ITALUS HORTUS
Brief Research Report
Vol. 32 (2025), Pages 44-51
doi: 10.26353/j.itahort/2025.1.4451

Rootstock age affects growth and nutrient uptake from apple trees in the nursery

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Received: 15 October 2024; Accepted: 3 June 2025; Available on line: 15 July 2025

Abstract: The objective of this study was to evaluate the effect of the rootstock age (one and two years) on the vegetative growth and nutrient uptake from apple trees (cv. 'Galaxy' on 'M.9' EMLA rootstock). The study was conducted in a commercial nursery in central valley Chile, at a density of 40,000 trees ha^{-1}. Biomass and nutrient concentrations (N, P, K, Ca, Mg, Mn, Cu, Zn and B) were determined at the end of the growing season, in separate samples of leaves, shoots (wood above the root collar) and roots. The results showed that rootstock age had a significant effect on biomass accumulation and thus on plant nutrient extraction. Apple trees with two-year-old rootstock reached twice the total fresh biomass compared to one-year-old rootstock. Dry biomass was distributed as follows: 33% leaves, 57% shoots and 10% roots. The uptake of macroelements from apple trees with two-year-old rootstock was twice as high as that of one-year-old trees, according to the following descending order: N>Ca>K>Mg>P. Apple trees on two-year-old rootstock took up 3.3 times more Zn, twice more Mn and Cu, and 1.7 times more B compared to those on one-year-old rootstock, with Zn and B as the main elements in the shoots. Differences in the concentration of certain mineral elements were significant at shoot and root level according to rootstock age, being directly related to plant biomass.

Keywords: nutrition; nursery; leaves; roots; shoots; *Malus* × *domestica* Borkh.

1. Introduction

Biomass production is highly dependent on the species, conduction system, nutrient supply, irrigation and tree health (Ebel et al., 2000; Bi et al., 2004; van Hooijdonk et al., 2015; Sorrenti et al., 2017). In the nurseries, trees are planted at much higher densities than in a fruit orchard, which may generate high nutrient uptake. In order to ensure rapid vegetative growth of plants, nursery operators tend to overfertilize the crop, ignoring its real nutrient demands, causing higher cost and environmental pollution (Natale et al., 2018; Świerczyński and Antonowicz, 2021; Zanotelli et al., 2022).

The amount of solar radiation intercepted by the canopy is positively correlated with biomass production in fruit trees. In recent decades, apple tree production has been intensified through the adoption of training systems that favor light interception and orchard precocity. Moreover, new conduction systems and plantation design have been incorporated to facilitate orchard agricultural work. This has been possible through the use of dwarfing rootstocks, which allow the control of tree height and vigor (Lezzer et al., 2022).

The rootstock influences varietal vigor and affect nutrient needs (Fallahi et al., 2001, 2002; Gjamovski et al., 2017; Kviklys et al., 2017). In apple trees, the rootstock 'M.9' has been widely used as a dwarfing rootstock, as it provides precocity and ease in canopy management (Reginato et al., 2008; Yuri et al., 2011; Anthony and Musacchi, 2021). In Chile, nurseries deliver apple trees with the variety grafted mostly on one-year-old rootstocks; if the growers require a plant with early shoots or "knip-type" plants, two-year-old rootstocks are used.

Currently, there are several studies that relate nutrient allocation to the leaves and the rootstock vigor (Gjamovski et al., 2017; Kviklys et al., 2017). However, the nutrient uptake by the whole plant in nursery production of apple trees has been object of very few studies (Zanotelli et al., 2022). Such info would allow calculating the amount of nutrient needed to support tree growth in the nursery. The objective of this study was to determine the biomass and the amount of nutrients in leaves, shoots, and roots of one-year-old 'Galaxy' apple trees grafted on one- and two-year-old 'M.9' rootstock.

