



Container volume and subirrigation schedule influence *Quercus variabilis* seedling growth and nutrient status in the nursery and field

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ABSTRACT

Container volume and irrigation management affect seedling growth in the nursery and field. We evaluated the effects of container volumes (D40, 656 ml; D60, 983 ml) and subirrigation schedules (85%, 75%, 65%, and 55% of 100% total substrate moisture content, TSMC) on seedling growth in a greenhouse and outplanting performance of Chinese cork oak (*Quercus variabilis* Blume) for one growing season. In the greenhouse, morphological attributes of seedlings grown at 85% and 75% TSMC in D60 were greater than those grown at 65% and 55% TSMC in D40. After outplanting, seedlings grown at 75% TSMC in D60 were tallest but not different than those grown at 65% TSMC. Shoot (9.2 g), root (28.0 g), and total (37.2 g) biomass of seedlings subirrigated at 55% TSMC in D60 reached maximum values, but shoot biomass for seedlings grown at either 65% or 55% TSMC was similar. Root and total N and K contents of seedlings subirrigated at 65% and 55% TSMC were greater than those grown at 85% and 75% TSMC. Our results suggest that reducing the subirrigation schedule threshold and using a container with more volume could improve oak seedling growth and nutrient accumulation during the first growing season of outplanting.

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Introduction

The genus *Quercus* is an important clade of woody angiosperms because of its rich species diversity, ecological and economical values (Nixon 2006), and widespread distribution throughout the northern hemisphere (Manos et al. 1999; Kanno et al. 2004; Nixon 2006). A series of problems, however, impede natural regeneration of *Quercus*. Animal predation, insect pests, diseases, and acorn inactivation may reduce germination. Insects, mammals, and climatic factors such as freezing damage young seedlings (Lorimer 1992; Chirino et al. 2008). Intensive vegetative competition, high fire frequency (Lorimer 1992), large canopy opening size (Humpgrey and Swaine 1997), and human-induced factors such as excessive grazing (Morrissey et al. 2010) also limit natural regeneration and growth.

Outplanting container or bareroot seedlings can effectively mitigate lack of natural regeneration (Dey et al. 2008). Because container seedlings experience less root damage during lifting than do bareroot seedlings, they often have higher survival and faster early growth (Crunkilton et al. 1992), especially on poor quality sites (Wilson et al. 2007). And container seedlings have a wider planting window (Ruehle et al. 1981).

Nursery culture is a complex technique that involves various factors, such as seed quality, container type, growing medium, irrigation, fertilization, environmental control, pests, and diseases. Related to the species (Carlson and Endean 1976), root characteristics (Dominguez-Lerena et al. 2006), and environmental conditions (Tsakalimi et al. 2005), container volume is one of most important determinants of

seedling quality (Landis 1990; Aphalo and Rikala 2003). Compared to seedlings with a small root volume, seedlings with a large root volume are more effective at water and nutrient uptake, particularly in a droughty environment (Peman et al. 2006; Jelic et al. 2016).

Irrigation management is a major factor affecting container seedling growth (Gingras et al. 1999) and has a direct effect on the water and aeration condition of substrates (Lamhamedi et al. 2001). Excess water that reduced root rhizosphere aeration inhibited gas exchange and the absorption of water and mineral nutrients (Lamhamedi et al. 2006), whereas deficit-irrigation decreased the growth resulting in poor quality (Arreola et al. 2006). Consequently, the container volume and the irrigation schedule are among the main factors to consider in the production of high quality seedlings.

Seedlings can be irrigated using overhead, drip, or sub-irrigation. Numerous studies have demonstrated that subirrigated plants, such as blue spruce (*Picea pungens* Engelm.) (Landis et al. 2006), northern red oak (*Quercus rubra* L.) (Bumgarner et al. 2008, 2015; Davis et al. 2008), koa (*Acaia koa* A. Gray) (Dumroese, Davis, et al. 2011), and Prince Rupprecht's larch (*Larix principis-rupprechtii* Mayr) (Xi 2015) are morphologically and physiologically similar to, or even better than, overhead-irrigated ones. Moreover, subirrigated koa seedlings grew better than overhead irrigated ones after one year of outplanting (Davis et al. 2011). Currently, much more is known about the main effects of container volume and irrigation schedule on seedlings development than it is known about their interactions on seedling growth and successive

field performance, such as growth and nutrient status. For example, during nursery production, nutrient loading adds advantage to early plantation establishment of Chinese fir (*Cunninghamia lanceolata* L.) and holm oak (*Quercus ilex* L.) by increasing root growth, biomass, and proportion of new leaves after outplanting (Xu and Timmer 1999; El Omari et al. 2003; Olet et al. 2011).

