

Normal nutrient ranges and nutritional monitoring of 'Pêra' orange trees based on the CND method in different fruiting stages

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Abstract – The objective of this work was to determine reference values for evaluating the nutritional status of 'Pêra' orange (*Citrus sinensis*) trees in different fruiting stages, using the compositional nutrient diagnosis (CND) method. The study used 243 leaf samples from 81 plots, with samplings performed in plants bearing fruits with three and six months of age, and also in the main harvest (nine months). Plots with productivity higher than 30 Mg ha⁻¹ were selected for the establishment of reference standards. Three normal ranges were evaluated for each nutrient in the reference population, comprehended by the confidence interval (CI) of the foliar content averages, by the mean±standard deviation (±SD), and by the mean± $\frac{2}{3}$ SD. Regardless of the normal range of nutrients used, the leaf sampling periods interfered with the nutritional status of 'Pêra' orange tree. The normal ranges obtained from the criteria CI and ± $\frac{2}{3}$ SD of the nutrient contents observed in the reference population frequently provided similar nutritional diagnoses. Zn, Ca, and Fe are the elements that most often limit the production of 'Pêra' orange in the state of Amazonas, Brazil.

Index terms: *Citrus limonia*, *Citrus sinensis*, DRIS, leaf sampling, nutritional balance index, reference population.

Faixas normais de nutrientes e monitoramento nutricional de laranjeiras 'Pêra' com uso do método CND em diferentes estádios de frutificação

Resumo – O objetivo deste trabalho foi determinar valores de referência para avaliação do estado nutricional de laranjeiras 'Pêra' (*Citrus sinensis*) em diferentes estádios de frutificação, com uso do método de diagnose da composição nutricional (CND). Foram utilizadas 243 amostras foliares, provenientes de 81 glebas, com coletas em plantas com frutos de três e seis meses de idade e, também, na colheita principal (nove meses). Glebas com produtividade superior a 30 Mg ha⁻¹ foram selecionadas para o estabelecimento dos padrões de referência. Foram avaliadas três faixas normais para cada nutriente, na população de referência, abrangidas entre o intervalo de confiança (IC) das médias dos teores foliares, a média±desvio-padrão (±DP) e a média± $\frac{2}{3}$ DP. Independentemente da faixa normal de nutrientes utilizada, a época de amostragem foliar interferiu no estado nutricional da laranjeira 'Pêra'. As faixas normais obtidas a partir dos critérios IC e ± $\frac{2}{3}$ DP dos teores nutricionais observados na população de referência proporcionaram diagnósticos nutricionais frequentemente semelhantes. Zn, Ca e Fe são os elementos que mais frequentemente limitam a produção da laranja 'Pêra' no Amazonas.

Termos para indexação: *Citrus limonia*, *Citrus sinensis*, DRIS, amostragem foliar, índice de balanço nutricional, população de referência.

Introduction

The citrus sector of Brazil contributes substantially to the economy of the country and is the largest exporter of concentrated orange juice in the world (Perez & Santos, 2014). The state of São Paulo is the greatest orange producer in the country, with a harvested area

of 46,043 ha, while the state of Amazonas ranks 11th, with a harvested area of 2,651 ha (IBGE, 2014).

In Amazonas, orange production is concentrated in the municipality of Rio Preto da Eva, which accounts for more than 70% of the state production. The orange variety 'Pêra' [*Citrus sinensis* (L.) Osbeck] is the most commonly cultivated, due to its easy adaptation to

the regional soil-climatic conditions (IBGE, 2014). However, information on the nutritional status of fruit trees in the region is scarce, and nutritional standards established in other states are still commonly used (Fernandes et al., 2010). However, this procedure can affect the efficiency of nutritional assessment due to variations, mainly, in non-nutritional factors (Jarrel & Beverly, 1981).

The sufficiency range (SR) and critical level (CL) are the most widely used methods for interpretation of leaf analysis. These reference values are traditionally obtained in calibration tests in which the nutrient rates are varied and all the other production factors are maintained constant (Kurihara et al., 2005). However, the long time and high costs required to establish the standard values are the main drawbacks of these methods, besides the limitations arising from the requirement that the cultivation conditions of the plants to be diagnosed must be similar to those of the plants used to obtain the calibration curve (Fageria et al., 2009).

