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# Nitrogen nutrition of guava trees in response to byproduct of fruit processing

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**ABSTRACT**
Studies of the effect of organic fertilization of perennial crops are scarce due to the time necessary for the plants to present the first results. The objective of this study was to assess the leaf nitrogen (N) content, the direct and indirect measures of chlorophyll content, fruit yield and their correlations in an orchard of adult guava trees of the Paluma cultivar, through five consecutive harvests, in function of application of a byproduct of guava processing. The following doses of the byproduct were applied: 0, 9, 18, 27 and 36 t $ha^{-1}$. The material was applied on the surface, without incorporation, once a year between 2006 and 2010. The byproduct doses influenced the nitrogen concentrations, SPAD index, chlorophyll b level and fruit production.

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**KEYWORDS**
*Psidium guajava*; chlorophyll a; chlorophyll b; SPAD index; leaf diagnosis

## Introduction

Nitrogen (N) fertilization influences the production of a series of intracellular compounds and components of plants. Independent of the nitrogen source utilized, increased N doses have positive reflections on the chlorophyll concentration of the leaves, due to the participation of this macronutrient in their structure (Arnon, 1949).

Besides the direct method of measuring chlorophyll content, by its fractionation, which requires destruction of the plant material, there are indirect methods employing portable devices, such as the chlorophyll meter, which allow instant determination of the value corresponding to the concentration of this pigment in the leaves, without destroying them. The relationship is attributed to the fact that 50 to 70% of the total N in leaves forms enzymes that are associated with the chloroplasts (Argenta et al., 2001). According to Buzetti et al. (2008), the indirect measurement of chlorophyll is a promising technique to obtain useful information to make recommendations on the need for nitrogen fertilization of crops.

Various studies have assessed the effects of N fertilization on fruit yield, correlating these effects with the nutritional status and chlorophyll levels, obtaining positive results, such as Leal et al. (2007) for carambola (starfruit) trees; Souza et al. (2011b) for citrus trees; Santos et al. (2011) for passion fruit trees; Amarante et al. (2009) for grape vines; and Shaahan et al. (1999) for several fruiting plants. Thus, it is clear that there are few studies assessing the nutritional status of nitrogen in fruit, and guava mainly.

Byproducts of fruit processing can be a good source of nitrogen due to the richness of organic matter and nutrients, besides the economic and environmental advantages of recycling these materials. The byproduct of the guava processing industry studied here contains reasonable levels of nitrogen,

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11.6 g kg$^{-1}$ (Souza et al., 2011a), enabling its use as a source of this element, as demonstrated in the studies of Mantovani et al. (2004), Corrêa et al. (2005) and Souza et al. (2011a). However, these studies were conducted in controlled or few years of evaluation in field conditions.

The residue of the guava processing industry is used in orchards empirically without knowledge by effects that it promotes in the production and especially in the nutritional status, since it may allow a reduction in the use of mineral fertilizers. It must be emphasized that nitrogen with potassium are the nutrients most exported by guava plants.

Based on the above observations, the aim of the present study was to assess the leaf nitrogen content, direct and indirect measures of chlorophyll content (SPAD index), critical N level, fruit yield and their correlations during five consecutive harvests in a guava orchard planted with the 'Paluma' cultivar.

## Materials and methods

### Experimental setup

The experiment was conducted in a guava orchard containing eight-year-old guava orchard (*Psidium guajava* L., Paluma cultivar), propagated vegetatively from seedlings. This cultivar is the most widely planted in Brazil. The orchard was irrigated by revolving microsprayers (31 L h$^{-1}$) and monitored by tensiometry (60%) (CC), with water taken from a semi-artesian well. The trees are spaced five meters apart in rows seven meters apart, the standard for this cultivar. The experimental area is located in the municipality of Vista Alegre do Alto (21° 08′ south latitude, 48° 30′ west longitude, altitude 602 m), in the main guava producing region of the state of São Paulo.

