

## GRAIN YIELD AND KERNEL CRUDE PROTEIN CONTENT INCREASES OF MAIZE HYBRIDS WITH LATE NITROGEN SIDE-DRESSING

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**ABSTRACT:** Physiological changes incorporated into current maize hybrids suggest the occurrence of modifications in the nitrogen uptake dynamics, improving plant ability to uptake N during grain filling. This may justify late N side-dressing whenever environmental constraints prevent adequate nitrogen supply during crops vegetative development. This study evaluates effects of nitrogen fertilization at booting and silking on grain yield and kernel crude protein contents of commercial maize hybrids. Two experiments were set up in Eldorado do Sul, RS, during the 2001/2002 and 2002/2003 growing seasons. In 2001/2002, treatments were composed of two hybrids (Agrocères 303 and Pioneer 32R21), three nitrogen rates applied during maize vegetative development (30, 80 and 130 kg ha<sup>-1</sup>) and three nitrogen rates applied at silking (0, 50 and 100 kg ha<sup>-1</sup>). In 2002/2003, four hybrids (Agrocères 303, Pioneer 32R21, Dekalb 215 and Syngenta Penta) and four nitrogen rates side-dressed at booting (0, 50, 100 and 150 kg ha<sup>-1</sup>) were assessed. There were significant increments in grain yield and kernel crude protein content with nitrogen fertilization at booting and silking. Grain yield response to late N side-dressing differed among cultivars. The impact of nitrogen fertilization at silking was higher at the smallest rate of N during the plant vegetative development. Enhancements in grain yield with late N side-dressing resulted from increases in grain weight. Modern hybrids can uptake nitrogen during silking, contradicting the hypothesis that late N side-dressing is not efficient to improve maize grain yield.

Key words: *Zea mays*, nitrogen, fertilization timing, productivity

## RENDIMENTO E TEOR DE PROTEÍNA BRUTA NOS GRÃOS DE HÍBRIDOS DE MILHO COM ADUBAÇÃO NITROGENADA DE COBERTURA TARDIA

**RESUMO:** Alterações morfo-fisiológicas introduzidas nos híbridos modernos de milho sugerem mudanças na dinâmica de absorção do nitrogênio, aumentando a habilidade da planta de absorvê-lo durante o enchimento de grãos. Isto pode justificar a utilização de coberturas nitrogenadas tardias sempre que restrições climáticas impedirem o suprimento adequado de nitrogênio durante o desenvolvimento vegetativo da cultura. Este estudo foi conduzido objetivando avaliar os efeitos da fertilização nitrogenada no emborrachamento e espigamento no rendimento e teor de proteína nos grãos de híbridos de milho. Dois experimentos foram instalados em Eldorado do Sul, RS, nos anos agrícolas de 2001/2002 e 2002/2003. Em 2001/2002, os tratamentos foram compostos por dois híbridos (Agrocères 303 e Pioneer 32R21), três doses de N aplicadas durante o desenvolvimento vegetativo (30, 80 e 130 kg ha<sup>-1</sup>) e três doses de N aplicadas no espigamento (0, 50 e 100 kg ha<sup>-1</sup>). Em 2002/2003, quatro híbridos (Agrocères 303, Pioneer 32R21, Dekalb 215 e Syngenta Penta) e quatro doses de N aplicadas no espigamento (0, 50, 100 e 150 kg ha<sup>-1</sup>) foram testadas. A fertilização nitrogenada no emborrachamento e espigamento promoveu incrementos significativos no rendimento e teor de proteína bruta dos grãos. A resposta do rendimento de grãos à cobertura nitrogenada tardia diferiu entre as cultivares. O impacto da fertilização nitrogenada no espigamento foi maior quando se aplicou baixas doses de N na fase de desenvolvimento vegetativo. Os aumentos no rendimento de grãos obtidos com coberturas tardias deveram-se principalmente ao maior peso de grãos. Os híbridos contemporâneos são capazes de absorver N depois do espigamento, contradizendo a hipótese de que coberturas nitrogenadas tardias não são eficientes para aumentar o rendimento de grãos do milho.

