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ORIGINAL ARTICLE

Colonization by arbuscular mycorrhizal in mandarin 'Montenegrina': interaction between rootstocks and sazonality

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Abstract - The objective was to evaluate the influence of seasonality on mycorrhizal colonization in roots of six rootstocks in an orchard of 'Montenegrina' mandarin (*Citrus deliciosa* Tenore). Rootstocks evaluated were: 'Flying Dragon' [*Poncirus trifoliata* var. *monstrosa* (T. Ito) Swing.]; 'Troyer' citrange [*C. sinensis* (L.) Osb. × *P. trifoliata* (L.) Raf.]; 'Swingle' citrumelo [*C. paradisi* Macf. × *P. trifoliata*]; 'Rangpur' lemon (*C. limonia* Osb.); 'Volkamer' lemon (*C. volkameriana* Ten. & Pasq.); 'Caipira' orange (*C. sinensis*). Roots were collected in the four seasons. The density of structures, root colonization (number of infected segments/total analyzed) and spore density were determined. The rootstocks showed high percentages of colonization in the spring (above 96.60%). In winter, only 'Swingle' and 'Rangpur' showed high colonization rates (96.60 and 95.00 %, respectively). On the other hand, 'Flying Dragon', 'Troyer' and 'Volkamer' showed low colonization in winter (11.60 to 33.30 %). 'Flying Dragon' showed an increase in mycorrhizal colonization from the winter season (33.30 %) to spring (100.00 %) and a subsequent decrease from summer (95.00 %) to autumn (63.30 %). Mycorrhizal colonization occurs naturally and at a high rate on rootstocks, varying between rootstocks and seasons. There is a negative correlation between foliar nutritional content and the presence of AMFs.

Keywords: *Citrus deliciosa*. Seasons. Endomycorrhizal fungi.

Colonização por micorrizas arbusculares em tangerineiras ‘Montenegrina’: interação entre porta-enxertos e sazonalidade

Resumo - O objetivo foi avaliar a influência da sazonalidade na colonização micorrízica nas raízes de seis porta-enxertos em pomar de tangerineiras ‘Montenegrina’ (*Citrus deliciosa* Tenore). Os porta-enxertos avaliados foram: ‘Flying Dragon’ [*Poncirus trifoliata* var. *monstrosa* (T. Ito) Swing.]; citrangeiro ‘Troyer’ [*C. sinensis* (L.) Osb. × *P. trifoliata* (L.) Raf.]; citrumeleiro ‘Swingle’ [*C. paradisi* Macf. × *P. trifoliata*]; limoeiro ‘Cravo’ (*C. limonia* Osb.); limoeiro ‘Volkameriano’ (*C. volkameriana* Ten. & Pasq.); laranjeira ‘Caipira’ (*C. sinensis*). Realizou-se a coleta de radicelas em quatro estações. Determinou-se a densidade de estruturas, colonização radicular (número de segmentos infectados/total analisado) e densidade de esporos. Os porta-enxertos apresentaram porcentagens elevadas de colonização na primavera (acima de 96,60%). No inverno, apenas ‘Swingle’ e ‘Cravo’ apresentaram altas taxas de colonização (96,60 e 95,00 %, respectivamente). Já, ‘Flying Dragon’, ‘Troyer’ e ‘Volkameriano’ apresentaram baixa colonização nessa estação (11,60 a 33,30 %). ‘Flying Dragon’ apresentou uma redução gradativa de colonização com o decorrer da época estudada: na primavera (100,00 %), verão (95,00 %), outono (63,30 %) e inverno (33,30 %). A colonização micorrízica ocorre naturalmente e de forma elevada nos porta-enxertos, variando entre os porta-enxertos e as épocas do ano. Há uma correlação negativa entre conteúdo nutricional foliar e presença de FMAs.

Palavras-chave: *Citrus deliciosa*. Estações do ano. Endomicorrizas.

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Introduction

Brazil is the second largest citrus producer in the world (20.8 million tons/year), surpassed only by China. Brazil is the world's largest producer of oranges and the largest exporter of orange juice. The production in 2019 was over than 2.25 million tons, representing more than 50 % of the total orange juice produced in the world. It is estimated that the Brazilian citrus industry is responsible for the generation of 230 thousand jobs (ANUÁRIO, 2020; IBGE, 2021). These data show the importance of citrus farming for the economic and social development of several regions.

Despite the aforementioned importance, one of the limitations of Brazilian citrus farming is the low diversification of rootstocks. Making it very susceptible to the emergence of new diseases, as well as the survival of plants in case of biotic stresses, resulting in a vulnerability in this sector. Thus, different assessments of potential rootstocks are essential, in order to overcome this limitation. According to Sulzbach *et al.* (2016), it is estimated that 74 % of citrus plants grown in Rio Grande do Sul are grafted on 'Trifoliata' (*Poncirus trifoliata*). In São Paulo, 50 % of the orchards are grafted on 'Swingle' citrumelo (*Citrus paradisi* × *P. trifoliata*) and 33 % on 'Rangpur' lemon (*C. limonia*) (CARVALHO *et al.*, 2019).

Another challenge for the agricultural sector refers to the scarcity and/or increase in prices of supplies for production, such as fertilizers. The replacement or reduction of the use of resources on a non-renewable basis is essential. Because of that, the increase in the bioavailability of nutrients can ensure the maintenance of productivity and food security (CELY, 2014; EMBRAPA, 2018).

