

Performance and nutrient dynamics of holm oak (*Quercus ilex* L.) seedlings in relation to nursery nutrient loading and post-transplant fertility

Juan A. Oliet · María Tejada · K. Francis Salifu ·
Aleyda Collazos · Douglass F. Jacobs

Received: 21 August 2008 / Revised: 9 December 2008 / Accepted: 3 February 2009 / Published online: 26 February 2009
© Springer-Verlag 2009

Abstract Holm oak (*Quercus ilex* L.) seedlings were exponentially (E) nutrient loaded using incremental increases in fertilizer addition or conventionally (C) fertilized using a constant fertilizer rate during nursery culture. The fertility treatments (mg N plant⁻¹) were control (0), 25E, 100E, and 100C. Subsequently, 1-year-old plants were transplanted under simulated soil fertility gradients in a greenhouse to evaluate effects of nutrient loading and post-transplant fertility on seedling performance. Post-transplant fertility consisted of fertilizing plants at two rates (0 vs. 200 mg N plant⁻¹). A water-soluble fertilizer 20-20-20 was supplied in both nursery and post-transplant experiments. Nutrient loading increased plant N content by 73% in 100E and by 75% in 100C relative to controls, although no significant differences were detected between constant and exponential fertilization regimes at the 100 mg N plant⁻¹ rate. When transplanted, nutrient loading promoted post-transplant root growth relative to shoot, implicating potential to confer competitive advantage to loaded holm oak seedlings after trans-planting. In contrast, post-transplant fertility increased new shoot dry mass by 140% as well as N, P and K content relative to controls. Results suggest that holm

oak seedlings can be successfully nutrient loaded in the nursery at higher fertility rates, improving its potential to extend new roots, but alternative fertilization regimes and schedules that better fit nutrient availability to the growth rhythm and conservative strategy of this species must be tested.

Keywords Exponential fertilization · Forest restoration · Nitrogen · Mineral nutrition · Remobilization

Introduction

Holm oak (*Quercus ilex* L.) is an important evergreen tree species widely used for forest restoration in Mediterranean ecosystems (Rodà et al. 1999; Rey Benayas and Camacho-Cruz 2004). Holm oak survives and grows from warm and arid areas to dry and cold highlands and mountains in Spain and the western Mediterranean basin (Ruiz de la Torre 2006). This is mainly due to its ability to cope with summer drought by different water stress avoidance and tolerance mechanisms (Hinckley et al. 1983; Romane and Terradas 1992; Terradas and Savè 1992). However, seedlings are vulnerable to transplanting stress and to summer drought, exhibiting high mortality and slow growth compared with other Mediterranean species (Rodà et al. 1999). Water deficits (Gakis et al. 2004; Villar-Salvador et al. 2004a, b), low site fertility (Pardos et al. 2005; Valdecantos et al. 2006; Sanz-Perez et al. 2007), and poor seedling quality (Villar-Salvador et al. 2004a) are key factors that could explain poor survival and growth of holm oak seedlings in restoration plantings in Mediterranean ecosystems.

Field fertilization has the potential to ameliorate nutrient limitations on low-fertility soils and enhance seedling growth (Vilà and Terradas 1995; Jacobs et al. 2005;

Communicated by C. Ammer.

J. A. Oliet (✉) · M. Tejada · A. Collazos
Departamento de Silvopascicultura,
E.T.S. Ingenieros de Montes,
Universidad Politécnica de Madrid,
Ciudad Universitaria s/n, 28040 Madrid, Spain
e-mail: juan.oliet@upm.es

K. F. Salifu · D. F. Jacobs
Department of Forestry and Natural Resources,
Hardwood Tree Improvement and Regeneration Center,
Purdue University, West Lafayette, IN 47907-2061, USA

Sardans et al. 2006a, b). However, field fertilization may inadvertently stimulate growth of competing vegetation rather than target trees (Staples et al. 1999; Jobidon et al. 2003), and has been shown to be detrimental in dry areas of some Mediterranean ecosystems (Oliet et al. 2003). Therefore, storing nutrients in seedlings through nursery nutrient loading (Timmer and Aidelbaum 1996; Salifu and Timmer 2003a) may provide a better rationale to build plant nutrient reserves, which are subsequently retranslocated to promote seedling field survival and growth. Nursery nutrient loading has been successfully applied to several conifer species (Burgess 1991; McAlister and Timmer 1998; Qu et al. 2003; Rytter et al. 2003), subtropical and tropical species (Xu and Timmer 1998; Imo and Timmer 1992; Close et al. 2005), and temperate deciduous species (Birge et al. 2006; Salifu and Jacobs 2006). Nutrient loaded seedlings may exhibit superior survival, growth and competitiveness over non-loaded cohorts when transplanted in a variety of habitats (Timmer and Munson 1991; Malik and Timmer 1996; Thompson and Pitt 2003; Salifu et al. 2009). Retranslocation of internal nutrient reserves to support new growth is a key mechanism to explain improved performance of nutrient loaded plants (Malik and Timmer 1996; Salifu and Timmer 2003b).

Despite evidence of nutrient limitations in Mediterranean ecosystems, relatively little information is currently available on the nutritional status of nursery-grown seedlings used in restoration plantings (Oliet et al. 2006; Valdecantos et al. 2006). Although it is suggested that high nitrogen (Villar-Salvador et al. 2004a; Sanz-Perez et al. 2007) and phosphorus (Sardans et al. 2006b) fertilization of holm oak seedlings in the nursery can improve early field survival and growth on abandoned croplands, seedlings of evergreen oak species are often poorly or not fertilized at all because it is assumed that acorn nutrient reserves will meet annual nutrient demand (Peñuelas and Ocaña 1996). Additionally, potential implications of using exponential nursery fertilizer additions (Timmer and Aidelbaum 1996) to nutrient load holm oak seedlings to enhance field performance in Mediterranean ecosystems have received little attention. Generally, holm oak is a conservative species and exhibits low phenotypic plasticity to resource availability (Mayor and Roda 1994; Valladares et al. 2000; Sardans et al. 2006a) compared to other Mediterranean species. However, increased nutrient storage without stimulating dry mass production in response to nutrient availability (El Omari et al. 2003; Sanz-Perez et al. 2007), suggests the potential to successfully nutrient load holm oak seedlings. Characterizing how holm oak seedlings respond to nutrient loading and subsequent effects on post-transplant performance will improve our understanding of how to manipulate nutrition at the nursery stage to enhance

success of holm oak under field conditions. Additionally, studies are needed to evaluate whether nutrient enrichment at transplanting can further benefit post-transplant performance of holm oak seedlings. Therefore, the objectives of the current study were (1) to determine if holm oak seedlings could be nutrient loaded at the nursery stage to build reserves to benefit future growth using constant and (or) exponential addition schedules, (2) to evaluate effects of nursery nutrient loading on seedling post-transplanting performance, and (3) to examine post-transplant response of nutrient-loaded stock to fertilized or unfertilized (UF) soils under controlled greenhouse conditions. Although the post-transplant response is studied under controlled greenhouse environments, we propose that results may have portability to field conditions. For example, based on a similar approach, black spruce (*Picea mariana* Mill BSP) seedling responses on bioassays under controlled greenhouse conditions corresponded well with field performance (Munson and Timmer 1989; Malik and Timmer 1996).

