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ORIGINAL PAPER

Root growth dynamics of Aleppo pine (*Pinus halepensis* Mill.) seedlings in relation to shoot elongation, plant size and tissue nitrogen concentration

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Abstract Large and high nitrogen (N) concentration seedlings frequently have higher survival and growth in Mediterranean forest plantations than seedlings with the opposite traits, which has been linked to the production of deeper and larger root systems in the former type of seedlings. This study assessed the influence of seedling size and N concentration on root growth dynamics and its relation to shoot elongation in Aleppo pine (*Pinus halepensis* Mill.) seedlings. We cultivated seedlings that differed in size and tissue N concentration that were subsequently transplanted into transparent methacrylate tubes in the field. The number of roots, root depth, and the root and shoot elongation rate (length increase per unit time) were periodically measured for 10 weeks. At the end of the study, we also measured the twig water potential (ψ) and the mass of plant organs. New root mass at the end of the study increased with seedling size, which was linked to the production of a greater number of new roots of lower specific length rather than to higher elongation rate of individual roots. Neither plant size nor N concentration affected root depth. New root mass per leaf mass unit, shoot elongation rate, and pre-dawn ψ were reduced with reduction in seedling size, while mid-day ψ and the root relative growth rate were not affected by seedling size. N concentration had an additive effect on plant size on root growth but its overall effect was less important than seedling size. Shoot and roots had an antagonistic elongation pattern through time in small seedlings, indicating that the growth of both organs depressed each other and that they competed for the same resources. Antagonism between shoot and root elongation decreased with plant size, disappearing in large and medium seedlings, and it was independent of seedling N concentration. We conclude that root and shoot growth but not rooting depth increased with plant size and tissue N concentration in Aleppo pine seedlings. Since production of new roots is critical for the establishment of planted seedlings, higher absolute root growth in large seedlings may increase their transplanting performance relative to small seedlings. The lack of antagonism between root and shoot growth in large seedlings suggests that these plants can provide resources to sustain simultaneous growth of both organs.

Keywords Nitrogen content · Rhizotron · Root elongation · Shoot growth · Sink-source relations · Water potential

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Introduction

Abiotic and biotic factors have a profound influence on root growth and structure (Alvarez-Uria and Korner 2007; Andersen et al. 1986; Lopushinsky and Max 1990; Lyr 1996; Munro et al. 1999). Root growth and structure also vary across plant species and are linked to species functional and ecological characteristics (Comas and Eissenstat 2004; Schenk and Jackson 2002). By contrast, fewer studies have analysed how plant functional characteristics affect root growth and structure within a given species. Nursery cultivation conditions may determine future root growth and architecture. For instance, root restriction by containers in nursery-cultivated plants can affect root

morphology for many years after transplanting (Halter and Chanway 1993; Lindström and Rune 1999). Similarly, new root growth capacity in greenhouse or growth chamber experiments is positively related to shoot size and nitrogen (N) concentration in forest species (van den Driessche 1992; Villar-Salvador et al. 2004).

New root growth after transplanting is essential to ensure seedling survival in forest plantations (Burdett 1990; Burdett et al. 1983; Grossnickle 2005; Ritchie and Dunlap 1980) as new roots allow seedlings to access soil water and mineral nutrients in the surrounding soil (Grossnickle 2005; Lyr and Hoffmann 1967; Padilla and Pugnaire 2007). Villar-Salvador (2003) suggested that higher root growth after transplanting in containerized plants might explain the frequently improved survival and growth of large, N-rich seedlings relative to seedlings with the opposite traits in Mediterranean plantations (Luis et al. 2009; Oliet et al. 2009; Puértolas et al. 2003; Tsakaldimi et al. 2005; Villar-Salvador et al. 2004; Villar-Salvador et al. 2008). Cuesta et al. (2010) observed that large Aleppo pine (*Pinus halepensis* Mill.) seedlings had greater new root biomass in the field than small seedlings at the onset of summer drought. However, they did not examine the effect of seedling size and N concentration in root growth dynamics and rooting depth.

Plants usually have limited resources to simultaneously support their main functions (growth, defence, maintenance, storage, and reproduction) (Chapin 1990; Herms and Mattson 1992; Obeso 2002). Trade-off in resource allocation among organs or functions increases with limiting environmental conditions and varies with organ type (Obeso 2002; Thaler and Pagès 1996b). In some studies, roots and shoots alternate their growth through time suggesting that both organs compete for the same pool of resources, and therefore, occur at the expense of the other (Langlois et al. 1983; Thaler and Pagès 1996a; Willaume and Pagès 2006). Other studies, however, have not reported an alternating growth between roots and shoots (Corchero-de la Torre et al. 2002; Harmer 1990; Lyr and Hoffmann 1967). Differences among studies suggest different sink/source relations among species or individuals within a species. Consistent with this argument, defoliation of *Quercus pubescens* seedlings amplified the decrease in root growth concomitant with leaf expansion (Willaume and Pagès 2006). Therefore, it can be expected that antagonist growth between organs in a given species will be lower in individuals with high photosynthetic capacity and/or amount of nutrients stored for remobilization.

