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Influence of container type and substrate composition on photosynthetic productivity and chlorophyll content in the leaves of container-grown apple plants

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Container production of apple nursery trees is increasingly used in intensive horticulture because it improves transplant survival and allows flexible marketing; however, restricted root volume makes plant performance highly dependent on container design and the physical and agrochemical properties of the growing medium. This study evaluated how container type and substrate composition affect photosynthetic performance of ‘Champion’ apple (*Malus domestica* Borkh.) nursery trees grafted on two contrasting rootstocks (‘M.9’ and ‘MM.106’) in Kyiv region (Ukraine). A field container experiment was conducted in 2024–2025 at a commercial nursery site. Trees were grown in 12-L containers of two designs (air-pruning Air-Pot type and standard plastic container C12) filled with three substrates: peat; peat + conifer bark (1:1); and peat + conifer bark + sandy loam (2:1:1). Net photosynthetic productivity was determined in vivo as the 24-h increase in leaf dry matter per unit area, and chlorophyll *a* and *b* contents were quantified spectrophotometrically; total chlorophyll and the chlorophyll *a/b* ratio were calculated. Substrate composition was the dominant factor controlling net photosynthetic productivity across rootstocks and ages, with peat ensuring the highest rates and the peat + bark (1:1) mixture causing a significant decline, while container type and interactions were non-significant. Chlorophyll accumulation was rootstock-dependent: ‘MM.106’ showed consistently higher total chlorophyll and greater pigment stability across treatments than ‘M.9’. Maximum pigment concentration was recorded in the peat + bark substrate in the C12 container, whereas the chlorophyll *a/b* ratio was significantly influenced by rootstock and container design, reflecting genotype-specific regulation of light-harvesting complexes. Overall, photosynthetic efficiency in container-grown apple nursery trees is primarily determined by substrate composition, and appropriate substrate optimization can compensate for differences in container design, supporting stable physiological performance and nursery tree quality.

Keywords: nursery trees; container cultivation; growing; photosynthetic productivity; chlorophyll; rootstock.

Introduction

Modern intensive technologies for the production of apple nursery trees (*Malus domestica* Borkh.) increasingly rely on container-based cultivation systems, which provide greater marketing flexibility and improve transplant survival after planting. However, the restricted root volume inherent to container systems increases plant dependence on the physical and agrochemical properties of the growing medium as well as on container design, since these factors determine water regime, aeration, and nutrient availability (Alexander & Bragg, 2014). In regions with unstable moisture supply, particularly under the climatic conditions of Kyiv region, container-grown plants may be exposed to thermal and osmotic stress, which can adversely affect their physiological status and the viability of scion–rootstock combinations, including the cultivar ‘Champion’ (Danjon et al., 2025).

Traditional visual assessment methods do not always allow the timely detection of early physiological disorders in plants. Therefore, the use of objective physiological indicators that reflect the efficiency of the photosynthetic apparatus and the adaptive potential of plants under specific growing conditions is of particular importance (Havryliuk et al., 2019, 2022, 2023). Despite the widespread adoption of container cultivation, uncertainty remains in nursery practice regarding the optimal combinations of container types and substrates for rootstocks with different vigor levels (‘M.9’ and ‘MM.106’) (Mezhenskyj et al., 2024).

This necessitates the development of scientifically grounded recommendations aimed at standardizing production technology and improving the quality of ‘Champion’ apple nursery trees (Burnett et al., 2016; Ellis, 2024).

The agricultural sector of Ukraine has suffered substantial losses as a result of military actions: a number of orchards and nurseries have been located in combat zones, while some areas have been occupied by defensive infrastructure or contaminated with explosive remnants and toxic residues of munitions (Ignatenko et al., 2024). Territories affected by active hostilities may remain unsuitable for conventional agricultural use for an extended period (Gilman & Paz, 2014; Gilman et al., 2016). Under such conditions, the implementation of alternative technological approaches in horticulture becomes particularly relevant.

One of the promising solutions is container-based cultivation of fruit crops, which is widely applied in Western Europe and other regions of the world (Sovakova et al., 2014; Telepenko & Silenko, 2019). Over recent decades, international research has addressed various aspects of container production systems, including the use of a broad range of growing media (Kim & Kim, 2011; Raviv, 2017; Majsztrik & Owen, 2020). In Ukraine, this approach has also been investigated; however, the level of its practical implementation still lags behind international practice.

In leading nurseries worldwide, container production systems have been used for several decades (Larcher & Scariot, 2009; Alexander & Bragg, 2014; Sovakova et al., 2014), ensuring more efficient use of space, water resources, and nutrients compared with open-field cultivation (Larcher & Scariot, 2009; Amoroso et al., 2010; Venig et al., 2025). Both organic and inorganic materials, predominantly of local origin, are used for the preparation of growing media (McGrath et al., 2021; Tuxun et al., 2025), including high-moor peat, coconut coir, composted bark, rice husks, expanded clay, vermiculite, perlite, and mineral wool (Danjon et al., 2025; Tuxun et al., 2025).

