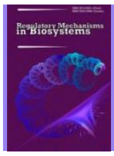

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Root system development of container-grown apple nursery trees as affected by rootstock, container type, and substrate composition

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The quality of planting material is a key factor determining the successful establishment and long-term productivity of intensive orchards. In container nursery systems, plant development largely depends on the interaction between rootstock genotype, container design, and substrate composition, which influence root system architecture, nutrient and water uptake, and biomass accumulation. The aim of this study was to determine the effect of rootstock, container type, and substrate composition on the formation of belowground and aboveground biomass of two-year-old apple nursery trees grown in container culture. The experiment included two rootstocks differing in vigor, two container types, and three substrate variants with different physical properties. Plant growth was evaluated at the end of the growing season based on root system biomass and aboveground biomass formation. The results showed that rootstock genotype significantly affected plant biomass formation. Trees grafted onto the more vigorous rootstock formed greater total biomass compared with those on the dwarfing rootstock, with differences reaching 25–35% depending on growing conditions. Container type also influenced plant development: containers with improved root-zone aeration stimulated the formation of a more branched fibrous root system and increased root mass by 15–20% compared with standard containers. Substrate composition affected the balance between root and shoot development. Substrates with higher air-filled porosity promoted more intensive root growth, whereas mixtures with higher water-holding capacity stimulated the formation of aboveground biomass. The study demonstrates that the interaction between rootstock, container type, and substrate composition plays an important role in optimizing container nursery technologies and improving the quality of planting material for intensive fruit production systems.

Keywords: container-grown apple nursery trees; root air-pruning; nursery container design; root biomass allocation; fibrous root system; peat–bark substrate; rootstock–substrate interaction; root–shoot balance.

Introduction

The quality of planting material is one of the key factors determining the stability, productivity, and longevity of modern intensive fruit orchards. The morphological and physiological characteristics of nursery trees largely determine their adaptation after transplanting into the orchard, the rate of canopy formation, and the subsequent productivity of trees (Atkinson, 2000). With the intensification of fruit production systems, the demand for technologies that enable the production of uniform planting material with a well-developed root system has increased (Savvas et al., 2013; Burnett et al., 2016; Ellis, 2024).

In modern nursery systems, the production of container-grown plants is becoming increasingly widespread, as it allows better control over plant growth, nutrient supply, and the formation of the morphological structure of nursery trees. Container-based technologies make it possible to regulate the parameters of the root environment, including substrate composition, aeration, and water-holding capacity, which directly affect plant growth and development (Gruda, 2019; Sabatino, 2020; Bulygin et al., 2025). Recent studies also confirm that substrate composition and container volume significantly influence leaf area formation, biomass accumulation, and root system development in apple rootstocks (Schafer & Lerner, 2022).

The formation of the root system under conditions of limited substrate volume depends on the complex interaction between rootstock genotype, the physical characteristics of the container, and the properties of the substrate. Root system architecture is determined both by the genetic traits of plants and by spatial constraints of the growth environment (Kormanek et al., 2023; Larrey et al., 2025). Recent studies confirm that container type can substantially alter root morphology by influencing root branching, length, and functional efficiency (Larrey et al., 2025).

In the production of container-grown plants, the problem of root spiraling becomes particularly relevant. This phenomenon occurs as a result of root contact with the container walls and may reduce the functional quality of nursery trees as well as their survival after transplanting into the soil (Gilman & Paz, 2014; Gilman et al., 2016; Dehodiuk et al., 2024). To address this issue, modern nursery production increasingly employs containers with an air-pruning system, which stimulate the formation of a more branched fibrous root system. Such technologies enhance the efficiency of water and nutrient uptake, thereby positively affecting plant biomass formation (Kim & Kim, 2011; Raviv, 2017; Majsztrik & Owen, 2020).

An important factor determining the morphological structure of nursery trees is the rootstock genotype. Apple rootstocks differ significantly in their morphogenetic potential, root system architecture, and the intensity of growth processes (Havryliuk et al., 2023; Pavlichenko et al., 2023; Ignatenko et al., 2024). In particular, the dwarfing rootstock 'M.9' forms a relatively compact root system, whereas the rootstock 'MM.106' is characterized by more vigorous growth and a more extensive root development (Atkinson, 2000). Recent studies also confirm that the rootstock genotype largely determines the formation of root architecture and the ability of plants to adapt to different growing conditions (Havryliuk et al., 2022; Ivanova et al., 2022).

Despite the considerable number of studies devoted to the container production of fruit planting material, the interaction between rootstock genotype, container type, and substrate composition, as well as their combined effect on biomass formation in apple nursery trees grown as container-grown plants, remains insufficiently studied (Larcher & Scariot, 2009; Amoroso et al., 2010; Venig et al., 2025). In particular, determining the optimal combination of these factors for the production of high-quality planting material remains an important research objective.

The aim of this study was to determine the role of rootstock genotype, container type, and substrate composition in the formation of root biomass in two-year-old apple nursery trees of the cultivar 'Champion' grown as container-grown plants.

