from both natural stands and from afforested populations outside the natural distribution range, in Syria. Cold, intermediate and hot temperature regimes were simulated (8/4 °C, 20/10 °C and 32/20 °C) along with cold stratification, drought stress (–0.2 and –0.4 MPa), salt stress (50 and 100 mMol l^{-1}), and deionized water (control) conditions. In addition, we tested the effects of seed weight and climatic conditions on seed germination. In general, intermediate temperatures were optimal for both population types. Afforested *P. brutia* populations outperformed natural ones under drought stress levels at hot and/or intermediate temperatures. Conversely, in *C. sempervirens*, cold stratification at all temperatures and higher salt stress at intermediate temperatures significantly decreased germination in afforested populations. Seed weight did not significantly affect germination percentages, which were however significantly negatively related to annual precipitation in *P. brutia*, and to annual temperature in *C. sempervirens*. We infer that silvicultural processes led to divergent outcomes in our species: local adaptation to drought stress and hot temperatures in afforested *P. brutia* populations and lower salt-stress tolerance in *C. sempervirens*.

Keywords cold stratification; drought stress; nursery; local adaptation; salt stress; silviculture; Syria

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1 Introduction

Afforestation involves the cultivation of a forest at a site where forests do not grow naturally (FAO 2003). Planted forests (including afforestations) account for 6.6% of the world's total forest area (FAO 2010), and they have important ecological and socio-economic benefits (Lal 2004; FAO 2005; Metzger and Hüttermann 2009).

Trees selected for afforestation have to be tolerant to considerable abiotic and biotic stress, particularly if they are planted outside their natural geographic distribution range. However, in tree domestication, the silvicultural practice of raising and planting seedlings may lead to contrasting selective outcomes for stress tolerance. First of all, trees are usually germinated in nurseries under relatively moderate and controlled conditions that differ strongly from natural field conditions, both in terms of abiotic and biotic factors (van den Driessche 1991). As such, selection processes are much reduced in nurseries compared to natural habitats (Muona and Harju 1989; Rajora 1999). This lack of exposure to stress may undermine species' natural selection in respect of stress tolerance at the seed and seedling stages (White et al. 2007), resulting in a loss of stress tolerance among afforested populations. In addition, trees cultivated under artificial conditions are subject to a variety of treatments such as seed storage and seed sizing (Silen and Osterhaus 1979; Chaisurisri et al. 1993; El-Kassaby et al. 1992), both of which can lead to an erosion of genetic variation in plantation stock (El-Kassaby and Thomson 1996; Davidson et al. 1996). Furthermore, inbreeding can lead to declining seed viability and poor seed germination (Wilcox 1983; Muona and Harju 1989), which becomes ever more pronounced as populations become smaller (Ferriol et al. 2011). For example, in a study by Salzer and Gugerli (2012), small peripheral populations of *Pinus cembra* showed lower germination success than central core populations, indicating higher inbreeding depression among smaller, scattered populations. However, it is also possible that the practice of planting trees in stressful conditions beyond their natural range may select for higher stress tolerance among afforested populations. As a species becomes established in a stressful environment, it experiences divergent selection pressures (Espinoza et al. 2013). Selection pressures on phenotypes could lead to local adaptation (Pigliucci et al. 2006) and the development of local landraces (Espinoza et al. 2013). The fast formation of landraces in a species indicates a high potential to evolve and adjust its performance to changing climatic conditions (Bigras and Colombo 2000). Indeed, the development of land races has occurred in several conifer species under more stressful environments (Zobel and Talbert 1984; Ennos et al. 1998; Espinoza et al. 2013).

The performance of a progeny shows a high phenotypic plasticity in response to prevailing environmental conditions during seed maturation (maternal effect) (Skrøppa and Johnsen 2000; Donohue et al. 2009). For example, species may acquire resistance to cold only during embryo development under lower temperatures in the field, and they may lose this ability if temperatures rise during seed maturation (Finkeldey and Hattermer 2007). However, seeds from favorable maternal environments outperformed those from stressful maternal environments in terms of their germination response (Cendàn et al. 2013). In addition, genotypes developed in unfavorable

maternal environment have aborted a higher proportion of their seeds than the genotypes from a favorable maternal environment (Kärkkäinen et al.1999).

Seed germination is a crucial life stage (Fenner and Thompson 2005) that is strongly influenced by abiotic factors such as temperature, water stress and salinity, as well as their interaction (Simak and Kamra 1970; Rao and Singh 1985; Bewley and Black 1994; Baskin and Baskin 1998). However, very scarce studies have compared germination performance between natural and afforested populations. In a germination experiment, Zhu et al. (2006) found that afforested *Pinus sylvestris* var. *mongolica* populations lost their tolerance towards increased drought stress compared to that of natural populations. In general, increased germination performance under unfavorable germination conditions, such as low or high temperatures as well as drought or salinity stress, could be due to larger seed weight and/or the establishment and development of the parent populations under more stressful conditions (Skrøppa and Johnsen 2000; Croser et al. 2001; Zhu et al. 2006).

For the present study, we addressed the question as to whether afforested populations develop lower or higher stress tolerances at the seed stage compared to natural populations. We focused on *P. brutia* (Brutia pine) and *C. sempervirens* (Common cypress) stands in the Eastern Mediterranean. In the Mediterranean region, germination is often restricted to short periods in the wetter spring or autumn seasons, but it is unlikely to occur during the dry summer (García-Fayos et al. 2000; Quilichini and Debussche 2000). We chose these species because their general germination ecology is well known (e.g. Skordilis and Thanos 1995; Boydak et al. 2003; Piotto et al. 2003; Tilki and Dirik 2007; Giannini et al. 1999). In addition, our natural and afforested populations originated from locations with contrasting climatic regimes, which enabled us to explore the role of climatic conditions as co-variables.