2. Materials and Methods

2.1. Site and experimental desing

The study was conducted in the commercial nursery "Vivero Los Olmos" located in the central valley of the O'Higgins Region (34º 37' S, 70º 58' W; 364 m a.s.l.) in Chile. The climate is semi-arid Mediterranean, with a seven-month water deficit, an annual rainfall of 753 mm, and an average annual air temperature of 14.5 ºC. The soil has a sandy loam texture, alluvial origin and is flat. The soil chemical analysis was carried out at the end of the growing season, at a 0-30 cm depth, based on Chilean analysis methods, according to Sadzawka et al. (2007). The mineral content and chemical characteristics of the soil are summarized in Table 1.

Table 1. Soil chemical properties at the experimental site.

Available N (ppm)	Olsen P (ppm)	Exchangeable Ca (cmol kg^{-1})	Exchangeable Mg (cmol kg $^{-1}$)	Exchangeable Na (cmol kg^{-1})	Exchangeable K (cmol kg^{-1})	Organic matter (%)	pH	Electrical conductivity (dS m^{-1})
15	19	10.32	1.24	0.19	0.26	1.2	5.5	0.12

Two treatments were evaluated: one-year-old apple trees cv. 'Galaxy' grafted on one- and two-year-old 'M.9' EMLA rootstock. The rootstocks were grown in the nursery for one to two years at a density of 1.0 × 0.25 m (40,000 trees ha^{-1}). They were subsequently grafted with the commercial variety using a two-bud scion. A randomized complete split plot experimental design was used, with rootstock age as main factor and soil status as the subplot. Each treatment consisted of three replicates.

The trees were drip irrigated using one irrigation line per row. Both plots received a single soil application of 115 kg N ha^{-1} (as urea) prior to establishment and a foliar application of 145 kg N ha^{-1} in spring, following leaf development of the commercial variety.

The evaluations were carried out at the end of the growing season (early autumn still with a completely green canopy), harvesting homogeneous trees to measure their biomass and mineral concentration, differentiating among leaves, shoots (wood above the root collar) and roots.

2.2. Evaluations of plant material

The trees were defoliated individually to measure the fresh weight (g) of leaves, while the woody parts were cut to determine the fresh weight of shoots and roots, separately. Dry weight (g) of each organ was measured after the samples were dried to constant weight in a forced-air oven (D-6450, Heraeus GmbH & CO. Hanau, Germany). Dry matter (%) was calculated using the fresh and dry weigh data. Leaf area (cm^2) was measured with a leaf area meter (LI-3100, LI-COR Biosciences Inc., Lincoln, USA).

To analyze macro and microelements, six random subsamples of leaves, shoots, and roots were taken from the trees. The total nitrogen (N) was determined by the Kjeldahl procedure, whereas total potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), zinc (Zn), cupper (Cu) by atomic absorption spectrophotometry in an air-acetylene flame. The phosphorus (P) content was measured by the nitro-vanado-molybdate method, and boron (B) by the H-azomethine colorimetry method accorded to Sadzawka et al. (2007). The concentration of macronutrients was expressed as a percentage and the micronutrients as ppm.

The uptake of mineral elements (kg ha^{-1}) in each organ was determined using the following equation:

$$\text{Amount of nutrient (kg/ha)} = \text{Element concentration (\%)} / 100 \times \text{DM (kg/tree)} \times \text{No trees/ha}$$

Where DM is the total tree dry matter.

2.3. Statistical analysis

The data were subjected to a simple variance analysis (p ≤ 0.05) using Statgraphics Plus 4.0 software (Warrenton, Virginia, USA). The figures were generated using SigmaPlot 10 software (WPcubed GmbH, München, Germany).

3. Results

3.1. Mineral concentration

The highest concentration of macro and microelements was found in leaves, followed by roots and then by shoots (Table 2). Leaves tended to show a higher concentrations of minerals in trees grafted on two-year-old rootstock, although without significant differences. Shoots of trees on two-year-old rootstock reached lower P, K and B concentrations and higher Zn concentration than those grafted on one-year-old rootstock. In the roots, trees on two-year-old rootstock showed a higher N and lower K and Cu concentrations than those on one-year-old rootstock.

Table 2. Mineral concentration of leaves, shoots and roots of ‘Galaxy’ apple trees on one- and two-year-old ‘M9’ rootstock. The different letters indicate significant differences in the values between rootstock age (* = significantly different at $p \leq 0.05$, ns = not significant), $n = 6$.

