# Determination of leaf sampling techniques to assess the nutritional status of Barbados cherry (*Malpighia emarginata* D.C.)

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## Determination of leaf sampling techniques to assess the nutritional status of Barbados cherry (*Malpighia emarginata* D.C.).

**Abstract** — **Introduction**. Research on the mineral nutrition of Barbados cherry is scarce. Leaf sampling techniques for this crop are unknown. Leaf analysis, associated with the determination of the nutrient availability in the soil, can provide reliable orientation for the establishment of fertilizer programs. For a correct interpretation of the nutritional status, it is important (1) to evaluate the seasonal variation of nutrient contents in the leaves, (2) to determine the appropriate time for leaf sampling and analysis, and (3) to indicate the best part of the plant for determination of the nutritional status. These subjects were the three objectives of this study. **Materials and methods**. The experiment was conducted from March 1997 to February 1998 in Visconde do Rio Branco, Minas Gerais, Brazil. The dynamics of the elements N, P, K, Ca, Mg, S, Fe, Cu, Zn and Mn were studied in leaves at the apical, median and basal positions of branches located in the upper, median and lower portions of the plant canopy. **Results**. The minimum variation in the foliar contents occurred at the medium third of the branches located in the upper portion of the canopy. So, these parts are the most appropriate for leaf sampling. Moreover, December was characterized as the appropriate time for leaf sampling. **Conclusion**. The leaf sampling techniques developed will allow better study of the mineral nutrition of *M. emarginata* in order to establish reliable fertilizer programs and to improve the yields of this fruit tree.

**Brazil / *Malpighia emarginata* / plant nutrition / fertilizer application / leaves / tissue analysis / seasonal variation / nutritional status**

## Détermination de techniques d'échantillonnage de feuille pour évaluer l'état nutritionnel de la cerise des Antilles (*Malpighia emarginata* D.C.).

**Résumé** — **Introduction**. Les recherches sur la nutrition minérale de la cerise des Antilles sont rares. Les techniques d'échantillonnage de feuille pour cette production ne sont pas connues. L'analyse de feuille, associée à la détermination de la disponibilité en éléments minéraux du sol, peut constituer une aide fiable pour l'établissement de programmes de fertilisation. Pour une interprétation correcte de l'état alimentaire, il est important (1) d'évaluer la variation saisonnière de la teneur en éléments minéraux dans les feuilles, (2) de déterminer le meilleur moment pour prélever et analyser les feuilles, et (3) d'identifier le matériel végétal le plus apte à être échantillonné pour la réalisation de ces analyses minérales. Ces trois points ont été étudiés lors de ces travaux. **Matériel et méthodes**. Les expérimentations ont été poursuivies de mars 1997 à février 1998 à Visconde do Rio Branco, Minas Gerais, Brésil. La dynamique des éléments N, P, K, Ca, Mg, S, Fe, Cu, Zn et Mn a été étudiée dans des feuilles situées à l'extrémité, au milieu et à la base de branches insérées dans les parties supérieures, médianes et inférieures de la frondaison de plants de *M. emarginata*. **Résultats**. La teneur foliaire a été la plus stable pour les feuilles situées dans la partie médiane des branches localisées dans la partie supérieure de la frondaison. Ces feuilles sont donc les plus appropriées pour le prélèvement de feuille. Par ailleurs, décembre s'est révélé être le mois le plus approprié pour cet échantillonnage. **Conclusion**. Les techniques d'échantillonnage de feuille développées permettront de mieux étudier la nutrition minérale de *M. emarginata* afin d'établir des programmes fiables de fertilisation et donc d'améliorer les rendements de cet arbre fruitier.

**Brésil / *Malpighia emarginata* / nutrition des plantes / fertilisation / feuille / analyse de tissus / variation saisonnière / état nutritionnel**

## 1. Introduction

Barbados cherry (*Malpighia emarginata* D.C.) cropping is expanding in Brazil, the world's main producer, and in other countries too. There is great interest in this crop because of its high content of vitamin C which can reach 4 000 mg of ascorbic and dehydroascorbic acids in 100 g juice. The trees develop well in tropical and subtropical climates. The plant grows continuously if the temperature and water supply are favorable all the time. In areas with drought and cold periods (March to August in the present location), the growth apparently ceases until the temperature and precipitation rise (September to February), when the vegetative and reproductive growths increase [1]. The flowers appear after vegetative growth flushes. Flower buds, flowers, and green and mature fruits occur simultaneously in the same plant. The fruits are drupes which can be round, oval or conical; their weight varies from (3 to 16) g. The fructification of plants propagated by cutting starts about the fifth to twelfth month after planting [2].

Research on the mineral nutrition of Barbados cherry is scarce, mainly in relation to seasonal variation of the nutrients. Leaf sampling techniques for this crop are unknown.

The diagnosis of the nutritional status is based on a relationship between the nutrient contents in the leaves or in the sap of the vascular tissues, and the growth of the plants [3].

Leaf analysis, associated with the determination of the nutrient availability in the soil, can provide reliable orientation for the establishment of fertilizer programs [4]. The visual aspect and the plant development stage, the occurrence of plagues and diseases and the water availability are factors that should also be considered in evaluating the nutritional status [5–8].