Materials and methods

Nursery phase

Holm oak acorns from ES-45-1 “Región Extremadurese” provenances were sown at the University of Córdoba (37°51'N, 4°50'W, 123 m above sea level) greenhouse on 3 January 2004 in 300 ml Forest Pot containers (50 containers per tray; Nuevos Sistemas de Cultivo, S.L., Girona, Spain) filled with peat moss. Fertilization started on 14 May, and was applied weekly for 21 weeks, terminating on 1 October. Four fertilization treatments were tested: (1) control (no fertilization, 0), (2) 25 mg N plant⁻¹ applied exponentially (25E), (3) 100 mg N plant⁻¹ applied exponentially (100E), and (4) 100 mg N plant⁻¹ applied conventionally (100C).

Exponential fertilization delivered nutrients at exponential addition rates (Ingestad and Lund 1986; Timmer and Aidelbaum 1996) according to Eq. 1:

$$N_T = N_S(e^{rt} - 1) \quad (1)$$

where r is the relative addition rate required to increase N_S (initial N content in seed) to a final N content ($N_T + N_S$), and N_T was the desired amount of fertilizer to be added over the number of fertilizer applications ($t = 21$). Average N_S was determined to be 10 mg N seed⁻¹. The quantity of fertilizer to apply on a specific week (N_t) was calculated using Eq. 2:

$$N_t = N_S(e^{rt} - 1) - N_{t-1} \quad (2)$$

where N_{t-1} is the cumulative amount of N added up to and including the previous application.

Each fertilization treatment was applied to three trays, resulting in a total of 12 trays. Trays were completely randomized and their position rotated every 15 days to minimize edge effect. Fertilization treatments were applied via the water-soluble fertilizer 20N–20P₂O₅–20K₂O (Scotts Co., Marysville, OH, USA). The desired amount of fertilizer was added to a watering can and applied to individual trays once per week. Supplemental irrigation was applied to field capacity based on gravimetric methods (Timmer and Armstrong 1989). Plants were cultured in the greenhouse from sowing to 30 July, after which time the trays were moved to an exterior environment under 80% shade cloth until the end of nursery culture. On 13 October, eight plants were harvested per tray. Plants were separated into roots and shoots (leaves plus stem), dried (48 h at 65°C), and then weighed for dry mass determination. Plants were composited by treatment replication for nutritional analysis (three trays per treatment). Nutrient analysis was conducted in the Laboratorio Agroalimentario de Córdoba (Junta de Andalucía, Spain) according to Official Methods of Spanish Department of Agriculture (Ministerio de Agricultura 1994): Kjeldahl procedure for N, colorimetry for P (blue-molibdate method), and K was determined with flame photometry.

Transplanting phase

The transplanting experiment was conducted under controlled greenhouse environments to eliminate potential confounding of other factors on treatment responses. On 25 November 2004, 57 plants per nursery treatment (19 plants per tray) were transplanted into 5.3 l pots filled with a mixture of peat moss, bark pine and vermiculite (60:20:20) plus Ca (6 g l⁻¹ of Dolokal, R.H.P., The Netherlands) to raise pH. Pine bark and vermiculite were included in the mixture to improve the stability of the growing media considering the duration of the transplanting study and the dimensions of the pot. At planting, 27 plants per nursery treatment were randomly chosen to be fertilized with 200 mg N plant⁻¹ (fertilized, F) at two split applications (22 December 2004 and 4 May 2005) of 100 mg N plant⁻¹ at each application. A total of 30 plants per nursery treatment were not fertilized (UF) during the entire transplanting experiment. The experimental design in this phase was a 4 × 2 factorial, testing four nursery fertilization (0, 25E, 100C, and 100E) and two post-transplant fertility levels (F and UF). Thus, eight treatments were evaluated during the transplanting phase. The plants were arranged in a randomized complete block design in three replicate blocks (with 9 or 10 seedlings per nursery fertilization treatment replicate for fertilized and UF seedlings, respectively). Temperature during planting experiment was registered with a HOBO H8 Pro Series (Onset Computer

Co., Cape Cod, MA, USA). Mean temperature was 21.1°C (mean maximum 31.5°C, mean minimum 13.6°C). The same 20-20-20 fertilizer was dissolved in solution and supplied to the plants at each fertigation. Supplemental irrigation was applied to container capacity based on gravimetric methods (Timmer and Armstrong 1989).

Plants were sampled at two time periods after transplanting (H1 and H2). The first sampling after transplanting was conducted on 13 April 2005 (5 months after planting, H1), and the second at the end of the study on 30 June 2005 (7 months after planting, H2). Between five and six plants were harvested per treatment replicate during the first sampling and between three and four plants during the second. Plants were partitioned into new shoots, old shoots and roots, dried (48 h 65°C), and weighed for dry mass determination. Plants were composited by treatment replication for nutritional analysis. Nutritional analysis was conducted as described in the nursery phase experiment.

Statistical data analysis

Morphological and nutritional data were evaluated by separate analysis of variance (ANOVA) for the nursery and transplanting trials using SAS Software (SAS Institute Inc., Cary, NC, USA). Treatment means computed from individual seedlings for each tray (nursery experiment) and for each block (transplanting trial) were used for morphological data analysis. For mineral nutrition, data were analyzed from a composite sample for each treatment from a tray (nursery experiment) or a block (transplanting trial). Significant treatment means were ranked according to Tukey's HSD test at $\alpha = 0.05$. Data not presented in tables such as summary ANOVA for seedling shoot height, shoot basal diameter and component P and K concentration at the nursery phase were declared significant at $P < 0.05$ and significant treatment means ranked according to Tukey's HSD test at $\alpha = 0.05$.

Results

Nursery phase

Component nutrient concentration increased with the amount of fertilizer applied and differed significantly ($P < 0.05$) between treatments, except for shoot K (Fig. 1; Table 1). However, 100E and 100C had similar and higher component nutrient concentration levels than in 25E. With exception of root K and root and plant P concentrations, no significant differences appeared between 25E and non-fertilized (control) seedlings. Nursery fertilization significantly affected component dry mass except for roots (Fig. 2; Table 1). Shoot dry mass was stimulated 68% in