Materials and methods

The experiment was conducted during the 2024–2025 growing seasons at the commercial nursery facilities of the "Pisardi" Garden Center located in the Bucha district of Kyiv region, Ukraine (50.5044° N, 30.1888° E). Additional laboratory analyses were performed at the Educational and Scientific Laboratory "Analytical Research in Crop Production" of the Faculty of Agrobiology, National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine).

Two-year-old apple nursery trees (*Malus domestica* Borkh.) of the cultivar 'Champion' grafted onto two rootstocks differing in vigor, namely 'M.9' (dwarf) and 'MM.106' (semi-vigorous), were used as plant material. These rootstocks were selected due to their contrasting growth characteristics and widespread use in modern intensive orchard systems.

The plants were cultivated in two container types differing in design and aeration properties. The first system consisted of Air Pot containers (Ercole SP30, 12 L capacity), characterized by perforated walls that promote air pruning of roots and enhanced aeration of the root zone. The second system involved standard plastic nursery containers C12 (model B28, 12 L capacity), which represent a commonly used conventional container in commercial nurseries.

Three substrate formulations differing in component composition were evaluated (Table 1). The first substrate consisted of high-moor peat with a particle size of 0–40 mm and a pH range of 5.5–6.5. The second substrate was prepared by mixing high-moor peat with shredded conifer bark (particle size 0–5 mm) in a 1:1 volume ratio. The third mixture included high-moor peat, conifer bark and sandy loam soil combined in a 2:1:1 proportion. These substrates were selected to represent growing media with different physical properties and aeration capacity.

Substrate mixtures were prepared using a Urbinati substrate mixer (Italy) to ensure homogeneous blending of components. To adjust substrate reaction to the optimal range for apple cultivation, liming was carried out based on agrochemical analyses performed in the NULES laboratory. The applied lime rates were 0.24 g/L for the peat substrate, 0.90 g/L for the peat–bark mixture and 0.62 g/L for the peat-bark-soil substrate.

Table 1
Experimental design for container-grown apple nursery trees ('Champion')

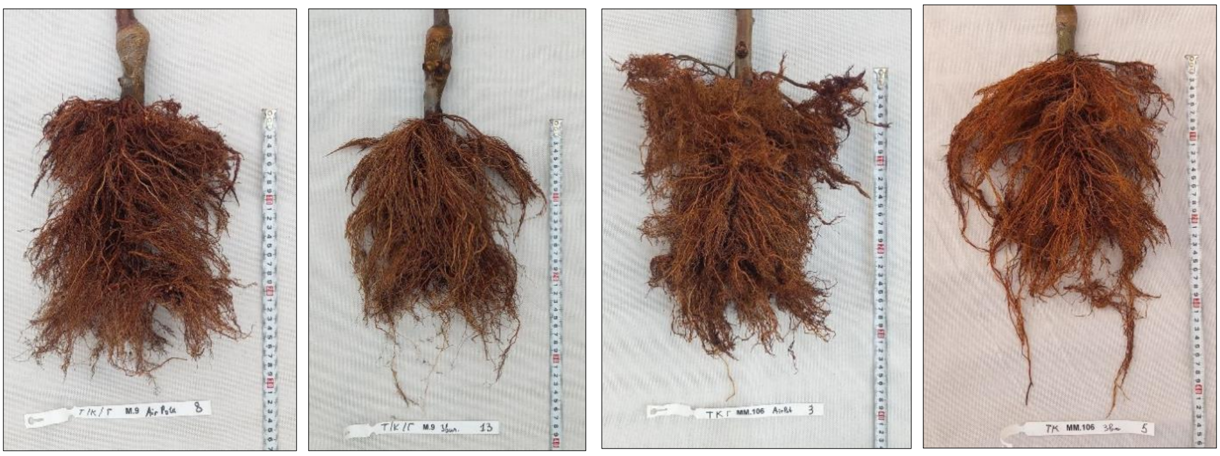
Rootstock	Container type	Substrate
MM.106 / M.9	Air Pot / C12	High-moor peat (Substrate 1)
		High-moor peat + conifer bark (Substrate 2)
		High-moor peat + conifer bark + soil (Substrate 3)

Prior to the onset of active vegetative growth, the nursery trees were maintained in a polyethylene greenhouse. In early May, the plants were acclimatized for three days under a shading net to reduce transplant stress and gradually adapt them to outdoor conditions. Subsequently, the containers were transferred to an open experimental area, where the experiment was conducted under field conditions.

The containers were connected to a drip irrigation system providing regular moisture supply to the root zone. Each plant was supported with bamboo stakes attached to the trellis to maintain upright growth. Containers were arranged according to a spacing scheme of 1.0 × 0.5 m, which ensured sufficient light interception and air circulation around the plants.

The experiment was arranged as a three-factor factorial design including the following factors: rootstock ('M.9', 'MM.106'), container type (Air Pot and C12) and substrate composition (three variants). Each treatment combination was replicated within the experimental layout.

At the end of the growing season, the plants were removed from the containers and the root systems were carefully washed to remove substrate residues (Fig. 1). The roots were then separated into diameter fractions: <1 mm, 1–2 mm, 2–4 mm, and >4 mm. The roots of each fraction were oven-dried to constant weight and weighed using laboratory scales to determine dry biomass. The total root mass was calculated as the sum of all fractions.



