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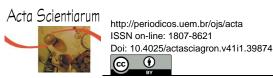
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# Can an increase in nitrogen rate mitigate damages caused by uneven spatial distribution of maize plants at the sowing row?

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**ABSTRACT.** An uneven distribution of maize plants at the sowing row can decrease grain yield. This work was carried out to evaluate the effect of an increasing nitrogen rate on maize agronomic performance under different variation coefficients of plant spatial distributions at the sowing row. The experiment was conducted in Lages, Santa Catarina State, South Brazil. Three variation coefficients (VC) of plant spatial distribution (0, 50, and 100%) were tested. At the 0 level, seeds were evenly distributed 17 cm from each other. In the other treatments with uneven spatial distributions, the distances between two neighbouring seeds ranged from 2 to 54 cm. Four nitrogen side-dress rates (0, 125, 250, and 375 kg N ha<sup>-1</sup>) were evaluated. The increase in VC of plant spatial distribution decreased the leaf area and the relative chlorophyll content of the index leaf at silking, regardless of the nitrogen rate. When the variation coefficient of seed placement was enhanced from 0 to 100%, such behaviour contributed to a decrease in the number of kernels per ear and the grain yield. Therefore, increasing the amount of N was not an efficient strategy for preventing yield losses caused by irregular plant spatial distribution at the sowing row.

Keywords: Zea mays; spatial variability; nitrogen side-dress; grain yield.

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#### Introduction

Maize is a crop that is very sensitive to intra-specific competition. The temporal and spatial uniformity of plant distribution at the sowing row is important for mitigating this kind of competition (Sangoi et al., 2012a). The space among seeds, the evenness in depth of seed deposition, the tractor speed during sowing, and the soil temperature and moisture from the time of sowing to the emergence of crops are factors that affect maize stand uniformity (Lauer & Rankin, 2004; Liu, Tollenaar, Stewart, & Deen 2004a).

The spatial variation between neighbour plants in the sowing row can affect maize development and grain yield. Works carried out by Andrade and Abbate (2005), Sangoi et al. (2012a), and Tollenaar, Deen, Echarte, and Liu (2006) reported that the presence of some spots with crowded plants within the row and the presence of other spots with empty spaces lacking plants reduced the ability of crops to use solar radiation, water, and nutrients, even when the required density was achieved. This situation enhanced the number of dominated individuals that had delayed phenological growth, reduced leaf area and compromised ear development.

The impact of uneven spatial distribution on maize grain yield depends on the unevenness intensity, plant density, hybrid type, solid fertility and desired productivity level (Luque, Cirilo, & Otegui, 2006; Pagano & Maddonni, 2007; Tollenaar & Lee, 2002).

Uneven stands are common in Brazilian maize crops. Several experiments have been conducted around the world by Andrade and Abbate (2005), Ciampitti and Vyn (2011), Lauer and Rankin (2004), Liu, Tollenaar, Stewart, and Deen (2004b), Martin et al. (2005), Sangoi et al. (2012a), and Tollenaar et al. (2006), to measure the consequences of irregular plant emergence on maize agronomic performance. However, few studies have focused on identifying management strategies that mitigate such stress or on elevating grain yield of crops with sowing problems.

An adequate nutrient supply is fundamental to achieving high productivity and attenuating biotic and abiotic stresses (Vargas et al., 2012). Nitrogen (N) is the mineral element taken up in the highest amounts by plants of

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the *Poaceae* family. Nitrogen directly affects the leaf area dimension and duration of *Poaceae* (Sangoi et al., 2015). Furthermore, nitrogen has a fundamental role in protein accumulation and grain yield determination (Ferreira, Oliveira, Von Pinho, & Queiroz, 2007).

The increment of nitrogen rate after plant emergence can help mitigate plant competition for environmental resources and reduce grain yield variability when compared to conditions of low N availability (Boomsma, Santini, Tollenaar, & Vyn, 2009). As nitrogen availability increases, maize biological yield and harvest index also increase (Ciampitti & Vyn, 2011; Echarte & Andrade, 2003).