Organ	Rootstock age	N	P	K	Ca	Mg	Mn	Zn	Cu	B
	(years)	(%)					(ppm)			
Leaves	1	3.63	0.23	1.75	1.12	0.31	70.7	88.4	11.0	46.7
	2	3.88	0.22	1.84	1.27	0.30	66.7	103.5	11.7	41.7
	Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Shoots	1	0.97	0.13 a	0.67 a	0.97	0.12	20.7	55.0 b	8.3	38.7 a
	2	0.86	0.11 b	0.56 b	0.80	0.12	19.7	109.3 a	8.7	27.7 b
	Significance	*ns*	*	*	*ns*	*ns*	*ns*	*	*ns*	*
Roots	1	1.06 b	0.18	0.68 a	1.02	0.18	57.7	36.3	32.7 a	47.7
	2	1.29 a	0.17	0.60 b	1.21	0.17	52.0	38.0	20.0 b	38.3
	Significance	*	*ns*	*	*ns*	*ns*	*ns*	*ns*	*	*ns*

3.2. Biomass partitioning

Rootstock age significantly affected the total biomass accumulation of apple nursery trees (Figure 1). Four months after grafting, dry biomass production of trees on two-year-old rootstock was 2.3 times greater than those on one-year-old rootstock, mainly due to greater root growth, which was 7.5 times higher in the former trees (Figure 1A). At the end of the vegetative growth, trees grafted on two-year-old rootstock showed 2.8 times as much total dry biomass accumulation as those on one-year-old rootstock (Figure 1B). The percentage distribution of dry biomass in the tree was very similar between treatments, with an average value of 33% for leaves, 57% for shoots and 10% for roots. In both treatments, leaf dry matter reached 36%, while in the permanent structures it was 44%.

Trees grafted on two-year-old rootstock showed twice leaf area index (LAI) compared to those on one-year-old rootstock (Figure 2), given their greater height and number of laterals that produced a greater number of leaves per plant (115 vs. 53). However, leaf size showed no differences between treatments (an average of 45 cm^2/leaf).

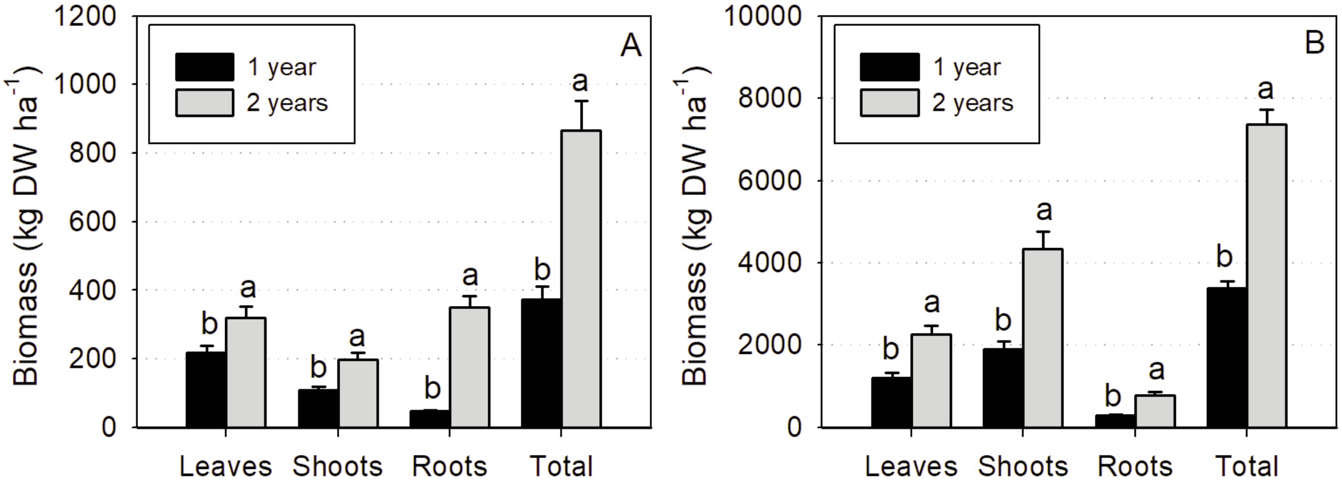

Figure 1. (**A**) Dry weight at the beginning and (**B**) end of the vegetative growth (kg ha^{-1}) in leaves, shoots, roots and in the entire plant of nursery apple trees cv. 'Galaxy' according to rootstock age. Values are means + standard error (*n* = 12). The different letters indicate significant differences in the values between rootstock ages ($p \leq 0.05$).