Container production sites can be established on areas poorly suited for conventional agriculture, including soils with low fertility, high groundwater levels, stony substrates, or reclaimed lands (Pavlichenko et al., 2023; Dehodiuk et al., 2024; Bulygin et al., 2025). At the same time, the implementation of this technology requires substantial initial investments associated with site preparation, covering the surface with geotextile fabric, installing irrigation systems, and setting up support structures.

Cultivation of plants in a confined root environment requires the use of specially formulated substrates containing nutrient components and regulatory amendments (e.g., liming materials and fertilizers) (Salash & Valtera, 2005; Kim et al., 2021; Ellis, 2024). High-moor peat is the most commonly used base component of such mixtures (Nazim & Gruda, 2019; Elsysy & Einhorn, 2020; Vasylenko et al., 2021), as it is characterized by high water-holding capacity, good aeration, low bulk density, and phytosanitary safety (Gruda et al., 2013; Gilman & Paz, 2014).

Container cultivation of apple trees is gaining increasing importance in intensive orchard systems (Mamonova et al., 2018; Nazim & Gruda, 2019), as it enables effective regulation of mineral nutrition, water supply, and root zone volume (Kormanek et al., 2024). However, the combination of container type and substrate composition may either optimize or constrain physiological processes, including photosynthetic activity and plant adaptive responses.

The primary functions of a green plant leaf include photosynthesis, transpiration, and the synthesis of various organic compounds, including certain phytohormones such as auxins, gibberellins, and abscisic acid (Mathes, 1989; Blanco & Folegatti, 2003). In addition, leaves possess adaptive properties that enable plants to respond to environmental conditions. Among the key biological indicators of photosynthetic apparatus performance are net photosynthetic productivity and the content of green pigments in leaves, namely chlorophyll *a* and chlorophyll *b*.

Absorption of light quanta in the red and blue spectral regions (400–700 nm) is mediated by chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*). Chlorophyll *a* is directly involved in the conversion of solar energy and transfers the absorbed energy to the reaction centers. Chlorophyll *b* is mainly localized in the light-harvesting complexes of photosystems I and II, as well as in the minor antenna complex of photosystem II (Mathes, 1989). It participates in transferring approximately half of the energy absorbed by carotenoids to chlorophyll *a* (Riabtseva, 2005; Andrusyk & Kytaiev, 2007), with energy flow proceeding from carotenoids to chlorophyll, then from Chl *b* to Chl *a*, and subsequently through a chain of Chl *a* molecules to the reaction centers of photosystems I and II (Yamasato et al., 2005).

The efficiency of photosynthesis depends on the quantitative content of green pigments in the leaf and on the Chl *a*/Chl *b* ratio, which reflects the structural organization of chloroplasts and the plant's adaptive potential to environmental changes. This ratio is typically higher under high light intensity than under low light conditions (Formaggio et al., 2001), and regulation of Chl *b* synthesis plays an important role in plant adaptation to different light regimes (Finch et al., 2014). Measurement of chlorophyll content is therefore an effective indicator of plant tolerance to abiotic stress.

Chlorophyll is characterized by a specific optical property – fluorescence (Tanaka et al., 1998). This phenomenon involves the re-emission of absorbed light at a longer wavelength, whereby part of the energy captured by chlorophyll molecules is released as red fluorescence. Spectral fluorescence analysis enables rapid assessment of plant tolerance to various stress factors and facilitates the identification of the most sensitive segments of the photosynthetic electron transport chain.

According to Havryliuk et al. (2024), the intensity of photosynthesis in apple trees is largely enhanced by the combined influence and magnitude of environmental factors such as temperature, light, moisture, and mineral nutrition, as well as by leaf position within the canopy, leaf size, age, and structural characteristics (Ivanova et al., 2022). According to Ovsyannikov (1969), the effect of annual environmental conditions on photosynthetic intensity (net photosynthetic productivity) accounts for 52–62%, whereas the varietal effect contributes 11–19%. The cited author also demonstrated that higher photosynthetic productivity and greater allocation of assimilates to yield formation reduce the number of days required to produce a unit of marketable yield, thereby increasing the cultivar's productive value.

The aim of this study was to determine the net photosynthetic productivity of the assimilating leaf surface and the content of green pigments (chlorophyll *a* and chlorophyll *b*) in leaves as affected by container type and substrate composition.

Materials and methods

The study on container cultivation of apple nursery trees (*Malus domestica* Borkh.) was conducted in 2024–2025 at the production sites of the "Pisardi" Garden Center (Bucha district, Kyiv region, Ukraine; 50.5044º N, 30.1888º E). 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. The experimental material consisted of 'Champion' apple nursery trees grafted onto two rootstocks, 'M.9' and 'MM.106'.

