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Monitoring and Evaluation of Need for Nitrogen Fertilizer Topdressing for Maize Leaf Chlorophyll Readings and the Relationship with Grain Yield

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ABSTRACT

The study was carried out for two years in maize in succession to the wheat using no tillage system in a distroferric Red Latosol (Hapludox). Methods of management nitrogen fertilizer (120 kg ha^{-1}) with ammonium sulphate were studied; the fertilizer was applied in maize sowing or in maize topdressing, and N with previous application in wheat sowing. In addition, leaf chlorophyll reading was used as an indicator for the need for topdressed nitrogen fertilizer. Nitrogen supply index (NSI) was shown to be effective at predicting need for topdressed nitrogen fertilizer for maize. The application of N improved the yield of the maize independent of the management system. The flowering stage was carried out at the appropriate time in order to estimate the nitrogen nutrition state and yield of maize using the relative chlorophyll level (RIC).

Key words: nitrogen fertilized, leaf chlorophyll, crop rotation, no till, *Zea mays, Triticum aestivum*

INTRODUCTION

Nitrogen is one of the most important nutrients for the crops, since it is in the makeup of proteins, enzymes, vitamins, and other organic compounds essential for growth and production, such as the chlorophyll molecule.

In no-tillage farming, nitrogen losses are mainly related to microbial activity, such as N volatilization (Lara Cabezas et al., 2004; 2005; 2006; 2008) and immobilization (Sangoi et al., 2007). Sangoi et al. (2007) reported that temporary N immobilization didn't reduce the yield of maize grain when N wasn't applied before, pre-planting or during maize planting in the soil with high level of organic material. The process of nitrogen loss by lixiviation can be minimized by dividing the application, partly during the planting and partly at topdressing, since it increases the efficiency of the N in the fertilizer. Favorable maize yield results were confirmed in the studies carried out by Da Ros et al. (2003); Aita et al. (2004); Villas Boas et al. (2005); Gomes et al. (2007); Lange et al. (2008); Silva et al. (2009) when N was supplied in parts as above.

On the other hand, in no-tillage farming, already stabilized and adequately fertile, organic material improves the efficiency of N use from ammonia

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and its availability in nitric form. For this reason, the efficiency of nitrogen fertilizer can be different, in function of losses, time in which no-tillage farming has been practiced, quantity of organic material, and crops in the rotation system, as well as dose and period of nitrogen application.

One way to evaluate the need for nitrogen is to determine its level indirectly through leaf chlorophyll content (Blackmer and Schepers, 1995; Chapman and Barreto, 1997; Graeff and Claupein, 2003). Using leaf chlorophyll has the advantage of being a viable technique that is able to predict N nutritional level, according to Booij et al. (2000), since its quantity seems positively correlated with the nutrient. According to Godoy et al. (2007) and Bueno et al. (2009), determination of the leaf chlorophyll is sufficiently sensitive to the variations in nitrogen supply in dry material or coming from the fertilizer, thus enabling quicker detection of N deficiency.

Carvalho et al. (2003) observed good perspectives for preventing N deficiency in the beans with the aid of a chlorophyll measuring device, as well as the viability of adequately correcting lack of N. Results have indicated the need for various collections of samples throughout the plant's life cycle in order to determine the variations in chlorophyll content and its relationship with leaf N levels (Waskon et al., 1996; Zebarth et al., 2002).

The objectives of the present work were: a) use of chlorophyll meter to evaluate the level of foliar N through the relative indexes of chlorophyll (RIC) and Nitrogen Supply Index (NSI) in maize rotation with wheat in no-tillage system; b) evaluate the NSI of 0.95 as a indicative minimum index of need for nitrogen topdressing in maize; and, c) relate nitrogen fertilizer management to absorbed N, evaluated by the chlorophyll content and maize yield.