In order to reduce the use of these supplies, the importance of Arbuscular Mycorrhizal Fungi (AMF) has been widely studied as a way to optimize the absorption of nutrients from the soil by plants. This is possible because plants colonized with AMF explore larger volumes of soil, the fungus hyphae act as an extension of the plant roots, generating greater nutrient absorption (SMITH *et al.*, 2011), tolerance to water deficit (JAYNE; QUINGLEY, 2014) and even tolerance to phytopathogens (MOREIRA *et al.*, 2016), in addition to other beneficial effects.

Citrus are generally highly dependent and responsive in relation to mycorrhizal association because the root system has poorly developed root hairs in most species (ORTAS, 2012). However, this mycorrhizal dependence may vary between citrus rootstock genotypes, AMF species, as well as soil fertility, mainly in relation to phosphorus levels in soil (SMITH; SMITH, 2011).

In soils with high phosphorus content, plants tend to allocate less carbon to AMF, harming the symbiosis, since the host absorbs the nutrient that is available in the soil. Thus, the greater the amount of phosphate fertilization, the lower the diversity and colonization of roots by AMFs (SCHNEIDER *et al.*, 2015; WILLIAMS *et al.*, 2017).

The plant x AMF symbiosis is also influenced by the season. According to Vivas *et al.* (2003), the plant response to mycorrhization can vary, due to the edaphoclimatic conditions influencing the development of native species in the environment where they are found.

The symbiotic association between AMF and citrus cultivation has been widely studied in the seedling production phase in greenhouses. However, it is still poorly understood in relation to the dynamics of the

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AMF community under field conditions.

Therefore, the objective of this work was to evaluate the influence of seasonality on population fluctuation and natural colonization of AMFs in radicles of six rootstocks grafted under canopy of 'Montenegrina' mandarin trees.

Material and methods

The study was carried out at Panoramas Citrus Farm (latitude 30° 07' 12 S, longitude 51° 57' 45'' W, altitude of 35 m), located on BR 290, Km 164, in the municipality of Butiá, Rio Grande do Sul. The climate of the region, according to the Köppen classification, is Cfa (humid subtropical) with an average annual temperature of 18.8 ºC and an average precipitation of 1,455 mm (BERGAMASCHI *et al.*, 2013). The soil is classified as a typical Dystrophic Red Argisol, with 19 % clay in the surface layer (0-20 cm depth) (SANTOS *et al.*, 2018).

The experiment was conducted in a 'Montenegrina' mandarin orchard (*Citrus deliciosa* Ten.) grafted onto six rootstocks: 'Swingle' citrumelo [*C. paradise* Macf. × *Poncirus trifoliata* (L.) Raf.], 'Caipira' orange (*C. sinensis* Osb.), 'Troyer' citrange (*C. sinensis* × *P. trifoliata*), 'Rangpur' lemon (*C. limonia* Osb.), 'Volkamer' lemon (*C. volkameriana* Ten. & Pasq.) and 'Flying Dragon' trifoliate [*P. trifoliata* var. *monstrosa* (T. Ito) Swing.]. The orchard was implanted in 1997 and the spacing was 6.0 x 2.5 m, totaling 667 trees per hectare. Phytosanitary and fertilization management was carried out according to usual management practices in commercial orchards, following the recommendations of Koller (2009), and the management of weeds between the rows was carried out through periodic mowing.

Soil chemical analysis and root hairs analysis has been done from of soil samples (500 up to 1,000 g). These samples were collected in the projection of the canopy of the trees, in the four seasons of the year: winter (08/24/2015), spring (12/01/2015), summer (03/21/2016) and autumn (05/30/2016). The samples were composed of four subsamples collected at a depth of 0-20 cm from the surface of the soil, taken from the two central plants of each experimental unit, with three replications.

The root hairs were separated from the soil samples using sieves with a mesh size of 53 µm (0.053 mm). Subsequently, the soil samples were shade drying. Part of these samples (50 g of soil) was separated and used for spore collection from AMF, in order to verify the population fluctuation throughout the year. The other part of the soil (in summer) was sent for chemical analysis at the Laboratory of Soil and Tissue Analysis of the Faculty of Agronomy – UFRGS (LAS), according to the methodology of Tedesco *et al.* (1995) and Teixeira *et al.* (2017).

The collection of leaves for foliar analysis was carried out on 03/21/2016 (summer), where the second and third leaves were removed from the fruit, from branches of the spring flow. Each sample consisted of 80 – 200 leaves per experimental unit (the same plants from which the soil samples were collected) at a height of approximately 1.5 m from the soil, in the four quadrants of each canopy (TEDESCO *et al.*, 2004). The leaf samples were sent to the Laboratory of the Horticulture and Forestry Department of the Faculty of Agronomy (DHS) - UFRGS, where they were dried in an oven at 60 ºC until reaching constant mass. Then, they were sent to the LAS for evaluation of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn) and boron (B), according to the methodology of Tedesco *et al.* (1995).