M.9 – Air Pot *M.9 – C12* *MM.106 – Air Pot* *MM.106 – C12*

Fig. 1. Morphology of the root system of apple nursery trees grafted onto M.9 and MM.106 rootstocks depending on the container type (Air Pot, C12)

The obtained data were processed using analysis of variance (ANOVA) to determine the significance of the effects of rootstock, container type and substrate composition, as well as their interactions. Mean values were presented as mean ± standard error (SE). Differences between treatments were evaluated using Tukey's HSD test at $P \leq 0.05$. Statistical calculations were performed using standard statistical software.

Results

The rootstock is the principal genetic factor determining the morphogenesis of the apple root system. Rootstocks differing in vigor are characterized by distinct root architecture, depth of penetration, branching density, and the proportion of root fractions of different diameters. Analysis of the fractional structure of the root system makes it possible not only to assess total biomass but also to evaluate its functional quality. It is well known that rootstocks with different growth vigor form distinct patterns of branching, density, and the proportion of fine and structural roots (Atkinson, 2000). In this context, the fractional composition of the root system of the rootstocks 'M.9' and 'MM.106' was analyzed (Table 2).

The total root system mass of the rootstock 'MM.106' was 52.54 ± 1.66 g, which was 5.75 g (≈12.3%) higher than that of 'M.9' (46.79 ± 1.35 g). This indicates a higher morphogenetic potential of

'MM.106' for the formation of belowground biomass. The most pronounced differences between the rootstocks were observed in the fractions of fine roots: in the <1 mm fraction, the root mass in 'MM.106' exceeded that of 'M.9' by 4.65 g (an increase of ≈23%), while in the 1–2 mm fraction the difference reached 3.67 g (an increase of ≈41%). These fractions constitute the main absorbing surface of the root system. Fine roots are characterized by a high specific length, active uptake of water and mineral nutrients, and rapid tissue turnover. An increase in their proportion directly enhances the physiological activity of the plant.

Table 2
Mean values of root mass by thickness fractions depending on rootstock (g, mean ± SE, n = 60)

Rootstock	<1 mm	1–2 mm	2–4 mm	>4 mm	Total mass
M.9	20.11 ± 0.78	8.92 ± 0.55	9.78 ± 0.37*	7.98 ± 0.51	46.79 ± 1.35*
MM.106	24.76 ± 1.09*	12.59 ± 0.71*	7.95 ± 0.67	7.23 ± 0.46	52.54 ± 1.66

Note: * indicates statistically significant differences between rootstocks within the same column ($P \leq 0.05$).

In the 2–4 mm root fraction, an opposite trend was observed: 'M.9' formed a slightly higher mass (9.78 vs. 7.95 g). This may indicate a different biomass allocation pattern, where under less intensive development of the fibrous component, part of the assimilated resources is redistributed toward roots of intermediate diameter. The >4 mm fraction was similar in both rootstocks (7.98 g in 'M.9' and 7.23 g in 'MM.106'), suggesting that the formation of structural transport roots at this stage of development is a relatively stable trait and less sensitive to genetic differences.

When analyzed in relative terms, the proportion of fine roots (<1 mm) in 'MM.106' accounted for approximately 47% of the total root mass, whereas in 'M.9' it was about 43%. Thus, both absolute and relative indicators confirm the greater fibrosity of the root system in 'MM.106'. The increased proportion of fine roots in 'MM.106' implies a larger contact surface with the substrate, higher capacity for water uptake, potentially improved responsiveness to mineral nutrition, and greater adaptability during the post-transplant establishment period.

From a functional perspective, the <1 and 1–2 mm root fractions largely determine the "quality" of the root system, whereas thicker roots primarily perform transport and mechanical functions. The obtained results are consistent with the findings of Atkinson (2000), who reported that more vigorous rootstocks tend to form more intensively branched root systems. According to the concept of the hierarchical organization of root systems (Guo et al., 2008), first- and second-order fine roots are the most metabolically active components. McCormack et al. (2015) further emphasize that these roots largely determine the efficiency of resource acquisition.

In the production of container-grown plants, spatial constraints and the aeration regime of the root zone are key factors determining root system architecture. The container type influences root growth patterns, branching intensity, the degree of root spiraling, and the proportion of functional root fractions. Of particular interest are containers employing air-pruning technology (Air Pot), which alter the direction of root growth and stimulate the formation of a more fibrous root structure (Table 3).

Table 3
Root mass by fractions depending on container type (g, mean ± SE, n = 60)

Container	<1 mm	1–2 mm	2–4 mm	>4 mm	Total mass
Air pot	24.97 ± 0.88*	11.83 ± 0.79*	9.60 ± 0.51*	7.80 ± 0.57*	54.20 ± 1.16*
C12	19.90 ± 0.95	9.68 ± 0.66	8.13 ± 0.60	7.41 ± 0.40	45.13 ± 1.34

Note: * see Table 2.