MATERIALS AND METHODS

Maize was sowed employing a no-tillage system using the crop rotation with a succession of wheat at the Experimental Farm of the Agroindustrial Cooperative (COAMO) in Campo Mourão, PR. The soil is classified as a Latossolo Vermelho distroférrico (Hapludox) with 600 g kg^{-1} of clay (Embrapa, 1999). The experimental area (Table 1) was sowed with oats, wheat and lupinis in winter and soy and maize in the summer using no-tillage system. The chemical analysis of the soil before implantation of the preparation, at a depth of 0 to 0.10 m had the following chemical attributes: pH (H_2O) 5.7; pH ($CaCl_2$) 5.1; MO (38.74 g dm^{-3}); H+Al (4.28 $cmol_cdm^{-3}$); Al (0.00 $cmol_cdm^{-3}$); K (0.33 $cmol_cdm^{-3}$); Ca (4.38 $cmol_cdm^{-3}$); Mg (1.66 $cmol_cdm^{-3}$); CTC (10.65 $cmol_cdm^{-3}$); V (59.81%); P (18 mg dm^{-3}); Fe (66.0 mg dm^{-3}); Zn (22.62 mg dm^{-3}); Cu (8.39 mg dm^{-3}); and Mn (53.72 mg dm^{-3}).

At a depth of 0.10 to 0.20 m, the soil had the following chemical attributes: pH (H_2O) 5.5; pH ($CaCl_2$) 4.9; MO (35.39 g dm^{-3}); H+Al (4.61 $cmol_cdm^{-3}$); Al (0.00 $cmol_cdm^{-3}$); K (0.26 $cmol_cdm^{-3}$); Ca (4.06 $cmol_cdm^{-3}$); Mg (1.61 $cmol_cdm^{-3}$); CTC (10.54 $cmol_cdm^{-3}$); V (56.26 %); P (11 mg dm^{-3}); Fe (79.43 mg dm^{-3}); Zn (16.31 mg dm^{-3}); Cu (9.33 mg dm^{-3}); and Mn (53.52 mg dm^{-3}). The methodology was carried out as in Embrapa (1997): extractable P and K by Mehlich 1; extractable exchangeable Ca and Mg by KCl 1 mol L^{-1}; organic C by $K_2Cr_2O_6$ 0.4 mol L^{-1} oxidation by methodology proposed by Walkley-Black; Fe, Zn, Cu and Mn, by Mehlich 1.

In the first year, wheat (CD 104) was sowed in April 2003 using 300 kg ha^{-1} 08-30-20 formula in all of the preparations (Table 1). The wheat plots were composed of 30 rows with 4.0 m long, spaced 0.17 m apart and 40 to 50 plants per meter (400.000 plants ha^{-1}). In October 2003, the maize (Pioneer-30P70) was sowed, applying P and K during the planting (300 kg ha^{-1} (00-20-10). In November 2003, the side dressing fertilization with ammonium sulphate was applied at the 34th day. Preparations with nitrogen fertilizer (120 kg ha^{-1} ammonium sulphate) were applied during the sowing in topdressing or both, divided into two applications (Table 1).

In the second year, the wheat (CD 104) was sowed in May and received 300 kg ha^{-1} 08-30-20 (Table 1). The sowing of maize (Garra, Syngenta) took place in November 2004. In December 2004, the side dressing fertilization with ammonium sulfate was applied to the maize plants at the 32nd day. The plot area of 40 m^2 (4x10m) had five lines of maize 0.80m apart (40.000 to 50.000 plants ha^{-1}).

Randomized block experimental design was used, made up of six treatments and four repetitions, totaling 24 parcels. Preparations with the management of nitrogen fertilizer were applied during wheat sowing, maize sowing and in maize topdressing, as follows: T1: 24-120-0; T2: 24-0-120; T3: 24-40-80; T4: 24-30-90; T5 144-0-0; T6: 0-0-0, respectively. The useful area of each parcel was 32 m^2, with evaluations carried out in tree central lines.

The need for N fertilizer in the topdressing in the monitored treatment (T4) was evaluated by the means of chlorophyll meter readings (SPAD) and Nitrogen Supply Index (NSI), which was determined from the quotient of the relative chlorophyll indexes (IRC) obtained in the parcel studied (T4) and in the control parcel (T1), in which all of the nitrogen fertilizer was done during sowing, as follows:

NSI = IRC pe / IRC pr

IRC pe = average of readings from the device in the parcel studied;
IRC pr = average of readings from the device in the control parcel.

The fertilization was made when NSI <0.95. In other treatments was made reading using SPAD and RIC, but there was not monitoring the need of N applied at side dressing.