The orchard's soil is classified as Ultisol, or dystrophic Red-Yellow Argisol, with medium sandy texture, according to Embrapa (1999). The results of the chemical analysis with respect to soil fertility are in Table 1. According to van Raij et al. (1997), these fertility conditions are within the ranges considered adequate for perennial cultures in the state of São Paulo.

The industrial byproduct employed in this study consisted basically of ground seeds together with a small fraction of the skin and pulp not separated during the pulping process, carried out after washing the fruits. The byproduct was applied at the start of each year in 2006 (March), 2007 (January), 2008 (January), 2009 (January) and 2010 (January).

The chemical content measurements (Table 2) were carried out according to the method described by Bataglia et al. (1983). The total organic carbon content was determined according to Abreu et al. (2006).

The experiment is randomized complete block design with five treatments and four replications. The doses of the byproduct were (dry weight): zero, 9, 18, 27 and 36 t ha$^{-1}$. The doses applied in the orchard were established in function of the nitrogen content of the byproduct. Since N is the most expensive element in terms of energy cost for production of fertilizers, the relatively high nitrogen content of the material makes it a promising alternative. The trees did not receive any mineral fertilizer during the experimental period. The experimental plots were composed of five trees, with only the three central ones used in the evaluations. The plants of extremity were considered borders.

### Sampling and chemical analysis

In the harvests from July to September 2007 (1st harvest), February to April 2008 (2nd harvest), January to March 2009 (3rd harvest), November 2009 to January 2010 (4th harvest) and August to October

**Table 1.** Chemical properties of the soil in the experimental area.

| | pH | M.O. | P | S-$SO_4^{-2}$ | K | Ca | Mg | (H+Al) | SB | T | Al | v |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ($CaCl_2$) | | (Resin) | | | | | | | | | |
| | | g dm$^{-3}$ | mg dm$^{-3}$ | | | | | mmol$_c$ dm$^{-3}$ | | | | % |
| Crown projection | 5.3 | 11 | 8 | 1 | 2.7 | 18 | 6 | 16 | 26.7 | 42.7 | 0.0 | 63 |

SB = sum of bases (K + Ca + Mg) / T = cation exchange capacity (SB + H+Al).

Table 2. Nutrient concentrations in the guava processing byproduct.

| Sample | N | C | P | K | Ca | Mg | S | B | Cu | Fe | Mn | Zn | C/N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ------------------------g $kg^{-1}$----------------------------- | | | | | | | ----------mg $kg^{-1}$---------- | | | | | |
| Byproduct | 11.6 | 290 | 2.1 | 2.3 | 0.8 | 0.9 | 1.3 | 10 | 10 | 150 | 12 | 28 | 25 |

2010 (5th harvest), the leaf N content was measured, according to Bataglia et al. (1983), along with the SPAD index, using a portable chlorophyll meter (Minolta SPAD 502, Minolta, Osaka, Japan). For both evaluations, four pairs of leaves (3rd recently expanded pair) were picked from the three middle trees of each plot, at the peak of flowering, according to the indications of Natale et al. (1996). This period corresponds to phenological stage F:65 (at least 50% of flowers open and first petals falling) for guava trees according to Salazar et al. (2006). The fruit production was determined from the five harvests by weighing all the fruits harvested from the three middle trees in the period when they were suitable for processing, corresponding to stage K:89 (fruit reached final volume and become completely yellow, releasing pleasant aroma, and fruit right for consumption) (Salazar et al., 2006).

Additionally, in the fourth and fifth harvests, the levels of chlorophyll a, b and total were measured in the same leaves picked for diagnosis of the nutritional status and SPAD reading. The extractions were carried out by the method of Arnon (1949), as modified by Linder (1974). The chlorophyll a/b ratio was also calculated.