For correct interpretation of the nutritional status, it is important to characterize the sampling time, due to the existence of seasonal variation in the tissue nutrient contents [9].

According to Maier *et al.* [10], when sampling the tissues of the perennial species, three factors should be considered. First, the tissue indicated for sampling should be easily identified to minimize sampling errors. Second, it is important to observe the physiological age of the tissue to be sampled since it can influence the tissue chemical composition. Last, as a function of the existence of seasonal variation in the composition of the nutrients, the determination of a sampling date is recommended when this variation is minimum.

The objectives of this study were: (1) to evaluate the seasonal variation of the contents N, P, K, Ca, Mg, S, Fe, Cu, Zn and Mn in the leaves at three positions in the branches and at three heights in the canopy of the trees; (2) to determine the appropriate time for leaf sampling and analysis; and (3) to indicate the best part of the plant for determination of the nutritional status.

## 2. Materials and methods

The experiment was carried out, during the period from March 1997 to February 1998, at an experimental area belonging to the Universidade Federal de Viçosa, located in Visconde do Rio Branco county, Minas Gerais, Brazil, with geographical coordinates of 21° 07' S, 43° 57' W and 349 m altitude.

According to the classification of Köppen, the local climate corresponds to the Cwa category, characterized as a subtropical climate with the average temperature of the coldest month inferior to 18 °C and that of the hottest months higher than 22 °C, with the dry period in winter. The annual precipitation ranges from (1 100 to 1 700) mm.

The soil where the experiment was conducted is classified as a "distrophic A yellow red latosol", moderate, with specific chemical characteristics (*table I*).

Seventy-seven Barbados cherry plants spaced at 3 m × 2 m were used. The plants were propagated by cuttings and were 2 years old. They were bearing trees. The plants were fertilized every 60 days starting from March 1997. The fertilizers were applied in shallow furrows, opened as a half moon at the canopy projection, in the most elevated part of the land. The first three fertilizer amounts were 30 g per plant ammonium sulfate and 80 g per plant potassium chloride. The last three doses each constituted 170 g per plant ammonium sulfate, 200 g per plant simple superphosphate and 90 g per plant potassium chloride. The increment in the doses was necessary because the intense vegetative and reproductive growth occurred in spring and summer (September to February), due to the higher rainfall, higher temperatures and longer days. The fertilization was done immediately after sampling the leaves to avoid collecting leaves with elevated nutrient contents.

The leaf samples were collected fortnightly for 12 months, beginning from March, and taken from the apical, medium and basal thirds of the branches located in the upper, medium and lower portions of the canopy in all plants of each block. The sampled leaves were identified, conditioned in paper bags, washed with deionized water, according to Jones [11] and Moranghan [12], and oven-dried with air-forced circulation at 70 °C until reaching a constant weight. Subsequently, the samples were crushed in a stainless steel Wiley-type mill and conditioned in appropriate paper bags.

The organic nitrogen content was determined, after a sulfuric digestion, by the colorimetric method of Nessler [13] and the nitrate was extracted in a water-bath at 45 °C for 1 h and its content was determined by colorimetry [14]. The P, K, Ca, Mg, S, Fe, Cu, Zn and Mn contents were determined after nitroperchloric digestion [15], using the vitamin C method modified by Braga and Defelipo [16] for P, photometry for K, atomic absorption spectrophotometry for Ca, Mg, Fe, Cu, Zn and Mn [17], and sulfate turbidimetry for S [13].

The experiment was set up on a randomized block design with three replicates and 25 plants in each replicate, on a split-plot scheme. Three effects were considered in the plot which was characterized by the division of the aerial part into upper, medium and lower thirds. Three parts of the branches (apical, medium and basal thirds) were studied in the subplot. The subsubplot was characterized by the months of the year.

**Table I.**
Chemical characteristics of the soil in the experimental area (Visconde do Rio Branco county, MG, Brazil) analyzed in order to determine leaf sampling techniques to assess the nutritional status of Barbados cherry.

| Chemical characteristic | Analytical result |
|---|---|
| pH | 5.20 |
| P[1] ($mg \times dm^{-3}$) | 10.20 |
| K ($mg \times dm^{-3}$) | 30.0 |
| Al[2] ($cmol_c \times dm^{-3}$) | 0.00 |
| Ca[2] ($cmol_c \times dm^{-3}$) | 1.60 |
| Mg[2] ($cmol_c \times dm^{-3}$) | 0.70 |
| H + Al[3] ($cmol_c \times dm^{-3}$) | 1.80 |
| Base sum ($cmol_c \times dm^{-3}$) | 2.35 |
| Effective CEC ($cmol_c \times dm^{-3}$) | 2.35 |
| Total CEC ($cmol_c \times dm^{-3}$) | 4.15 |
| Base saturation (%) | 56.70 |
| Aluminum saturation (%) | 0.00 |

[1] Extractor Mehlich 1.
[2] Extractor KCl 1 mol × $L^{-1}$, pH 7.0.
[3] Extractor $Ca(OAc)_2$ 0.5 mol × $L^{-1}$, pH 7.0.

