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# Seasonal nutrient content variation in avocado leaves of ‘Geada’, ‘Fortuna’ and ‘Quintal’ genotypes

Renan Moisés Paneghini Zanata<sup>1</sup>, Jairo Osvaldo Cazetta<sup>1</sup>, Paula Wellen Gonçalves Barbosa<sup>1</sup>

<sup>1</sup> Faculty of Agricultural and Veterinary Sciences – Unesp, Jaboticabal-SP, Brazil.

\*Corresponding author: [renanzanata20@gmail.com](mailto:renanzanata20@gmail.com)

**Abstract** - Avocado is an important fruit for fresh consumption and as raw material for several industries. In order to obtain good productivity and fruit quality, adequate plant nutrition is essential. However, information on the relationship between time of year, phenology and nutritional status of plants of different genotypes is difficult to compare, as most data are obtained from plants of different ages, planted in soils with different fertility levels, different management strategies and most cultivated under edaphoclimatic conditions different from those of Brazil. This study evaluated the seasonal nutrient content variation of avocado leaves, genotypic differences, the relationship with phenological stages and the periods of greater nutrient content stability in ‘Fortuna’, ‘Geada’ and ‘Quintal’ avocado genotypes cultivated in the municipality of Jardinópolis-SP, Brazil. All plants aged 30 years, were randomly planted within the same area and submitted to the same cultural treatments and management. Nutrient contents are more stable during the flowering phase (July to September) and in the final phase of fruit formation (February to March). In most of the cycle, ‘Geada’ plants have lower S, P and Zn levels compared to ‘Fortuna’ and ‘Quintal’ genotypes. ‘Fortuna’ genotype has higher Ca, Mg and Mn levels than the others at the time of fruit harvest (April to June). In the budding phase of the three genotypes (October), increase in N, P, K, S, Cu, Zn contents and decrease in Ca, Mg and Mn contents were observed.

**Index terms:** *Persea americana*, phenology, flowering, fruiting, macronutrients, micronutrients.

## Variação sazonal do teor de nutrientes em folhas de abacateiros dos genótipos Geada, Fortuna e Quintal

**Resumo** - O abacate é uma fruta importante como alimento *in natura* e como matéria-prima para várias indústrias. Para ter boa produtividade e qualidade de frutos, a nutrição adequada das plantas é fundamental. Entretanto, informações sobre a relação entre a época do ano, a fenologia e o estado nutricional de plantas de distintos genótipos são de difícil comparação, pois a maioria dos dados é obtida

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de plantas com diferentes idades, plantadas em solos com diferentes fertilidades, distintas formas de manejo e a maioria em condições edafoclimáticas diferentes das brasileiras. Neste trabalho, foram estudados a variação sazonal do teor de nutrientes nas folhas, as diferenças genotípicas, a relação com os estádios fenológicos e os períodos de maior estabilidade dos teores, em plantas dos genótipos: 'Fortuna', 'Geadá' e 'Quintal', cultivadas em Jardinópolis-SP, Brasil. Todas as plantas tinham 30 anos de idade, plantadas aleatoriamente dentro de uma mesma área e submetidas aos mesmos tratamentos culturais e manejo. Os teores dos nutrientes ficam mais estáveis durante a fase de florescimento (julho a setembro) e na fase final de formação dos frutos (fevereiro e março). Na maior parte do ciclo, as plantas do 'Geadá' apresentam menores teores de S, P e Zn que 'Fortuna' e "Quintal". O 'Fortuna' apresenta maiores teores de Ca, Mg e Mn que os demais na época da colheita dos frutos (abril a junho). Na fase de brotação dos três genótipos (mês de outubro), ocorre elevação dos teores de N, P, K, S, Cu e Zn, e diminuição dos teores de Ca, Mg e Mn.

**Termos para indexação:** *Persea americana*, fenologia, florescimento, frutificação, macronutrientes, micronutrientes.

## Introduction

The knowledge of the nutritional behavior of the avocado tree is essential to optimize the production and quality of fruits (LEONEL et al., 2012; ROSANE, 2022). The increase in fresh consumption and other demands of this fruit has occurred especially due to its richness in oil, minerals, proteins, vitamins, carotene, antioxidants, among other important compounds for human health and industry (NASCIMENTO et al., 2021; OCHOA-ZARZOSA et al., 2021). Although avocado is a fruit widely produced in Brazil, it occupies only the seventh position in the list of the largest world producers, with production corresponding to only 3.5% of the world's production (FAO, 2020). This demonstrates the great potential of Brazil to expand its market share of this fruit. In the Brazilian scenario, the states of São Paulo and Minas Gerais stand out, which account for about 80% of the national production (AGRIANUAL, 2018), with large participation of 'Fortuna', 'Geadá' and 'Quintal' genotypes (MOUCO & LIMA, 2014).

It is known that different genotypes flower and produce fruits at different times (DUARTE et al., 2016, NASCIMENTO et al., 2021; OCHOA-ZARZOSA et al., 2021), and have fruits with different sizes, shapes and chemical composition (KOLLER, 2002; PEREIRA, 2015; DUARTE et al., 2016;

NEWETT et al., 2018; SAMPAIO & WHATELY, 2022). It has also known that avocado fruits are much stronger nutrient sinks compared to leaves (SILBER et al., 2018), which could change the leaf nutrient content during the phase of fruit formation.

Although there are some studies in literature on the nutrient content of avocado plants, the vast majority were carried out in countries with edaphoclimatic conditions very different from those of Brazil and with genotypes not commonly cultivated in Brazil (NEWETT et al., 2018, ROZANE, 2022). The latest recommendations on adequate leaf nutrient levels and fertilization of avocado trees in the State of São Paulo refer to avocado plants in general, not considering differences among genotypes (TEIXEIRA et al., 2022). In addition, information in literature usually comes from plants with different ages and from genotypes cultivated separately both in temporal and spatial terms (CAMPISI-PINTO et al., 2017; TAMAYO et al., 2018; SILBER et al., 2018). This impairs the comparison of possible nutritional differences among genotypes and the probable variations in leaf nutrient content at different phenological stages and times of the year. Therefore, there is a hypothesis that different genotypes must present differences in leaf nutrient content over time, even when cultivated under the same edaphoclimatic and management conditions. The only study

with 'Fortuna', 'Geada' and 'Quintal' genotypes together in the same work was carried out by Mouco et al. (2012), who evaluated the nutrient content of these plants when grown in the Brazilian semiarid region, being evaluated only at the flowering time of each genotype. Therefore, there is no information about how the leaf nutrient content of these genotypes varies at other times of the year, nor how this occurs under the edaphoclimatic conditions of the state of São Paulo.

The reproductive phenology of the avocado tree was well described by Cabezas et al. (2003), and it has been well established that the best way to characterize the nutritional status of perennial plants is through foliar diagnosis (MARCANTE et al., 2010). However, there are indications that the leaf nutrient content of fruit plants varies depending on the time of year and phenology (LEONEL et al., 2012; CRUZ et al., 2019), which must occur at different times for different genotypes and different edaphoclimatic conditions. Studies carried out to identify the best time for leaf sampling in various avocado cultivars suggest that leaf collection should be carried out in the period of greater nutrient content stability (SALAZAR-GARCÍA et al., 2015).

