Article

Effect of rootstock by approach on the nutritional status of 'Brookfield Gala' apple tree

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Abstract

In high-density apple orchards, dwarfing rootstocks play a key role, as they promote or stimulate early production, increase yield and quality of fruit per unit surface area. However, their horticultural performance depends on the capability of soil to supply water and nutrients to the root system. Under shallow soil limiting conditions some dwarfing rootstocks are not able to meet water and nutrient requirements for the variety. In Aquixtla, Puebla, growth and development in 'Brookfield Gala'/Bud.9 apple trees are weak, with symptomatology of mineral deficiencies, in contrast to the trees grafted in Emla7, which are producing fruits normally. This work reports the nutritional status of 'Brookfield Gala' /Bud.9 trees, supported by the rootstock Emla7. The foliar analysis in 2018 showed a higher concentration of Mn and B in 'Brookfield Gala' /Bud.9 trees with approach graft performed in 2016 and 2017, respectively. In 2019 the concentration of Cu and B was higher in the trees with approach graft performed in 2017. In all trees the concentration of Zn and K was low and the concentration of Ca and B was high. The effect of approach grafting with the rootstock Emla7 was incipient in the development and growth of the 'Brookfield Gala'/Bud.9 apple tree.

Keywords: *Malus pumila* Mill., dwarfing rootstocks, high densities.

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Introduction

For the growth and development of fruit trees, rootstocks play an important role in the absorption and transport of water and nutrition, as well as the translocation to their different organs (Fallahi *et al*., 2000), which is influenced by the morphology and spatial distribution of roots (Gregory *et al*., 2013). Rootstocks can be selective in nutrient absorption, so they should be carefully selected to overcome unfavorable conditions (Fazio *et al*., 2013). In high-density plantations, the nutritional requirement differs from conventional plantations because dwarfing rootstocks induce precocity, fruit quality and higher yield (Cheng and Raba, 2009) and control tree vigor due to its efficiency in reducing or delaying competition for available space (Chalmers *et al*., 1981; Lo Bianco *et al*., 2003).

Rootstocks M.27, M.9 and M.26 can reduce trunk cross-section area and canopy volume, with positive effects on fruit quality compared to vigorous rootstocks (Autio *et al*., 1996; Fallahi *et al*., 2002).

Dwarfing rootstocks in limiting soil and water conditions do not always have the same efficiency with the grafted cultivar, due to nutrient deficiency. In the establishment of an orchard with a cultivar adapted to the environmental conditions of the place, but not to those of the rootstock, the productive potential can be seriously affected. Such is the case of an orchard in Aquixtla, Puebla, in which the Brookfield Gala cultivar is grafted onto the rootstocks Bud.9 and Emla7. The trees in the first rootstock have temporary nutritional deficiencies and low vigor. An alternative to this problem is to replant with a suitable rootstock; however, this is expensive and means waste of time.

In fruit trees, techniques such as intergraft have been used for resistance to pests and diseases or to increase yield, with positive results in some cultivars. Also, the use of approach graft in replanting with phytopathogenic incidences has been observed to improve the absorption of nutrients.

Approach grafting consists of planting young seedlings from another rootstock next to a grafted tree and then grafting them onto the trunk of the trees of the variety (Bové and Ayres, 2007). It has also been used with dwarfing rootstocks to improve the vigor of the variety by supporting vigorous rootstocks (Hartmann *et al*., 2002). In deficient boron conditions (B), in 'Newhall' orange this technique increases the concentration of B in foliage and improves the development of tree roots (Wang *et al*., 2016).

Therefore, the objective of this study was to evaluate the nutritional status and vigor of the apple tree of the cv. Brookfield Gala grafted onto the rootstock Bud.9, supported by the rootstock Emla7 by approach graft, on a shallow soil of the municipality of Aquixtla, Puebla. The rootstock Emla7 was selected because it works well with the *cv* Brookfield Gala, so it is expected to significantly improve the nutritional status and vigor of the 'Brookfield Gala'/Bud.9 trees.

Materials and methods

Experimental site

The work was ca rried out in 2018 and 2019 in a commercial apple orchard located in the Municipality of Aquixtla, which is in the northeastern part of the state of Puebla, with geographic coordinates of 19°48’ 05’’ north latitude and 97° 56’ 32’’ west longitude. The climate is temperate subhumid with rains in summer (600-900 mm) and annual temperature of 12-18 °C, at an altitude of 2 291 m (INEGI, 2009).