Palavras-chave: *Zea mays*, nitrogênio, época de aplicação, produtividade

## INTRODUCTION

Breeding plants that uptake and use N more efficiently, and developing management practices that synchronize N fertilization and stages of maize with higher N demand are important strategies to increase efficiency of nitrogen use (Raun & Johnson, 1999). The best period for N application depends on the degree of N deficiency, linked to the balance between quantity of N supplied by the soil and maize internal demand (Binder et al., 2000).

Recommendations for nitrogen fertilization rates in southern Brazil range from 10 to 30 kg ha<sup>-1</sup> on sowing, and the remaining side-dressed when the plant has three to eight expanded leaves (Amado et al., 2002). Postponing part of nitrogen fertilization to more advanced growth stages, when plants have greater capacity to take up nutrients, increase nitrogen efficiency use (NEU) (Indicações Técnicas para a Cultura do Milho no RS, 2001).

Because modern commercial hybrids are more productive and require a greater amount of N to express their yield potential the strategy of postponing part of the N fertilization to later growth stages is now more feasible (Sangoi et al., 2001). Huber et al. (1994) and Rajcan & Tollenaar (1999a) showed that modern North American hybrids presented high N absorption peaks during silking and grain filling. This response shows that there were changes in the N uptake dynamics and assimilation, in comparison to genotypes released in previous decades.

Nitrogen fertilization at more advanced phenological stages may allow the maintenance of root growth and the integrity of leaf area for a longer period of time, delaying the absorption of nitrogen and other nutrients required for high yield (Hageman & Below, 1984; Earl & Tollenaar, 1997). This may be important under circumstances that impair nitrogen side-dressing at the recommended time, such as rainy springs. The objective of this study was to assess the effect of nitrogen side-dressing at the booting and silking stages on agronomic traits of maize hybrids commercially released at different eras.

## MATERIAL AND METHODS

Two experiments were carried out in Eldorado do Sul, RS, southern Brazil (30°01'S, 51°40'W and 46m of altitude). The climate of the region is wet subtropical with hot summers (Bergamaschi et al., 2003). The soil in the experimental area is classified as Rhodic Kandudults. The soil attributes during the experimental period were: clay content - 290 g kg<sup>-1</sup>; water pH - 5.1; P - 14 mg kg<sup>-1</sup>; K - 125 mg kg<sup>-1</sup>; organic matter - 23 g kg<sup>-1</sup>; and cationic exchange capacity: 1.1 cmol L<sup>-1</sup> (Tedesco et al., 1995). Phosphorus and potassium soil contents were assessed through the Mehlich I and Mehlich II methods, respectively. Total nitrogen contents in the soil at planting were 3,204 kg ha<sup>-1</sup> at the 0-20 cm depth layer (Bayer et al., 2000).

In the 2001/2002 growing season, treatments consisted of two maize cultivars: Pioneer 32R21 (P 32R21), a single-cross, very early hybrid, released in the mid 90's; and Agrocerees 303 (AG 303), a double-cross early hybrid, released in the mid 80's. For each genotype, three rates of N were side-dressed during the vegetative period (30, 80 and 130 kg ha<sup>-1</sup>) and three rates (0, 50 and 100 kg ha<sup>-1</sup>) at silking. Trials were set up in a complete randomized block design, 2 × 3 × 3 factorial scheme (n = 4).

In the 2002/2003 growing season, in addition to the two genotypes tested early, two additional maize hybrids were tested: Dekalb 215 (DKB 215) and Syngenta Penta (PENTA), both single-cross early hybrids, released along the last five growing seasons. Each cultivar was submitted to four N rates, side-dressed at booting (0, 50, 100 and 150 kg ha<sup>-1</sup>), in a randomized split-plot design (n = 4). Hybrids were located in the main plots and the N rates at booting in the subplots.