The total root system mass in the Air Pot container was 54.20 ± 1.16 g, which was 9.07 g (≈20.1%) higher than in the C12 container (45.13 ± 1.34 g). Such a difference is biologically significant for container-grown plants, where the limited substrate volume may restrict root development. The main increase in root mass in the Air Pot treatment occurred due to the fine root fractions: the <1 mm fraction increased by 5.07 g (≈25%), while the 1–2 mm fraction increased by 2.15 g (≈22%). Thus, more than 70% of the total root mass increase was associated with fine roots. This indicates that the container architecture directly influences the formation of the absorbing component of the root system.

The 2–4 mm root fraction was also higher in the Air Pot treatment (+1.47 g), although the difference was less pronounced than in the fine root fractions. The >4 mm fraction showed only a minimal difference (+0.39 g), confirming the relative stability of structural roots regardless of container type. In relative terms, the proportion of <1 mm roots accounted for 46% of the total root mass in Air Pot and 44% in C12. Thus, the Air Pot container not only increases the absolute root mass but also enhances the proportion of the functionally active fine-root fraction.

The Air Pot container operates according to the principle of root air pruning. When the apical meristem of a root reaches the perforated container wall and comes into contact with air, its elongation growth ceases, which stimulates the formation of lateral roots. This process results in more intensive branching, the development of a fibrous root structure, reduced root spiraling, and improved aeration of the root zone.

In a conventional C12 container, roots reaching the container wall continue to grow along its surface, forming spiral structures. Such root architecture limits the efficiency of resource uptake and may negatively affect plant establishment after transplanting.

NeSmith & Duval (1998) demonstrated that containers with an air-pruning system significantly increase the density of lateral roots in ornamental and fruit crops. O'Connor et al. (2018) reported that plants with a more fibrous root system exhibit improved establishment after transplanting. Similar results have been described in studies on container systems for woody species, which emphasize that fine roots are the most sensitive to changes in root-zone aeration (McCormack et al., 2015).

In the production of container-grown plants, the substrate determines the water-air regime, nutrient availability, bulk density, and porosity of the growing medium, which together create the conditions for root growth and branching (Carlile et al., 2015; Barrett et al., 2016). In theory, substrates with improved aeration and optimal water-holding capacity should stimulate the development of the absorbing (fine) component of the root system, whereas excessive compaction or unstable moisture conditions may limit the formation of fine roots (Freschet et al., 2021; Zhu et al., 2021). Fine roots are functionally critical because they ensure the uptake of water and mineral nutrients and largely determine the functional quality of the root system in nursery trees (Table 4).

According to the total root mass, the following gradient was observed: Substrate 1 (51.46 ± 1.90 g) ≥ Substrate 2 (50.06 ± 2.07 g) > Substrate 3 (47.47 ± 2.06 g). The difference between Substrate 1 and Substrate 3 was approximately 3.99 g (≈7.7%), which can be considered a biologically noticeable trend; however, its statistical significance requires confirmation (see integration with ANOVA below). From an interpretative perspective, the slightly higher values observed in Substrate 1 may indicate a more stable water-holding regime and more favorable aeration of the root zone, whereas Substrate 3 exhibited the lowest level of root biomass accumulation.

Table 4
Root mass by fractions depending on substrate type (g, mean ± SE, n = 40)

Substrate type	<1 mm	1–2 mm	2–4 mm	>4 mm	Total mass
High-moor peat (substrate 1)	23.91 ± 1.28[a]	10.46 ± 0.77[ab]	9.43 ± 0.60[a]	7.66 ± 0.66[a]	51.46 ± 1.90[a]
High-moor peat + conifer bark (substrate 2)	22.45 ± 1.50[ab]	11.94 ± 0.90[a]	8.54 ± 0.69[ab]	7.12 ± 0.56[a]	50.06 ± 2.07[ab]
High-moor peat + conifer bark + soil (substrate 3)	20.95 ± 1.16[b]	9.86 ± 1.08[b]	8.63 ± 0.84[b]	8.03 ± 0.59[a]	47.47 ± 2.06[b]

Note: different letters within the same column indicate statistically significant differences between treatments according to Tukey's HSD test ($P \leq 0.05$).

The mass of the finest roots was highest in Substrate 1 (23.91 ± 1.28 g), slightly lower in Substrate 2 (22.45 ± 1.50 g), and lowest in Substrate 3 (20.95 ± 1.16 g). The difference between Substrate 1 and Substrate 3 was approximately 2.96 g (≈14.1%). Since roots <1 mm in diameter are the most metabolically active and responsible for resource acquisition, their greater mass in Substrate 1 may indicate more favorable conditions for the formation of an active absorbing surface. This observation is consistent with current concepts suggesting that fine roots largely determine the efficiency of resource uptake and play a key role in plant growth under controlled environments (McCormack et al., 2015).

In contrast to the previous fraction, the highest values in the 1–2 mm fraction were observed in Substrate 2 (11.94 ± 0.90 g), whereas in Substrate 1 the value was 10.46 ± 0.77 g, and in Substrate 3 it was 9.86 ± 1.08 g. This shift in the leading treatment (Substrate 2) may indicate a structural adjustment of the root system toward roots of slightly larger diameter, possibly in response to mechanical resistance of the substrate or differences in moisture and aeration regimes.