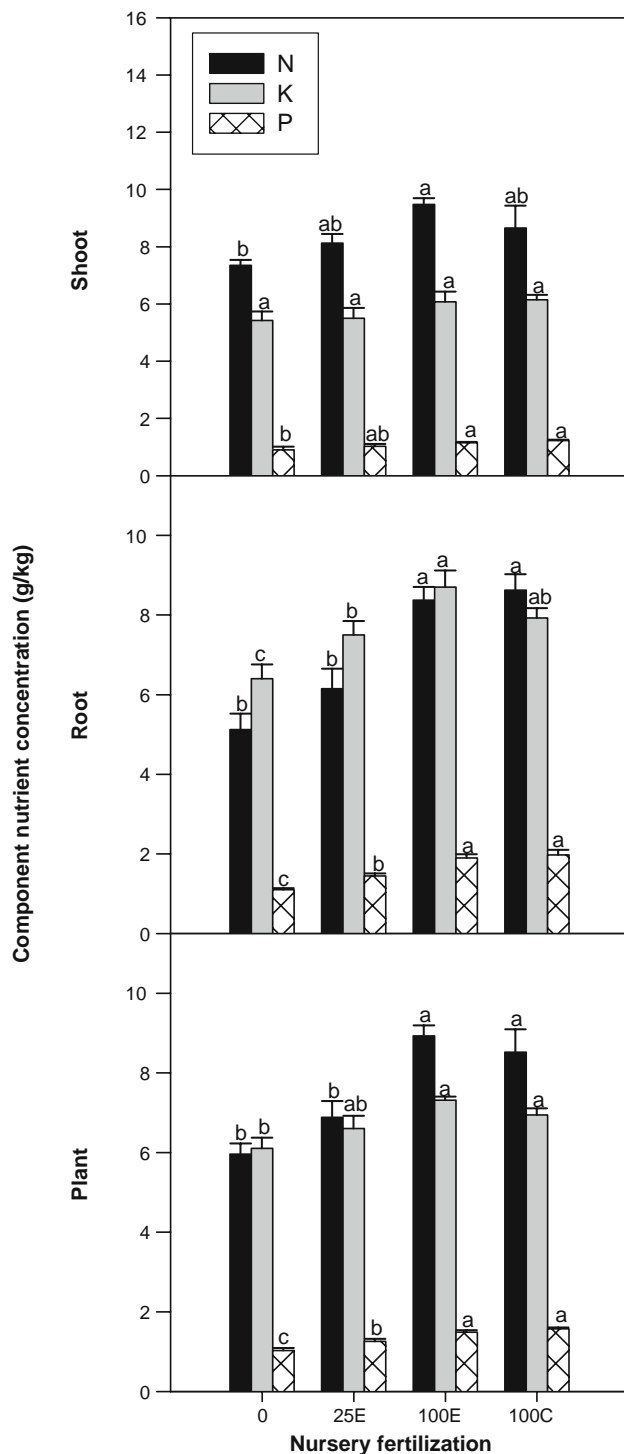


Fig. 1 Holm oak seedling component nutrient concentration following nursery growth in relation to nursery fertilization applied exponentially (E), conventionally (C) or unfertilized (0) for one growing season. Similar bars marked with different letters differ statistically according to Tukey's HSD test $\alpha = 0.05$

100E and 83% in 100C compared with the UF treatment. Similarly, plant dry mass increased by 15% in 100E and 23% in 100C relative to the control. With the exception of

root K, component nutrient content increased with fertilization rate and differed significantly (Fig. 2; Table 2). For example, compared with control, 100E increased N content by 116% in shoots, by 38% in roots, and by 73% in plant. Similarly, N content increased by 110% in shoots, by 47% in roots and by 75% in plant for 100C relative to control seedlings.

Post-transplant phase

At the end of transplant experiment, nursery fertilization significantly affected root and whole plant dry mass production (Fig. 3c, d; Table 1). Root dry mass was stimulated by 67% in 100C and by 42% in 100E relative to the control. Similarly, plant dry mass increased by 73% in 100C and by 35% in 100E relative to the control, although differences between 100E, 25E and control were not significant for these traits. In contrast, post-transplant fertility significantly stimulated new shoot dry mass production only (Fig. 3e; Table 1). Post-transplant fertility increased new shoot dry mass 140% compared to UF plants. Old shoot dry mass increased during the transplant experiment, with the exception of 100E that maintained a relatively constant pattern (Fig. 3b). Post-transplant fertility did not affect old shoot growth, which followed the same trend irrespective of nutrient availability within the growing media (Fig. 3f; Table 1). As a result of old shoot growth, N content 7 months after planting increased 8.7, 6.5 and 2.5 mg for 100C, 25E and control, respectively, while P and K content only increased in old shoot 0.2 and 2.8 mg, respectively, for the 25E nursery treatment (Figs. 2, 4).

Nursery fertilization significantly affected N and K accumulation in roots during the transplant experiment, but not P (Fig. 4; Table 2). Root nutrient content responses in 100C and 100E were generally greater than those noted for 25E. Root N content increased 60% in 100C and 56% in 100E relative to the control, while root K content also increased 63 and 44% compared to the non-fertilized nursery stock. Nursery fertilization did not affect N, P or K content in new shoots after planting (Fig. 4; Table 2). Post-transplant fertility significantly promoted N, P, and K accumulation in new shoots and roots, while accumulation in old shoots was only found for P (Fig. 4; Table 2). For example, post-transplant fertility increased N, P, and K content 160, 183, and 123%, respectively, in new shoots relative to UF plants. Nitrogen concentration declined rapidly in roots of nursery fertilized 100E and 100C plants during the growing season (Fig. 5c, d; Table 1), and stabilized by the first sampling event. Post-transplant fertilization maintained stable tissue N concentration in both roots and whole plant following an initial drop, but levels for UF seedlings continued to decrease throughout the study (Fig. 5g, h; Table 1). Generally, both the E and C

Table 1 ANOVA summary testing effects of nursery fertilization (NF) (see Figs. 1, 2) and subsequent effects of NF and fertilization at transplanting (FT) (see Figs. 3, 5) on *Q. ilex* seedling dry mass and nitrogen concentration

Source	Component dry mass				Component nitrogen concentration			
	New shoot	Old shoot	Root	Plant	New shoot	Old shoot	Root	Plant
Nursery culture phase								
NF	–	0.003	0.279	0.050	–	0.033	<0.001	<0.001
Transplanting phase								
First destructive sample (H1)								
NF	0.053	0.001	<0.001	<0.001	0.589	0.011	0.514	0.195
FT	<0.001	0.070	0.754	0.423	0.279	0.060	0.283	0.0750
NF × FT	0.190	0.416	0.749	0.561	0.957	0.808	0.783	0.965
Second destructive sample (H2)								
NF	0.520	0.018	0.012	0.018	0.995	0.034	0.183	0.254
FT	0.001	0.729	0.238	0.127	0.199	0.155	0.018	0.010
NF × FT	0.567	0.932	0.935	0.960	0.817	0.467	0.779	0.762

Fig. 2 Holm oak seedling component dry mass (left) and nutrient content (right) following nursery growth in relation to nursery fertilization applied exponentially (E), conventionally (C) or unfertilized (0) for one growing season. Similar bars marked with different letters differ statistically according to Tukey’s HSD test $\alpha = 0.05$