Plants were grown in two types of 12-L containers: Air-Pot (ERCOLE SP30) and a standard plastic container C12 (B28). Three substrate variants differing in component composition were used (Table 1):

– high-moor peat (particle size 0–40 mm, pH 5.5–6.5);
–peat and composted conifer bark (0–5 mm) mixed at a ratio of 1:1;
– peat, conifer bark, and sandy loam soil mixed at a ratio of 2:1:1.

Table 1
Experimental design for container cultivation
of 'Champion' apple nursery trees

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

Substrates were prepared using a mechanical mixer (Urbinati, Italy). To adjust pH to the optimal level, liming was performed according to the results of agrochemical analysis conducted at the laboratory of the National University of Life and Environmental Sciences of Ukraine. The application rates of the liming material were 0.24 g/L for the peat-based substrate, 0.90 g/L for the peat–bark mixture, and 0.62 g/L for the three-component substrate.

Before the onset of active vegetation, the nursery trees were maintained in a plastic-covered greenhouse. Beginning in early May, plants were hardened for three days under shading netting and subsequently transferred to an open trellis. Containers were connected to a drip irrigation system, and plants were secured to the trellis using bamboo stakes. The container spacing was 1.0 × 0.5 m.

Net photosynthetic productivity was determined in vivo without detaching the leaf from the plant. In the morning hours, two leaf discs (1.0 cm² each) were excised with a punch from fully expanded leaves located in the middle canopy layer. The discs were immediately weighed, dried to constant weight at 105 °C, and reweighed to determine dry matter content. After sampling, the leaf petiole was clamped to prevent assimilate export, and the leaf remained attached to the plant under natural conditions for 24 h. Following this exposure period, discs of the same area were again collected from the same leaves and processed according to the same procedure to determine dry mass. Net photosynthetic productivity was then determined according to Koshelev's (1987) method, based on the 24-h increase in leaf dry matter per unit leaf area.

Fully developed, physiologically mature leaves were sampled from the middle part of current-season shoots during the active vegetation period. Sampling was performed in the morning hours to minimize diurnal fluctuations in pigment content. Immediately after collection, leaf samples were transported to the laboratory for analysis. Chlorophyll pigments were extracted from fresh leaf tissue using 96% ethanol as a solvent. Approximately 0.19–0.20 g of fresh leaf tissue was weighed (analytical balance accuracy ± 0.001 g), homogenized, and the extract volume was adjusted to 10 mL.

Optical density of the extracts was measured using a spectrophotometer UNICO 1205 at wavelengths: λ = 665 and 649 nm.

Chlorophyll concentrations in the extract (mg/L) were calculated according to the equations of Lichtenthaler (1987) for ethanol extraction:

$$\text{Chl } a = 13.95 \times A_{665} - 6.88 \times A_{649} \qquad (1)$$

$$\text{Chl } b = 24.96 \times A_{649} - 7.32 \times A_{655} \qquad (2)$$

Total chlorophyll content was determined as the sum (Chl *a* + Chl *b*).

To express pigment content per unit fresh mass, the following formula was used:

$$\text{Chl (mg/g)} = (C \times V) / m \qquad (3)$$

where: C – chlorophyll concentration in extract (mg/L), V – extract volume (L), m – fresh leaf mass (g).

The chlorophyll *a/b* ratio was calculated as an indicator of pigment balance and physiological status.

Statistical processing of the experimental data was performed using descriptive statistics and analysis of variance. The significance of the effects of rootstock, container type, and substrate on fluorescence parameters of the photosynthetic apparatus was evaluated using three-way ANOVA followed by Tukey's post hoc test ($P < 0.05$). Relationships among the measured parameters were assessed using correlation analysis.

Results

The study of the effects of container type and substrate composition on the net photosynthetic productivity of leaves in two- and three-year-old apple nursery trees demonstrated a significant role of substrate in determining plant physiological activity, whereas container type did not exert a decisive influence. According to Table 2, net photosynthetic productivity of leaves in two-year-old 'Champion' apple trees grafted onto 'M.9' varied depending on the substrate within the range of 9.34–13.80 units. The highest values were recorded in the Air-Pot × Substrate 1 treatment (13.80 ± 1.67). Similar values were obtained in the C12 container with substrate 1 (13.36 ± 0.54), indicating the high effectiveness of this substrate regardless of container type.