According to WRB (2006), the soil is a Technosol characterized by its original tuff material (tepetate) modified by the farmer, which through subsoiling was broken and fragmented and with the mixture of soil from another source, organic matter and fertilizers was possible to use it in agriculture. The tuff material contains 45-60% silicon oxide, so there is a greater presence of olivine, amphiboles and pyroxenes that easily degrade making their elements available for plant nutrition.

Regarding the physical study, the soil profile showed five strata at the depths of 0-18, 18-30, 30-41, 41-57 and 57-80 cm, respectively. From the second to the fourth stratum, it is composed of fragmented tepetate with mixed materials, compact that has no porous spaces. The texture is clay-loam. At the depth of 0-30 cm high concentrations of K, Mg, Fe and Cu and low concentrations of N and Zn were found; 30-60 cm deep high concentrations of Fe and Mn and low levels in N, P, Ca, Zn and Na were found.

Plant material

The orchard was established in March 2014 with the Brookfield Gala cultivar grafted onto the rootstocks Bud.9 and Emla7 in an intensive production system. Brookfield Gala cultivar is a natural mutation of the Royal Gala cultivar, originated in New Zealand. Bud.9 is a dwarfing rootstock, of low vigor with fewer branches and short spurs. It induces early production in the second to third year and is resistant to pests and diseases, as well as low temperatures. Rootstock Emla7 is semi dwarf, it emits many sprouts and long spurs, also induces precocity to the third or fourth year with high yield. It is tolerant of neck rot, resistant to fire blight and tolerant of cold and drought.

Treatments and experimental design

Four treatments were defined according to the cultivar grafted onto the rootstock and the year in which the approach graft was performed, as follows: 1) ‘Brookfield Gala’/Bud.9; 2) ‘Brookfield Gala’/EMLA7; 3) ‘Brookfield Gala’/Bud.9+Emla7-2016; and 4) ‘Brookfield Gala’/Bud.9+Emla7-2017. The four treatments were distributed in the field according to a randomized complete block experimental design with three repetitions. The blocks were formed in relation to the vigor of the trees by measuring the diameter (cm) of the trunk (low, medium and high vigor). At a density of 4 000 trees ha, the experimental unit was a tree. Treatment 2 was considered as witness; that is, ‘Brookfield Gala’/Emla7, since it presents the best horticultural performance.

Management and conduction of the experiment

The trees are set at a planting distance of 1 m between plants and 2.5 m between rows. The trees had a height of 1.5 m in the rootstock Bud.9 and 2.25 m in the rootstock Emla7, they are formed with the Tall Spindle conduction system, which aims to take advantage of all lateral branches for fruiting (Robinson *et al*., 2014).

Irrigation was done by dripping, nutrition through fertigation applying 40 kg of nitrogen and 60 kg of potassium per ha, one application before vegetative sprouting, and the application of micronutrients via foliar with the formula 20-20-20 and 0.5 kg of magnesium sulfate in 100 L of water, every 20 days from vegetative sprouting until before harvest (approximately 30 days). In addition, two boron applications are carried out, at the beginning of the opening of the vegetative buds and at the opening of flower buds. Pollination is done with *cv* Granny Smith and placing of 'vases' with cultivars from the region.

Nutritional status

It was determined by foliar analysis, for which two samplings were performed in the 2018 cycle; the first at 24 days after full bloom (DDPF) and the second at 106 DDPF; that is, twenty days before harvest. A sample composed of mature leaves was obtained from the middle of the tree crown in spurs shoots of growth of the year, cutting 30 leaves with petiole of the east part and 30 leaves of the west. The samples were processed prior to analysis.

The leaves were washed with running, distilled and deionized water, then they were dried in a convection stove (Imperial V Mechanical and gravity convention ovens Dubuque Iowa, USA) at 68 °C for 72 hours. Later, they were ground into a stainless-steel mill (General Electric Model AC 160, USA). Nitrogen (N) was determined through the Micro-Kjeldahl method (Bremmer, 1965). The concentration of P, K, Ca, Mg, Fe, Mn, Zn, Cu and B was determined by inductively coupled plasma atomic emission spectrometry (ICP-OES Varian 725ES, Australia).