Chinese cork oak (*Quercus variabilis* Blume.) is the principle species in deciduous broadleaf, evergreen broadleaf, and mixed conifer-broadleaf forests of temperate, warm temperate, and subtropical zones of China. It is also an important species for transforming monoculture conifer plantations into conifer-broadleaf forests in China (Luo et al. 2009). Our study objectives were to assess the effects of container volume and subirrigation schedule on Chinese cork oak seedling production in a greenhouse and observe outplanting performance after one growing season in the field. We hypothesized that (1) larger container volume with higher substrate moisture content in the greenhouse would produce higher quality seedlings compared to seedlings grown in smaller container volumes with lower substrate moisture content; and (2) high quality seedlings would have an adequate morphology and nutrient status for this species to improve its phenotype and nutrition response to field conditions. Therefore, the aim of the study was (1) to compare the morphological characteristics of Chinese cork oak seedlings cultivated in 2 container volumes with 4 subirrigation schedules and (2) to assess seedling outplanting performance for one growing season in response to nursery culture management.

Materials and methods

Nursery experiment

To test our hypotheses, we grew Chinese cork oak 6 months in a greenhouse, stored them overwinter, outplanted seedlings in the field, and evaluated them after one growing season (from March to November). Our experiment was a completely randomized design with 2 container volumes $\times$ 4 subirrigation schedules $\times$ 3 replications. In April 2013, the 2 types of containers (D40, diameter 6.4 cm $\times$ depth 25 cm, volume 656 ml, density 174 seedlings·m⁻²; D60, diameter 6.4 cm $\times$ depth 36 cm, volume 983 ml, density 174 seedlings·m⁻²) were filled with 3:1 peat: perlite (V: V) medium (Pindstrup, Denmark) amended with 125 mg nitrogen (N) plant⁻¹ (Li et al. 2012) of controlled-released fertilizer (D40, 0.977 kg m⁻³; D60, 1.469 kg m⁻³) (5 to 6 month release rate, 13N-13P₂O₅-13K₂O with micronutrients; LUXECOTE, Jinan, China). Acorns, collected in September 2012 in Henan Province (N34.52°, E110.85°), were sorted to homogeneous size and sown on

26 April 2013. We cultivated 2 trays with 20 plants each for each container volume $\times$ subirrigation schedule $\times$ replication combination (960 seedlings total) inside a greenhouse (28°C day/16°C night) at Jiu Feng Forest Station (N40.05°, E116.08°) of Beijing Forestry University in Beijing, China.

All containers were overhead irrigated as needed until shoots emerged. On 26 May, subirrigation was initiated at 4 levels: 85, 75, 65, and 55% of 100% total substrate moisture content (TSMC) determined gravimetrically (Dumroese et al. 2015). We rotated trays every 14 days to minimize edge effects. From 20 September to 15 December, subirrigation for all treatments was applied at about 60% TSMC. On 20 October, we moved seedlings out of the greenhouse to acclimate to ambient conditions and to encourage leaves to senesce naturally. On 30 November, we measured height and root-collar diameter (RCD) on 8 seedlings per treatment per replication, separated them into shoots and roots, and dried those 72 h at 68°C to determine biomass. Because of dry and cold winter conditions in Beijing, we dug a root cellar 3 m long, 2 m wide, and 1 m deep outside the greenhouse. On 15 December, remaining plants were thoroughly irrigated and stored in the cellar until the following February.

Field experiment

We outplanted seedlings on 26 March 2014 in a field at Changping District, Beijing, China (N40.13°, E116.20°). Soil at this site has a sandy clay texture (Table 1). It is a temperate continental monsoon climate. The average monthly temperature and precipitation from March to November 2014 is shown in Figure 1.