For each study species, we compared the germination performance of seeds from natural and afforested populations and tested the following hypotheses: (1) Compared to natural *Pinus brutia* and *Cupressus sempervirens* populations, afforested populations have either lost or acquired their tolerance for cold and hot temperatures as well as for drought and salt stress; (2) The germination response of *Pinus brutia* and *Cupressus sempervirens* populations is influenced by seed weight, i.e. heavier seeds exhibit higher germination; and (3) Germination responses in natural and afforested populations of both species are influenced by climatic provenance, i.e. mean annual temperature and annual precipitation values of the seed collection sites. Specifically, we expected populations located in more arid regions to show enhanced germination.

2 Materials and methods

2.1 Study region and study species

Syria is an Eastern Mediterranean country with variable geographic, geological and climatic conditions that have influenced the abundance and structure of forest vegetation (Nahal and Zahoueh 2005). Semi-arid and arid habitats prevail in Syria (Ghazal 2008) with about 50% of soils being Aridisols, which occur particularly in arid areas with a mean annual precipitation of < 250 mm (Ilaiwi 2001). In addition, saline soils are widely distributed across coastal and inland areas in Syria (Al-Oudat and Qadir 2011). As such, both natural vegetation and man-made plantations grow in conditions of drought stress and salinity.

Pinus brutia and *C. sempervirens* are two important tree species in Syrian natural forests, whose natural ranges overlap along different zones in the coastal mountains (Nahal and Zahoueh 2005). *Pinus brutia* occurs naturally in a variety of bioclimatic zones (Thermo-Mediterranean, Eu-Mediterranean and Supra-Mediterranean) and altitudinal belts (from sea level to 1100 m)

throughout the coastal mountains of Syria (Nahal and Zahoueh 2005; Ghazal 2008). It is dominant in the Eu-Mediterranean humid zone, where it forms pure stands at 100–450 m a.s.l in the Baer-Bassit Mountains of Latakia, and it occurs as natural forest in northwest Syria in the Aleppo (or "Kurds") Mountain (Ghazal 2008).

Cupressus sempervirens natural forests grow in pure stands in the coastal mountains of Syria, or they are frequently associated with *P. brutia* (Zohary 1973). Beyond their natural distribution range, the two species are frequently planted to protect against soil and wind erosion in semi-arid and arid climate zones with mean annual precipitation levels of < 350 mm (Nahal and Zahoueh 2005). Previous studies have shown that an intermediate temperature of 20 °C is optimal for seed germination in both species, and that relatively lower temperatures along with decreasing water potentials significantly inhibit germination (e.g. Skordilis and Thanos 1995; Piotto et al. 2003; Tilki and Dirik 2007; Giannini et al. 1999). Cold stratification has been found to have a beneficial effect on the subsequent germination performance of *P. brutia* seeds (Skordilis and Thanos 1995; Tilki and Dirik 2007). For *C. sempervirens*, cold stratification over 3–4 weeks is recommended to improve germination (Piotto et al. 2003). So far, no research has been recorded on salt tolerance in both study species.

For the present study, we compared seed germination in natural and afforested populations originating from contrasting climates in Syria (Table 1). The afforested populations were established in the 1980s from seeds originating from older plantations that had previously been established in the 1950s in the vicinity of our studied sites (Fig. 1) [see Al-Hawija et al. (2014b) for more details on seed origins and geographic distances among sampled populations]. Thus, our afforested populations had gone through both a process of artificial selection in nurseries and they had been exposed to stressful environmental conditions at the afforestation sites (Table 1).

2.2 Seed collection

Cones from afforested and natural populations were collected in April–May in 2009 and 2010, respectively. We collected cones from six natural and five afforested *P. brutia* populations and three natural and six afforested *C. sempervirens* populations across different bioclimatic regions in Syria (Table 1). *Pinus brutia* cones were collected from natural populations in Latakia [coastal mountains; Eu-Mediterranean humid zone, electrical conductivity (henceforth: EC) generally less than 1 dS m^{-1}; Jamal et al. (2005)] and from the Aleppo Mountain [Eu-Mediterranean, semi-arid zone; EC is 5 dS m^{-1} (Jamal et al. 2005)]. Seeds from afforested populations were collected in the Manbij district of Aleppo province (Eu-Mediterranean, semi-arid zone), where the EC ranged between 2 and 16 dS m^{-1} (Schweers et al. 2005). We also collected seeds from afforested populations in the Irano-Turanian arid zone in Deir Ezzor and Raqqa, where the EC ranged between 2 and 5 dS m^{-1} (Jamal et al. 2005). Salinity levels were included in relation to the respective study regions as we lacked specific data on soil salinity at our study populations.

Cupressus sempervirens cones were also collected from natural populations in Latakia and from afforested populations in the Manbij district and Deir Ezzor city. The original seed source for the Aleppo afforestations was the 'Afrin' afforestation in the Eu-Mediterranean semi-arid zone of northwest Syria, while the seed origin of Deir Ezzor afforestations was the 'Al-Hasaka' afforestation in the Irano-Turanian zone of northeast Syria (Fig. 1). These seed origins are geographically and climatically comparable to the studied afforested populations. Ultimately, the seed source for these afforestations can likely be traced back to natural forests of Syria, from which afforestation stock was collected when forestry began in the 1950s. Consequently, natural forests in Latakia and the Aleppo Mountain may represent the primary origin of afforested *P. brutia* populations, whereas Latakia could represent the primary natural origin of afforested *C. sempervirens* populations.

Table 1. An overview of Syrian *Pinus brutia* and *Cupressus sempervirens* study populations, incl. population type, geographic location, climatic conditions, population area, seed weight and percentage of seed viability, empty seeds and degraded embryos before the start of the experiment.