The foliar chlorophyll readings were carried out using a SPAD-502 chlorophyll meter (Minolta, 1989), always late in the afternoon (after 4 o'clock pm). Those readings provided values called as "Relative Chlorophyll Index" (RIC), determined in maize following wheat for two years (2003 and 2004). An NSI value of 0.95 was adopted (Peterson et al., 1993) as a minimum indicator for the evaluation of need for nitrogen fertilizer, which was necessary whenever NSI was less than or equal to 0.95.

In 2003, the first chlorophyll reading in maize was taken during stage V6 and the second reading was during flowering stage (V12). Six points were read in the central part of recently-mature leaves in the fourth leaf totally open to the top, and five plants per parcel. In the second year (2004), the first reading of maize took place during stage V4 and the second reading during stage V8 with the objective of evaluating necessity of nitrogen fertilizer for topdressing. The third reading was taken during the flowering.

Relative chlorophyll index (RIC) read by the SPAD device was transformed into chlorophyll content which was calculated by the means of a regression equation found by Zotarelli et al. (2002), following the calibration of the chlorophyll meter device with the maize crop in various soils from Paraná. Determination of chlorophyll content directly provides leaf chlorophyll concentration, since chlorophyll reading by this device provides an indirect relative chlorophyll index.

The experiment was statistically analyzed using the Scott-Knott Test (1974), through the program SISVAR (Ferreira, 2000).

Table 1 - Relative chlorophyll index (RIC) and nitrogen supply index (NSI) in leaves of maize in rotation after wheat, with nitrogen fertilizer

2003								
Treatments					**V6**		**V12 (flowering)**	
T	WS	MS	SD	Total	RIC	NSI	RIC	NSI
1	24	120	0	144	46.61 a	1.00	53.65 a	1.00
2	24	0	120	144	41.94 b	0.90	53.25 a	0.99
3	24	40	80	144	42.78 c	0.92	50.57 a	0.94
4	24	30	90*	144	40.96 b	0.88	50.73 a	0.95
5	144	0	0	144	43.86 c	0.94	43.23 b	0.81
6	0	0	0	0	41.96 b	0.88	39.20 b	0.69
Average with nitrogen					43.23	0.93	50.29	0.94
CV (%)					5.64		9.78	

2004										
Treatments					**V 4**		**V8**		**V12 (flowering)**	
	WS	MS	SD	Total	RIC	NSI	RIC	NSI	RIC	NSI
1	24	120	0	144	47.85 a	1.00	40.39 a	1.00	55.56 a	1.00
2	24	0	120	144	33.50 b	0.70	43.79 b	1.08	55.37 a	1.00
3	24	40	80	144	47.35 a	0.99	40.12 a	0.99	54.26 a	0.98
4	24	30	0*	54	47.15 a	0.99	39.21 a	0.97	46.10 b	0.83
5	144	0	0	144	33.87 b	0.71	36.65 c	0.91	51.17 b	0.92
6	0	0	0	0	33.80 b	0.71	35.61 c	0.88	50.81 b	0.91
Average with nitrogen					41.94	0.88	40.03	0.99	52.49	0.95
CV (%)					6.73		10.22		9.43	

Averages with different characters were significant to 5% probability with the Scott Knott test
WS = wheat sowing fertilizer; MS = maize sowing fertilizer; CO = maize topdressing fertilizer;
*T4 = in the first year, maize topdressing fertilizer was monitored by SPAD chlorophyll meter readings; in the second year, it was read with a chlorophyll meter, though nitrogen fertilizer was not applied. In other treatments the fertilization at side dressing not depended of foliar chlorophyll reading.

RESULTS AND DISCUSSION

Using Relative chlorophyll index (RIC) and Nitrogen Supply Index (NSI) to predict the need for nitrogen topdressing of maize following wheat

The relative chlorophyll index (RIC) values obtained through the chlorophyll meter readings (Table 1) differed in function of the nitrogen management, and of the phenological stage of maize and the culture in rotation system, which supported the findings by Godoy et al. (2003); Argenta et al. (2001); Argenta et al. (2002) that it was not possible to adopt the critical RIC for a maize crop as an indicator of nitrogen deficiency. The chlorophyll indexes found were similar to those described by Strieder et al. (2007) in which higher chlorophyll levels were found when maize was cultivated after peas and wild turnip, compared to maize following oats.