From a practical perspective, this may represent a form of structural compensation: when the increase in the finest roots is not maximal, part of the biomass is allocated to the transitional 1–2 mm fraction, which also actively participates in resource uptake but is characterized by greater structural stability and a longer lifespan. Conceptually, this interpretation is consistent with approaches distinguishing functional root orders and their different roles in resource acquisition and transport (Guo et al., 2008).

For the 2–4 mm root fraction, the values were relatively similar: Substrate 1 (9.43 ± 0.60 g) > Substrate 3 (8.63 ± 0.84 g) ≈ Substrate 2 (8.54 ± 0.69 g). In this case, the substrate effect was less pronounced, which is logical since roots of intermediate diameter are generally less responsive to short-term changes in the microenvironment than fine roots.

Interestingly, in the >4 mm fraction, the highest mean value was recorded in Substrate 3 (8.03 ± 0.59 g), whereas Substrate 1 and Substrate 2 showed lower values (7.66 and 7.12 g, respectively). This may indicate a structural shift in Substrate 3, where under less favorable conditions for the formation of fine roots, a relatively larger proportion of biomass is allocated to thicker roots. From a functional perspective, however, this is not necessarily advantageous, since an increase in the structural component of the root system without a proportional development of the absorbing fraction may reduce the efficiency of water and nutrient uptake.

The three-factor ANOVA for total root system mass showed that the effect of substrate was not statistically significant ($P = 0.128$), whereas the effects of rootstock and container type were significant or highly significant, respectively.

From a practical perspective, this represents an important conclusion: within the conditions of the present experiment, the substrate as a factor did not determine the total accumulation of root dry mass, although it may have influenced the distribution of biomass among root fractions (in particular, the relative shift between the <1 mm and 1–2 mm fractions, as well as the tendency toward a higher proportion of the >4 mm fraction in Substrate 3). Such a result is consistent with the concept that, within the restricted volume of a container, the spatial and aeration effects associated with the container system tend to dominate, whereas the substrate more often affects finer mechanisms of growth and biomass allocation, which do not always manifest in total biomass.

International reviews of growing media emphasize that the physical properties of the substrate (such as porosity, aeration, and water-holding capacity) are often critical determinants of plant growth in container-grown plants, and their effects may strongly depend on irrigation regimes and overall crop management practices. For peat-based and peat-substitute substrates, proper water management has been shown to be particularly important due to the water-air balance: excessive moisture reduces aeration and may limit the development of fine roots, whereas insufficient moisture can also restrict the active growth of the absorbing zone.

Moreover, contemporary fine-root concepts emphasize that fine roots are the primary organs responsible for resource uptake. Therefore, even in the absence of statistically significant differences in total root biomass, changes in the <1 and 1–2 mm fractions have high biological and interpretative significance (McCormack et al., 2015).

The results of the comparison of container type × substrate type combinations in terms of root mass across different diameter fractions are presented in Table 5. The obtained data indicate a substantial influence of the growing conditions on the formation of the root system in apple nursery trees grown as container-grown plants.

Table 5
Comparison of Container × Substrate treatments by root mass (g, mean ± SE, n = 20)

Container × Substrate	<1 mm	1–2 mm	2–4 mm	>4 mm	Total mass
Air Pot × substrate 1	26.10 ± 1.32[a]	11.20 ± 0.84[ab]	9.90 ± 0.66[a]	7.85 ± 0.71[a]	55.05 ± 1.92[a]
Air Pot × substrate 2	25.20 ± 1.41[ab]	12.60 ± 0.95[a]	9.10 ± 0.74[ab]	7.30 ± 0.60[a]	54.20 ± 2.04[a]
Air Pot × substrate 3	23.60 ± 1.15[b]	11.70 ± 1.02[ab]	9.80 ± 0.81[a]	8.25 ± 0.63[a]	53.35 ± 1.98[ab]
C12 × substrate 1	21.70 ± 1.24[c]	9.70 ± 0.70[bc]	8.95 ± 0.63[ab]	7.45 ± 0.61[a]	47.80 ± 1.86[bc]
C12 × substrate 2	19.70 ± 1.39[d]	11.30 ± 0.86[ab]	7.98 ± 0.64[b]	6.95 ± 0.53[a]	45.92 ± 1.95[cd]
C12 × substrate 3	18.30 ± 1.10[d]	8.02 ± 0.92[c]	7.45 ± 0.77[b]	7.80 ± 0.55[a]	41.59 ± 1.87[d]

Note: see Table 4.

The highest mass of fine roots (<1 mm) was observed in the Air Pot × Substrate 1 treatment (26.10 ± 1.32 g), which was statistically higher than in most other treatments. Similar values were also recorded in the Air Pot × Substrate 2 treatment (25.20 ± 1.41 g), whereas the use of the C12 container resulted in a substantial reduction in the mass of this root fraction (18.30–21.70 g). The lowest values were observed in the C12 × Substrate 2 and C12 × Substrate 3 treatments (19.70 and 18.30 g, respectively).

A similar trend was observed for roots with a diameter of 1–2 mm. The maximum value was recorded in the Air Pot × Substrate 2 treatment (12.60 ± 0.95 g), which significantly exceeded the values observed in some treatments with the C12 container, particularly C12 × Substrate 3 (8.02 ± 0.92 g).