Figure 2. Leaf area index (LAI; m^2 m^{-2}) in nursery apple trees cv. 'Galaxy' according to rootstock age. Values are means + standard error (*n* = 12). The different letters indicate significant differences in the values between rootstock age ($p \leq 0.05$).

3.3. Mineral uptake

The amount of plant macroelement accumulated per hectare of nursery was twice as high in trees grafted on two-year-old rootstocks compared to those on one-year-old rootstocks. The amount extracted of mineral elements had the following descending order according to element: N>Ca>K>Mg>P, independent of rootstock age (Figure 3).

N, K, and Mg uptake was higher in leaves, representing 66%, 59% and 54%, respectively, in relation to the total plant. P uptake in leaves was equivalent to that in shoots, reaching 44%. In the case of Ca, the percentage extracted in the permanent structures represented 61% in relation to the total plant (Figure 3).

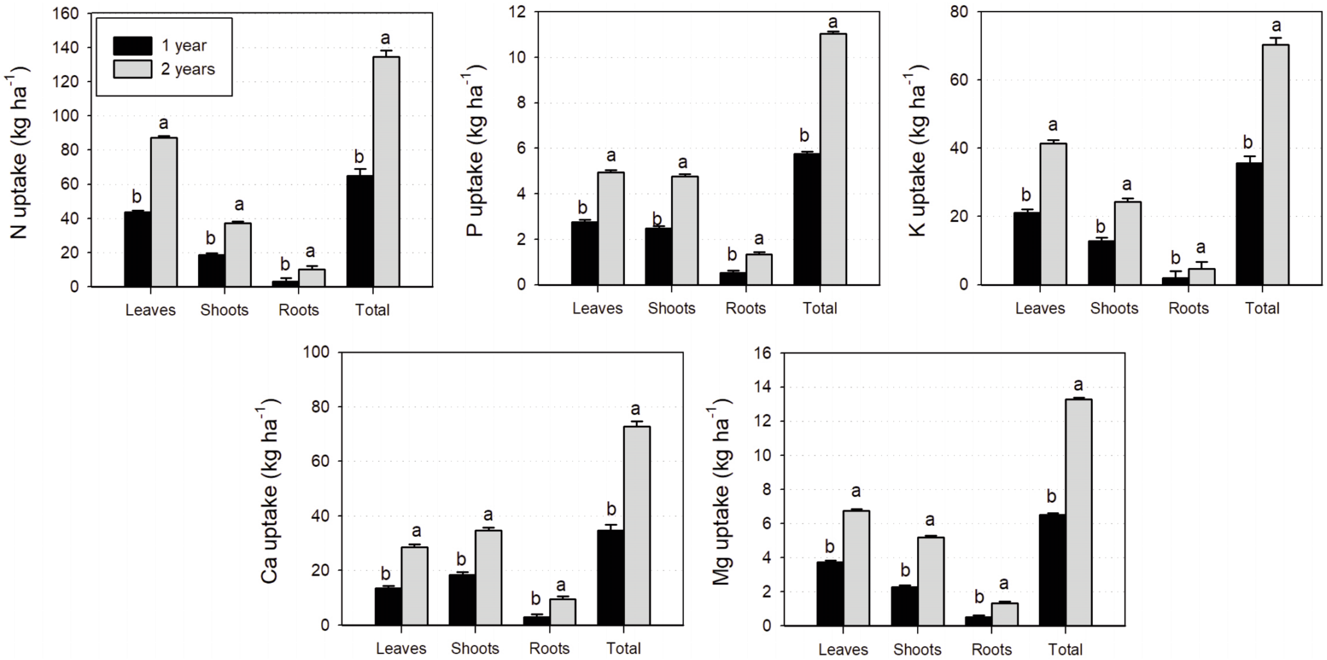

Figure 3. Macroelement uptake (kg ha^{-1}) in leaves, shoots, roots and in the entire plant of nursery apple trees cv. 'Galaxy' according to the rootstock age. Values are means + standard error (n = 12). The different letters indicate significant differences in the values between rootstock age ($p \leq 0.05$).