In the first year (2003), the readings during the stage V6 corresponded to NSI = 0.88 (Table 1) in the treatment monitored (T4), less than the NSI proposed by Peterson et al. (1993), a value of 0.95. This result pointed out the need for the first topdressing with nitrogen, which was carried out on the same day as the reading. In the same state (V6) in the other treatments, NSI values were lower than 0.95, which showed low N supply, possibly because readings were taken before topdressing. On the other hand, N supply of 0.98 was proposed by Rozas and Echeverria (1998) and in this case, the fertilizer was necessary for NSI less than 0.98.

After a reading during V8, nitrogen topdressing was applied, which made the NSI rise in the second reading (flowering). Rambo et al. (2007) reported the lower relative chlorophyll indexes and N supply indexes in maize undergoing doses from 120 to 480 kg ha^{-1} N in topdressing, monitored by the chlorophyll meter at stages V3, V6, V10 and during the formation of ears. These indexes corresponded to elevated grain yield.

During the flowering, the parcels in which nitrogen fertilizer was used in the wheat (T5) and to which fertilizer was not applied (T6) had low NSI, unlike the maize plants fertilized during the earlier stages, showing that N applied to wheat wasn't more available to be absorbed by maize.

In the second year (2004), NSI monitored in maize following wheat (T4) in the stages V4 and V8 was higher than the pre-established index (NSI=0.95), dispensing topdressing, which later diminished N supply index during the flowering (Table 1). Possibly, N in the initial stages could have been provided by the organic material or come from the sowing fertilizer, because of good distribution of rain (Figure 1) during the period.

In the two years studied, the relative chlorophyll index (RIC) values in V8 justified the fertilization with nitrogen in topdressing in the non-monitored treatment, which increased the NSI during the flowering. Argenta et al. (2004) reported chlorophyll meter readings higher than 45.4; 52.1; 55.3 and 58.0, respectively at the stages 3 to 4 leaves, 6 to 8 leaves, 10 to 11 leaves and during the flowering stage. The readings were adequate and it was related to high yield of maize. According to the authors, variation in the readings was higher in the initial stages of the maize crop. Schepers and al. (1992); Piekielek et al. (1992) and Rambo et al. (2008) considered the characteristics of a plant to be efficient in predicting the necessity of applying N in topdressing, among them N accumulated in the plant and relative chlorophyll index (RIC), which varied from 40.0 to 52.8 for N dose from zero to 300 kg ha^{-1} in the first year and from 36.0 to 49.5 in the second year. The authors identified greater potential for using the chlorophyll index rather than N accumulated in the leaves for the reason of practicality and availability.

In the second year during the stage V4, only when maize received fertilizer during planting (T1 = 120 kg ha^{-1} and T3 = 40 kg ha^{-1}) NSI > 0.95 was obtained (Table 1). In the other preparations, plants had low N supply, which might have happened due to sample collection taking place before the topdressing (T2).

Similarly, in stage V8, maize maintained a high NSI after receiving the topdressing (T2). The supply of the nutrient was maintained until the flowering stage, except in the preparation in which maize only received fertilizer during the sowing (T4). In this treatment, the monitoring didn't point out the need for nitrogen topdressing, which diminished the leaf chlorophyll index (Table 1).