In order to study the variations of the nutrient contents in each third of the branch and in each portion of the plant's aerial part, the following statistical analyses were accomplished during the evaluation period:

(a) Variance and regression analyses, involving the treatment-portions of the aerial part, portions of the branch and months, which were located in the plot, subplot and subsubplot, respectively.

(b) Calculation of the variation index: for indication of the plant part where the lowest variations occurred in nutrient contents, a variation index of the mean square values referring to the sources of the month variation within each portion of the aerial part and the month variation within each third of the branch was determined for each nutrient. This variation index for each nutrient was calculated by the expression: $VI = MQ_1 / MQ_2$, where *VI* is the variation index, $MQ_1$, the mean square for each source of variation and $MQ_2$, the least mean square among the three portions of the aerial part or among the three portions of the branches.

So, the variation index of the least value of the mean square for each nutrient assumes a unitary value. Finally, an average variation index for each part of the plant was obtained by considering all nutrients, and so allowing the selection for leaf sampling of the one that presented the least variation mean index.

(c) Polynomial regression analysis: The models of polynomial regression were adjusted (lineal, quadratic and cubic) for each variable, by taking the data of each third of the branch and each portion of the aerial part.

The appropriate time for leaf sampling was determined by derivation of the regression equations found for each nutrient in the different studied parts of the plant. The data were divided into two subsets, one formed by data referring to the months from March to August, and another subset composed of data from September to February.

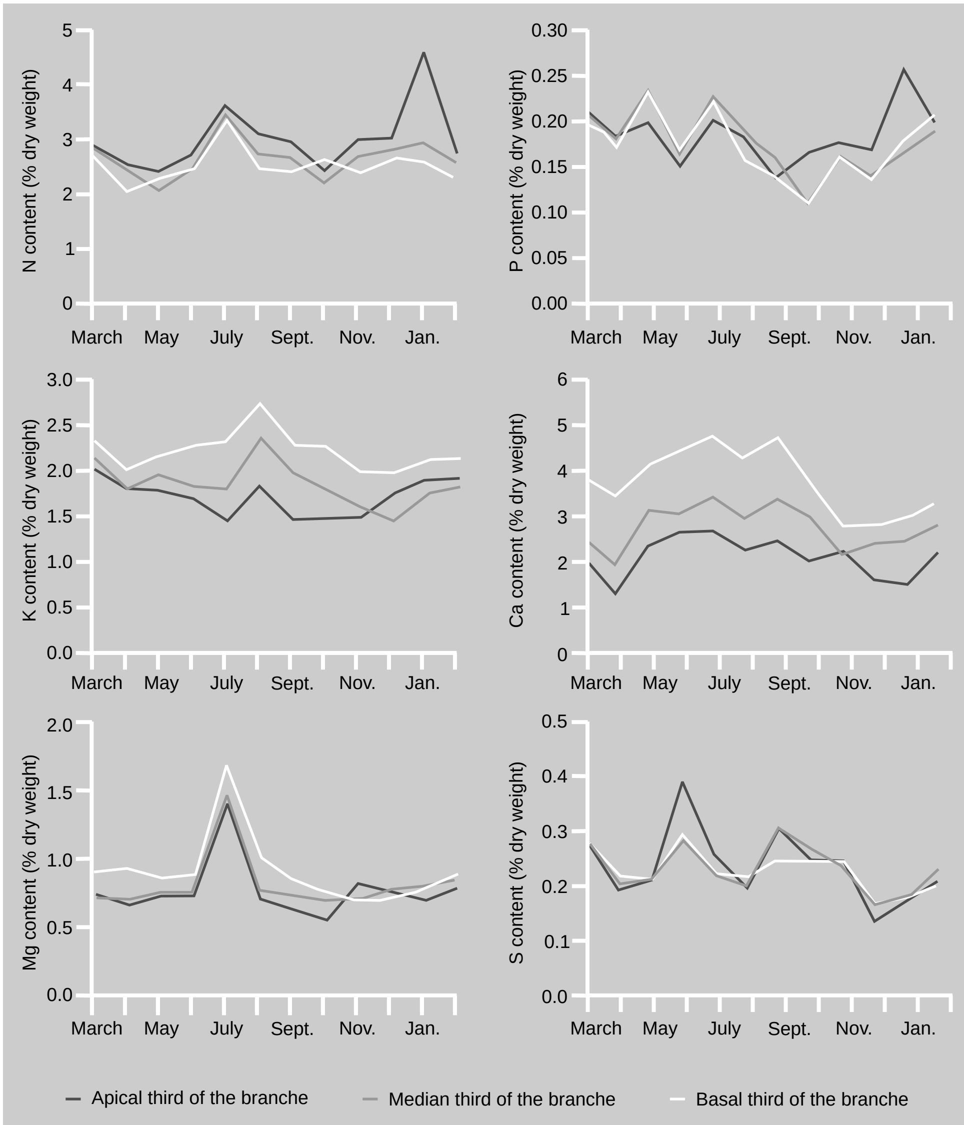


**Figure 1.**
Average monthly contents of the macronutrients N, P, K, Ca, Mg and S in the leaves of the apical, median and basal thirds of the branches of 2-year-old Barbados cherry, from March 1997 to February 1998, in Visconde do Rio Branco, MG, Brasil.

