

Effect of chemical root pruning on stem growth, root morphology and field performance of the Mediterranean pine *Pinus halepensis* Mill.

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Abstract

The objective of this study was to determine the influence of chemical root pruning on the nursery growth of (1 +0) *Pinus halepensis* seedlings and assess their outplanting performance. The experiment was conducted in an open-air nursery and the seedlings were grown in Quick-pots coated with 0, 0.0083 and 0.033 kg/l $\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$. At the end of the nursery growth period, seedling above-ground and below-ground morphological characteristics and nutritional status were assessed. Following, the seedlings were planted in the field and survival and growth were recorded for two growing seasons after planting. The most significant effect of chemical root pruning was on seedling morphology. Increased copper concentration on the container walls led to seedlings with significantly greater height, diameter, shoot and root biomass and quality index. However, there were no differences among treatments for field survival which was high, in all treatments, 2 years after planting. Seedlings field height was not affected by copper treatment, in contrast to the diameter and stem volume which were significantly greater in the seedlings subjected to higher copper concentration treatment.

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1. Introduction

Pinus halepensis has been widely used in reforestation and landscape restoration along the Mediterranean basin. In Greece, plantations of *P. halepensis* have been established on a great scale during the last century. The aims of these plantations were mainly to increase forest productivity, protect watersheds, conserve landscapes along the Mediterranean coastal areas, and also to secure species existence in the cases where its natural regeneration was not successful (Tsitsoni, 1997; Pausas et al., 2004; Zagas et al., 2004). However, the techniques for a successful establishment of this species are not fully developed, especially in the case of adverse site conditions such as those found in Mediterranean climate (long summer dry period).

The primary goal of applying suitable nursery cultural practices is to produce planting stock capable of tolerating stresses. In the Mediterranean regions the stresses are caused by drought, heat, intense radiation during the summer (Oliveira

and Penuelas, 2000) and mechanical damage during transportation and field planting as well.

Well-developed and well-structured root systems with numerous laterals roots are one of the most essential attributes of high quality seedlings (Aldhus, 1994). Moreover, the form of root development of seedlings largely affects the plantation performance (Sutton, 1980; Burdett et al., 1983). Densely matted, kinked and downward-deflected surface roots are common in vigorous plant species grown in containers (Ruter, 1994) or in plants held too long in a given size container. Transplanting container grown stock with undesirable root form results in poor establishment (Struve, 1993) and reduced shoot growth (Arnold et al., 1993).

Root pruning, before transplanting, is often required to correct root malformation of container grown plants. Positive results have been achieved by coating the interior walls of the containers with various copper compounds. Copper inhibits root tip growth, increases secondary root branching, reduces root circling, tends to reduce root to shoot ratio and promotes root regeneration after transplanting (Arnold and Struve, 1993; Arduini et al., 1995; Crawford, 1997).

Most container types allow lateral roots to turn downwards, resulting in the accumulation of most of the active growing tips

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at the base of the plug. Copper-treated containers prevent lateral roots from turning downward, effectively “pruning” them at the walls. The lateral root egress from the upper part of the plug increases the seedling stability and the access of the plants to the nutrients nearer the field soil surface, resulting in high survival and growth (Burdett et al., 1983; Wenny et al., 1988). Besides the number of laterals, the chemical root pruning with a copper compound increases the biomass and root collar diameter of *Pinus* seedlings (Romero et al., 1986; Arduini et al., 1995).

Even though many studies have shown the benefit of producing seedlings in copper-treated containers, no research has examined the growth of *P. halepensis* seedlings, in copper-coated containers and to our knowledge, little is known about the effect of chemical root pruning on the reforestation success in the Mediterranean region.

The objective of this study was (i) to determine the influence of copper-treated containers on stem growth, root morphology and nutritional status of (1 + 0) *P. halepensis* seedlings and (ii) to assess the survival and the subsequent growth of these seedlings during the 2 years after outplanting in the field.