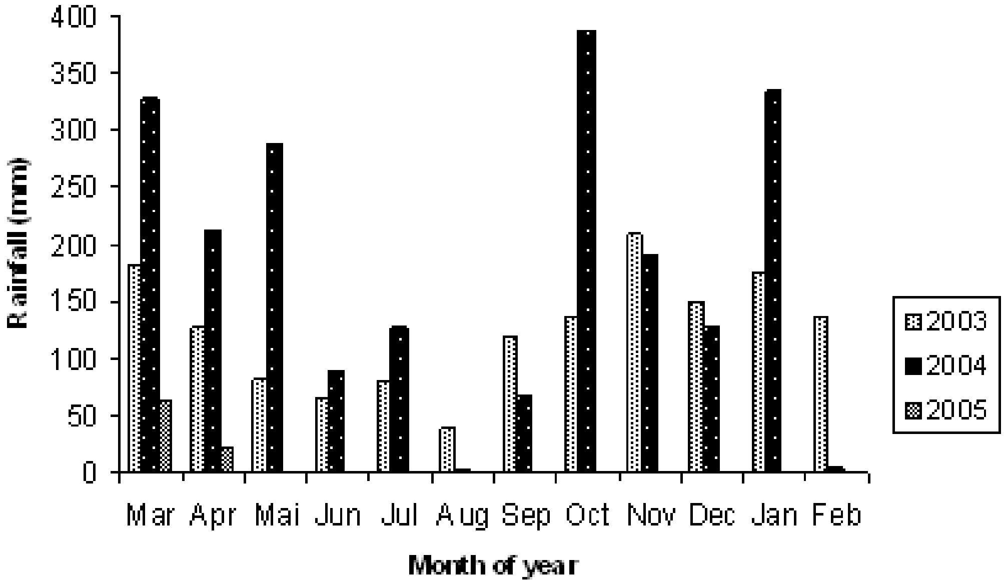

Figure 1 - Rainfall during the experimental period at the COAMO Research Farm, Campo Mourão, Paraná State, Brazil (2003 - 2005).

Relationship between nitrogen management with chlorophyll content (CC) and maize yield

The management of N fertilization caused highly significant differences in the chlorophyll content and maize yield and stages of crop development (Table 2). These results confirmed that there was a direct relationship between N absorbed and leaf chlorophyll content, similar to Fox et al. (2001); Godoy et al. (2007) and Godoy et al. (2008), who monitored green color index of grass leaves and use of green color intensity as an indicator of the need for the topdressing in maize. According to the authors, monitoring of green color index of leaves could help evaluate N availability to maize during its life cycle.

The control of nitrogen fertilizer expressively influenced maize yield (Table 2), since the nutrient was required by the plant for important functions such as protein synthesis, nucleic acid and nitrogen base synthesis.

Table 2 - Leaf chlorophyll content (g dm^{-2}) and maize yield (kg ha^{-1}) in succession of the wheat for two years related to nitrogen fertilizer management.

	Chlorophyll content of maize (1)		**Chlorophyll content of maize (2)**			**Yield of maize after wheat**	
	V6	V12	V4	V8	V12	2003	2004
Value F	11.38**	21.18**	127.63**	9.03**	8.99**	8.29**	37.42**
DMS	0.6807	1.4705	0.7268	1.0441	1.5789	820.64	1073.16
CV (%)	5.64	9.78	6.73	10.22	9.43	5.39	12.80
Average	4.16	5.19	3.73	3.52	5.78	7281.2	3911.8

(1) leaf chlorophyll reading for the first year of maize cultivation;(2) leaf chlorophyll reading for the second year of maize cultivation.

The management of N fertilization influenced the yield as well as chlorophyll content in maize leaves (Figure 2). In the first year (2003), the highest chlorophyll foliar contents (CC) corresponded to the highest maize yield (Figure 2A) for the treatments using 24-120-0 (8029 kg ha^{-1}) and 24-0-120 (8183 kg ha^{-1}) during wheat sowing, maize sowing and maize topdressing, respectively.

Maize yields during the first year were higher than those described by Santos et al. (2007), who reported the production of 7043 kg ha^{-1} for maize strain AG 1051, and 6865 kg ha^{-1} for AG 9010, when all nitrogen (120 kg ha^{-1}) was applied during the sowing. However, the maize yield found by Pauletti et al. (2010) varied from 7.328 to 10450 to maize fertilized with $NP_2O_5K_2O$ (30-60-60) and 90 kg ha^{-1} of N at top dressing.

In the second year just as the first, the chlorophyll reading (Figure 2B) followed maize yield and was higher in the treatments that received all N during the planting, 24-120-0 (4420 kg ha^{-1}), with all N in topdressing, 24-0-120 (4324 kg ha^{-1}), and when N was parceled during the planting and in topdressing, 24-40-80 (4344 kg ha^{-1}), agreeing with Bertolini et al. (2008). Arf et al. (2007) and Duete et al. (2008) also reported better dry mass production and yield when nitrogen fertilizer was used in the sowing or parceled in the sowing and topdressing. According to Arf et al. (2007), maize that received nitrogen fertilizer during the sowing for two years had yields of 7719 and 6686 kg ha^{-1}.