Alternatively, the use of SR derived from the diagnosis and recommendation integrated system (DRIS) applied directly to commercial plantations has been proposed in the literature, requiring no experimentation, with a significant reduction in cost and time demand necessary to establish the standards. This possibility has stimulated more and more research on fruit trees such as: banana tree (Teixeira et al., 2007), coconut tree (Santos et al., 2004), guava tree (Souza et al., 2013), mango tree (Politi et al., 2013), and mandarin (Srivastava & Singh, 2008). For orange trees, SRs have been proposed for the state of São Paulo (Camacho et al., 2012) and Central Amazon (Dias et al., 2013).

The application of orange tree fertilization in Brazil is best done at the beginning of fruiting (Mourão Filho, 2005; Santana et al., 2007; Fernandes et al., 2010). However, the crop leaf sampling period proposed in the literature prevents the nutritional diagnosis from being timely made for use in fertilization recommendation in the same year, since leaf sampling is only recommended when the fruits are six months old (Quaggio et al., 2005). This limitation has made nutritional diagnosis little widespread as a tool for the management of orange tree fertilization (Mourão Filho, 2005; Camacho et al., 2012; Dias et al., 2013).

The DRIS method varies according to the nutritional relationships used, which can be bi- or multivariate.

As an approach to a distinct diagnostic system, Parent & Dafir (1992) proposed DRIS based on multivariate relationships, called compositional nutrient diagnosis (CND). The CND corresponds to the natural logarithm of the relationship between the evaluated nutrient and the geometric mean of the nutritional composition in the leaf sample (Parent, 2011). Therefore, unlike the original DRIS method (Beaufils, 1973), CND does not consider the relationship between the contents of two discretionary nutrients.

The establishment of normal nutrient ranges for different fruiting stages of orange tree is essential so that the foliar analysis can be used as a decision tool in the management of the crop's main fertilization.

The objective of this work was to determine reference values for evaluating the nutritional status of 'Pêra' orange trees in different fruiting stages, using the compositional nutrient diagnosis (CND) method.

Materials and Methods

The study was carried out in the municipality of Rio Preto da Eva (02°41'56"S, 59°42'00"W), in the state of Amazonas, Brazil, using 243 leaf samples of 'Pêra' orange tree [*Citrus sinensis* (L.) Osbeck]. The trees were grafted on 'Cravo' lemon (*Citrus limonia* Osbeck) tree and sampled in the 2011/2012 growing season, in three fruiting stages. The samples were collected from 27 representative commercial orchards of this orange-producing region.

Each orchard was divided into three plots, representing the different micro soil-climatic conditions within the orchard, in order to maintain visual uniformity within the areas, with four orange trees per plot, to ensure representativeness. Therefore, 81 plots were monitored. The evaluated orchards were between 5 and 15 years old, producing yields between 10.9 and 55.8 Mg ha⁻¹. The orchards were planted at a spacing of 7x4 m, with a total of 357 plants per hectare, and at 8.5x5 m, with 236 plants per hectare. The evaluated orchards were cultivated in a dry system, and the main harvest occurred in June, while untimely fruits were harvested in September and October.

The climate of the region is Af type, according to Köppen's classification, with tropical humid conditions and annual means of 26°C and 2,550-mm precipitation. The rainy season lasts from December to April, with

rainfalls concentrated in the first quarter of the year, and the warmest period lasts from August to October. Latossolos Amarelos distróficos (Xanthic Oxisols) are predominant in the region (IBGE, 2010).

Sampling periods for nutritional diagnosis were selected to identify the ideal fruiting stage to evaluate possible nutritional imbalances. In the first period, leaf sampling was carried out when the plants showed three-month-old fruits (marble-sized fruit, corresponding to stage 6), in December 2011. In the second period, sampling was performed in March 2012, when the fruits were six months old (final size fruit, green, stage 8), according to Quaggio et al. (2005). In the third sampling period, the leaves were taken at the time of the main harvest (green to yellow fruit, stage 9), in June 2012. Each sample was composed of 100 freshly ripened leaves taken from four trees (25 leaves per plant), selected at random within the field. The leaves were removed from the third node from the apex of fruiting branches.