Despite the progress that has been made on the identification of mechanisms involved in intra-specific competition in maize, the response of plants that are poorly spatially distributed at the sowing row to nitrogen fertilization has not been properly addressed in the literature (Kovács & Vyn, 2014). This intra-specific competition can affect plant biomass and harvest index. Nonetheless, it is not clear how maize grain yield is affected by nitrogen fertilization in crops with uneven stands.

Nitrogen side-dress is a management practice used in nearly all Brazilian maize crops to supply this very unstable nutrient during the phenological stage when the plant has a higher capacity to uptake N (Vargas, Sangoi, Ernani, Picoli, & Cantarella, 2015). Therefore, changing the rate of nitrogen side-dress can also be used as a strategy to alleviate the stress caused by the irregular plant spatial distribution.

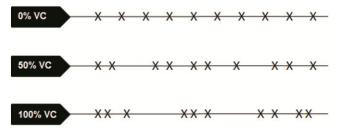
This work was carried out to evaluate the effect of increasing N side-dress rates on maize agronomic performance under different variation coefficients of plant spatial distribution at the sowing row.

#### Material and methods

The study was conducted in the city of Lages, Santa Catarina State, in the highlands of southern Brazil, during the growing seasons of 2012/2013 and 2013/2014. In the first growing season, the experiment was conducted under natural rainfall conditions. In the second growing season, the experiment was irrigated whenever soil tension was below -0.04 MPa. The experimental site is located at a latitude of 27° 52' S, a longitude of 50° 18' W, and an elevation 900 m above sea level. The climate of the region is classified as Cfb, presenting mild summers, cold winters and adequate rainfall throughout the whole year.

The soil at the study site was an Oxisol (Hapludox) with the following chemical characteristics: clay content: 560 g kg<sup>-1</sup>; organic matter content: 60.0 g kg<sup>-1</sup>; water pH: 5.2; SMP pH: 5.7; phosphorus: 4.4 mg dm<sup>-3</sup>; potassium: 186 mg dm<sup>-3</sup>; calcium: 5.79 cmol<sub>c</sub> dm<sup>-3</sup>; magnesium: 2.47 cmol<sub>c</sub> dm<sup>-3</sup>; aluminium: 0.2 cmol<sub>c</sub> dm<sup>-3</sup>; and CTC: 8.94 cmol<sub>c</sub> dm<sup>-3</sup>.

A randomized block design arranged into split plots was used, with four replicates per treatment. Three levels of plant spatial distribution at the sowing row were tested in the main plots and were equivalent to 0, 50, and 100% of the variation coefficient. At the 0 level, seeds were evenly distributed 17 cm from each other. In the other treatments with uneven spatial distribution, the distances among seeds ranged from 2 to 54 cm. They were calculated using the software Minitab and the randomising function with a normal data distribution. In this function, the average, standard deviation and number of data (i.e., plants per row) were provided. For each variation coefficient percentage, the software randomly generated the plant position at the sowing row. According to the calculated position, strings were marked so that seeds were placed exactly at the desired position. Figure 1 presents a schematic view of plant distribution for each level of plant spatial distribution. Seeds were placed at a depth of 5 cm in all treatments.



**Figure 1.** Maize seed position according to the variation coefficient of plant spatial distribution at the sowing row. In the treatment with 0% CV, the distance between two adjacent seeds was 17 cm. In the treatments with 50% CV and 100% CV, the distances between two adjacent seeds ranged from 2 cm to 54 cm.