In the 2019 cycle, sampling was only carried out at 24 DDPF using the same procedure of 2018, just as temporary deficiencies in trees appeared, the same nutrients were determined with the procedure used in 2018.

Trunk cross section area (ASTT)

It was determined in 2018 and 2019, measuring the diameter (cm) of the trunk of the cultivar, 15 cm above the point of junction between the cultivar and the rootstock of each treatment: 'Brookfield Gala'/Bud.9, 'Brookfield Gala'/Emla7 and 'Brookfield Gala' /Bud.9+Emla7 established in 2016 and 2017 (Figure 1), using a digital vernier of the brand Truper Stainless and the formula ASTT= $(C)^2/4\ 16\ \pi$, in which, ASTT= area of the cross section of the trunk in cm^2 and C= circumference of the trunk in cm.

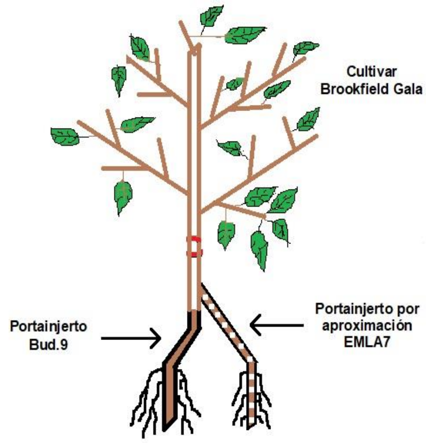

Figure 1. Brookfield Gala cultivar grafted onto Bud.9, with the rootstock Emla 7 grafted by approach.

Statistical analysis

The data were subject to an analysis of variance (Anova), using the SAS version 9.4 statistical analysis program and the comparison of means with the Tukey test with $p \leq 0.05$.

Results and discussion

Nutritional status

In 2018, at 24 days after full bloom (DDPF) the concentration of N, P, K, Ca, Mg, Zn, Mn and B was not significantly affected by the treatments, but Fe and Cu were (Table 1). The results of the nutrient concentrations were compared with the values of the *cv* Gala/Emla7 reported by Nachtigall and Dechen (2006) and *cv* Gala/M.26 by Cheng and Raba (2009), which coincide with the same sampling dates. In the Fe concentration, treatment 2 (222.25 mg kg^{-1}) was superior with respect to the rest of treatments, whose concentration ranged from 156.33 to 170.65 mg kg^{-1}.

Table 1. Effect of approach graft on the mineral concentration of macro and micronutrients on young leaves of 'Brookfield Gala' apple trees, 24 days after full bloom in 2018.

Treatments	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn	B
	(mg g^{-1})						(mg kg^{-1})			
1	27.3 a	3.95 a	12.1 a	7.33 a	2.9 a	156.33 b	9.5 ab^{y}	20.03 a	58.73 a	76.2 a
2 (witness)	27.75 a	3.7 a	13.15 a	6.95 a	3 a	222.25 a	12.3 a	16.95 a	56.8 5a	72.2 a
3	25.73 a	3.63 a	12.25 a	6.96 a	2.73 a	170.65 b	8.5 b	19.06 a	68.16 a	73.63 a
4	25.15 a	3.86 a	11.13 a	7.13 a	2.76 a	156.8 b	8.43 b	18.56 a	64.9 a	73.86 a
DHS	5.52	0.54	4.06	2.59	0.52	66.98	3.41	4.26	19.81	11.81

^{y}= different letters within the columns indicate significant differences ($p \leq 0.05$). Treatments: 1) 'Brookfield Gala'/Bud.9; 2) 'Brookfield Gala'/Emla7; 3) 'Brookfield Gala'/Bud.9+Emla7-2016; and 4) 'Brookfield Gala'/Bud.9+Emla7-2017.

This indicates that the rootstock Emla7 is more efficient at absorption of iron and is due to the greater number of roots it has, which allows a further exploration of the soil. These values are higher than those found by Nachtigall and Dechen (2006); Cheng and Raba (2009). The concentration of Cu, treatment 2 (witness) was higher (12.30 mg kg^{-1}) with respect to treatments 3 and 4 (8.43 and 8.5 mg kg^{-1}, respectively in 2016 and 2017), treatment 1 resulted in an intermediate value (9.5 mg kg^{-1}). These values are higher than those reported by Nachtigall and Dechen (2006), which could be due to foliar applications of micronutrients.