Table 2
Effect of container type and substrate composition on net photosynthetic productivity of leaves in two-year-old apple nursery trees (n = 10)

Rootstock	Container type	Substrate	mean ± SD
'M.9'	Air Pot (A)	Substrate 1	13.80 ± 1.67^{a}
		Substrate 2	9.34 ± 0.89^{c}
		Substrate 3	12.10 ± 2.74^{ab}
	C12 (B)	Substrate 1	13.36 ± 0.54^{ab}
		Substrate 2	9.90 ± 0.82^{c}
		Substrate 3	12.20 ± 0.21^{b}
'MM.106'	Air Pot (A)	Substrate 1	12.26 ± 1.02^{a}
		Substrate 2	10.34 ± 1.42^{b}
		Substrate 3	11.58 ± 0.77^{a}
	C12 (B)	Substrate 1	12.48 ± 0.80^{a}
		Substrate 2	9.06 ± 0.52^{b}
		Substrate 3	12.66 ± 1.31^{a}

Note: means followed by the same letters do not differ significantly within each rootstock (Tukey test, $P < 0.05$).

Substrate 3 provided intermediate values of net photosynthetic productivity (12.10–12.20 units). In contrast, the use of Substrate 2 resulted in a significant reduction in leaf photosynthetic activity (9.34–9.90 units), forming a distinct statistical group. Thus, for apple trees grafted onto 'M.9', substrate 1 proved to be the most favorable, whereas Substrate 2 significantly suppressed photosynthetic productivity.

In apple nursery trees grafted onto 'MM.106', net photosynthetic productivity values were more uniform and ranged from 9.06 to 12.66 units. The highest values were observed in the C12 × substrate 3 treatment (12.66 ± 1.31), as well as in treatments with substrate 1 (12.26–12.48 units). This indicates a similarly high level of photosynthetic activity when substrates 1 and 3 were used. In contrast, trees grown in substrate 2 exhibited significantly lower net photosynthetic productivity (9.06–10.34 units), confirming its lower effectiveness in supporting plant physiological functioning. Compared with trees grafted onto 'M.9', plants on the 'MM.106' rootstock exhibited a more stable response to growing conditions, without pronounced fluctuations in net photosynthetic productivity.

Two-way ANOVA results (Table 3) confirmed that substrate composition was the determining factor influencing net photosynthetic productivity of leaves in trees grafted onto both rootstocks.

Table 3
Two-way ANOVA of the effects of container type and substrate composition on net photosynthetic productivity of leaves in two-year-old apple nursery trees

Rootstock	Source of variation	df	F	P	Effect
'M.9'	Container (A)	1	0.25	0.620	not significant
	Substrate (B)	2	3.98	0.033	significant
	A × B	2	0.19	0.830	not significant
'MM.106'	Container (A)	1	0.00	0.990	not significant
	Substrate (B)	2	4.21	0.027	significant
	A × B	2	0.31	0.740	not significant

For trees grafted onto 'M.9', the effect of substrate was statistically significant ($F = 3.98$; $P = 0.033$), whereas container type ($P = 0.62$) and the interaction between factors ($P = 0.83$) did not have a significant influence.

A similar pattern was observed for trees on 'MM.106': substrate composition significantly affected leaf net photosynthetic productivity ($F = 4.21$; $P = 0.027$), while container type ($P = 0.99$) and the interaction between factors ($P = 0.74$) were not statistically significant. The absence of significant interaction effects indicates a consistent influence of substrate regardless of container type. The obtained results indicate that the physiological status of apple leaves is largely determined by substrate properties. The highest net photosynthetic productivity was observed in substrates 1 and 3, whereas substrate 2 significantly reduced photosynthetic activity in both rootstocks. Container type did not have a statistically significant effect on net photosynthetic productivity, suggesting that container selection may be based on technological or economic considerations without compromising plant physiological performance. Trees grafted onto 'M.9' exhibited greater sensitivity to substrate composition, whereas those on 'MM.106' were characterized by more stable physiological performance.

Net photosynthetic productivity of leaves in three-year-old trees on 'M.9' ranged from 9.36 to 12.32 units depending on growing conditions (Table 4).

Table 4
Effect of container type and substrate composition on net photosynthetic productivity of leaves in three-year-old apple nursery trees (n = 10)

Rootstock	Container type	Substrate	mean ± SD
'M.9'	Air Pot	Substrate 1	12.32 ± 0.30^{a}
		Substrate 2	9.88 ± 0.27^{c}
		Substrate 3	11.40 ± 0.59^{b}
	C12	Substrate 1	10.94 ± 0.68^{b}
		Substrate 2	9.36 ± 0.60^{c}
		Substrate 3	11.38 ± 0.38^{b}
'MM.106'	Air Pot	Substrate 1	11.94 ± 0.31^{a}
		Substrate 2	9.38 ± 0.38^{b}
		Substrate 3	10.36 ± 0.81^{ab}
	C12	Substrate 1	10.70 ± 0.74^{ab}
		Substrate 2	10.00 ± 0.72^{ab}
		Substrate 3	11.44 ± 0.45^{a}

Note: see Table 2.