Hybrid AG 303 was first sown at 60,000 plants per ha, on September 20, 2001, and hybrid P32R21 at 75,000 plants per ha, on October 10, 2001. Differences between sowing periods targeted matching the silking stage of both hybrids. Rates of 100 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O were applied at the AG 303 sowing date. When 50% of maize seedlings of each hybrid have emerged, 30 kg ha<sup>-1</sup> of N were applied. The mean and high rates of N in the vegetative period (80 and 130 kg ha<sup>-1</sup>) were divided in three equal doses, applied at the stages of three to four, seven to eight and ten to 11 expanded leaves. The rates of 50 and 100 kg ha<sup>-1</sup> N at silking were applied in a single operation, when 75% of the plants had visible silks (0.5 to 1.0 cm long) on the ear.

In the second growing season, hybrid AG 303 was sown on October 01, 2002, at 50,000 plants per ha; other hybrids were sown at 70,000 plants per ha. Phosphorus, potassium and nitrogen were banded in the rows during sowing, at rates of 25 kg ha<sup>-1</sup> N, 100 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 100 kg ha<sup>-1</sup> K<sub>2</sub>O. Two N side-dressing were performed in all plots during the vegetative stage: 40 kg ha<sup>-1</sup> when plants had five to six leaves, and 30 kg ha<sup>-1</sup> when plants presented ten to 11 expanded leaves. Rates of N at booting were applied in a single dose, on the same day for all the hybrids, when 75% of the earliest hybrid plants (P 32R21) had 15 expanded leaves, corresponding to a period of five to 15 days before silking for the earliest (P 23R21) and the latest (AG 303) hybrid, respectively.

Both trials were hand-sown, in a no-till sowing system, 0.7 m row spacing. Urea was used as the N source. Weeds, pests and diseases were controlled throughout the cropping cycle so that they did not interfere in grain yield. A spray system, with 10 mm h<sup>-1</sup> flow, was used to irrigate the trials whenever the water potential in the soil was lower than -0,04 MPa.

Each subplot comprised five, 6-m long rows, a total area of 21 m<sup>2</sup>. Grain yield, yield components and kernel crude protein contents were determined. Grain yield was assessed in the three central rows, excluding 0.5 m at each row end, comprising an experimental area of 8.4 m<sup>2</sup>. It was corrected to standard moisture of 13%, and extrapolated to one hectare. Individual kernel weight was determined by counting and weighing 400 grains per experimental unit. Each value was divided by 400 and corrected to the moisture of 13%. The number of grains per m<sup>2</sup> was obtained through the ratio between the grain mass produced per m<sup>2</sup> and the mass of one grain. A grain sample of 20 g was ground in a centrifuge-type grinder to determine crude protein contents. The N contents of grains were obtained by the Kjeldahl method, (Tedesco et al, 1995) and multiplied by 6.5 (1% N corresponds to 6.25 g protein), to calculate grain protein contents.

An analysis of variance was performed separately for each growing season (F teste;  $\alpha = 0.05$ ). Comparison among means was carried out using the Tukey's test ( $\alpha = 0.05$ ) during the first growing season. In the second experiment, data from significant effects were submitted to regression analysis, testing linear and quadratic models.

## RESULTS AND DISCUSSION

In 2001/2002, grain yield was affected by interactions between N levels in the vegetative period  $\times$  N rates at silking and hybrids  $\times$  N rates at silking (Tables 1 and 2). Grain yield response of the two hybrids to nitrogen side-dressing at silking depended on the rate of N supplied during the vegetative period (Table 1). At the lowest N rate, grain yield increased 2.8 and 3.6 t ha<sup>-1</sup>, with the application of 50 and 100 kg ha<sup>-1</sup> N at silking, respectively, compared to the control. At the highest N level,

Table 1 - Grain yield and number of grains per ear at three nitrogen levels (N) side-dressed in the vegetative period and two N rates applied at silking, in the average of two maize hybrids<sup>1</sup>.

Nitrogen levels applied in the vegetative period	Rates of nitrogen applied at silking		
	0	50	100
----- kg ha <sup>-1</sup> -----			
Grain yield (t ha <sup>-1</sup> )			
30	B 4.4 c	A 7.2 c	A 8.0 c
80	A 10.4 b	A 11.1 b	A 11.3 b
130	B 11.4 a	AB 12.1 a	A 13.1 a
Grains per ear (n <sup>o</sup> )			
30	B 282 b	A 363 b	A 369 b
80	A 540 a	A 528 a	A 524 a
130	A 556 a	AB 528 a	A 548 a

<sup>1</sup> Means followed by the same lower case letter in the column and preceded by the same upper case letter on the line - not differ (Tukey's test,  $\alpha = 0.05$ ).

increases were 0.7 and 1.7 t ha<sup>-1</sup>, respectively, with the application of 50 and 100 kg ha<sup>-1</sup> N at silking. There were no responses to N application at silking for the intermediary N rate in the vegetative period.