Despite the growing importance and great national potential for avocado cultivation, there is still lack of precise information about the nutritional status of the most genotypes cultivated in Brazil (LEONEL et al., 2012;

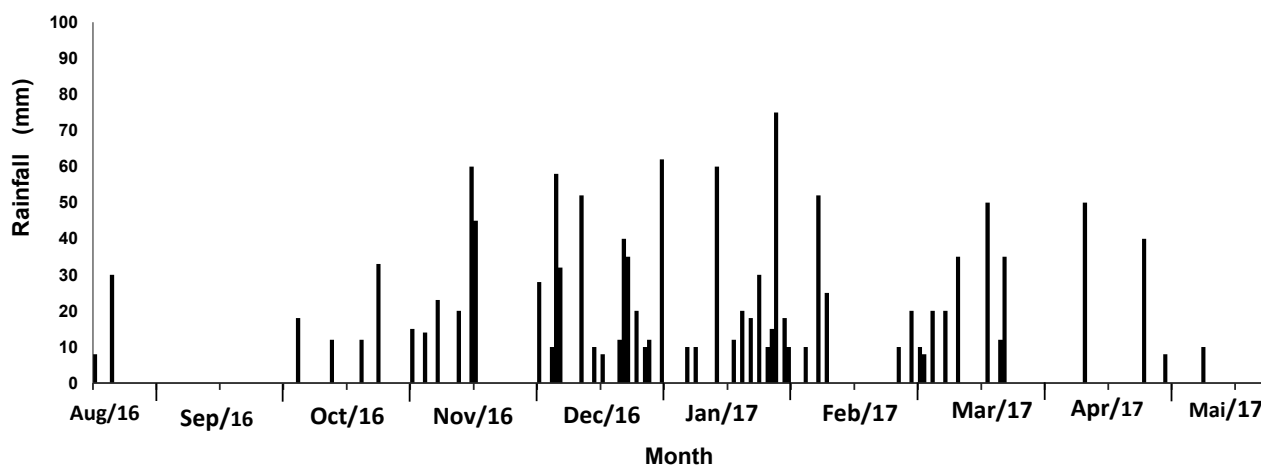
ROSANE, 2022). Such information is essential for a less empirical and more efficient management planning of each genotype in a given cultivation site (SILVA et al., 2014).

Given the above, the aim of the present work was to evaluate variations in leaf nutrient content as a function of genotypic difference, time of year and phenological stage, aiming to detect the best time for leaf sampling for foliar diagnosis of the main avocado genotypes grown under the edaphoclimatic conditions of the state of São Paulo.

## Material and methods

The experiment was carried out in the municipality of Jardinópolis, western state of São Paulo, at Mata da Chuva farm, 590 meters a.s.l. and flat relief. The experimental area is located at coordinates 20°55'28"S and 47°55'45"W. According to Köppen's classification, the climate in the region is tropical (Aw), characterized by hot and humid summers and cold and dry winters. The average annual temperature is 21.8 °C, with maximum monthly average of 23.7 °C in January and minimum of 18.4 °C in July. Annual precipitation is 1450 mm, with January being the month with the highest rainfall, with the lowest rainfall in August (ROLIN, 2020).

During the experiment, a rain gauge was installed in the experimental area, whose collected data are shown in Figure 1.



**Figure 1.** Rainfall index in the experimental area located in the municipality of Jardinópolis-SP. Data collected between Jun/2016 and May/2017, with rain gauge installed inside the experimental area.

The soil where the experiment was conducted is classified as Eutroferric Red Latosol (SANTOS et al., 2018). To characterize soil fertility in the experimental

area at the beginning of the experiment, soil samples were collected from 0-20 and 20-40 cm layers, whose data are shown in Table 1.

**Table 1.** Soil chemical characterization in the experimental area in June 2016. Jardinópolis - SP.

Depth (cm)	pH	OM g dm <sup>-3</sup>	P	S	Ca	Mg	K	H+Al	CTC	V	B	Cu	Fe	Mn	Zn
			mg dm <sup>-3</sup>			mmol <sub>c</sub> dm <sup>-3</sup>			%		mg dm <sup>-3</sup>				
0-20	5.7	38	19	6	56	20	1.7	22	99.7	78	0.18	6.1	15	19	0.8
20-40	5.8	28	9	6	50	16	2.0	20	88.0	85	0.21	4.1	12	13	0.3

In the orchard where the experiment was carried out, plants of 'Geada', 'Fortuna' and 'Quintal' genotypes were originally planted at random, with spacing of 8 × 12 m. The orchard is composed of adult 30-year-old plants in full production.

The application of fertilizers and pesticides in the experimental period (between June 2016 and May 2017) took place according to the following schedule: 07/30/2016 - foundation fertilization with 5-25-25 formulation, obtained by mixing monoammonium phosphate and potassium chloride at dose of 2 kg plant<sup>-1</sup>; 08/04/2016 - spraying with phosphite fertilizer, mancozeb fungicide, lambda-cyhalothrin insecticide; 09/16/2016 - spraying with phosphite fertilizer, lambda-cyhalothrin insecticide and thiamethoxam, mancozeb fungicide; 10/23/2016 - top dressing fertilization with 20-00-20 formulation, obtained by mixing ammonium nitrate and potassium chloride at dose of 1 kg plant<sup>-1</sup>; 11/18/2016 - spraying with phosphite fertilizer, lambda-cyhalothrin insecticide and thiamethoxam, mancozeb and copper oxychloride fungicides; 01/20/2017 - spraying with mancozeb and copper oxychloride fungicides. During the experimental period, cultivation was developed without irrigation and weed control was carried out by mowing and herbicide application.

The experiment consisted of three main treatments (three genotypes: 'Fortuna', 'Geada' and 'Quintal') with 10 replicates, totaling 30 experimental units. Each experimental unit consisted of an adult plant located at random within the orchard. As each plant (experimental unit) was inserted in the middle of the orchard, they were considered

as the useful area of the plot, while all the other surrounding plants were considered as border. Since the orchard was originally established by randomly planting the three genotypes in the same area, it was necessary to locate the position of each of the 30 experimental units, which were duly marked to be periodically evaluated. Therefore, it could be considered that the 30 experimental units were arranged in a completely randomized design.

All identified plants (experimental units) were monthly evaluated during one year (complete plant cycle) and, therefore, monthly evaluations were considered as secondary treatments. Since evaluations were always carried out in the same experimental units, for statistical purposes, secondary treatments (monthly evaluations) were considered as split-plots over time, as indicated by BARBOSA; MALDONADO JUNIOR (2015).

To determine the budding period, plants were weekly evaluated. For this, each plant was divided into eight quadrants, four on each side of the planting line. Each quadrant received a visual score indicating the budding percentage. The score assigned to each quadrant ranged from 0% (quadrant without buds) to 100% (quadrant in which all branches had new buds), and the mean scores of the eight quadrants were calculated for each plant. To assess the flowering period, the same assessment methodology was followed, using score scale ranging from 0% (no flower fully open) to 100% (all flower buds fully open), according to methodology similar to that proposed for the flowering assessment of citrus plants (GOTTWALD et al., 2007).