To determine the density and colonization by AMF in the root hairs, they were washed with distilled water and preserved in F.A.A. (5 % Formaldehyde, 5 % Acetic Acid and 90 % Ethyl Alcohol), according to the method by Honrubia *et al.* (1993). Afterwards, they were cut into segments of approximately one centimeter in length, clarified and stained with Trypan Blue, according to Phillips and Hayman (1970). Subsequently, 60 segments per treatment (20 segments per repetition) were arranged on microscope slides (total of 360 slides) and analyzed under a microscope to assess the presence and density of hyphae, vesicles and arbuscules. The percentage of colonized root hairs was obtained through the number of infected segments in relation to the total analyzed. To determine the hyphae density, the value 0 was assigned for absence of structures; 1, for low presence; 2, for medium presence; and 3, for high presence. The density of vesicles and arbuscules was also related to a scale from 0 to 3, where 0 was considered the absence of structures; 1, from 1 to 50 structures; 2, from 51 to 100; and 3, more than 100 (NEMEC, 1992).

To quantify AMF spores, an aliquot of 50 g was removed from each soil sample. The spores were isolated by wet sieving (GERDEMANN; NICOLSON, 1963), using 106 and 53 µm diameter sieves, followed by centrifugation at 2,000 rpm for three minutes with a solution of sterile distilled water and sucrose (50 %) according to Jenkins (1964). Subsequently, the spores were observed in a Petry dish, where they were counted using a stereoscopic microscope.

The experimental design used was a two-factor in randomized blocks, with 24 treatments (six rootstocks and four seasons) and three replications (blocks), where two plants constituted the experimental unit. Data collected in the same experimental unit throughout the different seasons of the year (root colonization by AMF; presence of hyphae; presence of vesicles; presence of arbuscules; and density of AMF spores in the soil) were analyzed as repeated measures, using if the PROC MIXED procedure in the SAS program, version 9.4 (SAS INSTITUTE INC., CARY, NC, USA). The covariance structure that presented the best fit for the data was selected according to the Akaike (AIC) and Bayesian (BIC) information criteria (SILVA; DUARTE; REIS, 2015). Interactions between factors were considered significant when $p \leq 0.25$ (PERECIN; CARGNELUTTI FILHO, 2008). For the other variables, an analysis of variance was done, with blocks as a random effect, also using the PROC MIXED procedure. When necessary, Tukey's test was performed at the 5 % probability level to compare means. In addition, the Pearson correlation coefficient (r) was calculated between the residues of the chemical composition data of the leaf tissue of the plants and the characteristics of the mycorrhizal association (root colonization by AMF; presence of hyphae; presence of vesicles; presence of arbuscules; and density of AMF spores in the soil) for the data collected in the summer.

The average air temperature and rainfall data from May 2015 to May 2016 from the meteorological station located at the UFRGS Agronomic Experimental Station (10 km away from the experiment), together with the climatological normals of the region (BERGAMASCHI *et al.*, 2013), are shown in Figure 1.

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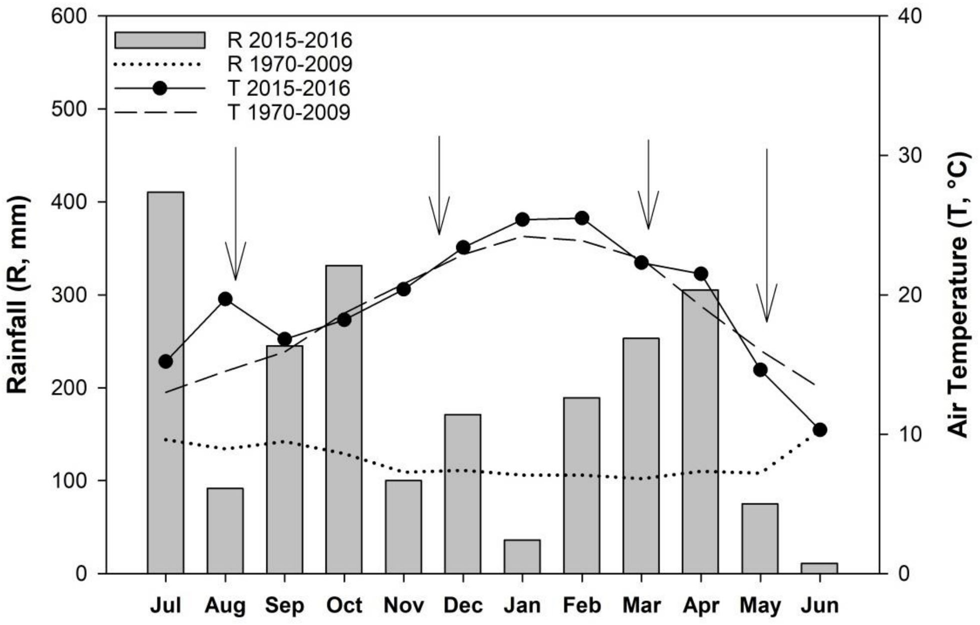

Figure 1. Accumulated precipitation and average monthly temperature between July 2015 and June 2016, and climatological normal values from 1970 to 2009 (BERGAMASCHI *et al.*, 2013). The arrows indicate the dates when the roots were collected.

Results and Discussion

The AMF colonized the roots of all rootstocks and at all evaluated times. However, the intensity of colonization differed between treatments. In the evaluation carried out during the winter, it was found that the rootstocks 'Swingle' and 'Rangpur' had a higher percentage of natural colonization (96.6 and 95.0 %, respectively), while 'Troyer', 'Volkamer' and 'Flying Dragon', had significantly the lowest values (11.6; 28.30 and 33.30 %, respectively) (Table 1).

During spring, all the rootstocks tested showed high percentages of natural mycorrhizal colonization, with values between 93.30 and 100 %. During this period, the average temperature remained high, with an average of 23 ºC (Figure 1).

