Improvements in the Root Morphology, Physiology, and Anatomy of *Platycladus orientalis* Seedlings from Air-root Pruning

Zhipei Feng and Xitian Yang
College of Forestry, Henan Agricultural University, Zhengzhou 450002, Henan, China

Hongyan Liang
College of Forestry, Henan Agricultural University, Zhengzhou 450002, Henan, China, and Sanmenxia Polytechnic, Sanmenxia 472000, Henan, China

Yuhua Kong
College of Forestry, Henan Agricultural University, Zhengzhou 450002, Henan, China

Dafeng Hui
Department of Biological Sciences, Tennessee State University, Nashville, TN 37209

Jiabao Zhao, Erhui Guo, and Beibei Fan
College of Forestry, Henan Agricultural University, Zhengzhou 450002, Henan, China

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Abstract. Air-root pruning (AP) has been identified as an effective technique for enhancing root growth and development. However, little information is available regarding the temporal changes in the root system of *Platycladus orientalis* (L.) Franco under AP. We performed integrated morphological, physiological, and anatomical analyses of the roots in *P. orientalis* seedlings that had been air-root pruned for 120, 150, and 190 days. Our results found that the whole root length, number of root tips, and root surface area of AP seedlings at 120, 150, and 190 days were higher than those of the non–root-pruned (NP) seedlings (*P < 0.05*), but the average root diameter did not differ significantly between the treatments. Compared with NP treatment, AP increased the root length, surface area, number of tips, and specific root length of *P. orientalis* during the experimental periods (*P < 0.05*), but those of 0.5- to 1-mm-diameter roots were only increased on day 190 (*P < 0.05*). The AP plants also exhibited higher root vitality and proportion of live fine roots than the NP plants (*P < 0.05*). Our anatomical evaluation of the ≤0.5 mm roots and taproots revealed features that could account for the morphological differences found between the AP and NP plants. In conclusion, our results indicate that air-root pruning induced changes in the roots that promote the root system development in *P. orientalis* compared with the NP treatment during the experimental period. These results thus provide experimental evidence to support the use of AP in *P. orientalis* seedlings.

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Root structure is crucial for plant adaptation to changing environments and plays a pivotal role in determining root functionality (Dura et al., 2015; Seago and Fernando, 2013; Sun et al., 2008). A well-developed and well-structured root system, consisting of a balanced combination of fine and coarse roots, is optimum for rapid growth and successful establishment after transplantation (Davis and Jacobs, 2005; Dey et al., 2010; Dumroese et al., 2013; Perrig et al., 2007). Restoration using container seedlings instead of bare-root seedlings has become increasingly common owing to its multiple advantages with respect to increased survival and growth benefits (Dumroese et al., 2013; Liu et al., 2016; Tsakalini et al., 2005; Villar-Salvador et al., 2012; Watson and Tombleson, 2002). For an individual plant grown in a container, however, root malformation (i.e., root spiraling, cycling, and matting) is a major limiting factor because it could result in partial or complete root death (Dumroese et al., 2013; Lindstrom and Rune, 1999; Liu et al., 2016; Ortega et al., 2006; Takoutsing et al., 2014), directly impeding a series of physiological processes (Dumroese et al., 2013). Eliminating root malformation in container seedlings is thus necessary for meeting the increased demands for vegetation restoration.

