

Short-term nitrogen deprivation increases field performance in nursery seedlings of Mediterranean woody species

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Received 23 May 2007; received in revised form 14 September 2007; accepted 12 November 2007
Available online 4 January 2008

Abstract

Tree seedling establishment in Mediterranean areas is strongly limited by water and nutrients. In this study, we address the effect of nitrogen deprivation during the late phase of nursery growth, in November 2003, on the morpho-functional traits and field performance of *Pistacia lentiscus*, *Rhamnus alaternus*, *Rhamnus lycioides*, *Quercus coccifera*, and *Tetraclinis articulata* seedlings. A field experiment was carried out in degraded semi-arid land in eastern Spain. To assess the effectiveness of nitrogen hardening of seedlings on their establishment in this area, we analyzed plant morphology, biomass partitioning, and field survival. The relationships between foliar nutrient concentrations and plant survival were also analyzed. Nitrogen hardening reduced shoot size, root collar diameter, leaf area, specific leaf area, and root growth potential. Seedlings deprived of N showed a higher survival range than those subjected to standard fertilization both 3 months after outplanting and 6 months later. Short-term field survival was highly dependent on the species and the nutritional conditions. We conclude that nutrient hardening may enhance plant resistance to drought.

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Keywords: Drought; Functional attributes; Seedling establishment; Nutrient status

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1. Introduction

In Mediterranean areas the establishment of woody seedlings is strongly limited by water and nutrients. Seedling mortality can be very high, especially during the first summer after outplanting (Cortina et al., 2004). Nursery techniques may help to improve seedling performance in the field (Howell and Harrington, 2004; Landis, 1985). However, desirable seedling morpho-functional traits, and cultural practices to obtain them, are still under discussion (Cortina et al., 2006). In these areas, nursery seedlings have traditionally been grown under stress to promote acclimation to unfavorable field conditions. More recently, several studies demonstrated that seedling survival was positively affected by nursery practices aimed at increasing seedling vigor (Oliet et al., 1997, 2004; Villar-Salvador et al., 2004). Nevertheless, exceptions have been found, particularly under semi-arid conditions (Seva et al., 2000; Trubat et al., 2004).

Hardening woody seedlings by exposing them to mild or short-term stress has been recommended to promote acclimatization to harsh environmental conditions in the field (Villar-Salvador et al., 1999). Several studies have evaluated the effect of drought preconditioning on field performance of Mediterranean woody species (Vilagrosa et al., 2003b; Villar-Salvador et al., 1999). In contrast, nutritional hardening, that is, a reduction in nutrient supply (particularly nitrogen) to promote stress-resistance mechanisms, has received less attention.

Some studies have shown that plants growing under high nutrient availability may be more able to sustain growth, and maintain photosynthesis and stomatal conductance at higher levels, than plants growing under low nutrient availability (Abrams, 1988). Other studies, however, have shown opposite results (Hechler et al., 1991). Seedling response to nutritional hardening may depend on the duration and intensity of the reduction in resource supply, and on the seedling developmental stage. When hardening is performed during winter, when active growth ceases, it may affect the assimilation and storage of nutrients and carbohydrates (Balsberg Pålsson, 1992; van den Driessche, 1983). When it is applied during the phase of active growth, we can expect seedlings to show more morpho-functional responses. These processes imply a high degree of feedback. For example, nutrient limitation may reduce aboveground growth rates, but promote biomass allocation belowground and root uptake rates (Trubat et al., 2006).

Seedlings subjected to low N availability may be better adapted to drought because of reduced leaf size and increased allocation of biomass and nutrients belowground (Chapin, 1991b; Liu and Dickmann, 1993). This shift in biomass allocation is a common response to drought as well (Mooney and Winner, 1991; Tan et al., 1995). On the other hand, soil nitrogen availability is frequently low in degraded Mediterranean soils, and may limit seedling establishment (Bottner et al., 1995; Martínez-Mena et al., 2002; Valdecantos et al., 2006). The depletion of seedling N reserves resulting from a reduction in the N application rate could compromise seedling performance in the field. However, a reduction in the N supply could also favor the luxury consumption of phosphorus and other macronutrients (Jupp and Newman, 1987) and improve seedling capacity to withstand the low P availability common in basic dryland soils (Valdecantos, 2003).