Population	Population type	Lat.	Long.	Alt.	MAT	Precip.	Population area (ha)	Seed weight (g) (n=300 seeds)	Seed viability	Empty seeds	Degraded embryos
Pinus brutia											
Latakia 1	Natural	35.776	35.902	143	18.38	1025	-	12.39	77%	0%	23%
Latakia 2	Natural	35.810	35.921	271	17.98	1085	-	16.138	70%	0%	10%
Latakia 3	Natural	35.815	35.972	425	17.98	1085	-	16.057	65%	0%	22.5%
Aleppo Mt 1	Natural	36.634	37.037	752	16.32	525	-	15.627	88.5%	1.4%	18.5%
Aleppo Mt 2	Natural	36.660	37.060	826	16.68	499	-	15.998	82%	0%	18%
Aleppo Mt 3	Natural	36.621	37.011	632	16.57	499	-	16.560	93%	0%	6.7%
Aleppo 1	Afforested	36.257	38.003	364	17.80	248	110	15.883	78%	0%	12%
Aleppo 2	Afforested	36.257	37.557	392	17.76	289	105	15.813	80%	0%	8.5%
Aleppo 3	Afforested	36.427	37.921	539	17.20	290	68.5	13.141	80%	0%	10%
Deir Ezzor 1	Afforested	35.244	40.035	238	19.54	166	9.6	10.093	75%	0%	20%
Raqqa	Afforested	35.970	39.022	312	18.61	183	40	13.033	75%	0%	15%
Cupresssus sempervirens											
Latakia 1	Natural	35.887	35.898	130	18.38	1025	-	2.627	57%	0%	40%
Latakia 2	Natural	35.919	35.916	450	17.98	1085	-	2.289	57%	0%	41%
Latakia 3	Natural	35.823	35.962	539	17.98	1085	-	2.827	61%	0%	37%
Aleppo 1	Afforested	36.426	38.009	540	17.80	248	110	2.483	57.5%	1.8%	29%
Aleppo 2	Afforested	36.440	37.809	535	17.76	289	105	1.785	69%	0%	13%
Aleppo 3	Afforested	36.508	37.962	474	17.68	295	1.5	2.086	49%	0%	43%
Deir Ezzor 1	Afforested	35.406	40.059	238	19.54	166	9.6	1.711	22.4%	8.6%	48.3%
Deir Ezzor 2	Afforested	35.347	40.152	205	19.54	166	5.7	3.042	48%	3.3%	29%
Deir Ezzor 3	Afforested	35.335	40.118	221	20.02	165	1.2	1.531	17.2%	53.4%	20.7%

Abbreviations used: Geographic location: Lat. = latitude (decimal degrees), Long. = longitude (decimal degrees) and Alt. = Altitude (m); Climatic conditions: MAT = mean annual temperature (°C) and Precip. = mean annual precipitation (mm).
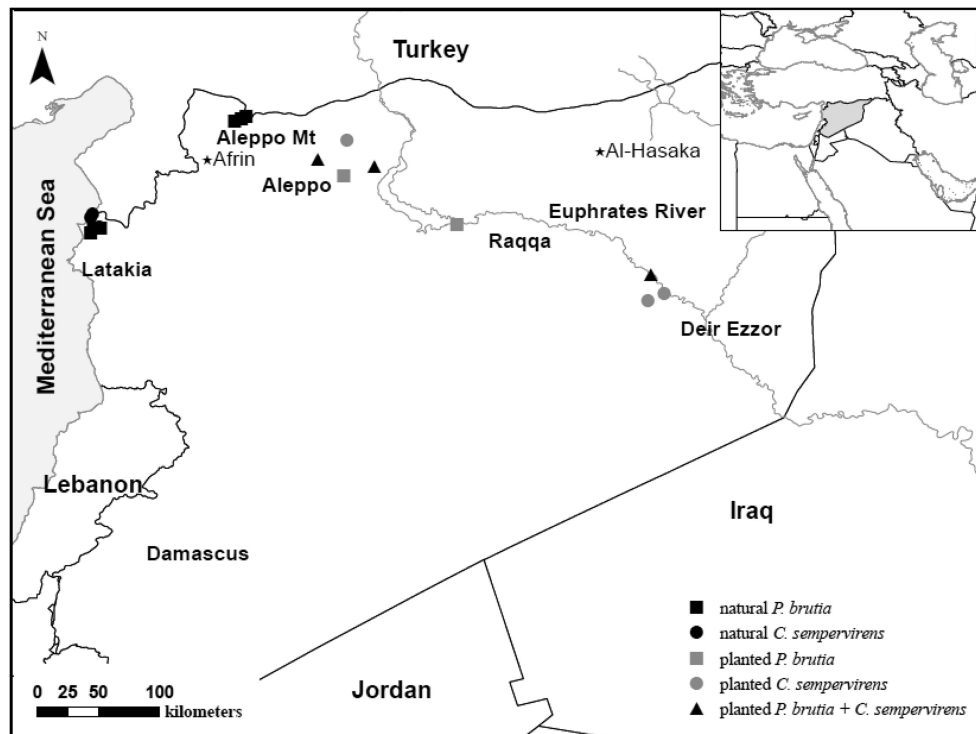
Fig. 1. Sample locations of natural and afforested *Pinus brutia* and *Cupressus sempervirens* populations in Syria. Seed origins of the afforestations are marked with a star.

A total of 75–100 cones were randomly collected from each population from at least 20 mother trees spread across the spatial distribution of each population, with distances between populations ranging from 3 to 426 km. Cones were then dried in the open air to stimulate opening, after which they were cracked mechanically. Seeds from each population were pooled and thoroughly mixed with 300 seeds from 30 bulk samples being weighed (Table 1). Seeds were then stored in paper bags under ambient laboratory conditions.

2.3 Germination experiment

The germination experiment was initiated in February 2010 and 2011, approximately one year after each collection phase. We used a full factorial design in which we crossed three different temperature regimes (8/4 °C, 20/10 °C and 32/20 °C; 12 h of warm, white light, 12 h of darkness) with six treatments (de-ionized water as a control, cold stratification, drought stress: –0.2 and –0.4 MPa, and salt stress: 50 and 100 mMol l^{-1}). All six treatments were applied for each temperature regime and for both species. In the case of the cold stratification treatment, we incubated seeds at 5 °C for four weeks under moist condition. Drought stress and salt stress effects were investigated using different concentrations (–0.2 and –0.4 MPa) of polyethylene glycol (PEG-4000) and sodium chloride NaCl (50 and 100 mMol l^{-1}) solutions. As no information was available on the soil water content or salinity of our study populations, the levels employed were in line with previous general findings for the East Mediterranean region (Thanos and Skordilis 1987; Boydak et al. 2003; Tilki and Dirik 2007).