To determine the variation in nutritional status over time, 12 monthly leaf samplings were carried out in each experimental unit, which took place between June 2016 and May 2017. Evaluation dates were: 06/12/16; 07/09/16; 08/14/16; 09/11/16; 10/16/16; 11/15/16; 12/14/16; 01/15/17; 02/16/17; 03/19/17; 04/16/17; 05/13/17. Sampled leaves always corresponded to the first completely expanded leaf of the last vegetative flow. In order to obtain a representative composite sample of each experimental unit, eight leaves were randomly collected (two from each of the four cardinal points in the middle part of the plant canopy), as indicated by Leonel et al. (2012). Leaves were washed with neutral detergent solution (1%), rinsed with running water and de-ionized water. Leaves were then dried in an air circulation oven (60-70°C) until reaching constant weight, ground in Willey-type mill, passed through a 0.5 mm sieve and submitted to chemical analysis to determine the nitrogen and phosphorus, potassium, sulfur, magnesium, calcium, manganese, zinc, copper and iron contents, according to methodology described by Bataglia et al. (1983).

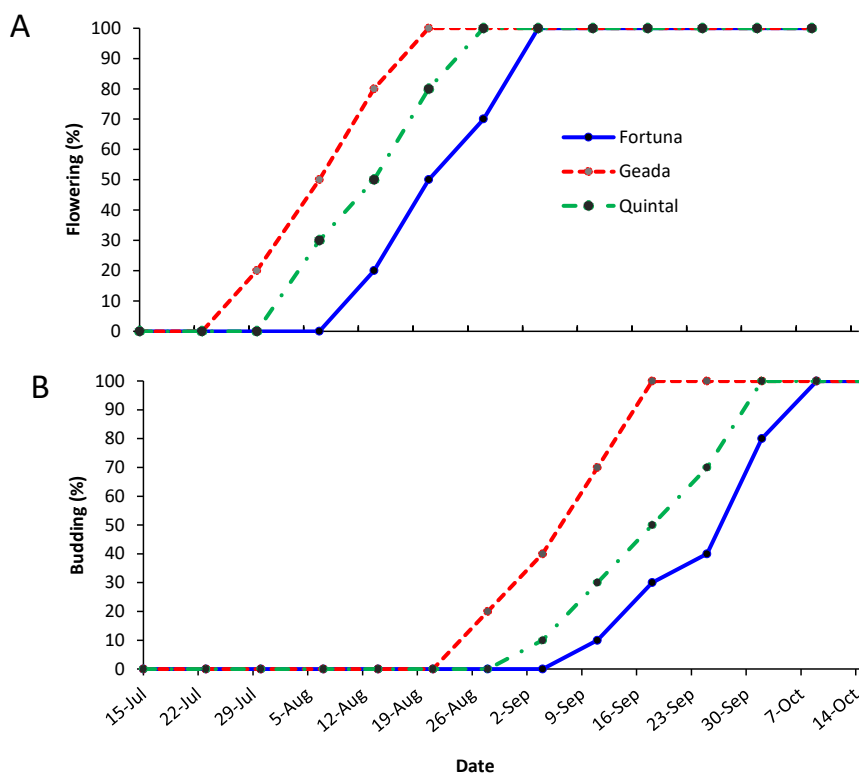
Data referring to nutrient content were submitted to analysis of variance by the F test, in a 3 × 12 factorial scheme (3 genotypes as main treatment and 12 monthly evaluations as split plots in time), with calculation of averages and respective statistics. For statistical analysis, the AGROESTAT software (BARBOSA; MALDONADO JUNIOR, 2015) was used.

## Results and discussion

Avocado genotypes started flowering with difference of seven days from each other (Figure 2A). 'Geada' began flowering on July 23, followed by 'Quintal' and finally 'Fortuna'. This difference was expected, as Oliveira et al. (2013) had already described this difference among these genotypes. Although the flowering of each genotype started at different times, it is interesting to observe that the flowering periods last-

ed four weeks for each of the three genotypes (Figure 2A). This resulted in a three-week overlap between flowering periods of 'Geada' and 'Quintal' genotypes, between 'Quintal' and 'Fortuna', as well as a two-week overlap between 'Geada' and 'Fortuna' genotypes. This overlap is important to ensure the pollination of plants within an orchard containing plants of various genotypes, as avocado trees present the phenomenon of protogyny dichogamy (FALCÃO, 2001). This means that, although flowers are hermaphrodite, the stigma of the flower of a variety is not receptive at the same time that the anther of that flower is releasing the pollen grain, making self-fertilization very difficult. Thus, according to Dymond et al. (2021), varieties are classified as belonging to floral group A or floral group B. In group A plants, the flower opens in the morning as a female, closes at night and opens again only in the afternoon of the following day, this time as a male. In group B plants, on the other hand, the flower opens as a female in the afternoon, closes at night and opens as a male the next morning (DYMOND et al., 2021). In this context, 'Geada' and 'Fortuna' genotypes belong to floral group A, while 'Quintal' genotype belongs to group B (OLIVEIRA, 2006). Such information is decisive when setting up a commercial orchard, since there must be plants from different floral groups to optimize pollination and, therefore, fruit productivity (FALCÃO, 2001).

'Geada' genotype started budding on August 20, followed by 'Quintal', on August 27 and 'Fortuna' on September 3 (Figure 2B). Similar to flowering (Figure 2A), complete budding was also reached after four weeks for 'Geada' genotype, but it took five weeks for 'Fortuna' and 'Quintal' genotypes (Figure 2B). It is noteworthy that this period occurred with total absence of rain (Figure 1), indicating the importance of healthy plants with deep and well-developed roots, as well as soil with cover that allows moisture maintenance, as these physiological phenomena of avocado trees coincide with the dry season in southeastern Brazil.



**Figure 2.** Flowering (A) and budding (B) development of 'Fortuna', 'Geda' and 'Quintal' genotypes, in 2016, Jardinópolis - SP.

The results of the analysis of variance (Test F) for nutrient content as a function of the effect of genotype (G), evaluation period (A) and interaction of these two factors (G×A) are presented in Table 2. The evaluation period (A) had a highly significant influence ( $P < 0.01$ ) on nutrient content, revealing intense seasonal variation.

The genetic factor (G) had no significant influence ( $P > 0.05$ ) only in the case of Mg, Cu and Fe contents, indicating that the contents of these nutrients in the three genotypes varied similarly, while the contents of the other nutrients differed for the three genotypes in at least one of the evaluations performed (Table 2).

Factors (genotype and evaluation period) had independent effects only on the Zn content and, therefore, only for this nutrient, it was possible to compare the mean of genotypes and the mean of evaluations independently. For the other nutrients, contents were significantly influenced ( $P < 0.05$ ) by the genotype x evaluation period interaction

(G×A). It is interesting to observe that the Mg, Cu and Fe data revealed an effect of interaction (G×A) even when no individual effect of the genotype factor was detected at 5% probability level (Table 2). This suggests that the genotypes must have influenced these nutrients to a lesser degree of reliability ( $P > 0.05$ ), but enough to interfere with the effect of evaluation period, leading to significant interactions (G×A) ( $P < 0.05$ ).

The significant effect of the interaction of factors (G×A) indicates that the genotypes had a distinct influence within each evaluation period, and vice versa. Therefore, in these cases, it makes no sense to analyze the individual effect of each factor (BARBOSA & MALDONADO JUNIOR, 2015). For this reason, in these cases, results were presented in the form of graphs to allow observing how variations in the nutrient content of each genotype occurred within each season, and vice versa, using MSD (minimum significant difference) by the Tukey test at 5% (bars) to verify in which situations means differed significantly (Figures 3 to 5). The independent mean effects of genotypes

and evaluation periods on Zn content were shown in Figure 6.