Four nitrogen side-dress rates were evaluated in the split plots and were equivalent to 0, 0.5 (125 kg ha<sup>-1</sup>), 1.0 (250 kg ha<sup>-1</sup>), and 1.5 (375 kg ha<sup>-1</sup>) times the rate recommended to achieve a grain yield of 18,000 kg ha<sup>-1</sup>. Nitrogen was side-dressed in three equal parts when plants were at the V4, V8, and V12 growth stages. Urea was used as the nitrogen source. Each split plot comprised four rows, which were 0.7 m apart and 7 m long. All measurements were taken from the two central rows, leaving margins of 0.5 m at the ends of each row.

The experiment was hand-planted on October 19th during both growing seasons. A no tillage system was used, over a layer of dead white oat (*Avena sativa*). On the sowing day,  $30 \text{ kg ha}^{-1}$  of N,  $295 \text{ kg ha}^{-1}$  of  $P_2O_5$ , and  $170 \text{ kg ha}^{-1}$  of  $P_2O_5$ , and  $P_2$ 

To control insects and diseases during the early stages of crop development, seeds were industrially treated with the insecticide tiametoxam (140 g a.i.  $100 \text{ kg}^{-1}$ ) and the fungicides fludioxonil + metalaxyl (25 +  $10 \text{ g a.i.} 100 \text{ kg}^{-1}$ ). Weeds were controlled with two herbicide applications. The first application included a combination of atrasine (1,400 g a.i. ha<sup>-1</sup>) and metolachlor (2,100 g a.i. ha<sup>-1</sup>) and was carried out immediately after sowing and prior to plant emergence. The second application was performed when plants were at the V4 stage and included tembotriona (100 g a.i. ha<sup>-1</sup>). The army worm (*Spodoptera frugiperda*) was controlled by spraying the insecticides lufenuron + lambda-cyhalothrin (15 + 7.5 g a.i. ha<sup>-1</sup>) when the crop reached the V6 and V12 growth stages. To prevent the incidence of foliar diseases, a mixture of the fungicides azixistrobina + ciproconazol (60 + 25 g a.i. ha<sup>-1</sup>) was sprayed over the canopy when plants were at the V12 and V18 stages.

Six neighbour plants were marked at the V4 stage and used to estimate leaf area and leaf relative chlorophyll content when maize reached the R1 (silking) stage. To determine leaf area (LA), the length (L), and the largest width (W) of all green leaves were measured, and LA was calculated using the following expression:  $LA = L \times W \times 0.75$ , where 0.75 is a correction coefficient to compensate for the fact that maize leaves do not present a rectangular shape. The index leaf (the leaf below the ear insertion node) was used to estimate leaf relative chlorophyll content. This evaluation was performed using the Minolta SPAD 502 chlorophyll meter.

Harvests were performed on 4/23/2013 and 4/24/2014, when all leaves were senesced and grain moisture ranged from 180 to 220 g kg<sup>-1</sup>. Ears from the six marked plants where leaf area was determined were collected separately, manually shelled, and used to estimate the number of kernels produced from each female inflorescence. The other ears were harvested, mechanically shelled, and kernels were oven dried at 65°C until they presented a constant mass. The grain yield and the weight of 1,000 grains were determined and expressed in a standard moisture of 130 g kg<sup>-1</sup>.

The data from each growing season were statistically evaluated by a variance analysis using the F test at significance levels of 1% and 5%. When the F values were significant, the averages were compared using Tukey's test and polynomial regression analysis. Both comparisons were conducted at the significance levels of 1% and 5%.

### **Results and discussion**

Grain yield ranged from 5,414 to 16,064 kg ha<sup>-1</sup> in 2012/2013 and from 7,801 to 15,321 kg ha<sup>-1</sup> in 2013/2014. Grain yield depended on plant spatial variability at the sowing row and nitrogen rate. During the first and second growing seasons, the experimental average yields were 11,201 and 12,326 kg ha<sup>-1</sup>, respectively.

The variance analysis indicated similar treatment effects on the experimental variables assessed during both growing seasons. The experimental variables were significantly affected by the main effects of the variation coefficient of plant spatial distribution at the sowing row and by nitrogen rate but not by the interaction between these two factors (Table 1).