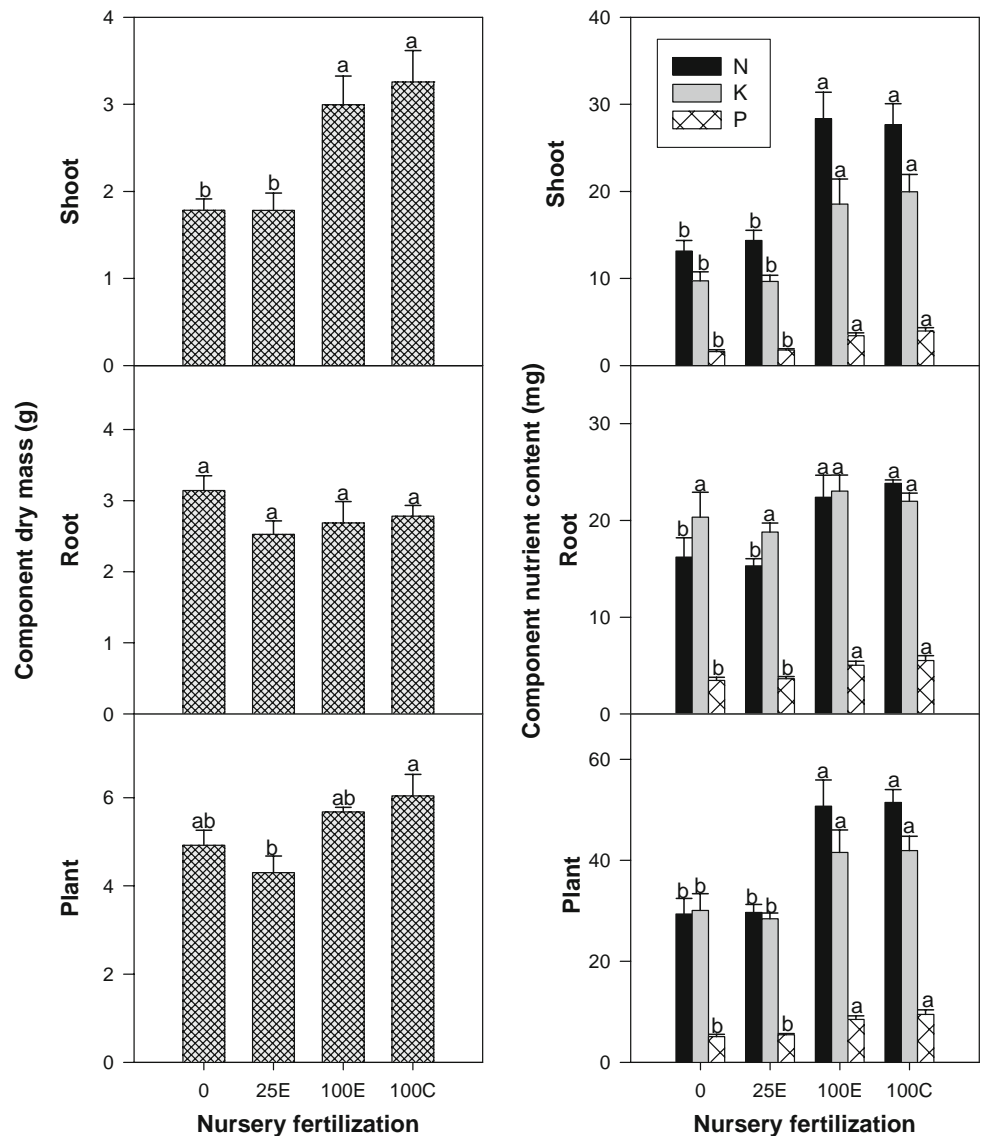


Table 2 ANOVA summary testing effects of nursery fertilization (NF) (see Fig. 2) and subsequent effects of NF and fertilization at transplanting (FT) (see Fig. 4) on *Q. ilex* seedling nutrient (N, P and K) content

	New shoot			Old shoot			Root		
	N	P	K	N	P	K	N	P	K
Nursery culture phase									
NF	–	–	–	<0.001	<0.001	0.002	0.004	0.005	0.331
Transplanting phase									
First destructive sample (H1 ^a)									
NF	0.299	–	–	<0.001	–	–	<0.001	–	–
FT	0.001	–	–	0.680	–	–	0.208	–	–
NF × FT	0.408	–	–	0.229	–	–	0.819	–	–
Second destructive sample (H2)									
NF	0.587	0.496	0.461	0.001	0.002	0.013	0.018	0.082	0.001
FT	0.001	<0.001	0.003	0.354	0.009	0.973	0.017	0.003	0.006
NF × FT	0.782	0.480	0.559	0.555	0.245	0.565	0.794	0.613	0.642

^a P and K in first sample could not be analyzed due to low amount of dry mass available

regimes that received the same fertilizer rate in the nursery (100 mg N plant⁻¹) produced similar effects in cultured plants, and post-transplanting response of 25E seedlings did not differ from control (non-fertilized) seedlings. Nursery × post-transplant fertility interaction effects were non-significant (Tables 1, 2).

Discussion

Nursery phase

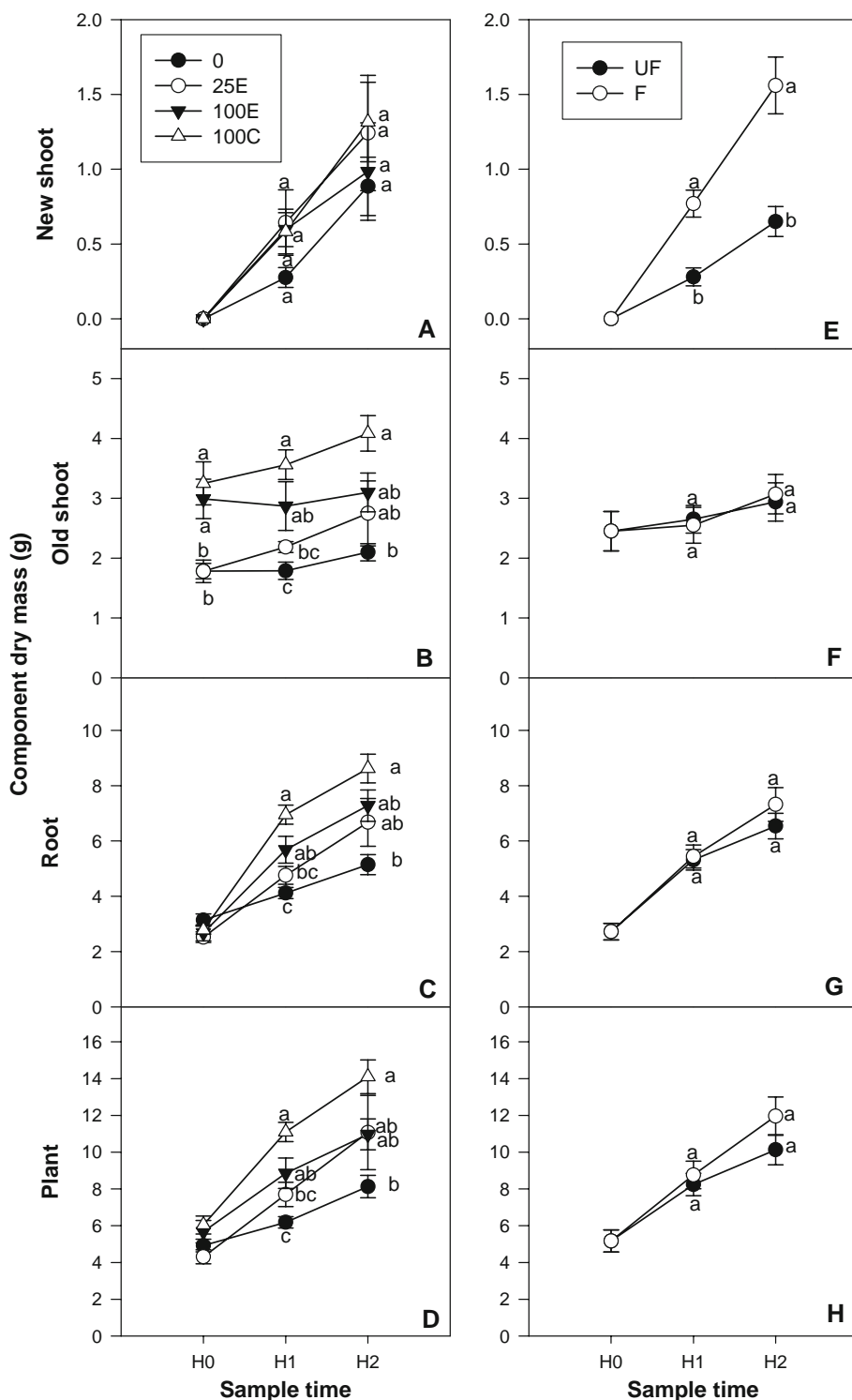
Increased growth and nutritional response of *Q. ilex* seedlings to fertilization rate (Figs. 1, 2) suggests that this species can be effectively nutrient loaded in the nursery, as confirmed by previous studies (Villar-Salvador et al. 2004a; Sanz-Perez et al. 2007). However, the relatively low N concentration (<10 g kg⁻¹) reached in both 100E and 100C is likely associated with the low plasticity of holm oak seedlings to resource availability (Sardans et al. 2006a), and has been observed in a similar nursery study with this species (Villar-Salvador et al. 2004a). In addition, the apparent lack of response to fertilization regime (exponential vs. constant) at the rates applied likely results from the differences between growth model and exponential fertilizer application pattern, as suggested by Everett et al. (2007). Similarities of 100E versus 100C, as well as 25E versus control, suggest that the exponential regime at these rates does not increase uptake efficiency in holm oak, as it does in other species (Timmer and Armstrong 1987; Salifu and Jacobs 2006). As a species exhibiting episodic growth through multiple flushes during a growing season (Terradas and Savè 1999) similar to other *Quercus* spp.

