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Effect of mini-plug container depth on root and shoot growth of four forest tree species during early developmental stages

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Abstract: The container type used in nursery culture is among the most important determinants of containerised seedling quality. The objective of the present study was to analyse root and shoot growth, root growth potential, and photochemical efficiency of seedlings of *Picea abies*, *Robinia pseudoacacia*, *Pinus brutia*, and *Pinus nigra* cultivated for 5 weeks in mini-plugs of 2 different cavity depths (37 and 60 mm). The results showed that precultivation of *P. nigra* and, to a lesser extent, of *R. pseudoacacia* in deeper mini-plug containers improved seedling morphological attributes and quality, but shallow containers produced better quality seedlings of *P. abies*. In *P. brutia*, the use of shallow containers led to the development of a superior root system, while the use of deeper containers produced seedlings with better above-ground characteristics.

Key words: Cavity depth, mini-plug container, root growth potential, seedling quality

Introduction

The use of low quality planting stock is often responsible for reforestation failure in Greece (Radoglou and Raftoyannis 2001). In addition, drought conditions, often encountered in Mediterranean ecosystems, pose a significant limitation to the successful establishment of transplanted material (Navarro et al. 2006; Plourde et al. 2009). The quality of the produced planting stock, as expressed by morphological and physiological characteristics, is actually the only factor that man can manipulate (Radoglou 1999) in order to ensure high seedling survival after outplanting.

Seedling morphological and physiological characteristics could be changed with the use of different cultural practices, leading to the production of planting stock better adapted to special environmental conditions (O'Reilly et al. 1989). Nursery practices are considered to have a large impact on seedling root development during the nursery phase; this effect is likely to be maintained during the early establishment phase (Costa et al. 2004). The characteristics of the nursery culture and particularly the container type are among the most important determinants of the cost of seedling production, as well as of seedling quality (Landis et al. 1990; Chirino et al. 2008). 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; Dominguez-Lerena et al. 2006). Many reports

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exist on the effect of container volume and seedling density (Howell and Harrington 2004; South et al. 2005; Tsakaldimi et al. 2005; Dominguez-Lerena et al. 2006), but very few articles deal with the effects of container depth (Pemán et al. 2006; Chirino et al. 2008). Container depth is often considered as one of the most important variables influencing seedling morphology because it is directly related to moisture holding capacity, humidity, and even the ventilation of the root ball (Landis et al. 1990). Container depth can determine the root system growth and tap root length of the transplanted seedling (Chirino et al. 2008). Growth of new roots out of the root plug to colonise the soil deeply is a critical factor for seedling survival, especially under Mediterranean climates in which root system characteristics (e.g. size, distribution, root-soil contact, and root hydraulic conductivity) can affect the water absorption capacity of the seedling after outplanting (Simpson and Ritchie 1996; Grossnickle 2005).

A promising new method in the field of nursery stock production is the growing of seedlings after a short precultivation period under close to optimal environmental conditions (Kostopoulou et al. 2009, 2010a, 2010b; Mattsson et al. 2010; Radoglou et al. 2011). Mini-plugs are very small container cells, usually less than 33 cm^3 in volume. Mini-plugs require a shorter production cycle compared to the standard container seedling culture, thus allowing 2 high quality crops with well developed fibrous root systems to be grown in a single season (Tanaka et al. 1988; Hahn 1990; Landis et al. 1990). The idea of mini-plug seedling production is based on the expectation that the combination of rapid root and shoot growth during seedling precultivation in mini-plugs will probably result in rapid establishment after transplanting and subsequent growth in the field. The advantages that mini-plugs offer are an almost 100% yield resulting in few culls, high plant density per unit production area, maximum use efficiency of seeds or cuttings, shorter crop rotation, and improved stock quality, since plants with large stem diameters and fibrous root systems can be produced (Landis 2007). Early results from several trials have indicated that this system shows promise as a replacement for the conventional method of stock production (Tinus 1996; McCreary and Lippitt 2000; Riley and Steinfeld 2005; Landis 2007).

This study is part of a series of studies in which the new method of precultivation of forest regeneration material in mini-plugs is evaluated (Bellarosa et al. 2007; Dini-Papanastasi et al. 2007; Kostopoulou et al. 2008a, 2008b, 2009, 2010a, 2010b; Radoglou et al. 2011). Previous studies have revealed that precultivation of *Cupressus sempervirens* (L.), *Pinus brutia* (Ten.), and *Pinus nigra* (Arnold) in mini-plugs could lead to higher quality seedlings compared to the standard planting stock currently produced by Greek nurseries (Kostopoulou et al. 2009, 2010a, 2010b; Radoglou et al. 2011).

In this study, a number of morphological and physiological traits were assessed in the planting material of 4 tree species that are ecologically and economically important in Greece, namely black locust (*Robinia pseudoacacia*), Norway spruce (*Picea abies* L.), brutia pine (*Pinus brutia*), and black pine (*Pinus nigra*), precultivated in mini-plugs of 2 different cavity depths. Our hypothesis was that the seedlings precultivated in deeper mini-plug containers would have a more developed root system that could quickly fill the plug of the bigger container into which the mini-plugs were transplanted, and thus have a higher probability of reaching deep soil horizons in the field after outplanting. To test this hypothesis, we evaluated the morphological and physiological characteristics of seedlings of the 4 species mentioned cultivated in shallow mini-plug containers (depth: 37 mm) compared to seedlings cultivated in deeper mini-plug containers (depth: 60 mm). For this purpose, we analysed root and shoot growth, root growth potential, and photochemical efficiency of seedlings resulting from the 2 different production methods.

Materials and methods

Plant material, mini-plug container design, and precultivation conditions

The seeds of *Robinia pseudoacacia* were of Hungarian origin and collected from a small seed orchard established near Thessaloniki in Central Macedonia, Greece (40°46′N, 22°21′E; 10 m above sea level) in 2006. *Picea abies*, *Pinus brutia*, and *Pinus nigra* seeds were provided by the Ministry of Rural Development and Food (Section of Forest Nurseries and Seed Production, Athens). The seeds of *P. abies* were collected from Drama in northeastern Greece (41°28′21″N, 24°19′51″E) in 1991. *P. brutia* seeds were collected from a natural stand on Thasos Island, northeastern Greece (40°46′40″N, 24°40′00″E; 50-130 m above sea level) in 2004 and *P. nigra* seeds were collected from the area of Kalabaka in central Greece (39°53′06″N, 21°15′07″E) in 2006. The seed germination ability, determined according to the policies of ISTA (2008), was 86%, 69%, 75%, and 78% for *R. pseudoacacia*, *P. abies*, *P. brutia*, and *P. nigra*, respectively. Prior to sowing, seeds of *R. pseudoacacia* were mechanically scarified for 75 min, while the conifer seeds were soaked in water for 24 h. Seeds were allowed to germinate under conditions recommended by ISTA (2008). Only pregerminated seeds were used to maximise the uniformity of germination.

One pregerminated seed was transferred into each cavity of the mini-plug plastic trays (QuickPot®, Herkuplast-Kubern GmbH, Ering, Germany). The mini-plug trays were of identical dimensions (310 × 530 mm, density: 1460 mini-plugs m^{-2}), except for cavity depth and consequently cavity volume. The more shallow type was 37 mm deep (volume: 13 cm^3) while the other was 60 mm deep (volume: 19 cm^3). All cavities were filled with stabilised growing medium (Preforma PP01, Jiffy Products International AS, Stange, Norway). In total, 240 seedlings per species and cavity depth were used.

After the placing of the pregerminated seeds, mini-plug trays were transferred to environmentally controlled growth chambers of 400 L (KB8000FL, Termaks AS, Bergen, Norway) for a cultivation period of 5 weeks. Environmental conditions in the chambers were set at a 14-h photoperiod, a photosynthetic photon flux density (PPFD) of 250 μmol m^{-2} s^{-1}, air relative humidity (RH) of 80 ± 10%, and a 20/15 °C day/night temperature. Watering was applied every second day, followed by full rotation of the trays in order to ensure uniform growth conditions.

Seedling morphology

After 5 weeks of precultivation in the growth chambers, 30 seedlings per species and container depth were randomly selected and the following growth parameters were measured: root length (RL), shoot height (SH), leaf area (including the shoot from the top of the root plug) (LA), root dry weight (RDW), and shoot dry weight (SDW). SH and RL were defined as the distance from the top of the root plug to the upper and lower end of a seedling, respectively, and were measured using a digital calliper. LA was measured using an area meter (AM100, ADC Bioscientific Ltd., Herts, UK). RDW and SDW were assessed after oven-drying at 70 °C for 48 h. The root-to-shoot ratio (R/S) was calculated on a dry weight basis.

Root growth potential (RGP) test

A root growth potential (RGP) test was carried out to determine the potential effect of plug depth on the capacity of seedlings to initiate new roots. This test was implemented immediately after the end of the precultivation period in the growth chambers (5 weeks) using the plant material that was derived from the precultivation phase. At random, 96 seedlings per species and container depth (8 replications of 12 plants) were selected and transplanted into mini-plug containers of same size, following the standardised RGP technique for containerised seedlings described by Mattsson (1986). The containers were placed on top of stainless steel boxes (35 × 26 × 8 cm) filled with equal volumes of peat (Klassmann Base Substrate 250I, Klassmann-Delmann GmbH, Geeste, Germany) and sand. The boxes were immersed in a stainless water bath. The seedlings remained in the RGP bath for 21 days at an air temperature of 21 ± 2 °C, RH of 40 ± 10%, and 14-h photoperiod, with a PPFD at plant level of 300 μmol m^{-2} s^{-1}. On day 12 and on day 21 of the RGP test, half of the seedlings per species and container depth (4 replications of 12 plants, total of 48 plants) were carefully removed from the RGP boxes. All new roots protruding from the root plug (new roots that were formed during the RGP test) were cut and cleared from the peat remains. The root growth potential (RGP) of each seedling was assessed by measuring the new root length (NRL) and new root dry weight (NRDW) of these roots. Shoot height (SH) and shoot dry weight were also measured. Leaf area (LA), including the shoot, was measured only for conifer species. All measurements were conducted as previously described.

Chlorophyll content and chlorophyll fluorescence

Chlorophyll content and fluorescence measurements were conducted by the end of the precultivation period (5 weeks) and at the end of the RGP test (day 21), when seedlings were 8 weeks old. The chlorophyll content index (CCI), which is the ratio of optical absorbance at 655 nm to that at 940 nm, was measured using a chlorophyll content meter (CCM-200, Opti-Sciences, Inc., Hudson, NH, USA) on 10 randomly selected samples per treatment. The ratio of variable to maximal fluorescence (F_v/F_m) (Maxwell and Johnson 2000) was measured on 5 dark-adapted (>20 min in the dark) samples per treatment, while the effective quantum yield of photosystem II ($\Delta F/F'_m$) (Maxwell and Johnson 2000) was measured on 10 light-adapted samples per treatment using a pulse-amplitude modulated photosynthesis yield analyser (Mini-PAM, Heinz Walz GmbH, Effeltrich, Germany). These 3 measurements were performed on a cluster of needles in the case of conifer species and on the first round leaf in the case of *R. pseudoacacia*. Due to a technical problem, results for the chlorophyll fluorescence and content of *R. pseudoacacia* acquired at the end of the RGP test were not considered reliable and, therefore, are not presented.

Statistical analysis

For the aims of the present study, a factorial experimental design was followed. Three samplings were performed in order to evaluate seedling morphology and physiology. The first was performed at the end of the precultivation phase in the growth chambers, when seedlings were 5 weeks old. The second sampling was performed on the 12th day after the initiation of the RGP test, when seedlings were 7 weeks old. The third sampling occurred at the end of the RGP test (21st day after the initiation of the test), when seedlings were 8 weeks old. The data were analysed using one-way ANOVA in SPSS (SPSS 15.0, Chicago, IL, USA) to determine the effects of the species and cavity depth on seedling morphological and physiological characteristics.

Results

Morphological characteristics of seedlings at the end of the precultivation period

At the end of the precultivation period in the growth chambers, *P. abies* seedlings grown in deep mini-plug containers had significantly higher values of RL, SH, and LA compared to seedlings grown in shallow containers (Table). However, container depth did not affect the seedling above- and below-ground biomass, as expressed by the shoot and root dry weight. In addition, seedlings grown in both container types showed similar allocation patterns to roots and shoots, as expressed by the root-to-shoot ratio values. Seedlings of *R. pseudoacacia* grown in both container types showed similar morphological characteristics (Table). Seedlings of *P. brutia* grown in deep mini-plug containers had significantly higher values of RL and SH (Table). Root and shoot biomass, as well as root-to-shoot ratio, were unaffected by container depth. Mini-plug container depth significantly affected the root length of *P. nigra* seedlings; longer roots were found in the deeper containers (Table).

Table. Mean values of morphological and physiological parameters per species and container depth at the end of the 5-week precultivation period.

Species	Container depth (mm)	RL (cm)	SH (cm)	LA (cm^2)	RDW (mg)	SDW (mg)	R/S	CCI	F_v/F_m	$\Delta F/F'_m$
P. abies	**37**	2.87***	3.84**	0.94***	1.09 ns	5.01 ns	0.225 ns	1.52 ns	0.67 ns	0.70 ns
	60	4.59	4.16	1.38	1.16	4.52	0.271	1.54	0.67	0.62
R. pseudoacacia	**37**	6.07 ns	6.56 ns	6.53 ns	5.81 ns	18.38 ns	0.312 ns	14.81 ns	0.79 ns	0.76 ns
	60	6.89	7.00	6.61	5.91	18.51	0.305	14.76	0.81	0.75
P. brutia	**37**	3.56***	4.88***	2.72 ns	3.70 ns	15.97 ns	0.244 ns	2.47 ns	0.67 ns	0.68 ns
	60	4.68	5.63	2.76	3.72	20.27	0.240	2.41	0.69	0.70
P. nigra	**37**	3.51***	7.30 ns	3.43 ns	3.53 ns	17.66 ns	0.209 ns	2.34**	0.72 ns	0.70 ns
	60	5.39	7.35	3.31	3.75	16.86	0.233	4.08	0.71	0.70

***P < 0.001, **P < 0.01, *P < 0.05, ns: not significant.

Root growth potential

Twelve days after the initiation of the RGP test (Figure 1), container depth significantly affected the shoot height of *P. abies* seedlings; deeper mini-plug containers produced higher seedlings. Container depth was found to significantly affect the below-ground characteristics of *R. pseudoacacia* seedlings. In fact, the roots produced from the shallow containers were longer and heavier compared to the roots produced in deep containers. In *P. brutia*, the depth of the mini-plug containers significantly affected the above-ground characteristics of the species. Deeper containers resulted in seedlings with significantly taller and heavier shoots (Figure 1) and higher LA (data not shown). In the same period, seedlings of *P. nigra* grown in the deeper mini-plug containers had significantly higher dry weights of new roots (Figure 1).

At the end of the RGP test (day 21), container depth significantly affected the length and the dry weight of new roots of *P. abies* seedlings. Longer and heavier roots were found in seedlings grown in shallow containers (Figures 2 and 3). The height difference between seedlings produced in deep and shallow mini-plug containers found on the 12th day of the RGP test (Figure 1) disappeared at the end of the RGP test (Figure 2). In *R. pseudoacacia*, the effect of container depth that was initially found on the below-ground characteristics of the seedlings (Figure 1) disappeared at the end of the RGP test (Figure 2). Seedlings of *R. pseudoacacia* grown in both container types showed similar values of new RL, new RDW, and SDW (Figures 2 and 3). However, seedlings cultivated in the deeper containers produced significantly taller shoots. Mini-plug container depth significantly affected the length of new roots, SDW, and LA of *P. brutia* seedlings. Longer roots were found in seedlings grown in the shallow containers (Figures 2 and 3). In contrast, seedlings cultivated in the deeper containers had higher SDW (Figure 2) and LA (Figure 4). In *P. nigra*, root and shoot biomass

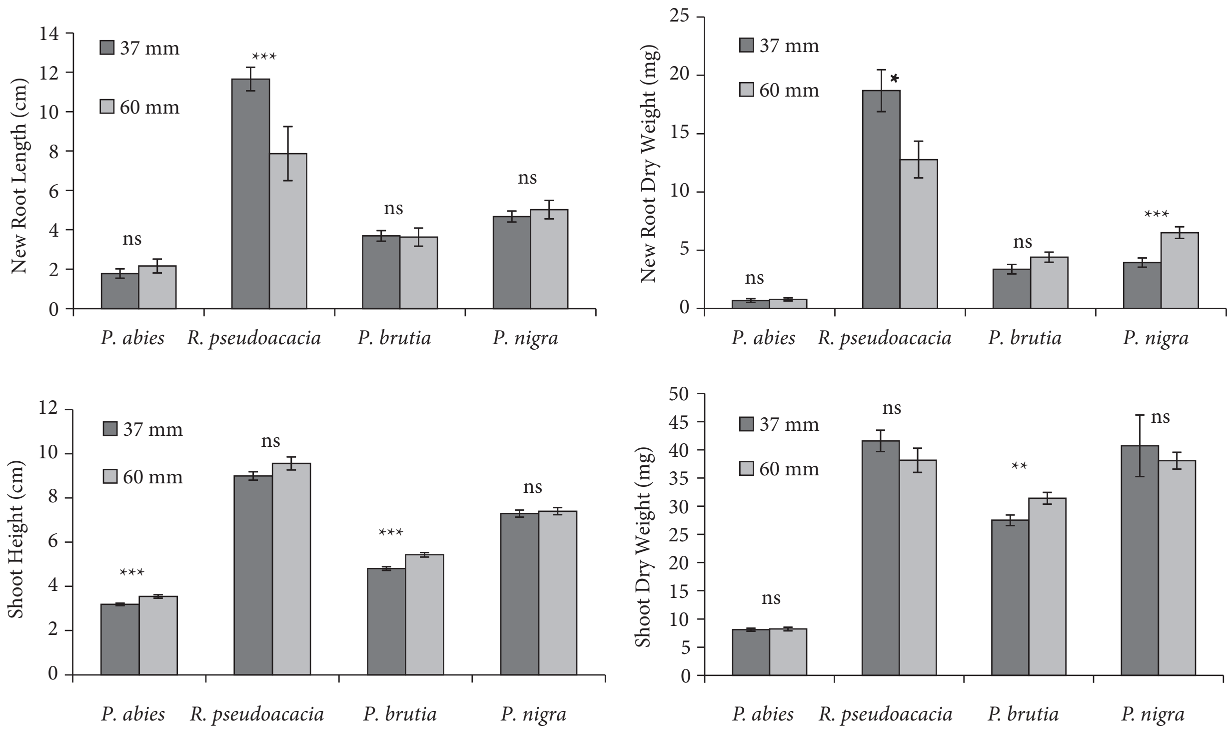

Figure 1. Length and dry weight of new roots, shoot height, and dry weight of *Picea abies*, *Robinia pseudoacacia*, *Pinus brutia*, and *Pinus nigra* seedlings cultivated in mini-plugs 37 and 60 mm deep, 12 days after the initiation of the RGP test, when seedlings were 7 weeks old (***P < 0.001, **P < 0.01, *P < 0.05, ns: not significant).

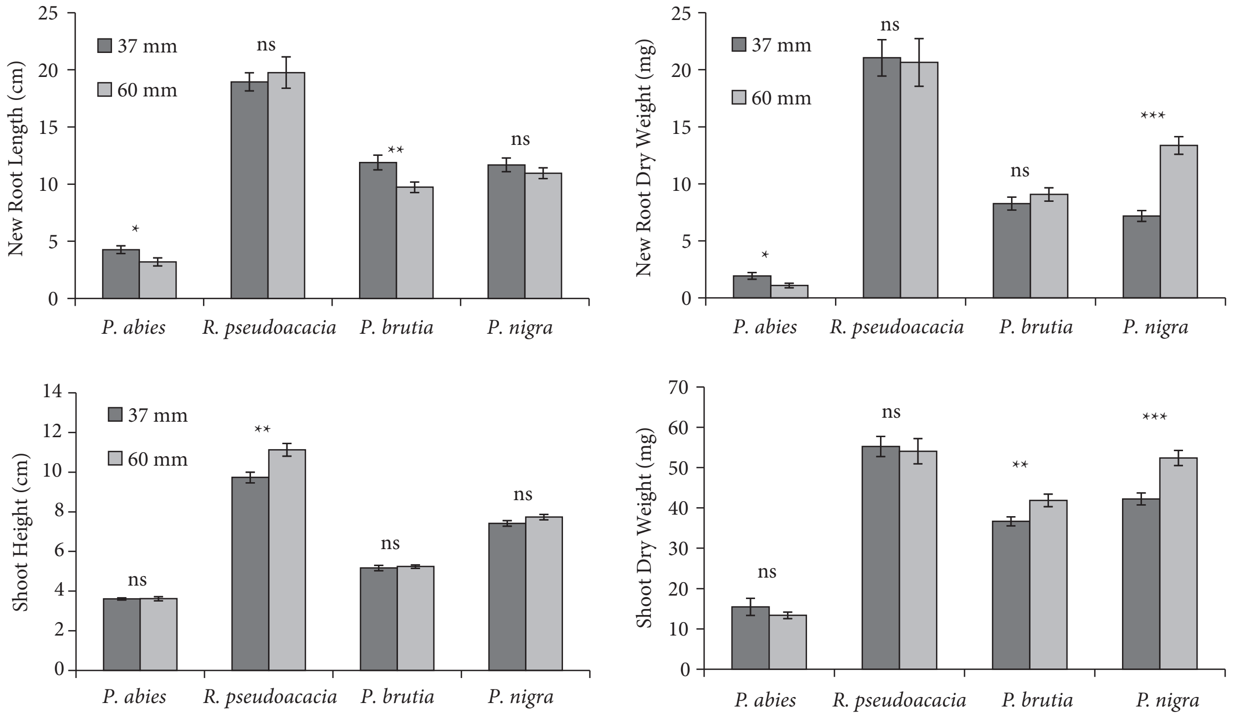

Figure 2. Length and dry weight of new roots, shoot height, and dry weight of *Picea abies*, *Robinia pseudoacacia*, *Pinus brutia*, and *Pinus nigra* seedlings cultivated in mini-plugs 37 and 60 mm deep at the end of the RGP test, when seedlings were 8 weeks old (***P < 0.001, **P < 0.01, *P < 0.05, ns: not significant).

(Figure 2), as well as LA (Figure 4), were significantly affected by the container type used; higher values of the above parameters were found in the deeper mini-plug containers.

Chlorophyll content and chlorophyll fluorescence

At the end of the precultivation period in the growth chambers, *P. abies*, *R. pseudoacacia*, and *P. brutia* seedlings grown in both container types showed similar values of chlorophyll content index (CCI), of ratio of variable to maximal fluorescence (F_v/F_m), and of effective quantum yield of photosystem II ($\Delta F/F'_m$) (Table). On the other hand, seedlings of *P. nigra* grown in the deeper containers had significantly higher values of CCI (Table).

At the end of the RGP test (day 21), seedlings of all species grown in both container types showed similar values of CCI (data not shown). At the same time, the ratio of variable to maximal fluorescence (F_v/F_m) and the effective quantum yield of photosystem II ($\Delta F/F'_m$) did not differ between the 2 container types in any of the 3 conifer species tested (Figure 4).

Discussion

The effect of container size has been the object of several studies of planting stock quality. In general, a relationship has been observed between container size or shape and seedling growth (Paterson 1996; Aphalo and Rikala 2003; South et al. 2005; Dominguez-Lerena et al. 2006). It is important to note that the majority of these studies refer to conventional, larger-sized containers (e.g. depths greater than 65 mm, volumes greater than 60 cm^3). Furthermore, most of the studies of the effect of container type on seedling morphological and physiological characteristics used containers that were very different in material, size, density, etc. In our study, the only difference between the 2 container types was depth, which influenced the final volume of the container. In addition, to our knowledge, no studies exist on the effect of mini-plug container depth on seedling quality.

Our results suggest that even at the early stage of growth, seedling morphological and root growth potential responses to container depth were species-

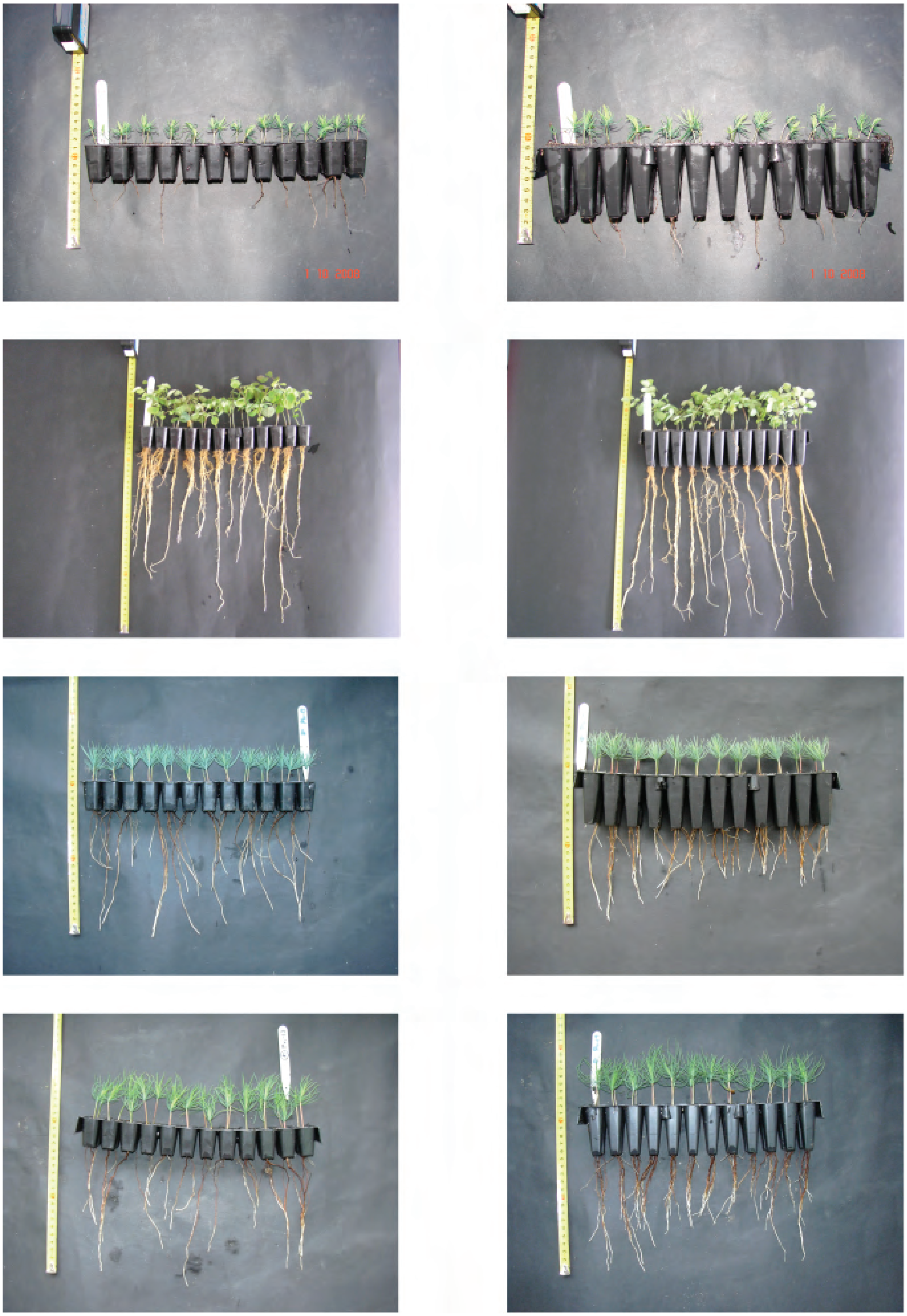

Figure 3. Example of root growth potential of a) *Picea abies*, b) *Robinia pseudoacacia*, c) *Pinus brutia*, and (d) *Pinus nigra* seedlings grown in 37 mm (left) and 60 mm (right) mini-plug containers at the end of the RGP test, when seedlings were 8 weeks old.

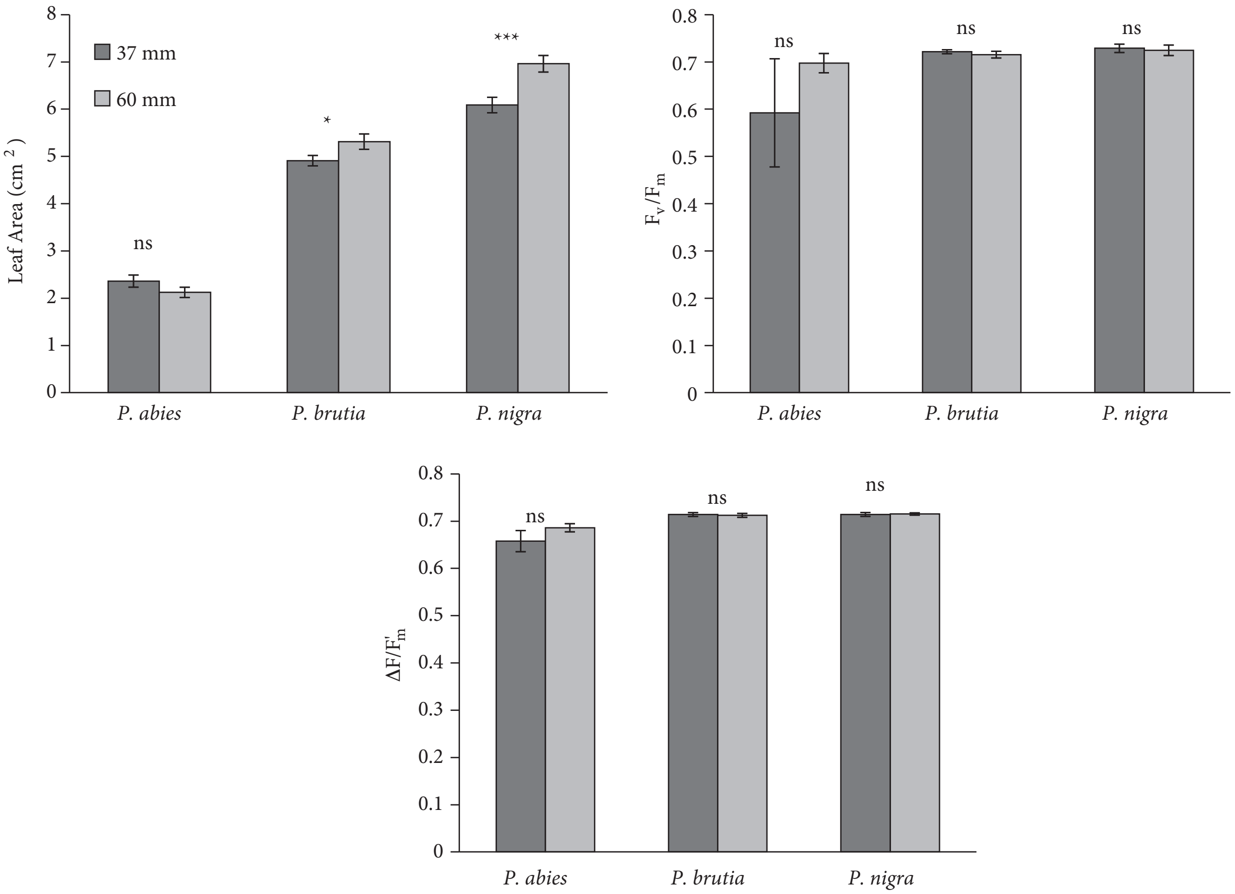

Figure 4. Leaf area, ratio of variable to maximum fluorescence (F_v/F_m), and effective quantum yield of photosystem II ($\Delta F/F'_m$) of *Picea abies*, *Pinus brutia*, and *Pinus nigra* seedlings cultivated in mini-plugs 37 and 60 mm deep at the end of the RGP test, when seedlings were 8 weeks old (***P < 0.001, **P < 0.01, *P < 0.05, ns: not significant).

specific. The knowledge of the specific demands and responses of each species is of particular importance in nursery culture. In this way, nursery cultivation regimes can strongly determine the functional characteristics of seedlings and their field performance (Villar-Salvador et al. 1999), giving the possibility to produce seedlings better adapted to special environmental conditions and thus increasing transplanting success.

During the precultivation phase and the RGP test, some of the morphological attributes tested were improved in seedlings grown in deep mini-plug containers. Container depth is believed to determine the length of the root system (Peñuelas and Ocaña 1996; Pemán et al. 2006; Al-Zalzaleh 2009). This could explain the longer root system found in seedlings of the 3 conifer species by the end of the precultivation phase. Furthermore, deep containers produced higher root and shoot biomass and LA of *P. nigra* seedlings by the end of the RGP test. This could probably be a result of the higher volume of the containers, resulting in higher water and nutrient availability for seedling growth. Small-volume containers can restrict water and nutrient availability and impose physical limitations on root system growth (Timmer and Armstrong 1989; McConnaughay and Bazzar 1991; Aphalo and Rikala 2003; South et al. 2005; Dominguez-Lerena et al. 2006). In a study with *Pinus radiata*, Ortega et al. (2006) found that seedlings grown in higher volumes per cavity (260-270 cm^3) showed significantly higher height values than seedlings grown in smaller containers (200 cm^3). Larger cell volume at a given density sustained faster growth rates of *Pinus* *contorta*, *Picea glauca*, and *Betula pendula* (Endean and Carlson 1975; Carlson and Endean 1976; Aphalo and Rikala 2003). Larger containers produced taller seedlings of *Pinus pinea* with larger diameter and biomass (Dominguez-Lerena et al. 2006). Similar findings were also reported for *Tsuga heterophylla* (O'Reilly et al. 1994), *Fraxinus americana*, *Quercus macrocarpa*, and *Q. rubra* (Cogliastro et al. 1995). South et al. (2005), in a study comparing different container types, reported that the lowest root growth potential of *Pinus palustris* was found in the container type with the smaller depth and volume (65 mm and 60 cm^3), while the greatest RGP was found in the container with the greatest depth (149 mm).

The relative allocation of resources to roots or shoots has been considered a key factor in plant strategies regarding water (Leiva and Fernández-Alés 1998) and it is very important for seedling performance and survival in the field (South 2000). However, at the end of the precultivation phase (5 weeks), the allocation patterns between above- and below-ground resources in the 4 species tested were unaffected by the type of container. Similar results were also reported for other species (Aphalo and Rikala 2003; Chirino et al. 2008).

It has been previously shown that new root growth of conifer seedlings largely depends on current photosynthesis (van den Driessche 1987; Dickson and Tomlinson 1996; Noland et al. 1996); most conifers are unable to efficiently mobilise reserve carbohydrates for root growth (Simpson and Ritchie 1996). Nevertheless, in our study, the effect of container depth on seedling photochemical efficiency or chlorophyll content was absent or negligible. Therefore, whenever a better root development was found, this was probably a result of different factors affecting seedling photosynthetic efficiency other than photochemistry.

Apart from seedling morphology and physiology, it is important to be able to predict seedling performance after transplanting. Root growth potential is generally considered as a measure of seedling vigour (Simpson and Ritchie 1996) and it often predicts seedling field performance in relation to overcoming planting stress (Grossnickle 2005). The ability of a species to develop new roots after transplanting is important, especially for areas like the Mediterranean, where drought stress is common (Plourde et al. 2009). Container depth has been found to affect the depth of the root system positioning after transplanting (Peñuelas and Ocaña 1996); a deeper positioning of the root system could help to avoid or reduce the competition for water with herbaceous plants in the top soil horizons, which can be an important factor limiting seedling performance in the field (Grossnickle 2005). In their study with *Quercus suber*, Chirino et al. (2008) found that seedlings cultivated in deep containers were the first to reach the deepest substrate layers and showed a higher number of new roots and more biomass at lower depths.

On the contrary, our RGP test results showed that *P. abies* root growth potential was favoured by the use of shallow mini-plug containers. This species appeared to have a very slow growth rate compared to the other species tested. Due to its slower growth, its roots probably reached the cavity bottom of the shallow container faster compared to the deeper container. At the cavity bottom, roots become air pruned, resulting in the formation of secondary roots at the top of the plug (Ortega et al. 2006) and leading to increased RGP values. Small containers are reported to produce a denser root system in some cases (Benoit de Coignac and Gruez 1987; Dominguez-Lerena et al. 2006). In their study, Pemán et al. (2006) found that the shortest tested container (200 mm) caused more intense root pruning of *Quercus ilex* seedlings, resulting in a greater length of roots with a diameter above 4.5 mm and a higher average root diameter. Furthermore, in a study testing different cavity sizes, Sword Sayer et al. (2009) found that the seedling size of *Pinus palustris* was increased by chemical root pruning only when small cavities were used. The observed increase in RGP of *P. abies* seedlings when grown in shallow containers could limit the drought risk common in Mediterranean regions after transplanting (Plourde et al. 2009).

Results similar to those for *P. abies* were also obtained in *R. pseudoacacia* at day 12 in the RGP test, where both RL and RDW were higher when seedlings were grown in shallow mini-plug containers. *R. pseudoacacia* is a fast growing species; it seems to be initially favoured by the use of shallow containers, because more roots can be developed and thus measured from the root plug at 12 days. But due to its faster growth rate, this difference is gradually lost in time and, therefore, both container types produce similar root systems in terms of length and dry weight. In addition, *R. pseudoacacia* is considered to be sensitive to interference from low grasses and herbaceous plants (Bridgen 1992; Hanover 1992). Therefore, higher shoots of this species produced with the use of deep mini-plug containers may offer seedlings an additional advantage after outplanting.

The use of shallow containers also resulted in the production of a longer root system of *P. brutia* seedlings, while the use of deeper containers resulted in improved above-ground characteristics of this species. Seedlings produced in shallow containers could be more suitable for transplanting to areas with dry soils, since a deep root system enables plants to access deeper soil water reserves, a factor considered critical for plant survival during summer droughts in Mediterranean areas (Canadell and Zedler 1995; Canadell et al. 1996; Costa et al. 2004). On the other hand, seedlings produced in deep mini-plug containers could be more suitable for areas in which water is not a limiting factor and weed competition is the most critical factor in initial seedling establishment and performance.

Conclusions

The results of this study confirm the importance of new investigation in considering container form and seedling production. This study demonstrates that precultivation of *Pinus nigra* and, to a lesser extent, *Robinia pseudoacacia* in deep mini-plug containers improves seedling morphological attributes and quality in general. For precultivation of *Picea abies* seedlings in mini-plugs, the use of shallow containers is recommended. In addition, according to our results, shallow mini-plug containers could be more promising for the production of *Pinus brutia* planting stock to be transplanted in dry areas, while deep containers show promise for producing seedlings to be transplanted in areas with strong weed competition. However, we think that this research needs to be continued at the scale of experimental plots in the field for a definitive assessment of the technique.

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