This study examines the root growth dynamics of Aleppo pine (*Pinus halepensis*) seedlings and how it is influenced by shoot growth, seedling size and tissue N concentration. We assessed the hypotheses that large seedlings and plants with high tissue N concentration (1) produce larger and deeper root systems because they produce more roots and individual roots elongate faster, and (2) have lower antagonism between root and shoot growth relative to plants with the opposite attributes. To test these hypotheses, we transplanted into transparent methacrylate tubes seedlings that differed in size and N concentration and periodically measured root and shoot growth. We selected *P. halepensis* because it is a structural species in many types of woodland throughout the Mediterranean basin and it is commonly used in reforestation projects.

Materials and methods

Seedling nursery cultivation

Seedlings were cultivated in the Centro Nacional de Recursos Genéticos Forestales "El Serranillo" (Ministerio de Medio Ambiente y Medio Rural y Marino). Seeds from an inland Spain provenance were sown in Plasnor 190/300-45 trays (45 cavities of 300 ml per tray; Plasnor, Spain) with unfertilized *Sphagnum* peat (Kekkilä B0, Finland). We cultivated 135 seedlings of six phenotypes that differed in size (small, medium and large types) and N concentration (high and low) (Table 1). To achieve differences in plant size we varied seeding date, which determined the length of the growing season. Seeds were sown on December 15, 2005 (large seedlings), March 13, 2006 (medium seedlings) and May 23, 2006 (small seedlings). After emergence, seedlings were fertilized weekly with a 100-ppm N fertilizer solution until September 27, 2006. Fertilization started in May 10, June 2 and August 2 for the seedlings sown in December, March and May, respectively. To achieve differences in tissue N concentration, half of the seedlings of each seeding date were fertilized weekly with 200 ppm N from early October until mid December 2006 (high-fertilized seedlings, N^+). The remainder half was not fertilized any more (low-fertilized seedlings, N^-). Fertilization was done with a Peters Professional® 20-7-19: $N–P_2O_5–K_2O$ fertilizer (Scotts Professional, The Netherlands). Seedlings were grown in a glasshouse, in which temperature ranged from 4 to 25°C and radiation was approximately 50% of that outside, until mid May 2006. Then plants were moved outside and kept under full sun during the rest of the experiment. Seedlings were kept well watered according to their requirements by irrigating them every 1–3 days. Cultivation finished when plants stopped their growth due to low winter temperature in late December 2006.

Morphology and N concentration measurements following cultivation

Fifteen plants of each seedling phenotype were randomly sampled in mid January 2007 for morphology and N

Table 1 Morphology and N concentration content of *Pinus halepensis* seedlings that were cultivated by sowing seeds on three distinct dates (December, March and May) and fertilized with nitrogen at two distinct rates (N^+ and N^-)

	December		March		May	
	N^-	N^+	N^-	N^+	N^-	N^+
Plant height (cm)	19.8 ± 0.6^{a}	19.1 ± 0.8^{ab}	15.2 ± 0.4^{c}	16.9 ± 0.4^{bc}	8.0 ± 0.5^{d}	8.4 ± 0.3^{d}
Plant diameter (mm)	4.0 ± 0.1^{a}	4.5 ± 0.1^{a}	3.4 ± 0.1^{b}	3.8 ± 0.1^{b}	2.1 ± 0.1^{c}	2.1 ± 0.1^{c}
Root mass (g)	3.0 ± 0.34^{a}	2.9 ± 0.16^{ab}	2.0 ± 0.09^{c}	2.2 ± 0.08^{bc}	0.5 ± 0.07^{d}	0.6 ± 0.06^{d}
Shoot mass (g)	3.2 ± 0.35^{a}	3.0 ± 0.25^{ab}	2.3 ± 0.18^{bc}	2.2 ± 0.14^{c}	0.5 ± 0.04^{d}	0.5 ± 0.05^{d}
Shoot/root mass ratio	1.12 ± 0.1^{a}	1.07 ± 0.1^{ab}	1.11 ± 0.05^{a}	1.01 ± 0.1^{ab}	1.12 ± 0.1^{a}	0.77 ± 0.1^{b}
Plant N concentration (mg g^{-1})	6 ± 0.3^{d}	10 ± 1.1^{bc}	7 ± 0.2^{cd}	10 ± 0.9^{b}	9 ± 0.6^{bcd}	14 ± 0.6^{a}
Plant N content (mg)	42 ± 2.5^{bc}	56 ± 3.6^{a}	30 ± 1.9^{c}	45 ± 4.7^{ab}	9 ± 2.1^{d}	15 ± 1.8^{d}