Over the past decade, AP has evolved into a particularly effective means of mitigating root malformation and regulating root growth in container-seedling production and functions by stopping root extension by exposing the roots to relatively dry air (Amoroso et al., 2010; Campbell et al., 2006; Mariotti et al., 2015; Marler and Musser, 2016; Rune, 2003; Ortega et al., 2006; Van Sambeek et al., 2016). Related experiments with *Pinus contorta* Doug. var. latifolia Engelm. and *Pinus radiata* D. Don seedlings have discovered that AP can modify root system architecture and ultimately enhance seedlings growth and survival in the field (Campbell et al., 2006; Ortega et al., 2006). Consistent with these results, Rune (2003) and Van Sambeek et al. (2016) found that AP seedlings exhibited improved lateral root growth and presented a greater root mass relative to the NP seedlings, which might confer a survival advantage after transplantation (Marler and Musser, 2016). Contrary to the positive reports of AP, Amoroso et al. (2010) documented that root biomass declined following AP due to root loss and reduced root regeneration. The observed differences between these results may be due to differences in the planting sites (Campbell et al., 2006) or the dimensions of the AP containers (Mariotti et al., 2015). The preceding studies have primarily focused on the root architecture and survival rate associated with AP. To date, little information is available on the temporal changes in root morphology, physiology, and anatomy of seedlings in response to AP. *Platycladus orientalis* (L.) Franco, a native Chinese Cupressaceae plant, is among the most intensively used cypress species for vegetation restoration projects in northern China (Jiang et al., 2014; Li et al., 2016). This indigenous species is a perennial evergreen coniferous tree with remarkable longevity that exhibits high tolerance to a diversity of adverse environmental conditions, including drought, freezing temperatures, hot weather, and nutrient imbalances (enrichment and depletion) (Jiang et al., 2014; Li et al., 2016; Wu et al., 2017; Zhang et al., 2017). Other studies have demonstrated that *P. orientalis* provided considerable ecological benefits for vegetation restoration, especially in fragile ecosystems such as arid and semiarid regions (Yu et al., 2015; Zhang et al., 2017). However, its survival rate is low due to the poorly developed root systems of container seedlings (Yang et al., 2010; 2011) coupled with the extreme environments in which they are transplanted (Cui et al., 2017). We previously found that AP could be used to develop a relatively well-grown root system that increases survival of *P. orientalis* seedlings.
compared with NP seedlings (Yang et al., 2010; 2011). Although we have also reported on considerably different biomass allocation patterns of AP and NP *P. orientalis* seedlings, the way in which the roots respond to AP has not yet been documented.

The main objectives of the present study were to 1) assess how the AP treatment influences changes in the morphology of whole root and fine roots, 2) evaluate the differences in the anatomical response of the taproots and fine roots to AP at 120, 150, and 190 d, and 3) evaluate the efficacy of AP in *P. orientalis*.

**Materials and Methods**

**Experimental facility**

This research was conducted in a greenhouse at the forestry ecological experimental station of Henan Agricultural University (lat. 34°43′N, long. 113°42′E), Henan Province, China. The study site conditions during the entire growth period were as follows: air temperatures ranged from 22 to 33 °C, the relative humidity was between 50% and 65%, and there was ≈12 to 14 h of daylight.

**Experimental design and plant material**

The *P. orientalis* seeds for this research were collected by the Jiyuan City Forestry Administration of Henan Province in China from Oct. to Nov. 2015. The seeds were transported to the forestry ecological experimental station of Henan Agricultural University (lat. 34°43′N, long. 113°42′E), Henan Province, China. The seeds were surface disinfected with 3% H2O2 for 30 min, rinsed several times in sterile water, and soaked in distilled water for 48 h. Then, the sterilized seeds were germinated in a plastic pot over a period 15 to 20 d at room temperature (18/24 °C, night/day). The germination rate was ≈94%. After germination, the *P. orientalis* seeds were sown into soil blocks of 10 cm × 10 cm × 10 cm (length × width × height) and ordinary plastic containers of the same dimensions. Each treatment had 180 seedlings. The soil blocks were specially designed for use in an AP system (Yang et al., 2010). The seedlings grown in the soil blocks were thus considered as the AP-treated plants, while those growing in ordinary plastic containers were the NP plants (Fig. 1). The growth medium for both treatments contained a 2:1:1:1 (by volume) mixture of organic soil, sand, loam, and vermiculite with 5 g L−1 of a slow-release 6:18:14 (N–P–K) water-soluble fertilizer and 1 g L−1 of a binding agent (Yang et al., 2010; Yang et al., 2011). All seedlings were evenly arranged on wire mesh (pore size 2 mm × 2 mm) placed 20 cm above the ground (Fig. 1), following a randomized complete block design. The planting space was 5 cm × 5 cm on the screen (Fig. 1). To ensure the same water availability, the growth medium water content was measured daily with a Theta Probe soil moisture sensor ML2x (Delta-T Devices, Cambridge, UK), and each treatment was watered to a certain weight every 2 d. Pest management and weed control were practiced during the growth period of *P. orientalis*. It took 30 d for the roots of seedlings in the soil blocks to grow beyond the growing medium and show AP effects (Feng et al., 2017). Healthy seedlings with an approximately uniform height were selected as the testing samples on the 120th, 150th, and 190th days during the AP period.

