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Field performance of *Pinus halepensis* planted in Mediterranean arid conditions: relative influence of seedling morphology and mineral nutrition

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Abstract In Mediterranean arid regions, relatively small planting stock has traditionally been used in an attempt to reduce drought susceptibility, though few studies have examined influences of initial seedling morphology and nutrition on long-term plantation establishment. We fertilized *Pinus halepensis* Mill. seedlings in the nursery with controlled release fertilizer (CRF) varying in formulations and rates; 9-13-18 and 17-10-10 (N-P-K) formulations at 3, 5 and 7 g l^{-1} substrate plus an unfertilized control and we evaluated growth and survival 7 years after planting in arid conditions in Almería province, southeast Spain. Interactions between initial height and fertilizer treatments occurred during the first 3 years; initial size advantages of specific fertilizer treatments (7 g l^{-1} of 9-13-18 and

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17-10-10 at 3 g l^{-1}) persisted after 7 years. The largest and most nutrient-rich seedlings from 9-13-18 at 7 g l^{-1} (41.0 cm tall, 4.4 mg of P per g of root tissue at time of planting) exhibited the highest survival after 7 years (79%), while survival declined to 42% for non-fertilized plants (12.9 cm tall and 0.6 mg of P per g of root tissue). Initial seedling morphological parameters were most consistently correlated with field performance. Root P concentration was the nutrient variable most closely related to survival. Our data emphasizes importance of longer-term experiments to accurately assess influences of nursery treatments on field responses, particularly in arid areas. We suggest that larger seedlings with greater nutrient reserves than are currently being used should be incorporated into Mediterranean plantations.

Keywords Aleppo pine · Fertilization · Forest restoration · Nitrogen · Phosphorus · Reforestation

Introduction

Use of large planting stock for regeneration of temperate forests in areas of dense vegetative competition has increased over the last three or more decades as a means to improve reforestation success (Tuttle et al. 1988; South et al. 2005). Several reasons have been cited to account for increased growth of larger compared to smaller stock. Greater foliar mass of larger stock allows for increased photosynthetic capacity, which may facilitate rapid growth and ability to quickly occupy site resources during establishment (Grossnickle 2000). In addition, larger stock has other attributes such as higher nutrient and carbohydrate content that can promote field performance. However, successful establishment of planted seedlings also requires an equilibrium between the evaporative demand of the shoot and water uptake capacity of the roots; high foliar mass of large seedlings may create a disadvantage under certain environmental circumstances (Lamhamedi et al. 1997; South et al. 2001). Accordingly, shoot:root has long been considered an important morphological quality index (Thompson 1985), particularly in dry areas (Birchler et al. 1998).

Container stock has been used for more than 30 years in Mediterranean areas as a means to help overcome frequent spring and summer droughts (Peñuelas and Ocaña 1996). Harsh conditions during the summer after planting led foresters to advise the use of small stock for decades (Villar-Salvador 2003). However, recent results from experiments carried out with several Mediterranean species refute this contention (Puértolas et al. 2003; Villar-Salvador et al. 2004, 2008). In these studies the survival and growth of large stock were greater than small stock. Rapid initial height growth reduces mortality and lessens the need to replant areas with high mortality or to remove competing vegetation (Roth and Newton 1996; South and Mitchell 1999). Therefore, either in temperate or Mediterranean areas, large seedling size appears to be generally advantageous for reforestation, although the point at which size becomes too great or unbalanced likely varies according to site and seedlings characteristics (Lamhamedi et al. 1997). There is a current need to develop target values for Mediterranean container forest tree seedlings that optimize field response, particularly with regard to shoot:root equilibrium under the driest conditions of Mediterranean areas (Navarro et al. 2006).

Along with morphology, studies show that nutrient status of nursery seedlings plays a significant role in postplanting response. However, most of these focus on wet, temperate forests, with an emphasis on nitrogen (N) (van den Driessche 1980; Imo and Timmer

1999). Foliar N concentration is related to photosynthetic assimilation after planting, which enhances root and shoot growth in many species (van den Driessche 1987; Green and Mitchell 1992; Jose et al. 2003), including those from Mediterranean areas (Villar-Salvador et al. 2004). In addition, N is the nutrient that usually causes the most pronounced effect on morphological parameters in the nursery (Villar-Salvador et al. 2005). Recent results also show that phosphorus (P) concentration influences survival and growth of outplanted seedlings in Mediterranean areas (Villar-Salvador et al. 2004; Oliet et al. 2005). Conversely, other studies from these areas have reported detrimental effects of high nutrient concentrations on survival of several species (Trubat et al. 2008). Therefore, improved knowledge of the roles of N and P after planting in Mediterranean species is needed (Villar-Salvador et al. 2008). Because nursery fertilization affects both morphology and nutrient status of planting stock, it is difficult to clearly identify the specific role of nutrients in field responses. Therefore, efforts to separate the relative importance of morphology and nutrient status could contribute to improved understanding of the importance of each effect in outplanting responses, particularly under Mediterranean arid and semiarid conditions; the use of statistical tools to separate these effects is relatively uncommon in this area of research.

In addition, many reforestation studies focus only on first year field results. However, some authors emphasize the importance of tracking development for longer timescales (Cain and Barnett 1996; Jacobs et al. 2004). This may sometimes alter the conclusions of a single season study, due to interactions between experimental treatments and time. Enhanced nutrient status, combined with early growth dominance and rapid attainment of free-to-grow status suggests that improved field performance of fertilized seedlings may continue beyond the first two seasons. Few studies, however, have verified prolonged dominance of seedlings characterized by increased mineral nutrient levels, especially when examining effects associated with nursery fertilization (Puértolas et al. 2003).