Grain yield of the two hybrids differed in relation to N application at silking, (Table 2). The double-cross hybrid AG 303 responded up to the application of 50 kg ha<sup>-1</sup> N, increasing 1.5 t ha<sup>-1</sup> in yield. The single-cross hybrid P 32R21 responded up to the application of 100 kg ha<sup>-1</sup> N at silking, with increases in yield of 2.7 t ha<sup>-1</sup>, in comparison to the treatment without N application at this stage.

In the experiment carried out in 2002/2003, interactions between hybrids and N rates applied at booting affected grain yield. The response of grain yield to N application at booting was linear for PENTA and quadratic for the other hybrids (Figure 1). For each kg of N applied at booting, an increase of 14 kg in the grain yield of PENTA was recorded. The estimated N rates at boot-

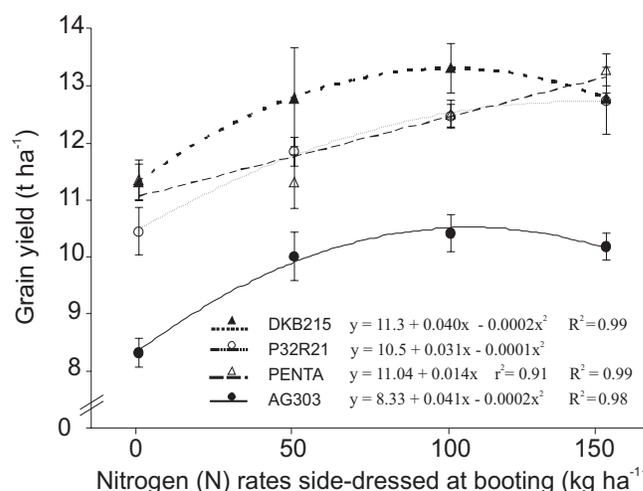


Figure 1 - Grain yield of four maize hybrids as a function of four nitrogen (N) rates side-dressed at booting. Error bars represent the standard error of mean (Tukey's test;  $\alpha = 0.05$ ).

Table 2 - Grain yield and kernel crude protein content of two maize hybrids and three rates of nitrogen (N) applied at silking, in the average of three N rates side-dressed during the vegetative period<sup>1</sup>.

Hybrids	Rates of nitrogen applied at silking		
	0	50	100
----- kg ha <sup>-1</sup> -----			
Grain yield (t ha <sup>-1</sup> )			
Agroceres 303	B 7.9 b	A 9.4 b	A 9.2 b
Pioneer 32R21	C 9.7 a	B 10.8 a	A 12.4 a
Kernel crude protein content (%)			
Agroceres 303	B 6.9 a	B 8.2 a	A 10.5 a
Pioneer 32R21	AB 7.3 a	B 7.1 a	A 8.5 b

<sup>1</sup> Means followed by the same lower case letter in the column and preceded by the same uppercase letter on the line - not differ (Tukey's test,  $\alpha = 0.05$ ).

ing that optimized grain yield were 103, 150 and 100 kg ha<sup>-1</sup> for AG 303, P 23R21 and DKB 215, respectively. Confirming the results of the first experiment, the double-cross hybrid AG 303 presented lower grain yields than the more recent single-cross hybrids, regardless of nitrogen rates applied at booting.

Enhancements in grain yield with late nitrogen side-dressing in 2001/2002 can be attributed to increasing number of grains per ear, when 50 kg ha<sup>-1</sup> of N were applied at silking during the vegetative period (Table 1). Conversely, the increase in grain weight was responsible for higher yields when the rate of N applied at silking increased from 0 to 100 kg ha<sup>-1</sup>, regardless of the amount of N applied in the vegetative period or the kind of hybrid tested in the trial (Table 3). The number of ears produced per area was not affected by N application at silking or booting (data not presented). This may have occurred because this yield component had already been defined previously to the timing of N application. Another factor that may have prevented the response of this component was that the rate of N available in the vegetative period was relatively high (95 kg ha<sup>-1</sup>) in the experiment with N application at booting.