The collection was rigorously standardized, since the leaf patterns differ according to the type of the leaf samples (Kurihara et al., 2013). The samplings were performed on the sides of the trees facing the four cardinal points, and always at medium plant height, as recommended by Quaggio et al. (2005). The collected plant material was packed in paper bags and transported to the laboratory. For the analyses, the samples were washed in running water, dried in a forced air circulation oven at 60°C, ground in a Willey mill, and the total nutrient contents were determined.

To analyze the P, K, Ca, Mg, B, Cu, Fe, Mn, and Zn contents, the samples were solubilized in

nitric-perchloric solution (65% nitric acid and 70% perchloric acid), while nitrogen was subjected to sulfuric solubilization and determined by the semi-micro Kjeldahl method (Carmo et al., 2000).

In 51 of the 81 plots monitored, the fruit yields exceeded 30 Mg ha⁻¹ in the main harvest (June 2012). These 51 plots were considered as the reference population to determine the nutritional standards. The other 30 plots had low yields and were used to perform the nutritional diagnoses. The yield of 30 Mg ha⁻¹ was used as a cutoff criterion for being more than 50% higher than the mean yield of orange trees in the state of Amazonas, in 2012 (IBGE, 2014).

In the plots of the reference population, the following mean values were found in the soil chemical analyses (0.0–0.20 m layer): pH in CaCl₂ 5.7; P (Mehlich-1) 5.6 mg dm⁻³; Ca, Mg, K, and potential acidity of 2.3, 1.2, 0.2, and 2.9 cmolc dm⁻³, respectively; oxidizable organic carbon of 3.0 dag kg⁻¹; and clay content of 59.3 dag kg⁻¹. In the low-yield plots (≤30 Mg ha⁻¹), the results of the soil analysis were: pH in CaCl₂ of 5.4; P of 1.2 mg dm⁻³; Ca, Mg, K, and potential acidity of 1.7, 1.1, 0.2, and 4.5 cmolc dm⁻³, respectively; oxidizable organic carbon of 3.2 dag kg⁻¹; and clay content of 49.2 g kg⁻¹. The analyses were performed as proposed by Donagema et al. (2011).

Once the reference population was defined, the CND method (Parent, 2011) was used to identify the leaf nutrient concentrations in the samples that were sufficiently high, for each fruiting stage. To this end, the leaf concentration of nutrients in all plots, regardless of the sampling time, was adjusted to a same measure unit (dag kg⁻¹).

Table 1. Mean and standard deviation from the nutritional standards used for the compositional nutrient diagnosis of 'Pêra' orange (*Citrus sinensis*) trees in different fruiting stages.

Norm	zN	zP	zK	zCa	zMg	zB	zCu	zFe	zMn	zZn	zMS
3-month-old fruits											
Mean	3.47	0.44	2.51	3.22	1.42	-2.64	-4.67	-2.58	-4.31	-3.95	7.09
Standard deviation	0.20	0.22	0.31	0.34	0.43	0.23	1.07	0.26	0.53	0.46	0.18
6-month-old fruits											
Mean	3.44	0.44	2.10	3.27	1.36	-2.55	-4.64	-2.53	-4.09	-3.83	7.04
Standard deviation	0.21	0.24	0.28	0.31	0.43	0.23	1.00	0.21	0.34	0.44	0.24
Main harvest											
Mean	3.32	0.53	2.31	3.38	1.38	-2.47	-4.83	-2.52	-4.19	-3.98	7.07
Standard deviation	0.21	0.19	0.24	0.25	0.30	0.28	0.66	0.27	0.36	0.33	0.19

The supplementary nutrient value for the total leaf biomass (R value) was calculated according to the expression: $R = 100 - (vN + vP + vK + vCa + vMg + vB + vCu + vFe + vMn + vZn)$, in which R is the complementary value for 100 dag kg⁻¹ dry matter, in relation to the sum of nutrient contents v_i ($i = N, \dots, Zn$), in dag kg⁻¹. From the geometric mean (mGeo) calculated for the values of each sample, the multinutrient variable (zX) was computed by the expression: $zX = \ln(vX/mGeo)$, where zX represents the value of the multivariate relation of each one of the evaluated nutrients (vX). With the zX values of each reference plot, the descriptive parameters – arithmetic mean (mX) and standard deviation (sX) – and the CND standards were calculated for each fruiting stage (Table 1).