**Root sampling and measurements**

**Root sampling and measurements of root morphology.** Nine *P. orientalis* seedlings in each treatment were harvested, numbered, and wrapped in sealable plastic bags to prevent water loss from 09:00 AM to 11:00 AM on the 120th, 150th, and 190th days of AP. The samples were promptly taken to the laboratory for measurements of morphological characteristics. Each plant was separated into shoots and roots at the root collar using a sharp razor blade. The roots were immersed in deionized water to remove soil particles and then washed with tap water to remove all the remaining soil. The roots were then carefully divided into the following three root diameter classes: ≤0.5 mm, 0.5–1 mm, and 1–2 mm. All sampled roots were scanned using a scanner (EPSON PERFECTION V700 Photo, Japan) with a transparency adapter at 300 dpi. The total root length (RL, cm), root surface area (RSA, cm2), average root diameter (ARD, mm), and number of root tips (RT) of each sample were directly analyzed using specific computer image analysis software (WinRhizo Pro 2007d, Regent Instruments, Canada). After root scanning, all root samples were oven-dried at 75 °C for 48 h and carefully weighed to the nearest 0.1 mg. Specific root length (SRL, m g−1) was calculated as the ratio of root length and root dry weight of the corresponding root system.

**Root vitality measurement using the triphenyltetrazolium chloride method.** Nine fresh fine root (diameter ≤2 mm) samples were used for separating live roots from dead roots and nine fresh fine roots were used for root vitality analysis for each treatment at each sampling day. The live fine roots were separated from dead roots based on color and tensile strength (McClaugherty et al., 1982; Reinmann and Templer, 2015). Root vitality analysis was used the 2,3,5-triphenyltetrazolium chloride (TTC) method according to the standard procedures of Ruf and Brunner (2003) with some modifications. This vitality assay is based on the reduction of TTC to water-insoluble reddish triphenylformazan (TF) by mitochondrial dehydrogenase activity, which is regarded as an index of root tissue vitality (Comas et al., 2000). Nine roots were sampled (≈500 mg fresh weight) from each treatment at each sampling day, including three independent replications with three individuals in each replication. The fresh root samples were overdried at 60 °C for 48 h and carefully weighed to the nearest 0.1 mg. Root

![Fig. 1. *P. orientalis* seedlings between the air-root-pruning (AP) and non-root-pruning (NP) treatments.](image-url)
the plants subjected to AP for 120, 150, and 190 d (Northampton, MA), respectively. 