The highest net photosynthetic productivity was recorded in the Air-Pot × substrate 1 treatment (12.32 ± 0.30), which was significantly higher than the treatments with Substrate 2. Substrate 3 provided an intermediate level of net photosynthetic productivity (≈11.4 units), whereas the use of SSbstrate 2 resulted in a statistically significant reduction in photosynthetic activity.

In the C12 container, net photosynthetic productivity values were slightly lower or comparable to those observed in the Air-Pot system; however, the differences between container types did not reach statistical significance. In trees grafted onto 'MM.106', net photosynthetic productivity ranged from 9.38 to 11.94 units. The highest values were recorded with substrate 1 in the Air-Pot system (11.94 ± 0.31) and with substrate 3 in the C12 container (11.44 ± 0.45). Substrate 2 reduced photosynthetic intensity, forming a statistically lower group. Compared with trees grafted onto ‘M.9’, those on ‘MM.106’ exhibited a more uniform response to growing conditions, indicating greater physiological stability of this rootstock.

Two-way analysis of variance confirmed that substrate composition was the determining factor influencing net photosynthetic productivity of leaves in three-year-old nursery trees (Table 5).

Table 5
Two-way ANOVA of the effects of container type and substrate composition on net photosynthetic productivity of leaves in three-year-old apple nursery trees

Rootstock	Source of variation	df	F	P	Effect
‘M.9’	Container (A)	1	1.21	0.280	not significant
	Substrate (B)	2	8.34	0.002	significant
	A × B	2	0.94	0.410	not significant
‘MM.106’	Container (A)	1	0.18	0.670	not significant
	Substrate (B)	2	5.12	0.014	significant
	A × B	2	1.03	0.370	not significant

For nursery trees grafted onto ‘M.9’, the effect of substrate was highly significant (P = 0.002), whereas container type and the interaction between factors did not show a significant influence. A similar pattern was observed for trees on ‘MM.106’: substrate composition significantly affected net photosynthetic productivity (P = 0.014), while container type and factor interaction were not statistically significant. The absence of significant interaction effects indicates that substrate influence was consistent regardless of container type. The obtained results confirm that substrate composition is the key factor determining leaf photosynthetic productivity in three-year-old apple nursery trees. Substrate 1 provided the highest net photosynthetic productivity values, Substrate 3 resulted in comparable levels, whereas Substrate 2 consistently reduced photosynthetic activity. Container type did not exert a statistically significant effect, indicating that its selection may be based on technological or economic considerations.

The obtained results indicate that substrate composition is the key factor determining the intensity of photosynthetic processes in leaves of apple nursery trees, regardless of plant age. At the same time, the response of the photosynthetic apparatus to the physical and agrochemical properties of the substrate exhibits age-related features associated with changes in root system functioning, water regime, and source-sink relationships within the plant. In all experimental treatments, Substrate 1 provided the highest net photosynthetic productivity values in both two- and three-year-old nursery trees, indicating an optimal combination of its physical and chemical properties.

The high levels of photosynthetic activity are likely associated with optimal substrate porosity and structural stability, ensuring efficient gas exchange in the root zone; adequate water-holding capacity without the risk of waterlogging; balanced nutrient supply; and a stable plant water regime. Under such conditions, the root system functions actively, promoting intensive uptake of water and mineral nutrients, which supports cell turgor, stomatal opening, and efficient CO_2 diffusion to chloroplasts. This, in turn, creates favorable conditions for photochemical reactions and assimilate synthesis. High net photosynthetic productivity in plants grown in Substrate 1 was maintained regardless of plant age, indicating its versatility and technological reliability. In contrast, plants cultivated in Substrate 2 consistently exhibited the lowest net photosynthetic productivity values across all treatments, suggesting less favorable conditions for the functioning of the photosynthetic apparatus. The reduction in photosynthetic activity may be attributed to insufficient root-zone aeration, excessive substrate density or particle compaction, unstable water regime, limited oxygen availability to roots, and reduced efficiency of nutrient uptake. Under such conditions, root system performance is impaired, leading to partial stomatal closure, decreased transpiration, and restricted CO_2 diffusion into the leaf mesophyll. Consequently, photochemical reactions are inhibited and assimilate synthesis is reduced.

Nursery trees grown in Substrate 3 consistently exhibited high net photosynthetic productivity, although slightly lower than that observed in Substrate 1. The effectiveness of Substrate 3 can be attributed to an optimal balance between air porosity and water-holding capacity, ensuring a well-regulated air–water regime in the root zone. In two-year-old plants, Substrate 3 provided values close to the maximum, whereas in three-year-old trees it showed a tendency toward greater stability of photosynthetic activity. This may be associated with the increasing demand for stable water supply and structural stability of the substrate as plants age.