The calculation of the critical level of a particular nutrient by the continuous probability distribution, proposed by Maia et al. (2001), is based on the reduced normal distribution. For this purpose, it is necessary to have data on productivity (P) and Q, where Q is defined as the ratio between P and $n_i$ ($Q = P/n_i$), in which $n_i$ is the level of the nutrient of interest. The critical level is the value representing 90% of its maximum value. The critical level is obtained by the formula:

$$\mathrm{CLi} = \frac{1.281552\ s_1 + m_1}{1.281552\ s_2 + m_2}$$

where $m_1$ and $s_1$ are the are the arithmetic mean and standard deviation of P, and $m_2$ and $s_2$ are the mean and standard deviation of Q, respectively. The basic assumption to find the critical level by the continuous probability distribution is that P and Q are normally distributed. For that purpose, we tested the normality of the variables by the Shapiro-Wilk test ($p > 0.01$). Data that were not normally distributed were transformed into natural logarithm (ln).

### Statistical analysis

We submitted the results to analysis of variance and the F-test, and used regression and correlation analysis when the statistics were significant, employing the SISVAR statistical program (Ferreira, 2008).

### Results and discussion

It can be seen from Figure 1 that in the first harvest, the level of N and the SPAD index increased linearly in function of the doses of the guava processing byproduct. However, there was no difference in fruit yield. The explanation is that guava need time for different management techniques to be reflected in production, as also observed by Natale et al. (2007; 2008). There is slow release of N by the byproduct (Mantovani et al., 2004), so the influence of the treatments is only gradual. In the other four harvests, the changes in the variables were significant. The results are presented in Figures 2, 3, 4 and 5. In all cases, the best response model was a rising line. Rozane et al. (2013) observed good results in soil when apply mix of mineral fertilization and byproduct of guava.

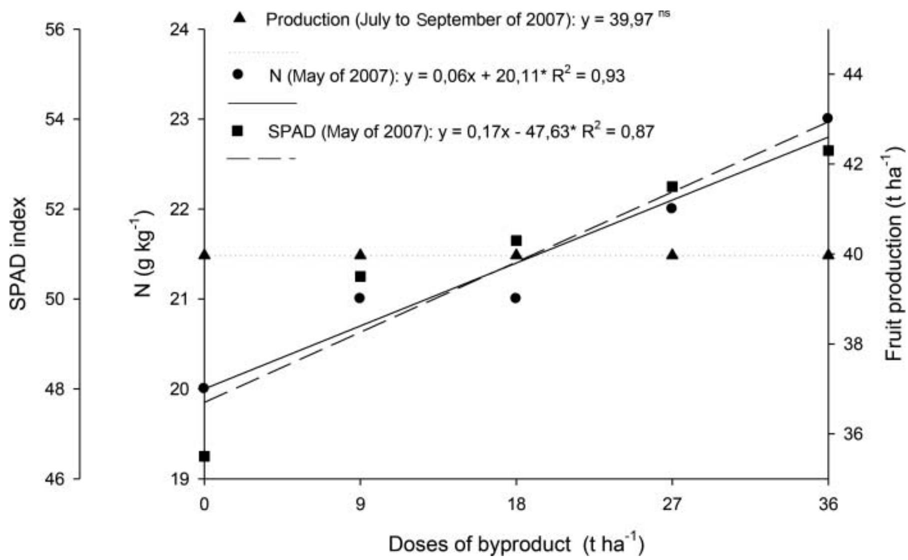


**Figure 1.** SPAD index, leaf N content and fruit production in function of doses of the byproduct of guava processing (1st harvest) in an orchard of adult trees. [ns] and * - Not significant and significant at 5%.

Natale et al. (1995) concluded that the guava tree is responsive to nitrogen fertilization in an experiment involving three consecutive harvests. The results here corroborate those findings and indicate that the guava processing byproduct tested here can be used as a source of N.

We tested the correlation between the SPAD index vs. N content and SPAD index vs. fruit yield. The results are displayed in Table 3. There was a significant positive correlation between the SPAD index and leaf N content, except in the fourth harvest. There was also a significant and positive relation between the SPAD index and yield for the second, third and fifth guava harvests.