From the standards, the CND indices were calculated by the log-centered multivariate correlation (Parent, 2011), for each of the evaluated nutrients: $I_X = (zX - mX)/sX$; in which I_X represents the CND index; mX is the mean norm; and sX is the standard deviation norm.

For the interpretation of the CND indices, the DRIS dry matter method (M-DRIS) was used (Hallmark et al., 1987), and each nutrient was classified into two categories – deficiency and sufficiency – based on the comparison of the value of the CND index of each nutrient (I_n) with the CND dry matter index (DMI), where deficiency occurs when $I_n < 0$ and $I_n < DMI$; and sufficiency when $I_n > 0$ or $I_n > DMI$.

The normal range for each nutrient, irrespective of the fruiting stage, was estimated from the mean nutritional contents in the monitored plots with nutritional sufficiency, for each element analyzed, according to the thresholds: limits of the confidence interval (CI); of the standard deviation ($\pm SD$); and of $\pm \frac{2}{3}SD$. The CI was computed as: $CI = mX \pm t\alpha \cdot s_{mX}$; where: mX and s_{mX} represent the mean content and standard deviation for the nutrients evaluated in the nutritionally balanced plots, and $t\alpha$ is the value of bilateral t, at 1% probability, with n-1 degrees of freedom, where n refers to the total number of data.

The SD was obtained by the expression: $SD = [\sum(yX - mX)^2]^{0.5}/n$; where: y and m represent the content of each sample and mean value, respectively; and n, the total number of samples.

The amplitude of the CI, SD and $\frac{2}{3}SD$, expressed in grams per kilogram (g kg⁻¹) for macronutrients, and in

milligrams per kilogram (mg kg⁻¹) for micronutrients, were considered as the normal range. Values below and above the lower and upper limits of CI, SD and $\frac{2}{3}SD$ were considered as deficient and excessive nutritional contents (luxury consumption or toxicity), respectively.

In the low-yield plots, the nutritional diagnoses, carried out in the three fruiting stages, were compared with each other, and the concordance cases were computed for each leaf and nutrient sample. Therefore, concordant diagnoses were the cases in which the normal ranges – obtained with the different criteria at each fruiting stage – provided the same nutritional diagnosis of the samples (deficiency, balance and excess). The obtained results were expressed in percentage of agreement.

The norms, the CND indices, the M-DRIS, and the chi-square test ($p \leq 0.01$) were calculated in a spreadsheet. The descriptive statistics and Tukey's test, at 5% probability, were processed with the statistical software Assisat, version 6.2 (Silva & Azevedo, 2002).

Results and Discussion

Of the 81 commercial 'Pêra' orange plots monitored in the different fruiting stages, nutritional sufficiency was confirmed in the plots 45, 41, 50, 38, 49, 38, 38, 43, 40, and 32 for N, P, K, Ca, Mg, B, Cu, Fe, Mn and Zn, respectively, in evaluations when the trees had three-month-old fruits. Similar results were found for the plots 60, 44, 59, 35, 50, 34, 33, 38, 31, and 27, when the fruits were six months old, and for the plots 51, 45, 53, 36, 58, 42, 37, 41, 33, and 28 in the main harvest. Regardless of the fruiting stage evaluated, the N, P, K, and Mg contents were found to have an adequate nutritional sufficiency in most of the monitored plots. However, in more than 60% of the plots, Zn was the most yield-limiting element (Table 2).

The distributions of the frequencies at which the plots were classified as nutrient-deficient and sufficient were similar for all nutrients, regardless of the fruiting stage (Table 2). This similarity in distribution can be explained by the low variations in the nutritional status between plots during fruiting.

From the CND results, the normal nutrient concentration was estimated based on the assumption that plants with higher nutritional CND indices than dry matter CND index were not affected by any yield-limiting nutritional restriction (Hallmark et al., 1987).

Table 2. Frequency at which a state of nutritional sufficiency and insufficiency was diagnosed in the 81 commercial 'Pêra' orange (*Citrus sinensis*) orchards, in the fruiting stages, evaluated by the method of compositional nutrient diagnosis⁽¹⁾.