In Figures 1-5, the points in graphs correspond to the actual leaf nutrient contents, while in Figure 6A, they correspond to the general average of each genotype (consid-

ering all evaluation periods) and in Figure 6B, each point corresponds to the average of each evaluation period (considering the three genotypes). Therefore, in the case of Zn, results were presented in both Figure 5D and Figure 6.

**Table 2.** Results of the analysis of variance by the F test of nutrient content in the leaves of 30-year-old avocado trees of 'Fortuna', 'Geada' and 'Quintal' genotypes (G) cultivated in Jardinópolis - SP, determined in 12 monthly assessments (A).

Factor	Nutrients									
	N	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn
Genotype (G)	*	**	**	**	ns	**	ns	ns	*	**
Assessment (A)	**	**	**	**	**	**	**	**	**	**
Interaction (G×A)	**	**	**	**	**	**	**	**	**	ns

\*\* =  $P < 0.01$ ; \*  $P < 0.05$ ; ns =  $P > 0.05$

The N contents in the leaves of 'Fortuna' and 'Quintal' genotypes increased significantly in October from 16.7 and 18.1 g kg<sup>-1</sup>, verified in September to 23.2 and 23.3 g kg<sup>-1</sup> in October, respectively, while those of 'Geada' genotype remained around 17.0 g kg<sup>-1</sup> in the same period. In November, N contents in 'Fortuna' and 'Quintal' genotypes returned to levels around 18.0 g kg<sup>-1</sup>, even so with higher values compared to 'Geada' genotype (Figure 3A). Such sudden variations in the N content coincided with the budding phase of plants (Figure 3A). In this phase, the leaves of the different genotypes are in the formation phase, when their constitution changes significantly within a few days. Immediately after the budding phase, when leaves are not yet fully mature, fruit formation begins at the same time (see indication of phenological phases in the upper part of Figures 3A and 3B), which would explain such fluctuations in this period. Therefore, it would not be recommended to sample leaves for foliar diagnosis purposes in periods within or close to this phenological phase. According to Salazar-García et al. (2015), the most suitable period for collecting leaves for foliar diagnosis is the one where the levels of the nutrients of interest are more stable.

In the period that goes from the intermediate phase of fruit formation to complete fruit formation (which occurred in the period

from February to April), N levels remained quite stable and with little difference among the three genotypes (Figure 3A). This stability and similarity of N content in the leaves of different genotypes is important, as a single reference could be established to categorize the nitrogen nutritional status of adult avocado plants without the need to create specific N content classes to characterize the nutritional status of each genotype.

From the month of April onwards, N contents begin to vary again, including differences between genotype averages (Figure 3A). Such variation must be an indication that leaves are beginning to enter the senescence phase. In the December sampling, it was already possible to observe that 'Fortuna' leaves had the lowest N content, followed by 'Geada' with intermediate value, and 'Quintal' with the highest values. Since intense N remobilization occurs during the leaf senescence (SALAZAR-GARCÍA et al., 2015; HAVÉ et al., 2017), the sharpest reduction in N content in 'Fortuna' leaves in May and June suggest that leaf senescence must have started first in this genotype, followed by 'Geada' and 'Quintal' genotypes. Therefore, leaf sampling for nutritional diagnosis purposes would also not be recommended from the end of April, as differences in leaf senescence should lead to different values for different genotypes.

In general, considering the three genotypes and all evaluation periods, N contents were between 16 and 24 g kg<sup>-1</sup> (Figure 3A). There are no consistent references to categorize N levels for plants of these genotypes under the Brazilian edaphoclimatic conditions. The limits described in Brazilian references such as Leonel et al. (2012) and Rosane (2022) are mostly based on works developed from the 1960s to the 2000s in Australia, South Africa and North America, which are practically the same as those observed in reviews by Newett et al., (2018) and Mohale et al. (2022). In these references, N contents between 22 and 26 g kg<sup>-1</sup> are considered adequate, while the most recent recommendations for the state of São Paulo report levels from 16 to 20 mg kg<sup>-1</sup> (TEIXEIRA et al., 2022). In this context, it could be considered that the N content in leaves of the three genotypes would be within the adequate range, considering evaluations carried out from February to April. Taking into account evaluations of June and July, only the N contents of 'Quintal' genotype could be considered as sufficient, while those of the other genotypes would be classified as low. This is another justification that the best period for collecting leaves from adult avocado trees cultivated under the conditions of this trial (state of São Paulo) would be between the months of February and April, which corresponds to the period that goes from the intermediate phase of fruit formation until complete fruit formation. This sampling period coincides in part with that suggested by Teixeira et al. (2022).

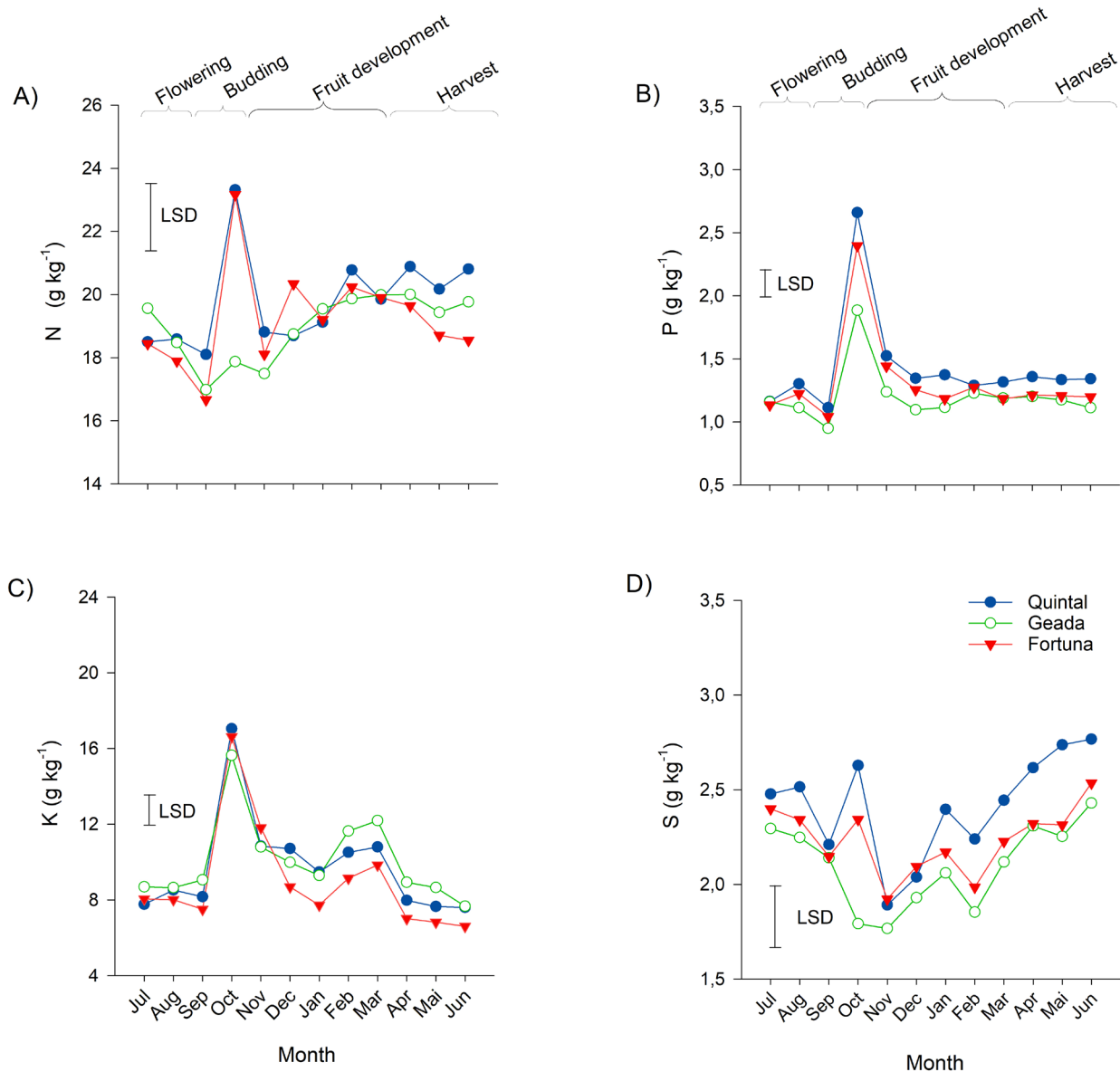
Significant increase in P levels in the leaves of the three genotypes was observed in October (Figure 3B), in the same way as observed for N (Figure 3A). At that time, the P content in leaves reached 2.6 g kg<sup>-1</sup> for 'Quintal' genotype, 2.4 g kg<sup>-1</sup> for 'Fortuna' genotype and 1.9 g kg<sup>-1</sup> for 'Geadá' genotype. After the month of October, P levels decreased to values between 1.0 and 1.5 g kg<sup>-1</sup> in the other evaluations and varied little within each genotype (Figure 3B). Such P values would be considered sufficient,