The aim of this experiment is to evaluate the effect of late-season N deprivation on the morpho-functional traits and field performance of nursery seedlings of five Mediterranean woody species. To our knowledge, nutritional hardening has not been evaluated in Mediterranean woody species. Our hypotheses are: (1) N hardening will affect plant

morphology and nutritional status by reducing seedling size, increasing biomass allocation belowground, and reducing the N-to-P ratio in leaves, (2) the magnitude of the changes may be species-dependent, and (3) morpho-functional changes resulting from N hardening may improve seedling performance in the establishment phase.

2. Materials and methods

Seedlings of *Pistacia lentiscus* (L.), *Quercus coccifera* (L.), *Rhamnus lycioides* (L.), *Rhamnus alaternus* (L.), and *Tetraclinis articulata* (L.) were grown in a public nursery (Santa Faç, Alicante, SE Spain; 38°23'N, 0°26'E, 50 m a.s.l.), under semi-arid climate, with a 30-yr average annual precipitation and average temperature of 353 mm and 18 °C, respectively. Seedlings were grown from February 2002 to February 2003 in 305 cm³ forest containers (Super-leach[®]) filled with a mixture of peat and coco-peat (1:1). Seeds from the five species were collected from local provenances (Region of Valencia, Eastern Spain) by the Forest Services (Generalitat Valenciana Forest Seed Bank). Seedlings were cultivated in the open air and fertilized every two days with 40 mL of a modified Hoagland's solution containing 150 mg N L⁻¹ [as Ca (NO₃)₂ and K (NO₃)], 80 mg P L⁻¹ (as KH₂PO₄), and 100 mg K L⁻¹ (Control, hereafter C).

In November 2003 another fertilization level was differentiated. Nutritional hardening was imposed on half of the seedlings, selected randomly. The nitrogen concentration was reduced from 150 to 40 mg N L⁻¹ while the supply rate of the other nutrients was kept constant (Nutritional hardening, hereafter NH). Nitrogen deprivation lasted from November 2002 to February 2003.

Measurements of stem height, root collar diameter (RCD), leaf area, biomass accumulation, and foliar nutrient content were carried out at the end of the nursery period (February 2003). Measurements were taken in 10 randomly selected seedlings per species and treatment. Leaf area was measured by scanning the leaves (EPSON Expression 1680 Pro scanner with transparency adapter) and analyzing the image by means of specific image analysis software (WinRhizo, Régent Instruments Inc., Que., Canada). All biomass fractions were measured after drying at 65 °C for 48 h. The root weight ratio (RWR, g g⁻¹) was evaluated by calculating the ratio between root dry weight and total dry weight. Specific leaf area (SLA, cm² g⁻¹) was calculated as the ratio between leaf area and foliar dry weight. Dry leaves were fragmented by hand and digested in a heating block at 250 °C with a mixture of H₂SO₄ and H₂O₂ (1:1, v/v). Total N concentration was determined by semi-micro Kjeldahl distillation (Tecator Kjeltac Auto 1030 Analyzer, Hogana, Sweden), and P and K concentration by ICP spectrometry (Perkin Elmer Optima 3000, Perkin Elmer Corporation, Norwalk, CT, USA).

In February 2003, root growth potential (RGP) was measured on 10 seedlings per species and treatment. *T. articulata* was excluded from this analysis because of the lack of seedlings. Seedlings were transplanted to 3 L PVC pots filled with vermiculite, and watered every 2 days with a standard nutrient solution. The growth chamber provided day conditions of 20 °C for 16 h with a photosynthetic photon flux density of 400 μmol m⁻² s⁻¹. The number of newly elongated white roots longer than 5 mm was counted on each seedling at the end of a 21-day period.