In this study, we examined variation in *Pinus halepensis* Mill. seedling stock characteristics associated with nursery fertilization treatments consisting of different rates of two formulations of controlled release fertilizer (CRF). Incorporating CRF into the growing media provides a means for nutrient loading in the nursery as well as for continued fertilization after outplanting (Oliet et al. 2004; Haase et al. 2006). We tested the null hypothesis that no differences occurred in survival or growth over the course of 7 years after planting in Mediterranean arid conditions when using larger, nutrient-rich stock. *Pinus halepensis* is one of the most common tree species in the arid lands of the Mediterranean basin and plays a critical role in the restoration of degraded lands and abandoned croplands in this region (Ne'eman and Trabaud 2000).

Materials and methods

Seedling production and characterization

Seedling production was conducted in the Agriculture Research and Training Centre of La Mojonera (2°41′W, 36°47′N, elevation 160 m, Almería, Spain). Plants were grown in 230 cm^3 containers (75 containers per tray; density of 308 m^{-2}) filled with a 3:1 (v:v) sphagnum peat moss-vermiculite growing medium in which fertilizer treatments were mixed. Fertilizer treatments consisted of two Osmocote® (Scotts Co., Marysville, OH, USA) controlled release fertilizer (CRF) formulations: 9-13-18, and 17-10-10 applied at 3, 5 and 7 g l^{-1} substrate. The two fertilizers vary in N:P, which was used to create a broad

Table 1 N, P and K quantities applied per seedling by fertilizer treatments

Formulation	9-13-18			17-10-10		
Rate ($g\ l^{-1}$)[a]	3	5	7	3	5	7
N (mg $seedling^{-1}$)	77.2	128.7	180.2	145.9	243.1	340.3
P (mg $seedling^{-1}$)	48.6	81.0	113.5	37.4	62.4	87.4
K (mg $seedling^{-1}$)	128.2	213.7	299.1	71.2	118.7	166.2

[a] To calculate effective nutrients supplied within the 230 cm^3 container, a 1.243 shrinkage empirical coefficient was used

gradient in these macronutrients within the plants. Each formulation had an equivalent stated nutrient release period: 12–14 months at 21°C. Micronutrients were incorporated by adding 0.2 g l^{-1} substrate of Micromax (Scotts Co.). A non-fertilized treatment was also included. N, P and K rates applied per plant are presented in Table 1. Four trays per treatment were installed and the 7 (treatments) × 4 (trays) = 28 trays were randomly arranged in the nursery. Trays were sown on 9 November 1994 with seed collected at the natural pine forests in Sierra de Lúcar, southeastern Spain (region of provenance 15, "Bética meridional"; Gil et al. 1996). Mean daily temperature for the 11 month nursery stage was 20.1°C. Irrigation was applied two to three times per week. The volume of water supplied at each irrigation event varied from 25 to 70 ml $seedling^{-1}$ (18 ml $seedling^{-1}$ average daily water supply). Irrigation water pH was 8.2, electrical conductivity at 25°C (EC) was 0.56 dS m^{-1}, and chemical composition (mg l^{-1}) was NO_3-N 7.0, P 0.0, K 5.9, Ca 27.9, Mg 31.6, SO_4 72.0, Na 24.8, Cl 35.5, and CO_3H 183.0. Additional details regarding growing conditions can be found in Oliet et al. (2004).

Eleven months after sowing (19 October 1995) seven interior plants (excluding edges) per tray (28 plants per treatment total) were randomly selected for destructive sampling. Roots were separated from the growing medium using pressurized water and plants were then dipped in distilled water for 5 min prior to chemical analysis. In addition, plant height and root collar diameter were measured. The different plant fractions were separated (needles, stem and roots) and oven-dried at 65°C for 48 h to determine dry mass. Needles and roots from each tray were combined in a composite sample (four samples per treatment and fraction in total) to determine mineral nutrient concentrations. Nitrogen was determined using a Leco analyzer (model CHN-600, St. Joseph, MI, USA); P and K were determined by atomic emission spectophotometry (spectrometer ICP model 400, Perkin Elmer, Waltham, MA, USA).

Outplanting site and experiment

The planting site was in the southeast of Almería province, southeast of Spain (2°0′W, 36°58′N, elevation 230 m), on a hillside with a moderate slope and NW aspect. The previous stand was a twenty-year-old *P. halepensis* plantation (on terraces) destroyed by a 1994 forest fire. The natural vegetation is typical of degraded areas from the arid to semiarid Mediterranean region, dominated by thyme (*Thymus* sp.) and alpha grass (*Stipa tenacissima* L.) (Ruiz de la Torre 1990) with scattered mastic (*Pistacia lentiscus* L.) and wild olive trees (*Olea europaea* var. *sylvestris*). The soil profile is rocky, of the unit calcaric regosol (FAO), and developed on calcareous parent material (MAPA 1989). The climate is arid Mediterranean, with extremely hot and dry summers. Average annual rainfall is 181 mm (MAPA 1989).

Table 2 Annual and per season rainfall (mm) during the study period

	1996	1997	1998	1999	2000	2001	2002
January–March	44.2	18.2	23.6	75.0	28.0	40.4	53.2
April–June	18.8	48.4	27.2	2.4	25.6	19.1	50.0
July–September	55.6	70.2	11.0	6.6	0.6	7.6	27.4
October–December	54.4	54.0	16.4	58.6	120.4	100.0	41.2
Total year	173.0	190.8	78.2	142.6	174.6	167.1	171.8

Planting was accomplished on 22 November 1995, in mechanically opened pits 0.95 m $\times$ 0.95 m $\times$ 0.95 m upon the 3.8 m wide terraces. Two staggered rows of pits were opened per terrace. Pits were 1.95 m apart. The seven treatments were arranged as a randomized complete block design with three replications. The experimental unit consisted of a row of 30 seedlings. Experimental units in a block were placed on four to five consecutive terraces along the slope, with one or two experimental units per block and terrace.

