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# Growth Responses of Chemically Root-pruned Cork Oak Seedlings in the Nursery<sup>1</sup>

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## – Abstract –

Cork oak (*Quercus suber* L.) seedlings of two Iberian provenances (PA-SR and SM-Lp) were grown for one growing season in nontreated containers or in containers treated on their interior surfaces with white exterior latex paint containing 80 g CuCO<sub>3</sub>/liter. Copper carbonate-treated containers effectively prevented root deformation and decreased the amount of circled, kinked and matted roots formed at the container wall-medium interface. Root morphology was altered by the copper coating, so elongation of lateral roots contacting CuCO<sub>3</sub>-treated surfaces was more reduced than that of the tap root (82.7% vs 1.5%). These lateral roots showed higher branching frequency than roots contacting untreated container walls. CuCO<sub>3</sub> treatment decreased root collar diameter, but did not influence seedling height, leaf area and tap root length. No sign of copper toxicity was observed in any seedling treated with CuCO<sub>3</sub>. Provenance had a significant effect on height, root collar diameter, tap root length and root weight per unit length; these results may reflect differences in growth habit of the two provenances. Other growth parameters measured were affected by a copper × provenance interaction. Seedlings of the PA-SR provenance produced less root and plant dry wt when grown in CuCO<sub>3</sub>-treated than in control containers, but shoot:root ratio was not influenced.

Index words: Quercus suber L., root morphology, provenance, container production, reforestation.

#### Significance to the Nursery Industry

Copper compounds applied to the interior surfaces of containers have been shown to be effective in controlling root deformation and increasing lateral branching of woody plants during production. Results of this study indicate that a copper carbonate (CuCO<sub>2</sub>) coating was effective in reducing root circling, matting and kinking of cork oak seedlings. This experiment expands the list of species for which coppertreated containers appear to reduce root deformation without detrimental effects on shoot growth or quality. As shoot growth was not affected by the copper coating, there is not likely to be any significant economic effect. No sign of copper toxicity on above-ground organs was observed in any seedling treated with CuCO<sub>2</sub>. The dose tested (80 g/liter) was enough to prevent root deformation, but as various species exhibit a different dose response, the effective range for chemical root pruning must be tested for each one. The altered and improved root morphology in the treated seedlings suggest that chemical root pruning is an adequate nursery practice to improve the performance of seedlings used for reforestation purposes in xeric sites. Even when seedlings are well planted, the relative reduction in root-soil contact due to transplanting can be a cause of reduced water uptake and transplanting stress. Thus, only when roots resume growth does a seedling recovers its water status, and this appears to occur more rapidly and effectively with copper coating due to a higher presence of root tips. Provenances highly adapted to drought conditions and more responsive to copper coating must be selected.

### Introduction

Since the 1960s, coating interior surfaces of containers with copper salts (CuCO<sub>3</sub> or Cu(OH)<sub>2</sub>, mainly) has been shown to be a viable method of controlling circling roots of many containerised woody species. Among them are mainly conifers (*Pinus taeda* and *P. palustris* (18), *P. pinea* and *P. pinaster* (1)), but also woody landscape species (*Quercus rubra* (2), *Acer rubrum, Ligustrum japonicum, Quercus laurifolia*, etc. (5), *Eucalyptus viminalis, Jacaranda acutifolia* (12), *Rhododendron, Magnolia grandifola*, etc. (6)), tropical species (*Averrhoa carambola, Dimocarpus longan*, etc. (17)), and species used for afforestation (*Acacia holosericea, Eucalyptus camaldulensis*, etc. (10)).

Successful afforestation relies on selecting the appropriate species, planting adequately conditioned seedlings and employing efficient establishment techniques. Well developed and structured root systems with numerous lateral roots are an essential attribute of high quality seedlings (20). This attribute is even more important in Mediterranean sites, where rainfall varies yearly and is unpredictable. Under such conditions seedlings must develop an adequate root system, capable of reaching the deep water table (25). After outplanting, seedlings must show the ability to quickly reestablish soilroot contact and to rapidly produce new roots. The best way to achieve this is with a root system large enough at the time of outplanting to supply water in amounts that cover transpiration loss (8), to provide physical support, and with sufficient laterals to resume growth.