At 106 days after full bloom in the same year, the concentration of N, P, K, Ca, Fe, Cu and Zn was not significantly affected by treatments, but Mg, Mn and B were (Table 2). The concentration of magnesium, treatment 2 was higher (4.7 mg g^{-1}) compared to treatments 1 and 4 (3.83 and 3.86 mg g^{-1}). In treatment 3 the concentration was intermediate (4.03 mg g^{-1}), which reflects the effect of the year in which the graft by approach was performed. These results are similar to those reported on the *cv* Gala/Emla7 (Nachtigall and Dechen, 2006); however, in treatments 1 and 4 the values are lower, which may suggest that the rootstock Bud.9 has low efficiency in the absorption of Mg.

Table 2. Effect of approach graft on the mineral concentration of macro and micronutrients on mature leaves of 'Brookfield Gala' apple trees, 106 days after full bloom in 2018.

Treatments	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn	B
	($mg\ g^{-1}$)						($mg\ kg^{-1}$)			
1	22.83 a	2.43 a	10.63 a	17.63 a	3.83 b[y]	152.25a	8 a	17.8 a	101.07 b	63.8 ab
2 (witness)	24.6 a	2.2 a	10.8 a	17.15 a	4.7 a	163.95 a	9.9 a	16.15 a	98.35 b	58.05 b
3	24.96 a	2.2 a	9.63 a	17.6 a	4.03 ab	175.8 a	7.4 a	15.43 a	153.5 a	62.73 ab
4	24.46 a	2.4 a	10.53 a	17.73 a	3.86 b	162.2 a	7.4 a	18.16 a	105.4 b	66.03 a
DHS	5.16	0.6	2.02	2.67	0.92	64.49	2.94	10.04	43.35	7.59

[y]= different letters within the columns indicate significant differences ($p\leq 0.05$). Treatments: 1) 'Brookfield Gala'/Bud.9; 2) 'Brookfield Gala'/Emla7; 3) 'Brookfield Gala'/Bud.9+Emla7-2016; and 4) 'Brookfield Gala'/Bud.9+Emla 7-2017.

The concentration of manganese, treatment 3 was higher (153.5 mg kg^{-1}) compared to the other treatments whose concentration ranged from 98.35 to 105.4 mg kg^{-1} respectively. These results indicate that by approach graft the concentration of Mn in the canopy of the tree can be increased. The concentrations of these treatments are higher than those reported by Cheng and Raba (2009), in *cv* Gala/M.26; however, on *cv* Gala/Emla7, these values are lower except for treatment 3 that is within the reported range.

In the case of boron, treatment 4 was higher (66.03 mg kg^{-1}) with respect to treatment 2 (witness) (58.05 mg kg^{-1}), indicating that the year in which the graft was performed by approach did not influence. However, treatments 1 and 3 had an intermediate concentration (63.80 and 62.73 mg kg^{-1}), so it can be inferred that the rootstock Bud.9 is efficient in boron absorption. These concentrations of B are higher than those reported in *cv* Gala/Emla7 (Nachtigall and Dechen, 2006) and 'Gala'/M.26 (Cheng and Raba, 2009).

The results of the foliar analysis indicate normal levels for N, P, Mg, Fe, Cu, and Mn; low minerals for K and Zn and high levels for Ca and B (Jones *et al*., 1991). Potassium deficiency coincided with the symptomatology observed in the field at 24 DDPF, from a reddish to purple or bronzed coloration, with greater pronouncement on the underside of mature and new shoots with subsequent development of necrotic margins, which coincides with what was reported by Burrell and Boynton (1943); Basile *et al*., (2003).

Low potassium levels have an impact on development and growth, as the initial concentration in young leaves is higher than in old leaves (Huber, 1985), thus it also decreases as their phenology and extraction by the fruit progress (Faust, 1989; Brown, 1994). Potassium deficiency affects carbohydrate translocation and regulation in plant-water ratios (Grace *et al*., 2012). Mpelasoka *et al*. (2003) mention that variations in foliar K content may be due to the root absorption capacity and variations in the incorporation of K ions into the xylem and its translocation from the root to the shoots. Also, this deficiency could be due to antagonism with boron, as reported in olive tree (*Olea europea*) (Chatzissavvidis *et al*., 2005).