Finally, in February 2003, one 50 m × 50 m plot was established in a degraded area close to Albatera (Alicante, SE Spain; 38°14'N, 0°56'E, 350 m a.s.l.; 24° slope; 240° SW aspect). Thirty-five randomly selected seedlings per species and treatment were planted in

40 × 40 × 40 cm³ manually dug planting holes. The species and treatment distribution was also random. The climate is semi-arid, with a 30-yr average annual precipitation and average temperature of 277 mm and 18.2 °C, respectively. Average annual rainfall and temperature in 2003 were 151 mm and 17.2 °C. Soils are loamy–silty loam, Lithic Calciorthid (Soil Survey Staff, 1990). Seedling survival, stem height and RCD were monitored in all seedlings immediately after outplanting, and before and after the summer of 2003.

Analysis of variance (ANOVA) was used to evaluate the effect of species (random factor with five levels), and N deprivation (fixed factor with two levels) on morphological traits, RGP and nutrient concentration. Only four species were considered for RGP analysis, as previously mentioned. When the ANOVA results showed a significant effect, we performed Student–Newman–Keuls post hoc test for multiple pair-wise comparisons between species. Log-linear analysis was used to test the effect of different fertilization levels on seedling survival. Survival was analyzed separately for each sampling period. RGP was log transformed to meet requirements of normality and homoscedasticity. Statistical analyses were performed with the SPSS 11.0 statistical package (SPSS Inc., Chicago, USA).

3. Results

Reduction in nitrogen availability had a strong effect on foliar nutrient concentration, but the effect differed among species (Table 1). Nitrogen deprivation reduced foliar N concentration in all species. The magnitude of the reduction ranged from 21% (*P. lentiscus*) to 33% (*R. alaternus*). Foliar P concentration in N-deprived *P. lentiscus* seedlings was lower than in well-fertilized seedlings ($F = 8.9$; $p < 0.01$). The same trend was observed for the other species, but it was not statistically significant. Nitrogen deprivation resulted in a decrease in foliar K concentration in *Q. coccifera* ($F = 0.14$; $p < 0.01$), and a marginally significant decrease in *T. articulata* ($F = 0.01$; $p = 0.72$). The N:P ratio in leaves decreased in all species, except for *R. alaternus* ($F = 0.5$; $p = 0.4$). A significant decrease in

Table 1

Foliar nutrient concentration (mg g⁻¹) of *P. lentiscus* (Pl), *R. alaternus* (Ra), *Q. coccifera* (Qc) and *R. lycioides* (Rl) seedlings grown under standard nutritional conditions (C), and under late-season N deprivation (NH)

	<i>P. lentiscus</i>	<i>R. alaternus</i>	<i>Q. coccifera</i>	<i>R. lycioides</i>	<i>T. articulata</i>
C					
N	13.3 ± 0.4a	22.6 ± 1.2a	14.6 ± 1.1a	21.1 ± 1.1a	15.7 ± 1.2a
P	6.4 ± 0.7a	2.8 ± 0.4	3.7 ± 0.4	4.1 ± 1.1	3.5 ± 0.4
K	8.3 ± 0.5	7.5 ± 0.7	5.7 ± 0.6	6.4 ± 1.4	7.8 ± 0.5a
N:P	3.3 ± 0.4a	8.9 ± 0.8	4.9 ± 0.5a	7.4 ± 1.1a	5.5 ± 0.7a
N:K	2.2 ± 0.2a	3.2 ± 0.2a	3.3 ± 0.4	4.4 ± 0.9a	2.2 ± 0.1
NH					
N	10.5 ± 0.6b	15.1 ± 1.3b	10.6 ± 0.9b	13.6 ± 0.5b	11.5 ± 1.2b
P	3.9 ± 0.5b	1.9 ± 0.3	2.9 ± 0.4	2.4 ± 0.4	3.3 ± 0.4
K	6.7 ± 0.5	7.1 ± 0.8	3.5 ± 0.3	6.4 ± 1.4	5.9 ± 0.8b
N:P	2.2 ± 0.2b	10.4 ± 1.7	3.6 ± 0.3b	5.1 ± 0.8b	3.5 ± 0.5b
N:K	1.6 ± 0.3b	2.2 ± 0.1b	3.1 ± 0.4	2.1 ± 0.2b	2.3 ± 0.1