During the summer, the percentage of colonization remained high, although less intense than in spring, with an average of 79.20 % (Table 1), possibly due to the high temperature associated with the water deficit that occurred between January and February (Figure 1).

In the collection of autumn, the rootstocks 'Volkamer' and 'Troyer' showed 100 % colonization, statistically differing only from 'Swingle', which showed lower values (51.70 %) (Table 1).

The rootstock 'Flying Dragon' showed an increase in mycorrhizal colonization from the winter season (33.30 %) to spring (100.00 %) and a subsequent decrease from summer (95.00 %) to autumn (63.30 %) (Table 1). This can be explained by the fact that this rootstock presents well-marked dormancy in winter, a period of temporary inhibition of plant growth and development, being characterized, basically, by the reduction of metabolic activity, regulated by phytohormones and influenced by external factors

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(environmental conditions) (PES; ARENHARDT, 2015), which according to Rieth (2012), can affect the potential for emergence and development of roots.

Table 1. Percentage of natural colonization of arbuscular mycorrhizae fungi, density of colonization structures (hyphae, vesicles and arbuscules) and density of viable spores (number/gram) in 'Montenegrina' mandarin orchard grafted onto six rootstocks and analysed at four times of the year. Porto Alegre, 2017.

Colonization (%)	*Rootstocks*	Winter	Spring	Summer	Autumn	Average
	'Caipira'	51.70 ABb	100.00 a	85.00ABab	80.00 ABab	79.20
P pe 0.0246	'Swingle'	96.70 Aa	96.70 a	83.30 ABab	51.70 Bb	82.10
P t <0.0001	'Rangpur'	95.00 A	96.70	88.30 A	83.30 AB	90.80
*P pe*t <0.0001*	'Flying Dragon'	33.30 Bb	100.00 a	95.00 Aa	63.30 ABab	72.90
	'Troyer'	11.70 Bb	100.00 a	38.30 Bb	100.00 Aa	62.50
	'Volkamer'	28.30 Bb	93.30 a	85.00 Aa	100.00 Aa	76.70
	Average	*52.80*	*97.80*	*79.20*	*79.70*	
Hyphae (%)	*Rootstocks*	Winter	Spring	Summer	Autumn	Average
	'Caipira'	0.65 ABb	2.13 a	1.60 ab	0.87 Bab	1.31
P pe 0.0518	'Swingle'	1.82 A	1.80	0.97	0.85 B	1.36
P t 0.0002	'Rangpur'	1.88 A	2.20	1.75	1.37 AB	1.80
*P pe*t 0.0089*	'Flying Dragon'	0.85 ABb	2.55 a	1.03 b	1.03 ABb	1.37
	'Troyer'	0.13 Bb	1.91 a	0.43 b	1.30 ABab	0.95
	'Volkamer'	0.28 Bb	1.90 a	1.27 ab	2.42 Aa	1.47
	Average	*0.94*	*2.08*	*1.18*	*1.31*	
Vesicles (%)	*Rootstocks*	Winter	Spring	Summer	Autumn	Average
	'Caipira'	0.00 B	0.17	0.00	0.35 AB	0.13
P pe 0.3908	'Swingle'	0.52 AB	0.47	0.00	0.00 B	0.25
P t 0.0007	'Rangpur'	0.65 Aa	0.40 ab	0.00 b	0.07 Bb	0.28
*P pe*t 0.0052*	'Flying Dragon'	0.22 AB	0.00	0.00	0.28 AB	0.13
	'Troyer'	0.00 B	0.00	0.00	0.00 B	0.00
	'Volkamer'	0.05 Bb	0.07 b	0.00 b	0.73 Aa	0.21
	Average	*0.24*	*0.18*	*0.00*	*0.24*	
Arbuscules (%)	Rootstocks	Winter	Spring	Summer	Autumn	Average
	'Caipira'	0.48	1.35	0.43	0.95	0.80
P pe 0.5915	'Swingle'	1.07	0.98	0.77	0.40	0.80
P t 0.0121	'Rangpur'	1.18	1.45	1.12	0.98	1.18
*P pe*t 0.2899*	'Flying Dragon'	0.57	1.00	0.95	0.80	0.83
	'Troyer'	0.05	1.29	0.12	1.40	0.71
	'Volkamer'	0.12	0.87	0.52	1.23	0.68
	Average	*0.58 b*	*1.16 a*	*0.65 b*	*0.96 ab*	
Spores (number/gram)	*Rootstocks*	Winter	Spring	Summer	Autumn	Average
	'Caipira'	14.50 AB	3.50	16.20	15.00 AB	12.30
P pe 0.0252	'Swingle'	3.30 B	6.20	15.00	3.50 B	7.00
P t <0.0001	'Rangpur'	3.70 Bb	2.40 b	18.30 a	2.90 Bb	6.80
*P pe*t 0.0625*	'Flying Dragon'	21.30 Aa	6.40 b	19.10 ab	21.80 Aa	17.20
	'Troyer'	18.10 Aba	2.70 b	7.50 ab	14.50 ABab	10.70
	'Volkamer'	18.70 ABa	3.50 b	16.00 ab	14.70 ABab	13.20
	Average	*13.30*	*4.10*	*15.30*	*13.30*	

Different capital letters within the column (rootstock in each season) and different lowercase letters within the rows (season in each rootstock) indicate significant differences at 5% probability. P = probability; t = time (time of collection); pe = rootstock.