Seedlings measurement

A sample of 10 plants per treatment combination (2 container volumes $\times$ 4 subirrigation schedules = 8 treatments) was randomly allocated and outplanted in 10 rows, 1.0 m $\times$ 1.0 m apart with three replications (240 plants total). We weeded twice every month. No additional irrigation or fertilizer was applied during the outplanting experiment.

We measured initial seedling height and RCD immediately after outplanting, and determined survival on 15 September. On 20 November, after leaves had senesced, we harvested 3 plants per treatment per replication and processed them as described for the greenhouse experiment. We composited the shoots and roots from each treatment $\times$ replicate combination and milled them for chemical assays. We determined N, phosphorus (P), and potassium (K) concentrations using a distillation and titration unit (UDK-159, VELP Scientifica, Usmate Velate MB, Italy), a UV-Vis Spectrophotometer (Agilent 8453, Agilent, Santa Clara, CA, USA), and an atomic

Table 1. Characteristics of the sandy clay soil at the outplanting site.

Depth (cm)	Soil water content (g kg ⁻¹)	Maximum water holding capacity (g kg ⁻¹)	Capillary water holding capacity (g kg ⁻¹)	Minimum water holding capacity (g kg ⁻¹)	pH	Organic matter (g kg ⁻¹)
0-10 cm	132	392	352	223	7.5	20.3
10-20 cm	137	313	282	180	7.4	19.5
20-30 cm	142	320	281	170	7.6	11.2

These data was measured in March 2014.

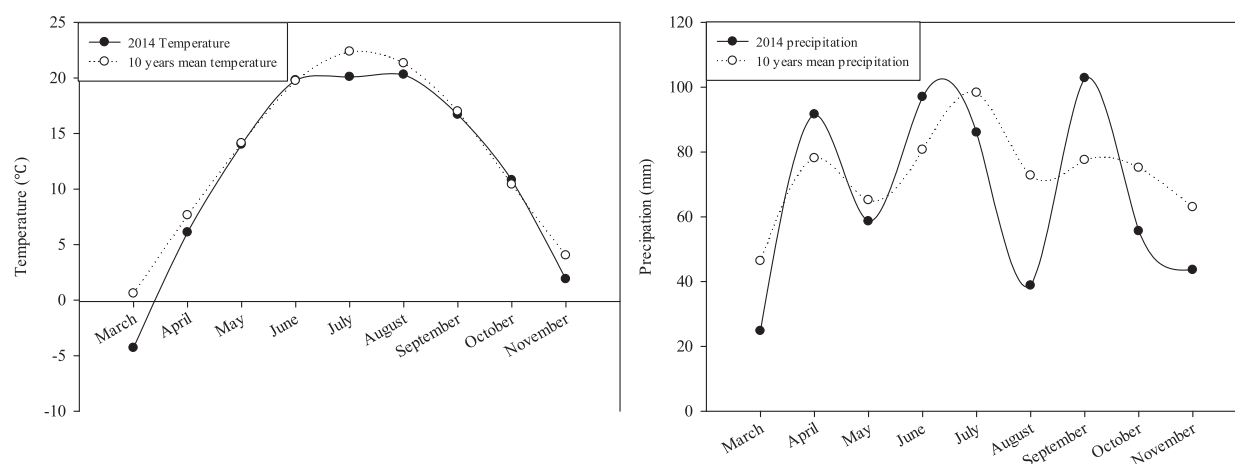


Figure 1. The average monthly temperature (left) and precipitation (right) from March to November in the field.

absorption spectrophotometer (Varian Spectrophotometer AA 220FS, Agilent, Santa Clara, CA, USA), respectively.

Statistical analysis

Analysis of variance (two-way ANOVA) was used to examine the effect of independent variables (container volume and subirrigation schedule). Interaction of the independent variables on seedling morphological attributes in a greenhouse, and morphology, nutrient status in the field were subjected to analysis using PASW Statistics (SPSS Inc., Chicago, Illinois, USA). Subirrigation schedules and the interactions were compared using the Duncan's tests. Results were considered significant at $\alpha = 0.05$.