No weed control was applied during the experiment. At the beginning of years 1996 (first measurement), 1997, 1998, 1999, 2001 and 2003 (January), seedlings were measured for survival, height and groundline stem diameter (GSD). Stem volume was calculated using the formula for a cone: $0.2618(\mathrm{GSD}^2 \times h)$, where h is the height. Precipitation during the study period was recorded with an automatic pluviograph (DATA-RAIN 128 Geonica S.A., Spain) installed on the planting site. Annual rainfall from 1996 to 2002 averaged 157 mm, with an extremely dry season in 1998 (Table 2).

Data analysis

Seedling morphology and nutritional status before planting were assessed using a one-way analysis of variance (ANOVA) for a completely randomized design. For analysis of height, GSD, height growth and stem volume after planting, a general complete block design was used, and a corresponding ANOVA, with treatment and block as fixed effects, was conducted. For this ANOVA, treatment $\times$ block interaction mean square was included in the error term. For each analysis, when ANOVA was significant, statistically significant differences between means were identified using Fisher's LSD test adjusting the overall α-level by Bonferroni correction (Sit 1995). Some of the data from seedling characterization and outplanting response were previously log transformed to meet ANOVA assumptions of equal variance, though data were reported as original means.

The effect of fertilization treatment on seedling survival in each planting measurement was analyzed using a Chi-Square test. In addition, effect of fertilization treatment on seedling survival during the study period was analyzed by the Mantel–Haenszel test. When this test was significant, differences in seedling survival among groups of fertilization were tested with a log-rank test on the survival curves obtained for each treatment (Doménech 1999), and Bonferroni's correction for multiple comparisons was applied.

Analysis of covariance (ANCOVA) was performed to examine fertilizer treatment effects. In previous studies it has proven useful to evaluate whether treatment differences remained after adjusting for seedling size (South and Skinner 1998). In our study, ANCOVA was conducted with height growth as a dependent variable, fertilizer treatment and block as factors and initial height at the beginning of each measurement period as a covariate. For each period and fertilizer treatment, a linear regression between initial height (at the beginning of that period) and height growth was conducted. The regression coefficient (slope) of the model reflects the Transplant Stress Index (TSI), which indicates the effect of plant size on subsequent growth. A negative slope indicates that seedlings incurred post-planting stress (South and Zwolinsky 1997). The *P*-value for the initial height × fertilizer interaction in the ANCOVA was used for comparison of TSI among fertilizer treatments (parallelism hypotheses).

To assess the relationships between certain variables, Pearson correlation coefficients were calculated. Effects were considered significant when $P < 0.05$. Statistical analyses were performed with SPSS Version 14.0 (2005).

Results

Seedling nursery attributes

Eleven months after sowing, height, RCD, shoot dry mass and shoot to root ratio (g g^{-1}) differed significantly among fertilizer treatments ($P < 0.001$). All these attributes increased with rate applied within a formulation: height ranged from 12.9 ± 0.3 for non-fertilized pines to up to 40 cm height for maximum rates of both formulations (data not shown). Similarly, shoot dry mass increased with rate for both formulations (Fig. 1). The greatest root dry mass was obtained with 5 and 7 g l^{-1} of 9-13-18 formulation, while no significant differences appeared between the other fertilized treatments (Fig. 1). Shoot:root was maximized at the highest rates of 17-10-10 fertilizer, with values over 3 for 7 g l^{-1} of this formulation (Fig. 1), which differed significantly from 7 g l^{-1} of 9-13-18 (2.5 ± 0.1).

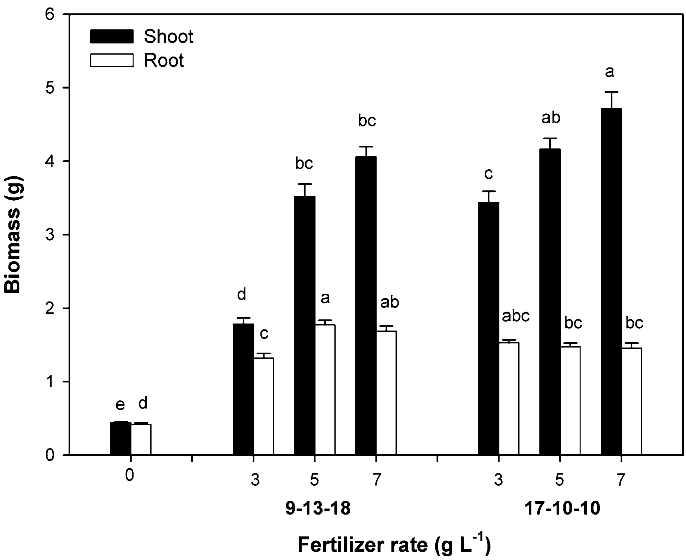

Fig. 1 Preplanting shoot and root dry mass (mean ± SE, $n = 28$) of containerized *Pinus halepensis* seedlings as affected by fertilizer treatments in the nursery (rates of each formulation per liter of substrate). *Bars* with different letters indicate significant differences among treatments, according to LSD test with Bonferroni correction. LSD test was performed with log transformed data for root mass

Table 3 Preplanting N, P and K (mg g^{-1}) concentrations in needles and roots ($n = 4$) of containerized *Pinus halepensis* seedlings as affected by fertilizer treatments in the nursery (rates of each formulation per liter of substrate)