The treatments with plants that were evenly distributed at the sowing row presented higher grain yields than those with 100% VC of plant spatial distribution, regardless of the nitrogen side-dress rate. For each 10% increase in the variation coefficient of plant spatial distribution, there was a significant linear decrease

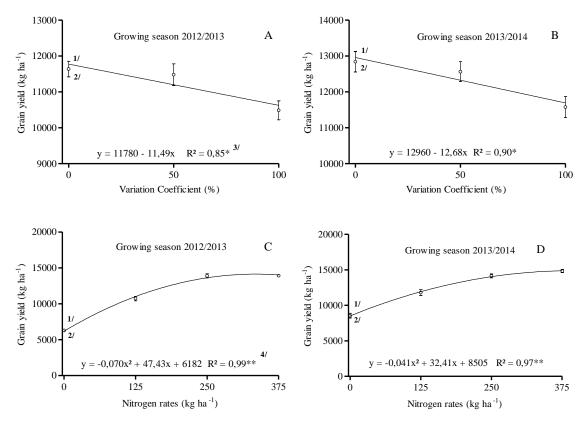
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in grain yield. This decrease was equivalent to 115 kg ha<sup>-1</sup> in the first growing season (Figure 2A) and 127 kg ha<sup>-1</sup> in the second growing season (Figure 2B), and these values were based on the average of four nitrogen rates. In both years, the increase in the nitrogen side-dress dose enhanced the grain yield. The estimated N rates that maximized grain yield were 338 kg ha<sup>-1</sup> in 2012/2013 (Figure 2C) and 395 kg ha<sup>-1</sup> in 2013/2014 (Figure 2D), according to the quadratic equations that were adjusted to the data.

**Table 1.** Calculated F values according to the variance analysis and variation coefficients for the main plots and split plots for the agronomic traits assessed in the experiment. Lages, Santa Catarina, Brazil.

Agronomic trait	Plant spatial variability (V)	Nitrogen rate (R)	V × R	CV main plot (%)	CV split plot (%)		
		Growing season of 2012/2013					
Grain yield	5.54 *	287.8 **	1.28 ns	8.18	5.7		
Grains per ear	5.33 *	19.03 **	0.52 ns	10.18	13.79		
Mass of 1,000 grains	20.02 **	74.17 **	2.14 ns	1.35	2.88		
Leaf area	5.69 *	24.86 **	2.33 ns	10.49	10.39		
SPAD reading	1.93 ns	75.57 **	2.12 ns	8.86	6.54		
	Growing season of 2013/2014						
Grain yield	5.44 *	104.7 **	0.33 ns	8.02	6.74		
Grains per ear	6.21 *	10.62 **	0.79 ns	12.13	15.23		
Mass of 1,000 grains	3.23 *	34.22 **	2.71 ns	4.38	3.41		
Leaf area	28.58 **	47.05 **	1.58 ns	6.01	5.36		
SPAD reading	7.11 *	78.70 **	1.19 ns	6.5	5.1		

<sup>\*</sup>Significant values at 5% (p < 0.05); \*\*significant values at 1% (p < 0.01); \*svalues not significant at the 5% or 1% significance levels.



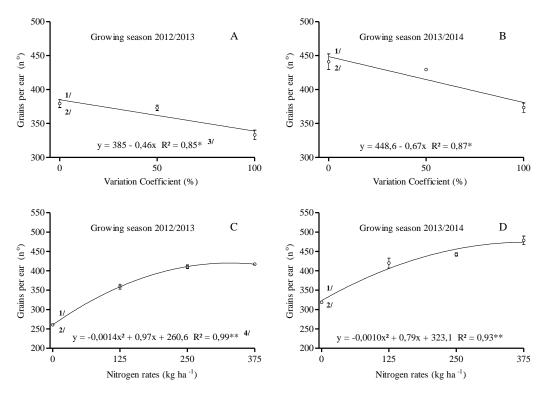
**Figure 2.** Grain yield of maize as affected by the variation coefficient of plant spatial distribution at the sowing row (A, B) and nitrogen side-dress rates (C, D). Lages, Santa Catarina, Brazil. <sup>1</sup>Each point represents the average of four nitrogen rates (A, B) or three variation coefficients (C, D). <sup>2</sup>Vertical bars indicate each average standard error; <sup>3</sup>\*Equation significant at the significance level of 5%.