Data are mean ±1 SE. $n = 15$ except for N concentration where $n = 3$

Seedling types with different letter indicate significant differences at $P < 0.05$ after Tukey's post-hoc test

concentration determinations. Shoots were cut at the cotyledon insertion point and root plugs were cleaned from the peat. Shoot height was measured as the length of the stem while shoot diameter was measured as the stem thickness at the cotyledon insertion point. Then, all plant parts were washed with tap water, rinsed in distilled water and dried in a ventilated oven at 60°C for 48 h to measure their mass (Table 1). Plants of each seedling phenotype were randomly distributed into three groups and finely ground to assess tissue N concentration. N concentration was determined by Kjeldahl analysis with K–$SeSO_4$–Se_2Cu_4 as catalyst in a Tecator DS-40 digestion system (FOSS Tecator, Sweden) and a SAN++ auto-analyser (Skalar, Netherlands).

Experimental design and field measurements

The experiment was carried out in a flat plot located in the Centro Nacional de Recursos Genéticos Forestales "El Serranillo" (Ministerio de Medio Ambiente y Medio Rural y Marino). The site is in Guadalajara, central Spain (38°38′ N, 3°28′ W) at 650 m a.s.l. on a flat plot. The climate is Mediterranean continental with mean annual precipitation and temperature of 400 mm and 13.4°C, respectively. A pronounced dry and hot season occurs from June to late September and frosts in winter are frequent.

Six seedlings per seedling phenotype were transplanted into transparent methacrylate tubes (here after rhizotrons) on April 18, 2007. One seedling was planted in each rhizotron. Rhizotrons were 1 m in length, 13.5 cm in exterior diameter, and had a wall thickness of 0.5 cm. Rhizotron bottom end was closed with a perforated PVC lid, which drainage holes were covered with a mesh to prevent substrate loss. The bottom of the rhizotron was filled with gravel (*ca.* 10 cm in height) and the remainder was filled with washed and sieved sand. Subsequently, we planted the seedlings placing the root plug against the rhizotron wall. Rhizotrons were inserted into the soil to maintain roots as close as the plot soil temperature. To facilitate periodic extraction, each rhizotron was inserted into a second opaque plastic tube 1.1 m in length and 15 cm in interior diameter that was buried in the soil with a 30° inclination to force root growth against the rhizotron wall. As the top of the opaque tube and of the rhizotron protruded 10–15 cm from the ground, we wrapped an insulating sheet around the protruding portion of the opaque tube to prevent rhizotron overheating. Seedlings were thoroughly irrigated at planting and then again on April 23, May 10 and May 18, with 200 ml per plant to simulate standard spring rainfall at the experimental site. During the rest of the experiment, seedlings were not irrigated to simulate the summer drought typical of Mediterranean climate.

When most seedlings had visible roots, we measured root elongation and depth as well as shoot height every 6–12 days from April 30 to July 11. On each date, all new roots were drawn on the same acetate sheet, which was placed over the rhizotron wall on identical position, and their length was measured after correcting for root curvatures. We counted the number of new growing roots. The roots that stopped their growth for at least 15 days were not counted. The mean root elongation rate was calculated as the mean elongation of each individual root per time unit. We also calculated the total new root elongation rate of a plant as the sum of elongations of all individual new roots per time unit, which estimates plant effort to expand its root system. Root depth was measured as the vertical distance from the plug bottom to the tip of each drawn root. Shoot height was measured as the distance between the cotyledon insertion point and the shoot apex, and shoot elongation rate was calculated as the differences in seedling height between two dates. We also measured the stem diameter at the cotyledon insertion point with a calliper in the first day of the field experiment to calculate the initial stem volume assuming the stem to be a cone. Stem volume was highly correlated with plant mass ($r = 0.92$, $P < 0.001$, $n = 36$) and we used it as a proxy of initial seedling size.

On the last day of the field experiment, we measured the twig water potential at predawn (ψ_{pd}) and at midday (ψ_{md}) in all seedlings with a pressure chamber.