Data are means ± SE ($N = 10$ plants). Results of a two-way ANOVA are shown.

the foliar N:K ratio was observed in *P. lentiscus* ($F = 4.1$; $p = 0.05$), *R. alaternus* ($F = 10.6$; $p < 0.01$), and *R. lycioides* ($F = 5.4$; $p = 0.03$).

Nitrogen deprivation had a significant effect on aboveground seedling size in *P. lentiscus* ($F = 72.8$; $p < 0.001$), *R. alaternus* ($F = 8.6$; $p < 0.01$) and *R. lycioides* ($F = 14.6$; $p < 0.01$), and, to a lower extent, in *Q. coccifera* ($F = 0.38$; $p = 5.7$; Table 2). Reductions in stem height ranged from 13% to 54%, in *R. lycioides* and *P. lentiscus*, respectively. *P. lentiscus* also showed the highest reduction in RCD when deprived of N ($F = 83.4$; $p < 0.001$). *Quercus coccifera* showed no changes in stem height, but it experienced a weak decrease in RCD with NH ($F = 0.54$; $p = 0.02$) (Table 2) in the nursery period, February 2003.

Belowground biomass accumulation showed a similar trend, with significant decreases in *P. lentiscus* ($F = 6.1$; $p = 0.02$), *R. alaternus* ($F = 5.1$; $p = 0.03$) and *R. lycioides* ($F = 39.4$; $p < 0.001$), and no significant changes in *Q. coccifera* ($F = 0.15$; $p = 0.7$) and *T. articulata* ($F = 0.11$; $p = 0.7$) (Table 3). Changes in leaf area paralleled those in aboveground biomass in all species except *T. articulata* ($F = 3.8$; $p = 0.07$). In this species, a significant reduction in leaf area with no significant reduction in aboveground biomass resulted in a decrease in SLA. *R. alaternus* ($F = 6.1$; $p = 0.02$) and *Q. coccifera* ($F = 6.4$; $p = 0.02$) also showed decreases in SLA in response to N deprivation, whereas this effect was absent for *P. lentiscus* ($F = 0.006$; $p = 0.94$) and the opposite effect, i.e., a significant increase in SLA in preconditioned seedlings, was observed in *R. lycioides* ($F = 17.5$; $p = 0.001$). Belowground biomass accumulation was reduced by N deprivation in *R. alaternus* ($F = 5.01$; $p = 0.03$) and *R. lycioides* ($F = 11.6$; $p = 0.003$), but not in the other species. Interestingly, only one species, *R. lycioides*, showed a significant increase in the root weight ratio in response to the reduction in N supply ($F = 31.9$; $p < 0.001$).

Despite the increase in belowground biomass allocation in N-deprived *R. lycioides* seedlings, RGP was lower in these seedlings than in seedlings receiving standard fertilization throughout the nursery period ($F = 22.63$; $p = 0.001$; Fig. 1). A decrease in RGP was also observed in preconditioned *P. lentiscus* ($F = 15.23$; $p = 0.002$) and *R. alaternus* ($F = 9.37$; $p = 0.007$). In contrast, *Q. coccifera* responded to the decrease in N availability by increasing RGP ($F = 26.01$; $p = 0.001$).