For the 2–4 mm fraction, the greatest root mass was formed in the Air Pot × Substrate 1 and Air Pot × Substrate 3 treatments (9.90 and 9.80 g, respectively), whereas the lowest values were observed in the combinations involving the C12 container. With respect to thick roots (>4 mm), no statistically significant differences were detected among the treatments, as the values varied within a relatively narrow range (6.95–8.25 g). This indicates that the growing conditions had a stronger influence on the formation of fine and intermediate roots, which play a key role in the uptake of water and mineral nutrients.

The highest total root system mass was observed in the Air Pot × Substrate 1 and Air Pot × Substrate 2 treatments (55.05 and 54.20 g, respectively), which significantly exceeded the values recorded in most treatments involving the C12 container. In contrast, the lowest total root mass was observed in the C12 × Substrate 3 treatment (41.59 ± 1.87 g).

The obtained results confirm that the use of the Air Pot container system promotes more intensive development of the root system, particularly of fine roots. This effect can be explained by the specific design of the container, which ensures improved substrate aeration and stimulates the process of root air pruning. As a result, a more highly branched and functionally active root system is formed. Similar effects have been reported in studies on the container production of fruit crops, where improved substrate aeration enhanced the growth of absorbing roots.

At the same time, substrate composition also played an important role in the formation of root biomass. The highest values of total root mass were obtained in the treatments with Substrate 1, which may be associated with an optimal combination of water-holding capacity and aeration. In contrast, the use of Substrate 3 in combination with the C12 container resulted in the lowest values of root system development.

Thus, the results of the study indicate that the combination of the Air Pot container with Substrate 1 or Substrate 2 creates the most favorable conditions for root system formation in apple nursery trees grown as container-grown plants, whereas the use of the standard C12 container, particularly in combination with Substrate 3, restricts root development.

To evaluate the relative contribution of rootstock genotype, container type, and substrate composition to the formation of the total root system mass in two-year-old apple nursery trees, a three-factor analysis of variance (ANOVA) was performed. The aim of the analysis was to determine which factors represent statistically significant determinants of root dry matter accumulation in container-grown plants (Table 6).

Table 6
Results of three-factor ANOVA for the effects of rootstock, container type and substrate composition on total root mass of container-grown apple nursery trees (n = 120)

Source of variation	F	P	Significance level
Rootstock	13.56	0.001	significant
Container	33.78	<0.001	highly significant
Substrate	2.25	0.128	not significant
Rootstock × container	<1.0	>0.05	not significant
Rootstock × substrate	<1.0	>0.05	not significant
Container × substrate	<1.0	>0.05	not significant
Rootstock × container × substrate	<1.0	>0.05	not significant

The factor "rootstock" was statistically significant (F = 13.56; P = 0.001), indicating a substantial contribution of the genotype to the formation of the total root system mass. This confirms the previous analysis of mean values (Table 1), where the rootstock 'MM.106' produced a greater root mass compared with 'M.9'. From a biological perspective, this finding is consistent with the morphogenetic characteristics of the rootstocks: the semi-vigorous 'MM.106' possesses a higher potential for branching and the formation of an absorbing root surface, whereas 'M.9' is characterized by a more compact root architecture. Thus, genetically determined traits define the baseline level of root biomass accumulation, regardless of other environmental factors.

The container factor exhibited the highest F-value (33.78; P < 0.001), indicating its dominant role in determining the total root system mass. Such a high level of statistical significance suggests that the spatial and aeration conditions created by the container are key regulators of root formation in container-grown plants. The Air Pot container resulted in a significantly greater root mass compared with the C12 container, which can be explained by the mechanism of root air pruning, the stimulation of lateral branching, and the prevention of root spiraling.

From a physiological perspective, this indicates that within the confined volume of a container, the container architecture itself becomes the determining factor in the formation of an active root system. The substrate factor was statistically non-significant for the total root mass parameter (F = 2.25; P = 0.128). This indicates that, within the range of treatments studied, changes in the substrate composition did not lead to a statistically significant difference in the total accumulation of root dry matter. Although the mean values showed certain trends (Table 3), the variability within groups did not allow a statistically confirmed effect to be detected. Thus, in the present experiment, the substrate did not act as a key factor regulating the overall root biomass. International studies on the container production of fruit crops confirm that container design and spatial constraints often exert a stronger influence on root system architecture than variations in substrate composition (Poorter et al., 2012). In particular, systems employing root air-pruning technology have been shown to increase the number of fine roots and promote greater overall root system biomass.

To identify the factors determining the formation of aboveground biomass in two-year-old apple nursery trees of the cultivar 'Champion' grown as container-grown plants, a three-factor analysis of variance (ANOVA) was performed. In contrast to the root system, shoot growth under container cultivation largely depends on the water regime, nutrient availability, and the physicochemical properties of the substrate, which necessitated a separate statistical evaluation of these factors (Table 7).