2. Materials and methods

2.1. Nursery phase

2.1.1. Experimental treatments

The experiment was conducted in an open-air nursery of the Forest Service (N. Chalkidona, North Greece). The containers used for seedling production were the Quick pots T18; these are plastic containers with 12 cavities each. The cavities are 18 cm deep, with top opening 8 cm and volume 650 cm³. Each cavity has four vertical ribs in the interior surfaces and an open crossed base.

The interior surfaces of 72 cavities were coated with basic cupric carbonate compound (CuCO₃·Cu(OH)₂) 0.0083 kg/l, in a water-based paint solution (distilled water and neutral white emulsion) (treatment T1) and another 72 cavities were coated with 0.033 kg/l (treatment T2). The remaining 72 cavities were left uncoated, as a control.

The cavities were filled with Lithuanian sphagnum peat of medium structure and coarse perlite (3:1, v/v). The potting medium was fertilized with 1.3 kg mixed fertilizer (N:P:K 15:30:15 + micronutrients), 0.6 kg potassium sulfate, 1.0 kg super-phosphate (0-20-0), 0.4 kg magnesium sulfate and 2 kg lime (CaO) per m³ peat. Finally, the total amount of N, P and K per seedling was 0.09 g for N, 0.13 g for P and 0.20 g for K.

Seeds of *P. halepensis* were sown in containers on 15 March. The three treatments were arranged in a randomized complete block design with three replications. There were 24 seedlings per treatment per replication (total 216 seedlings). All seedlings were irrigated with an overhead irrigation system, as needed. During the growth period, seedlings were watered with ammonium nitrate solution (2.5 g/l water) every 15 days. In early September, seedlings were subjected to one-cycle moderate water stress (– 1.5 MPa) in order to prevent their late season shoot elongation

(lammas growth), induce dormancy and promote terminal buds set (Duryea, 1984). The water stress was monitored by measuring the leaf predawn water potential of the seedlings using a portable Skye Plant moisture system (pressure chamber technique). After the drought, the seedlings well watered and then fertilized with 1.5 g potassium sulfate, 2.0 g super-phosphate and 0.15 g ammonium nitrate per liter water, every 20 days.

At the end of the growth period, the pH of the growing media was determined in three random samples of each treatment and it was found 7.1 for the control treatment and 6.5 and 6.4 for the T1 and T2 treatments, respectively.

2.1.2. Growth measurements and destructive sampling

At the end of the growth period in the nursery (in November) the shoot height (h), the diameter (d) (0.5 cm above root collar) of all seedlings were measured with an accuracy of 0.1 cm and 0.1 mm, respectively. Based on the above measurements the sturdiness index (h/d) and the stem volume were calculated for each seedling. The stem volume was calculated using the equation for the volume of a cone (Kooistra and Bakker, 2002; Oliet et al., 2004):

$$V = \frac{\pi \times h \times d^2}{12}.$$

Twelve randomly selected seedlings per treatment (four seedlings x three replications) were collected for biomass and seedling quality index (Dickson et al., 1960) estimations as well as nutrient analysis. From these selected seedlings, five random root samples per treatment were chosen for the root morphology estimations.

The root system was separated from the soil, under a gentle water jet, using a sieve to collect any root fragments detached from the system. Then, each root system was put into a glass box and covered with a white plastic sheet to keep it in a fixed position and improve the contrast of the root image. The box was placed on a scanner (Hewlett Packard, ScanJet 6100C) connected to a computer and an image analysis system (DT-Scan, Delta T-Devices) was used to determine the total root length, the root surface area, the total root volume (Fitter et al., 1991; Barnett and McGilvray, 2001). The number of laterals (d > 0.2 mm) was recorded by the scanned root images.