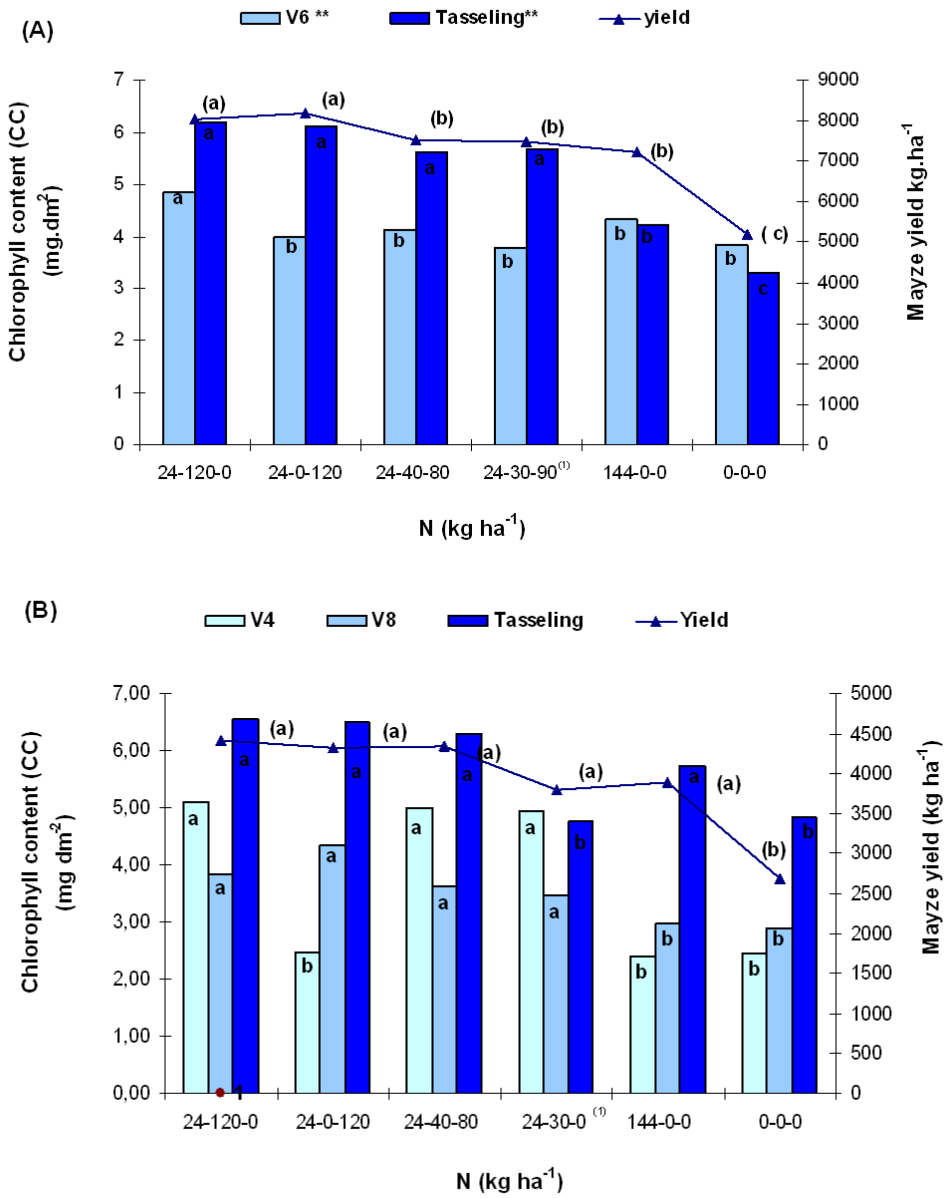

Figure 2 - Chlorophyll content (mg dm^{-2}) in column and maize yield (kg ha^{-1}), in line, cv Pioneer 30P70 in 2003 year (A) and Garra Syngenta, in 2004 (B), cultivated in succession of the wheat in no-tillage system using N management: N in wheat sowed; N in maize sowed; and N in maize topdressing. The different characters were significant using the Scott-Knott test to 5.0% probability.[(1)] treatment with monitoring of chlorophyll content as indication of the N fertilization need at side dressing. Other treatments was not monitoring to N fertilization at side dressing.