(Hanson et al. 1986; Sloan and Jacobs 2008), holm oak may have non-continuous nutrient demands along the season. Apparently, a need exists for additional studies to encompass broad N rates ranging from deficiency to toxicity to characterize and quantify targets rates for effective nutrient loading of holm oak. Moreover, different fertilization delivery regimes and schedules that better match growth rhythm of this species must be tested. Although previous studies have confirmed that other oak species can be nutrient loaded by exponential regimes (Birge et al. 2006; Salifu and Jacobs 2006), the ability of exponential fertilization regimes to nutrient load seedlings may be species-specific, as shown for red pine (*Pinus resinosa* Ait.) (Timmer and Armstrong 1989), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) (Hawkins et al. 2005) and Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) seedlings (Everett et al. 2007). On the other hand, the lack of significant differences in root dry mass in the nursery (Fig. 2) may be associated with the relatively small container size, which limits capacity for root growth and extension (Oliet et al. 2004; Villar-Salvador et al. 2004a).

Post-transplant phase

The nursery fertilizer regimes of 100C and 100E had similar effects on post-transplant growth and nutrition of holm oak seedlings (Figs. 3, 4, 5), which were greater than those noted for 25E. This suggests that constant and exponential regimes at higher fertility rates can produce plants that are equally competitive in the field (Timmer and Armstrong 1989; Hawkins et al. 2005). Exponentially and conventionally fertilized white pine (*Pinus monticola*

Fig. 3 Holm oak seedling component dry mass following transplant in relation to nursery fertilization applied exponentially (*E*), conventionally (*C*) or unfertilized (*U*) for one growing season (*left*), and transplanted into fertilized (*F*) or unfertilized (*UF*) soils (*right*) under controlled greenhouse conditions. Points marked with different letters at each sampling event differ statistically according to Tukey’s HSD test $\alpha = 0.05$. Plants were sampled at 5 (*H1*) and 7 (*H2*) months after transplanting. *H0* corresponds to basal data determined at the end of nursery experiment

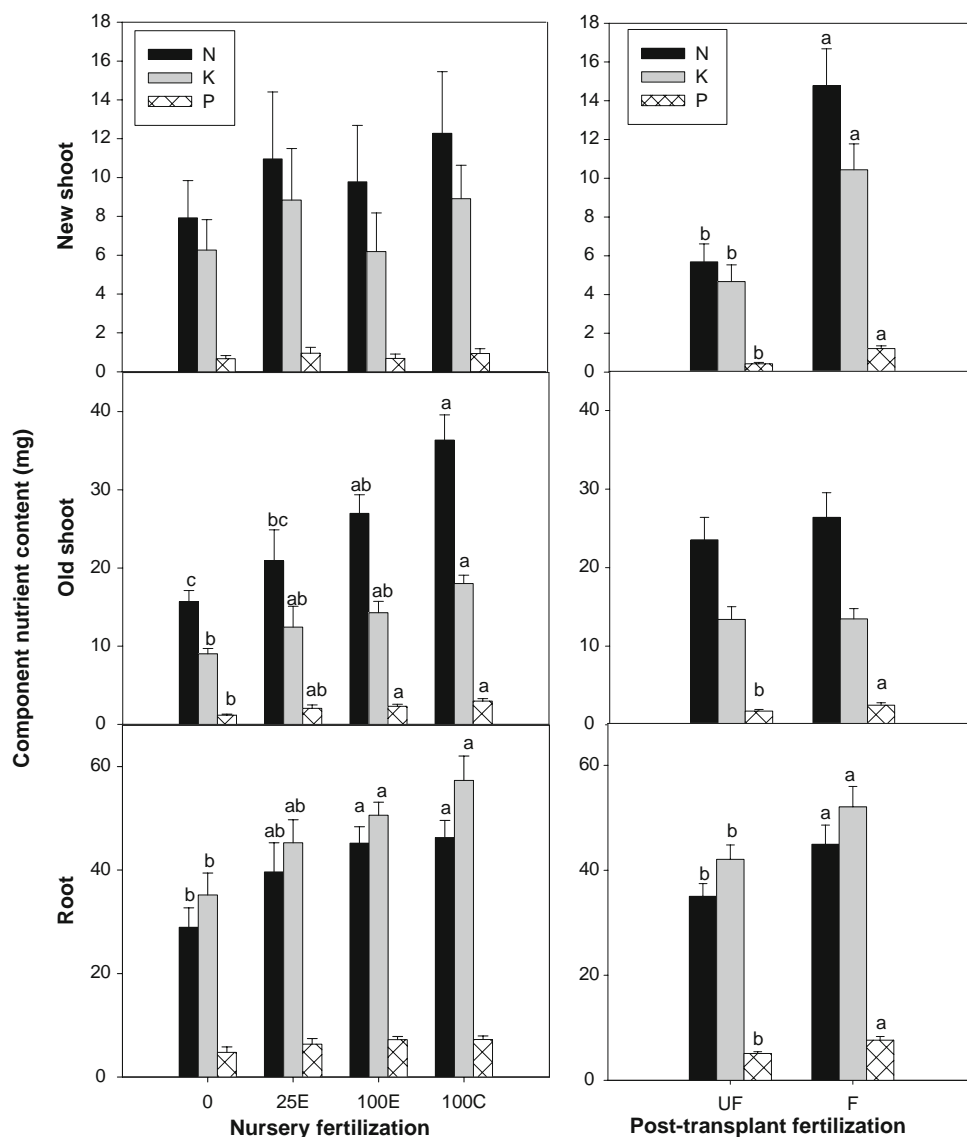


Doug. ex D. Don.) seedlings exhibited similar performance in the field (Dumroese et al. 2005), although conventionally fertilized seedlings received higher applications rates (i.e., 36 vs. 20 mg N plant kg⁻¹).