Morphological analyses following the field experiment

At the end of the field experiment, seedlings were extracted from the rhizotrons and separated into leaves, stems and old and new roots, which were washed with tap water and dried in a ventilated oven at 60°C for 48 h to measure dry mass. Roots protruding out of the root plug were designated as new roots while those in the root plug were classified as old roots. Absolute increments in shoot and old and new root mass, and the root relative grow rate (RGR_{root}) were calculated. RGR_{root} was calculated as

$$(\log_e m_2 - \log_e m_1)/(t_2 - t_1)$$

where m_1 and m_2 are the total root mass at the end of the nursery cultivation and at the end of the field experiment, respectively.

Standardization of new root mass by leaf mass (NR/*L*) was used as a proxy of the plant's water balance potential. We also calculated the production of new root mass per total plant mass (NR/*P*). Before drying the new roots, we measured new root length according to methodology proposed in Marsh (1971). We calculated the specific root length (SRL) as the new root length to new root mass ratio.

Data analysis

Differences in seedling attributes following nursery cultivation were analysed by two-factorial ANOVA, with sowing date and fertilization as main factors. Root and shoot mass increment, RGR_{root}, NR/*L* and NR/*P* at the end of the field experiment were analysed by ANCOVA. Mean and total root elongation rate and depth, the number of roots and ψ were analysed by repeated measure ANCOVA. In these analyses, fertilization was the categorical predictor and seedling stem volume at the beginning of the field experiment was the covariable.

Antagonism between root and shoot growth was measured by calculating the slope of the linear regressions between the standardized shoot and total root elongation rate. Negative slopes mean that roots and shoot have an antagonistic growth pattern, while regressions with zero or positive slope values mean no antagonism between roots and shoot growth. The effect of fertilization and plant size on the slope values between root and shoot elongation rate was analysed by ANCOVA, where plants size was the covariable. To assess if the slope values in each seedling type were different from zero, we carried out a *t* test for single samples. Data were checked for normality and homogeneity of variances and were transformed when necessary to correct deviations from these assumptions. In ANCOVA, we also checked the homogeneity of slopes across fertilization treatments, and in all cases treatment slopes were similar (no significant covariable × Fertilization interaction). All statistical analyses were performed with the Statistica 6.1. Package (StatSoft, Inc., Tulsa, OK, USA). For simplicity, we classified plants into three size categories according to sowing date in the nursery (small, medium and large; see Table 1), to show the results of the effect of the stem volume covariable on studied variables.

Results

Seedling attributes following nursery cultivation

Seedling height increased with the delay in sowing date ($F = 214$, $P < 0.001$), and increased with fertilization, but only in seedling sown in March (sowing date × fertilization interaction, $F = 5.3$, $P = 0.011$). Delay in sowing date and fertilization increased seedling diameter ($F = 190$, $P < 0.001$ and $F = 7.5$, $P = 0.01$, respectively). Shoot and root mass decreased with the delay in sowing date ($F = 116$, $P < 0.001$ and $F = 103$, $P < 0.001$, respectively), while fertilization had no effect. Seedling total mass in plants sown in December was 1.5 and more than six times larger than that in plants sown in March and in May, respectively, while plants sown in March were more than four times larger than those sown in May. By contrast, seedling mass did not differ between fertilization treatments (Table 1). Seedling shoot/root ratio was close to 1 and did not differ among sowing dates, but it was lower in N^+ than in N^- seedlings ($F = 7.09$, $P = 0.009$).

Fertilization increased seedling N concentration ($F = 53.4$, $P < 0.001$), which was on average 35% higher in N^+ than in N^- seedlings. Delay in sowing date also increased seedling N concentration ($F = 15.3$, $P < 0.001$); seedlings sown in May had 26 and 30% higher N concentration than those sown either in March or in December, respectively. Both sowing date ($F = 81.5$, $P < 0.001$) and fertilization ($F = 23.5$, $P < 0.001$) affected seedling N content, with seedlings sown in December and May having the highest and lowest N content, respectively, while seedlings sown in March had intermediate values. N content was 30% higher in N^+ than in N^- seedlings.

Root and shoot growth in the field

Shoot elongation rate decreased with seedling size. Small seedlings had two shoot elongation rate peaks through time while medium and large seedling had only one peak, which

did not coincide in time with those of small seedlings (time × stem volume interaction; Table 2). Over all dates, mean elongation rate of individual roots was not affected by seedling size (Table 2; Fig. 1). This occurred because seedling size increased mean root elongation rate during the first weeks, but it reduced it at the end of the experiment (time × stem volume interaction; Table 2). Fertilization significantly reduced the mean elongation rate of individual roots. Total new root elongation rate increased with seedling stem volume, but there were no differences between large and medium plants at the end of the study (time × stem volume interaction; Table 2). Fertilization did not affect total root elongation rate. The number of new roots increased with initial seedling stem volume, except in the first measurement date (time × initial stem volume interaction; Table 2). Nursery fertilization significantly increased the number of roots, N^+ and N^- plants having 20.2 ± 1.3 and 14.5 ± 1.4 roots (mean ± 1 SE), respectively. Mean and maximum root depth increased through time, ranging from 32 to 37 cm and from 51 to 61 cm, respectively, at the end of the experiment. Neither initial seedling size nor fertilization significantly affected mean or maximum root depth.

At the end of the experiment, both initial seedling stem volume and nursery fertilization significantly increased new root mass and length (Table 2; Fig. 2). New root mass was 29% higher in N^+ seedlings than in N^- seedlings. Large and medium seedlings had 52 and 48% higher new root mass than small seedlings, respectively. New root length was 7.9 ± 0.92 and 5.6 ± 0.54 m (mean ± 1 SE) in N^+ and N^- seedlings, respectively, whereas large, medium and small seedlings had 8.0 ± 1.03, 8.1 ± 0.89 and 4.2 ± 0.52 m of new roots, respectively. Seedling stem volume and fertilization significantly enhanced growth of both shoot and old root mass. Root to shoot mass ratio at the end of the experiment and RGR_{root} was not affected either by initial seedling stem volume or nursery fertilization (Table 2, means not shown). Increase in seedling stem volume significantly diminished NR/*L*, NR/*P* and SRL, although the effect was marginal in the latter ($P = 0.065$). Medium and large seedlings had 22 and 30% lower NR/*L*, respectively, than small seedlings. NR/*P* was 43 and 30% lower in large and medium seedlings, respectively, than in small seedlings. Nursery fertilization did not significantly affect either NR/*L*, NR/*P* or SRL.

Mass of new roots was positively related to the seedling N content (Fig. 3) and the number of roots ($r^2 = 0.32$, $P < 0.001$, $n = 36$) at the end of the experiment, but not with either the mean elongation rate of individual roots ($r^2 = 0.05$, $P = 0.19$, $n = 36$) or the plant N concentration at the beginning of the study ($r^2 = 0.05$, $P = 0.67$, $n = 6$).

Table 2 Effects of initial stem volume (covariable), fertilization and time (within effect in repeated measure ANCOVA) on root and shoot growth variables and twig water potential (ψ) in *P. halepensis* seedlings

	Initial stem volume	Fertilization	Time	Initial stem volume × Time	Fertilization × Time
Shoot elongation rate	9.98***	0.074	4.24***	6.64***	0.79
Mean root elongation rate	0.32	3.49†	16.4***	2.66*	0.73
Total root elongation rate	17.5***	0.09	6.44***	4.44***	1.65
New root number	29.7***	4.91*	2.32*	6.40***	1.24
Average root depth	1.76	0.01	116***	0.42	0.18
Maximum root depth	2.00	1.57	137***	1.18	1.46
New root mass	23.1***	4.42*	–	–	–
New root mass/leaf mass	4.44*	0.41	–	–	–
New root mass/total plant mass	13.7***	1.48	–	–	–
Shoot mass to root mass	0.06	0.19	–	–	–
RGR_{root}	0.0002	2.02	–	–	–
New root length	11.4**	4.67*	–	–	–
New root specific length	3.51†	0.14	–	–	–
Root-shoot growth interference	19.5***	0.01	–	–	–
Old root increment	25.3***	3.99†	–	–	–
Shoot increment	25.5***	10.28**	–	–	–
ψ	5.08*	3.64†	580***	5.61*	0.04

Data are *F* ratios

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, † $P < 0.07$

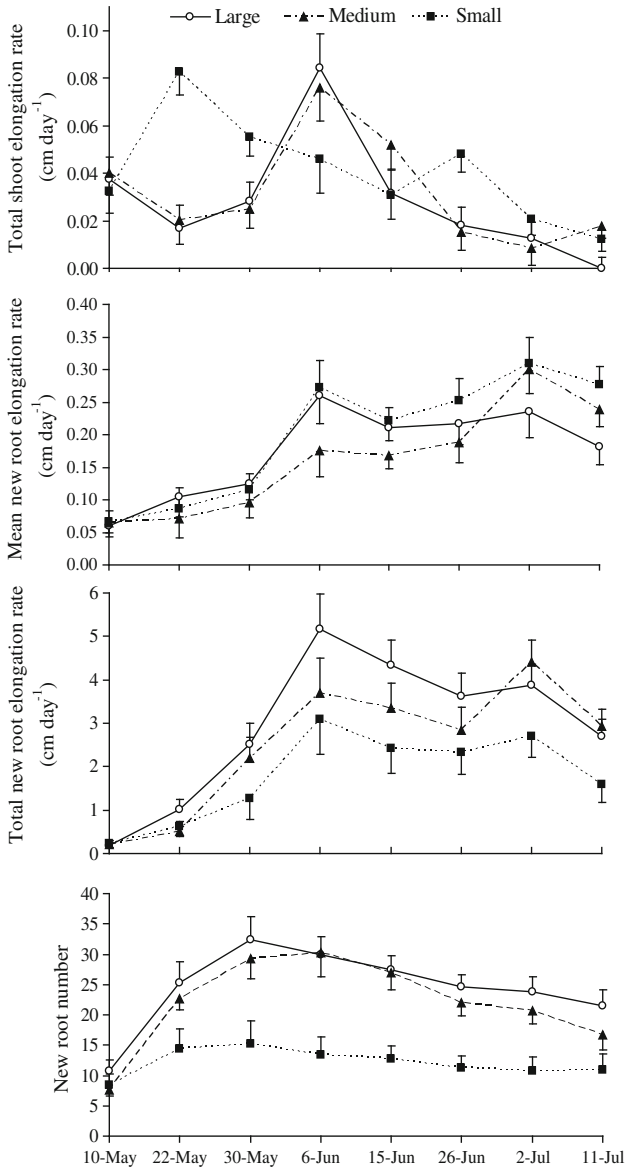

Fig. 1 Variation of shoot elongation rate, mean elongation rate of individual new roots, total new root elongation rate and number of new roots in *Pinus halepensis* seedlings of three size types along the experiment. Data are mean ± 1 SE. $n = 6$

Seedling water potential and relation between root and shoot elongation rate

Seedling ψ_{pd} was higher than ψ_{md}. Stem volume reduced ψ_{pd} whereas it did not affect ψ_{md} (time × initial stem volume interaction, Table 2). ψ_{pd} for large, medium and small seedlings was -1.21 ± 0.04, -1.05 ± 0.04 and -0.96 ± 0.04 MPa, respectively, whereas ψ_{md} was -2.53 ± 0.08, -2.53 ± 0.08 and -2.48 ± 0.08 MPa, respectively. Nursery fertilization did not affect ψ.

The slope of the regression of total root elongation rate against shoot elongation rate was significantly and positively related to stem volume while fertilization had no effect (Table 2; Fig. 4). Similarly, no interaction between fertilization and stem volume on the slope of the regression between the elongation rates of both organs was observed ($F = 0.18$, $P = 0.68$). Slopes of small seedlings were significantly lower from zero [-0.26 ± 0.046 (mean ± 1 SE), $t = -5.63$, $P < 0.001$]. On the contrary, the slope of large plants was significantly greater than zero (0.20 ± 0.089, $t = 2.25$, $P = 0.045$), whereas the slope of medium seedlings did not significantly differ from zero (0.12 ± 0.09, $t = 1.30$, $P = 0.22$).

Discussion

Root growth and structure

Large Aleppo pine seedlings produced larger new root system than small seedlings. Similar results have been reported in several forest species both in field experiments (Cuesta et al. 2010; Martínez-Sanz 2006) and in root growth capacity tests performed in controlled environments (van den Driessche 1992; Villar-Salvador et al. 2004). In agreement with our first hypothesis, large seedlings produced larger new root systems because they produced higher number of roots than small seedlings. Furthermore, new roots in large seedlings had lower SRL than in small seedlings. However, contrary to our hypothesis individual roots did not grow faster in larger than in smaller plants. Unlike Aleppo pine, elongation rate of individual roots increased with seedling size in *J. thurifera* (Martínez-Sanz 2006).

Seedling survival in dry ecosystems depends on the development of large and deep root systems (Burdett et al. 1983; Grossnickle 2005; Padilla and Pugnaire 2007). Greater root systems of large seedlings could explain their frequently improved post-transplanting performance relative to small seedlings in Mediterranean plantations (Luis et al. 2009; Oliet et al. 2009; Tsakaldimi et al. 2005; Villar-Salvador et al. 2008). Contrary to our expectations, we did not find differences in root depth among seedlings phenotypes, which differs with the result found for *J. thurifera*, where roots of large seedlings grew deeper than those of small seedlings (Martínez-Sanz 2006). Absence of differences in root depth among seedling types is consistent with their lack of differences in the mean elongation rate of individual roots among seedling types. Padilla and Pugnaire (2007) did not find any relationship between initial seedling size and either root extension rate or maximum root depth in a comparison of several Mediterranean woody species. This suggests that functional processes occurring across species may not coincide with those occurring at the intraspecific level. In accordance