since they are in the range from 0.8 g kg<sup>-1</sup> to 2.5 g kg<sup>-1</sup> (LEONEL et al., 2012; NEWETT et al., 2018; MOHALE et al., 2022; ROSANE, 2022, TEIXEIRA et al, 2022). Although the P levels in the leaves of the three genotypes remained within a very narrow range (between 1.0 and 1.5 g kg<sup>-1</sup>), P levels in 'Quintal' genotype tended to be around 20% higher than those of 'Geadá' genotype, with those of 'Fortuna' genotype remained at intermediate values between those of 'Quintal' and 'Geadá' genotypes. Considering the stability of P contents in leaves between February and April, this period would be the best time for leaf sampling which, similarly to what was observed for N, corresponds to the period between the intermediate phase and complete fruit formation. This result partially corroborates those of TEIXEIRA et al. (2022), who recommended sampling leaves in February or March.

Similar to leaf N and P contents found, K content also showed a sharp increase in October when the content reached values around 16 g kg<sup>-1</sup> for the three genotypes. In the following months, values tended to decrease until January, reducing to values between 8.0 and 10.0 g kg<sup>-1</sup>. From January to March, increases of 28%, 16% and 22% were observed in K contents for 'Geadá', 'Quintal' and 'Fortuna' genotypes, respectively. In turn, in the period between April and September, K contents stabilized in the range from 7.0 to 9.0 g kg<sup>-1</sup> in the three genotypes (Figure 3C).

Literature references indicate that the sufficiency range for K content in leaves can be quite wide, with values between 7.5 and 20 g kg<sup>-1</sup> (LEONEL et al., 2012; MOHALE et al., 2022; ROSANE, 2022, TEIXEIRA et al, 2022). In this context, it could be considered that practically all data obtained in the present study (Figure 3C) can be considered as sufficient, even taking into account the large oscillations that occurred between October and January. However, due to the large fluctuations observed in K contents in the months between October and December, both for K and for N and P, leaf sampling during this period should be avoided.





**Figure 3.** Variation in nitrogen (N), phosphorus (P), potassium (K) and sulfur (S) content (Figures A, B, C and D, respectively) in avocado leaves of 'Fortuna', 'Geada' and 'Quintal' genotypes throughout the production cycle (July/2016 to May/2017, in Jardinópolis - SP). Bars correspond to LSD (Least significant difference) by the Tukey test (5%).

One explanation for the large variation in N, P and K contents in October is the first fertilization (carried out on July 30), which must have reflected in the increase observed in the October sampling. This finding is supported by the fact that in plants without irrigation, the response was not observed in August, since it was in the dry season, which may have delayed the uptake of elements. Thus, the occurrence of two rain events in the second half of August (Figure 1) must have induced the uptake of nutrients from

fertilizers, resulting in increase in the N, P and K concentration in leaves sampled in October. It was also observed that this is a month of emission of new buds, in which the concentration of mobile nutrients in leaves is high (Salazar-García et al., 2015).

The lower N, P and S levels presented by 'Geada' genotype, especially in the months from September to November (Figure 3), may be due to the greater dilution of these nutrients within the plant of this genotype, since the crown architecture of this geno-

type is much more voluminous than that of the other genotypes. In addition, data shown in Figure 2 indicate the more advanced flowering and budding stage of this genotype when compared to the others, which should drain more nutrients and may also have contributed to this difference. However, values found in the present study are very similar to N and K contents reported by Mouco et al. (2012), even under climate and soil conditions very different from those prevailing in the present study.

As for the N and K supply by the second fertilization (carried out on October 23), there was only a subtle increase in leaf levels, since, at this point, fruit development is observed, which are the most important drains at the moment and should divert the flow of nutrients to fruits to the detriment of leaves, so as not to induce large leaf content variations. This finding corroborates results obtained by TAMAYO et al. (2018) and by SELLADURAI; AWACHARE (2019), who concluded that N and K are the nutrients most extracted by avocado fruits.

Sulfur values varied between 1.77 g kg<sup>-1</sup> and 2.77 g kg<sup>-1</sup> (Figure 3D). It is interesting to observe that since 1964 until the present moment, exactly the same range of suitable sulfur content has been suggested, from 2.0 g kg<sup>-1</sup> to 6.0 g kg<sup>-1</sup> (LEONEL et al., 2012; NEWETT et al., 2018; MOHALE et al., 2022; ROSANE, 2022, TEIXEIRA et al., 2022), despite the change in genotypes and management that occurred during this period. In this context, the levels detected in the present study (Figure 3D) are below or close to the lower limit of the range considered sufficient (2.0 to 6.0 g kg<sup>-1</sup>).

The increase in S contents observed in October also occurred for 'Quintal' and 'Fortuna' genotypes, but not for 'Geada' genotype, which continued to decrease from around 2.4 g kg<sup>-1</sup> in June to 1.9 g kg<sup>-1</sup> in October. From October onwards, the three genotypes began a trajectory of increase in S content, only with a slight decrease in January, and again an increase until the end of the cycle (in June), when 'Quintal',

'Fortuna' and 'Geada' genotypes ended up the cycle with leaf contents of 2.8 g kg<sup>-1</sup>, 2.5 g kg<sup>-1</sup> and 2.4 g kg<sup>-1</sup>, respectively (Figure 3D).