For biomass measurements the seedlings were divided into two parts: shoot (stem + needles) and root system. Both parts were oven-dried at 70 °C for 48 h and weighed. The root to shoot ratio was calculated by the root and shoot dry weights (Thompson, 1985). After biomass estimations, the same sampled shoots and roots of each treatment were subjected to nutrient analysis. All samples were pooled at a mill with a sieve 40 mesh. Total N was determined by Kjeldahl method. Total concentrations of P, K, Ca, Mg and Cu were determined after dry ashing at 500 °C for 5 h. The ash was diluted by HCl 1:1 (v/v) and filtered. Then, P was determined by visible spectrophotometry and molybdenum blue method and total K, Ca, Mg and Cu were determined by atomic absorption spectrophotometry (Perkin-Elmer A Analyst 300) (Faithfull, 2002).

2.2. Field experiment

In early December, 8-month-old seedlings were outplanted to the field in ‘Kassandra’ Peninsula, Chalkidiki (North Greece), which is located 80 km south-east of Thessaloniki at 25°30'E and 40°N. The climate of the area is Mediterranean type with mild winters and dry hot summers. The mean annual rainfall is 581 mm, while the mean annual air temperature is 16.3 °C and the mean maximum air temperature of the warmest month (July) is 30.1 °C. The dry period begins in the middle of April and lasts until the middle of September (Tsitsoni, 1997; Tsakaldimi, 2001). The vegetation of the area belongs to Quercetalia illicis floristic zone. The soil of the area, where the experiment was conducted, is characterized as deep, sandy–clay loam, neutral to moderate alkaline and rich in organic matter at the surface horizons (Tsakaldimi, 2001).

Twenty seedlings per treatment per replication were planted in a randomized complete block design with three replications. The seedlings were spaced 2 m apart. Height and diameter growth of each seedling were assessed (with an accuracy of 0.1 cm and 0.1 mm, respectively) at the end of the growth period for 2 successive years after planting. Based on these measurements, the stem volume growth was calculated. Furthermore, the survival was recorded for each seedling for 2 successive years after planting and within each year for two periods (before and after summer).

2.3. Statistical analysis

All statistics were calculated with SPSS software. Distribution was tested for normality by Kolmogorov–Smirnov criterion and the homogeneity of variances was tested by Levene’s test. Significant differences among treatment means were tested using analysis of variance (one-way ANOVA). Wherever treatment effects were significant the Duncan’s Multiple Range Test was carried out to compare the means (Snedecor and Cochran, 1988).

Diagnostic interpretations of plant growth and nutrient status employed vector analysis (Timmer and Armstrong, 1989). The

nutrient and weight values for the control were normalized to 100 in order to facilitate comparisons with a common base (Haase and Rose, 1995). The magnitude and direction of the vectors are used to interpret the effect of treatment (Koricheva, 1999; Proe et al., 1999). Pearson’s correlation coefficient was used to examine the relationship between initial seedling characteristics and seedling size in the field.

3. Results

3.1. Nursery performance

Seedlings in copper-treated containers were significantly taller and had greater diameter and stem volume than control seedlings (Table 1). However, the higher values of the seedlings height, diameter and stem volume (29.6 cm, 4.1 mm and 1.4 cm³, respectively) were obtained in the treatment with the higher copper concentration (T₂). Neither copper treatments affected the h/d ratio. The copper-treated containers greatly affected seedlings biomass and as a consequence the seedlings quality index (Q.I.). Shoot and root dry weights and Q.I. of the seedlings from coated containers were significantly greater than that of the control seedlings. No significant differences among treatments ($P > 0.05$) were found for root to shoot ratios, which fluctuated between 0.41 and 0.49.

The differences in root surface area, volume and length were not statistically significant. The root surface area ranged from 162.7 to 189.5 cm², the root volume from 3.4 to 5.2 cm³ and the total root length from 1009.4 to 1135.2 cm. However, seedlings subjected to the higher copper concentration treatment (T₂) had a more fibrous root system with significantly greater number of laterals (186.8) than control seedlings (153.8) (Table 1).