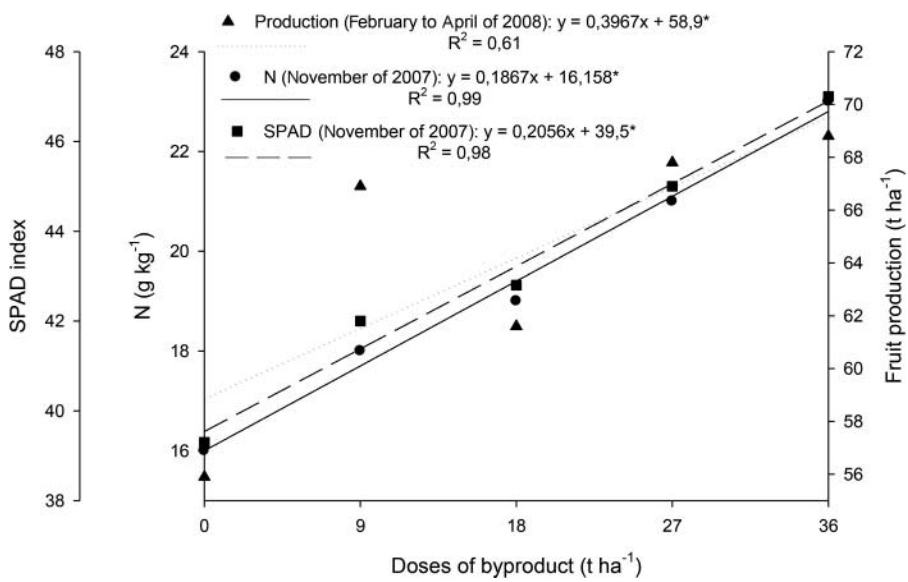


**Figure 2.** SPAD index, leaf N content and fruit production in function of doses of the byproduct of guava processing (2nd harvest) in an orchard of adult trees. * - Significant at 5%.

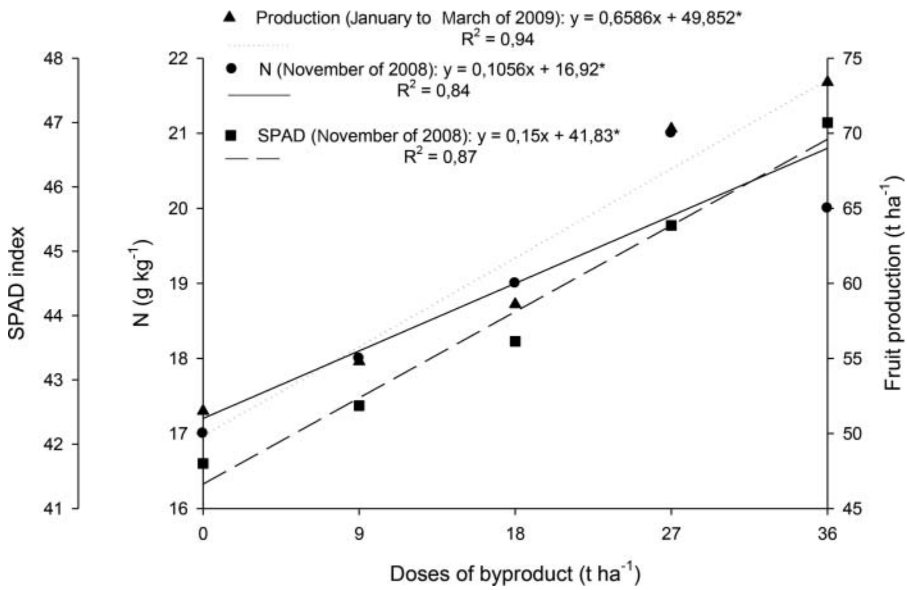


Figure 3. SPAD index, leaf N content and fruit production in function of doses of the byproduct of guava processing (3rd harvest) in an orchard of adult trees. * - Significant at 5%.

As observed in this study, there is evidence in the literature of the possibility of using portable chlorophyll meters in perennial fruit cultures (Leal et al., 2007; Shaahan et al. 1999). According to these authors, the SPAD index can also be used to evaluate the nitrogen nutritional status and to help predict fruit production, due to the positive influence of nitrogen on the yield of orchards.