Formulation		9-13-18			17-10-10		
Rate (g l^{-1})	0	3	5	7	3	5	7
Needles							
N	12.3e	12.0e	13.5de	14.9cd	16.3bc	18.2ab	18.5a
P	0.8e	2.2a	1.9abc	1.8bc	1.3d	1.5cd	1.5cd
K	8.8a	5.8d	6.7bcd	7.5ab	5.9cd	7.1bcd	7.5abc
Roots							
N	10.1de	9.4e	13.1cd	14.7c	15.0bc	18.0ab	20.2a
P	0.6d	2.4c	3.5ab	4.4a	1.8c	2.6bc	3.6a
K	5.5ab	5.1ab	5.7ab	6.0a	4.4b	5.2ab	4.9ab

Within a row, values with different letter indicate significant differences. ANOVA $P > F < 0.001$ for all variables, except K in roots ($P < 0.05$)

Needle N and K as well as root N and P concentrations increased with rate applied within a formulation (Table 3). Highest P concentration in needles was found in 3 and 5 g l^{-1} of 9-13-18 treatments while roots of the highest rate of 9-13-18 had the greatest P concentrations. Non-fertilized seedlings had the lowest P concentrations (Table 3). Conversely, K needle concentration was significantly higher (Table 3) when no CRF was added to the substrate.

All morphological seedling attributes were correlated, except shoot:root and root dry mass (Table 4). Needle and root N concentrations were significantly correlated to shoot dry mass and shoot:root, and root N was also correlated with height and RCD. Needle P concentration was not correlated significantly with any attribute, while root P concentration was correlated to both shoot and root attributes (height, RCD, shoot and root dry mass). Among nutrient concentration variables, only needle N was correlated to root N. All correlations were positive.

Planting response: survival

One year after planting (January 1997), *P. halepensis* mean survival decreased to 80% (Fig. 2), with values ranging from 72% (non-fertilized plants) to 84% (5 and 7 g l^{-1} of 9-13-18), although differences between treatments were not significant (Fig. 2). In 1997 and 1998, the decline in survival was slight (2.5 and 3% per year in average, respectively) although during 1998 differences became significant among treatments (Chi-Square test $P = 0.259$ for January 1998 and 0.050 for January 1999 survival). During 1999 and 2000, mortality was more severe (mean of 13.5%, Fig. 2) and differences between treatments became more pronounced (Chi-Square test $P < 0.001$), with survival ranging from 46% (non-fertilized seedlings) to 79% (7 g l^{-1} of 9-13-18) in January 2001. From then until January 2003, mortality was again very low (less than 2% in 2 years), and differences between treatments remained significant. As a result, 7-year survival curves representing each treatment were significantly different (Mantel–Haenszel test $P < 0.001$, Fig. 2). A log-rank test on the survival curves showed that survival was highest at 7 g l^{-1} of 9-13-18 (79%), followed by 3 g l^{-1} of 17-10-10 (71%), and lowest for non-fertilized plants (42%), with significant differences between the former two treatments and non-fertilized seedlings

Table 4 Pearson correlation matrix for coefficients between main seedling nursery attributes of *Pinus halepensis* ($n = 7$)

	Height	RCD	Shoot mass	Root mass	Shoot:root	[N] needles	[P] needles	[K] needles	[N] roots	[P] roots	[K] roots
Height	1										
RCD	***	1									
Shoot mass	***	***	1								
Root mass	**	**	*	1							
Shoot:root	**	*	**		1						
[N] needles			*		**	1					
[P] needles							1				
[K] needles								1			
[N] roots	*	*	**		***	***			1		
[P] roots	*	*	*	*						1	
[K] roots											1

All coefficients were positive

* Correlation at $P < 0.05$; ** Correlation at $P < 0.01$; *** Correlation at $P < 0.001$

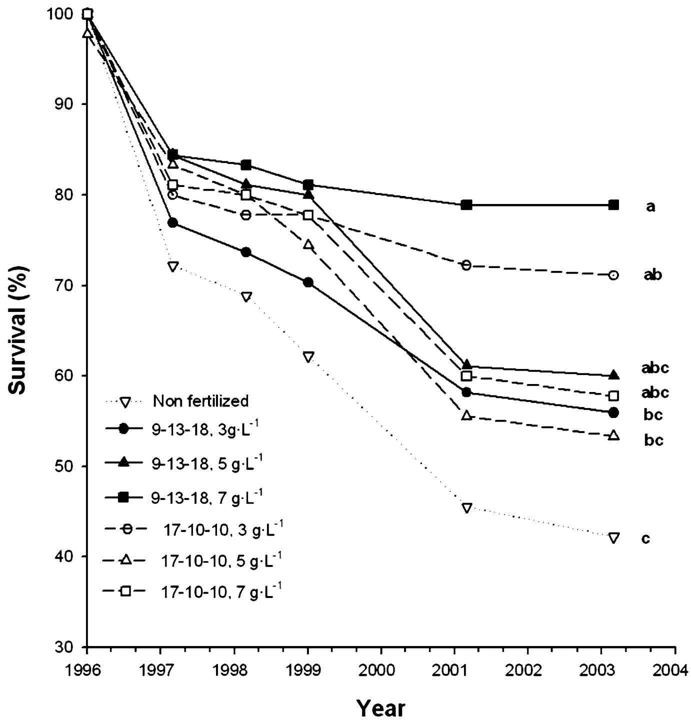

Fig. 2 Survival of *Pinus halepensis* over 7 years following outplanting in an arid area as affected by nursery fertilization. Initially, $n = 90$ seedlings per treatment. *Letters* indicate significant differences among means (log-rank test of survival curves, $P < 0.05$ after Bonferroni's correction for multiple comparisons)

(Fig. 2). Additionally, differences in survival among the maximum rate of 9-13-18 and 3 g l^{-1} of last formulation (56%) and 5 g l^{-1} of 17-10-10 (53.3%) were significant according to a log-rank test.