Class	3-month-old fruits	6-month-old fruits	Main harvest	Expected frequency (%)
Nitrogen				
Sufficient	56	74	63	64
Insufficient	44	26	37	36
Chi-square	3.25	4.24	0.07	-
Phosphorus				
Sufficient	51	54	56	53
Insufficient	49	46	44	47
Chi-square	0.33	0.03	0.17	-
Potassium				
Sufficient	62	73	65	67
Insufficient	38	27	35	33
Chi-square	1.10	1.71	0.07	-
Calcium				
Sufficient	47	43	44	45
Insufficient	53	57	56	55
Chi-square	0.17	0.11	0.01	-
Magnesium				
Sufficient	60	62	72	65
Insufficient	40	38	28	35
Chi-square	0.74	0.36	2.14	-
Boron				
Sufficient	47	42	52	47
Insufficient	53	58	48	53
Chi-square	0.00	0.98	0.98	-
Copper				
Sufficient	47	41	46	44
Insufficient	53	59	54	56
Chi-square	0.25	0.56	0.06	-
Iron				
Sufficient	53	47	51	50
Insufficient	47	53	49	50
Chi-square	0.33	0.43	0.01	-
Manganese				
Sufficient	49	38	41	43
Insufficient	51	62	59	57
Chi-square	1.77	0.84	0.17	-
Zinc				
Sufficient	39	33	35	36
Insufficient	61	67	65	64
Chi-square	0.60	0.27	0.07	-

⁽¹⁾None of the chi-square values was considered significant at 5% probability.

Thus, the nutritional content in leaf tissues of the plots with sufficiency for each of the nutrients was used to establish the optimum concentration in the different fruiting stages of 'Pêra' orange tree.

In the 81 plots, the mean levels of P, Mg, B, Fe, and Zn were considered adequate; those of K, Ca and Mn were low; and those of N and Cu were excessive for orange trees with 6-month-old fruits (Quaggio et al., 2005) (Table 3). However, in contrast to the normal nutrient range proposed by Camacho et al. (2012), with the use of CND in 'Pêra' orange trees cultivated in municipality of Bebedouro, state of São Paulo, Brazil, the mean K and Fe levels were considered adequate; those of Ca, Mg and Mn were low; and those of N, P, B, Cu, and Zn were excessive, also for plants with 6-month-old fruits.

For the elements evaluated in the stage of fruit establishment (after three months), the leaf concentrations of N, K and Cu were higher than those after six months, while those of Mg and Zn remained stable, and those of P, Ca, B, Fe, and Mn were lower. In 'Hamlin' orange trees, Mattos Junior et al. (2003) also observed a decrease in the N and K leaf concentrations as the fruiting stages advanced. These results can be explained by the fact that N and K are, in this order, the elements removed at highest levels in orange fruits (Boaretto et al., 2007). Thus, since the reproductive organs are preferential drains, these elements tend to

Table 3. Leaf contents in 'Pêra' orange (*Citrus sinensis*) trees in orchards with nutrient sufficiency (reference population), in samples taken at different fruiting stages⁽¹⁾.

Nutrients	Sampling time			Coefficient of variation (%)
	3-month-old fruits	6-month-old fruits	Main harvest	
N (g kg ⁻¹)	31.27a	28.46b	27.66b	10.3
P (g kg ⁻¹)	1.35b	1.38b	1.47a	7.7
K (g kg ⁻¹)	12.53a	8.48c	10.13b	18.5
Ca (g kg ⁻¹)	20.77c	23.23b	24.71a	10.3
Mg (g kg ⁻¹)	3.73a	4.03a	3.73a	16.8
B (mg kg ⁻¹)	64.02b	78.63a	84.85a	16.5
Cu (mg kg ⁻¹)	33.74a	43.58a	19.50b	64.8
Fe (mg kg ⁻¹)	70.85b	77.19ab	80.98a	18.9
Mn (mg kg ⁻¹)	19.45b	26.31a	26.34a	43.2
Zn (mg kg ⁻¹)	29.46b	46.51a	37.70ab	52.5

⁽¹⁾Means followed by equal letters, in the columns, do not differ by Tukey's test, at 5% probability.

be transferred from the leaf to the fruits. In the case of Ca, due to its restricted mobility in the phloem, accumulation of the nutrient occurs in the leaf tissue throughout plant development.