Results

Nursery study

The container volume $\times$ subirrigation schedule interaction was not significant for any seedling growth variables (all $P > 0.2$; Table 2). Container volume ($P < 0.01$), and subirrigation schedule ($P < 0.01$) significantly affected height, RCD, and seedling biomass (Table 2). Height, RCD, and shoot, root, and total biomass of seedlings cultivated in D60 were 6.6, 7.1, 13.9, 21.5, and 19.4% greater than those cultivated in D40, respectively. The morphological attributes generally

increased with increasing substrate moisture content. Height, RCD, and shoot, root, and total biomass of seedlings with higher subirrigation thresholds (85% and 75% TSMC) were similar but significantly greater (7.1–10.5%, 8.0–9.9%, 13.5–28.8%, 12.5–22.3%, and 13.4–23.2%, respectively) than that of seedlings with lower subirrigation thresholds (65% and 55% TSMC), which were also similar to each other.

Field performance

After one growing season, neither container volume, subirrigation schedule, nor their interaction significantly affected seedling survival and RCD ($P > 0.1$) (Table 3); outplanting survival exceeded 88%. Both container volume and subirrigation schedule significantly affected final height ($P < 0.01$). After one growing season, the height growth of seedlings in D60 (17.6 cm) were greater than that of seedlings in D40 (13.1 cm). Seedlings cultivated with 75% TSMC in the greenhouse were significantly taller (83.4 cm) than those cultivated with 85% and 55% TSMC (76.4 cm, 71.8 cm) after one growing season (Table 3).

Container volume $\times$ subirrigation schedule significantly interacted to affect shoot ($P = 0.004$), root ($P = 0.012$), and total plant biomass ($P = 0.001$) in the field. After one growing season, shoot, root, and total biomass of seedlings cultivated in D60 increased with decreasing substrate

Table 2. The effect of container volume and irrigation schedule on the morphology of *Quercus varabilis* seedlings in the nursery.

Treatments	Height (cm)	RCD (mm)	Shoot biomass (g)	Root biomass (g)	Total biomass (g)
<i>Container volume</i>					
D60	64.0 $\pm$ 0.6 a	4.69 $\pm$ 0.05 a	2.95 $\pm$ 0.07 a	7.57 $\pm$ 0.25 a	10.52 $\pm$ 0.31 a
D40	60.4 $\pm$ 0.5 b	4.38 $\pm$ 0.04 b	2.59 $\pm$ 0.06 b	6.23 $\pm$ 0.17 b	8.81 $\pm$ 0.21 b
<i>Irrigation schedule</i>					
85%	64.9 $\pm$ 0.8 a	4.78 $\pm$ 0.07 a	3.09 $\pm$ 0.10 a	7.47 $\pm$ 0.36 ab	10.56 $\pm$ 0.45 a
75%	65.1 $\pm$ 0.9 a	4.70 $\pm$ 0.06 a	3.02 $\pm$ 0.09 a	7.56 $\pm$ 0.35 a	10.57 $\pm$ 0.42 a
65%	60.6 $\pm$ 0.8 b	4.35 $\pm$ 0.07 b	2.66 $\pm$ 0.09 b	6.64 $\pm$ 0.32 bc	9.31 $\pm$ 0.39 b
55%	58.9 $\pm$ 0.7 b	4.35 $\pm$ 0.05 b	2.40 $\pm$ 0.06 c	6.18 $\pm$ 0.22 c	8.58 $\pm$ 0.26 b
<i>Analysis of variance $P > F$</i>					
Container volume (C)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Irrigation schedule (I)	<0.0001	<0.0001	<0.0001	0.003	<0.0001
C $\times$ I	0.443	0.301	0.207	0.384	0.376

Means for measured response variables (height, RCD, shoot biomass, root biomass, and total biomass) and the associated standard error are listed. Column means followed by different letters within a given treatment differ significantly according to Duncan's significant difference at $\alpha = 0.05$.

Table 3. Effect of container volume and irrigation schedule on *Quercus variabilis* survival and morphological attributes during the first growing season in the field.