Zinc deficiency is due to low content in soil Swietlik (2002) mentions that zinc deficiency occurs greatly because it is not available, or it is motionless in the soil. The high calcium concentration can be explained by the accumulation according to the phenological stage of the leaves, as well as this high concentration of Ca can explain potassium deficiency, since calcium blocks potassium causing Ca/K antagonism.

These results are not related to the content of Ca in the soil, because the analyses showed medium to moderately low content and in the profile strata the presence of $CaCO_3$ was medium to low. However, Fallahi *et al*. (2001), they also found higher concentrations of Ca with the rootstock Bud.9 than in EMLA7. The importance of Ca is because of its role in cell enlargement and cell division (Bush, 1995).

The high concentration of boron may be due to the two applications that are made during the opening of vegetative and flower buds, together with foliar applications of micronutriments. Likewise, the concentration in plants is influenced by the rootstocks used (Wojcik, 2003). Boron plays an important role in perspiration, as it is transported through the xylem to the upper organs (Paparnakis *et al*., 2013). Wojcik *et al*. (2003) found high concentrations of B with the rootstocks M.9 and M.26, similar to what was found in the rootstocks of this study. Also, the high concentration may be due to the pH of the soil which is moderately acidic (Paparnakis *et al*., 2013).

Deficiency or excess of B usually reduces the harvest load as observed in the reduction of the percentage of olive flowers (Chatzissavvidis *et al*., 2005), in addition to synchronization with pollination. Symptoms of excess of boron are reflected in the low vigor of trees, delayed development and reduction in the number and weight of fruits (Reid and Fitzpatrick, 2009; Herrera-Rodríguez *et al*., 2010), as well as the decrease of chlorophyll in the leaf, alteration of metabolism and necrosis of mature tissues (Reid *et al*., 2004; Paparnakis *et al*., 2013).

The concentration of N, P and K at 24 DDPF decreased at 106 DDPF, while that of Ca, Mg and Mn increased. This result coincides with what was reported on the cv. Gala/EMLA7 on similar sampling dates (Nachtigall and Dechen, 2006); however, the concentration of the elements Fe, Cu, Zn and B decreased. Nachtigall and Dechen (2006); Mengel and Kirby (2001) mention that young leaves tend to have higher concentrations of N, P and K and mature leaves have higher concentrations of Ca, Mn, Fe and B, mainly. Decrease in concentration of nutrients throughout the vegetative cycle may be related to a dilution effect that occurs in leaves and in redistribution to other organs in the tree.

In 2019, at 24 days after full bloom, the concentration of N, K, Ca, Fe, Zn and Mn was not significantly affected by treatments, but that of the elements P, Mg, Cu and B (Table 3) was. The concentration of P was higher in treatments 1 and 4 (2.38 and 2.33 mg g^{-1}) with respect to treatment 2 (2.07 mg g^{-1}), which indicates the effect that rootstock Bud.9 has on the concentration of P, although the effect of the approach graft is also observed regardless of the year of its application. The concentration of P in this study is higher than that reported in *cv* Gala/Emla7 (Nachtigall and Dechen, 2006) and lower than that of *cv* Gala/M.26 (Cheng and Raba, 2009).

Table 3. Effect of approach graft on the mineral composition of macro and micronutrients on young leaves of 'Brookfield Gala' apple trees, 24 days after full bloom in 2019.

Treatments	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn	B
	(mg g^{-1})						(mg kg^{-1})			
1	22.15 a	2.38 a	10.61 a	3.7 a	1.56 b[y]	64.9 a	6.8 b	7.85 a	30.71 a	39.33 a
2 (witness)	20.35 a	2.07 b	11 a	3.47 a	1.87 a	67.75 a	6.46 b	8.95a	34.32 a	26.2 b
3	23.11 a	2.26 ab	9 a	3.36 a	1.63 b	72.2 a	5.13 b	7.98 a	34.2a	30.84 ab
4	22.26 a	2.33 a	10.76 a	3.46 a	1.63 b	68.38 a	11.86 a	8.33 a	33.05 a	35.38 a
DHS	3.62	0.25	2.46	0.53	0.16	11.63	3.62	2.57	5.81	12.99

[y]= different letters within the columns indicate significant differences ($p \leq 0.05$). Treatments: 1) 'Brookfield Gala'/Bud.9; 2) 'Brookfield Gala'/Emla7; 3) 'Brookfield Gala'/Bud.9+Emla7-2016; and 4) 'Brookfield Gala'/Bud.9+Emla7-2017.