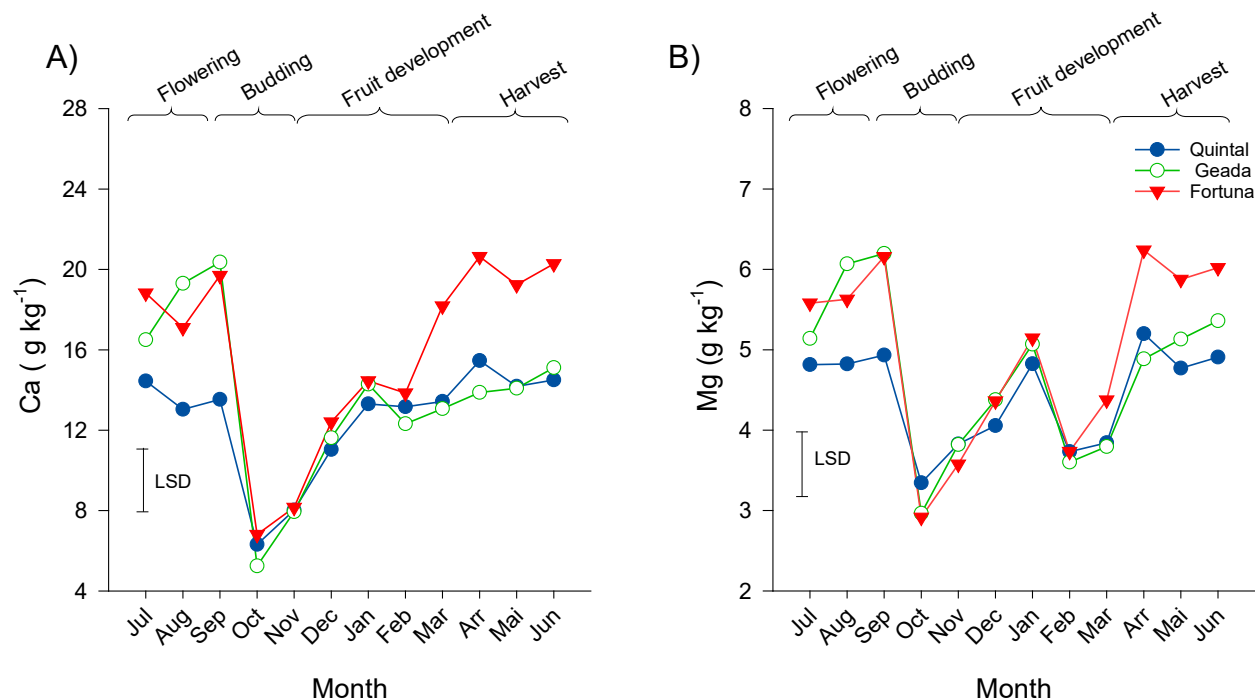
The irregular behavior of the S content in leaves during the production cycle is probably due to its close connection with soil organic matter, since this nutrient was not supplied in the form of fertilizers in that period. Tiecher et al. (2013) reported that more than 90% of sulfur present in soil originates from organic matter, and the process of mineralization of this nutrient is essential for plants to absorb it. Considering that organic matter mineralization only occurs properly when the soil has adequate humidity and temperature, it is possible that the increases in S levels from October onwards are due to the increase in the frequency and amount of rainfall, combined with the increase in ambient temperature that occurred in this period (Figure 1), which would lead to greater mineralization and explain the increases in S levels.

The Ca and Mg contents showed very similar behavior among themselves (Figure 4), but opposite to the other macronutrients (Figure 3), because while N, P, K and S contents increased in October, Ca and Mg contents decreased in that month. The Ca contents, which were close to 20.0 g kg<sup>-1</sup> in 'Geada' and 'Fortuna' leaves and 13.0 g kg<sup>-1</sup> in 'Quintal' leaves from July to September, showed reduction of about 70% in the budding phase (October), dropping to values around 6.0 g kg<sup>-1</sup> (Figure 4A). Then, Ca concentration increased again in the leaves of the three genotypes, and from the month of March onwards, Ca levels tended to return to levels observed at the beginning of samplings (months of July and August). The lowest Ca levels observed in October and November were below the range considered adequate (LEONEL et al., 2012; NEWETT et al., 2018; MOHALE et al., 2022; ROSANE, 2022, TEIXEIRA et al., 2022).

In the month of October, Mg levels decreased by almost half, compared to values observed in the previous three months (Figure 4B). Although increasing in November and

December, a new decrease was observed in January for the three genotypes. As in February, Mg levels resumed the upward

trend until April, when they reached levels similar to those observed at the beginning of evaluations (Figure 4B).



**Figure 4.** Variation of calcium (Ca) and magnesium (Mg) content (Figures A and B, respectively) in avocado leaves of 'Fortuna', 'Geada' and 'Quintal' genotypes throughout a production cycle (July/2016 to May/2017, in Jardinópolis – SP. Bars correspond to LSD (Least significant difference) by the Tukey test (5%).

The decrease in Ca and Mg contents and the increase in N, P, K contents in younger leaves, shortly after budding, was also observed by Salazar-García et al. (2015) for 'Hass' genotype. An important factor that leads to such a discrepancy between values in young leaves is that Ca and Mg are not remobilized within the plant, while N, P and K are translocated and directed to young leaves to support their development, with consequent increase in the content of these mobile nutrients (Salazar-García et al., 2015; Campisi-Pinto et al., 2017). Another factor that may have contributed to the discrepancy in Ca and Mg values in relation to other macronutrients in young leaves is the antagonistic interaction between the absorption of these nutrients and potassium (SILVA; TREVISAM, 2015; BONOMELLI et al., 2019). In this context, July and October fertilizations must have increased the soil K concentration, which may have contributed to the decrease in the Ca and Mg uptake by plants