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The lower colonization values observed in winter may be a consequence of the lower temperatures, characteristic of this season in the southern region of Brazil (Figure 1). According to Smith and Smith (2011), in the winter period there is a reduction in the metabolic activity of AMF and, consequently, their presence in the root system of plants, reducing symbiosis.

The rootstock *Poncirus trifoliata* has roots with a high amount of root hairs (WU *et al.*, 2012), which can result in a more efficient absorption of water and nutrients, reducing its symbiotic dependence on AMF. Cao, DeJong and Kirkpatrick (2013), similarly, observed that in conditions of nutrient deficiency, this rootstock modifies the anatomy of the root system, adapting to this condition by increasing the amount of root hairs and, consequently, the absorption. This genetic behavior justifies the lower mycorrhizal colonization in 'Flying Dragon' and also in 'Troyer', since both have *P. trifoliata* genotype.

According to Nunes *et al.* (2006), although there are differences between the adaptation of fungi and also their genotype, most AMF species have an ideal temperature range for colonization to occur, which is between 20 and 25 ºC.

The symbiosis between AMF and the plant depends on several factors, one of the most important being the genetic and anatomical characteristics of the host root, as well as soil and climate conditions. Thus, there are plants that are more dependent on AMF than others (SMITH; SMITH, 2011). This mycorrhizal dependence, according to Nunes *et al.* (2006), it can vary with the plant species and particularly, with the morphology of the root and also the conditions of soil and climate. According to Zambrosi *et al.* (2011), differences in root morphology and physiology alter the characteristics of nutrient absorption and are determined by the rootstocks, explaining the results found in the present study.

Table 1 shows that hyphae density has a significant interaction between rootstock and season. In the rootstocks 'Caipira' and 'Flying Dragon' the presence of hyphae was higher in spring. In 'Swingle' and 'Rangpur' colonization did not vary significantly along the evaluated seasons. On the other hand, 'Troyer' and 'Volkamer' showed a tendency towards greater presence of hyphae in spring and autumn.

The presence of vesicles also suffered a significant interaction between the factors evaluated (Table 1). In general, the presence of these structures was low in all rootstocks and evaluated times, never reaching the average index of 1 (up to 50 structures per centimeter). In some rootstocks, such as 'Caipira', 'Flying Dragon' and 'Troyer', the presence of vesicles was negligible in some seasons, with absence in others. In 'Troyer' it did not exist in all stations evaluated. There was a statistical difference in the presence of vesicles between rootstocks in winter and autumn. In winter the rootstocks 'Rangpur', 'Swingle' and 'Flying Dragon' stood out, in the autumn the rootstocks 'Volkamer', 'Caipira ' and 'Flying Dragon'. There was only statistical difference in the presence of vesicles between seasons for 'Rangpur' and 'Volkamer' rootstocks. In 'Rangpur', a significant presence of vesicles was found in winter and spring. Otherwise, in 'Volkamer' a significant presence of vesicles was found in autumn. No vesicles were found on any rootstock in summer.

These results can be explained by the high levels of phosphorus found in the soil samples (Table 2), which did not prevent root colonization, however, may have inhibited the development of AMF structures inside them. Vesicles are structures that contain storage compounds such as glycogen and lipids. These

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structures, which have a globular shape, are usually formed under conditions where there is low metabolic activity of the AMF and the host too, such as periods of low or high temperatures and drought (SMITH; SMITH, 2011). Normally, under the conditions of southern Brazil, these structures are formed from April/May (when there is a drop in temperature and a reduction in the photoperiod) to provide reserves for the AMFs during the winter period (BACK; ALTMANN; SOUZA, 2016). Thus, in the period evaluated, it is assumed that the AMFs did not need to form a large number of vesicles, because there are not adverse periods in a short therm.

The presence of arbuscules was similar in the root hairs of all rootstocks (Table 1). The presence of arbuscules on rootstocks was statistically higher in spring, intermediate in autumn and lower in winter and summer.

Table 2. Chemical attributes of soils collected in the summer in the area of the cultivar Montenegrina in different rootstocks of mandarin. Porto Alegre, 2017.

Rootstocks	pH H_2O	Index SMP	O. M.	P	K
			---%---	----------mg dm^3	------------
‘Caipira’	5.24 b	5.98 bc	2.90 a	52.94 a	137.38 ab
‘Swingle’	5.51 a	6.10 b	2.81 a	51.37 a	155.47 a
‘Rangpur’	5.23 b	5.89 c	2.47 ab	39.98 b	136.97 ab
‘Flying Dragon’	5.63 a	6.30 a	2.78 a	34.97 b	93.60 c
‘Troyer’	5.63 a	6.25 a	2.43 ab	31.91 b	125.51 b
‘Volkamer’	5.24 b	6.01 bc	2.22 b	16.85 c	152.93 a
P	*< 0.0001*	*< 0.0001*	*0.0154*	*< 0.0001*	*< 0.0001*

Rootstocks	Ca	Mg	Zn	Cu
	---------$cmol_c$ dm^3	-------------	-----------------mg dm^3	-----------------
‘Caipira’	5.45 abc	1.91 bc	6.16 ab	14.35 a
‘Swingle’	6.05 a	2.60 a	3.84 cd	10.02 ab
‘Rangpur’	4.51 c	1.79 c	5.30 b	10.58 ab
‘Flying Dragon’	5.88 ab	1.29 d	4.31 c	10.47 ab
‘Troyer’	4.90 bc	1.86 c	5.71 ab	13.74 a
‘Volkamer’	4.49 c	2.20 bc	3.42 d	6.62 b
P	*0.0017*	*< 0.0001*	*< 0.0001*	*0.0103*

Lowercase followed by the same letter, in the column, do not differ from each other by Tukey's test ($p < 0.05$).