Table 7
Effects of rootstock, container type and substrate composition on shoot biomass of container-grown 'Champion' apple nursery trees according to three-factor ANOVA (n = 120)

Source of variation	F	P	Significance level
Rootstock	3.74	0.065	trend
Container	1.79	0.193	not significant
Substrate	7.82	0.002	significant
Container × substrate	4.38	0.024	significant
Rootstock × container	>1.0	>0.05	not significant
Rootstock × substrate	>1.0	>0.05	not significant
Rootstock × container × substrate	–	>0.05	not significant

The rootstock factor showed a marginal (trend-level) effect (F = 3.74; P = 0.065). This indicates that genotype may influence the formation of aboveground biomass, although in the present experiment the effect did not reach the level of statistical significance (P < 0.05). From a biological perspective, this result is reasonable: although the rootstock determines the potential for root formation and resource supply, its direct influence on shoot growth intensity in container-grown plants may be moderated by agronomic conditions such as irrigation and nutrient management. Therefore, in this case the genotype plays a secondary role in regulating aboveground biomass compared with the substrate factor.

The container factor did not show a statistically significant effect on aboveground biomass (F = 1.79; P = 0.193). This indicates that although the container strongly influences the root system, its direct effect on the accumulation of shoot biomass is not decisive. It is likely that the influence of the container on shoot growth is expressed indirectly through the root system, but this effect was insufficient to produce a statistically significant difference in aboveground mass.

Such a result is consistent with current understanding that container volume limitations primarily modify root architecture rather than directly affecting shoot growth rates.

The substrate factor was statistically significant (F = 7.82; P = 0.002), indicating a substantial contribution of the physicochemical properties of the growing medium to the formation of aboveground biomass. Shoot growth directly depends on several key parameters, including nitrogen and other macronutrient availability, the water-holding capacity of the substrate, the air–water balance in the root zone, and the buffering capacity of the medium. Thus, the substrate acts as a key regulator of photosynthetic activity, transpiration, and growth processes in the aboveground part of the plant.

The statistically significant interaction between container type and substrate (F = 4.38; P = 0.024) indicates that the effect of the substrate depends on the container system. From an interpretative perspective, this means that in a container with improved aeration (Air Pot) a particular substrate may realize its potential more fully, whereas in a less aerated container (C12) the same substrate may function differently due to changes in the water–air regime of the root zone. Therefore, the effectiveness of the substrate is not autonomous but is modified by the spatial conditions of root system development.

Other two-factor and three-factor interactions were statistically non-significant. This indicates that the effect of the rootstock did not depend on a specific substrate or container type, and that the combined influence of all three factors did not produce an additional effect beyond the sum of their individual contributions.

Discussion

Role of rootstock genotype in root system formation. The comparison of rootstocks showed that 'MM.106' formed a greater total root system mass and a higher proportion of fine roots, whereas 'M.9' exhibited a more compact root formation pattern. Importantly, the largest differences between the rootstocks were observed in the <1 and 1–2 mm fractions, i.e., in the roots that determine the absorptive capacity of the root system and the plant's responsiveness to substrate conditions and moisture availability.

This result is consistent with the concept of genetically determined differences in the root system architecture of apple rootstocks and their varying morphogenetic potential (Atkinson, 2000). From the perspective of the functional classification of roots, the increase in fine-root fractions represents a key indicator of planting material quality, since these roots provide the largest contact surface with the substrate and make the greatest contribution to resource acquisition (Guo et al., 2008; McCormack et al., 2015).

Influence of container type on root system architecture. International studies on the container production of fruit crops confirm that container design and spatial constraints often exert a stronger influence on root system architecture than variations in substrate composition (Poorter et al., 2012). In particular, systems employing root air-pruning technology have been shown to increase the number of fine roots and promote greater overall root system biomass.

The most pronounced technological effect observed in the experiment was associated with the container type. The advantage of the Air Pot system in terms of total root mass, and particularly the development of fine root fractions, is critical for container-grown nursery trees, as they are especially prone to the formation of defective root systems (e.g., spiraling, deformation, and low fibrosity), which represent one of the major limitations after field planting.

The mechanism of root air pruning redirects root growth from continued apical elongation toward the formation of lateral roots, thereby stimulating branching, reducing root spiraling, and increasing root fibrosity. This observation is consistent with the concept of spatial constraints as a regulator of root system architecture (Lynch, 2013), as well as with evidence on the negative effects of root spiraling on the functional quality of nursery trees (Gilman et al., 2016) and reports on enhanced root branching in air-pruning container systems (NeSmith & Duval, 1998).

These findings also have practical implications: even with the same substrate volume, container architecture can effectively redirect biomass allocation toward the root fractions that determine rapid plant establishment and early growth after transplanting.

Influence of substrate composition on plant growth. The effect of the substrate on total root mass was less pronounced and was not always statistically confirmed; however, the trends observed in the root fractions (particularly <1 and 1–2 mm) indicate that the substrate can modify the distribution of biomass among functional components of the root system. This is logical, since the substrate determines the water-air balance, mechanical resistance, and nutrient availability, while fine roots are the most sensitive to such changes.

International studies on the container production of fruit and ornamental crops demonstrate that substrate composition is often the principal factor determining the intensity of aboveground growth, particularly during the early stages of plant ontogeny (Carlile et al., 2019). Numerous studies have shown that:

– an optimal balance between air-filled and water-filled porosity directly correlates with shoot growth;

– increased water-holding capacity of the substrate may stimulate photosynthetic activity;

– insufficient aeration restricts the transpiration flux and reduces the accumulation of aboveground biomass.