Seedlings often have root morphologies drastically different from those grown from seed in place (23). Container grown seedlings frequently have long lateral roots directed downward along the container wall until air-pruned at the drainage hole, and a restricted root system which is shaped by the container, while naturally regenerated seedlings show abundant lateral roots from the upper portions of the plug (26). Root system malformation from container production leads to increased mortality or poor initial transplant growth and mechanical instability (17).

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One succesful strategy for reducing or eliminating these root distortions produced in container-grown seedlings is the use of copper salts. These salts are found to increase root system density by inhibiting root elongation and stimulating lateral root branching (3). The elongation of the lateral roots ceases when their growing tips come into contact with the copper coating on the interior of the containers (10). Secondary lateral root growth develops from further back and when these laterals reach the container wall they are also inhibited by the copper. Overall root distribution is more efficient and natural than in untreated seedlings, and the young feeder roots tend to be located within the container media volume rather than on the outside; this leads to more effective utilisation of water and nutrients (14) and a quicker establishment after outplanting, when the arrested root tips resume growth (5).

Little is known about the response to copper coating of Mediterranean containerised species used for reforestation purposes. Among them is cork oak (*Quercus suber* L.), a xerophytic and endemic species of the western Mediterranean area with 0.5 million hectare of coverage in Spain (19). Cork oak has a special relevance in the forestry and rural economy of the Iberian Peninsula and has been widely used in the reforestation of former agricultural land. The objectives of this study were to test the effectiveness of CuCO<sub>3</sub>-treated containers in altering root distribution patterns of two different provenances of cork oak, a coarse-rooted species, during container production. The effect of the copper compound on other morphological parameters was also investigated.

#### **Materials and Methods**

On December 10, 1996, 200 pre-germinated acorns of *Quercus suber* L. from two Iberian provenances, Parque de los Alcornocales-Serranía de Ronda (PA-SR) and Sierra Morena occidental- Llanuras pacenses (SM-Lp), were sown in Forest Pot® block containers (truncated square pyramid cell, dimensions of cell:  $4.6 \times 4.8 \times 18.0$  cm  $(1.8 \times 1.9 \times 7.1 \text{ in})$ , 300cc (18.3 cubic in); 50 cells per container, 390 cell/m<sup>2</sup> (36.2 cell/ft<sup>2</sup>)), containing a medium of Spaghnum peat moss:vermiculite (3:1 by v/v). Provenance details are presented in Table 1.

Before seeding, half of the containers used for each provenance had the interior surfaces coated with 80 g CuCO<sub>3</sub>/ liter (2.67 oz/qt) of flat white acrylic latex exterior house paint. The other containers were left unpainted as controls. A  $2 \times 2$  factorial experiment that included all combinations of provenance (PA-SR/SM-Lp) and +/– copper coating (+Cu/ –Cu) was set up. Containers were placed in the greenhouse until April 15, 1997, when the seedlings were grown outdoors (40°25'N, 3°44'W, 590 m (1920 ft) elevation). Plants were completely randomized under the following greenhouse conditions: 30/10C (86/50F) day/night temperature, under natural photoperiod, 450 to 600 µmol/m<sup>2</sup>·s of light intensity at midday, and watered to field capacity as needed. Conditions outdoors from mid-April to mid-October were: 18.9C (66F) average temperature (26.9 to 10.8C (80.4–51.4F)), 1000 to 1500 µmol/m<sup>2</sup>·s of light intensity at midday, 157.3 mm (61.9 in) of rainfall and watered as needed.

At the completion of the growing season (October 10, 1997) stem height and diameter at ground level of all seedlings were recorded. Components of height growth (mean length and number of stem units) were measured and related to shoot height by the following equation:

$$SH = NSU \times MSUL$$

where SH is total shoot height (cm), NSU is number of stem units and MSUL is mean stem unit length (cm).

The exteriors of the root ball were examined visually for circling or matting of roots. Root ratings were recorded for each seedling. The following rating scale was used (4): 0 =many matted, circled, and kinked roots present at the container wall-medium interface; 1 = several roots elongating > 1 cm (0.4 in) after contacting a container surface; 2 = noescaped lateral roots, but taproot(s) continuing to elongate more slowly after contacting a container surface; 3 = no escaped lateral roots and taproot(s) ceasing to elongate after contacting a container surface. After harvest, the following measurements for individual seedlings were recorded: leaf area (in cm<sup>2</sup>, with the use of a Delta-T image analyzer), root volume (in cc, by displacement of the cleaned root system dipped into a vessel of water standing on a top-loading balance (7)), length of tap root and lateral roots (in cm), root density (estimated by dividing the average dry weight by the average root length, in g/cm) and dry weight (shoot, root, leaves and plant; in g; seedlings were oven dried to constant weight at 70C (158F)). Shoot to root dry weight ratio (S/R, g/g) and sturdiness quotient (cm shoot height/mm root collar diameter) were computed.