The results from nutrient analysis (Table 2) and the interpretation of vector nomogram for seedlings shoot (Fig. 1) would suggest that N, P and K, in all cases, were available for sufficiency uptake, because their vectors matched direction “B” in the diagnostic scheme of Timmer and Armstrong (1987), indicating that growth differences between

Table 1
Effects of chemical root pruning on seedling above-ground growth, biomass and root morphology at the nursery phase

Seedling characteristics	Treatments		
	Control	T ₁	T ₂
Shoot height (<i>h</i>) (cm)	21.1 (0.76) b	29.0 (0.76) a	29.6 (0.81) a
Diameter (<i>d</i>) (mm)	3.0 (0.07) c	3.9 (0.07) b	4.1 (0.07) a
<i>h/d</i>	7.1 (0.20) ns	7.4 (0.20) ns	7.2 (0.17) ns
Stem volume (cm ³)	0.5 (0.37) c	1.2 (0.58) b	1.4 (0.73) a
Shoot dry weight (g)	2.4 (0.18) b	3.7 (0.32) a	4.1 (0.36) a
Root dry weight (g)	1.2 (0.13) b	1.5 (0.12) ab	1.7 (0.18) a
Total dry weight (g)	3.5 (0.29) b	5.2 (0.38) a	5.8 (0.50) a
Root to shoot (R/S)	0.49 (0.03) ns	0.43 (0.04) ns	0.41 (0.03) ns
Quality index (Q.I.)	0.34 (0.03) b	0.48 (0.03) a	0.59 (0.07) a
Root surface area (cm ²)	162.7 (19.06) ns	189.5 (26.29) ns	186.7 (29.36) ns
Root volume (cm ³)	3.4 (0.50) ns	4.7 (0.60) ns	5.2 (0.96) ns
Total root length (cm)	1009.4 (143.6) ns	1135.2 (183.75) ns	1024.7 (127.84) ns
Number of lateral roots	153.8 (24.65) b	180 (23.12) ab	186.8 (19.34) a

Values are means ± standard error (in parenthesis). Within a row, means followed by different letters are significantly different ($P < 0.05$, $n = 45$ for above-ground growth characteristics, $n = 12$ for biomass and $n = 5$ for root parameters). ns, non-significant differences ($P > 0.05$).

Table 2
Mineral nutrient concentrations in the shoots and roots of *Pinus halepensis* seedlings, in the three treatments, at the end of the growth period in the nursery

Treatment	N (%)	P (mg/g)	Mg (mg/g)	K (mg/g)	Ca (mg/g)	Cu (ppm)
Shoot						
Control	0.91 (0.05)	1.12 (0.12)	2.63 (0.41) a	6.53 (0.13)	11.2 (0.47)	10.3 (4.99)
T ₁	0.89 (0.16)	1.25 (0.24)	1.69 (0.04) b	6.27 (0.39)	4.81 (0.23)	16.4 (4.85)
T ₂	0.85 (0.10)	1.38 (0.17)	1.59 (0.15) b	6.98 (0.57)	5.90 (0.41)	24.0 (3.00)
Root						
Control	0.71 (0.03)	0.80 (0.05)	1.78 (0.16)	3.16 (0.14)	7.64 (0.23) a	11.7 (4.83) b
T ₁	0.61 (0.09)	0.87 (0.16)	1.43 (0.18)	2.70 (0.46)	5.30 (0.27) b	52.0 (4.00) b
T ₂	0.70 (0.08)	0.80 (0.24)	1.66 (0.11)	3.70 (0.23)	5.36 (0.52) b	168.3 (29.5) a

Values are means \pm standard error (in parenthesis). Means followed by different letters, within a column, are significantly different ($P < 0.05$).

treatments occurred without apparent limitation of these micronutrients. Although similar in orientation, vector size varied between copper treatments reflecting a nutritional response to copper concentration. The vectors of Mg matched direction "A", indicating that Mg was diluted but remained non-limiting. However, Ca content, mainly in T₁ treatment, declined suggesting that Ca concentration in the control was not optimal. Still, it is important that the roots of the seedlings which were subjected to high copper concentration treatment (T₂) presented significantly greater Cu concentration (168.3 ppm) in relation to the roots of the T₁ (52.0 ppm) and control treatment (11.7 ppm) (Table 2). However, this did not result in comparable shoot Cu concentrations.