Short-term field survival was highly dependent on species ($\chi^2 = 26.61$; $N = 30$; $p = 0.014$) and nutritional conditions ($\chi^2 = 12.66$; $N = 29$; $p = 0.0004$; Fig. 2). Survival in control seedlings ranged from 61% to 85% in *Q. coccifera* ($\chi^2 = 9.39$; $N = 28$; $p = 0.002$) and *R. lycioides* ($\chi^2 = 3.89$; $N = 29$; $p = 0.04$), respectively. The effect was independent of the species, as the species \times treatment interaction was not statistically

Table 2

Stem height (cm) and root collar diameter (mm) of seedlings of five Mediterranean woody species grown under standard nutritional conditions (C), and under late-season N deprivation (NH)

	<i>P. lentiscus</i>	<i>R. alaternus</i>	<i>Q. coccifera</i>	<i>R. lycioides</i>	<i>T. articulata</i>
C					
Height (cm)	19.5 \pm 1.3a	13.3 \pm 1.3a	17.3 \pm 1.3	10.3 \pm 0.8a	9.8 \pm 0.6
RCD (mm)	4.2 \pm 0.3a	3.1 \pm 0.2a	3.9 \pm 0.2a	2.3 \pm 0.1a	2.2 \pm 0.1
NH					
Height (cm)	9 \pm 0.5b	9.1 \pm 0.7b	16.1 \pm 1.4	9 \pm 0.5b	9.5 \pm 0.6
RCD (mm)	2 \pm 0.1b	2.7 \pm 0.1b	3.7 \pm 0.2b	2 \pm 0.1b	2.4 \pm 0.1

Data are means \pm SE ($N = 10$ plants). Results of a one-way ANOVA are shown.

Table 3

Morphological traits of seedlings of five Mediterranean woody species grown under standard nutritional conditions (C), and under late-season N deprivation (NH)

	Shoot dry weight (g)	Root dry weight (g)	Leaf area (cm ²)	RWR (g g ⁻¹)	SLA (cm ² g ⁻¹)
<i>P. lentiscus</i>					
C	1.9 ± 0.02a	1.1 ± 0.02	37.5 ± 1.4a	0.7 ± 0.01	32.8 ± 0.36
NH	0.9 ± 0.04b	1.1 ± 0.01	28.8 ± 1.1b	0.8 ± 0.01	32.6 ± 1.28
<i>R. alaternus</i>					
C	0.9 ± 0.01a	0.9 ± 0.03a	87.2 ± 3.22a	0.6 ± 0.01	96.7 ± 2.4a
NH	0.6 ± 0.03b	0.6 ± 0.04b	36.2 ± 4.9b	0.7 ± 0.01	73.1 ± 3.36b
<i>Q. coccifera</i>					
C	1.3 ± 0.04	4.5 ± 0.2	62.8 ± 4.45	2.1 ± 0.06	51.4 ± 4.2a
NH	1.2 ± 0.02	4.7 ± 0.11	55.5 ± 1.56	2.3 ± 0.02	44.2 ± 1.24b
<i>R. lycioides</i>					
C	1.2 ± 0.03a	0.9 ± 0.03a	62.4 ± 2.9a	0.6 ± 0.01a	54.1 ± 2.16a
NH	0.3 ± 0.08b	0.6 ± 0.04b	23.4 ± 3.9b	1.1 ± 0.05b	65.5 ± 1.01b
<i>T. articulata</i>					
C	0.3 ± 0.01	0.3 ± 0.01	20.6 ± 0.92	0.7 ± 0.01	68.2 ± 2.82a
NH	± 0.02	0.2 ± 0.02	14 ± 0.58	0.6 ± 0.03	50.7 ± 1.25b

Data are means ± SE ($N = 10$ plants). Results of a one-way ANOVA are shown.

RWR, root weight ratio (g g⁻¹); SLA, specific leaf area (cm² g⁻¹).

significant. The average survival rate increase associated with N deprivation was 25%. *R. alaternus* showed the largest difference in survival rates in April ($\chi^2 = 5.64$; $N = 35$; $p > 0.001$) between control (67%) and N-deprived seedlings (92%). Seedling mortality increased before and after the first summer in the field. The differences between control and N-deprived seedlings were maintained and even increased afterwards.

4. Discussion

Seedlings receiving low N during the last phase of nursery growth showed lower field mortality than seedlings growing under standard conditions. These results support our hypothesis that short-term nitrogen deprivation at the end of the nursery period would enhance seedling performance under semi-arid conditions. However, the morphological response of the different species was not uniform.