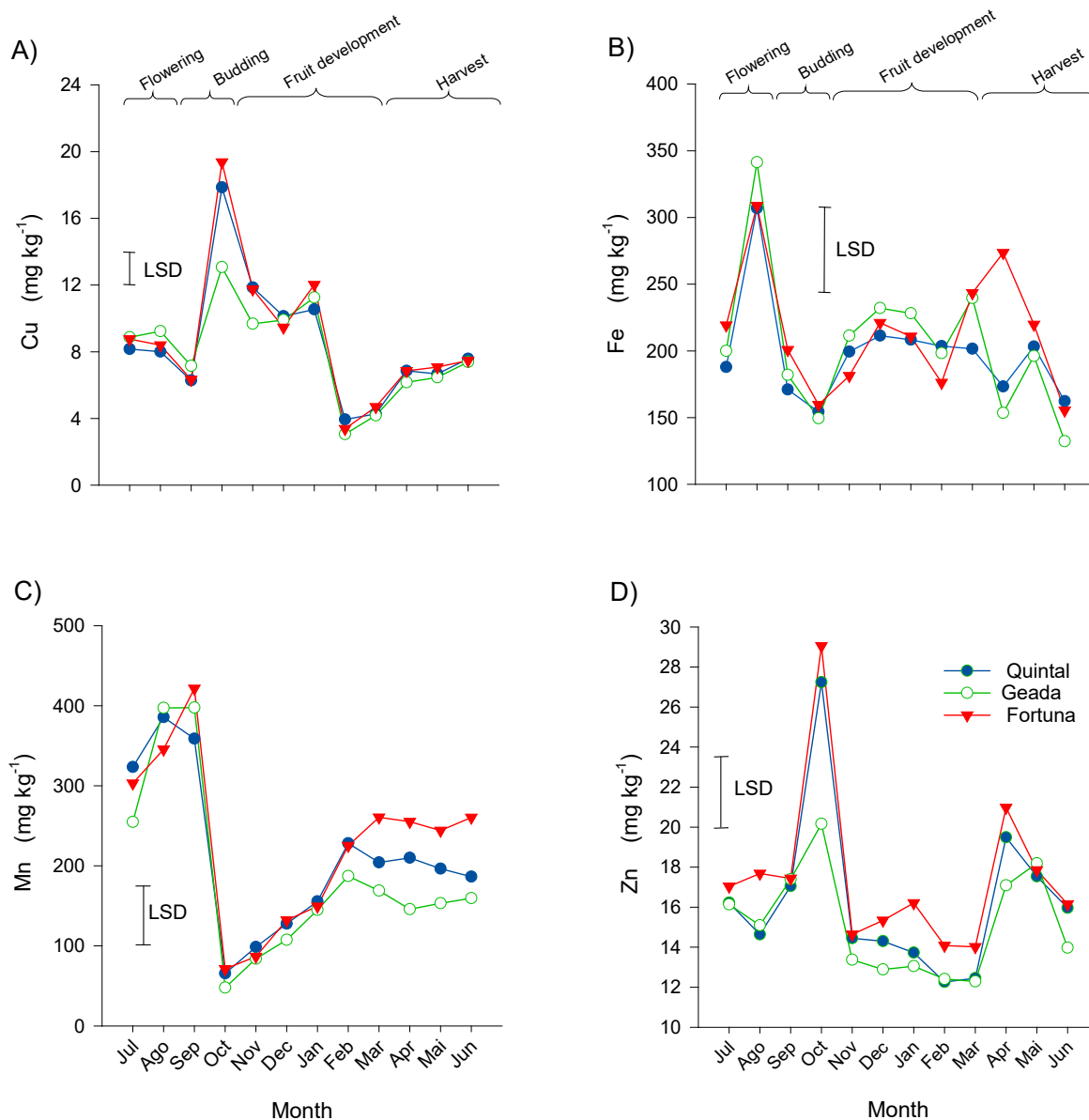
due to competitive inhibition (BEHERA et al., 2021). In addition, Ca is indispensable for pollen grain germination and pollen tube growth (YANG et al., 2021), whose demand in this process may have directed the flow of Ca to flowers, which may also have contributed to the decrease in the content of this nutrient in leaves in September. Finally, the intense growth of leaves at the beginning of their development with little Ca and Mg absorption and translocation must also lead to the dilution of these nutrients in the dry matter of leaves in this period, which can also lead to lower concentration of these nutrients, compared to other macronutrients.

It is noteworthy that, despite the high seasonal variation in Mg contents, all values were considered within the sufficiency range between 2.5 and 8.0 g kg<sup>-1</sup> (LEONEL et al., 2012; NEWETT et al., 2018; MOHALE et al., 2022; ROSANE, 2022, TEIXEIRA et al., 2022). On the other hand, based on field observations in California - USA, Crowley et al.

(2015) presented a proposal to change the appropriate range to values between 6.0 and 9.0 g kg<sup>-1</sup>.

Cu (Figure 5A) and Fe (Figure 5B) contents increased in October, similarly to what occurred with N, P, K and S (Figure 3), while Mn content (Figure 5C) had reduction in October, as occurred for Ca and Mg (Figure 4). The great Cu and Fe mobility in the avocado plant,

which leads to translocation to developing tissues (Salazar-García et al., 2015; Campisi-Pinto et al., 2017) may explain the similarity of data of these micronutrients with those of N, P, K and S. In turn, the low Mn mobility in the avocado plant (Salazar-García et al.; 2015; Campisi-Pinto et al., 2017) justifies the similarity between data on this nutrient and Ca and Mg (Figure 4).



**Figure 5.** Variation of copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) content (Figures A, B, C and D, respectively) in avocado leaves of 'Fortuna', 'Geadá' and 'Quintal' genotypes throughout the production cycle (July/2016 to May/2017, in Jardinópolis - SP). Bars correspond to LSD (Least significant difference) by the Tukey test (5%).

For Cu (Figure 5A), in addition to its translocation in the plant, the increases found and the oscillations in the period from October

to December must also be due to spraying with copper oxychloride-based fungicides (as described in material and methods),



which were performed due to the great demand for this nutrient by plants that enter the reproductive phase (Silber et al., 2018). The organic matter mineralization that occurs with the increase in soil moisture in this period may also have contributed, since the main source of this nutrient in the soil is the organic matter mineralization (KUMAR et al., 2021), which occurs mainly in the period with higher soil moisture (Figure 1). Considering that all plants were conducted under exactly the same soil, climate and management conditions, everything indicates that the differences between 'Geada' and the other genotypes, in the month of October, are of a genetic nature. Despite oscillations in Cu (from 4 mg kg<sup>-1</sup> to 20 mg kg<sup>-1</sup>) and iron (from 50 mg kg<sup>-1</sup> to 350 mg kg<sup>-1</sup>) contents, the values observed in periods of greater stability remained between 5 mg kg<sup>-1</sup> and 15 mg kg<sup>-1</sup> for Cu and between 50 mg kg<sup>-1</sup> and 200 mg kg<sup>-1</sup> for Fe, which correspond to ranges of adequate nutritional levels pointed out by LEONEL et al. (2012). However, the limit values of Cu sufficiency concentration range (0.05% to 0.065%) pointed out by Mohale et al. (2022), would correspond to Cu content between 500 mg kg<sup>-1</sup> and 650 mg kg<sup>-1</sup>, values that are about one hundred times higher when compared with those found in this work, and with sufficiency limits pointed out by Leonel et al., 2012; Newett et al., 2018; Rosane, 2022 and Teixeira et al., 2022. The fact that the same type of difference also seems to occur for the other micronutrients but not for macronutrients indicates that the micronutrient contents described by Mohale et al. (2022) may be mistaken regarding the expression unit of results.

Leaf manganese content (Figure 5C) was about 10 times higher than zinc content (Figure 5D). This difference was also observed in the concentration of this element in the soil of the experimental area (Table 1), which would explain such differences in the levels of these nutrients in leaves. However, Mn levels observed throughout the entire plant cycle were between 50 mg kg<sup>-1</sup> and

420 mg kg<sup>-1</sup>, which are considered adequate when compared to levels described by Leonel et al., 2012; Newett et al., 2018, but levels above 200 mg kg<sup>-1</sup> would already be considered excessive when compared to levels from 30 mg kg<sup>-1</sup> to 200 mg kg<sup>-1</sup> reported by Teixeira et al. (2022).

Zn contents in leaves of the three genotypes, as a function of time, are shown in Figure 5D, which shows that in all evaluation periods, the Zn content in 'Fortuna' leaves was always the highest, contents in 'Geada' leaves were always the lowest and those of 'Quintal' remained at intermediate values. Therefore, in the specific case of this nutrient, an effect was observed regardless of genotype and evaluation period (Table 2, Figure 6).

