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Effects of a deep container on morpho-functional characteristics and root colonization in *Quercus suber* L. seedlings for reforestation in Mediterranean climate

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ABSTRACT

In the last decades, reforestation and afforestation programs are being carried out mainly with containerized seedlings. Container design determines the morphological and physiological characteristics of seedlings. However, container characteristics are often the same for plant species with very different growth strategies. The most commonly used nursery containers are relatively shallow and limit tap root growth; consequently, species relying on the early development of a long tap root to escape drought, such as those of the *Quercus* genus, might need to be cultivated in deep containers. The aim of this paper was to compare the morphological and physiological characteristics of *Quercus suber* L. seedlings cultivated in shallow containers (CCS-18, depth 18 cm) with seedlings cultivated in deep containers (CCL-30, depth 30 cm). Both container types used were made of high-density polyethylene, cylindrical in shape, open-bottomed, with a diameter of 5 cm, two kinds of vertical ribs on the inside wall showing a cultivation density of 318 seedlings/m^2. At the end of nursery culture, the seedlings cultivated in the CCL-30 deep container presented a longer tap root, higher shoot and root biomass and higher Dickson Quality Index (DQI). Moreover, the CCL-30 seedlings showed a higher root growth capacity (RGC), they reached deep substrate layers faster and they presented higher root hydraulic conductance. These morpho-functional advantages improved the CCL-30 seedling water status, which was expressed by higher stomatal conductance during an imposed drought period.

1. Introduction

In the Mediterranean region, the success of forest restoration actions is frequently related to the annual rainfall, soil type and seedling quality (Tsakaldimi et al., 2005; Cortina et al., 2006). Planting seedlings in drylands and degraded soils is often discouraging because of high mortality rates and poor growth (Vilagrosa et al., 1996; Cortina et al., 2004). On the other hand, direct seeding is generally not successful due to water limitations and animal predation (Leyva and Fernández-Alés, 1998; Merouani et al., 2001). Thus, the use of seedlings produced in containers or forest trays is the most common technique for introducing native species in dry and semiarid ecosystems (Cortina et al., 2004).

Forest restoration through the planting of resprouting shrubs and trees on degraded lands is recommended to increase the resilience of Mediterranean degraded ecosystems (Vallejo et al., 2000; Vilagrosa et al., 2003a). Forest restoration is a complex technique that covers various fields of activity, such as species selection, nursery culture and soil preparation among others. In this context, the characteristics of the nursery culture, and particularly the container type, are among the main factors to consider in the production of seedlings of quality; thus, the container utilized should be according to morpho-functional characteristics of the species. Moreover, plant species show different root system development patterns due to different biotic and abiotic factors (Cairns et al., 1997), and abiotic factors can often be critical in the plantation establishment phase. Therefore, nursery cultivation should try to favour the particular root development strategy of a given species in order to optimise its potential for establishment in harsh field conditions.

Container design determines the morphological and physiological characteristics of seedlings, mainly in terms of their root systems (Landis et al., 1990; Aphalo and Rikala, 2003; Domínguez-Lerena et al., 2006). The literature offers frequent reports on the

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effects of container volume and seedling density (Howell and Harrington, 2004; South et al., 2005; Tsakaldimi et al., 2005; Domínguez-Lerena et al., 2006), but very few articles deal with the effects of container depth (Chirino et al., 2005; Pemán et al., 2006). Container depth can determine root system growth and tap root length, and thus it can modify soil colonization in deep soil horizons. Growth of new roots out of the root plug for deep soil colonization is a critical factor for seedling survival, especially under Mediterranean climate, where root system size and distribution, root–soil contact, and root hydraulic conductivity can affect the capacity of the seedling to take up water after outplanting (Simpson and Ritchie, 1997; Grossnickle, 2005). On the other hand, new advances in container design have been carried out in the last decades. In general, forest trays commonly used in reforestation programs in subhumid, dry and semiarid Mediterranean ecosystems have cell volumes between 250 and 400 cm^3, and a maximum depth of 18 cm (Peñuelas and Ocaña, 1996). Containers and forest trays with depths close to 30 cm have very large volumes (more than 1000 cm^3), which are necessary in these cases to prolong the culture time (Howell and Harrington, 2004).

Cork oak (*Quercus suber* L.) is a typical resprouting Mediterranean species of great interest for restoration in fire-prone ecosystems (Pausas, 2004; Vallejo et al., 2006; WWF, 2006). This evergreen oak develops a strong tap root during the nursery culture period (Tsakaldimi et al., 2005; Chirino et al., 2005). The upper part of the tap root (about 10–15 cm) is considered a lignotuber, a specialized structure with dormant buds that have resprouting capacity after perturbations (Verdaguer et al., 2001; Pascual et al., 2002). The true root system in this species starts under this lignotuber structure. Consequently, the use of shallow containers with a maximum depth of 18 cm for cork oak cultures will limit the seedling capacity to develop an adequate root system.