3.2. Field performance

Survival in the field was high 2 years after outplanting and it did not show any significant difference among treatments ($P > 0.05$). At the end of the first growth period and after a hot-dry summer period, the seedlings survival ranged from 96.7% (control seedlings) to 98.3% (copper-treated seedlings); however after the second growth period the survival of the control seedlings was slightly reduced to 95.0% while the survival of the copper-treated seedlings was remained unaffected (98.3%).

One year after outplanting, the seedlings subjected to the higher copper concentration treatment (T₂) had significantly

greater height and diameter than the ones from T₁ and control treatment, while in the second year, they were bigger only in diameter (Fig. 2). However, during the 2 years after outplanting, neither height growth nor diameter growth were significantly different between seedlings from T₂ and control treatment. On the contrary, the stem volume and its annual growth were always significantly greater in the seedlings subjected to the higher copper concentration treatment (T₂). Pearson's correlate coefficients showed that the initial seedling size had significant ($P = 0.01$) positive but relatively low effect on seedling size 1 year after field planting ($R = 0.35$ for the seedling height, $R = 0.45$ for the seedling root collar diameter and $R = 0.37$ for the stem volume), while strong significant ($P = 0.01$) correlation was found between seedling size at the first year and at the second year after outplanting ($R = 0.82$ for the seedling height, $R = 0.74$ for the seedling root collar diameter and $R = 0.81$ for the stem volume).

4. Discussion

In our study the comparison of *P. halepensis* seedlings from adjacent treated and untreated containers indicated that the copper paint (0.0083 and 0.033 kg/l, basic cupric carbonate) significantly improved nursery seedling shoot height, diameter, stem volume, dry weights and Dickson's quality index. Similar results were reported by Barnett and McGilvray (2001) for *Pinus palustris* and by Aldrete et al. (2002) for *Pinus pseudostrobus*, who found that shoot and root dry weights were greater in copper-treated seedlings. Burdett and Martin (1982), McDonald et al. (1984) and Ruehle (1985) also found that nursery performance of many conifer species was not inhibited by coating nursery containers with CuCO₃ (0.05 kg/l) and in some instances that average seedling shoot height and root collar diameter were improved.

The fact that copper-treated seedlings grew bigger than control seedlings, can be attributed to their rich and well distributed root system, which helped them to uptake nutrients and water sufficiently. Although, copper paint had no significant effect on the root volume, surface area and total length, the root system of copper-pruned (T₂) *P. halepensis* seedlings was found more fibrous with higher order laterals. This could be important for *Pinus* seedlings which usually have large woody taproots that form few laterals and seem unable to develop adventitious roots (Arduini et al., 1995). Our above

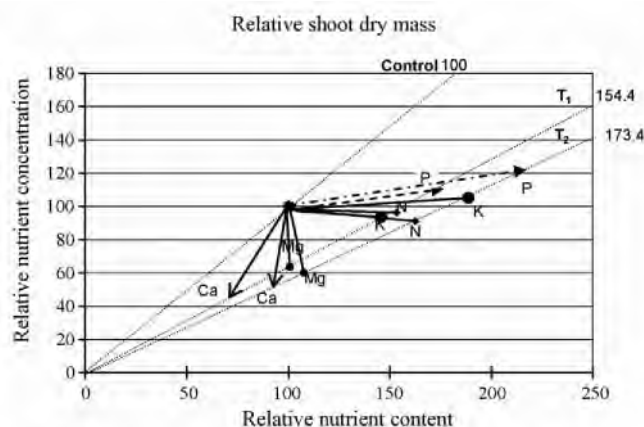


Fig. 1. Vector nomogram for the effects of treatments on shoot nutrients and biomass of seedlings.