Assessments of root anatomy

On each sampling day, nine taproots were carefully harvested per treatment, as close as possible to 10 cm from stem-root connection, as well as the first-order roots in the proximity of the root tips at room temperature to calculate the anatomical parameters of the root system. Each root sample was carefully dissected using forceps according to the branch order, following the approach described in Pregitzer et al. (2002) and Wang et al. (2006), regarding the distal nonwoody roots as first-order roots. Subsequently, a 5-mm-long segment of each root sample was immediately fixed for 72 h in FAA (5 mL of 37% formalin, 5 mL of 100% glacial acetic acid, 90 mL of 70% ethanol). Samples were then rinsed successively in gradient ethanol (70%, 50%, and 30%, v/v) and distilled water (30 min in each), stained with 1% safranin O for 48 h, dehydrated in a graded alcohol series (70%, 85%, 95%, 100%, v/v; 30 min each), and then embedded in cerasin wax. Ten-micrometer-thick transverse sections were sliced off from each root sample by a Leica SM 2400 microtome fitted with a dry glass knife. All the sections were dewaxed twice with dimethylbenzene for 15 min and 25 min, dehydrated with an equivalent volume mixture of ethanol and dimethylbenzene for 15 min and 5 min. Then, the stelae were dehydrated using a light microscope (BX43, Olympus, Japan) under transmitted light and were photographed using a digital camera (Camedia C4040, Olympus, Germany). All digitized photographs were observed at ×20 objective magnification. The CellSens Standard software (Olympus) was used to measure the root cross section, cortical thickness, and stele diameter. The ratio of the stele to root diameter was estimated as the stele diameter of root samples divided by the corresponding diameter.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY). A single multivariate analysis was used to assess significant differences among the treatments at the three sampling points in terms of root morphology (RL, RSA, ARD, RT, and SRL), root vitality and anatomy (root cross section, cortical thickness, stele diameter) characteristics. Means were compared for significance using the least significance difference test at a 5% significant level. All data and graphs were postprocessed in Excel 2010 and Origin (Version 8.5, OriginLab Software Corp., Northampton, MA), respectively.

Results

Root morphological characteristics of P. orientalis under AP. The RL, RT, and RSA of the plants subjected to AP for 120, 150, and 190 d were higher than those of the NP plants (P < 0.05). The RL of AP was ≈0.57, 0.37, and 0.21 times longer than that of the NP treatment on the corresponding sampling days (P < 0.05; Fig. 2A). Similarly, RT followed the same pattern, increasing from 180 to 578, and was higher in AP than in the NP treatment (P < 0.05; Fig. 2C). After 120, 150, and 190 d of the AP treatment, RSA was higher (i.e., 17.86, 38.49, and 57.20 cm²) than the NP treatment (10.07, 22.06, and 37.13 cm²) (P < 0.05; Fig. 2D). However, ARD was marginally lower in the AP treatment, but this difference was not statistically significant (Fig. 2B).

Effects of AP on fine root system morphology of P. orientalis. The AP treatment significantly stimulated the growth of the smallest fine roots (≤0.5 mm diameter) in terms of several root morphological characteristics (RAL, RT, RSA, and RSL) (P < 0.05), but this was more pronounced in the 0.5- to 1-mm roots (only at 190 d). The average RL values of the ≤0.5 mm roots ranged from 78 to 149.9 cm; RT ranged from 170 to 537; RSA ranged from 6.3 to 12 cm²; and SRL ranged from 7.3 to 9.4 m·g⁻¹ in the AP treatment from 120 to 190 d. Compared with the NP seedlings, those treated with AP had an enhanced RL for the ≤0.5 mm roots at 120, 150, and 190 d (P < 0.05; Fig. 3A). Similarly, the RT of the ≤0.5 mm roots was higher at 120, 150, and 190 d of the AP treatment when compared with the NP treatment (P < 0.05; Fig. 3D). The RSA of the ≤0.5 mm roots was 62%, 32%, and 14% higher in the AP treatment at 120, 150, and 190 d relative to the NP treatment (P < 0.05; Fig. 3G). The SRL of the ≤0.5 mm roots tended to be longer in the AP treatment compared with the corresponding roots in the NP treatment, with differences observed only at 150 and 190 d (P < 0.05; Fig. 3J). Indeed, all of the investigated morphological parameters for 0.5- to 1-mm roots under AP had higher values than those under NP at the end of the experiment (190 d) (P < 0.05), but no such significant differences were detected on days 120 and 150 (Fig. 3B, E, H, and K). No significant changes were observed for 1- to 2-mm roots (Fig. 3C, F, I, and L). Differences in the proportion of fine roots and root vitality between the AP and NP treatments. The proportion of live fine roots (≤2 mm) increased to 77%, 84%, and 87% in the AP treatment at 120, 150, and 190 d (P < 0.05; Fig. 4A). The P. orientalis seedlings showed higher root vitality in the ≤2 mm roots at 120, 150, and 190 d after AP compared with those of NP (P < 0.05; Fig. 4B).
**Discussion**