A strong positive correlation appeared between some morphological nursery attributes (height, RCD, shoot and root dry mass) and survival from January 1997 to January 1999 (data not shown). From 1999 to January 2003, differences became non significant. Shoot:root was positively correlated with survival only for the January 1998 measurement, although correlations were nearly significant in the previous and following years. The association between nutritional status in the nursery and survival was weaker: only root P concentration was significantly correlated with survival from January 1997 to 1999 ($P < 0.016$). No significant correlations were detected between all combinations of ratios of N, P, and K and response variables.

Height, groundline stem diameter, volume and growth after planting

During the first year after planting, *P halepensis* seedlings fertilized with 3 g l^{-1} of 9-13-18 and non fertilized plants grew significantly more in height than most of the other treatments (Fig. 3). However, differences in height growth among treatments diminished during the following years. At the end of the study period, greatest height (94.1 ± 2.6 cm) was attained by 7 g l^{-1} of 9-13-18, which differed significantly from non-fertilized plants and from the maximum rate (7 g l^{-1}) of 17-10-10 (Fig. 3). With regard to GSD, absolute

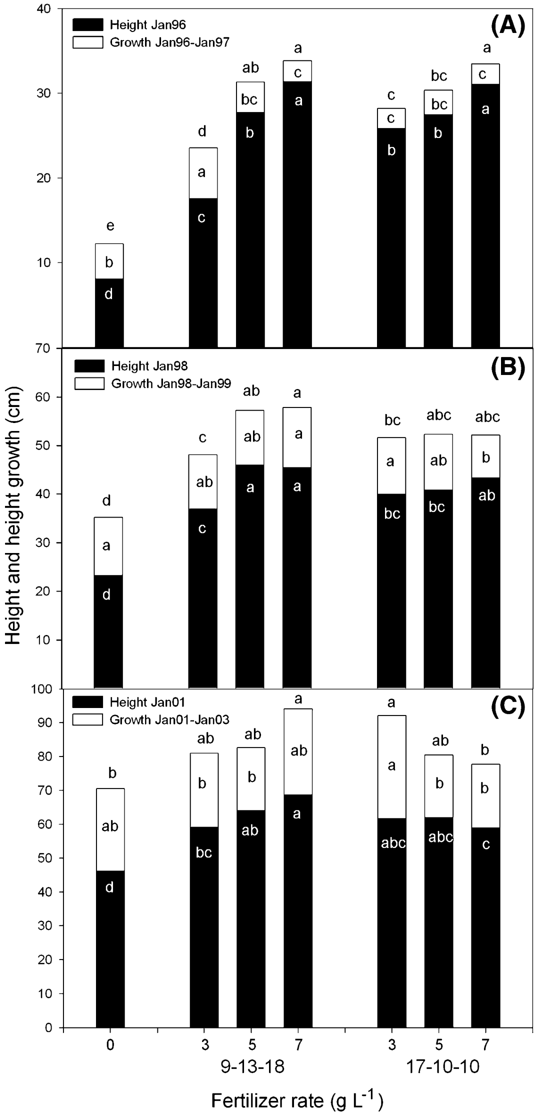

Fig. 3 Height (*full and whole columns*) and height growth (*white portion column*) of *Pinus halepensis* in an arid area as affected by nursery fertilization in the nursery during: 1996 (**a**), 1998 (**b**) and 2001–2002 (**c**). *Different letters* among treatments for total height or height growth component indicate significant differences, according to LSD test with Bonferroni correction. LSD test was performed with log transformed data for January 1996 height. For all dates ANOVA $P < F$ was <0.001 with the exception of height growth during 1998, when $P = 0.005$

values of planted seedlings in January 1996 were slightly lower than that of the nursery stock, as occurred with height (data not shown). After 7 years, maximum GSD corresponded to 7 g l^{-1} of 9-13-18 treatment, which differed significantly from non-fertilized and 17-10-10 at 7 g l^{-1} rate treatments by 34 and 25%, respectively (Table 5). Stem volume was greatest for 7 g l^{-1} of 9-13-18, although variability within groups (see SE in Table 5) increased the ANOVA P-value to a marginally significant level (0.054).

ANCOVA analysis showed that initial height at planting negatively affected height growth during the first year for most treatments, except for 7 g l^{-1} of 9-13-18 and 5 g l^{-1} of 17-10-10, for which TSI did not differ significantly from 0 (Table 6). The lowest values corresponded to non-fertilized plants, followed by 9-13-18 at 3 g l^{-1} rate. However, during 1997 initial height in January did not negatively affect height growth for any treatment,

Table 5 Mean values (±SE) for groundline stem diameter and stem volume at planting (January 1996) and 7 years after planting (January 2003) of *Pinus halepensis* as affected by nursery fertilizer treatments (rates of each formulation per liter of substrate)