With this procedure, it was possible to study the variation in nutrient concentration over two different periods, that is, in the dry season (March to August) and the rainy season (September to February).

## 3. Results and discussion

The Barbados cherry produces flowers and fruits simultaneously in the same branch, and its fruits become completely developed about (3 to 4) weeks after anthesis. Thus, the competition for nutrients among plant tissues causes changes in the foliar composition mainly during flowering and fructification (September to February).

It was observed that the foliar contents of K, requested at high concentrations in fruits, declined from August to November, re-establishing its levels after a potassium fertilization performed at the end of the year (*figure 1*).

The foliar contents of N, P, and Mg declined with the arrival of the spring in September (*figure 1*). These elements possess high mobility in phloem [3], so those variations can be attributed to retranslocation of the nutrients from the mature to young leaves, flowers and fruits which are considered to be preferential drains.

The foliar concentrations of Ca and Fe increased from April until flowering in the middle of September (*figures 1, 2*). During fructification, the contents of these nutrients were reduced, probably due to the competition imposed by the source/drain relationship. Taking Ca as an example (*figure 1*), it is observed that starting from the development of the fruit, the leaves did not accumulate this nutrient so much. So, during the vegetative growth phase (March to August), the contents of these elements increased due to lower competition.

The average foliar contents of Mn were practically constant in each portion of the aerial part and each third of the branch from March to January, while the foliar contents of S, Cu and Zn did not follow a defined model (*figures 1, 2*).

The elements provided with low to intermediate mobility in phloem such as Fe, Cu,

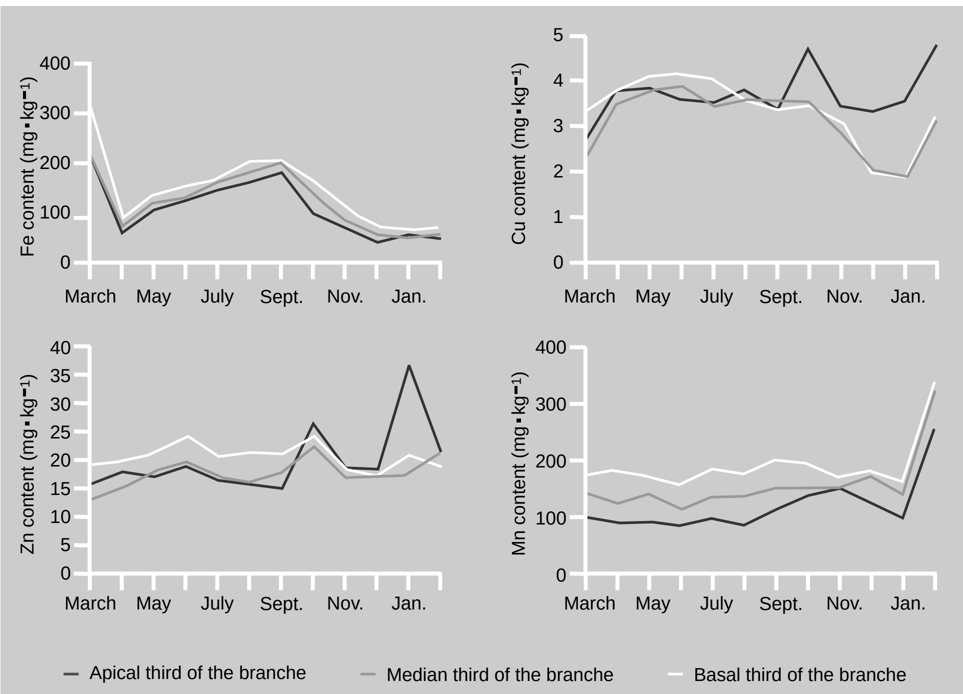


**Figure 2.**
Average monthly contents of the micronutrients Fe, Cu, Zn and Mn in the apical, median and basal thirds of the branches of 2-year-old Barbados cherry, from March 1997 to February 1998, in Visconde do Rio Branco, MG, Brazil.

**Table II.**
Medium squares of the deviations relative to the effects: month in three different portions of the aerial part and month in three parts of the branches referring to contents of macronutrients and micronutrients evaluated in 2-year-old Barbados cherry, over the months from September 1997 to February 1998, in Visconde do Rio Branco, MG, Brazil.