Treatments	Survival (%)	Morphological attributes	
		Height (cm)	RCD (mm)
<i>Container volume</i>			
D60	88.3 ± 2.6	81.6 ± 1.9 a	8.29 ± 0.27
D40	93.3 ± 2.6	73.5 ± 1.6 b	7.69 ± 0.27
<i>Irrigation schedule</i>			
85%	98.3 ± 3.7	76.4 ± 2.4 b	7.76 ± 0.38
75%	90.0 ± 3.7	83.4 ± 3.0 a	8.73 ± 0.38
65%	86.7 ± 3.7	78.4 ± 1.9 ab	7.76 ± 0.38
55%	88.3 ± 3.7	71.8 ± 2.5 b	7.71 ± 0.38
<i>Analysis of variance P > F</i>			
Container volume (C)	0.198	0.001	0.126
Irrigation schedule (I)	0.165	0.006	0.185
C × I	0.162	0.084	0.209

Means for measured response variables (survival, height, and RCD) and the associated standard error are listed.

Column means followed by different letters within a given treatment differ significantly according to Duncan's significant difference at $\alpha = 0.05$.

moisture content in the greenhouse (Figure 2(A–C)), whereas, shoot and total biomass of seedlings cultivated in D40 appeared to sharply decrease when subirrigation threshold was below 65% TSMC in the greenhouse (Figure 2(A,C)). There was no significant difference on root biomass of seedlings grown in D40 among 4 subirrigation schedules in the greenhouse after outplanting. Container volume had a significant effect on shoot biomass of seedlings ($P < 0.05$), and subirrigation schedule had a significant effect on root and total biomass of seedlings ($P < 0.01$). Shoot (9.2 g), root (28.0 g), and total (37.2 g) biomass of seedlings subirrigated with 55% TSMC in D60 in a greenhouse reached maximum values after one growing season.

Nutrient concentration was largely unaffected ($P > 0.05$) by container volume and subirrigation schedule after outplanting. Container volume significantly affected ($P < 0.05$) shoot P concentration. Shoot P concentration of seedlings cultivated in D40 in the greenhouse was greater than that of seedlings cultivated in D60 (0.28% vs. 0.23%) after outplanting. Compared to nutrient concentration of seedlings in the greenhouse (2013), N and K concentration of stems and roots decreased, whereas P concentration increased after one growing season (2014) (Figure 3).

Although container volume did not significantly affect nutrient content ($P > 0.05$), subirrigation schedule did significantly affect root and total plant N and K contents ($P < 0.05$) one growing season after outplanting. Root and total plant N and K contents increased after outplanting as the subirrigation schedule thresholds in the nursery decreased. Root and total plant N content of seedlings cultivated with 55% TSMC in the greenhouse was greater than those of seedlings cultivated with other subirrigation schedule thresholds by 17–41%, and 13–35% after one growing season. No significant difference on root N content was observed on seedlings grown at 55% and 65% TSMC. Root and total plant K contents of seedlings grown at 65% and 55% TSMC in the greenhouse were greater than those of seedlings grown at 85% and 75% TSMC after one growing season, by 44–73% and 36–58%, respectively. Compared to the nutrient content of seedlings in the greenhouse, total seedling and root N contents and P

and K contents in the stem, root, and for the entire seedling increased after outplanting (Figure 4).

Discussion

Nursery study

In our study, height, RCD, shoot, root, and total biomass of Chinese cork oak seedlings grown in larger containers were greater than those seedlings grown in smaller containers (Table 2). Our results correspond well with those from studies on a variety of species (Dumroese, Davis, et al. 2011; Pinto et al. 2011; Mariotti et al. 2015; Jelic et al. 2016). Compared to seedlings grown in smaller containers, seedlings grown in larger containers have larger root systems that are probably more efficient in transporting water (Chirino et al. 2008) and assimilating nutrients in the greenhouse (Dominguez-Lerena et al. 2006). Other researchers working with *Pinus* larch (*Larix olgensis* Henry) (Xu 2010), poplar (*Populus × euroamericana*) (Gao 2011), ponderosa pine (*Pinus ponderosa* Douglas ex C. Lawson) (Dumroese, Page-Dumroese, et al. 2011), and Prince Rupprecht's larch (Xi 2015) reported that seedlings that received a higher rate of irrigation (70–90% TSMC) during the nursery period were morphological larger than those that received a lower rate of irrigation (60–50% TSMC), and our research shows Chinese cork oak seedlings responded similarly. Because nutrients move toward roots via soil water, sufficient soil water provided by more frequent irrigation (i.e. irrigating at higher TSMC thresholds) likely improved the effectiveness of nutrient uptake (Chen 2015).