The presence of structure-forming components likely contributes to improved aeration under substrate compaction, stabilization of moisture content, reduced fluctuations in water potential, and decreased root system stress. Therefore, Substrate 3 can be considered an effective compromise between aeration and water retention, which is particularly important for older plants.

Age-related analysis indicates that the negative effect of Substrate 2 was evident in both age groups; however, in older plants its impact may be more pronounced due to the increased demand of the root system for oxygen and stable water supply. The obtained results confirm that the physical properties of the substrate play a key role in shaping the photosynthetic productivity of apple nursery trees. Substrate 1 provides optimal conditions for root system functioning and photosynthetic apparatus performance in plants of different ages. Substrate 3 maintains stable physiological activity and may be particularly effective in older plants due to its structural stability and water-regulating properties. In contrast, Substrate 2 creates conditions of root stress, leading to a systematic decline in photosynthetic activity.

Table 6 presents the content of chlorophyll *a*, chlorophyll *b*, their total amount (*a* + *b*), and the chlorophyll *a/b* ratio in leaves of two-year-old apple nursery trees grown under different combinations of rootstocks, substrates, and container types.

Table 6
Effect of rootstock–substrate–container combinations on chlorophyll content in leaves of two-year-old apple nursery trees (mg/g FW, mean ± SD, n = 10)

Rootstock	Substrate	Container	Chl *a*	Chl *b*	Total (*a*+*b*)	*a*/*b*
‘M.9’	Substrate 1	Air Pot	0.655 ± 0.103^{bc}	0.111 ± 0.028^{c}	0.766 ± 0.130^{c}	5.975 ± 0.578^{a}
		C12	0.688 ± 0.012^{b}	0.129 ± 0.004^{ab}	0.817 ± 0.008^{bc}	5.336 ± 0.264^{a}
	Substrate 2	Air Pot	0.638 ± 0.031^{c}	0.121 ± 0.001^{bc}	0.758 ± 0.030^{c}	5.286 ± 0.309^{a}
		C12	0.740 ± 0.019^{a}	0.132 ± 0.002^{ab}	0.871 ± 0.021^{a}	5.615 ± 0.069^{a}
	Substrate 3	Air Pot	0.707 ± 0.111^{ab}	0.134 ± 0.024^{a}	0.842 ± 0.135^{ab}	5.270 ± 0.108^{a}
		C12	0.664 ± 0.009^{bc}	0.124 ± 0.007^{bc}	0.788 ± 0.016^{bc}	5.373 ± 0.250^{a}
‘MM.106’	Substrate 1	Air Pot	0.651 ± 0.016^{bc}	0.126 ± 0.010^{bc}	0.778 ± 0.026^{c}	5.173 ± 0.293^{b}
		C12	0.714 ± 0.038^{ab}	0.095 ± 0.039^{d}	0.809 ± 0.077^{bc}	8.100 ± 2.940^{a}
	Substrate 2	Air Pot	0.666 ± 0.042^{bc}	0.122 ± 0.001^{c}	0.787 ± 0.041^{bc}	5.469 ± 0.372^{b}
		C12	0.807 ± 0.007^{a}	0.143 ± 0.010^{a}	0.951 ± 0.017^{a}	5.640 ± 0.346^{b}
	Substrate 3	Air Pot	0.738 ± 0.087^{ab}	0.126 ± 0.016^{bc}	0.864 ± 0.104^{ab}	5.886 ± 0.073^{b}
		C12	0.709 ± 0.012^{ab}	0.127 ± 0.004^{bc}	0.835 ± 0.016^{bc}	5.596 ± 0.083^{b}

Note: see Table 2.

The highest values of chlorophyll *a* (0.807 ± 0.007 mg/g FW), chlorophyll *b* (0.143 ± 0.010 mg/g FW), and total chlorophyll (0.951 ± 0.017 mg/g FW) were recorded in the ‘MM.106’ × Substrate 2 × C12 treatment. This indicates a favorable combination of substrate physical properties (improved aeration and structural stability due to bark addition) and stable air–water conditions in the standard container, supporting root system development and, consequently, the photosynthetic apparatus.

For the 'M.9' rootstock, maximum pigment values were also observed in the Substrate 2 × C12 treatment, confirming the positive role of bark-containing substrates. In most treatments, the chlorophyll *a/b* ratio ranged from 5.1 to 5.9, which is typical of well-illuminated leaves with a predominance of chlorophyll *a*.

An elevated chlorophyll *a/b* ratio (8.100 ± 2.940) observed in the 'MM.106' × Substrate 1 × C12 treatment was associated with a reduced chlorophyll *b* concentration and increased variability, which may reflect a physiological response to specific substrate conditions or the microclimate within the container. Overall, Table 6 shows that the highest pigment levels were formed under the combination of 'MM.106' and Substrate 2 in the standard container, whereas the lowest values were observed in treatments with pure peat and the Air-Pot system. Thus, even at the stage of two-year-old nursery trees, a clear influence of agronomic factors on plant pigment status is evident. Analysis of the main effects of the factors (averaged across other factors) allowed evaluation of the individual contribution of each variable (Table 7).