We used four Petri dishes per treatment (72 Petri dishes per population). For the cold stratification treatment, we used 25 seeds of *P. brutia* and 30 seeds of *C. sempervirens* per Petri dish. For all other treatments, we chose 20 seeds of *P. brutia* and 25 seeds of *C. sempervirens* per Petri

dish. Seeds were chosen randomly and placed on filter paper in standard glass Petri dishes (7 cm diameter). Dishes were placed in three programmed incubators, each with one of the aforementioned temperature regimes that simulated temperature fluctuations in winter, spring and autumn in the study region. Although it has been shown that the intermediate temperature is optimal for seed germination in both species (see above), the cold and hot temperature regimes were applied to explore the differences in responses between the two population types to relatively non-optimal conditions.

Seeds were regarded as having germinated when they showed a visible radicle, and they were subsequently scored and removed every second or third day. The location of the dishes in the chambers was rotated after each census, and dishes with PEG and NaCl solutions were covered with Parafilm to prevent evaporation. Filter papers and solutions were changed as needed in order to keep water potentials and salt concentrations steady throughout the germination experiment. The experiment lasted for 45 days.

Before the experiment, we tested 100 randomly chosen seeds per population for viability (with triphenyl tetrazolium chloride; Hendry and Grime 1993), degradation and emptiness (Table 1). Shortly after the end of the experiment, we checked non-germinated seeds under the microscope and recorded whether seeds were filled or had degraded embryos (data not shown).

2.4 Data analysis

We estimated the final germination percentage as the number of seeds germinated from the total seeds (i.e. 20 or 30) per Petri dish during the experiment. Germination rates were calculated using a modified Timson's index (Timson 1965; Pérez-Fernández et al. 2006): the cumulative sum of all germinated seeds per Petri dish during the course of the experiment divided by the number of days ($n = 45$). However, as the germination percentage and rate were highly correlated (data not shown), we included only germination percentage in all subsequent analyses. It is noted that one afforested population (Deir Ezzor 3) did not show any germination, and it was consequently not include it in the statistical analysis. We extracted climatic variables (Table 1) per population from the WORLDCLIM database (resolution of 2.5 arc minutes, Hijmans et al. 2005).

We used linear mixed-effects models (LMMs) to analyze the effect of population type (afforested *vs*. natural populations), temperature regime and treatment combinations and their interactions, and we used seed weight (ln-transformed) as a covariate on germination percentage (arcsine square-root – transformed). The arcsine square-root transformation was performed on our data before statistical analysis to ensure homogeneity of variance. For each species, we performed three individual models, with each model testing different treatment combinations (cold stratification, drought stress and salt stress). The results for the water control were always included as an additional level in the treatment combinations.

We started with a full model using population type, temperature regimes and treatment combinations and their interactions as a fixed effect, and seed weight as a covariate. To account for spatial clustering, we used population as a random effect (justified following model comparisons). Estimates in our models were fit with restricted maximum likelihood (REML). All analyses were performed separately for the two species using the 'nlme' package (Pinheiro et al. 2012) in R (R Core Team 2013). We also implemented the 'multcomp' package (Hothorn et al. 2008) to perform a Tukey's pairwise post hoc test ($P < 0.05$) for the significant interactions.

For both species, we used general linear models to test the influence of mean annual temperature and mean annual precipitation at the seed collection site (henceforth: annual precipitation) on mean germination percentage under the intermediate temperature regime (20/10 °C) and the water control. This resulted in a model comprising the effects of population types (natural *vs*. afforested)

and climatic variables. In order to generate a minimal adequate model for each response variable, we generated a full model and simplified it by removing all non-significant terms ($P > 0.05$) in a stepwise-backward procedure. Model selection was based on the corrected Akaike Information Criterion (AIC; Akaike 1974) using the 'sme' package (Berk et al. 2013).

3 Results

3.1 *Pinus brutia*

Seed viability was relatively high in natural and afforested population types of *P. brutia* (mean $= 87.5 \pm 7.80\%$). Most non-viable seeds in both population types contained degraded embryos (Table 1). In general, there was no difference in seed germination between the two types of *P. brutia* populations (Table 2; A-C: Population type). The temperature regimes significantly influenced germination percentage (Table 2; A-C: Temperature). The intermediate temperatures produced the highest germination while the cold temperatures and, to a lesser extent, the hot temperatures produced lower germination percentages (Fig. 2). In all treatments, afforested and natural populations differed in their germination percentage under different temperature regimes (Table 2; A-C: Population type × Temperature, Fig. 2).

   Cold stratification significantly increased germination percentage in *P. brutia* (Table 2; A: Treatment). This effect was revealed in the natural population type only at the cold temperatures (Table 2; A: Temperature × Treatment, Fig. 2). By comparison, both drought and salt stress significantly decreased germination percentage in *P. brutia* (Table 2; B and C: Treatment, Fig. 2). Under the drought stress treatment, differing responses were revealed in relation to the population types (Table 2; B: Population type × Treatment). A Pairwise-Tukey test showed that afforested populations outperformed natural ones under drought stress level (–0.2) at the intermediate temperatures and under the drought stress level (–0.4 MPa) at both intermediate and hot temperatures (Table 2; B: Population type × Temperature × Treatment; Fig. 2). Moreover, salt stress (50 and 100 mMol l^{-1}) inhibited seed germination in *P. brutia*, which occurred similarly in both population types under the hot temperature regime (Table 2; C: Temperature × Treatment, Fig. 2).

   Seed weight had no significant influence on the germination percentage of *P. brutia* (Table 2; A-C: Seed weight). According to the minimum adequate model, annual precipitation (AIC:

Table 2. Results of linear mixed-effects models performed for *Pinus brutia*. Models tested whether Population type (natural *vs.* afforested), Temperature (8/4 °C, 20/10 °C and 32/20 °C), Treatment (including, cold stratification, drought stress: –0.2 MPa and –0.4 MPa, salt stress: 50 mMol l^{-1} and 100 mMol l^{-1} and the "deionized water" as a control), and their interactions had a significant effect on seed germination. Population was used as a random effect and seed weight as ln-transformed.