4/\*\*Equation significant at the significance level of 1%.

The results of the present work corroborate the data reported by Sangoi et al. (2012a), who observed yield losses of 128 kg ha<sup>-1</sup> and 83 kg ha<sup>-1</sup> for each 10% increment in the variation coefficient of plant spatial distribution at the sowing row. On the other hand, Lauer and Rankin (2004) and Liu, Tollenaar, Stewart, and Deen (2004c) carried out experiments in the United States and Canada and did not detect decreases in yield caused by irregularities in plant spatial distribution.

The divergences found in the literature regarding the effects of plant spatial variability at the sowing row on maize agronomic performance can be related to the traits of the genotype used in each experiment. Echarte and Andrade (2003) noted that, compared to hybrids cultivated in the previous century, hybrids grown in the XXI century presented higher reproductive plasticity, more stability and greater harvest index when subjected to stresses. The soil temperature and moisture, the meteorological conditions and the methodology used to simulate spatial variability can also contribute to explaining the contrasting effects of irregular plant distribution on maize grain yield (Liu et al., 2004c).

The reduction in grain yield caused by unevenness in plant spatial distribution is due to the increase in intra-specific competition for environmental resources, such as water, solar radiation and nutrients. This situation favours the appearance of dominated plants. The dominated plants are very sensitive to intra-specific competition, producing small ears with few kernels (Pagano & Maddonni, 2007). The results presented in Figure 3 confirm this hypothesis. Though the increase in nitrogen side-dress augmented the number of kernels harvested per ear (Figure 3C and D), based on the average of nitrogen side-dress rates (Figure 3A and B), there was a linear decrease in the number of grains produced per ear when the variation coefficient of plant spatial distribution was enhanced from 0 to 100%. The ears produced by plants at the highest spatial variability level had 46 and 67 fewer grains than did the ears produced by plants that were evenly distributed at the sowing row in 2012/2013 and 2013/2014, respectively.



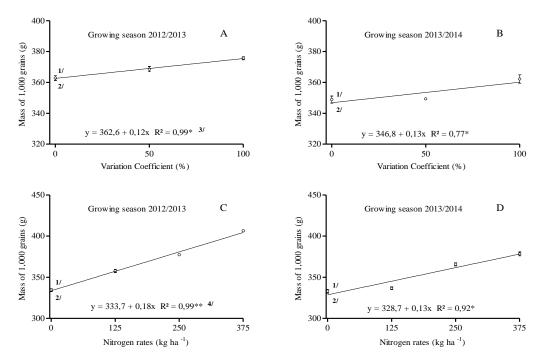
**Figure 3.** Grains per ear of maize as affected by the variation coefficient of plant spatial distribution at the sowing row (A, B) and nitrogen side-dress rates (C, D). Lages, Santa Catarina, Brazil. <sup>1</sup>Each point represents the average of four nitrogen rates (A, B) or three variation coefficients (C, D). <sup>2</sup>Vertical bars indicate each average standard error. <sup>3/\*</sup>Equation is significant at the significance level of 5%. <sup>4/\*\*</sup>Equation is significant at the significance level of 1%.