| Variation source | Nutrient | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | P | K | Ca | Mg | S | Fe | Cu | Zn | Mn |
| Portion of the aerial part | | | | | | | | | | |
| Upper | 1.2932$^{**}$ | 0.0019$^{ns}$ | 0.1497$^{ns}$ | 0.0455$^{ns}$ | 0.0027$^{ns}$ | 0.0052$^{ns}$ | 231.31$^{ns}$ | 1.2362$^{ns}$ | 309.76$^{**}$ | 7751.78$^{ns}$ |
| Median | 0.6294$^{*}$ | 0.0059$^{**}$ | 0.1400$^{ns}$ | 0.1103$^{ns}$ | 0.0193$^{ns}$ | 0.0079$^{*}$ | 57.44$^{ns}$ | 0.4641$^{ns}$ | 453.13$^{**}$ | 16973.77$^{**}$ |
| Lower | 0.2607$^{ns}$ | 0.0083$^{**}$ | 0.1076$^{ns}$ | 0.3314$^{ns}$ | 0.0575$^{**}$ | 0.0069$^{ns}$ | 271.57$^{ns}$ | 0.4991$^{ns}$ | 79.39$^{*}$ | 8609.64$^{ns}$ |
| Part of the branches | | | | | | | | | | |
| Apical third | 5.6655$^{**}$ | 0.0144$^{**}$ | 0.0374$^{ns}$ | 0.8677$^{ns}$ | 0.1496$^{**}$ | 0.0154$^{**}$ | 795.76* | 2.1866$^{ns}$ | 1044.30$^{**}$ | 7475.91$^{ns}$ |
| Median third | 0.2683$^{ns}$ | 0.0064$^{**}$ | 0.1247$^{ns}$ | 0.5534$^{ns}$ | 0.0038$^{ns}$ | 0.0022$^{ns}$ | 125.06$^{ns}$ | 0.0391$^{ns}$ | 108.39$^{*}$ | 14173.40$^{*}$ |
| Basal third | 0.2564$^{ns}$ | 0.0064$^{**}$ | 0.1062$^{ns}$ | 0.2925$^{ns}$ | 0.0006$^{ns}$ | 0.0041$^{ns}$ | 414.50$^{ns}$ | 0.3483$^{ns}$ | 32.48$^{ns}$ | 8182.05$^{ns}$ |

**, * Significant at 1 and 5% probability by *F*-test, respectively; ns: non-significant at 5% probability by *F*-test.

Zn and Mn tend to accumulate in the leaves. The influx of these nutrients results a liquid gain in the leaves as they grow.

The variance and regression analyses involving the months of March to August did not allow the adjustement of a polynomial model that could explain the phenomenon. In most cases, the deviation regression was significant, indicating the inadequacy of the model and/or the lack of important information, considered as aleatory causes. However, the data of the rainy season (September to February) made it possible to adjust a polynomial model in 65% of the cases whose deviation mean squares were not significant (*table II*).

The regression analysis of the data referring to the months of September to February allowed the establishment of regression equations for all studied nutrients, in each third of the branch (*figures 3, 4*). The variance analysis including the treatments of the canopy parts, branch parts and months

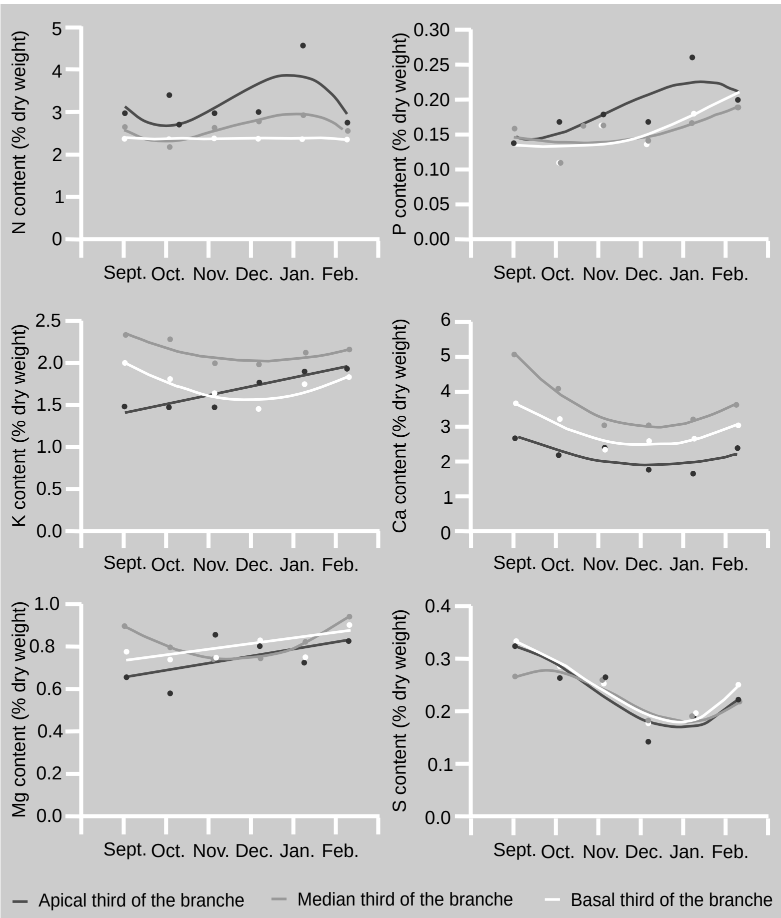


**Figure 3.**
Graphic representation of the seasonal variation of the macronutrients N, P, K, Ca, Mg and S in the leaves at the apical, median and basal thirds of the branches of 2-year-old Barbados cherry, from September 1997 to February 1998, in Visconde do Rio Branco, MG, Brazil.