Since the value of the standard deviation (SD) defines the amplitude of the normal nutrient range, it is expected that the greater the dispersion of nutritional contents in the set of plants of the reference population, the lower the sensitivity of the method to detect nutritional imbalances (Table 4). The amplitude of the CI corresponds to an estimated range, in which the probability that the leaf concentrations of the reference population are included in this range is 99% ($p \leq 0.01$) (Coutinho & Cunha, 2005). Therefore, the normal nutrient range estimated from the CI usually provides a small amplitude, and is very rigorous in the nutritional diagnosis, as already pointed out for coffee trees (Farnezi et al., 2010) and orange trees (Dias et al., 2013).

In contrast, optimum nutrient contents corresponding to $\pm SD$ and $\pm \frac{2}{3}SD$ of the nutritional indices of the nutritionally balanced population, using DRIS and CND, have already been estimated for mango trees (Politi et al., 2013), cotton plants (Serra et al., 2010; Kurihara et al., 2013), coffee trees (Partelli et al., 2007), and guava plants (Souza et al., 2013), with higher amplitudes than those estimated by CI, but lower amplitudes than the sufficiency ranges (SR) proposed in traditional calibration tests (Quaggio et al., 2005).

In the nutritional diagnosis, the use of small-amplitude SR increases the capacity of the method to identify plants in situations of nutritional imbalance, compared to the use of large-amplitude SR (Partelli et al., 2007). On the other hand, possible minor nutritional variations in the leaf tissue, due to non-nutritional factors, may be interpreted inappropriately (e.g., as nutritional deficiency or excess) when very narrow ranges are used (Jarrel & Beverly, 1981).

Regardless of the criteria used to establish the normal nutrient ranges in this study, the ranges obtained for N, P, B, Cu, and Zn exceeded the threshold established by Camacho et al. (2012) for 6-month-old fruits in São Paulo orange orchards; while those of Ca and Mn were below the threshold, and that of Fe was contained in the range proposed by the authors. However, the critical level established in 'Pêra' orange orchards in the same region of Central Amazônia, with low technological

Table 4. Normal nutrient ranges in 'Pêra' orange (*Citrus sinensis*) leaves, in three fruiting stages, calculated by the compositional nutrient diagnosis method based on different criteria.

Criterion ⁽¹⁾	3-month-old fruits	6-month-old fruits	Main harvest
Nitrogen (g kg ⁻¹)			
Standard deviation	28–35	26–31	25–30
$\frac{2}{3}$ of the standard deviation	29–34	27–30	26–29
Confidence interval	30–33	28–29	27–29
Phosphorus (g kg ⁻¹)			
Standard deviation	1.2–1.5	1.3–1.5	1.4–1.5
$\frac{2}{3}$ of the standard deviation	1.3–1.4	1.3–1.4	1.4–1.5
Confidence interval	1.3–1.4	1.3–1.4	1.4–1.5
Potassium (g kg ⁻¹)			
Standard deviation	10–15	7–10	9–12
$\frac{2}{3}$ of the standard deviation	11–14	8–9	9–11
Confidence interval	12–13	8–9	10–11
Calcium (g kg ⁻¹)			
Standard deviation	18–24	22–25	23–26
$\frac{2}{3}$ of the standard deviation	19–23	22–24	24–25
Confidence interval	19–22	22–24	24–25
Magnesium (g kg ⁻¹)			
Standard deviation	2.8–4.7	1.1–7.0	3.1–4.4
$\frac{2}{3}$ of the standard deviation	3.1–4.4	2.1–6.0	3.3–4.2
Confidence interval	3.4–4.1	3.0–5.0	3.5–3.9
Boron (mg kg ⁻¹)			
Standard deviation	57–71	67–92	68–97
$\frac{2}{3}$ of the standard deviation	59–69	71–88	73–93
Confidence interval	61–67	74–85	77–89
Copper (mg kg ⁻¹)			
Standard deviation	15–53	16–71	7–32
$\frac{2}{3}$ of the standard deviation	21–47	25–62	11–28
Confidence interval	26–42	31–56	14–25
Iron (mg kg ⁻¹)			
Standard deviation	56–86	68–86	64–98
$\frac{2}{3}$ of the standard deviation	61–81	71–83	70–92
Confidence interval	65–77	73–81	74–88
Manganese (mg kg ⁻¹)			
Standard deviation	10–29	19–33	13–39
$\frac{2}{3}$ of the standard deviation	13–26	22–31	18–35
Confidence interval	15–23	23–30	20–32
Zinc (mg kg ⁻¹)			
Standard deviation	14–45	27–66	14–62
$\frac{2}{3}$ of the standard deviation	19–40	34–59	22–54
Confidence interval	23–36	37–56	26–49