However, when N was applied at wheat sowing, the chlorophyll reading (Figure 2B) followed maize yield and they were higher in this treatments, probably because there was liberation of N that was absorbed during the maize development. This agreed with Rodrigues et al. (2000) who reported that 70% of the N accumulated into wheat plants was translocated from the vegetative parts of the plant to the grains and 30% was maintained in the wheat shoots and then released into the soil during the decomposition.

Higher chlorophyll readings were during flowering, which coincided with Waskom et al. (1998) and Argenta et al. (2001) and Argenta et al. (2004), who reported that the chlorophyll reading, mainly in initial stages, varied more than in later stages, causing errors due to the reason that there was lower demand for the nutrient.

It was noted that in the preparation monitored by the chlorophyll meter (T4) in which N in topdressing wasn't applied at V8, for NSI=0.97, there was lower yield and also lower chlorophyll content during tasseling stage (Figure 2B), which showed the direct relationship between the leaf chlorophyll and maize yield.

Chlorophyll content followed maize yield in the two years studied, corroborating Zotarelli et al. (2002) and Rambo et al. (2007) who monitored leaf N level using relative critical leaf chlorophyll level to indicate when nitrogen should be applied in the topdressing.

However, adjusting the SPAD readings for specific leaf weight improved the estimation of N in dry mass, up to 88% (Esfahani et al., 2008). SPAD readings predicted about 80% of leaf N concentration changes on the basis of leaf area. It seemed that the chlorophyll meter provided a simple, rapid, and non destructive method to estimate the leaf N concentration based on leaf area, and could be reliably exploited to predict the exact N fertilizer side dressing in rice.

Maize yields in rotation with wheat in the first year (Figure 2A) were higher than in the second year (Figure 2B), possibly because during the first year, though there was less rain, it was distributed better, which caused reduction in N loss by lixiviation and better utilization of the nutrient by the crop. In addition, topdressing was carried out in November, the season in which rain was expected. In the second year, maize was sowed in November and nitrogen topdressing was carried out in December/January, with more rains, which could justify the reduced yield grain.

Relationship among relative production and concentration of foliar N with chlorophyll content

In a similar way, when chlorophyll content was compared to the relative maize production, a lower relationship was observed between them in the initial stages, as seen in stage V4 (Figure 3A) and V8 (Figure 3B), but a higher relationship was found at tasseling (Figure 3C). Similarly, tasseling stage was the best stage to predict the concentration of N in the leaves in the blooming period (Figure 3D), corroborating with the reports by Rozas and Echeverria (1998) and Carvalho et al. (2003), in the studies with maize and bean, respectively. The positive relationship showed that when N content increased, there was an increase in the chlorophyll content.

The best correlation, at tasseling stage, hindered the prediction regarding the productivity and leaf content of N through the chlorophyll reading. The evaluation of N content by the chlorophyll had the advantage of permitting correction when necessary in the same harvest (Rocha et al., 2005), since there was a good correlation with N in leaf up to stage V8. Besides being a non-destructive method, the use of chlorophyll meter presents the advantage of not reading the absorbed N, as a 'luxurious' consumption, but reading just the content of N that is associated to the chlorophyll molecule. Thus, the measurement performed by the chlorophyll meter has been considered as a high indicative of nitrogen level in the plant than its own content (Blackmer and Schepers, 1995).