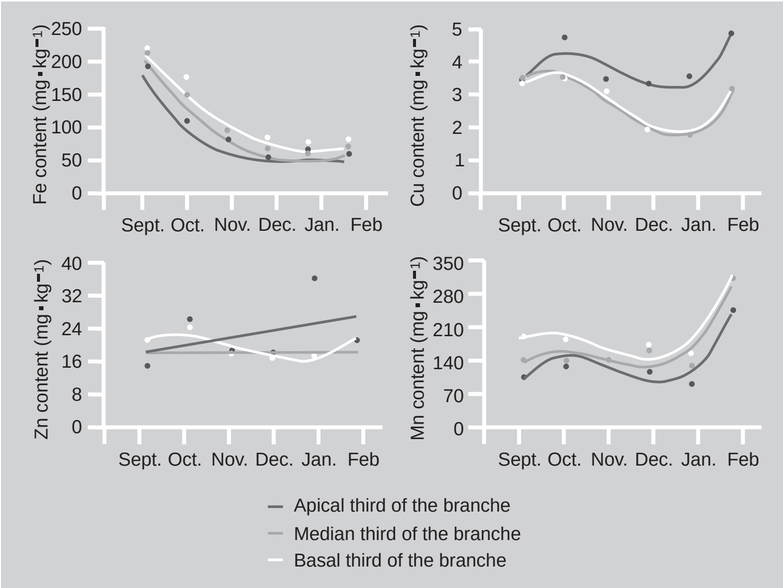


**Figure 4.**
Graphic representation of the seasonal variation of the micronutrients Fe, Cu, Zn and Mn, in mg × $kg^{-1}$, in the leaves at the apical, median and basal thirds of the branches of 2-year-old Barbados cherry, from September 1997 to February 1998, in Visconde do Rio Branco, MG, Brazil.

(September to February), associated with the regression analysis of these data, allowed the aquisition of comparisons for nutrient behavior in the plant, with time variation.

By comparison of the mean square values corresponding to each third of the aerial part and those of the branch, as well as by derivation of the regression functions, it was possible to determine the plant part and the lowest variation time in the contents of the nutrients. According to Mayer *et al.* [10], the tissues should be sampled at that time, when the variation rate in the nutrient contents is minimum.

There was a larger frequency of the variation indexes relative to the effects of the month within each portion of the aerial part and within each third of the branch, with a unitary value in the medium portion of the canopy, although, for P and Mn, these indexes were very high: 4.05 and 8.16, respectively (*table III*). The lowest average index (1.49) was observed in the upper portion of the plant. Considering the thirds of the branches, there was a higher frequency of the variation indexes with unitary value in the apical third. However, the indexes related to N and Zn were also high: 19.97 and 11.88, respectively (*table III*). The lowest average index (1.41) was verified for the medium third of the branches. Therefore, it is recommended to sample the leaves of the medium third of the branches located in the upper portion of the canopy.

The regression models that did not exhibit significant effect for the F-test, $\alpha \leq 0.05$, were not included in the *table IV*. The derivation of these regression functions, referring to the seasonal variation in the foliar nutrient contents at the three positions of the branches allows the definition of the times when the variation in concentrations is minimum.

The best time for collecting the leaves for nutritional diagnosis was established on the basis of the statistical analysis. The first derivative of the regression functions of the medium portion of the branches (*table IV*), in which the lowest variation in nutrient content of the leaves exists, indicates the maximum and/or minimum point, when the variation in these contents are also minimum. For N, the lowest variation occurred on the 210th and the 295th days after the first sampling date, at the beginning of October and middle of December, respectively. For P, the lowest variation happened on the 227th day after the first sampling date, in the middle of October. However, it should be distinguished that in this case the regression deviation was significant, and $R^2$

**Table III.**
Medium squares and respective variation indexes (= medium square / lower medium square) relative to the effects: month in three different portions of the aerial part, month in three different parts of the branches, referring to macro- and micronutrient contents evaluated in 2-year-old Barbados cherry during the months from September 1997 to February 1998, in Visconde do Rio Branco, MG, Brazil.