Field performance

In our trial, seedlings had similar outplanting survival during the first growing season in the field regardless of container volume or subirrigation schedule. Because of the protection of the container, the roots were not harmed during storage, lifting, transportation, and the afforestation process, yielding high survival (Liu 1999; Wilson et al. 2007). For example, long-leaf pine (*Pinus palustris* M.) (Boyer 1989), Douglas-fir (*Pseudotsuga menziesii* M.) (McDonald 1991), and northern red oak (Wilson et al. 2007) container seedlings survived and grew better than bareroot seedlings after outplanting. Although Jelic et al. (2016) reported that holm oak seedlings cultivated in larger containers (923–1205 ml) had a slightly better survival rate compared with seedlings cultivated in smaller containers (120–220 ml) (associated with larger root volume, larger surface area, and greater total root length that possibly contributed to greater water and mineral nutrient consumption in the early development stage), we observed no differences among our container sizes, probably because the absolute difference in our container sizes was less than that used by Jelic et al. (2016).

Our Chinese cork oak seedlings cultivated in the larger D60 had greater height growth and final height during the first growing season of outplanting than those cultivated in the smaller D40, a result similar to that found for holm oak after 6 years in the field (Jelic et al. 2016), which may be attributed to deeper root penetration into the soil by seedlings

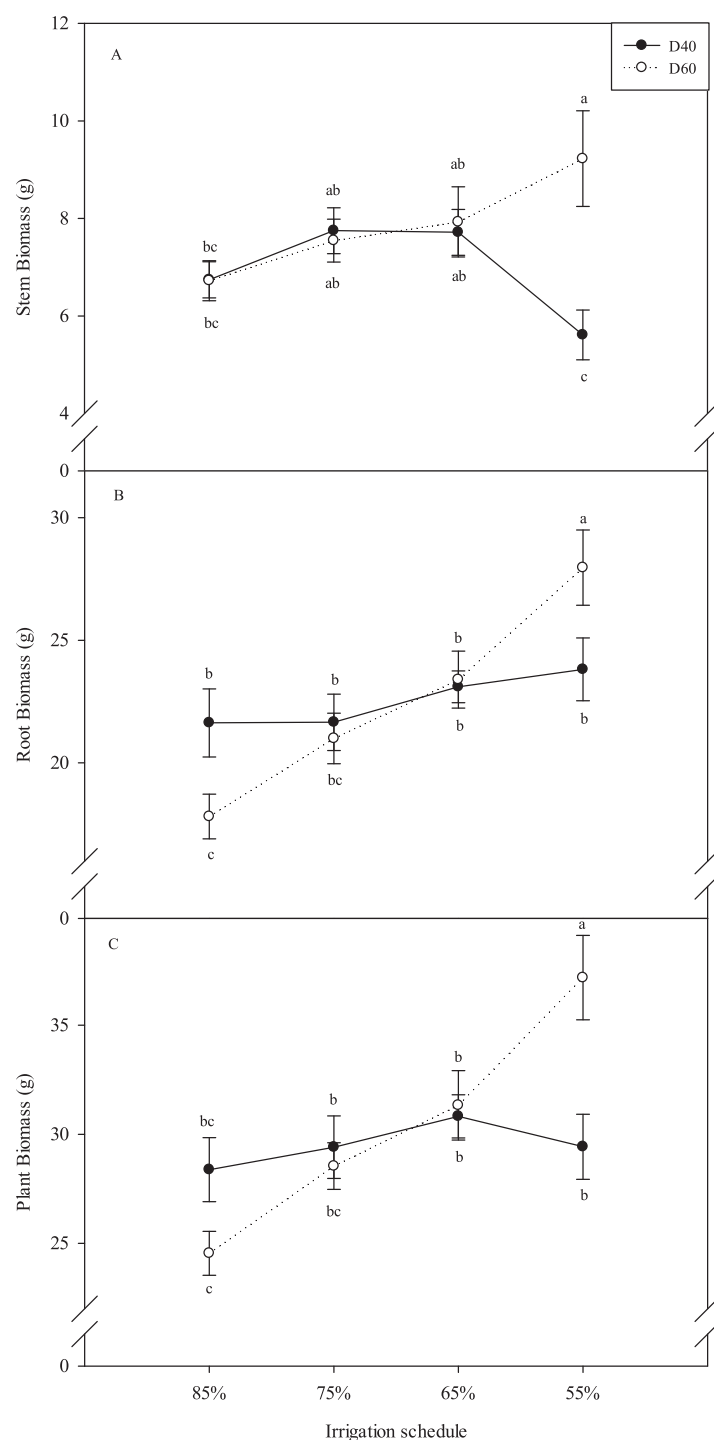