Reductions in N availability had a strong effect on foliar N concentration in all species. But it must be noted that N-deprived seedlings showed foliar N concentration values far above the levels that have been commonly associated with N deficiency (Boardman et al., 1997; Bonneau, 1988; Trubat et al., 2004). These seedlings also showed a trend towards lower foliar P and K concentrations. This is in contrast with studies showing a luxury consumption of other nutrients when the N supply rate is low (Jupp and Newman, 1987), but it is in agreement with studies showing that a weak P deficiency may develop in plants subjected to deficient N availability (Ashraf et al., 2001; Gutierrez-Boem and Thomas, 2001). Decline in foliar K concentration in N-deprived seedlings may reflect a reduced demand for K resulting from the decrease in growth rate (Jarvis et al., 1990; Subasinghe, 2006). The increase in root K concentration resulting from a reduction in growth rate may

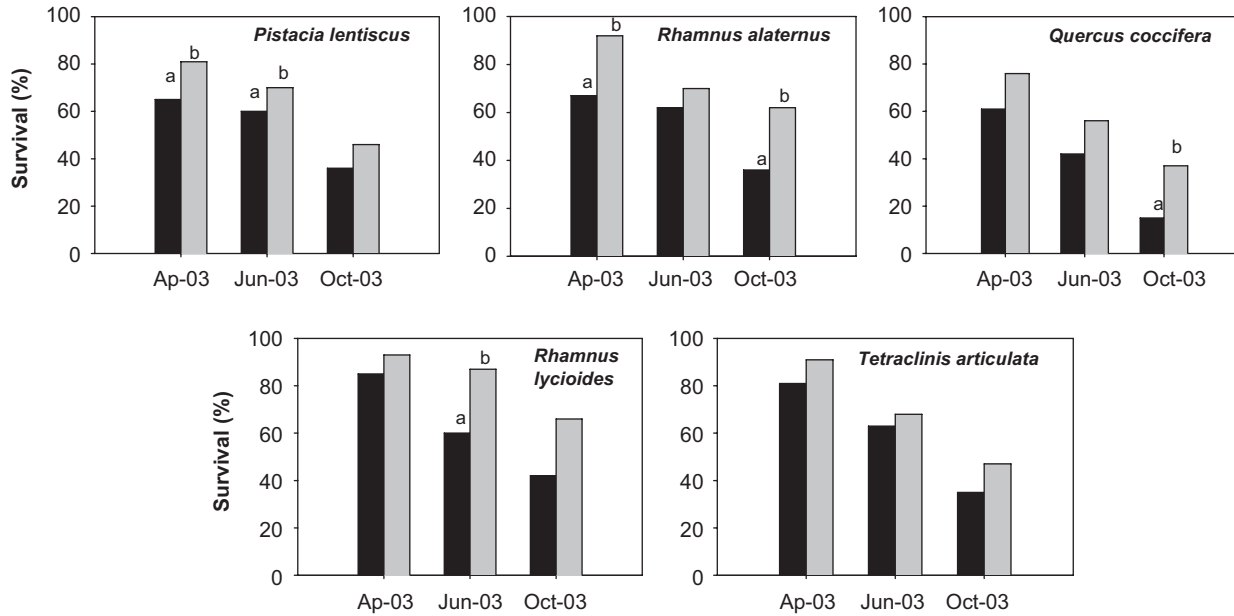


Fig. 1. Temporal changes in field survival of seedlings of five Mediterranean woody species grown under standard nutritional conditions in the nursery (black bars), and under late-season N deprivation (gray bars). Different letters indicate significant differences between treatments at $p < 0.05$.