Table 3 - Grain weight at three nitrogen rates (N) applied at silking, in the average of two maize hybrids and three N rates side-dressed in the vegetative period<sup>1</sup>.

Rates of nitrogen applied at silking kg ha <sup>-1</sup>	Grain weight mg
0	280 c
50	300 b
100	320 a

<sup>1</sup>Means followed by the same lower case letter in the column did not differ (Tukey's test;  $\alpha = 0.05$ ).

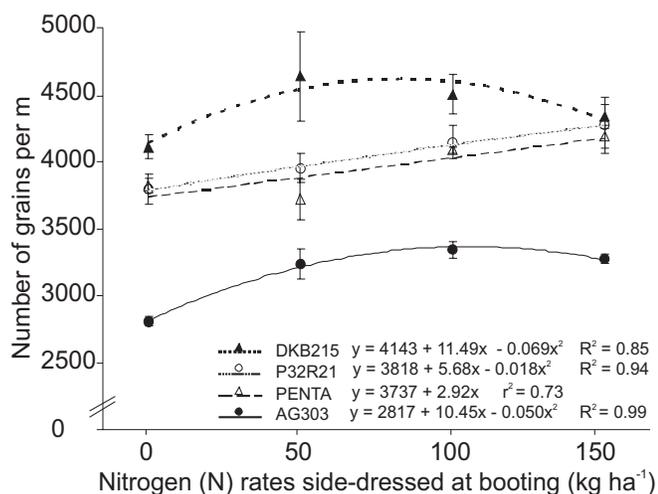


Figure 2 - Number of grains of four maize hybrids as a function of four nitrogen (N) rates side-dressed at booting. Error bars represent the standard error of mean values (Tukey's test;  $\alpha = 0.05$ ).

The number of grains m<sup>-2</sup> (Figure 2) and grain weight (Figure 3) of PENTA increased linearly with increasing rates of N fertilization at booting. These increases were 2.92 grains m<sup>-2</sup> and 0.13 g, for each kg of applied N, respectively. The response of these yield components in the other hybrids was quadratic. The N rates side-dressed at booting that optimized the number of grains m<sup>-2</sup> were 104, 125 and 83 kg ha<sup>-1</sup> for the hybrids AG 303, P 32R21 and DKB 215, respectively. Rates of nitrogen fertilization that resulted in heavier grains were 111, 125 and 48 kg ha<sup>-1</sup> for the hybrids AG 303, P 32R21 and DKB 215, respectively.

The greater grain yield of more recent hybrids P32R21 and Penta, in comparison to hybrids released in the mid 80's, AG 303 for instance, can be associated to the higher number of grains per area (Figure 2) in 2002/2003, and to the combined effect of grain weight and number of grains per ear (Tables 1 and 3) in 2001/2002. In both trials, the weight of 1,000 grains was the yield component that showed the most consistent response to N fertilization at booting and silking.

Nitrogen application at silking also increased kernel crude protein content (Table 2), up to the application of 100 kg ha<sup>-1</sup> N, grain protein percentage being 52 and 16% higher than the control for AG 303 and P 32R21, respectively. This response showed that N applied during flowering was taken by the plant and accumulated in the grains. Nitrogen side-dressing at booting affected grain protein contents of tested hybrids in a different way (Figure 4). The response was linear for AG 303, P 32R21 and DKB 215, with increases of nine, 15 and 41% in protein content for each 100 kg N applied at booting. The optimal rate of N at booting to maximize the PENTA crude protein content was 100 kg ha<sup>-1</sup>.