Souza et al. (2011b) evaluated the sensitivity of the indirect measurement of chlorophyll as a way to monitor the levels of N in citrus trees. Based on complete harvests, the authors reported that the chlorophyll meter can be used as a fast and nondestructive method to assess the availability of N in citrus

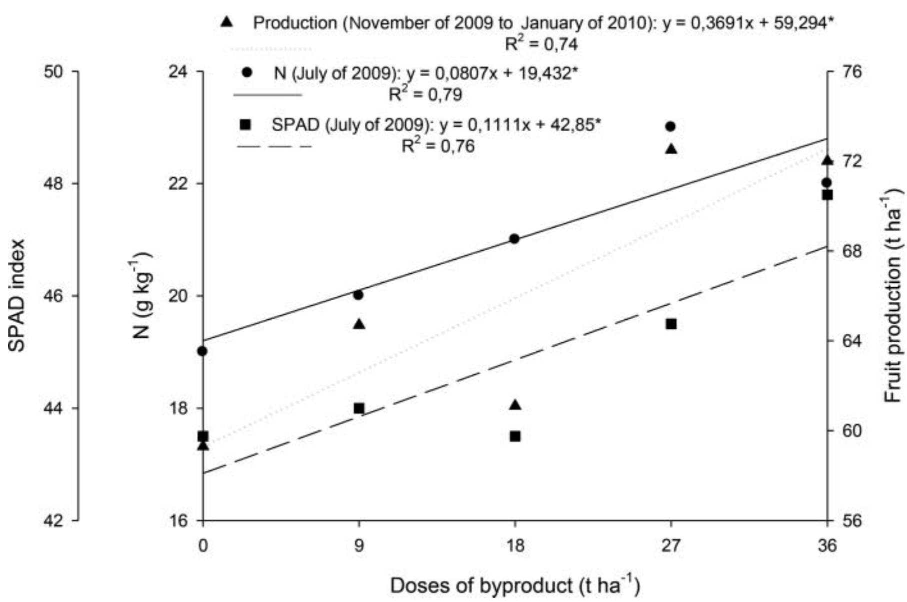


Figure 4. SPAD index, leaf N content and fruit production in function of doses of the byproduct of guava processing (4th harvest) in an orchard of adult trees. * - Significant at 5%.

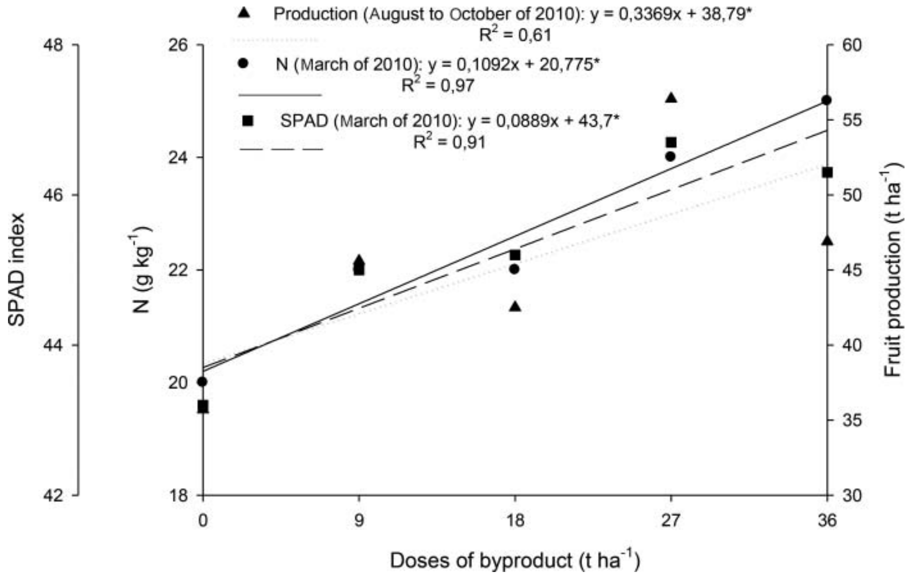


**Figure 5.** SPAD index, leaf N content and fruit production in function of doses of the byproduct of guava processing (5th harvest) in an orchard of adult trees. * - Significant at 5%.

trees. Wang et al. (2005) concluded that the SPAD-502 could be used as an instrument to provide quick, nondestructive, and objective estimation of quality for green-leaved foliage plants.