| Variation source | Statistical parameter | Nutrient | | | | | | | | | | Average index |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N | P | K | Ca | Mg | S | Fe | Cu | Zn | Mn | |
| Portion of the aerial part | | | | | | | | | | | | |
| Upper | Medium square | 1.1785 | 0.0021[1] | 0.4098 | 1.8668 | 0.0749 | 0.0181[1] | 30,437.07 | 5.6133 | 141.7809 | 10,014.74[1] | – |
| | Variation index | 1.51 | 1.00 | 1.42 | 1.05 | 2.41 | 1.00 | 1.17 | 2.91 | 1.51 | 1.00 | 1.49 |
| Median | Medium square | 0.7790[1] | 0.0085 | 0.2880[1] | 1.7807[1] | 0.0360 | 0.0246 | 25,933.12[1] | 1.9270[1] | 243.5674 | 81,776.77 | – |
| | Variation index | 1.00 | 4.05 | 1.00 | 1.00 | 1.16 | 1.36 | 1.00 | 1.00 | 2.59 | 8.16 | 2.23 |
| Lower | Medium square | 1.1791 | 0.0260 | 0.2976 | 7.5972 | 0.0311[1] | 0.0483 | 32,488.48 | 5.7012 | 93.96990[1] | 41,089.14 | |
| | Variation index | 1.51 | 9.81 | 1.03 | 4.27 | 1.00 | 2.67 | 1.25 | 2.96 | 1.00 | 4.10 | 2.96 |
| Part of the branches | | | | | | | | | | | | |
| Apical third | Medium square | 4.0863 | 0.0156 | 0.4489 | 1.4475[2] | 0.1067 | 0.0374 | 24,883.95[2] | 4.5494[2] | 544.2175 | 29,216.13[2] | – |
| | Variation index | 19.97 | 2.36 | 2.51 | 1.00 | 2.92 | 2.67 | 1.00 | 1.00 | 11.88 | 1.00 | 4.63 |
| Median third | Medium square | 0.5713 | 0.0066[2] | 0.3059 | 2.0860 | 0.0365[2] | 0.0297 | 33,760.25 | 5.2428 | 45.8065[2] | 45,873.50 | – |
| | Variation index | 2.79 | 1.00 | 1.71 | 1.44 | 1.00 | 1.1 | 1.36 | 1.15 | 1.00 | 1.57 | 1.41 |
| Basal third | Medium square | 0.2046[2] | 0.0113 | 0.1786[2] | 5.6285 | 0.0591 | 0.0140[2] | 31,309.15 | 4.5956 | 71.5461 | 39,698.00 | – |
| | Variation index | 1.00 | 1.71 | 1.00 | 3.89 | 1.62 | 1.00 | 1.26 | 1.01 | 1.56 | 1.36 | 1.54 |

For each nutrient: [1] the lower medium square referring to the three portions of the aerial part; [2] the lower medium square referring to the three thirds of the branches.

**Table IV.**
Regression equations and the corresponding determination coefficients, referring to the apical, median and basal thirds of the Barbados cherry branches, for macronutrients and micronutrients, relative to months from September 1997 to February 1998, in Visconde do Rio Branco, MG, Brazil.

| Nutrient | T | Equation | $R^2$ |
|---|---|---|---|
| N | Apical third | $y = 59.2591 – 0.7156\ d + 0.00295\ d^2 – 0.0000039\ d^3$ | 44.54 |
| | Median third | $y = 33.7878 – 0.3933\ d + 0.0016\ d^2 – 0.0000021\ d^3$ | 84.04 |
| | Basal third | $y = \bar{Y} = 2.46$ | – |
| P | Apical third | $y = 1.4567 – 0.0175\ d + 0.0000754\ d^2 – 0.000000102\ d^3$ | 62.88 |
| | Median third | $y = 0.4068 – 0.002369\ d + 0.00000521\ d^2$ | 57.62 |
| | Basal third | $y = 0.3523 – 0.002147\ d + 0.0000052\ d^2$ | 76.67 |
| K | Apical third | $y = 0.7768 + 0.0037\ d$ | 86.73 |
| | Median third | $y = 6.0370 – 0.0335\ d + 0.00006349\ d^2$ | 83.48 |
| | Basal third | $y = 5.0460 – 0.0219\ d + 0.00004055\ d^2$ | 75.91 |
| Ca | Apical third | $y = 8.9509 – 0.0513\ d + 0.0000937\ d^2$ | 57.31 |
| | Median third | $y = 13.8099 – 0.0839\ d + 0.000156\ d^2$ | 89.39 |
| | Basal third | $y = 20.6727 – 0.1285\ d + 0.000233\ d^2$ | 97.67 |
| Mg | Apical third | $y = 0.4394 + 0.001189\ d$ | 37.53 |
| | Median third | $y = 0.5548 + 0.000974\ d$ | 89.73 |
| | Basal third | $y = 2.7611 – 0.0161\ d + 0.0000322\ d^2$ | 99.18 |
| S | Apical third | $y = 1.4614 + 0.0261\ d – 0.000121\ d^2 + 0.00000017\ d^3$ | 83.52 |
| | Median third | $y = 1.6552 + 0.0286\ d – 0.000132\ d^2 + 0.00000019\ d^3$ | 96.87 |
| | Basal third | $y = 2.5784 + 0.0368\ d – 0.000154\ d^2 + 0.00000021\ d^3$ | 88.56 |
| Fe | Apical third | $y = 2292.38 – 22.753\ d + 0.07666\ d^2 – 0.00008582\ d^3$ | 98.72 |
| | Median third | $y = 1044.45 – 6.6845\ d + 0.01119\ d^2$ | 99.85 |
| | Basal third | $y = 890.15 – 5.2837\ d + 0.00844\ d^2$ | 98.89 |
| Cu | Apical third | $y = 74.5669 + 0.9927\ d – 0.0041\ d^2 + 0.00000551\ d^3$ | 80.77 |
| | Median third | $y = 57.8141 + 0.8117\ d – 0.0035\ d^2 + 0.00000477\ d^3$ | 99.70 |
| | Basal third | $y = 65.6234 + 0.8977\ d – 0.0038\ d^2 + 0.00000514\ d^3$ | 96.97 |
| Zn | Apical third | $y = 7.8937 + 0.0575\ d$ | 17.22 |
| | Median third | $y = \bar{Y} = 18.9$ | – |
| | Basal third | $y = 225.316 + 3.2249\ d – 0.0136\ d^2 + 0.0000186\ d^3$ | 81.84 |
| Mn | Apical third | $y = 5122.11 + 66.5261\ d – 0.2755\ d^2 + 0.000373\ d^3$ | 89.76 |
| | Median third | $y = 3578.61 + 48.8077\ d – 0.2093\ d^2 + 0.000294\ d^3$ | 87.64 |
| | Basal third | $y = 3382.62 + 47.8824\ d – 0.2093\ d^2 + 0.000298\ d^3$ | 91.76 |