Arbuscules are exchange structures between the plant and the AMF, where the exchange of nutrients and photoassimilates occurs. Normally appearing in greater quantities in the periods favorable to the symbionts, which explains their greater presence in spring, when plants retake their development and increase their metabolism, resulting in greater symbiotic activity between the fungus and the host (BERBARA; SOUZA; FONSECA, 2006; SMITH; SMITH, 2011).

The spore density was high for most of the rootstocks and seasons evaluated, ranging from 2.4 to 21.8 spores g^{-1} of soil (Table 1). Soil management may have favored mycorrhizal sporulation. In the management of the orchard, coverage was used between the rows, controlled use of agricultural supplies (fertilizers and pesticides), and the use of a mower as a method to control the growth of the natural coverage

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between the rows. According to Säle *et al.* (2015) and Gottshall, Cooper and Emery, (2017) erosive processes, contamination, compaction and excessive use of soluble chemical fertilizers or pesticides tend to decrease the number of AMF species and spores in the soil. In addition, soil tillage, carried out using agricultural implements, can damage the propagules and break the hyphae network, reducing root colonization. Similarly, in less intensive crops there is a favoring of AMF community and species diversity (OEHL *et al.*, 2010).

'Flying Dragon' rootstock showed higher spore density in autumn and winter (21.80 and 21.30 spores g^{-1} soil, respectively), but did not differ statistically from 'Troyer', 'Volkamer' and 'Caipira' rootstocks. In the winter period, the 'Flying Dragon' is dormant, which may have caused greater formation of spores, as these are resistance structures with thick walls and have a long-term survival capacity, as is the case with winter period. With increasing temperature and more appropriate humidity conditions, they become quiescent and able to germinate and initiate colonization (SMITH; READ, 2008).

In spring and summer, there was no statistical difference between the rootstocks studied in terms of spore density. Also, in spring, all rootstocks showed low spore density, a time of year that was marked by intense rains, especially in the months of September and October (Figure 1). According to Ijdo, Cranenbrouck and Declerck (2011), both host plants and AMFs must have access to sufficient water, but at the same time excess water must be avoided, as there is a shortage of oxygen, which can result in less spore production. Also, according to Durazzini, Teixeira and Adami (2009), high humidity and temperature favor the germination of spores and the appearance of other structures, such as hyphae, resulting in high colonization and low spore production, as observed in the present work.

Rootstocks 'Swingle' and 'Rangpur' showed lower spore density in autumn and winter when compared to other rootstocks, only showing an increase in the summer period for both rootstocks. This behavior is erratic, because spores tend to form in greater quantity in adverse periods of temperature and photoperiod, coinciding with autumn and winter (Table 1).

Table 2 shows that there are no important variations in the nutritional levels in the soil samples between the different rootstocks analyzed in this study. This homogeneity is probably related to fertility corrections, which producers normally make to obtain better yields.

The levels of hydrogenic potential (pH) in water according to the CQFSRS/SC (2004), were considered medium for the 'Flying Dragon', 'Troyer' and 'Swingle' rootstocks, and low for the 'Caipira' rootstocks, 'Volkamer' and 'Rangpur'. However, these levels are considered appropriate for the development of symbiosis, since many AMF species prefer acid soils (NUNES *et al.*, 2008).

The levels of organic matter (OM), which is a source of nitrogen for plants, according to CQFSRS/SC (2004), were low for 'Rangpur' 'Troyer' and 'Volkamer' rootstocks, and medium for 'Flying Dragon', 'Swingle' and 'Caipira' rootstocks.

High levels of phosphorus (P) were observed in the soil, and the plots with the rootstocks 'Swingle' and 'Caipira' stood out with the highest levels. However, even the rootstocks that were in the intermediate range, 'Rangpur', 'Flying Dragon' and 'Troyer', according to CQFSRS/SC (2016), are in the very high range of availability of this nutrient. Only the plots with the 'Volkamer' rootstock, which had the lowest amount of P in

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the soil, were in the high range. Even with the high values, they did not prevent the intense colonization of the roots observed in this work.

The relationship between AMF colonization and the amount of phosphorus has been extensively studied, which generally shows a negative influence for mycorrhizas when there are high levels of P in the soil, differently what happened in the present study. A similar result was observed by Nunes *et al.* (2006), who verified a high natural colonization of mycorrhizae in the field on citrus rootstocks in areas under the influence of fertilization and with high doses of phosphorus.

Siqueira (1994) reports that phosphorus levels influence root colonization by AMF, but that the effects of this nutrient on colonization differ among species. Thus, the amount of phosphorus needed to inhibit colonization may vary according to the host's ability to absorb and translocate the nutrient, which is confirmed in the present study.

However, when the amount of P in the soil is high, the plant prefers root absorption than mycorrhizal, because there is less energy expense, inhibiting AMF phosphate transporters (NAGY *et al.*, 2009). Thus, it can be inferred that AMFs which are in symbiosis with citrus plants would have a more parasitic nature, since the plant needs to supply carbohydrates to the fungus. According to Kahiluoto, Ketoja and Vestberg (2012), there is a greater benefit of mycorrhizal colonization for the plant in systems with lower supplies, such as in the organic system.