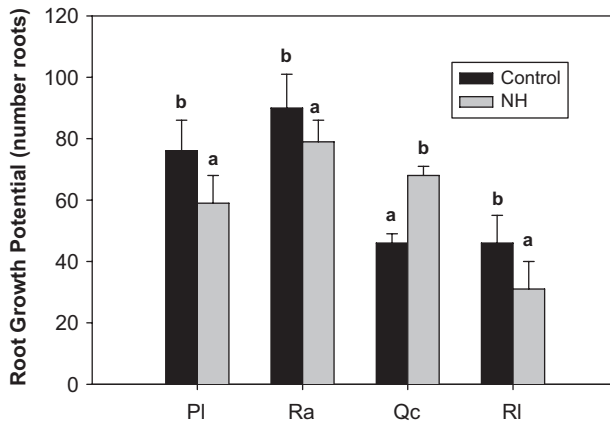


Fig. 2. Root growth potential of *P. lentiscus* (PI), *R. alaternus* (Ra), *Q. coccifera* (Qc) and *R. lycioides* (RI) seedlings growing under standard nutritional conditions (black bars), and under late-season N deprivation (gray bars), as measured under high water and nutrient availability. Data are means \pm 1 SE ($N = 10$ plants). Different letters for a given species indicate significant differences at $p < 0.05$.

act as a negative feedback for K uptake and translocation aboveground (Zsoldos et al., 1990).

The role that decreases in foliar nutrient concentration had on the observed field performance is not clear. Some plant strategies to cope with drought, such as increased biomass allocation belowground, increased water use efficiency or changes in root morphology, have been described for N and P limited plants (Forde and Lorenzo, 2001; Trubat et al., 2006). Potassium deficiency commonly hampers plant resistance to drought (Marschner, 1995). But K levels in the Mediterranean seedlings tested were probably not so low. On the contrary, K limitation may have promoted an increase in the water uptake rate, and thus the observed improvement in field performance (as described in Quintero et al., 1998).

The reduction in N supply resulted in smaller seedlings in three out of five of the species tested. Reduced growth rates are common in plants living in low-resource environments, including those characterized by drought (Chapin, 1991a). Previous studies have shown that plant growth cycles have important implications with respect to transplant and fertilization timing (Marler and Willis, 1996). The timing of cycle events through the year is a decisive feature determining plant adaptation to seasonally changing environments (Orshan, 1989). Plant phenological patterns in semi-arid regions have been related to seasonality (Castro-Díez and Montserrat-Martí, 1998). However, individual species growing under the same climate may differ widely in the arrangement of their phenophases. All the species tested showed higher relative growth rates in the spring, although they differed widely in the start and end dates of their growing period. Castro-Díez et al. (2003) showed that the earliest onset of spring growth was detected in February (*R. alaternus*); the latest, in *P. lentiscus*, occurred between late April and early May, coinciding with the cessation of growth in *Q. coccifera* and *R. lycioides* (Castro-Díez et al., 2003). In addition, the magnitude of the response probably depended on species growth and resource allocation patterns. We did not build a complete nutrient balance for each species. But we can hypothesize that *Q. coccifera*, a rather conservative species in terms of

growth rate and water use (Vilagrosa et al., 2003a), probably stored higher amounts of nutrients in woody tissues, particularly the tap root, which were mobilized as nutrients became limiting. Finally, nutrients accumulated in *Q. coccifera* acorns may have supported the phase of low N supply. Internal remobilization allows growth to be partly independent of external nutrient availability (Chapin, 1991a). Decreases in total leaf area commonly enhance convective heat loss and facilitate the maintenance of leaf energy balance. A smaller leaf area may reduce transpiration rate in leaves exposed to high radiation, improving water use efficiency under drought (Miller and Stoner, 1979). Additional effects of nitrogen deprivation, such as a reduction in water transport capacity (Ewers et al., 2000; Trubat et al., 2006), may also play a role in the water economy of N-limited plants by promoting a conservative use of water (Sperry, 2003).