Root morphological characteristics are associated with root function (Alameda and Villar, 2012; Perrig et al., 2007; Rao et al., 2016; Tian et al., 2014). Well-developed and well-structured root systems are associated with increases in water and nutrient absorption and the photosynthetic rate (Alameda and Villar, 2012; Davis and Jacobs, 2005; Dey et al., 2010; Perrig et al., 2007), thereby positively influencing plant adaptation and field performance after transplantation (Davis and Jacobs, 2005; Dumroese et al., 2013; Marler and Musser, 2016; Rao et al., 2016). In our study, the root morphological characteristics (i.e., RL, RT, and RSA) of *P. orientalis* were significantly higher in the AP-treated seedlings than the NP seedlings during the experimental periods (Fig. 2), suggesting that root growth was improved under AP. Our findings were similar to those in *Serianthes nelsonii* Merr. (Marler and Musser, 2016) and *Quercus bicolor* Willd. (Van Sambeek et al., 2016) seedlings. Both Yang et al. (2011) and Feng et al. (2017) reported that AP had no effect on the root-to-shoot ratio of *P. orientalis* compared with the NP treatment but did increase the aboveground and total biomass (Feng et al., 2017).
Therefore, the improved root system of *P. orientalis* after AP might contribute to the higher total biomass (Close et al., 2010; Feng et al., 2017). Higher total biomass is linked to larger carbohydrate stores available (Close et al., 2010), thus suggesting the growth of the AP seedlings would be higher than NP seedlings (Tsakaldimi et al., 2013; Yang et al., 2011).

Fine roots, especially ≤0.5-mm roots, perform important physiological functions in terms of water and nutrient uptake (McCormack et al., 2015; Perrig et al., 2007). Some investigators have also found that fine roots are involved in the synthesis of certain growth hormones and associated with root adaptations for survival in changing environments (Di Iorio et al., 2016; Makita et al., 2016; Mosca et al., 2017). We found that the fine roots (≤0.5 mm) of *P. orientalis* seedlings under AP had significantly higher RL, RT, RSA, and SRL (except for 120 d) compared with NP at 120, 150, and 190 d (Fig. 3), with all responses being driven mainly by the enhanced formation of ≤0.5 mm roots. These results suggest that AP-treated seedlings of *P. orientalis* might possess a greater ability to adapt to increased competition and exploit belowground resources (Di Iorio et al., 2016; Perrig et al., 2007). In parallel, we only observed a significant increase in RL, RT, RSA, and SRL of 0.5- to 1-mm roots after AP on the 190th day, thus associating it to the long-term effect of AP. Taken together, these results suggest that *P. orientalis* can optimize its root morphology and structure via the differential alteration of fine roots under AP. This type of strategy is consistent with the findings of several published reports (Dong et al., 2016; Sudmeyer et al., 2004).

In addition to the modified root morphology, we further found that the proportion of all fine roots (≤2 mm) in *P. orientalis* was slightly stimulated by AP (Fig. 4A). This result may be interpreted as a thigmomorphogenetic response to increase plant stability (Sun et al., 2008). Additionally, the root vitality of fine roots (≤2 mm) was greater under the AP treatment (Fig. 4B), implying that AP may promote the competitive and metabolic activities of fine roots (Comas et al., 2000; Dong et al., 2016; Ruf and Brunner, 2003).