The concentration of Mg was higher in treatment 2 (1.87 mg g^{-1}) compared to the rest of the treatments, which ranged from 1.56 to 1.63 mg g^{-1}. Therefore, the rootstock Emla7 influenced magnesium concentration. However, these values are lower than those reported in the cultivar Gala/Emla7 (Nachtigall and Dechen, 2006) and in *cv* Gala/M.26 (Cheng and Raba, 2009). On the cv. BC-2 Fuji/Emla7, high concentrations and low with the rootstock Bud.9 are also reported, which may be due to low net photosynthesis (Fallahi *et al*., 2001).

The concentration of Cu in treatment 4 was higher (11.86 mg kg^{-1}) than the rest of the treatments (5.13 to 6.80 mg kg^{-1}). These values are lower than those reported in the *cv* Gala/Emla7 (Nachtigall and Dechen, 2006).

Regarding B, treatments 1 and 3 (39.33 and 35.38 mg kg^{-1}) were higher than treatment 2 (26.2 mg kg^{-1}), which could explain that the rootstock Bud.9 absorbs and concentrates more boron, as well as with approach graft. On the *cv* Gala/M.26, minor concentrations have been reported (Nachtigall and Dechen, 2006).

The concentration of nutrients reflects the factors that influence availability and supply of soil, as well as the variation in climate and harvest load (Faust, 1989). Nutritional deficiency limits growth and development in fruiting and thereby reducing quality, while high concentrations produce symptoms of toxicity (Hoying *et al*., 2004). Also, the absorption of nutrients by rootstocks can be explained by the differences in the distribution and function of roots and the possible differences in anatomy of root and stem that affect the absorption rate and movement towards the xylem and leaves (Zarrouk *et al*., 2005).

Trunk cross section area (ASTT)

In 2018, the area of the cross section of the trunk was higher in treatment 2 (12.8 cm^2) compared to the rest of the treatments that ranged from 6.9 to 7.6 cm^2, respectively. This reflects the vigor of the rootstock Emla7. In 2019, the results were similar, treatment 2 (13.6 cm^2) exceeded the rest of the treatments (Table 4). Treatments with less ASTT could be explained by the smaller size and foliar area that correspond to the vigor of the trees.

Table 4. Area of the cross section of the 'Brookfield Gala' apple tree in 2018, 2019.

Treatments	ASTT (cm^2)		
	2018	2019	Increase
1	7.6 b	8.51 b	0.91
2 (witness)	12.8 a	13.6 a	0.8
3	6.9 b	8.22 b	1.32
4	7.2 b	8.27 b	1.07
DHS	2.59	2.76	

[y]= different letters within the columns indicate significant differences ($p \leq 0.05$). Treatments: 1) 'Brookfield Gala'/Bud.9; 2) 'Brookfield Gala'/Emla7; 3) 'Brookfield Gala'/Bud.9+Emla7-2016; and 4) 'Brookfield Gala'/Bud.9+Emla 7-2017.

The increase in growth from one cycle to another in combinations without approach graft was less than 1cm; however, in treatments with approach graft of 2016 and 2017 it was greater than 1 cm. Although the difference is minimal, it reflects the effect of the year in which the approach graft was performed.

Meanwhile, the aggregate of ASTT showed significance in the treatments, with treatment 2 (25.36 cm^2) being superior to the other treatments. These results relate to what has been reported in several cultivars, as the vigor of rootstocks increases, the accumulative yield/tree increases, but accumulative yield efficiency decreases (Barrit *et al*., 1995). Webster and Wetheim (2003) indicate that dwarf rootstocks are more efficient than vigorous rootstocks because they induce the size of the fruits of the variety.

Conclusions

The response of the nutritional status and vigor of the tree, with the approach graft to improve the horticultural performance of apple trees grafted on Bud.9, the effect is incipient, although it shows a tendency to improve, which can be attributed to the year of its establishment based on the nutritional status and area of the cross section of the trunk, as well as yield (unpublished data).

Based on the degree of use of the rootstock Bud.9 in intensive plantations, under conditions of a fragmented tuff soil its behavior limits the growth and development of the cultivar. However, cultural practices such as the incorporation of organic matter, humus, constant irrigation and fertilization can contribute to the horticultural improvement of the trees.

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