The K content also showed great variation between the data according to the statistical analysis, where the 'Swingle' and 'Volkamer' rootstocks presented values statistically similar to those of the 'Caipira' and 'Rangpur' rootstocks, distinguishing statistically from 'Troyer' and 'Flying Dragon' rootstock plots. But according to the CQFSRS/SC (2004), except for the plots of the 'Flying Dragon', which was in the range considered high, all the others were in the range of very high levels of this nutrient. K has several functions in plant growth and development, with an important role in osmotic regulation, enzymatic activation and in the transport of solutes and water through the membrane. This element is key in maintaining stomatal guard cell turgor and consequently photosynthesis, regulating their opening and closing through changes in osmotic potential (TAIZ *et al.*, 2017).

There is evidence that a higher level of K favors root colonization by AMF, as confirmed in this work. Zhang *et al.* (2010), found that in corn plants inoculated with AMF, there was a higher chlorophyll content compared to the control, where there was no inoculation, suggesting that a higher level of K in the soil, together with symbiosis, positively interferes with the concentration of chlorophyll.

In all soil samples, independently of the rootstock studied, and according to CQFSRS/SC (2004), high availability of the nutrients calcium (Ca), magnesium (Mg), zinc (Zn) and copper (Cu) was observed.

In the foliar analysis (Table 3), it is observed that there was no statistical difference for N, Zn and B (boron) regardless of the rootstock studied. According to Mattos Jr. *et al.* (2020), the levels of N and Fe were normal in all rootstocks, while those of Zn were insufficient for all of them.

Foliar P levels were statistically equal and higher in plants grafted on 'Flying Dragon' and 'Caipira', intermediate in those grafted on 'Volkamer' and 'Troyer' and lower in those grafted on 'Swingle' and 'Rangpur'.

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Despite the relative differences, according to Mattos Jr. *et al.* (2020) all values found in the chemical composition of leaves are considered higher than the optimal levels for citrus (> 0.16 %). This behavior confirms the importance of the AMFs, because despite the differences found in the levels of P in the soil for the different rootstocks, due to the symbiosis, this element was supplied by the AMFs in those soils with lower availability.

Table 3. Analysis of leaf tissue collected in the summer in the area of cultivar Montenegrina on different rootstocks of mandarin. Porto Alegre, 2017.

Rootstocks	N	P	K	Ca	Mg	S
	---%---					
'Caipira'	2.53	0.21 ab	1.83 a	2.50 bc	0.38 b	0.44 a
'Swingle'	2.50	0.17 c	0.95 bc	3.16 a	0.40 b	0.31 b
'Rangpur'	Nd	0.17 c	1.26 b	2.93 ab	0.53 a	0.36 ab
'Flying Dragon'	2.53	0.22 a	1.13 bc	3.26 a	0.49 a	0.30 b
'Volkamer'	2.63	0.18 bc	1.66 a	2.30 c	0.40 b	0.41 a
'Troyer'	2.70	0.18 bc	0.87 c	2.66 abc	0.49 a	0.38 ab
IOCF[1]	2.5-3.0	0.12-0.16	1.2-1.6	3.5-5.0	0.35-0.5	0.2-0.3
P	*0.0742*	*0.0028*	*< 0.0001*	*0.0016*	*0.0003*	*0.0035*

Rootstocks	Cu	Zn	Fe	Mn	B
	--mg kg^{-1}--				
'Caipira'	41.30 a	34.00	149.0 a	52.6 ab	87.50
'Swingle'	29.6 b	30.67	121.3 ab	35.0 ab	80.67
'Rangpur'	18.30 cd	20.00	107.3 b	60.3 ab	Nd
'Flying Dragon'	14.3 d	25.67	147.0 a	28.3 ab	104.00
'Volkamer'	19.00 c	22.3	133.3 ab	53.3 ab	85.67
'Troyer'	21.30 c	17.67	118.3 ab	19.6 b	72.33
IOCF[1]	10-20	50-75	50-150	35-75	75-150
P	*< 0.0001*	*0.0654*	*0.0104*	*0.0198*	*0.1139*

Lowercase letters followed by the same letter in the column do not differ by Tukey's test ($p < 0.05$). [1]Optimal Foliar Concentration Interval (Mattos Jr. *et al.*, 2020). Nd – there was not enough plant material.

Foliar K levels were higher in plants grafted on 'Caipira' and 'Volkamer', considered excessive for citrus (MATTOS Jr. *et al.*, 2020), while the lowest levels were found in 'Troyer', considered insufficient, together with 'Flaying Dragon' and 'Swingle' (< 1.2 %). The rootstock 'Troyer' showed the lowest mycorrhizal colonization in the summer (38.30 %), when compared to the other rootstocks (Table 1), which may have influenced the levels of this nutrient.

Donha (2014), analyzing the interaction between foliar K levels and the presence or absence of AMF in bean plants (*Phaseolus vulgaris* L.), found lower levels of K in bean plants cultivated in the absence of AMF and greater lipid peroxidation, with formation of malondialdehyde. It was also found in that study that the higher concentration of K and the symbiosis of plants with these species of fungi can positively interfere in the concentration of chlorophyll.

The foliar levels of Ca showed variation between the scion/rootstock combinations, however all the values were insufficient according to Mattos Jr. *et al.* (2020) (< 3.5 %). For the foliar levels of Mg, the plants

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grafted on 'Rangpur', 'Flying Dragon' and 'Troyer' showed higher concentrations than the others, showing excess of this nutrient only for 'Rangpur' (> 0.5 %).