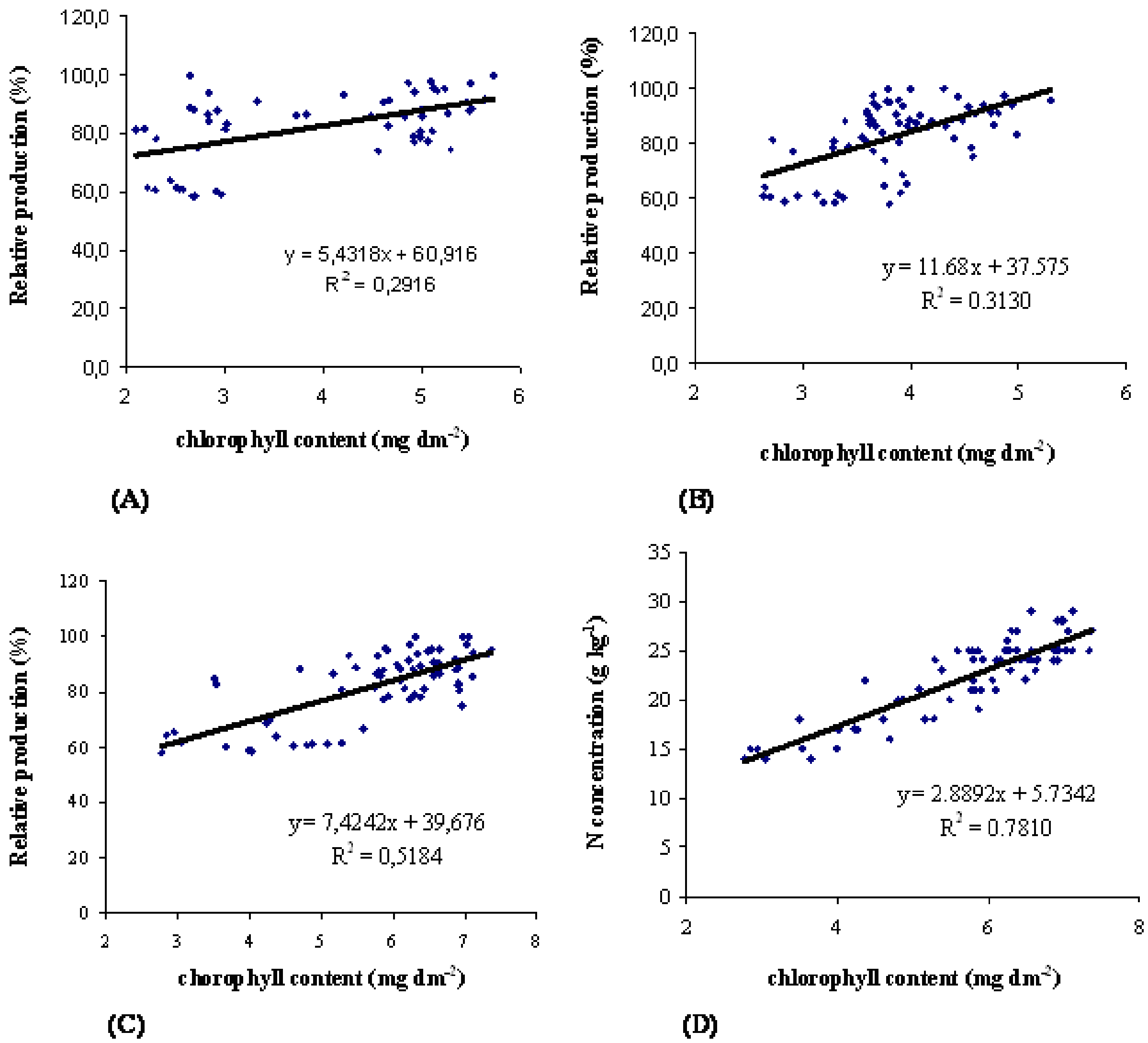

Figure 3 - Relationship among chlorophyll content and relative production of maize, in stages of development V4 (A), V8 (B), tasseling (C) and chlorophyll content and N concentration in blooming (D) in maize in succession of wheat and oat, in no tillage system.

CONCLUSIONS

The monitoring with relative index of chlorophyll (RIC) and Nitrogen Supply Index (NSI) was shown to be an efficient indicator of the need for nitrogen top dressing in maize. The N supply index (0.95) was low to indicate the need for N fertilization and compromised maize yield in the monitored treatment. The application of N increased the leaf chlorophyll content and improved maize yield, independent of fertilizer management. The foliar chlorophyll reading made during the tasseling stage related better with the nutritional state and maize yield and it showed an adequate indicative to predict this parameter.

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