Formulation		9-13-18			17-10-10			
Rate (g l^{-1})	0	3	5	7	3	5	7	$P > F$
Stem diameter (mm)								
January 1996	1.3 ± 0.0d	2.6 ± 0.1c	3.3 ± 0.1b	3.7 ± 0.1a	3.3 ± 0.1b	3.7 ± 0.1a	3.8 ± 0.1a	<0.001
January 2003	24.0 ± 1.8b	28.9 ± 1.3ab	28.6 ± 1.0ab	31.9 ± 1.1a	29.6 ± 1.1ab	28.2 ± 1.6ab	25.5 ± 1.1b	<0.001
Stem volume (cm^3)								
January 1996	<0.1 ± 0.0e	0.4 ± 0.0d	0.8 ± 0.0c	1.2 ± 0.0a	0.8 ± 0.0c	1.0 ± 0.0b	1.3 ± 0.1a	<0.001
January 2003	184.5 ± 57.1	218.4 ± 25.5	206.9 ± 26.7	302.1 ± 28.8	258.8 ± 26.8	248.0 ± 55.5	163.7 ± 19.8	0.054

Within a row, means with different letters indicate significant differences. LSD test was performed with log transformed data for January 1996 stem diameter

Table 6 Regression coefficient (±SE) and P values of the linear regression adjusted model between previous year height (initial) and following height growth (representing the Transplant Stress Index as per South and Zwolinsky 1997)

Formulation	Rate (g l^{-1})		Jan1996–Jan1997	Jan1997–Jan1998	Jan1998–Jan1999	Jan1999–Jan2001	Jan2001–Jan2003
Unfertilized (control)	0	Regression coefficient	−0.38 ± 0.14	0.80 ± 0.20	0.61 ± 0.08	0.37 ± 0.08	0.59 ± 0.12
		$P > F$	**0.008**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
9-13-18	3	Regression coefficient	−0.33 ± 0.06	0.32 ± 0.15	0.08 ± 0.08	0.14 ± 0.11	0.22 ± 0.11
		$P > F$	**<0.001**	**0.040**	0.349	0.194	**0.049**
	5	Regression coefficient	−0.25 ± 0.06	−0.02 ± 0.16	0.25 ± 0.07	0.15 ± 0.06	0.39 ± 0.12
		$P > F$	**<0.001**	0.887	**<0.001**	**0.009**	**0.002**
	7	Regression coefficient	−0.05 ± 0.03	−0.03 ± 0.10	0.14 ± 0.07	0.19 ± 0.07	0.31 ± 0.09
		$P > F$	0.164	0.787	**0.049**	**0.007**	**0.001**
17-10-10	3	Regression coefficient	−0.12 ± 0.04	−0.14 ± 0.13	0.37 ± 0.07	0.16 ± 0.06	0.44 ± 0.12
		$P > F$	**0.003**	0.299	**<0.001**	**0.008**	**<0.001**
	5	Regression coefficient	−0.08 ± 0.05	0.04 ± 0.12	0.41 ± 0.07	0.35 ± 0.07	0.58 ± 0.09
		$P > F$	0.093	0.714	**<0.001**	**<0.001**	**<0.001**
	7	Regression coefficient	−0.07 ± 0.03	0.03 ± 0.10	0.21 ± 0.07	0.17 ± 0.05	0.29 ± 0.11
		$P > F$	**0.034**	0.743	**0.003**	**<0.001**	**0.012**
				ANCOVA[a]			
Initial height × fertilizer		($P > F$)	**<0.001**	**0.003**	**0.003**	0.132	0.279
Initial height		($P > F$)				**<0.001**	**<0.001**
Fertilizer		($P > F$)				**<0.001**	**<0.001**

For each period ANCOVA results are presented for the interaction between initial height at the beginning of each period and fertilizer treatment. Statistically significant P values are shown in bold font

[a] Significant interaction precludes statistical analysis of covariable (initial height) and treatment variable (fertilizer)

while it positively influenced that of the non-fertilized and 3 g l^{-1} of 9-13-18 (Table 6) treatments. During 1998 there was a shift in TSI whereby the values became significantly positive for all treatments with exception of 9-13-18 at the 3 g l^{-1} rate. During these first 3 years after planting an interaction between initial height and fertilizer treatment appeared (Table 6). During the last 4 years (January 1999–January 2003) the relationship between height at the beginning of each year and subsequent growth was significant and positive (except for 3 g l^{-1} of 9-13-18 in 1999–2001 period) and did not interact with fertilizer treatment (Table 6). Values of TSI for January 1999 through January 2001 were lower than the previous year for most of the treatments, but increased to maximum positive values from January 2001 to January 2003. In addition, height growth during the last 4 years of the study was significantly affected by both nutritional status and initial height (Table 6).

Discussion

Seedling quality diagnosis in relation to fertilization

With exception of either non-fertilized plants or those fertilized with 3 g l^{-1} of 9-13-18, seedlings were taller than the maximum value (25 cm) required by European Community regulations for *P. halepensis.* RCD was also greater than the minimum (2 mm) recommended by these rules (European Community Council 2000) for all but non-fertilized plants (data not shown). In addition, plants fertilized with 7 g l^{-1} of 9-13-18 and with all rates of 17-10-10, had a greater shoot:root ratio than the 1.5–2 range recommended for this species (Navarro et al. 2006), with the 17-10-10 formulation promoting a less balanced morphology, with shoot dry mass relatively superior to root (Fig. 1). In general, shoot morphological attributes, as well as shoot:root were higher than that obtained in experiments using CRF for *P. halepensis* (Oliet et al. 1999; Puértolas et al. 2003) or even fertigation (Villar-Salvador et al. 1999), confirming that plants in these experiment were larger than those typically produced for this species. The longer cultural period, mild winters at the nursery area, and high rates of N applied in many treatments probably promoted development of these seedling attributes.