There was an inverse relationship in the quantitative and qualitative responses of maize kernels to nitrogen side-dress at booting. PENTA presented linear in-

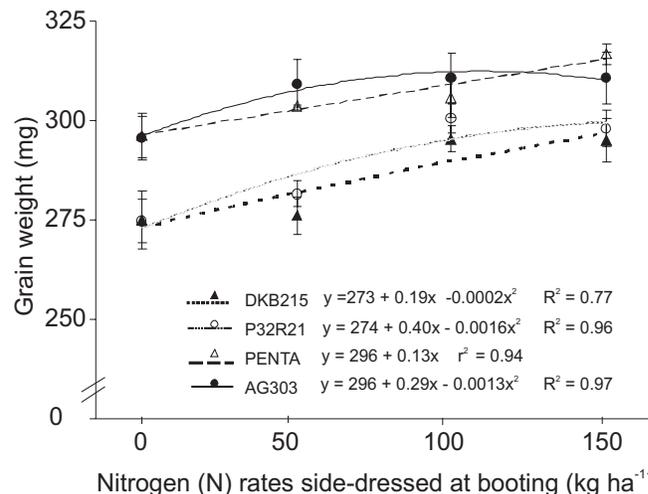


Figure 3 - Grain weight of four maize hybrids as a function of four nitrogen (N) rates side-dressed at booting. Error bars represent the standard error of mean values (Tukey's test;  $\alpha = 0.05$ ).

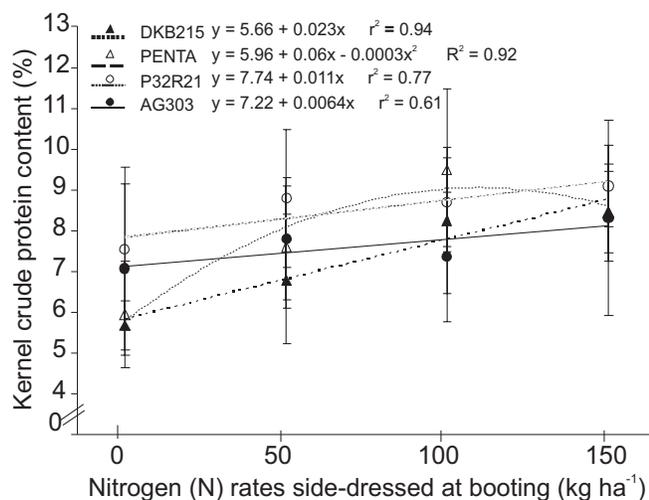


Figure 4 - Kernel crude protein content of four maize hybrids as a function of four nitrogen (N) rates side-dressed at booting. Eldorado do Sul, RS, Brazil, 2002/2003. The error bars represent the standard error of mean values (Tukey's test;  $\alpha = 0.05$ ).

crease in grain yield and quadratic response for grain protein content, while the other hybrids showed quadratic increases in grain yield and linear responses in grain protein contents (Figures 1 and 4). Although no increases in grain yield with high N rates were recorded, kernels of AG 303, P 32R21 and DKB 215 continued demanding N and storing the nutrient as protein.

N side-dressing at silking mitigates loss of grain yield potential, mainly when N supply during the vegetative stages is insufficient, and nitrogen deficiency more accentuated at flowering. According to Binder et al. (2000), when nitrogen deficiency is greater at the beginning of maize development, N should be side-dressed earlier to obtain maximum grain yield. However, the determination of an ideal period for N application differs from the definition of a phenological stage where it is still possible to get a yield positive response to fertilizer application. Grain yield and kernel crude protein contents increases can be obtained with late N application whenever there is deficiency of this nutrient during the vegetative period, although the crop yield potential has already been hindered and can no longer be reached. An economically viable response can be observed with the application of mineral nitrogen up to silking under undesirable climatic conditions that either delay N application, or favor high N losses during maize vegetative period (Scharf et al., 2002). This was the case in the first experiment. Application of 50 kg ha<sup>-1</sup> N at silking promoted 63.6% increment in grain yield over the control, receiving only 30 kg ha<sup>-1</sup> during the crop vegetative period.