⁽¹⁾Criteria underlying the normal range: CI, confidence interval of nutritional contents in leaves of plants of the reference population; SD, range between \pm the standard deviation of the nutritional contents in the reference population; and $\pm \frac{2}{3}SD$, range between $\pm \frac{2}{3}$ of the standard deviation of the nutritional contents in the reference population.

level and fruits of the same age (Dias et al., 2013), was higher than the normal ranges established here for P, Ca, and Fe; below those for K, B, Cu, and Zn; and within the ranges for N, Mg and Mn, in most cases.

The distribution frequencies of the nutritional classes of deficiency, balance and excess, observed in low-yield plots, were close to the expected frequencies for most of the nutrients, and random in the case of N using the normal range based on the criteria CI and \pm SD, and in the case of K using the \pm SD criterion (Table 5).

All normal ranges indicated Zn, Ca and Fe as the elements that most frequently limit yields of 'Pêra' orange in the Amazonas region, and in more than 65% of the monitored plots, at least one of these nutrients was deficient. Nonetheless, Mg most often fitted within the established normal nutrient ranges, in more than 92% of the diagnosed plots (Table 5).

The degree of agreement in the diagnoses based on normal ranges and the criteria CI and \pm $\frac{2}{3}$ SD in the different fruiting stages was higher than 79% for most nutrients and reached 100% for P and Ca (Table 6). However, the degree of agreement at different sampling times, regardless of the criterion adopted to establish the normal range, was less than 65% for most nutrients.

The variation in leaf nutrient contents according to the fruiting stage may be associated with several factors

Table 5. Frequency at which nutritional deficiency, balance and nutritional excess were detected in 30 plots with low yields, according to the Compositional nutrient diagnosis method using different criteria to establish the normal range, in 'Pêra' orange (*Citrus sinensis*) trees evaluated in three-month-old fruits.

Criterion ⁽¹⁾	Deficiency	Equilibrium	Excess	Chi-square
Nitrogen				
Standard deviation	4	24	2	**
$\frac{2}{3}$ of the SD	7	17	6	ns
Confidence interval	10	4	16	**
Expected frequency	7.0	15.0	8.0	-
Phosphorus				
Standard deviation	12	16	2	ns
$\frac{2}{3}$ of the SD	12	8	10	ns
Confidence interval	12	8	10	ns
Expected frequency	12.0	10.7	7.3	-

Continuation...

Continuation...				
Potassium				
Standard deviation	4	21	5	**
$\frac{2}{3}$ of the SD	10	8	12	ns
Confidence interval	10	8	12	ns
Expected frequency	8.0	12.3	9.7	-
Calcium				
Standard deviation	22	7	1	ns
$\frac{2}{3}$ of the SD	23	4	3	ns
Confidence interval	23	4	3	ns
Expected frequency	22.7	5.0	2.3	-
Magnesium				
Standard deviation	1	28	1	ns
$\frac{2}{3}$ of the SD	1	28	1	ns
Confidence interval	8	21	1	ns
Expected frequency	3.3	25.3	1.0	-
Boron				
Standard deviation	20	4	6	ns
$\frac{2}{3}$ of the SD	21	1	8	ns
Confidence interval	21	1	8	ns
Expected frequency	20.7	2.0	7.3	-
Copper				
Standard deviation	18	9	3	ns
$\frac{2}{3}$ of the SD	22	5	3	ns
Confidence interval	24	3	3	ns
Expected frequency	21.3	5.7	3.0	-
Iron				
Standard deviation	20	9	1	ns
$\frac{2}{3}$ of the SD	22	7	1	ns
Confidence interval	23	6	1	ns
Expected frequency	21.7	7.3	1.0	-
Manganese				
Standard deviation	21	7	2	ns
$\frac{2}{3}$ of the SD	21	5	4	ns
Confidence interval	22	3	5	ns
Expected frequency	21.3	5.0	3.7	-
Zinc				
Standard deviation	21	7	2	ns
$\frac{2}{3}$ of the SD	24	1	5	ns
Confidence interval	24	1	5	ns
Expected frequency	23.0	3.0	4.0	-