Figure 2. The interaction effect of container volume $\times$ irrigation schedule on shoot (A), root (B), and total biomass (C) in the field. Different letters indicate significant differences for container volume $\times$ irrigation schedule treatments.

produced in deeper containers (Chirino et al. 2008). Our seedlings that received the highest rate of irrigation during nursery production had less height growth after outplanting, which concurs with Hipps et al. (1996). Our seedlings that received the lowest rate of irrigation in the nursery also had less height growth after outplanting, but these seedlings, when grown in large containers, had more shoot, root, and total biomass than seedlings from other treatments (Figure 2). This greater biomass accumulation may be explained by greater and more rapid root growth after outplanting, as

reported by Franco et al. (2001), who concluded that plants provided less water in the nursery grew more roots faster than seedlings given more water during nursery production, especially when outplanted on dry sites.

In the field experiment, we noted a sharp N concentration reduction in the shoot and root after one growing season, which suggested nutrient dilution during the first growing season after outplanting. Such N dilution has been reported for Scots pine (*Pinus sylvestris* L.) (Troeng and Ackzell 1988) and was the impetus for much work on nutrient loading

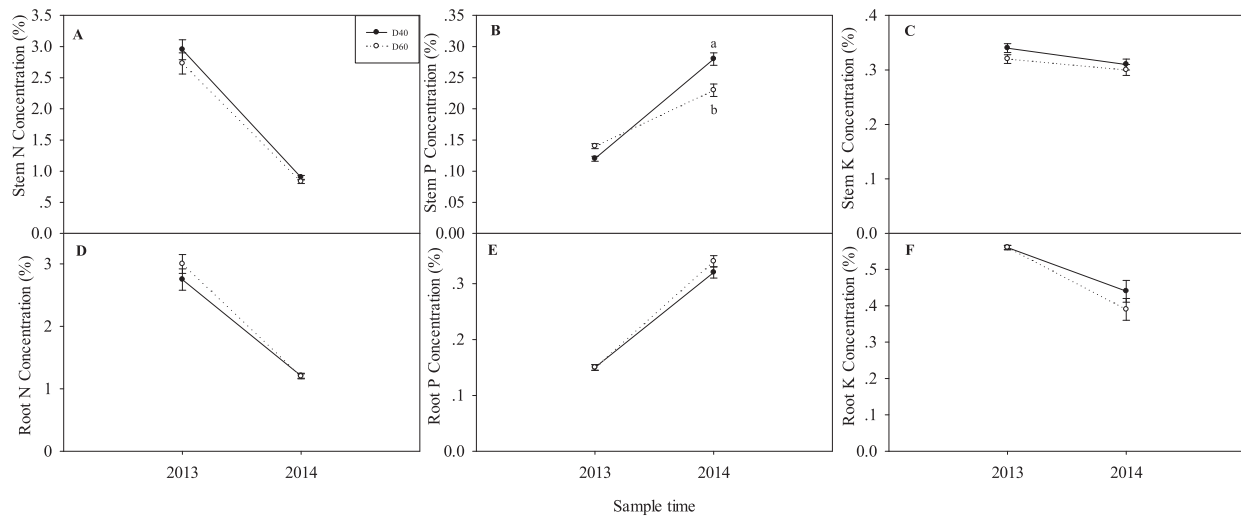


Figure 3. N, P, K concentration of stem (A–C) and root (D–F) of *Quercus variabilis* seedling in the nursery (2013) and field (2014). Different letters indicate significant differences for container volume treatments.