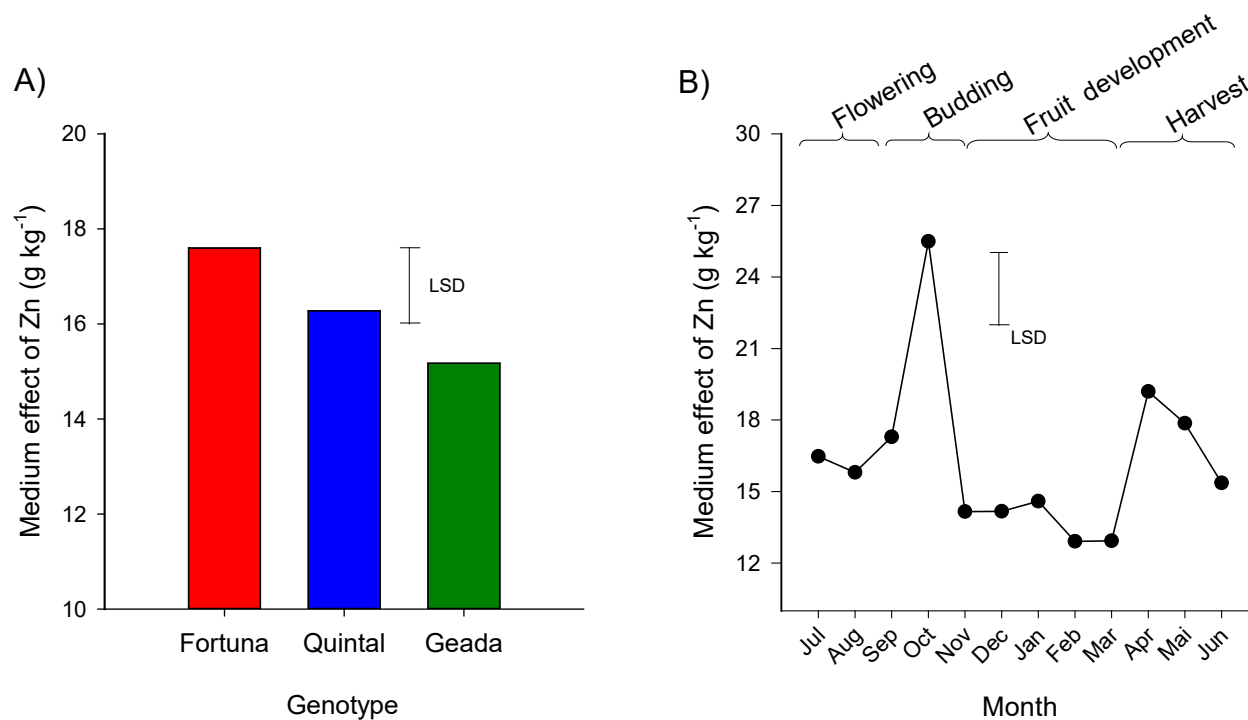
The analysis of data in Figure 6A revealed that the Zn content in 'Fortuna' leaves tended to show higher values (average of 17.6 mg kg<sup>-1</sup>); 'Geada' leaves showed the lowest values (average of 15.2 mg kg<sup>-1</sup>) and 'Quintal' leaves showed intermediate values (mean of 16.2 mg kg<sup>-1</sup>), which did not differ from the others by the Tukey's test (5%).

Considering that Zn is a nutrient that does not undergo translocation in the plant (Salazar-García et al.; 2015; Campisi-Pinto et al., 2017), seasonal variation similar to that of Ca, Mg and Mn was expected (Figures 4 and 5C). However, this was not observed, as there was a large increase in Zn content in October (Figure 5D), while Ca, Mg and Mn contents decreased. Therefore, the only justification for the increase in Zn content in the month of October, in the three genotypes, were the foliar applications of Mancozeb-based fungicides (described in the material and methods), a product containing zinc in its composition. Such applications were carried out due to the fact that Zn is in great demand during budding and flowering, as well as during intense protein synthesis in the phase of construction of new tissues, both vegetative and reproductive, and fruit filling (XIE et al., 2021). As the edaphoclimatic conditions and cultural treatments carried out in the three genotypes were exactly the same, it is believed that the lower leaf zinc content

found in September in the 'Geada' genotype in relation to 'Fortuna' and 'Quintal' (Figure 5D) stems from genetic factors.

Despite the foliar applications of products containing Zn and the increase in Zn levels

in the leaves of plants analyzed in October, April and May, all values were below the range (30 mg kg<sup>-1</sup> to 100 mg kg<sup>-1</sup>) considered as suitable for plants cultivated under the conditions of the state of São Paulo (TEIXEIRA et al., 2022).



**Figure 6.** Average effect of genotypes (A) and evaluation period (B) on the zinc content in the leaves of avocado trees of 'Fortuna', 'Geada' and 'Quintal' genotypes throughout the production cycle (July/ 2016 to May/2017, in Jardinópolis - SP). Letters in (A) and bar in (B) correspond to LSD (Least significant difference) by the Tukey test (5%).

Despite the fact that avocado is of great importance in Brazil and in the world, knowledge of its mineral nutrition aspect seems to have been stagnant for a long period, since the limits of sufficiency of nutrients have remained the same since the 1960s (ROSANE, 2022). Some minor changes have been suggested only recently, such as those by Crowley et al. (2015) and Teixeira et al. (2022), which basically constitute the narrowing of limits of content ranges considered adequate for several nutrients, indicating that the ranges that had been considered were too wide and not contemplate the current reality of the crop.

In the case of annual plants such as corn and soybeans, nutritional limits and fertilization recommendations are carried out

with a series of calibration tests distributed within a given uniform edaphoclimatic region (CANTARELLA et al., 2022), which is not so simple in the case of avocado. This culture is composed of perennial plants, which involves several genotypes that are distinguished in various aspects such as size and shape of plants, as well as number, size, shape, chemical composition and useful post-harvest time (KOLLER, 2002; PEREIRA, 2015; DUARTE et al., 2016; NEWETT et al., 2018; SAMPAIO & WHATELY, 2022). The present study found evidence that the nutritional aspect can also vary in some genotypes, in addition to significant changes depending on the time of year. In addition, it is a crop that takes several years after planting to reach full production (NEWETT et al., 2018; SAMPAIO; WHATELY, 2022), and

even so, harvest does not occur in a single moment as in annual crops, nor all fruits produced are of commercial quality, so that productivity cannot be measured only in terms of the total kg ha<sup>-1</sup> produced, as is done in the case of annual crops. Therefore, it seems that such difficulties are what would justify the delay to obtain consistent data to support recommendation updates for this crop.

In recent years, new ways of determining the nutritional status of cultivated plants have emerged, such as the application of multivariate statistical techniques and DRIS using databases collected from commercial plantations (ROSANE, 2022), which may contribute to circumventing the difficulties of obtaining recommendations based on tests traditionally used for other crops. In the case of avocado crop, it would be interesting if such techniques involved, in addition to nutritional data and productivity, data on the quality of fruits produced, which, ultimately, is what attracts consumers in their decision to buy or not the product.

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## Conclusions

The leaf nutrient contents are more stable during the flowering phase (July to September) and in the final phase of fruit formation (February to March), indicating that these are the most recommended times for assessing the nutritional status of plants.

'Geada' plants have lower S, P and Zn levels than 'Fortuna' and 'Quintal' in most of the plant cycle.

'Fortuna' genotype has higher Ca, Mg and Mn levels than the other genotypes at the time of fruit harvest (April to June).

In the budding phase of the three genotypes (October), the greatest variations in leaf nutrient contents occur, with increase in N, P, K, S, Cu, Zn levels, and decrease in Ca, Mg and Mn levels.

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