Data were tested by analysis of variance (22) in a two provenance  $\times$  two CuCO<sub>3</sub> factorial treatment arrangement in a completely randomized design. Mean separation of parameters was tested using Tukey's Multiple Range test for significance. When the interaction copper  $\times$  provenance was significant, each copper  $\times$  provenance level was analized by t-Student test.

#### **Results and Discussion**

Copper carbonate-treated containers significantly reduced circled, kinked and matted roots formed at the container wallmedium interfaces in the two provenances. Cork oak roots contacting treated surfaces were nearly completely inhibited (root rating: 2.7, +Cu vs 0.0, -Cu). Previous experiments

Table 1. Details of the two Iberian cork oak provenances used in the study in Spain.

Provenance	Name	Coordinates	Elevation (m)	Annual rainfall (mm)	Annual avg temp (C)	Soil type
Parque de los Alcornocales-Serranía de Ronda	PA-SR	36°34'16" N 5°28'00" W	600	1,500	18	Eutric cambisol
Sierra Morena occidental-Llanuras pacenses	SM-Lp	38°22'31" N 6°50'52" W	500	666	16	Distric and eutric cambisol

Morphological parameter	Container interio	r walls treatment	Provenance	
	+Cu	-Cu	PA-SR	SM-Lp
Height (cm)	22.84a <sup>y</sup>	22.52a	26.39a	18.71b
No. of stem units	23.7a	24.1a	26.0a	21.8b
Length of stem units (cm)	0.92a	0.94a	1.00a	0.85b
Root collar diameter (mm)	4.99b	5.68a	5.80a	4.88b
Tap root length (cm)	16.77a	17.02a	16.39b	17.40a
Lateral root length (cm)	2.55b	14.73a	8.58a	6.80a
Root weight per unit length (g/cm)	0.41a	0.43a	0.45a	0.39b

<sup>z</sup>Means are the average of 15 seedlings per treatment.

<sup>y</sup>Mean separation for main effects within columns by ANOVA at P < 0.05.

with other coarse-rooted species (*Quercus acutissima*, *Q. falcata*, *Q. alba*, *Q. stellata*) using  $Cu(OH)_2$ -treated containers showed similar results (4), so roots contacting treated surfaces were nearly completely inhibited, although total control was not complete. Taproots were elongated, but lateral root deformation was less in CuCO<sub>3</sub>-treated containers than in non-treated containers (Table 2). Beeson and Newton (5) also found incomplete control of deflected roots on some species using Cu(OH)<sub>2</sub>-treated containers. In general, root systems of seedlings grown in CuCO<sub>3</sub>-treated containers appeared more fibrous than those from non-treated containers, with numerous higher order lateral roots originating from several cm behind the inhibited roots.

Visual observations indicated cork oak root systems were more branched and evenly distributed throughout the medium in  $CuCO_3$ -treated containers than in controls. Similar results were reported by Arnold and Struve (2) in red oak (*Quercus rubra*).

While no sign of copper toxicity on above-ground organs (leaf chlorosis, senescent leaves and necrotic stems) was observed in any seedling treated with  $CuCO_3$ , the roots that contacted treated surfaces exhibited a mild copper toxicity confined to the apical few cm of the root tips (2). This toxicity was so low that the root thickening and the increase in the root dry wt per unit length, frequently observed in forest species grown in culture solutions containing heavy metals (1), was not shown (Table 2); but the toxicity was enough to affect the morphology of the root system by decreasing elongation of laterals more than that of the tap root (82.7% vs 1.5%, Table 2).

Copper treated containers effectively controlled (P < 0.05) root collar diameter and lateral root length (Table 2). All seedlings had similar height (22.6 cm (8.9 in)), leaf area (90.0 cm<sup>2</sup> (14.0 in<sup>2</sup>)) and tap root length (16.9 cm (6.7 in)). The rest of the morphological parameters were affected by a copper × provenance interaction (P < 0.005). Root volume, root system dry wt and plant dry wt were significantly greater for control seedlings; while sturdiness quotient was greater for copper treated seedlings, but only in the PA-SR provenance (Table 3). Leaf dry wt, shoot dry wt and S/R were greater for copper treated seedlings, but only in the SM-Lp provenance (Table 3). Height, root collar diameter, tap root length and root weight per unit length showed a provenance effect (Table 2); these results may reflect differences in growth habit of the two provenances.