Apple trees on two-year-old rootstocks took up 3.3 times more Zn, twice more Mn and Cu, and 1.7 times more B compared to those on one-year-old rootstocks. The amount of micronutrients occurred according to the following descending order: Zn>Mn>B>Cu (Figure 4).

Mn was found mostly in the leaves (60% in relation to the total plant). In contrast, most Zn, Cu, and B were in the shoots, with an average of 40%. The roots contained relatively lower amounts of microelements (<20%), compared to the rest of the organs evaluated (Figure 4).

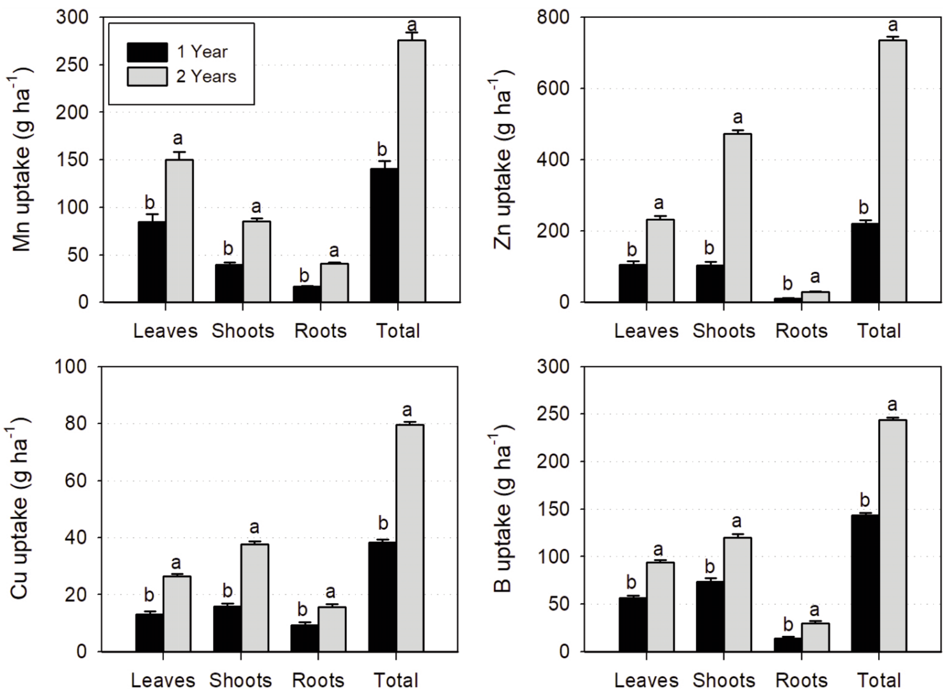

Figure 4. Microelement uptake (g ha^{-1}) in leaves, shoots, roots and in the entire plant of nursery apple trees cv. 'Galaxy' according to the rootstock age. Values are means + standard error (n = 12). The different letters indicate significant differences in the values between rootstock age according to Tukey test ($p \leq 0.05$).

4. Discussion

The rootstock regulates plant vigor and thus can influence planting density, which determines the nutritional requirements of the crop (Webster and Wertheim, 2003). Fallahi et al. (2001) observed significant differences in element concentration among apple rootstocks ‘Bud.9’, ‘Ottawa 3’, and ‘M.7’, with higher leaf concentration of Mg and K in the more vigorous rootstocks. Amiri et al. (2014) reported higher efficiency of rootstock ‘M.9’ in taking up and transporting N and Mn compared to high vigor rootstocks such as ‘MM.106’ and ‘MM.111’. The authors associated the differences to the various root distributions in each rootstock, as well as to the xylem anatomy and its hydraulic capacity to supply the mineral solution, whereas Gjamovski et al. (2017) noted that the differences between nine apple dwarfing rootstocks in foliar element concentration were not significant.