Table 7

Effects of rootstock, substrate and container type on chlorophyll content in leaves of two-year-old apple nursery trees (mg/g FW, mean ± SD, n = 10)

Factor	Factor level	Chl *a*	Chl *b*	Total *(a+b)*	*a/b*
Rootstock	'M.9'	0.682 ± 0.043^{b}	0.125 ± 0.009^{a}	0.807 ± 0.048^{b}	5.476 ± 0.264^{b}
	'MM.106'	0.714 ± 0.056^{a}	0.127 ± 0.019^{a}	0.841 ± 0.062^{a}	5.977 ± 1.008^{a}
Substrate	Substrate 1	0.677 ± 0.046^{b}	0.115 ± 0.024^{b}	0.792 ± 0.057^{b}	6.146 ± 1.492^{a}
	Substrate 2	0.713 ± 0.070^{a}	0.130 ± 0.009^{a}	0.843 ± 0.072^{a}	5.503 ± 0.203^{b}
	Substrate 3	0.705 ± 0.061^{ab}	0.128 ± 0.014^{a}	0.833 ± 0.068^{ab}	5.531 ± 0.258^{b}
Container	Air-Pot	0.676 ± 0.043^{b}	0.124 ± 0.010^{a}	0.800 ± 0.049^{b}	5.447 ± 0.337^{b}
	C12	0.720 ± 0.052^{a}	0.124 ± 0.021^{a}	0.844 ± 0.062^{a}	6.110 ± 1.095^{a}

Note: within each factor (rootstock, substrate or container), means followed by the same letter are not significantly different according to Tukey's honestly significant difference (HSD) test at $P < 0.05$.

The rootstock factor showed a consistent tendency toward higher chlorophyll content in plants grafted onto 'MM.106' compared with 'M.9'. The mean chlorophyll a content was 0.714 ± 0.056 mg/g FW for 'MM.106' versus 0.682 ± 0.043 mg/g FW for 'M.9'. A similar pattern was observed for total chlorophyll (0.841 ± 0.062 vs. 0.807 ± 0.048 mg/g FW, respectively), indicating a greater photosynthetic potential in plants grafted onto 'MM.106'.

The bark-containing substrate (Substrate 2) provided the highest average total chlorophyll content (0.843 ± 0.072 mg/g FW), which may be attributed to improved root-zone aeration and a more balanced water regime. In contrast, pure peat showed slightly lower values and greater variability in the chlorophyll *a/b* ratio. Container type had a moderate effect. The standard container provided slightly higher levels of chlorophyll *a* and total chlorophyll, which may be associated with more stable moisture retention compared with the Air-Pot system. Thus, among the main factors, the most pronounced effect was observed for the rootstock, whereas substrate and container type played a modulating role.

Three-way analysis of variance revealed that the chlorophyll *a/b* ratio was significantly influenced by rootstock ($P = 0.017$) and container type ($P = 0.026$). The effect of substrate was at the threshold of statistical significance ($P = 0.052$), indicating a tendency toward influence, although less pronounced than the genotypic effect (Table 8).

Table 8

Three-way ANOVA results (mg/g FW) – Chlorophyll *a/b* ratio

Source	F	P
Rootstock	6.87	0.017
Substrate	3.41	0.052
Container	5.72	0.026
Rootstock × Substrate	2.11	0.139
Rootstock × Container	3.02	0.071
Substrate × Container	1.89	0.168
Rootstock × Substrate × Container	1.55	0.213

Second- and third-order interactions (Rootstock × Substrate, Rootstock × Container, Substrate × Container, and the three-way interaction) did not reach statistical significance, indicating the predominance of additive effects of the main factors. Thus, the chlorophyll *a/b* ratio is primarily determined by the rootstock genotype and the characteristics of the container growing system.

Discussion

The present study demonstrates that, under container nursery conditions, the net photosynthetic productivity of apple leaves is driven primarily by the properties of the root-zone environment created by the substrate, whereas the container type plays a secondary role. This pattern is consistent with the broader concept that performance of container-grown plants largely depends on the physical–hydraulic characteristics of the growing medium (air-filled porosity, water-holding capacity, bulk density), which determine root aeration and water supply and, consequently, leaf gas exchange and carbon assimilation (Barrett et al., 2016; Nazim & Gruda, 2019).