Model/Treatment	A: Cold stratification			B: Drought stress			C: Salt stress		
Fixed effect	d.f.	F-*value*	*p-value*	d.f.	F-*value*	*p-value*	d.f.	F-*value*	*p-value*
Population type	1	0.66	0.440	1	4.11	0.077	1	0.63	0.450
Temperature	2	255.23	**<0.0001**	2	729.47	**<0.0001**	2	926.59	**<0.0001**
Treatment	1	111.05	**<0.0001**	2	158.34	**<0.0001**	2	37.25	**<0.0001**
Seed weight	1	0.24	0.637	1	0.24	0.634	1	0.04	0.864
Population type × Temperature	2	9.97	**0.0001**	2	33.56	**<0.0001**	2	5.91	**0.003**
Population type × Treatment	1	0.89	0.346	2	15.21	**<0.0001**	2	2.75	0.065
Temperature × Treatment	2	12.84	**<0.0001**	4	43.15	**<0.0001**	4	15.09	**0.0001**
Population type × Temperature × Treatment	2	0.06	0.935	4	2.90	**0.021**	4	0.938	0.441

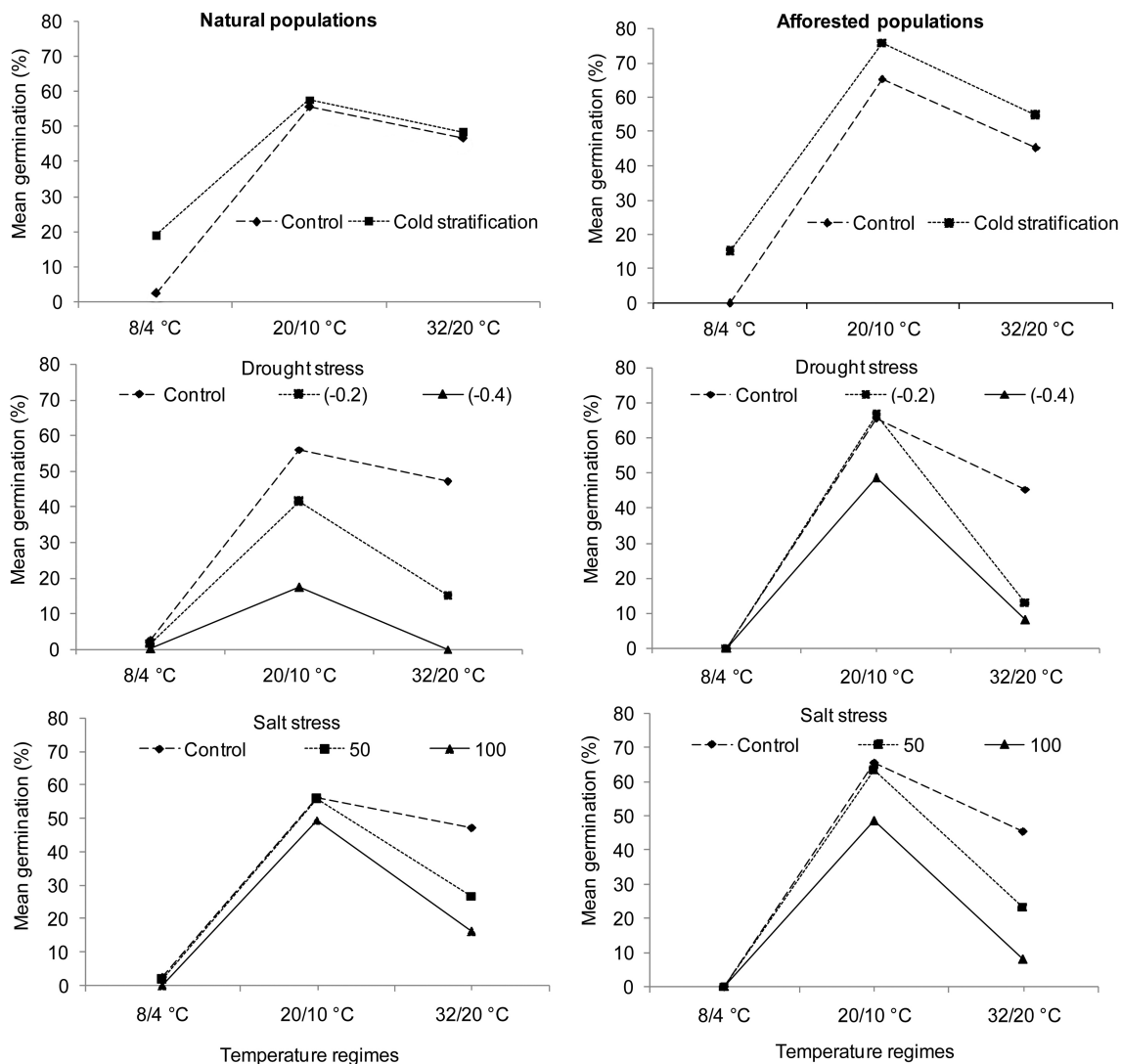Bold numbers indicate significant effects ($P < 0.05$).

Fig. 2. Interaction plots showing germination percentages of natural (left) and afforested (right) *Pinus brutia* populations across three different temperature regimes and treatments. Note that germination percentage is shown as not arcsine – transformed. Control = deionized water. Drought stress was tested at levels of –0.2 MPa and –0.4 MPa, and salt stress at concentrations of 50 mMol l^{-1} and 100 mMol l^{-1}.

10.37) was the only climatic predictor that significantly influenced mean germination percentage ($F_{(9, 1)}$ = 6.91, p = 0.034), and had a negative effect (Fig. 4). Adding population type (AIC: 15.84) or annual temperature (AIC: 16.69) did not improve the fit of the minimum adequate model showing that these variables do not affect germination percentage.