The results obtained in the present study are fully consistent with these findings: the substrate acts as the leading factor in the formation of aboveground biomass, whereas the container plays a supportive and modifying role.

Interaction between container system and substrate. Importantly, according to the present results, the aboveground part responded more strongly to the substrate, as evidenced by the significant effects of the substrate and the container × substrate interaction. This suggests the presence of different pathways of factor influence: the container primarily shapes root system architecture through aeration and spatial constraints, whereas the substrate exerts a stronger influence on the realization of photosynthetic potential and shoot growth through the regulation of the water regime, nutrient supply, and buffering properties of the growing medium (Alexander & Bragg, 2014).

The presence of a significant container × substrate interaction for the aboveground biomass indicates that the effectiveness of the substrate depends on the type of container system. This can be explained by the fact that the same substrate may create different water–air regimes in different containers, resulting from variations in aeration, drying rate, and moisture gradients within the root zone.

Therefore, the selection of a substrate should not be considered in isolation, but rather as part of an integrated technological combination of "container + substrate".

Technological implications for nursery production. The obtained results confirm that under conditions of container-grown plants, the formation of the morphological structure of two-year-old apple nursery trees is determined by a hierarchy of factors. Within this hierarchy, the rootstock defines the baseline morphogenetic potential, the container type substantially modifies root system architecture through spatial and aeration constraints, and the substrate primarily influences the realization of growth processes (particularly in the aboveground part) through the regulation of the water–air regime and resource availability.

Overall, the results allow a technologically important conclusion to be drawn: for two-year-old apple nursery trees grown as container-grown plants, the container type (Air Pot) is the key factor improving root system quality, whereas the substrate primarily determines the intensity of aboveground growth and expresses its effect mainly through interaction with the container system.

Practical implications for nursery tree quality. From a practical perspective, the key integrative criterion of container-grown nursery tree quality is the balance between the belowground and aboveground parts (root-shoot balance), which determines the plant's ability to maintain water status, transpiration, and stable growth after field planting. Excessively rapid shoot growth relative to weak root development increases the risk of water stress, delayed establishment, and uneven early growth, whereas the formation of a well-developed fibrous root system combined with moderate aboveground biomass enhances transplant resilience and ensures a more predictable initiation of growth processes. In this context, not only the total root mass but also the proportion of fine roots (<2 mm – the most functionally active fraction) are critical indicators, as they determine the actual absorptive capacity of the nursery tree.

Considering the obtained results, the application of the Air Pot container system in combination with Substrate 1 or Substrate 2 appears to be the most suitable option for nursery practice, whereas the C12 × Substrate 3 combinations may reduce the field resilience of nursery trees and require more careful irrigation management after planting.

Conclusion

The conducted study made it possible to determine the patterns of belowground and aboveground biomass formation in two-year-old apple nursery trees of the cultivar 'Champion' depending on rootstock genotype, container type, and substrate composition under conditions of container-grown plants. The results demonstrated that the rootstock significantly influenced root system morphogenesis. The rootstock 'MM.106' exhibited a higher morphogenetic potential and formed a greater total root mass (52.54 g), which was approximately 12% higher than that of 'M.9'. The main differences between the rootstocks were observed in the fine-root fractions (<1 and 1–2 mm), which determine the absorptive capacity of the root system.

The use of the Air Pot container system resulted in a substantial increase in total root mass (54.20 g) compared with the conventional C12 container (45.13 g), representing an increase of approximately 20%. The advantage of Air Pot was primarily associated with the more intensive formation of fine roots, which can be explained by improved substrate aeration and the root air-pruning effect, stimulating branching and the formation of a more fibrous root system.

The substrate composition had a less pronounced effect on total root mass but influenced the distribution of biomass among root fractions. The highest values of total root mass and fine-root fractions were observed in the treatments with Substrate 1, whereas Substrate 3 showed a tendency toward lower levels of root system development. The analysis of factor combinations revealed that the most favorable conditions for root system formation were observed in the Air Pot × Substrate 1 and Air Pot × Substrate 2 treatments, where the highest total root mass and the greatest proportion of functionally active fine roots were recorded.

The analysis of variance (ANOVA) confirmed that rootstock and container type were statistically significant factors determining root biomass formation, whereas the influence of the substrate on total root mass was less pronounced. In contrast, the formation of aboveground biomass was primarily influenced by the substrate factor, indicating different regulatory mechanisms governing the growth of belowground and aboveground plant components.

Overall, the results indicate that in the technology of container-grown apple nursery trees, the most effective approach is the use of containers with root air-pruning systems (Air Pot) in combination with optimized peat-based substrates, which promotes the formation of a more fibrous root system, improves the root–shoot balance, and enhances the establishment potential of nursery trees after transplanting into the orchard. The obtained results may be applied to improve container nursery production technologies for apple trees by selecting the optimal combination of rootstock, container system, and substrate composition for the production of high-quality planting material.

The authors declare they have no conflict of interests.

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