Pre-planting needle N concentration was below 20 mg g^{-1} (Table 3), considered by several authors as an optimum for many *Pinus* spp. (Ingestad and Kahr 1985; Larsen et al. 1988) and for *P. halepensis* based on field responses (Oliet et al. 1997; Puértolas et al. 2003). P concentration was also low, particularly in needles, in relation to the recommended levels for container conifer seedlings (Rook 1991; Landis and van Steenis 2004), and for *P. halepensis* in relation to growth responses (Cornelissen et al. 1997). Concentration of P in roots was similar to some studies (Oliet et al. 1999), or even high for some treatments like 7 g l^{-1} of 9-13-18. Low P availability, probably caused by high pH of irrigation water that immobilizes this element in the growing media, may help explain the relatively low P uptake (Landis and van Steenis 2004). Only when applied P exceeded 80 mg per plant did root concentrations reach 3 mg g^{-1}, which acted as a P reserve that could be used after planting. However, K concentrations in all treatments were within the general recommended 4.0–15.0 mg g^{-1} range of container conifers for reforestation (Landis 1989; Rook 1991) and for seedling growth of another *Pinus* species (Timmer and Armstrong 1987) (Table 3). Non-fertilized plants were N and P deficient, but K sufficient, according to the references provided above. The observed highest K concentration values for non-fertilized plants (especially in needles), may be associated with a dilution effect promoted by growth response to N addition (Ingestad and Kahr 1985; Grossnickle 2000).

Thus, based on foliar nutrient concentration, our seedlings can be described as P deficient and relatively low in N for all treatments, especially non-fertilized plants, which were highly deficient for these two elements.

Seedling outplanting response, size and nutrient status in relation to growth

The interaction between initial height and fertilization treatment on height growth during the first 3 years after planting indicated that the effect of initial height on subsequent height growth was differentially affected by plant nutritional status. For instance, during the first year after planting (1996), plants from 7 g l^{-1} of 9-13-18 exhibited height growth that was unrelated to initial height in January 1996, indicating the absence of planting check according to the theory of South and Zwolinsky (1997). However, for the rest of the treatments, taller plants within a treatment grew less in height than smaller ones. Two planting studies with *P. halepensis* in more humid zones showed positive TSI values after the first year for all treatments (Oliet et al. 2000; Puértolas et al. 2003). Positive TSI values in Table 6 from 1998 and thereafter indicated that planting check was overcome during 1998, despite the drought severity of that year. During subsequent years, there was no interaction between initial height and fertilizer treatment; taller plants grew more in height irrespective of fertilizer treatment and a significant fertilizer effect still persisted 7 years after planting regardless of initial size. A fertilization effect after adjusting for initial height was also found for first year height growth of *P. halepensis* in Puértolas et al. (2003), although this effect disappeared during the second year under the more humid conditions of this study. It seems that, at least for some treatments in our experiment, the fertilization effect is more persistent on harsh sites.

In many studies, larger seedlings tend to maintain size advantage over time compared to smaller seedlings (Rose and Ketchum 2003; Haase et al. 2006) partly due to the ability to outgrow competing vegetation (South et al. 2005), while others report that differences fade after a few years (South et al. 2001; Oliet et al. 2005). Lack of consistency between these results, apart from differences in study duration, are associated with variability of height growth response to a complex interaction between seedling attributes (size and nutritional status) and site characteristics such as soil fertility, drought, and competition (South et al. 2005). In our study, there was almost no competing vegetation after planting, primarily due to arid conditions.

Survival response in relation to size and nutrient status

The first steep declines in survival coincided with planting check that occurs in most plantations (South and Zwolinsky 1997). Mortality was particularly pronounced for non-fertilized seedlings and those fertilized with the lowest N rates (9-13-18 at 3 g l^{-1}, Fig. 2), which also exhibited the lowest TSI (Table 6); however, both treatments simultaneously showed the highest absolute height growth during the first year (Fig. 3). The second decline in survival occurred during 1999–2001 (Fig. 1), after the severe drought of 1998 (Table 2). It is noteworthy that the decline in survival in response to this drought did not occur immediately but rather at the end of the driest year (1998). Reduced TSI values of five treatments during 1999–2001 in relation to 1998 values (Table 6) also revealed the impact of the 1998 drought. Early survival values of our study are similar to several planting studies with *P. halepensis* in semiarid to arid areas of the Mediterranean Spain, albeit none of these studies were monitored as long as 7 years (Royo et al. 2001; Querejeta et al. 2001; Barbera et al. 2005). The importance of following planting response for longer periods is evident in this case, as the decline in survival during 1999 resulted in significant differences among treatments.

The significant positive correlation between morphological attributes and survival indicates that, under our study conditions, larger seedlings survived better. van den Driessche (1980) also found that shoot dry mass of *Pseudotsuga menziesii* (Mirb.) Franco accounted for more variation in survival after planting than nutrient concentration. South (2000) demonstrated that *Pinus taeda* L. seedlings with greater RCD survived better, particularly under droughty conditions. Similar results were obtained by South et al. (2001) with *P. radiata* and Rose and Ketchum (2003) with *P. menziesii* 7 and 4 years, respectively, after planting. This suggests that the apparent effect of nursery N (or P) fertilization could be associated with its influence on seedling size. However, the correlation between root P concentration and root dry mass in the nursery and between root dry mass and survival, and the fact that the only significant correlation between survival and nutrients corresponded to root P, suggests that this element plays an important role in promoting root growth after planting. Importance of P in promoting root growth is still debated (Landis and van Steenis 2004). Basic mechanisms relating root growth and this element are not direct, rather its key function is in growth and energy storage processes in general (Marschner 1995). This fundamental role could, under certain circumstances, affect root growth, as has been demonstrated in some studies (Bigg and Schalau 1990; Folk and Grossnickle 2000). Furthermore, significant relationships between P fertilization, root growth, and survival have been reported for Mediterranean species (Villar-Salvador et al. 2004; Oliet et al. 2005). The response we observed may be accentuated by the scarcity of P in degraded soils from arid calcareous areas. The role of P as an important limiting resource for growth of *P. halepensis* in Mediterranean calcareous ecosystems is widely acknowledged (Querejeta et al. 2008).