3.2 *Cupressus sempervirens*

Seed viability tested at the beginning of the experiment was relatively low in *C. sempervirens* (mean = 48.67 ± 17.57%). Moreover, most of the non-viable seeds were empty or had degraded embryos; an observation that was more pronounced in the afforested populations from the arid region (Table 1). We detected no effect of population type on seed germination of *C. sempervirens* (Table 3; A-C: Population type). Temperature regimes significantly influenced seed germination (Table 3; A-C: Temperature), with the highest germination percentages being revealed at the
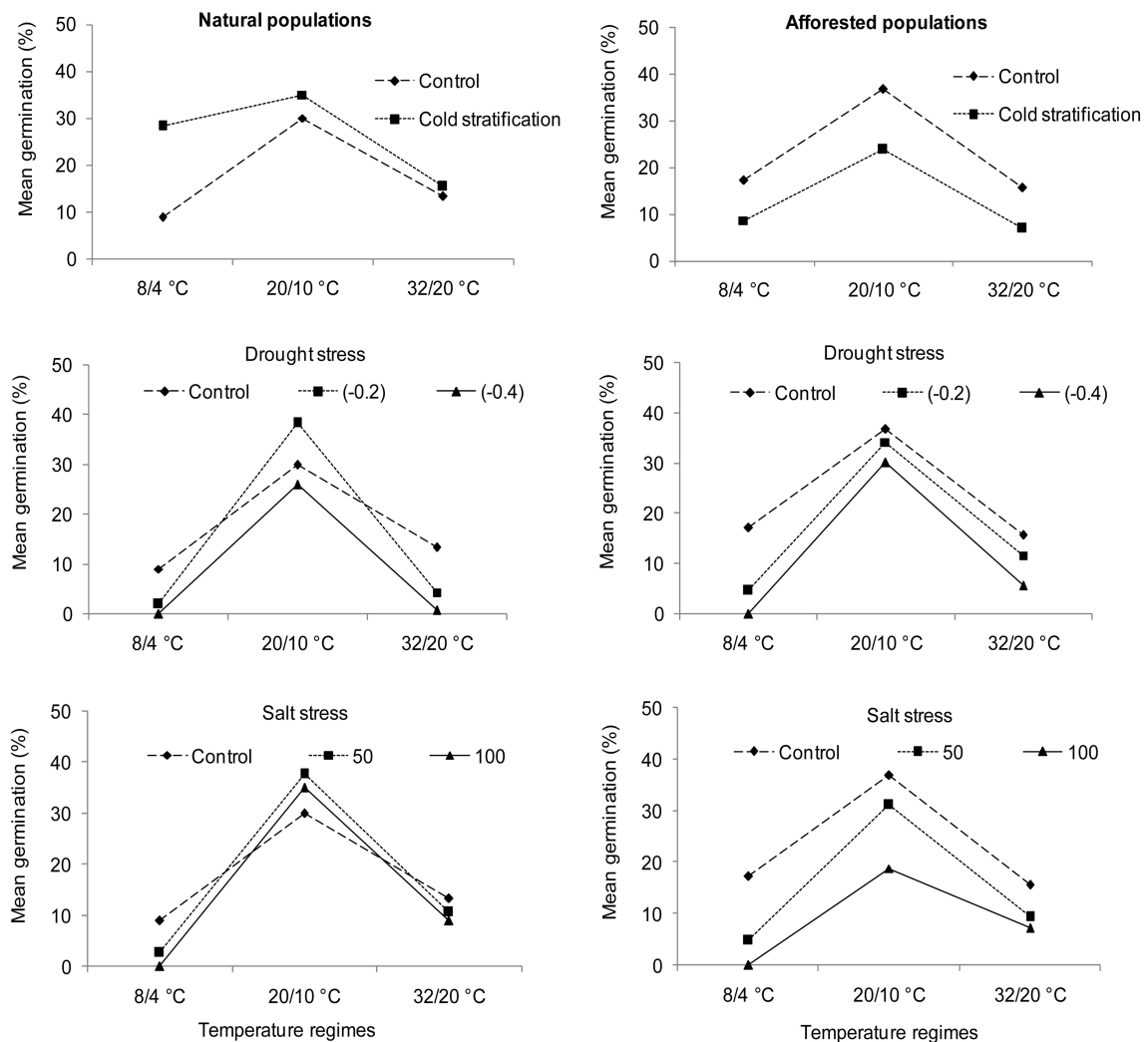
Fig. 3. Interaction plots showing germination percentages of natural (left) and afforested (right) *Cupressus sempervirens* populations across three different temperature regimes and treatments. Note that germination percentage is shown as not arcsine – transformed. Control = deionized water. Drought stress was tested at levels of –0.2 MPa and –0.4 MPa, and salt stress at concentrations of 50 mMol l[-1] and 100 mMol l[-1].

intermediate temperatures (Fig. 3). In all treatments except for cold stratification, population types reacted differently to temperature regimes (Table 3; A-C: Population type × Temperature). Germination percentage in *C. sempervirens* was significantly affected by cold stratification, with population types reacting differently to the treatment (Table 3; A: Population type × Temperature × Treatment). A Pairwise-Tukey test showed a decreased germination percentage in the afforested populations in response to this treatment at all temperature regimes but an enhanced germination of the natural populations under the cold temperature regime (Table 3; A: Population type × Temperature × Treatment, Fig. 3).

Drought and salt stress revealed significant effects on seed germination of *C. sempervirens* (Table 3; B and C: Treatment). Natural and afforested population types reacted similarly and differently to drought stress and salt stress treatments, respectively (Table 3; B and C: Population type × Treatment). A Pairwise-Tukey test showed that both natural and afforested population types exhibited decreased germination percentages compared to the control under a drought stress of –0.4 MPa at the cold and hot temperatures. Moreover, a drought stress level of –0.2 MPa affected

Table 3. Results of linear mixed-effects models performed for *Cupressus sempervirens*. Models tested whether Population type (natural *vs.* afforested), Temperature (8/4 °C, 20/10 °C and 32/20 °C), Treatment (including, cold stratification, drought stress: –0.2 MPa and –0.4 MPa, salt stress: 50 mMol l^{-1} and 100 mMol l^{-1} and the "deionized water" as a control), and their interactions had a significant effect on seed germination. Population was used as a random effect and seed weight as ln-transformed.