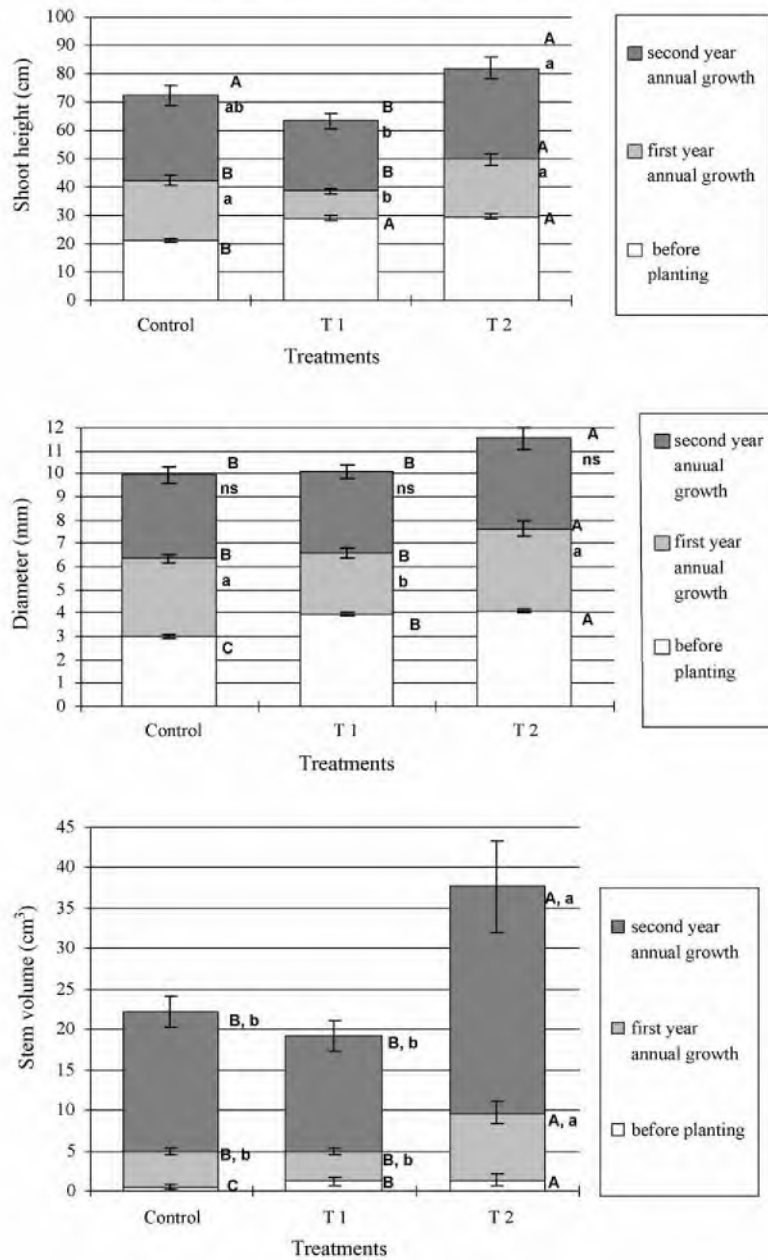


Fig. 2. Effect of chemical root pruning on *P. halepensis* shoot height, root collar diameter and stem volume before planting and 2 successive years after field planting. Values are means \pm standard errors. For the same growth period, means followed by different letter are significantly different ($P < 0.05$). Differences in final height, diameter and stem volume are indicated by capital letters, while differences in annual height, diameter and stem volume growth are indicated by lower case letters.

observation agrees with the earlier studies on other forest species (Burdett, 1981; Romero et al., 1986; Arnold and Struve, 1993; Barnett and McGilvray, 2001; Aldrete et al., 2002). A possible explanation for the lack of a significant increment of the root quantitative parameters is that the selected concentrations of the copper were not or scarcely toxic for the *P. halepensis* seedlings. This may result either from the low acidity of the growth medium or the lime amendment which both affected the copper bioavailability (Basta et al., 1993; Poschenrieder et al., 2001).