Higher values of net photosynthetic productivity recorded for Substrate 1 (peat-based) and the structurally improved Substrate 3 can be interpreted as the outcome of a more balanced water–air regime in the root zone, enabling stable transpiration, stomatal functioning and CO_2 diffusion to chloroplasts. Similar conclusions have been reported for container production systems, where optimization of substrate structure improves plant physiological performance by preventing extremes of waterlogging or drought within the container profile (Barrett et al., 2016; Burnett et al., 2016).

By contrast, the reduced photosynthetic productivity observed with Substrate 2 suggests that, in the tested configuration, this medium likely created suboptimal aeration and/or a less stable moisture regime, which can restrict root activity and indirectly downregulate photosynthesis. From a mechanistic perspective, limited oxygen diffusion in container substrates is known to constrain root respiration and nutrient uptake, which can induce partial stomatal closure and suppress photochemical and biochemical processes in leaves. This interpretation agrees with the emphasis in soilless-culture literature that substrate selection must prioritize rewetting capacity, pore architecture and oxygen availability to maintain stable plant function (Raviv, 2017; Nazim & Gruda, 2019).

The absence of a statistically significant container effect on net photosynthetic productivity indicates that, within the tested volume (12 L) and management regime, the substrate effects were dominant. Nevertheless, the literature shows that container design can substantially reshape root architecture (e.g., reduced circling, altered fine-root distribution) via air-pruning or wall geometry, which may translate into differences in canopy growth and physiology depending on crop, production stage and irrigation strategy (Amoroso et al., 2010; Elsysy & Einhorn, 2020). Therefore, the non-significant container effect in our experiment likely indicates that the tested container types did not override the substrate-driven differences in root-zone conditions, rather than indicating that container design is universally irrelevant.

The chlorophyll results further support the conclusion that both genotype (rootstock) and root-zone conditions shape photosynthetic capacity. The higher mean chlorophyll content observed in plants grafted on 'MM.106' compared with 'M.9' suggests a greater capacity to maintain or build the pigment apparatus under the same container environment, which may reflect differences in vigor and root system functioning between rootstocks. Genotype-dependent differences in physiological performance are well documented in perennial nursery production, particularly when root constraints and resource supply interact with scion demand (Venig et al., 2025).

At the same time, substrate structure clearly modulated pigment accumulation: the peat–bark substrate (Substrate 2) produced the highest average total chlorophyll, indicating that improved structural stability and aeration can favor chlorophyll synthesis and/or reduce pigment degradation under container stress. Evidence from growing-media research highlights that inclusion of renewable structural components (e.g., bark, wood fibers, composts) can improve aeration and hydrology of peat-based systems, supporting plant functioning while also addressing sustainability concerns (Barrett et al., 2016; Raviv, 2017).

Finally, the significant effects of rootstock and container on the chlorophyll *a*/*b* ratio indicate that pigment balance is sensitive not only to substrate composition but also to micro-environmental conditions linked to container configuration (e.g., evaporation rate, temperature dynamics, and moisture distribution). Prior work on container systems has shown that container design influences root morphology and post-planting performance, which can manifest as physiological differences, particularly under stress or variable irrigation (Gilman & Paz, 2014; Gilman et al., 2016). Collectively, these findings support a technology strategy where substrate optimization is the primary lever for stabilizing photosynthetic productivity, while container selection can be tailored for operational benefits and, in some cases, for fine-tuning physiological traits.

Conclusion

The study showed that net photosynthetic productivity of apple nursery trees is primarily determined by substrate composition, while container type does not exert a significant independent effect. Substrate 1 consistently promoted the highest photosynthetic performance across both rootstocks and age groups, whereas Substrate 2 led to a stable reduction in photosynthetic intensity. The ‘M.9’ rootstock exhibited higher photosynthetic rates at a younger age but was more sensitive to growing conditions, while ‘MM.106’ demonstrated greater physiological stability. Three-year-old trees tended to show lower photosynthetic activity than two-year-old plants, likely reflecting age-related shifts in growth dynamics and carbon allocation patterns. The lack of significant interaction between container type and substrate suggests that substrate properties exert a generally consistent effect on plant physiological status. For container-based apple nursery production, substrates with well-balanced air–water characteristics are essential to maintain active root function and maximize photosynthetic potential.

The results also indicate that pigment composition in leaves of two-year-old nursery trees is largely controlled by rootstock genotype and further influenced by container-growing conditions. Trees grafted on ‘MM.106’ accumulated higher levels of chlorophyll *a*, chlorophyll *b*, and total chlorophyll than those on ‘M.9’, reflecting a greater inherent photosynthetic capacity. The bark-amended substrate (Substrate 2) enhanced pigment accumulation, and the C12 container provided more stable pigment parameters compared with the Air-Pot system. The significant effects of rootstock and container type on the chlorophyll *a*/*b* ratio highlight the importance of integrating genotype selection with optimized substrate and container management to produce high-quality nursery trees with improved physiological performance.

The authors declare no conflict of interests.

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