Model/Treatment Fixed effect	A: Cold stratification			B: Drought stress			C: Salt stress		
	d.f.	F-*value*	*p-value*	d.f.	F-*value*	*p-value*	d.f.	F-*value*	*p-value*
Population type	1	1.81	0.226	1	0.67	0.448	1	0.10	0.758
Temperature	2	85.43	**<0.0001**	2	344.37	**<0.0001**	2	230.52	**<0.0001**
Treatment	1	3.79	**0.048**	2	78.69	**<0.0001**	2	56.08	**<0.0001**
Seed weight	1	1.96	0.219	1	1.34	0.298	1	0.39	0.557
Population type × Temperature	2	0.01	0.986	2	5.38	**<0.001**	2	7.34	**<0.001**
Population type × Treatment	1	28.91	**<0.0001**	2	0.23	0.790	2	4.29	**0.014**
Temperature × Treatment	2	4.37	**0.014**	4	7.21	**<0.0001**	4	6.21	**0.0001**
Population type × Temperature × Treatment	2	8.93	**<0.001**	4	3.42	**0.009**	4	0.34	0.846

Bold numbers indicate significant effects (*P*<0.05).

population types differently at the varying temperature regimes (Table 3; B: Population type × Temperature × Treatment). In comparison to the control, a reduction in germination percentage of the natural type occurred under the cold and hot temperature regimes but only under the cold temperature regime in the afforested population type (Fig. 3). Interestingly, under the intermediate temperature regime, natural populations of *C. sempervirens* showed enhanced germination under drought stress (–0.2 MPa) compared to the control (Fig. 3), albeit to a non-significant degree. Natural *C. sempervirens* populations showed higher salt tolerance than afforested populations. In this regard, a Pairwise-Tukey test demonstrated a significant decline in seed germination from afforested populations compared to natural ones under the higher salt stress (100 mMol l^{-1}) at the intermediate temperatures (Fig. 3). In afforested populations, both salt stress levels significantly decreased seed germination relative to the control under all temperature regimes (Fig. 3).

We did not find a significant relationship between germination percentage and seed weight under all treatments (Table 3; B-C: Seed weight). In addition, the minimum adequate model (AIC: 9.93) showed that annual temperature significantly negatively influenced the mean germination percentage ($F_{(7, 1)} = 5.77$, $p = 0.049$) (Fig. 4). Population type (AIC: 14.50) and annual precipitation (AIC: 14.25) did not have a significant effect on seed germination of *C. sempervirens*.
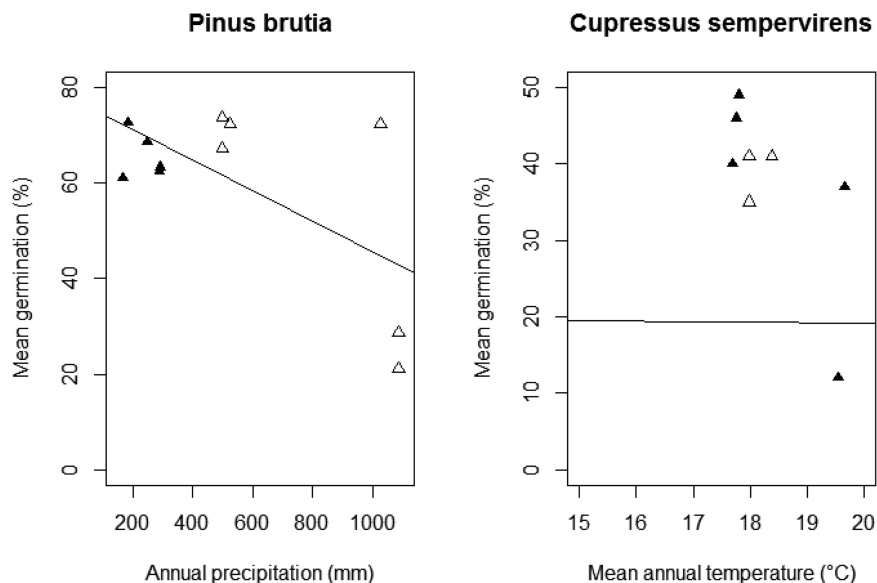
Fig. 4. Relationship between mean germination percentage and annual precipitation and mean annual temperature per population of *Pinus brutia* and *Cupressus sempervirens*, respectively (natural populations: empty triangles; afforested populations: solid triangles).

4 Discussion

Trees planted for afforestation need to tolerate considerable abiotic and biotic stress when they are planted outside their native range. Our study demonstrated that the silvicultural practice of raising and planting seedlings led to two different selective outcomes in our study species. In *P. brutia*, afforested populations acquired an ability to tolerate drought stress at intermediate and hot temperatures. The result may indicate a first sign of local adaptation to more stressful conditions and the development of well adapted land races. However, in *C. sempervirens*, afforested populations lost their ability to tolerate higher salt stress.

4.1 Local adaptation among afforested *Pinus brutia* populations

In the case of *P. brutia*, afforested populations showed significantly higher germination than natural populations under both drought stress levels (–0.2 and –0.4 MPa) and under both intermediate and hot temperature regimes. Afforested populations and their planted parent populations grow in areas with a more arid climate than natural populations. Our results indicate that within two generations, the more arid climate has selected for increased drought stress and higher temperature tolerance among afforested populations. Interestingly, Zhu et al. (2006) found contrasting results for *Pinus sylvestris* var. *mongolica* populations, where natural populations had higher germination percentages under increased simulated drought stress compared to afforested populations. The authors attributed their results to the fact that seeds from natural forests developed under lower precipitation, suggesting adaption to more drought conditions.