It appears that holm oak exhibits differential plasticity to resource availability. For instance, dry mass and nutrient

content of new shoots responded significantly only to post-transplant fertility, while root dry mass was significantly affected by nursery pre-planting fertilization treatments only (Figs. 3, 4). El Omari et al. (2003) also showed that increased root growth of 1-year-old holm oak seedlings after 2 months was associated with nutrient reserves

Fig. 4 Holm oak seedling component nutrient content following transplant in relation to nursery fertilization applied exponentially (*E*), conventionally (*C*) or unfertilized (*U*) for one growing season (*left*), and transplanted into fertilized (*F*) or unfertilized (*UF*) soils (*right*) under controlled greenhouse conditions. Similar bars marked with different letters differ statistically according to Tukey's HSD test $\alpha = 0.05$

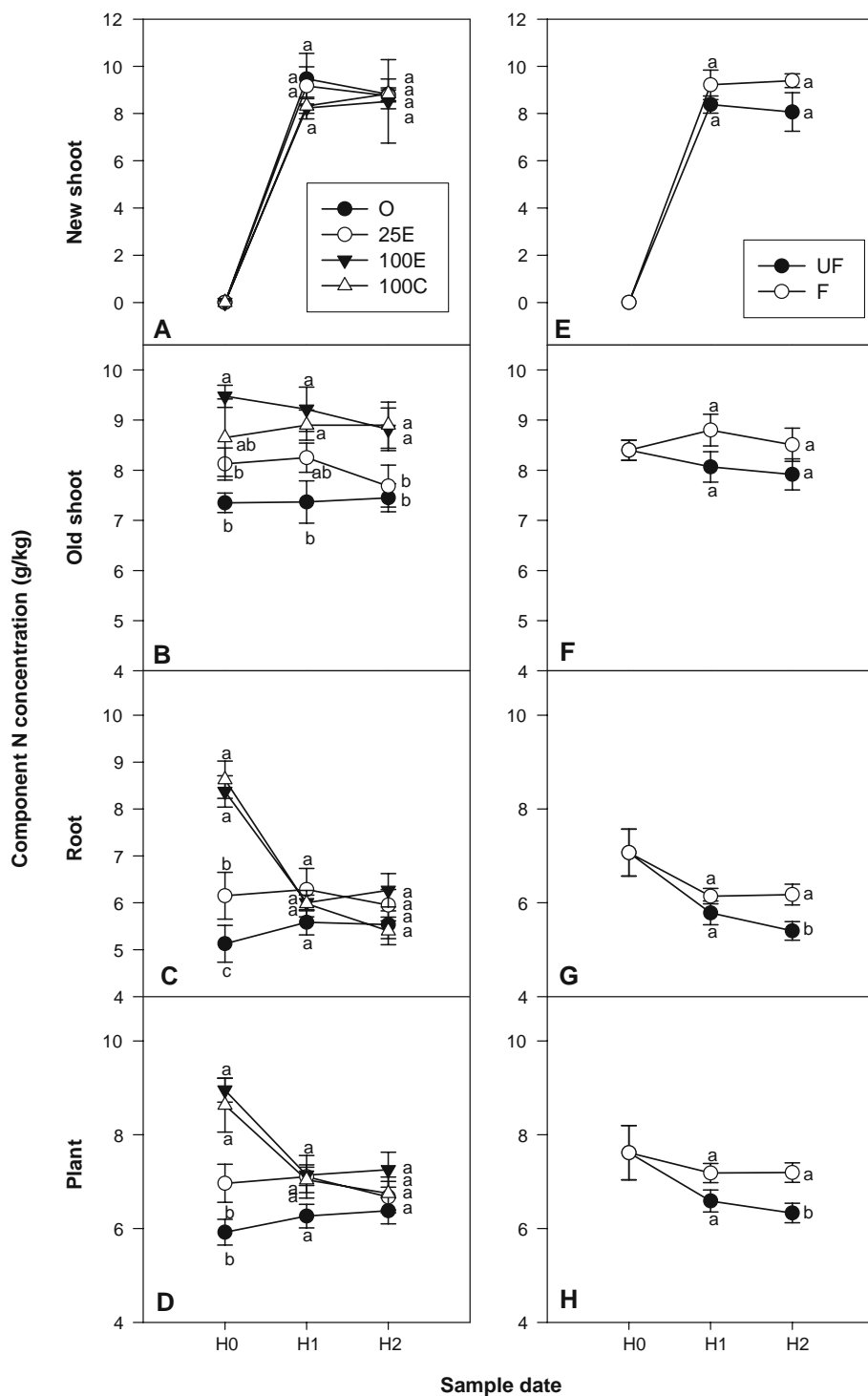


accumulated via fertilization treatments. This evidence suggests that nutrient loading promoted post-transplant root growth in this species. This relative response of holm oak component growth to current fertility of the soil compared to plant nutrient reserves demonstrates differential resource utilization strategies employed by this species and component plasticity to resources availability. Because nutrient content of new shoots was not related to pre-planting nutrient content but was associated with post-transplant fertility (Fig. 4), nutrients in new shoots seem to be derived mainly from external root uptake rather than from internal reserves. This was not the case for other species, like *P. mariana*, where new shoot growth was based on internal reserves of loaded seedlings (Boivin et al. 2004). Conversely, N and K content in old shoots after 7 months were not affected by post-transplant fertility (Fig. 4), suggesting

that internal reserves serve as a source of nutrients to support secondary shoot growth. Internal dynamics of N, P, and K seem to follow comparable patterns in most circumstances, with exception of P in some cases. This similarity is also true for other forest nursery species (Imo and Timmer 1999, 2001).

The similarity of root system size at planting (Fig. 2) suggests that retranslocation of nutrients from roots (Nambiar 1987) may partly explain increased root growth after transplanting (Fig. 3c). Increased root growth following nursery nutrient loading emphasizes the importance of stored nutrient reserves in promoting root growth, which may confer competitive advantage to seedlings when transplanted in the field. This contention is supported by published data where holm oak seedlings fertilized at high N–P–K rates in the nursery exhibited greater root growth

Fig. 5 Holm oak seedling component N concentration following transplant in relation to nursery fertilization applied exponentially (*E*), conventionally (*C*) or unfertilized (*O*) for one growing season (*left*), and transplanted into fertilized (*F*) or unfertilized (*UF*) soils (*right*) under controlled greenhouse conditions. Points marked with different letters at each sampling event differ statistically according to Tukey’s HSD test $\alpha = 0.05$. Plants were sampled at 5 (*H1*) and 7 (*H2*) months after transplanting. *H0* corresponds to basal data determined at the end of nursery experiment



potential as well as improved early field establishment and survival in the field (Villar-Salvador et al. 2004a). Root growth improves root–soil contact to exploit site resources, which enhances drought avoidance in *Q. ilex* seedlings (Villar-Salvador et al. 2004a, b).

Conclusions

We have shown that holm oak seedlings can be successfully nutrient loaded using either exponential or constant addition regimes at higher fertility rates confirming results

noted elsewhere (Timmer and Armstrong 1989; Hawkins et al. 2005). Nursery nutrient loading promoted post-transplant root growth but not shoot growth, suggesting that the approach has potential to increase competitive success and drought avoidance of holm oak seedlings when transplanted under field conditions. In contrast, post-transplant fertilization was effective in promoting new shoot growth but not root growth, which demonstrates the plasticity of this species to channel nutrient sources in support of different growth tissues. Holm oak seedlings fertilized conventionally at a high rate resulted in plants with similar post-transplant responses to those fertilized exponentially at the same rate. Due to the low plasticity of holm oak seedlings to nutrient availability, additional studies should be conducted using broad N rates ranging from deficiency to toxicity, along with varying specific nutrient ratios and fertilizer delivery regimes. These studies will quantify and develop optimum target rates for effective nutrient loading of holm oak to increase forest restoration success in Mediterranean ecosystems.

Acknowledgments We gratefully acknowledge the financial support of the University of Córdoba and Andalucía Research Programs to promote research groups activities. Additionally, this research was partially supported by MEC project AGL2006-12609-C02-01/FOR.

References

- Birge ZDK, Salifu KF, Jacobs DF (2006) Modified exponential nitrogen loading to promote morphological quality and nutrient storage of bareroot-cultured *Quercus rubra* and *Quercus alba* seedlings. *Scand J For Res* 21:306–316. doi:10.1080/02827580600761611
- Boivin JR, Salifu KF, Timmer VR (2004) Late-season fertilization of *Picea mariana* seedlings: intensive loading and outplanting response on greenhouse bioassays. *Ann For Sci* 61:737–745. doi:10.1051/forest:2004073
- Burgess D (1991) Western hemlock and Douglas-fir seedling development with exponential rates of nutrient addition. *For Sci* 37:54–67
- Close DC, Bail I, Hunter S, Beadle CL (2005) Effects of exponential nutrient-loading on morphological and nitrogen characteristics and on after-planting performance of *Eucalyptus globulus* seedlings. *For Ecol Manage* 205:397–403. doi:10.1016/j.foreco.2004.10.041
- Dumroese RK, Page-Dumroese DS, Salifu KF, Jacobs DF (2005) Exponential fertilization of *Pinus monticola* seedlings: nutrient uptake efficiency, leaching fractions, and early outplanting performance. *Can J For Res* 35:2961–2967. doi:10.1139/x05-226
- El Omari B, Aranda X, Verdagner D, Pascual G, Fleck I (2003) Resource remobilization in *Quercus ilex* L. sprouts. *Plant Soil* 252:349–357. doi:10.1023/A:1024792206369
- Everett KT, Hawkins BJ, Kiiskila S (2007) Growth and nutrient dynamics of Douglas-fir seedlings raise with exponential or conventional fertilization and planted with or without fertilizer. *Can J For Res* 37:2552–2562. doi:10.1139/X07-108
- Gakis S, Manzanos K, Alifragis D, Papanastasis VP, Papaioannou A, Seilopoulos D, Platis P (2004) Effects of understory vegetation on tree establishment and growth in a silvopastoral system in northern Greece. *Agrofor Syst* 60:149–157. doi:10.1023/B:AGFO.000013275.30617.ad
- Hanson PJ, Dickson RE, Isebrands JG, Crow TR, Dixon RK (1986) A morphological index of *Quercus* seedling ontogeny for use in studies of physiology and growth. *Tree Physiol* 2:273–281
- Hawkins BJ, Burgess D, Mitchell AK (2005) Growth and nutrient dynamics of western hemlock with conventional and exponential greenhouse fertilization and planning in different fertility conditions. *Can J For Res* 35:1002–1016. doi:10.1139/x05-026
- Hinckley TM, Duhme F, Hinckley AR, Richter H (1983) Drought relations of shrub species. Assessment of the mechanism of drought resistance. *Oecologia* 59:344–350. doi:10.1007/BF00378860
- Imo M, Timmer VR (1992) Nitrogen uptake of Mesquite seedlings at conventional and exponential fertilization schedules. *Soil Sci Soc Am J* 56:927–934
- Imo M, Timmer VR (1999) Vector competition analysis of black spruce seedling responses to nutrient loading and vegetation control. *Can J For Res* 29:474–486. doi:10.1139/cjfr-29-4-474
- Imo M, Timmer VR (2001) Growth and nitrogen retranslocation of nutrient loaded *Picea mariana* seedlings planted on boreal mixedwood sites. *Can J For Res* 31:1357–1366. doi:10.1139/cjfr-31-8-1357
- Ingestad T, Lund AB (1986) Theory and technique for steady state mineral nutrition and growth of plants. *Scand J For Res* 1:439–453
- Jacobs DF, Salifu KF, Seifert JR (2005) Growth and nutritional response of hardwood seedlings to controlled-release fertilization at outplanting. *For Ecol Manage* 214:28–39. doi:10.1016/j.foreco.2005.03.053
- Jobidon R, Roy V, Cyr G (2003) Net effect of competing vegetation on selected environmental conditions and performance of four spruce seedling stock sizes after eight years in Quebec (Canada). *Ann For Sci* 60:691–699. doi:10.1051/forest:2003063
- Malik V, Timmer VR (1996) Growth, nutrient dynamics, and interspecific competition of nutrient-loaded black spruce seedlings on a boreal mixedwood site. *Can J For Res* 26:1651–1659. doi:10.1139/x26-186
- Mayor X, Roda F (1994) Effects of irrigation and fertilization on stem diameter growth in a Mediterranean holm oak forest. *For Ecol Manage* 68:119–126. doi:10.1016/0378-1127(94)90143-0
- McAlister JA, Timmer VR (1998) Nutrient enrichment of white spruce seedlings during nursery culture and initial plantation establishment. *Tree Physiol* 18:195–202
- Ministerio de Agricultura (1994) Pesca y Alimentación. Métodos Oficiales de Análisis Secretaría General Técnica, Dirección General de Política Alimentaria, Madrid
- Munson AD, Timmer VR (1989) Site-specific growth and nutrition of planted *Picea mariana* in the Ontario Clay Belt. II. Effects of nitrogen fertilization. *Can J For Res* 19:171–178. doi:10.1139/x89-024
- Nambiar EKS (1987) Do nutrients retranslocate from fine roots? *Can J For Res* 17:913–918. doi:10.1139/x87-143
- Oliet J, Navarro R, Contreras O (2003) Evaluación de la aplicación de mejoradores y tubos en repoblaciones forestales. Consejería de Medio Ambiente de la Junta de Andalucía, Sevilla
- Oliet J, Planelles R, Segura ML, Artero F, Jacobs DF (2004) Mineral nutrition and growth of containerized *Pinus halepensis* seedlings under controlled-release fertilization. *Sci Hortic* 103:113–129. doi:10.1016/j.scienta.2004.04.019
- Oliet JA, Valdecantos A, Puértolas J, Trubat R (2006) Influencia del estado nutricional y el contenido en carbohidratos en el establecimiento de los plantones. In: Cortina J, Peñuelas JL, Puértolas J, Savé J, Vilagrosa A (Coords) Calidad de planta forestal para la restauración en ambientes mediterráneos degradados. Estado actual de conocimientos. Organismo Autónomo

