From Forest Nursery Notes Winter 2013

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Trees (2012) 26:311–320
DOI 10.1007/s00468-011-0593-3

ORIGINAL PAPER

Autumn fertilization with nitrogen improves nutritional status, cold hardiness and the oxidative stress response of Holm oak (*Quercus ilex* ssp. *ballota* [Desf.] Samp) nursery seedlings

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Received: 13 April 2011 / Revised: 2 July 2011 / Accepted: 8 July 2011 / Published online: 23 July 2011

Abstract Holm oak (*Quercus ilex* ssp. *ballota* [Desf.] Samp) is one of the most important species in forest communities of the western Mediterranean Basin, but is very vulnerable to environmental stress during the first years of its life. In particular, summer drought and winter frosts limit the distribution, survival, and growth of Holm oak. These two stress factors can lead to plasmolysis and/or oxidative stress. We hypothesized that autumn fertilization with nitrogen (N) can improve plant tolerance to these abiotic stress factors and improve plant quality and therefore reforestation success. A 12-week autumn application of 30 or 60 mg N (plus 70 mg N applied to both treatments during the previous 28 weeks in the nursery, i.e. 100 and 130 mg N in total, respectively) improved overall growth, root growth capacity, frost tolerance, and nutritional status of plants relative to plants given 0.0 and 1.5 mg autumnal N, and had no negative effect on seedling response to water stress. A very small increment in N doses during the autumn (1.5 vs. 0.0 mg N) improved some morphological parameters, such as stem diameter (*D*) and shoot dry weight, and physiological parameters, such as total antioxidant activity. The highest autumnal N dose (60 mg) increased leaf nutrient content without decreasing the concentration, but had a tendency to reduce frost tolerance relative to seedlings given a lower autumnal N dose (30 mg).

Keywords Holm oak · Water stress · Mineral nutrients · Frost tolerance · Oxidative stress

Communicated by R. Hampp.

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Introduction

Holm oak (*Quercus ilex* ssp. *ballota* [Desf.] Samp) is one of the most important species in forest communities of the western Mediterranean Basin (Palacios et al. 2009). This evergreen sclerophyllous species has been increasingly used for reforestation (MAPA 2006). However, Holm oak usually has poor early out-planting performance compared to other Mediterranean species, particularly in sites with unfavorable climatic conditions (Pausas et al. 2004). This poor performance has been attributed to water stress (Villar-Salvador et al. 2004a, b), low site fertility (Pardos et al. 2005; Sanz-Pérez et al. 2007; Valdecantos et al. 2006), and poor seedling quality (del Campo et al. 2010).

Drought and frosts are the main factors that limit the distribution, abundance, survival, and growth of this species in Mediterranean areas (Larcher 2000), and Holm oak is very vulnerable to stress conditions during early life (Ruiz de la Torre 2006). Afforestation projects conducted with this species are usually subjected to long periods of low temperatures and frosts and extreme summer drought, especially in continental areas (Aranda et al. 2005; Corcuera et al. 2005; García-Plazaola et al. 1999; Gimeno et al. 2009), limiting the survival and growth after field planting. The exposure to suboptimal non-lethal growth temperatures causes a depression of photosynthesis and