According to the data from direct measurement of chlorophyll, there was a significant result in function of the byproduct doses in the fourth harvest for the variables chlorophyll a and b, where the best response model was a rising line (Figure 6). For the fifth harvest there was significance only for the chlorophyll b content, with an increase of this pigment in function of the doses applied (Figure 7).

Besides the correlation between the N levels in the leaves and the SPAD index values, we also calculated the following correlations: N content vs. chlorophyll a, b, total and a/b ratio; and SPAD index vs. chlorophyll a, b, total and a/b ratio. There were no significant results for the correlations between chlorophyll and the N contents in the fourth harvest, while for the SPAD index, there was a positive and significant correlation between this index and the chlorophyll a/b ratio ($r = 0.87$ and $t = 3.10^{*}$). In the fifth harvest there was a positive and significant correlation between the leaf N content and the level of chlorophyll b ($r = 0.82$ and $t = 2.54^{*}$).

**Table 3.** Correlation coefficients and significance between SPAD index and leaf N content and SPAD index and fruit yield.

| N vs. SPAD | r | t |
|---|---|---|
| 1st harvest | 0.92 | 4.12* |
| 2nd harvest | 0.99 | 14.00** |
| 3rd harvest | 0.87 | 3.05* |
| 4th harvest | — | — |
| 5th harvest | 0.96 | 6.76** |
| SPAD vs. yield | | |
| 1st harvest | — | — |
| 2nd harvest | 0.92 | 4.12* |
| 3rd harvest | 0.98 | 10.98** |
| 4th harvest | — | — |
| 5th harvest | 0.92 | 4.10* |

ns, * and ** - not significant, significant at 5% and 1%, respectively.

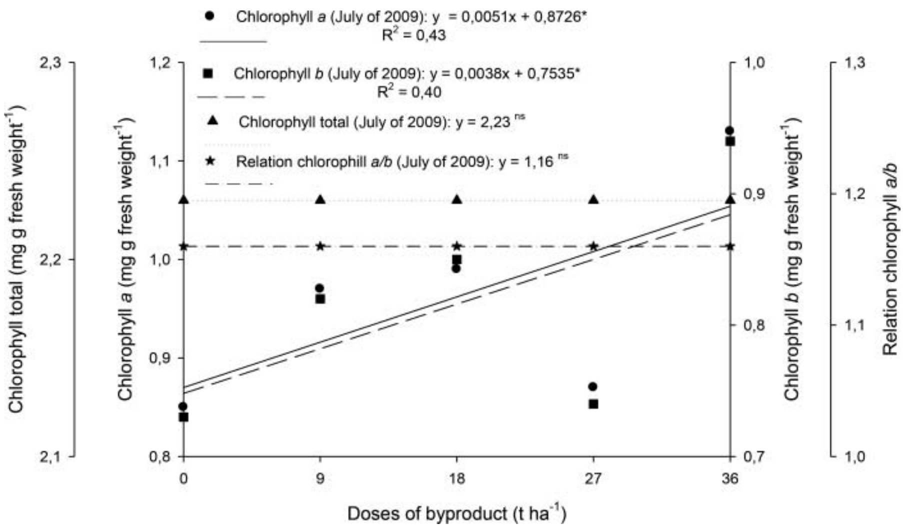


**Figure 6.** Chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll a/b ratio, in function of doses of guava processing byproduct (4th harvest) in an orchard of adult trees. ns and * - Not significant and significant at 5%.