Our results showing chemical root pruning with CuCO<sub>3</sub> inhibited root-collar diameter in the nursery, agree with those

of Dunn et al. (10) in five native Australian tree species, and Brass et al. (6) with different woody landscape plants. However, results of McDonald et al. (18) with pine seedlings, and Ruter (21) with four vigorous-rooted tree species, indicated that, in some instances, root-collar diameter was increased by copper treatment.

Root dry wt reduction in the PA-SR provenance in treated containers also affected total seedling dry wt, but not shoot:root ratio (Table 3). A reduction in root system dry wt from copper application has been reported for other species (5, 24). Meanwhile, root:shoot dry wt ratio was reduced in the SM-Lp provenance when seedlings were grown in  $CuCO_3$ -treated containers, while shoot growth was equal to the non-treated seedlings. Similar results with other tree species have been found (2, 3, 13).

Studies have shown copper to have positive (3, 18), negative (12) or no effects (9, 24, 26) on plant height. Height growth in cork oak is the result of the interaction between number and length of stem units, as has been described elsewhere (15). Our results showed no increase in the length or number of the stem units; which means that height was not affected by the copper treatment (Table 2). On the contrary, shoot growth showed a response to the provenance: PA-SR provenance had greater shoot growth than SM-Lp provenance

Table 3. Cork oak seedling morphological parameters affected by a copper  $\times$  provenance interaction.

Evaluation parameter	Copper	Provenance		
		PA-SR	SM-Lp	
Root volume (cc)	+	10.33aB	10.60aA <sup>z</sup>	
. ,	_	12.73aA	10.07bA	
Root dry wt (g)	+	6.62aB	7.24aA	
	_	8.21aA	6.10bA	
Leave dry wt (g)	+	1.00aA	1.13aA	
•	_	1.17aA	0.88bB	
Shoot dry wt (g)	+	1.98aA	2.15aA	
	_	2.32aA	1.63bB	
Plant dry wt (g)	+	8.60aB	9.37aA	
•	_	10.54aA	7.38bA	
S/R (g/g)	+	1.14aA	1.27aA	
	_	1.31aA	1.00bB	
Sturdiness quotient (cm/mm)	+	0.54aA	0.39bA	
,	_	0.40aB	0.40aA	

<sup>z</sup>Means separation within columns (lower case) and rows (upper case) by t-test, P < 0.05.

due to longer stem units and a higher proportion of them. Milder temperatures joined to more abundant rainfall (Table 1), absence of frosts, and major proximity to the sea in the more southern provenance (PA-SR provenance) lead to a longer growing season, which permits higher seedling growth (Table 3), and in particular higher shoot elongation (Table 2). This southern provenance is adapted to warmer fall and winter conditions and continue growth under shorter photoperiods than the SM-Lp provenance. Thus PA-SR provenance maintained higher shoot activity. Other species (*Pinus pinaster, P. ponderosa, Abies magnifica, A. concolor*) showed a similar increase on seedling height growth due to lower elevation and more rainfall at the seed source site (11, 15, 16).

The study clearly showed the CuCO<sub>2</sub> treatment stopped the growth of many lateral roots of cork oak at the wall interface, and that more higher order laterals formed in response to repeated pruning of tips. Thus cork oak, important species for reforestation purposes in the Mediterranean basin, can be added to the list of plants whose root growth can be controlled in CuCO<sub>2</sub>-treated containers without drastically affecting biomass production. Control of root development of woody plants in containers is potentially important because plants with temporarily inhibited first-order laterals should develop a well-distributed root system nearer the soil surface after planting. Moreover, the elimination of circling or downward-deflected roots in the container increases the stability and growth rate of planted trees once they are established, and, therefore, enhances establishment of transplanted cork oak. Various studies have shown how the differences in root morphology between CuCO<sub>2</sub>-treated seedlings and control seedlings that were observed in the nursery, persisted in the field (2, 10, 17, 26). Poor root morphology at the time of planting caused permanent and serious root deformation in the untreated seedlings.

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