In nursery studies, Sorrenti et al. (2017) showed that ‘Santa Maria’/’Quince A’ pear trees had higher N uptake than ‘Golden Delicious’/‘M.9’ apple trees (186 vs 106 kg N ha^{-1}), at equal planting density (30,000 trees ha^{-1}). However, the authors point out that this higher nutrient demand in pear trees would be related to the higher plant biomass (650 vs. 370 g DW/tree), which depended on the rootstock combination, rather than on the species difference. Water availability and soil temperature affect plant biomass partitioning and therefore nutrient uptake, with water limitations affecting particularly stems and leaves, and to a lesser extent roots (Valverdi et al., 2019).

Nitrogen is taken up by plants mainly in the period of greatest shoot and leaf growth, being essential in the formation of organic compounds (Bi and Fuchigami, 2004). Nursery trees have no fruit, which means that all element consumption is allocated to vegetative growth.

In the nurseries, the main mineral elements applied to the soil are N, P, and K, complemented with foliar applications of microelements. The high nitrogen input seeks to stimulate vegetative development, to obtain an adequate trunk thickness and shoot development that allows a successful crop establishment (Natale et al., 2018). P has special relevance given its role in meristematic activity during root and shoot growth. Similar to P, Zn is an element with low mobility in the soil, so its absorption depends on the surface area explored by the roots (Eissenstat et al., 2001).

Root growth is valued by apple growers, as they associate root size with a better initial development of their orchards. However, recent studies contradict this assertion, showing that soil preparation and irrigation during planting are similarly or even more relevant than root size (Yuri et al., 2024).

The nutrient uptake rate of “knip-type” apples was reported to be of 50 kg N ha^{-1} the first year after grafting, and of 120 kg N ha^{-1}, 16 kg P ha^{-1}, 81 kg K ha^{-1}, 112 kg Ca ha^{-1} and 23 kg Mg ha^{-1} at the end of the second year (Zanotelli et al., 2022), values slightly lower than those obtained in the present study. On 15-year-old apple trees cv. ‘Galaxy’, the amount of mineral uptake per hectare for the permanent structures (stem, shoots and roots) was mainly attributed to Ca, N and K, reaching 238, 153, and 86 kg, respectively (data not shown).

In the present study, apple trees reached a high vegetative growth, with foliar N levels of 3.6%-3.9%, considered above the norm (2.5%). In general for both treatments, the concentrations of the other macroelements in the leaves were within normal ranges for an adult plant (Neilsen and Neilsen, 2003).

The application of high doses of ammoniacal nitrogen to the soil can alter its physicochemical and biological properties, such as a decrease in pH and organic matter, imbalance in the availability of elements (particularly K, Mg, Ca), lower biological activity, among others (Balezentiene and Klimas, 2009; Pahalvi et al., 2021). On the other hand, previous studies have reported that excessive applications of fertilizers in agriculture generate an increase in the accumulation of heavy metals in the soil and water pollution, as well as harmful effects on the environment and human health (Dobermann et al., 2022; de Vries et al., 2024).

The nitrogen dose used in this research generated a very high accumulation in plants. However, the efficiency of fertilizer uptake was different between rootstocks of different age, with the apple trees on two-year-old rootstocks reaching 52%, while in plants on one-year-old rootstocks it reached 25%.

5. Conclusions

The present study carried out on 'Galaxy' apple trees grafted on 'M.9' rootstock showed that using two-year-old rootstocks doubled the biomass production per hectare expressed in dry matter and leaf area, with respect to those produced by plants on one-year-old rootstocks. This higher biomass resulted in a significant increase in the macro and microelements uptake rates. N was the most extracted element with a total of 135 kg ha^{-1}, followed by Ca and K with 72 and 70 kg ha^{-1}, respectively. Nursery trees have a high uptake of nutrients from the soil given their rapid growth. The results of this study could make it possible to adjust the nutrient dose for greater resource use efficiency and crop sustainability.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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