According to the anatomical investigations, AP also influenced the root (≤0.5 mm) anatomy of *P. orientalis* (Fig. 5). Changes in the layers of the root (≤0.5 mm) cortical cells by AP may alter the cortex, which might be responsible for modifications of the root diameter of *P. orientalis*. More importantly, the AP seedlings displayed a significantly shorter taproot than the NP plants (Table 1). One possible explanation for this is that the taproot was restricted under AP, and subsequently, the cortical cells of the root tips were also impaired, thereby inhibiting taproot elongation (Table 1) and thus causing an increase in the proportion of fine roots as a type of compensatory response due to the shorter taproot (Fig. 4A). Another explanation could be that the stimulation of signal molecules, such as the plant hormone indole-3-acetic acid (IAA), may have increased the proportion of fine roots of *P. orientalis* under AP. It was recently reported that high concentrations of IAA could stimulate the formation of fine roots when the taproot length was restricted (Pacholczak et al., 2017; Pierret et al., 2007; Remans et al., 2008; Spaepen et al., 2007). Furthermore, we suggest that the enlarged taproot cross-section in response to AP might be attributed to the increased cortical thickness within the root cross-sections of *P. orientalis*. In the present study, the stele diameter of the taproot remained unchanged under AP treatment for the first 120 d but increased at 150 and 190 d (Table 1). Because the root stele comprises vessels that form major conductive tissue, the proportion of the stele in root cross sections is mostly used as an indicator of water and nutrient transport potential from the root to shoots (Guo et al., 2008; Wang et al., 2016). Thicker taproots with a greater stele within a cross section are likely related to a higher potential for reducing various biotic and abiotic stresses in perennials (Alamed and Villar, 2012). These results collectively imply that air-root pruning of *P. orientalis* seedlings may provide more benefits to roots, possibly via the enhanced physiological and ecological functions, such as improved water and nutrient acquisition efficiencies (Zadzorny et al., 2015), enhanced an increased absorptive capacity.

![Fig. 5. Root cross section, cortical thickness, stele diameter, and ratio of stele to diameter at 120, 150, and 190 d of the air-root-pruning (AP) (left panel) and non–root-pruning (NP) (right panel) in *P. orientalis* seedlings. The bars indicate the standard error (n = 9). Different lowercase letters represent significant differences (P < 0.05) in root traits among treatments according to Fisher’s least significant difference test.](http://example.com/fig5.png)

Table 1. Changes in length and transverse sections of taproots on day 120, 150, and 190 by treatments.

| Variables                          | 120 d | 150 d | 190 d |
|-----------------------------------|-------|-------|-------|
| Taproot length (cm)              | AP    | NP    | P     |
| 10.11 ± 2.69 d                    | 11.78 ± 2.09 c | 0.03  |
| Cross section (mm)                | 0.44 ± 0.05 e  | 0.45 ± 0.07 de | 0.39  |
| Cortical thickness (μm)           | 137.54 ± 6.99 cd | 139.41 ± 10.75 cd | 0.73  |
| Stele diameter (μm)               | 170.62 ± 9.03 cd | 176.16 ± 2.89 cd | 0.18  |
| Ratio of stele to root diameter   | 0.48 ± 0.04 a  | 0.47 ± 0.05 b  | 0.01  |

AP = air root pruning (days 120–190); NP, non–root pruning (days 120–190). Values are mean ± SE (n = 9). Different lowercase letters in rows represent significant differences (P < 0.05) in root traits among treatments according to Fisher’s least significant difference test.
and greater mycorrhizal colonization (Mosca et al., 2017).

Conclusion

Morphological, physiological, and anatomical assessments revealed the response of *P. orientalis* roots to air exposure when grown in soil blocks. Our findings provide insights into the mechanisms associated with the reduced root growth of *P. orientalis* at an early stage of growth under AP treatment. The results suggest that *P. orientalis* seedlings of AP are likely to perform better after transplantation compared with NP plants. Further research should aim to ascertain the efficacy of this treatment following field transplantation to ensure practical application, as well as investigate the benefit of AP for other plant species in northern China.

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