d: days after the beginning of the evaluation (March 3).

was just 57.62% (*table IV*), so this information should be regarded with caution. For K, the lowest variation occurred on the 264th day, in the middle of November. For Ca, the best stability was verified at the end of November. For Mg, the increasing lineal behavior observed in the regression function (*figure 3*) makes impossible, at the beginning, the definition of a stable sampling time for this element. Even so, as the lineal coefficient is very low ($H_0$: $\beta = 1$, rejected), it can be inferred that the Mg content was relatively stable during the evaluated period. The stability in S contents happened on the 172nd and the 292nd days after the first sampling date, at the end of August and the end of December, respectively. The lowest variation in Fe contents happened in the middle of December, while, for Cu content, it occurred in the middle of September and in the middle of December. For Zn, a polynomial regression was not adjusted, so indicating a relatively invariable behavior over time, suggesting that the sampling could be performed at any time in the year. The lowest variation in Mn content happened on the 206th and the 268th days after the first sampling date, at the end of September and the beginning of December, respectively.

A sampling time over which the variation in foliar contents of all nutrients would be minimum could not be established. According to Cresswell and Wickson [9], it is difficult to select a sampling time that would be appropriate to all nutrients due to basic differences in their seasonal variations, and one alternative would be to choose a sampling date that provides a reasonable determination of the plant nutritional status for most nutrients.

So, a time interval was established with extremes just a little before and a little after for each date, with low variations in the element contents, by indicating the month of December as the time when the variation in the element contents is minimum.

## 4. Conclusions

1. The upper portion of the aerial part and the median third of the branches are the best plant parts indicated for leaf sampling when aiming to diagnose the nutritional state of the Barbados cherry plant.

2. In the area of the study (Visconde do Rio Branco county, Minas Gerais, Brazil), the month of December was the most appropriate for the accomplishment of leaf sampling aiming at the diagnosis of the nutritional status of the Barbados cherry plant.

3. These data will allow better study of the mineral nutrition of *M. emarginata* in order to establish reliable fertilizer programs. Thus, it will be possible to improve yields of this crop.

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## Determinación de técnicas de muestreo de hoja para evaluar el estado nutricional de la cereza de las Antillas (*Malpighia emarginata* D.C.).

**Resumen** — **Introducción**. Las investigaciones sobre la nutrición mineral de la cereza de las Antillas son escasas. Se desconocen las técnicas de muestreo de hoja para esta producción. El análisis foliar, asociado a la determinación de la disponibilidad de elementos minerales en el suelo, puede constituir una ayuda fiable para el establecimiento de programas de fertilización. Para una interpretación correcta del estado de nutrición, es importante (1) evaluar la variación estacional del contenido de elementos minerales en la hoja, (2) determinar el mejor momento para recolectar y analizar las hojas y (3) identificar el material vegetal más apto para el muestreo y realización de estos análisis minerales. Estos tres puntos fueron estudiados en estos trabajos. **Material y métodos**. Las experimentaciones se efectuaron de marzo de 1997 a febrero de 1998 en Visconde do Rio Branco, Minas Gerais (Brasil). Se estudió la dinámica de N, P, K, Ca, Mg, S, Fe, Cu, Zn y Mn en las hojas situadas en los extremos, en medio y en la base de ramas de las partes superiores, medias e inferiores de las copas de plantas de *M. emarginata*. **Resultados**. El contenido foliar fue más estable en las hojas situadas en la parte media de las ramas situadas en la parte superior de la copa. Estas hojas son, por tanto, las más adecuadas para efectuar los muestreos. Por otro lado, el mes de diciembre se mostró como el mes más apropiado para la toma de muestras. **Conclusión**. Las técnicas de muestreo de hoja desarrolladas permitirán estudiar mejor la nutrición mineral de *M. emarginata* para establecer programas fiables de fertilización mejorando así los rendimientos de este árbol frutal.

**Brasil / *Malpighia emarginata* / nutrición de las plantas / aplicación de abonos / hojas / análisis de tejidos / variación estacional / estado nutricional**