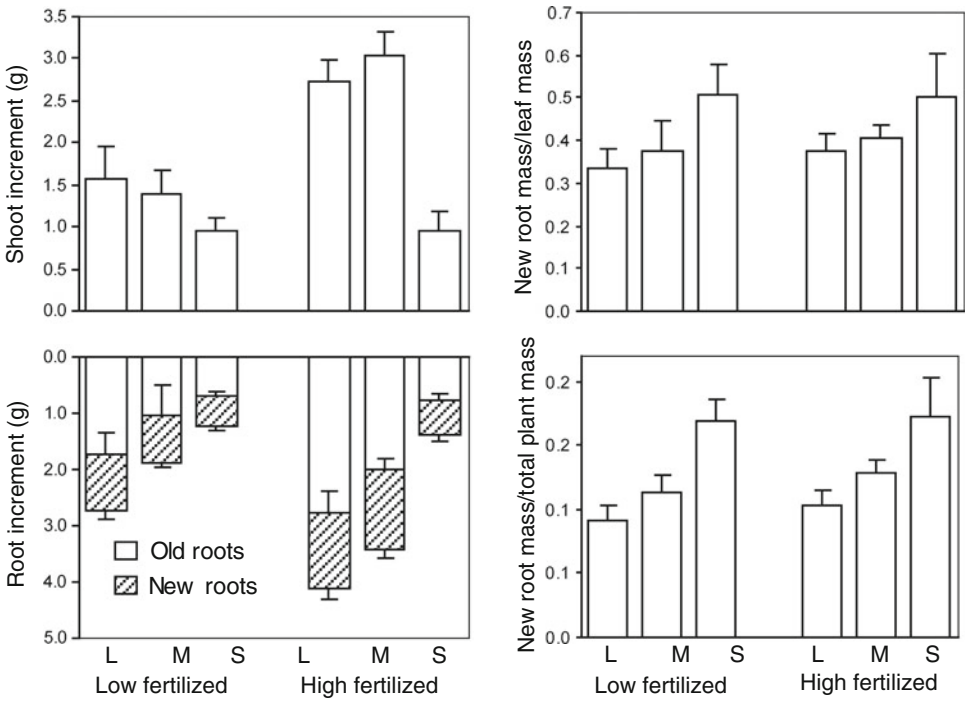

Fig. 2 Shoot and old and new root mass increment (*left*), and new root mass to leaf mass ratio and new root mass to total plant mass ratio (*right*) at the end of the rhizotron experiment in *Pinus halepensis* seedlings that differed in size at transplanting and were cultivated with contrasting fertilization rate in the nursery. Data are mean ± 1 SE. $n = 6$. *S* small seedling, *M* medium seedling, *L* large seedling

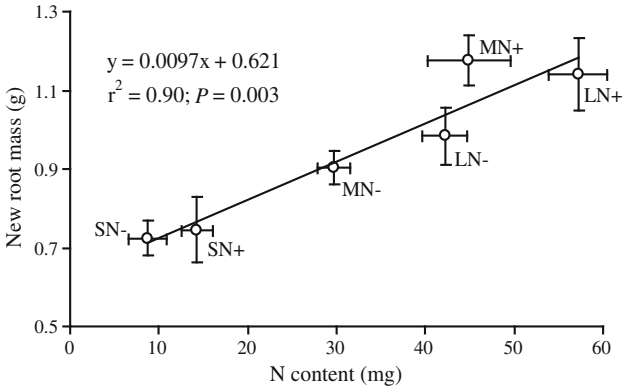

Fig. 3 Relationship between new root mass and N content in *Pinus halepensis* seedlings. Data are mean ± 1 SE. *S* small seedling, *M* medium seedling, *L* large seedling, N^- low fertilization and N^+ high fertilization

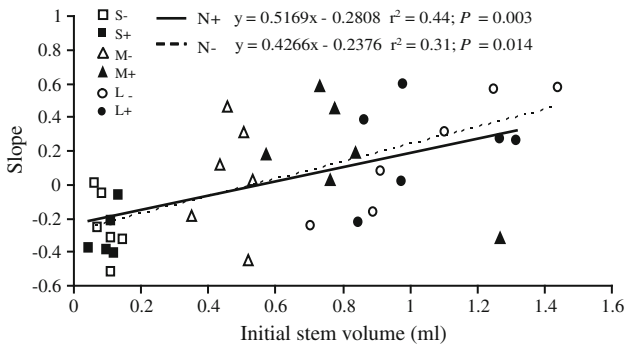

Fig. 4 Relationship between the slope of the regressions of shoot against root elongation rates and seedling size in high and low N fertilized plants. Seedling size was measured as the initial stem volume at transplanting. Each point represents an individual seedling. *S*− small seedling with low fertilization, *S*+ small seedling with high fertilization, *M*− medium seedling with low fertilization, *M*+ medium seedling with high fertilization, *L*− large seedling with low fertilization, *L*+ large seedling with high fertilization

with our first hypothesis, high-fertilized seedlings also produced greater root systems than low-fertilized plants. Nevertheless, root elongation rate of individual roots was just slightly higher in low-fertilized seedlings than in high-fertilized seedlings, which can explain the similar total root elongation rate between fertilization regimes.