Coating containers with basic cupric carbonate had no significant effect on shoot concentrations of Cu. However, as the $\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$ concentration in the containers increased

from 0 to 0.033 kg/l, the Cu concentration in shoots increased from 10.3 to 24 ppm, respectively. Although, shoot concentrations of Cu in T₂ seedlings were quite high (20–30 ppm is considered toxic for leaves and needles) (Dunn et al., 1997; Landis and Van Steenis, 2000), the seedlings showed no sign of copper toxicity (leaf chlorosis, senescent needles, necrotic stems). On the contrary, seedlings treated with the higher basic cupric carbonate concentration (0.033 kg/l) had significantly higher Cu concentration in their roots (168.3 ppm) than the seedlings of the other two treatments. Seedlings of all treatments accumulated higher Cu concentrations in roots than in shoots and exhibited shoot/root Cu ratios < 1 . Above results agree with earlier studies of Whitehead (1987), Arduini

et al. (1996), Poschenrieder et al. (2001). Interpretation by vector analysis would conclude that the status of the main nutrients (N, P, K) was not altered by the treatments. Exceptions to this were significant dilution of Mg and Ca in response to copper treatments.

Survival in the field plots did not show any significant difference among treatments and it was high during the first (96.7–98.3%) and the second year (95–98.3%) after planting. However, in a previous study, Royo et al. (2001) found that the survival of *P. halepensis* seedlings, planted in Muela de Cortes (70 km west of Valencia), was 92% the first year but by the end of the second summer survival decreased sharply to 52%.

During the 2 years after planting, the chemical root pruning had no significant effect on annual height and diameter growth. Moreover, besides the treatments, all seedlings showed significant positive relationships between final and initial dimensions. Thus, differences in final height and diameter between seedlings, in the first or second year after planting, were predominantly due to the differences of the initial size. Similarly, Burdett et al. (1983) working with lodgepole pine and Dunn et al. (1997) working with native Australian species found that 2 years after planting the seedling height and the stem diameter were not significantly affected by copper treatment (CuCO₃ 0.1 and 0.05 kg/l, respectively). Also, Royo et al. (2001) reported that 2 years after planting, differences among treatments in height growth of *P. halepensis* seedlings were not significant. However in our study, during the 2 years after planting, the stem volume and the annual growth of the stem volume were positively influenced when the higher copper concentration treatment (T2) was applied.

5. Conclusions

Coating containers with basic cupric carbonate significantly improved *P. halepensis* seedling quality without causing visible phytotoxicity symptoms. Two years after field planting, chemical root pruned seedlings survived no better than non-treated seedlings but exhibited higher stem volume and annual growth of the stem volume. The fast early growth of newly planted seedlings is the ideal because it decreases the risks from competing vegetation, mammals and frosts (Jinks and Kerr, 1999). Thus, although at these site conditions, *P. halepensis* species did not face special difficulty in artificial establishment, at harsher sites these copper-treated seedlings are expected to present better field performance than untreated seedlings, because the better the seedling quality in the nursery, the higher the seedling field performance (Duryea, 1984; Thompson, 1985). Nevertheless, the results from this study are to date the only published results concerning copper-coated containers and field performance of *P. halepensis*, and contribute valuable information to the continuing effort in order to investigate the benefit of chemical root pruning for other plant species, commonly used in reforestation and landscape restoration along the Mediterranean basin.

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