⁽¹⁾Criteria underlying the normal range: CI, confidence interval of nutritional contents in leaves of plants of the reference population; SD, range between \pm the standard deviation of the nutritional contents in the reference population; and $\pm\frac{2}{3}$ SD, range between $\pm\frac{2}{3}$ of the standard deviation of the nutritional contents in the reference population. nsNonsignificant. **Significant by the chi-square test, at 1% probability.

Table 6. Degree of agreement (%) between diagnoses based on normal ranges obtained from different criteria, of 'Pêra' orange (*Citrus sinensis*) trees sampled at different fruiting stages.

Diagnosis ⁽¹⁾	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
------(%)-----										
Three-month-old fruits (Frt3)										
SD vs. $\frac{2}{3}$ SD	83	77	73	83	77	90	50	83	87	93
SD vs. CI	63	77	47	83	47	77	57	63	77	90
$\frac{2}{3}$ SD vs. CI	80	100	73	100	70	87	80	80	90	97
Six-month-old fruits (Frt6)										
SD vs. $\frac{2}{3}$ SD	77	73	57	90	100	90	87	93	93	80
SD vs. CI	33	73	57	90	70	90	80	90	87	80
$\frac{2}{3}$ SD vs. CI	57	100	100	100	70	100	93	97	93	100
Main harvest (MH)										
SD vs. $\frac{2}{3}$ SD	87	100	83	93	73	87	67	67	97	93
SD vs. CI	80	100	60	93	57	63	57	53	97	93
$\frac{2}{3}$ SD vs. CI	93	100	77	100	83	77	90	87	100	100
Standard deviation (SD)										
Frt3 vs. Frt6	37	43	60	67	80	47	60	33	77	77
Frt3 vs. MH	57	47	53	73	63	60	60	57	83	90
Frt6 vs. MH	57	57	63	80	57	60	57	27	83	87
$\frac{2}{3}$ of the standard deviation ($\frac{2}{3}$ SD)										
Frt3 vs. Frt6	37	43	40	67	57	50	50	40	80	73
Frt3 vs. MH	47	60	37	77	47	53	53	63	90	90
Frt6 vs. MH	43	57	47	77	30	63	73	40	87	77
Confidence interval (CI)										
Frt3 vs. Frt6	27	43	47	67	53	53	57	57	73	77
Frt3 vs. MH	50	60	30	77	60	67	57	60	80	93
Frt6 vs. MH	20	57	43	77	43	80	80	53	90	77

⁽¹⁾Criteria underlying the normal range: CI, confidence interval of the nutritional contents in leaves of plants of the reference population; SD, range between \pm standard deviation of nutritional contents in the reference population; and $\pm\frac{2}{3}$ SD, range between $\pm\frac{2}{3}$ of the standard deviation of the nutritional contents in the reference population.

related to plant physiology, e.g., nutritional demands resulting from fruiting and the development of vegetative and reproductive branches. In addition, this variation may also be related to meteorological variables, which interfere with plant development (Fageria et al., 2009).

Conclusions

1. The nutritional status of 'Pêra' orange (*Citrus sinensis*) trees cultivated in Amazonas varies according to the season of leaf sampling, regardless of the normal nutrient range underlying the diagnosis.

2. Normal nutrient ranges obtained from the criteria of the confidence interval and $\pm\frac{2}{3}$ of the standard

deviation of the nutritional levels observed in the reference population often provided similar nutritional diagnoses.

3. The Zn, Ca, and Fe are the elements that most often limit the yield in the production of 'Pêra' orange in the state of Amazonas, Brazil.

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