Specific root length depends on root thickness and/or density and varies widely among species, although it usually has low variation in response to environmental conditions such as temperature or nutrient availability (Alvarez-Uria and Korner 2007; George et al. 1997; Pregitzer et al. 2002; Reich et al. 1998). We found that, at the intraespecific level, small seedlings tended to have higher SRL than large seedlings; this suggests that the former produced thinner and/or less dense roots, which have lower construction and maintenance cost than low SRL roots (Pregitzer et al. 2002).

NR/L provides an idea of the potential balance between the water transpiration and water uptake capacity in a plant. High NR/*L* may confer greater capacity of plants to maintain high water potential under drought conditions. In spite of the large differences in seedling size in our study, large seedlings were similarly water balanced than small seedlings. This argument is supported by the lack of differences in mid-day ψ among seedlings of different size. Higher root density (mass of new roots per soil volume

unit) and enhanced root hydraulic conductance in larger plants (Chirino et al. 2008; Wan et al. 1996) in comparison to smaller plants could explain why large seedlings, in spite of having lower NL/*R* and NR/*P*, had similar mid-day ψ than small seedlings. Small seedlings had lower pre-dawn ψ than large seedlings, suggesting that the former rehydrate faster than the latter at night or that night transpiration increased with seedling size.

Seedling size had greater effect on root growth than nursery fertilization as much more variables were affected by seedling size than fertilization (see Table 2). Differences in size among seedling phenotypes were higher than differences in N concentration, which probably explains the greater effect of seedling size on root growth. As root growth in *P. halepensis* and other conifers strongly relies on current photosynthesis (Moreno 2003; van den Driessche 1987), an increase in root growth with seedling size might be attributed to higher photosynthesis in larger plants than in small plants (Cuesta et al. 2010). Early growth of new organs in spring is also supported by remobilization of stored N, and growth is usually positively linked to the amount of remobilized N (Dyckmans and Flessa 2001; Malik and Timmer 1996; Millard 1996; Salifu and Timmer 2003). In our study, large plants had higher N content than small seedlings, which potentially increased the amount of remobilizable N. This might explain the positive relationship between the new root mass at the end of the experiment and seedling N content at planting (Fig. 3). In addition, nursery fertilization, which increased plant N concentration without promoting significant growth, had an additive effect on the effect of plant size on root growth. Increase in N concentration can stimulate growth by increasing the amount of remobilizable N and/or by enhancing photosynthetic rate (Field and Mooney 1986).

Relationship between root and shoot growth

As resources become limited, investment of resources to root growth can reduce shoot growth or reproduction and vice versa (Bloom et al. 1985; Chapin 1990). The relationship between root and shoot elongation through time differs among species and if lateral or taproots are considered (Harris et al. 1995; Lyr and Hoffmann 1967; Reich et al. 1980; Riedacker 1976; Thaler and Pagès 1996a). Our study demonstrates that antagonism between root and shoot elongation also varies among individuals of contrasted size at a within-species scale. In agreement with our second hypothesis, root and shoot growth did not have an antagonistic growth pattern in large and medium seedlings as the slope of the regression between shoot and root elongation rate was positive and zero, respectively. On the contrary, the slope was negative in small seedlings, indicating that the growth of both organs depressed each other probably because they competed for the same resources. Willaume and Pagès (2006) found that reduction of photosynthesis by defoliation increased the alternating growth pattern of roots and shoots in *Quercus pubescens*, highlighting the importance of carbohydrate sink/source relations in this process. Decrease in root and shoot growth antagonism with increase in seedling size suggests that large seedlings can provide resources to sustain high and simultaneous growth of both organs. Because increase in seedling N concentration did not have an additive effect over the plant size effect on the antagonism between shoot and root growth, we suggest that differences in carbon economy rather than N remobilization might be the mechanism underlying the differences in the shoot–root growth relationship in Aleppo pine seedlings. Future studies should investigate this hypothesis.

We conclude that large seedlings developed denser but not deeper root systems than small seedlings due to greater proliferation of new roots with lower specific root length. Seedling size had greater influence on root growth than N concentration, which had an additive effect over plant size. Root and shoot growth did not depress each other in large and medium seedlings, whereas it did occur in small seedlings. These findings provide insights for understanding root growth differences and transplanting performance among Aleppo pine stock-types in Mediterranean woodland plantations.

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