The stressful environmental conditions at afforestation sites can exert severe selection pressure on species, which is indicated by the differing mortality rates recorded among provenances (Schiller et al. 2004). Although there is no data on the mortality rate of outplanted *P. brutia* trees after afforestation establishment, investigations on different afforestation projects performed in semiarid areas suggest a mortality average of ca. 80% or 60% for *Pinus halepensis* (Maestre and

Cortina 2004; Schiller et al. 2004). If the same rate applies for *P. brutia*, it may be assumed that the surviving individuals could cope with stressful and variable environmental conditions at the afforestation sites. A parallel study (Al-Hawija et al. 2014b) showed that afforested populations are also genetically distinct, compared to natural populations, which may indicate that afforested populations are in the process of developing into different land races (White et al. 2007).

4.2 Loss in stress tolerance among afforested *Cupressus sempervirens* populations

In *C. sempervirens*, seeds from afforested populations showed significantly lower germination under cold stratification compared to the control. In addition, at the end of the cold-stratification experiment, most of the non-germinated seeds were non-viable and highly infested with fungi, suggesting that the stratification conditions may not have been optimal for afforested *C. sempervirens* populations. Likewise, in *Abies amabilis*, stratified seeds displayed more fungal growth than unstratified seeds and exhibited lower germination (Davidson et al. 1996). Edwards (1980) reviewed several studies and revealed that immature seeds tend to show reduced germination as a result of prechilling, and that they are more susceptible to disease. Maturity differences can be found among cones within the same tree and even among seeds within any one cone (Edwards 1982). Cones of *C. sempervirens* can remain on the tree as long as twenty years (Piotto et al. 2003); it may therefore be assumed that our afforested seeds maintain different levels of maturity and some of them may remain immature.

Our results showed that afforested populations lost their tolerance towards higher salt stress at the intermediate temperatures. The loss in salt tolerance among afforested populations could be attributed to different effects: First, high levels of inbreeding among the small and scattered afforested populations: In this study, the vast majority of non-viable seeds were empty or had degraded embryos. Conifer species are characterized by a high number of lethal equivalents (Savolainen et al.1992). Consequently, most selfed-embryos die during seed development (Charlesworth and Charlesworth 1987; Kärkkäinen et al. 1999). Further, Salzer and Gugerli (2012) found that 76% of seeds collected in small peripheral stands were empty; by comparison, seeds from large core populations showed an average of 30% embryo abortion. The authors considered the high embryo abortion rate is an indication of inbreeding depression at the earliest life stage. Second, the maternal environment could have affected salt tolerance: In conifer species, the maternal environment effect is more pronounced compared to other species because the seed tissues (i.e. the seed coat, megagametophyte and the embryo) that control germination are mainly maternal (El-Kassaby et al. 1992; Linkeis et al. 2010). Moreover, all hormones and provisioning transcripts that regulate germination processes are transmitted from the mother trees to seeds (Nakabayashi et al. 2005). It is possible that the more favorable maternal environment in natural habitats allows progeny seeds to resist higher salt concentrations. Similar results were revealed by Cendàn et al. (2013) for *Pinus pinaster*, where seeds from the favorable maternal environment germinated earlier and to a greater extent than seeds from the stressful maternal environment.

Last but not least, the result could be due to higher water content within seeds of natural populations: These populations grew under remarkably higher annual precipitation levels compared to afforested populations. Their seeds could have contained more water to withstand salt stress. Croser et al. (2001) similarly explained higher germination success in seeds of *Pinus banksiana* under salt stress by virtue of their larger seed size and more solutes.

4.3 The effect of seed weight and climatic provenance

Seed germination in *P. brutia* and *C. sempervirens* was not affected by seed weight. Likewise, no

relationship between seed mass and germination parameters was observed in *Pseudotsuga menziesii* (El-Kassaby et al. 1992) or *Pinus strobus* (Parker et al. 2006).

In addition, our results demonstrated for *P. brutia* that afforested populations from localities with higher annual precipitation showed decreased germination. This is in line with other studies that showed that seeds from humid provenances were more susceptible to high water stress than seeds from arid provenances (Boydak et al. 2003; Tilki and Dirik 2007). Conversely, in *C. sempervirens*, natural population seeds from localities with lower annual temperatures exhibited higher germination percentages. This can be interpreted again as a more favorable maternal environment in the natural area of the species (Cendàn et al. 2013).

In conclusion, the results of our study show that raising seedlings in nurseries and outplanting them into relatively more stressful environments at afforestation sites can be two-fold: Afforested *P. brutia* populations showed signs of local adaptation to increased drought and hot temperatures, while afforested *C. sempervirens* populations appeared to lose their tolerance to salt-stress. Our finding indicates that for *P. brutia*, the stressful environmental conditions at afforestation sites compared to those prevailing in natural forests induces selection pressures, which in turn result in local adaptation and a development of land races within the species.

The different trend in germination performance between natural and afforested *C. sempervirens* populations may have mainly resulted from inbreeding depression in the afforested populations, particularly arid regions, which was evidenced by the higher numbers of empty seeds and degraded embryos. In a previous study by Al-Hawija et al. (2014a), seedlings from the respective natural *C. sempervirens* populations outperformed the afforested ones under different temperature and moisture regimes. This result, together with the current study results, calls for an urgent protection of the natural forest resources of this species in Syria.

As the intermediate temperatures were demonstrated to be optimal for seed germination of population types in both species, it is practically beneficial when such conditions prevail during the germination phase in nurseries.

The positive effect of cold stratification on the activation of germination was more pronounced in afforested populations of *P. brutia*. This result substantiates the beneficial effect of cold stratification in terms of breaking dormancy and enhancing the germination percentage of *P. brutia* seeds. Such an approach can be used to facilitate nurseries for large scale afforestation programs. With regard to *C. sempervirens*, cold stratification improved seed germination in natural population seeds at cold temperatures, which is of relevance where plant sowing can be performed under cold conditions. The negative influence of cold stratification on the afforested populations suggests that this treatment is not recommended for the afforested *C. sempervirens* populations.

Acknowledgements

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