The differential response to late N side-dressing among cultivars developed in different periods may have occurred because many current hybrids keep the integrity of leaf area for a longer period, which contributes to the greater synthesis of photoassimilates during grain fill-

ing. The stay-green trait has been emphasized by many breeding programs because delay in leaf senescence is associated with higher grain yield (Wolfe et al., 1988; Dwyer & Tollenaar, 1989; Dwyer et al., 1991; McCullough et al. 1994). The prolonged maintenance of leaf area for photoassimilate production during grain filling and the ability to up take N from the soil after flowering characterize maize hybrids with highly efficient use of N (Dwyer et al. 1995). The capacity of maize to maintain N uptake during the filling period may be related to the supply of carbohydrates to the root (Rajcan & Tollenaar, 1999a). Therefore, considering that older hybrids, such as AG 303, established a weak drain during the beginning of kernel formation due to their lower yield potential, it is more likely that poor sinking lead to a reduction in N demand inside the plant, decreasing N uptake by roots after silking, and its remobilization from the leaves and stem to the grains (Sangoi et al., 2001). It can thus be speculate that many modern hybrids, such as P23R21 and PENTA, have greater capacity to uptake N from the soil and to mobilize it from the vegetative tissues to the grains after silking, resulting in delayed leaf senescence, prolonged filling period and heavier grain production. This physiological sequence of events elicits a more balanced source:demand ratio, resulting in higher N efficiency use (Rajcan & Tollenaar, 1999a; 1999b).

Grain protein quality was not assessed in the study. However, protein quality (type and quantity of amino acids present) is a relevant factor for producers and consumers, especially when grain quality determines the final price of the commodity. Tsai et al. (1992) showed that increases in protein content in maize grains driven by nitrogen side-dressing were derived from greater quantity of zein. This protein is deficient in lysine and triptophane, two essential amino acids for species such as pigs and poultry. Consequently, it is possible that protein quality is not improved with late N side-dressing. Another possible advantage of increasing grain protein content with late N side-dressing is reducing kernel susceptibility to breakage at harvesting, a feature that allows greater aggregation of commercial value to the product (Tsai et al., 1992).

Several modern hybrids can up take up and assimilate N efficiently during silking, contradicting the premise that the impact of nitrogen side-dressing after flowering on grain yield is negligible. The use of late nitrogen side-dressing is advantageous particularly when adequate quantities of N are not supplied during the vegetative period. This situation commonly occurs under water stress, caused either by deficient or excessive rainfall during the crop cycle. Another potential advantage of spreading nitrogen side-dresses in a longer period of time is the reduction of N losses from volatilization and leaching, lowering negative impact on environmental resources and human health. This is particularly important

Table 4 - Comparison of maize yield at identical total N rates and different times of nitrogen application, in the average of two maize hybrids.

Total N rate vegetative + silking kg ha <sup>-1</sup>	Grain yield		
	t ha <sup>-1</sup>		
80	10.4 (80 + 0) <sup>1</sup>	7.2 (30 + 50)	-
130	11.4 (130 + 0)	11.1 (80 + 50)	8.0 (30 + 100)
180	12.1 (130 + 50)	11.3 (80 + 100)	-

<sup>1</sup>The two values in parenthesis represent the N rate at vegetative stage and silking, respectively.

when high N rates are used during the entire crop cycle, a common situation in production systems designed to reach yield rates close to the crop's fall potential.

On the other hand, N use efficiency is usually higher when applications are carried out when maize has three to eight expanded leaves (Amado et al., 2002). At each total N rate, yield was always higher when all or most N was applied before silking than when part of total N was applied at silking (Table 4). This illustrates the importance of N fertilization during vegetative stage. As a result, N applications at booting or silking should not be recommended as part of routine N application scheme, but rather as a remedy in case of undesirable crop nutrition when entering reproductive phase. In addition to that, the availability of suitable machinery to apply N at late growth stages is a limiting factor to the adoption of this management strategy. Nonetheless, the offer of high-clearance applicators is increasing (Scharf et al., 2002). Furthermore, the demand for more modern agricultural equipment has been met by the industry whenever the effectiveness of a new technique is worthwhile. An example of this situation occurred with the development of sowing machinery designed specifically for the no-till sowing system. Ferti-irrigation is another alternative to carry out late N side-dressing in areas where irrigation is available. On small farms, this crop management practice can be performed manually by producers.

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