Leaf levels of manganese (Mn) were insufficient in plants grafted on 'Troyer' and 'Flying Dragon', and within the normal range for the other rootstocks. Only the 'Troyer' rootstock presented insufficient values of Boro (B), for the others it was normal (MATTOS Jr. *et al.*, 2020)

All plants showed excessive levels of sulfur (S). Copper was normal for 'Rangpur', 'Flying Dragon' and 'Volkamer', and the remainder was excessive. This is due to phytosanitary treatments, since most of the pesticides used have these elements in their composition.

Table 4 shows that the correlation between the structures (hyphae and arbuscules) of the AMF and foliar nutrients was not statistically significant ($p > 0.05$). However, significant negative correlations were observed between the macronutrients Ca and Mg and the micronutrient Fe with mycorrhizal colonization. This indicates that the greater the root colonization, the lower the absorption of these elements.

Table 4. Pearson's correlation coefficient between foliar nutrients, structures (hyphae and arbuscules), percentage of root colonization by AMF and spore density in the summer in the area of cultivar Montenegrina in different rootstocks of mandarin. Porto Alegre, 2017.

DF = 7	Hyphae	Arbuscules	Colonization	Spores
N	0.058891	0.284088	0.558898	0.636791
	0.889800	0.495300	0.149800	0.089500
P	-0.342688	0.073589	-0.680354	**-0.862668**
	0.406000	0.862500	0.063300	**0.005800**
K	-0.524359	-0.226120	-0.547009	-0.352030
	0.182200	0.590300	0.160600	0.392400
Ca	-0.528186	0.050058	**-0.764142**	**-0.759194**
	0.178400	0.906300	**0.027300**	**0.028900**
Mg	-0.402391	0.062753	**-0.742968**	**-0.733274**
	0.323000	0.882600	**0.034700**	**0.038500**
S	-0.520631	0.007797	-0.704793	**-0.768183**
	0.185900	0.985400	0.050900	**0.026000**
Cu	0.027121	0.031540	-0.638555	-0.614997
	0.949200	0.940900	0.088400	0.104600
Zn	0.322373	0.596836	0.109498	-0.389193
	0.436100	0.118300	0.796300	0.340600
Fe	-0.188745	-0.109651	**-0.724113**	**-0.810676**
	0.654400	0.796000	**0.042200**	**0.014600**
Mn	0.378906	0.279502	-0.105881	-0.160154
	0.354600	0.502600	0.803000	0.704800
B	-0.265908	-0.632434	-0.620861	-0.677117
	0.524400	0.092400	0.100400	0.065100
H	1.000.000	0.494189	0.605460	0.405946
		0.213200	0.111700	0.318300

The elements Ca and Mg are essential nutrients in several physiological processes. Ca acts on the structure of the plant, being necessary for the constitution of the middle lamella of the cell wall, and also for

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the absorption of nutrients. Mg, on the other hand, constitutes the central core of the chlorophyll molecule, and its deficiency is important in enzymatic activity and phosphate transfer (TAIZ *et al.*, 2017).

Several authors have demonstrated that AMFs have the power to reduce the absorption of Ca and Mg in the most diverse fruit species, such as 'P1103' vine rootstock (CARNIEL, 2004) and 'Aldrighi' peach rootstock (NUNES *et al.* al., 2008) inoculated with AMF. Corroborating with the results of the present study, Souza (2000), reports that the reduction in foliar levels of Ca and Mg of 'Carrizo' citrange inoculated with AMF is due to a buffering effect provided by fungi. This effect can be beneficial in alkaline soils, with an excess of these mineral elements, but it can be limiting in soils lacking them.

The macronutrients P, Ca, Mg and S and the micronutrient Fe showed a significant negative correlation with spore density (Table 4). This explains that the greater the absorption of these nutrients, the lower the spore density.

Several studies on root colonization by AMF have shown great variations regarding the influence of chemical characteristics. The relationships and interactions involving the AMF/host symbiosis are complex and depend on several factors. In production systems, agricultural practices can change the occurrence of AMF and root colonization rates, and the association can become beneficial or harmful to plants (SCHNEIDER *et al.*, 2015; WILLIAMS *et al.*, 2017).

The population of AMFs is related to edaphic, climatic and plant factors, as they influence their distribution and occurrence, in addition to soil management and use practices that can also change the quantitative and qualitative composition of AMFs and other microorganisms (FEILER, 2020). According to Gomide (2013), the ecological aspects of vegetation can better explain the diversity of AMFs than the chemical attributes, because this is an attempt to demonstrate the effects of local processes acting on the diversity of these fungi in the soil.

The period of greatest root colonization is spring with no difference between the rootstocks in this season of the year.

Mycorrhizal colonization occurs naturally and with high level in citrus rootstocks in the field. However, there are variations between the rootstocks and the seasons of the year. The AMF colonization is high even with fertilization with high doses of phosphorus.

There is a negative correlation between foliar nutritional content and the presence of mycorrhizal, indicating that in nutritionally rich soils mycorrhizal dependence is reduced.

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Conflict of interest

The authors declare that the research was conducted in the absence of any potential conflicts of interest.

Ethical statements

The authors confirm that the ethical guidelines adopted by the journal were followed by this work, and all authors agree with the submission, content and transfer of the publication rights of the article to the journal. They also declare that the work has not been previously published nor is it being considered for publication in another journal.

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