Q. coccifera, *T. articulata*, and *R. alaternus* showed a significant reduction in SLA with N deprivation. These may reflect different proportions of new leaves in control and N-deprived seedlings, although it must be noted that in *Q. coccifera*, the difference in foliar surface between both sets of seedlings was rather small, and was not statistically significant. In contrast, the increase in SLA in N-deprived *R. lycioides*, and the lack of response in *P. lentiscus*, cannot be attributed to new growth. Changes in SLA can be explained by variations in leaf thickness. These variations can be the consequence of several anatomical changes, such as a reduction in cell diameter, an increase in cell wall thickness, and an increase in the proportion of dense tissues (Castro-Díez et al., 1997). Reductions in SLA have frequently been associated with an increased capacity to withstand drought (Corcuera et al., 2002). Low SLA, leaf thickness and reduced leaf area (Castro-Díez et al., 1997) are features that improve drought resistance in Mediterranean species, decreasing photochemical damage to the photosynthetic system and reducing transpiration rates by lowering leaf temperature under water stress (Gratani and Bombelli, 2001).

Root to shoot allocation patterns did not follow the expected inverse relationship with nutrient supply. An increase in root:shoot ratio is predicted to be a better strategy for maintaining growth under water-limiting conditions, because it can increase water and nutrients absorption and return carbon and nutrient contents to a balance more favorable for storage (Vilela et al., 2003). But only *R. lycioides* showed higher biomass allocation belowground as a response to the decrease in N availability. Biomass allocation to roots in N-deprived seedlings may be limited by low levels of P (van den Driessche and El-Kassaby, 1991). However, in previous studies we found that the RWR of *P. lentiscus* seedlings showed no increase in response to low N and water availability (Cortina et al., 2007; Trubat et al., 2006), suggesting that increased biomass allocation belowground may not be a major strategy for coping with depletion of soil resources in some Mediterranean species.

Seedling ability to colonize fertile soils, as reflected by the RGP, was highly sensitive to N deprivation. The formation of new roots is critical for seedling establishment after transplanting (Kaushal and Aussenac, 1989). Three out of the four species tested showed a significant decrease in RGP when N was removed from the nutrient solution. This is in agreement with studies showing a strong relationship between seedling vigor and RGP (McCreary and Duryea, 1987; Villar-Salvador et al., 2004), including studies with the same species tested here (Trubat et al., 2004). In contrast, *Q. coccifera*, which showed a weak morphological response to N deprivation, increased RGP by 21% in response to this treatment. Differences in RGP did not translate into differences in field performance. This was somewhat surprising as, under Mediterranean conditions, the initial seedling response,

and particularly its ability to root, may be crucial to ensure water supply during the summer, and successful seedling establishment (Reader, 1993; Fonseca, 1999). Thus, one would expect that initial survival potential and field performance would be closely related. According to Folk and Grossnickle (1997), the ability of seedlings to produce new roots (RGP) shows a low capacity to predict field performance when conditions at the planting site are mild, or when they are harsh but seedlings are highly resistant to stress. The latter was probably the case in the present study.

In conclusion, seedlings deprived of N during the last phase of nursery growth showed higher field survival. The positive effect of N deprivation was related to a decrease in seedling size. In contrast to our expectations, the positive effect of N deprivation was not related either to increased biomass allocation belowground, or to luxury consumption of nutrients other than N. A short-term reduction in N availability prior to planting seems to be a promising technique to improve the establishment of woody species in semi-arid environments.

Acknowledgments

This research was funded by the CEAM Foundation (Project: “Selección de Procedencias y Producción de Planta en Vivero para la Restauración de la Cubierta Vegetal y Control de la Erosión en Clima Semiárido”). The CEAM Foundation is funded by Generalitat Valenciana and Fundació Bancaixa. We are grateful to Marian Pérez, Amina Gobbi, Tadas Bitinas, and Santi Soliveres for their help with the fieldwork, and to Felipe Gil and the Conselleria de Territori i Habitatge for the use of Sta. Faç public nursery. J.C. acknowledges support from the Ministerio de Educación y Ciencia (Programa Nacional de Ayudas para la Movilidad), and thanks ERI and the School of Forestry at NAU for their kind hospitality.

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