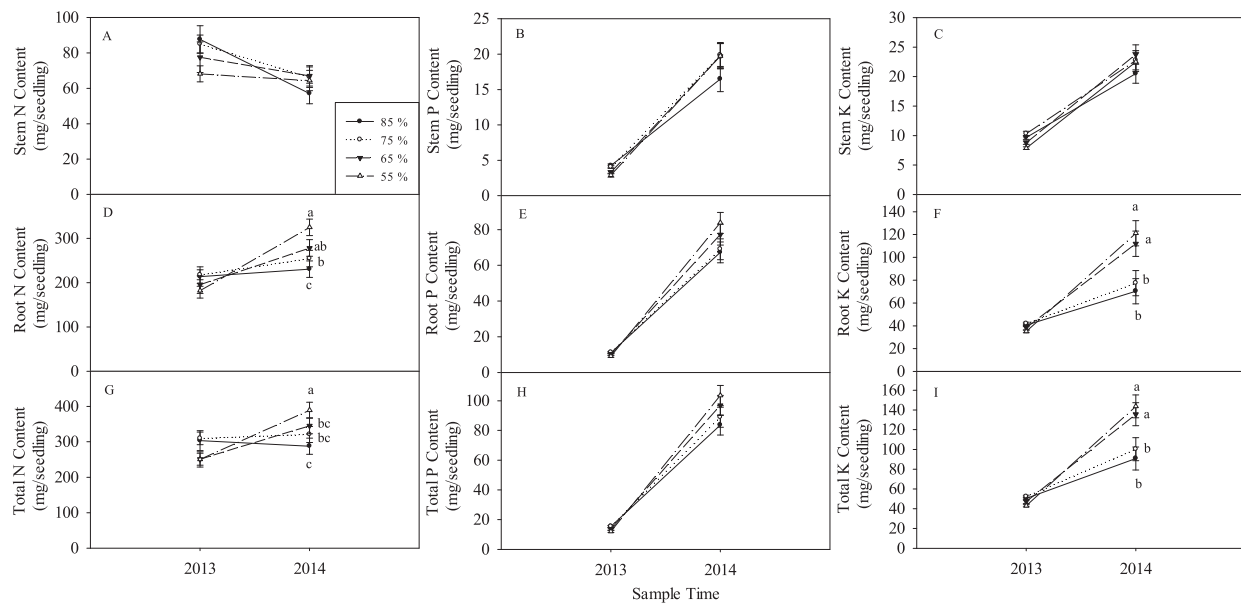


Figure 4. N, P, K content of stem (A–C) root (D–F), and total plant (G–I) of *Quercus variabilis* seedling in the nursery (2013) and field (2014). Different letters indicate significant differences for irrigation schedule treatments.

(Timmer 1996; Dumroese et al. 2005). Nutrient loading, sometimes referred to as fall fertilization, has been suggested to mitigate nutrient dilution and thereby reduce nutrient stress following outplanting (Imo and Timmer 1992). By contrast, P and K content of stems, and N, P, and K contents of roots and in the total seedling increased after one growing season in our research. Similarly, N loading of red oaks and white oaks (*Quercus alba* L.) increased nutrient content after outplanting (Salifu et al. 2009).

In this study, we examined container volume and irrigation schedule and interactions toward improving the nursery seedling quality and subsequent outplanting performance of Chinese cork oak. We found that this species responded best, in terms of height, RCD, biomass, and nutrient at 75% TSMC in the nursery and 55% TSMC in the field. Seedlings

performed well on morphology and nutrient status in large containers during the nursery and field periods. Seedlings in large containers with large root systems have shown excellent potential to grow in the field compared to seedlings in smaller containers. Cultivating seedlings in smaller containers has the advantages of lower price, less substrate requirement, and lower weight during transport for afforestation than larger containers. Seedling quality and economic cost should be considered simultaneously to ensure afforestation is done efficiently.

Thus, our results suggest that subirrigation at 65% TSMC in D60 could serve as a feasible option for producing quality oak seedlings while conserving resources during nursery production in the greenhouse. Seedling field performance depended on its morphological and physiological status

sometimes may vary with outplanting site conditions (del Campo et al. 2010; Pinto et al. 2011). Seedling performance in the nursery and field are paramount. Therefore, future studies should consider seedling outplanting performance to help adjust nursery cultural practices. Additional research is needed to establish the relation between outplanting environment and nursery culture in a variety of forest tree species to optimize nursery cultivate technique and improve seedling quality for outplanting.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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