The positive relation observed between the leaf N levels and the SPAD index can be explained by the participation of nitrogen in the chloroplasts of plant tissue, giving them their green color. Therefore, the intensity of this color, detected by the chlorophyll meter, has a direct relation with the nitrogen content, reflecting the plant's nutritional status regarding this element. The relationship is attributed to the fact that 50 to 70% of the total N in the leaves is associated with chloroplasts (Argenta et al., 2001).

The results indicate that the guava processing byproduct influenced the SPAD index and the chlorophyll concentrations, from which it can be inferred there was luxury consumption of nitrogen by the plants. Therefore, the nitrogen concentrations in the leaves, which rose with increasing doses of the byproduct, were translated into better nutritional status of the guava trees, with positive reflections on fruit yield.

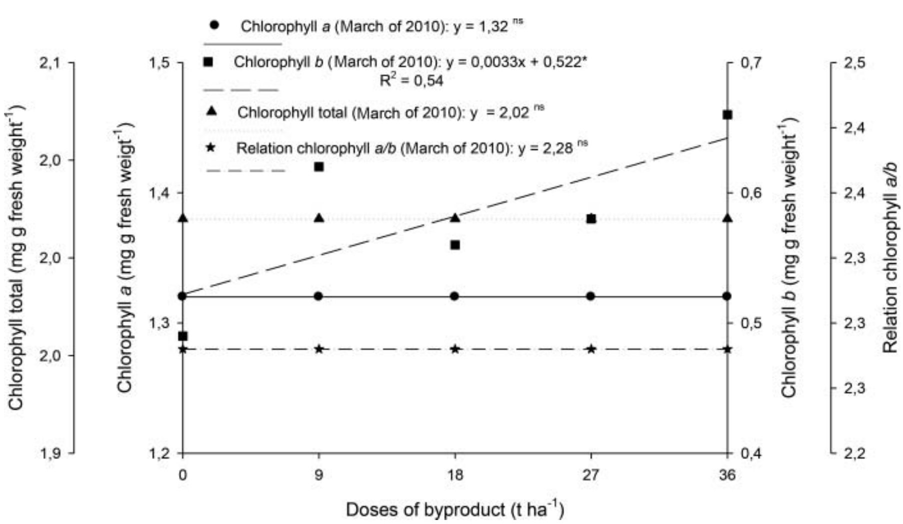


**Figure 7.** Chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll a/b ratio, in function of doses of guava processing byproduct (5th harvest) in an orchard of adult trees. ns and * - Not significant and significant at 5%.

Table 4. Mean, standard deviation and critical levels for nitrogen content and SPAD index for 'Paluma' guava trees, obtained by the continuous probability distribution method.

| Factors | Mean | Standard deviation | Critical level calculated | Sufficiency range in the literature | |
|---|---|---|---|---|---|
| | | | | Natale et al. (1996) | Quaggio et al. (1997) |
| Yield (t ha$^{-1}$) | 55.3 | 13.5 | — | — | — |
| N (g kg$^{-1}$) | 20.6 | 2.4 | 19.8 | 20.0-23.0 | 13.0-16.0 |
| SPAD Index | 45.5 | 3.6 | 42.4 | — | — |
| Productivity for 90% of the maximum (referring to the five harvests): 72.6 t ha$^{-1}$ | | | | | |

From the results of the N content, SPAD index and fruit production, we calculated the critical level by the reduced normal distribution criterion. The results are shown in Table 4.

The results of this study for the critical level are very near the lower range (or critical level) indicated by Natale et al. (1996), and higher than the values suggested by Quaggio et al. (1997). It is important to point out also that there are no indications in the Brazilian literature for the critical level in function of the SPAD index for guava trees.

## Conclusions

- The doses of the guava processing byproduct influenced the levels of nitrogen, SPAD index and chlorophyll b content and fruit yield of Paluma guava trees.
- There was a significant positive correlation between the N level and SPAD index and between SPAD index and fruit production.
- The critical levels for N and SPAD index are, respectively, 20 g kg$^{-1}$ and 42.4.
- Based on the five guava harvests evaluated, it can be inferred that indirect measurement of chlorophyll can be employed as a fast and nondestructive tool to evaluate the nutritional status of guava trees.

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