The hypothesis of this work was that the root system of cork oak seedlings cultivated in deep containers would develop a long tap root which could quickly reach deep soil horizons in the field after outplanting and thus have a higher probability of finding some soil moisture at the onset of the dry season. This pattern of growth would imitate the development strategy of this species for establishment in natural conditions. A seedling with a long tap root that colonises deep soil layers would have an adequate morphology and biomass distribution for this species, and this should improve its physiological response to water stress conditions. To test this hypothesis, the aim of this paper was to compare the morphological and physiological characteristics of *Q. suber* L. seedlings cultivated in shallow containers (depth of 18 cm), with respect to seedlings cultivated in deep containers (depth of 30 cm). For this purpose, we analyze biomass fractions, root system development and its capacity for water transport and the responses of seedlings to imposed drought conditions.

2. Materials and methods

2.1. Container design and nursery culture

Cork oak (*Q. suber* L.) seedlings were grown for a 1-year period in two kinds of containers manufactured by CETAP-Antonio Matos Lda. (Forestry Containers Manufacturer Company, Espinho, Portugal). The shallow container (CCS-18), with a depth of 18 cm and a volume of 353 cm^3, represented the container commonly used for cork oak culture in the nursery, while the deep container (CCL-30), with a depth of 30 cm and a volume of 589 cm^3, constituted a technological innovation (Fig. 1). Both container types used were made of high-density polyethylene, cylindrical in shape, open-bottomed, with a diameter of 5 cm and show a cultivation density of 318 seedlings/m^2. Inside, these containers have two kinds of vertical ribs (smaller section and larger section) to prevent root spiraling. At the bottom of each, there is a 3 cm-wide net to favour air root pruning. The CCL-30 deep container is patented by CETAP-Antonio Matos Lda. (No. ref. 9976. Boletim Propiedade Industrial no. 11-2004, Portugal).

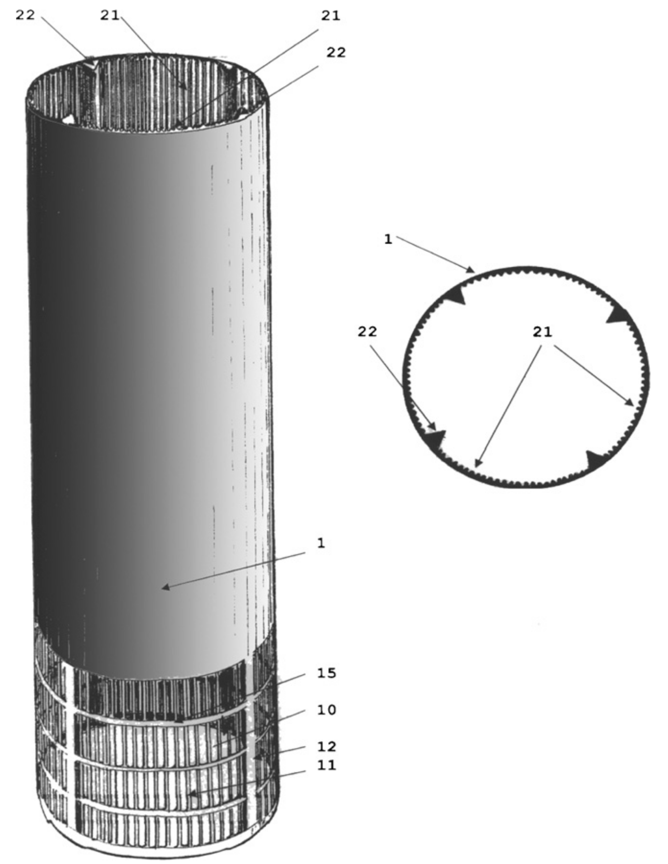

Fig. 1. Drawing of CCL-30 deep container (front and cross section). Legend—1: cylindrical shape, 21: vertical ribs of smaller section, 22: vertical ribs of greater section, 10: network in the lower part composed by horizontal hoops (15) and extensions of vertical ribs (11 and 12).

The culture activity was carried out at the Public Nursery of Santa Faz, Alicante Forest Service, Spain (38°23′N, 0°26′W; 80 m a.s.l.; 240° SW facing) with a mean annual rainfall of 353 mm and a mean annual temperature of 18 °C (Pérez Cueva, 1994). Cork oak acorns from the Espadàn mountain range (Castellón, Spain) were supplied by the Regional Government Forest Service (Banc de Llavors, Quart de Poblet) and were seeded in April 2004. The substrate was limed peat and coconut peat (1:1, v/v) fertilized with 57 mg NO_3; 69 mg NH_4; 60 mg P; 344 mg K per litre of substrate. Additional slow-release fertilizer (Osmocote plus©, N-P-K: 14-8-14; approximates longevity of 12 months at a mean temperature of 21 °C) was mixed with the substrate at a dose of 1 g/L of substrate. The watering regime was moderate in accord with the seedling water demand and to avoid excessive seedling development (15 mm in autumn, winter and spring, applied 2 days/week, and 25 mm in summer, applied 3 days/week).

2.2. Seedling morphology and biomass

At the end of nursery culture, at 10 months of cultivation, morphological characterization was carried out. Fourteen seedlings per treatment were randomly sampled, and shoot height (H_s) and root collar diameter (RCD) were measured. Seedlings were cut at the cotyledon insertion point and separated into four fractions: