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Effect of Container Design on Plant Growth and Root Deformation of Littleleaf Linden and Field Elm

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Abstract. **This experiment investigated the effect of different container design on growth and root deformation of littleleaf linden (*Tilia cordata* Mill.) and field elm (*Ulmus minor* Mill.). The trial was carried out over two growing seasons (2008 to 2009). In April 2008, 1-year-old bare-root seedlings of the two species were potted in three types of 1-L containers: Superoots® Air-Cell™ (The Caledonian Tree Company, Pathhead, UK), Quadro fondo rete (Bamaplast, Massa e Cozzile, Italy), and smooth-sided containers. At the beginning of the second growing season, the same plants were repotted in the following 3-L containers: Superoots® Air-Pot™ (The Caledonian Tree Company), Quadro antispiralizzante (Bamaplast), and smooth-sided containers. At the end of each growing season, a subset of the plants from each container type was harvested to determine shoot and root dry mass and root deformation (by dry weight of root deformed mass relative to the whole root mass). Chlorophyll fluorescence and leaf chlorophyll content were measured during the second growing season. For both species, at the end of first growing season, the poorest root architecture was observed in the smooth-sided containers, whereas Superoots® Air-Cell™ and Quadro fondo rete both reduced the percentage of deformed root mass. At the end of the second growing season, plants of both species grown in Superoots® Air-Pot™ showed less deformed root mass, whereas Quadro antispiralizzante provided good results only in littleleaf linden. A reduction of field elm root biomass and littleleaf linden shoot biomass was observed at the end of the trial in plants grown in Superoots® Air-Pot®. Plants grown in these containers showed less leaf chlorophyll content compared with plants grown in smooth-sided containers at the end of the second year.**

Nursery container design affects post-transplant growth of several species (Arnold and McDonald, 2006; Gilman, 2001; Struve, 1993). Plants grown in smooth-sided plastic containers for a long production cycle result in deformed roots (Gilman et al., 2003; Ruter, 1994) because lateral roots cannot extend horizontally and therefore they either circle within the container or grow vertically to the bottom (Lindström and Rüne, 1999). The impact of an unevenly spread and undistributed root system on the juvenile anchorage of a tree is well documented (Balisky et al., 1995; Lindgren and Örlander, 1978; Lindström and Håkansson, 1995). Deformed root systems can contribute to long-term tree growth problems in the landscape such as instability (Nichols and Alm, 1983), reduced shoot growth, tree decline, and mortality (Ortega et al., 2001, 2006). Tree survival and growth after outplanting are directly related to the ability of the root system to rapidly produce new roots that will grow into the surrounding soil; thus, plants are highly affected by initial shoot and root morphology (Paz, 2003; Schultz and Thompson, 1997; Tsakaldimi et al., 2005). Circling roots developing at the bottom of a container commonly fail to grow into the soil profile resulting in reduced root growth. Root deformation is common in plug-grown seedlings after planting (Blanusa et al., 2007; Landis et al., 1990). Loblolly pine (*Pinus taeda* L.) seedling growth after transplanting was significantly reduced when trees with malformed tap roots were planted (Harrington and Howell, 1998).

Mechanical remediation of circling roots at transplanting has become a standard practice, although correcting root malformation can cause transplant shock during field establishment (Arnold and Struve, 1989; Harris, 1992; Struve, 1993). Despite the short-term stress induced by root pruning, correcting root malformation is critical to successful long-term establishment of container-grown nursery stock (Chalker-Scott, 2005, Franco et al., 2006; Gilman et al., 2002; Kozlowski and Pallardy, 1997). Mechanical remediation of circling roots is typically accomplished at transplanting by making several cuts on the root ball periphery or splitting and splaying the bottom two-thirds of the root ball (butterfly pruning) (Gouin, 1983; Harris, 1992). Recently, root shaving has been introduced as a method of correcting root malformations (Gilman et al., 2010b).

To reduce the incidence of deformed roots induced during container production, many alternative container types have been designed (Appleton and Whitcomb, 1983, Gilman et al., 2010a). These containers use air root pruning, specialized container shapes, bottomless containers, woven or non-woven fabrics, mechanical deflection, or chemicals to control root growth (Brass et al., 1996; Gilman et al., 2003; Marshall and Gilman, 1998). Several studies indicate that these methods can improve the root system architecture avoiding mechanical root pruning at planting and long-term problems on plant growth (Arnold, 1996; Gilman et al., 2010b; Marshall and Gilman, 1998; Struve, 1993; Tsakaldimi et al., 2005; Tsakaldimi and Ganatsas, 2006).

When porous-walled plastic containers are used, root growth is stopped at the wall–substrate interface (Privett and Hummel, 1992): the tip roots reach the container wall hole and are desiccated (air-pruning). As a result of the loss of root apical dominance, more fine roots develop in the inner part of the root ball, allowing a more even root system (Marler and Willis, 1996). Air-pruning technology can be combined with mechanical deflection by including vertical ribs on the interior container surfaces (Rune, 2003).

Field elm and littleleaf linden are two widespread woody plants in Italy; however, no study about the influence of container typology on root conformation of these two species has been reported in the literature. Thus, the objective of this experiment was to evaluate the effect of three different containers on plant growth and root architecture of these two species. To provide further insight on the effect of container shape on plant stress, chlorophyll content and chlorophyll fluorescence were also studied.

Materials and Methods

First year experiment. The study was carried out in an experimental nursery at the

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Fondazione Minoprio located in Vertemate con Minoprio (Como, Italy; lat. 45°44' N, long. 9°04' E, elevation 342 m). A hoophouse (2.5 m tall in the middle) covered with antihail netting (10% of photosynthetically active radiation reduction) was used to grow the plants. Overhead irrigation was applied daily to maintain water content of the containers at near 100% water-holding capacity according to Sammons and Struve (2008). Irrigation was not supplied when rainfall exceeded 15 mm during a 24-h period.

One-year-old bare root seedlings of littleleaf linden and field elm purchased from a commercial nursery were used. Container substrate consisted of a 4:1 (v/v) mixture of sphagnum peat and pumice amended with 4 kg·m^{-3} of calcium carbonate; a controlled-release fertilizer, Ficote® (15N–3.5P–10K; 8- to 9-months formulation at 20 °C; Scotts, Marysville, OH), was preincorporated at the rate of 4 kg·m^{-3}. Weeds were controlled by periodic hand weeding.

In April 2008, plants were potted in three different 1-L containers filled with 0.9 L substrate (Fig. 1). No roots were pruned at planting. Containers tested were: 1) smooth-sided square container (Stop quadro; ARCA spa, Osio Sotto, Italy); 2) Superoots® Air-Cell™ (The Caledonian Tree Company, Pathhead, UK), a container characterized by cuspated walls made up of closed inward pointing cones and open-ended outward pointing cones and the container bottom consisted of a plastic net (1 cm × 1 cm); 3) Quadro fondo rete (Bamaplast, Massa e Cozzile, Italy), a square container with eight vertical interior ribs (two ribs per container wall) and a plastic net bottom (0.5 cm × 0.5 cm). Containers used were made of black plastic.

The experiment was set as a complete randomized block design. There were 40 containers in each of 12 blocks for a total of 480 plants per species. Within each block, containers were arranged in eight rows of five plants at a density of 25 plants/m^2.

Plant growth and root morphology were determined at the end of the growing season (Sept. 2008) on five plants per block and species, a total of 60 plants per species. Substrate was removed from the roots using compressed air; after cleaning, for each root, when deformations were detected (i.e., circling or ascending parts), the deformed root part was manually separated from the non-deformed root part at the first point of deflection. For each plant deformed roots, non-deformed roots and aboveground parts were oven-dried separately (104 °C) until constant weight (≈72 h), and then dry weights were recorded. The percentage of deformed root mass relative to the entire root mass was used as the determinate of root system quality where low deformed root percentage indicates a high-quality root system.

Percentage values were transformed through the formula: arcsine $\sqrt{x}$ (where x is the percentage value divided by 100) before data were subjected to one-way analysis of variance using SPSS® statistical package for Windows (Version 17.0; SPSS Inc., Chicago, IL). Data from the two species were analyzed independently. Differences between means were declared statistically different using Duncan's multiple range test ($P \leq 0.05$).

Second year experiment. The trial was carried out in the same hoophouse using similar cultural practices. Plants grown in 1-L containers during the first growing season were repotted into a similar type but larger containers (3 L) in May 2009 (Fig. 2). Three-liter Quadro antispiralizzante (Bamaplast), characterized by a square shape, four vertical ribs on the inside walls, and a wholly closed plastic bottom, was used instead of the 1-L Quadro fondo rete; a 3-L circular smooth-sided container (Cultistop; ARCA spa) was used instead of the 1-L square smooth-sided container. Plants grown in 1-L Superoots® Air-Cell™ were repotted into 3-L Superoots® Air-Pot™ (The Caledonian Tree Company).

All the containers were filled with 2.8 L of substrate and no roots were pruned at planting. Substrate composition was the same as the previous year.

The experimental design was a complete randomized block design with 35 containers per species in each of 12 blocks for a total of 420 plants per species. In each block, containers were arranged in seven rows of five plants at a density of 11 plants/m^2.

Data regarding plant growth and root morphology were collected like in Fall 2008. Moreover, during the growing season, an index of leaf greenness was made and chlorophyll fluorescence (CF) was determined. Leaf greenness index was measured with a chlorophyll meter (SPAD-502; Minolta, Osaka, Japan) on five randomly chosen plants per block and replicated in June, July, and August in littleleaf linden and in July, August, and October for field elm. A clear relationship between SPAD readings and total leaf chlorophyll concentrations was reported by Percival et al. (2008). Chlorophyll fluorescence was measured on five randomly selected plants per block and species in concomitance of leaf greenness index evaluation. Leaves were adapted to the dark for 30 min before CF measurements using shading clips. Determinations of CF were carried out using a portable fluorometer (Plant Efficiency Analyser; Handy Pea, Hansatech Ins., Norfolk, U.K.) and recorded for up to 1 s with data acquisition every 10 µs for the first 2 ms, and every 1 ms thereafter, with 12-bit resolution (Strasser et al., 2000). The fluorescence transients were induced by red light (peak at 650 nm) at 600 µmol photons/m^2/s provided by an array of six light-emitting diodes. All measurements were done on youngest fully expanded leaves. On the application of a saturating flash of actinic light, fluorescence raises from the ground state value (Fo) to its maximum value, Fm. This allows the determination of the maximal quantum yield of PSII (Fv/Fm) (Pinior et al., 2005). Fluorescence values at time intervals corresponding to the step O-J-P were recorded and used as original data in the OJIP-test (Strasser and Tsimilli-Michael, 2001). These include the Fm, the fluorescence intensity at 50 µs (F_0), 100 µs (F_{100}), 300 µs (F_{300}), 2 ms (step J), and 30 ms (Step I).

Data analyses were similar to the previous year.

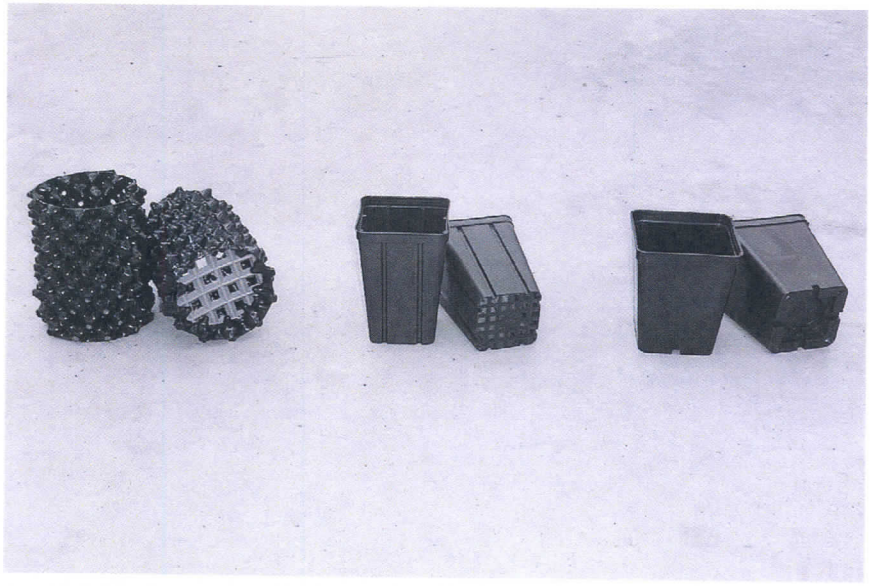

Fig. 1. From left to right (1-L containers): Superoots® Air-Cell™ (The Caledonian Tree Company, Pathhead, UK), Quadro fondo rete (Bamaplast, Massa e Cozzile, Italy), and smooth-sided square container (Stop quadro; ARCA spa, Osio Sotto, Italy).

Results and Discussion

Littleleaf linden. At the end of the first growing season (when plants were grown in 1-L containers), the highest percentage of deformed roots, more than one-fourth of total root weight, was observed when plants were grown in traditional smooth-sided containers, whereas there were no differences in the percentage of deformed roots between plants grown in Superoots® Air-Cell™ and Quadro fondo rete containers (Table 1). At the end of

the second growing season (when plants were grown into 3-L containers), littleleaf linden grown in traditional smooth-sided containers continued showing the highest percentage of deformed roots, whereas no differences were observed between the two other container types: Superoots® Air-Pot™ and Quadro antispiralizzante (Table 2).

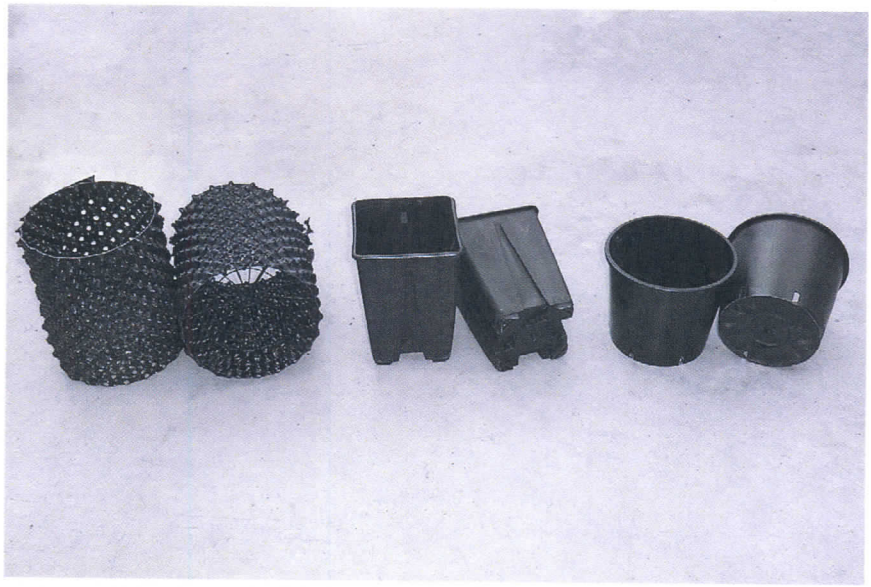

Fig. 2. From left to right (3-L containers): Superoots® Air-Pot™ (The Caledonian Tree Company, Pathhead, UK), Quadro antispiralizzante (Bamaplast, Massa e Cozzile, Italy), and circular smooth-sided container (Cultistop; ARCA spa, Osio Sotto, Italy).

Table 1. Shoot and root dry biomass (g) and percentage of deformed roots on relative to total root biomass (w/w) in littleleaf linden and field elm seedlings grown for one season (2008) in three 1-L containers.

Species and container typology	Shoot biomass (g)	Root biomass (g)	Deformed roots (%)
Littleleaf linden			
Superoots® Air-Cell™	12.7	14.6 a[z]	13.2 b
Quadro fondo rete	13.7	11.4 b	15.1 b
Smooth-sided square container	11.9	14.0 a	26.2 a
Significance	NS	*	**
Field elm			
Superoots® Air-Cell™	13.4	9.5	11.3 b
Quadro fondo rete	12.8	9.0	17.1 b
Smooth-sided square container	12.5	8.8	26.8 a
Significance	NS	NS	**

[z]For each species, means within the same column followed by different letters are significantly different from each other using the Duncan's mean separation test.
NS, *, **, *** indicates non-significant, significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively.

Table 2. Shoot and root dry biomass (g) and percentage of deformed roots on relative to total root biomass (w/w) in littleleaf linden and field elm seedlings grown for two seasons (2008 to 2009) in different containers.[z]

Species and container typology[y]	Shoot biomass (g)	Root biomass (g)	Deformed roots (%)
Littleleaf linden			
Superoots® Air-Pot™	35.9 b[x]	38.7	18.3 b
Quadro antispiralizzante	47.1 a	40.8	19.3 b
Circular smooth-sided container	41.9 a	40.7	34.6 a
Significance	**	NS	***
Field elm			
Superoots® Air-Pot™	66.6	39.4 b	25.0 c
Quadro antispiralizzante	76.1	50.4 a	48.0 b
Circular smooth-sided container	77.9	44.7 ab	58.9 a
Significance	NS	*	***

[z]Plants grown in 1-L containers during the first growing season were repotted in similar but larger (3-L) containers at the beginning of the second growing season (2009).
[y]In the 2009 growing season, Superoots® Air-Pot™; Quadro antispiralizzante and circular smooth-sided containers were used instead of Superoots® Air-Cell™, Quadro fondo rete, and square smooth-sided containers, respectively.
[x]For each species, means within the same column followed by different letters are significantly different from each other using the Duncan's mean separation test.
NS, *, **, *** indicates non-significant, significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively.

Our study show that air-pruning technology and mechanical impediments on the inside walls of container can be a useful tool to limit root deformation in container-grown littleleaf linden. The positive effect of air-pruning on root system architecture was also reported by Moore (2001) for Australian tree species grown in 20-cm diameter Superoots® Air-Pot™, whereas Marler and Willis (1996) reported that air-root pruning containers (Root-Builder® panels; Lacebark, Inc., Stillwater, OK) produced a more fibrous root system than conventional containers.

Shoot biomass was unaffected by the container design at the end of the first growing season (Table 1), whereas at the end of the second season, the lowest value was observed on plants grown in Superoots® Air-Pot™ (Table 2). At the end of the first year, the lowest root biomass was observed in plants grown in Quadro fondo rete (Table 1), whereas no differences were observed between plants grown in traditional container and Superoots® Air-Cell™. Root biomass at the end of the second year was unaffected by the container typology (Table 2). As reported by Ortega et al. (2006), seedlings grown in containers that permit air pruning had less growth and lower biomass production. This can be attributed to the moderate plant stress resulting from root tip replacement associated with new root regeneration. Moreover, Superoots® containers are designed to introduce air into substrate through both container walls and bottom net, whereas Quadro antispiralizzante is air-opened through the bottom net. This allows more water to evaporate from the substrate periphery (Arnold and McDonald, 2006; Owen and Stoven, 2008). As reported by Amoroso et al. (2010), a dry substrate is warmer in comparison with a well-watered substrate during the day, and this can lead to plant stress. Root tip replacement in plants grown in Superoots® Air-Pot™ and water evaporation through the open-air container walls could explain the observed lower plant growth.

In littleleaf linden, the container type did not affect chlorophyll content or chlorophyll fluorescence (Fv/Fm), except on 11 Aug. when plants grown in Superoots® Air-Pot™ showed a lower chlorophyll content compared with plants grown in smooth-sided containers (Table 3). Indeed, the leaves of these plants were more yellow and senescent. This finding can also be explained by the moderate plant stress resulting from porous walls and bottom in Superoots® Air-Pot™ containers. At every assessment, no differences in chlorophyll content were observed between plants grown in Quadro antispiralizzante and smooth-sided containers (Table 3). In this species, the polyphasic shape of the cholorophyll *a* fluorescence was unaffected by the container design at each assessment (thus, only the last measurement is reported in Figure 3).

Field elm. Also in this species, root architecture was affected by the container design by the end of the first growing season, when plants were grown in 1-L containers (Fig. 4). Plants grown in traditional smooth-sided containers

had poorer root architecture in comparison with plants grown in Superoots® Air-Cell™ and Quadro fondo rete containers (Table 1). At the end of the second growing season, field elm grown in traditional smooth-sided containers showed the highest percentage of deformed roots, whereas plants grown in Superoots® Air-Pot™ had significantly lower root deformation compared with field elm grown in Quadro antispiralizzante (Table 2). The vigorous root system of this species can explain the lower effect of Quadro antispiralizzante on root architecture in comparison with Superoots® Air-Pot™; indeed, as reported by Lindström (1994) and Lindström and Håkansson (1995), plants with a vigorous root system (i.e., *Pinus* spp.) grown in containers with vertical interior ribs reduced root spiraling when compared with plants grown in traditional smooth-walled containers but offered limited improvement in root structure.

In both years, field elm shoot biomass was unaffected by the container design (Tables 1 and 2). At the end of the second year, plants grown in Superoots® Air-Pot™ had the lowest root biomass compared with plants grown in Quadro antispiralizzante and traditional smooth-sided containers, whereas no differences among containers were observed during the first growing season (Tables 1 and 2). As observed in littleleaf linden, root tip replacement in plants grown in Superoots® Air-Pot™ and water evaporation through the open-air container walls and bottom net could explain the premature leaf senescence of the plants.

Plants grown in Superoots® Air-Pot™ had also lower chlorophyll content at the end of the growing season compared with plants grown in smooth-sided containers at the last assessment (6 Oct.) in addition to lower Fv/Fm ratio (Table 4). As reported in the literature, Fv/Fm ratio is a reliable indicator of the occurrence of environmental stresses, including water stress, on PS II of several woody and herbaceous species (Angelopulos et al., 1996; Lazár, 2006; Maxwell and Johnson 2000; Percival, 2005; Percival and Fraser, 2001; Percival et al., 2006; Yamada et al., 1996). This finding was also confirmed by OIJP chlorophyll *a* fluorescence transients, which provided evidence that field elm grown in Superoots® Air-Pot™ was more stressed at the end of the growing season, showing moderate changes in the redox state of plastoquinone (JI-phase) and of the acceptor side of PSI (IP-phase) (Fig. 5) (Tóth et al., 2007). The polyphasic shape of the cholorophyll *a* fluorescence was unaffected by the container design in the two previous assessments (data not shown).

Table 3. Chlorophyll content (Chl, unit SPAD) and chlorophyll fluorescence (Fv/Fm) in littleleaf linden grown in three different containers.

	25 June 2009		17 July 2009		11 Aug. 2009	
Container typology	Chl	Fv/Fm	Chl	Fv/Fm	Chl	Fv/Fm
Superoots® Air-Pot™	32.4	0.787	33.8	0.793	31.8 b[z]	0.741
Quadro antispiralizzante	33.9	0.783	34.3	0.799	32.2 ab	0.752
Circular smooth-sided container	34.5	0.772	37.4	0.795	35.7 a	0.744
Significance	NS	NS	NS	NS	*	NS

[z]Means within a column followed by different letters are significantly different from each other using the Duncan mean separation test.
NS, * indicates non-significant, significant at $P \leq 0.05$, respectively.

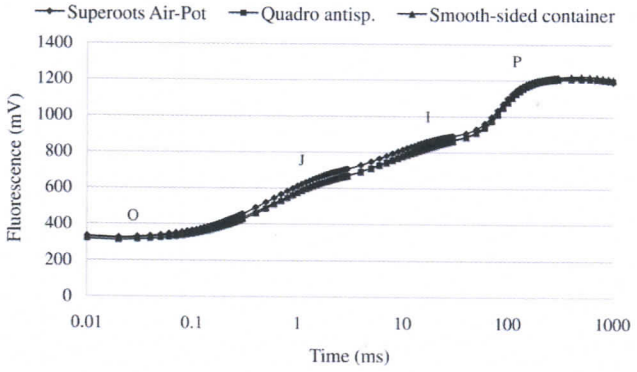

Fig. 3. Chlorophyll *a* fluorescence induction curves in littleleaf linden grown in three different containers. Data collected on 11 Aug. 2009. Each single curve represents the average of 20 independent chlorophyll fluorescence measurements.

Conclusion

The results of this study suggest that the container typology has a strong influence on root system conformation in the two tested species. Plants grown in traditional smooth-sided containers showed the highest percentage of deformed roots compared with plants grown in containers with air-pruning or mechanical impediments. As reported in the literature, a poor root conformation can seriously reduce seedling quality after outplanting in several species (Day and Parker, 1997; Landis et al., 1990; Lindström and Håkansson, 1995; Tsakaldimi et al., 2005). In contrast, a well-developed and well-structured root system is an essential attribute of quality of seedlings (Day and Parker, 1997; Tsakaldimi et al., 2005). Moreover, as suggested by Livingston (1990), another negative effect of root deformation is deteriorated plant vitality resulting from root disease caused by fungal infections. Finally, the ultimate consequence of root deformation can be an uprooting resulting from weak root anchorage (Lindström and Håkansson, 1995). The moderate lower root biomass observed at the end of the experiment in field elm grown in Superoots® containers can be repaid by a better plant root quality. To obtain field elm or littleleaf linden characterized by a well-structured root system, the use of traditional smooth-sided containers should be avoided in the nursery

Fig. 4. (**A**) Rootball of field elm grown in 1-L Superoots® Air-Cell™ (The Caledonian Tree Company, Pathhead, UK) at the end of the growing season. (**B**) Rootball of field elm grown in 1-L Quadro fondo rete (Bamaplast, Massa e Cozzile, Italy) at the end of the growing season. (**C**) Rootball of field elm grown in 1-L smooth-sided square container (Stop quadro; ARCA spa, Osio Sotto, Italy) at the end of the growing season.

Table 4. Chlorophyll content (Chl, unit SPAD) and chlorophyll fluorescence (Fv/Fm) in field elm grown in three different containers.[z]

	8 July 2009		7 Aug. 2009		6 Oct. 2009	
Container typology	Chl	Fv/Fm	Chl	Fv/Fm	Chl	Fv/Fm
Superoots® Air-Pot™	39.7 a[y]	0.783	36.7	0.761	34.6 b	0.798 b
Quadro antispiralizzante	38.8 a	0.768	39.0	0.750	40.0 a	0.811 ab
Circular smooth-sided container	34.6 b	0.779	36.5	0.762	40.4 a	0.823 a
Significance	*	NS	NS	NS	**	**

[z]Data collected three times during the second growing season.
[y]Means within a column followed by different letters are significantly different from each other using the Duncan mean separation test.
NS, *, ** indicates non-significant, significant at $P \leq 0.05$, $P \leq 0.01$ respectively.

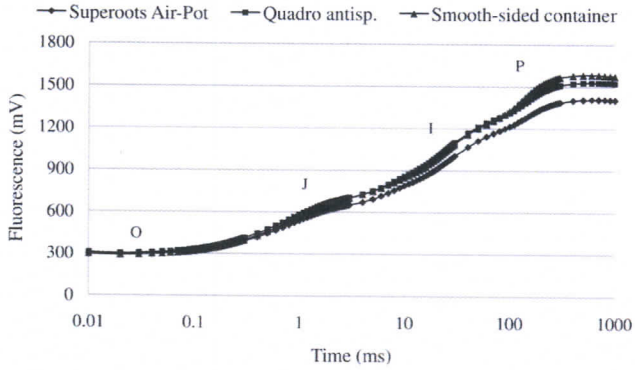

Fig. 5. Chlorophyll *a* fluorescence induction curves in field elm grown in three different containers. Data collected on 6 Oct. 2009. Each single curve represents the average of 20 independent chlorophyll fluorescence measurements.

phase. However, transplanting studies need to be conducted to determine if higher plant root quality increases transplant success or speeds establishment in the tested species.

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