- Parques Nacionales, Ministerio de Medio Ambiente, Madrid, pp 89–117
- Pardos M, Royo A, Pardos JA (2005) Growth, nutrient, water relations, and gas exchange in a holm oak plantation in response to irrigation and fertilization. *New For* 30:75–94. doi:[10.1007/s11056-004-2553-x](https://doi.org/10.1007/s11056-004-2553-x)
- Peñuelas J, Ocaña L (1996) Cultivo de plantas forestales en contenedor. MAPA-Mundi Prensa, Madrid
- Qu L, Quoreshi AM, Koike T (2003) Root growth characteristics, biomass and nutrient dynamics of seedlings of two larch species raised under different fertilization regimes. *Plant Soil* 255:293–302. doi:[10.1023/A:1026159709246](https://doi.org/10.1023/A:1026159709246)
- Rey Benayas JM, Camacho-Cruz A (2004) Performance of *Q. ilex* saplings planted in abandoned Mediterranean cropland after long-term interruption of their management. *For Ecol Manage* 194:223–233. doi:[10.1016/j.foreco.2004.02.035](https://doi.org/10.1016/j.foreco.2004.02.035)
- Rodà F, Retana J, Gracia C, Belloe J (eds) (1999) Ecology of Mediterranean evergreen oak forests. Ecological studies. Springer, Berlin
- Romane F, Terradas J (1992) *Quercus ilex* L. ecosystems: functions, dynamics and management. *Advances in vegetation science* 13. Kluwer, Dordrecht
- Ruiz de la Torre J (2006) Flora mayor. Organismo Autónomo Parques Nacionales, Madrid
- Rytter L, Ericsson T, Rytter R-M (2003) Effects of demand-driven fertilization on nutrient use, root:plant ratio and field performance of *Betula pendula* and *Picea abies*. *Scand J For Res* 18:401–415. doi:[10.1080/02827580310001931](https://doi.org/10.1080/02827580310001931)
- Salifu KF, Jacobs DF (2006) Characterizing fertility targets and multi-element interactions in nursery culture of *Quercus rubra* seedlings. *Ann For Sci* 63:231–237. doi:[10.1051/forest:2006001](https://doi.org/10.1051/forest:2006001)
- Salifu KF, Timmer VR (2003a) Optimizing nitrogen loading in *Picea mariana* seedlings during nursery culture. *Can J For Res* 33:1287–1294. doi:[10.1139/x03-057](https://doi.org/10.1139/x03-057)
- Salifu KF, Timmer VR (2003b) Nitrogen retranslocation response of young *Picea mariana* to nitrogen-15 supply. *Soil Sci Soc Am J* 67:309–317
- Salifu KF, Jacobs DF, Birge ZKD (2009) Nursery nitrogen loading improves field performance of bareroot oak seedlings planted on abandoned mine land. *Restor Ecol* (in press)
- Sanz-Perez V, Castro-Diez P, Valladares F (2007) Growth versus storage: responses of Mediterranean oak seedlings to changes in nutrient and water availabilities. *Ann For Sci* 64:201–210. doi:[10.1051/forest:2006104](https://doi.org/10.1051/forest:2006104)
- Sardans J, Peñuelas J, Roda F (2006a) Plasticity of leaf morphological traits, leaf nutrient content, and water capture in the Mediterranean evergreen oak *Quercus ilex* subsp. *ballota* in response to fertilization and changes in competitive conditions. *Ecoscience* 13:258–270. doi:[10.2980/i1195-6860-13-2-258.1](https://doi.org/10.2980/i1195-6860-13-2-258.1)
- Sardans J, Roda F, Peñuelas J (2006b) Effects of a nutrient pulse supply on nutrient status of the Mediterranean trees *Quercus ilex* subsp. *ballota* and *Pinus halepensis* on different soils and under different competitive pressure. *Trees (Berl)* 20:619–632. doi:[10.1007/s00468-006-0077-z](https://doi.org/10.1007/s00468-006-0077-z)
- Sloan JL, Jacobs DF (2008) Carbon translocation patterns associated with new root proliferation during episodic growth of transplanted *Quercus rubra* seedlings. *Tree Physiol* 28:1121–1126
- Staples TE, Van Rees KCJ, Van Kessel C (1999) Nitrogen competition using ^{15}N between early successional plants and planted white spruce seedlings. *Can J For Res* 29:1282–1289. doi:[10.1139/cjfr-29-8-1282](https://doi.org/10.1139/cjfr-29-8-1282)
- Terradas J, Savè R (1992) The influence of summer and winter stress and water relations on the distribution of *Quercus ilex* L. *Vegetatio* 99–100:137–145
- Terradas J, Savè R (1999) Holm oak and holm oak forests: an introduction. In: Rodà F, Retana J, Gracia C, Bellot J (eds) Ecology of Mediterranean evergreen oak forests. Ecological studies. Springer, Berlin, pp 3–14
- Thompson DG, Pitt DG (2003) A review of Canadian forest vegetation management research and practice. *Ann For Sci* 60:559–572. doi:[10.1051/forest:2003060](https://doi.org/10.1051/forest:2003060)
- Timmer VR, Aidelbaum AS (1996) Manual for exponential nutrient loading of seedlings to improve outplanting performance on competitive forest sites. NODA/NFP Tech. Rep. TR25. Nat. Res. Canadian Forest Service, Sault Ste Marie, ON, Canada
- Timmer VR, Armstrong G (1987) Growth and nutrition of containerized *Pinus resinosa* at exponentially increasing nutrient additions. *Can J For Res* 17:644–647. doi:[10.1139/x87-105](https://doi.org/10.1139/x87-105)
- Timmer VR, Armstrong G (1989) Growth and nutrition of containerized *Pinus resinosa* seedlings at varying moisture regimes. *New For* 3:171–180. doi:[10.1007/BF00021580](https://doi.org/10.1007/BF00021580)
- Timmer VR, Munson AD (1991) Site-specific growth and nutrient uptake of planted *Picea mariana* in the Ontario Clay Belt. IV. Nitrogen loading response. *Can J For Res* 21:1058–1065. doi:[10.1139/x91-145](https://doi.org/10.1139/x91-145)
- Valdecantos A, Cortina J, Vallejo VR (2006) Nutrient status and field performance of tree seedlings planted in Mediterranean degraded areas. *Ann For Sci* 63:249–256. doi:[10.1051/forest:2006003](https://doi.org/10.1051/forest:2006003)
- Valladares F, Martínez-Ferri E, Balaguer L, Pérez-Corona E, Manrique E (2000) Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy? *New Phytol* 148:79–91. doi:[10.1046/j.1469-8137.2000.00737.x](https://doi.org/10.1046/j.1469-8137.2000.00737.x)
- Vilà M, Terradas J (1995) Effect of nutrient availability and neighbours on shoot growth, resprouting and flowering of *Erica multiflora*. *J Veg Sci* 6:411–416. doi:[10.2307/3236240](https://doi.org/10.2307/3236240)
- Villar-Salvador P, Planelles R, Enriquez E, Peñuelas-Rubira JL (2004a) Nursery cultivation regimes, plant functional attributes, and field performance relationships in the Mediterranean oak *Quercus ilex* L. *For Ecol Manage* 196:257–266. doi:[10.1016/j.foreco.2004.02.061](https://doi.org/10.1016/j.foreco.2004.02.061)
- Villar-Salvador P, Planelles R, Oliet J, Peñuelas-Rubira JL, Jacobs DF, Gonzalez M (2004b) Drought tolerance and transplanting performance of holm oak (*Quercus ilex*) seedlings after drought hardening in the nursery. *Tree Physiol* 24:1147–1155
- Xu XJ, Timmer VR (1998) Biomass and nutrient dynamics of Chinese fir seedlings under conventional and exponential fertilization regimes. *Plant Soil* 199:313–322. doi:[10.1023/A:1004307325328](https://doi.org/10.1023/A:1004307325328)