Although both the 5 and 7 g l^{-1} of 17-10-10 had the greatest height, RCD and shoot mass at planting, survival and growth after 7 years was lower than other treatments. Initially lower P concentrations of these treatments, as well as high shoot:root that exceeded recommended values for this species (Navarro et al. 2006) could affect ability of seedling to overcome planting check and grow as successfully as those at the 9-13-18 at 7 g l^{-1} rate. This treatment exhibited the highest GSD from January 1998 (data not shown) through the remainder of the study period (Table 5). The GSD has been shown to be correlated to root development at planting in many studies (Haase and Rose 1993; South et al. 2005). In our study, larger seedlings with higher nutrients concentration and content probably grew roots more rapidly, improving water uptake efficiency from deeper soil layers (Villar-Salvador et al. 2008). This could also help explain better performance of the 7 g l^{-1} of 9-13-18 treatment after the intense drought of 1998 (Fig. 2). As no significant correlation between N concentration and survival was detected, our data suggest that, in this study, the role of N may be less important than that of other variables, such as root P concentration. This is in contradiction to studies showing a positive effect of N concentration on postplanting survival in temperate (van den Driessche 1980; 1985; Irwin et al. 1998) and Mediterranean species, such as *Pinus halepensis* (Oliet et al. 1997). In these studies, the role of N in promoting carbon assimilation (Grossnickle 2000; Jose et al. 2003) is related to rooting, survival and growth after planting. In our study, however, shoot:root, which was significantly related to N concentration, reached values as high as 3.3, and this ratio could create a water deficit that hinders carbon assimilation and rooting (Lamhamedi et al. 1997). In the Oliet et al. (1997) study mentioned above, *P. halepensis* shoot:root did not exceed 2.2 for any treatment, and in Larsen et al. (1986), one year survival of *Pinus taeda* L. decreased significantly for shoot:root above 2.2. Thus, the highest values of 3.3

reached by 7 g l^{-1} of 17-10-10 could be exceeding the required balance between both fractions for these drought conditions. Under these circumstances, the lower N concentration but corresponding smaller (i.e., 2.5) shoot:root of 7 g l^{-1} of 9-13-18, along with higher P concentrations and adequate root development, could help explain improved survival and growth after planting for this treatment. The results of Trubat et al. (2008) with five Mediterranean species (not including *P. halepensis*) under arid conditions showed that N deprived seedlings (short-term reduction of N availability prior to planting) survived better after the first summer, and this was related mainly to a decrease in shoot size and leaf area (i.e., reduced transpirational demand) but not to an increase of biomass allocation belowground. Both the control (not deprived) and N deprived seedlings had foliar nutrient concentration values far above deficiency levels and therefore, effects of nutrient treatments may have been limited only to morphological differences. However, N and P concentrations in our study had much broader ranges (i.e., from deficiency to sufficiency), which could condition plant response to a more complex set of effects that includes both morphological and physiological processes. A recent study with *Retama sphaerocarpa* (L.) Boiss shows that high fertilization during cultivation significantly increased shoot mass and shoot:root along with nutrient concentration in the nursery, but also promoted higher survival, growth rates and water use efficiency under Mediterranean planting conditions (Villar-Salvador et al. 2008).

The lack of growth and survival responses of outplanted seedlings to K concentration is relatively common. For instance, in our study, K values fell within the interval of sufficiency, with maximum values in poorly performing non-fertilized plants. On the contrary, the range of P concentrations used in the study that created a slight deficiency could further explain the significant planting response to initial P status. Thus, the relationship between nutrient concentration and outplanting response can be strongly affected by the range of nutrients inputs used. For instance, according to Zas and Serrada (2003) foliar P and K levels appeared to be related to improved growth and survival of *P. radiata,* while high foliar N was considered unfavorable in plantations where soil N is excessive and P levels are low. This also suggests that soil fertility can affect the relative role of nursery stock mineral nutrient reserves in survival and growth after planting.

Conclusions

Our study shows that large seedlings of *P. halepensis* (up to 40 cm shoot height) with shoot:root of 2.5 and moderate to high P and N concentrations outperformed other treatments in both survival and growth, and may help to improve seedling field performance under Mediterranean arid conditions. These results concur with those of Puértolas et al. (2003) and Villar-Salvador et al. (2004, 2008), suggesting that larger seedlings with greater nutrient reserves than are currently recommended should be incorporated into Mediterranean restoration plantations. However, our results contrast with those of Trubat et al. (2008) using a different species. Thus, we believe that: (1) a shift in a target toward larger, nutrient loaded seedlings should promote the need for corresponding changes in nursery cultural production regimes in these areas, at least for certain species; and (2) more studies specifically designed to ascertain the role of nursery fertilization and mineral nutrition on outplanting survival under dry Mediterranean areas are needed. Results of this and past studies suggest the importance of N and P in plantation establishment success, although further research is needed to more accurately distinguish the relative significance of these two elements (N and P), alone or in combination, under varying experimental conditions and species.

In addition, our data encompassing a 7-year period, illustrates the limitation of using results of outplanting performance from only the first year to identify seedling characteristics that are important for reforestation success, as differences between treatments became significant after an intense drought that occurred 3 years after planting. Therefore, more long-term reforestation studies, particularly in arid and semiarid areas exposed to periodic severe drought events, are needed. This may become particularly important considering the likely future impact of global climate change on Mediterranean arid and semi-arid forest development.

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