On the other hand, the data from Figure 4 indicate that there was an increase in 1,000 grain mass with the increment in nitrogen rate and the plant spatial variability at the sowing row. Such behaviour demonstrates that increasing kernel mass (Figure 4A and B) is a plant strategy used to compensate for the reduction in the number of grains produced per ear (Figure 3A and B). In agreement with that, Ciampitti and Vyn (2011) emphasised that the reduction in productive plants per area may be partially mitigated by the increment of yield components of dominated plants.

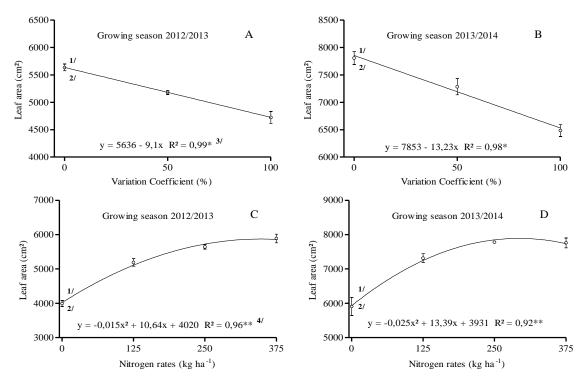
There was a significant reduction in maize leaf area at silking with the increment of plant spatial variability based on the average nitrogen rate (Figure 5). For each 10% of VC augmented at the sowing row, plants lost 91 and 132 cm<sup>2</sup> of green leaves at flowering in the first (Figure 5A) and second (Figure 5B) growing seasons, respectively. Sangoi et al. (2014) reported that damages to leaf area decrease the photosynthetic capacity of

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maize because the damage reduces the interception of solar radiation. Such effects diminished the stem carbohydrate concentration, changed the silage quality (Roth & Lauer, 2008), and negatively affected the number of fertilised ovules (Sangoi et al., 2012b). Therefore, the smaller leaf area presented by the crop when it was subjected to higher spatial variability at the sowing row (Figure 5) probably contributed to the decrease in the number of grains produced per ear and the grain yield (Figures 2 and 3).

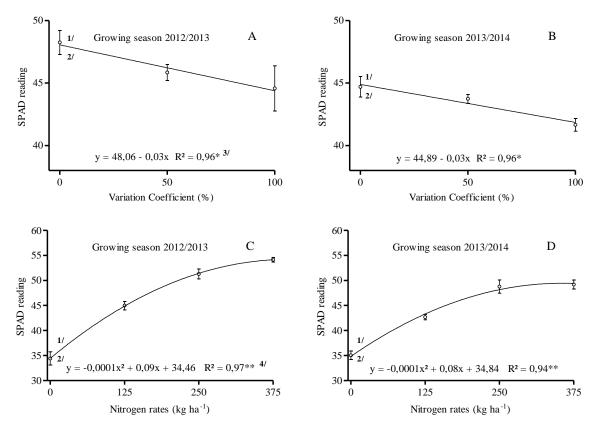


**Figure 4.** Mass of 1,000 grains of maize as affected by the variation coefficient of plant spatial distribution at the sowing row (A, B) and nitrogen side-dress rates (C, D). Lages, Santa Catarina, Brazil. <sup>1</sup>Each point represents the average of four nitrogen rates (A, B) or three variation coefficients (C, D). <sup>2</sup>Vertical bars indicate each average standard error. <sup>3/\*</sup>Equation is significant at the significance level of 5%. <sup>4/\*\*</sup>Equation is significant at the significance level of 1%.



**Figure 5.** Leaf area of maize at silking as affected by the variation coefficient of plant spatial distribution at the sowing row (A, B) and nitrogen side-dress rates (C, D). Lages, Santa Catarina, Brazil. <sup>1</sup>Each point represents the average of four nitrogen rates (A, B) or three variation coefficients (C, D). <sup>2</sup>Vertical bars indicate each average standard error. <sup>3</sup>\*Equation is significant at the significance level of 5%. <sup>4</sup>\*\*Equation is significant at the significance level of 1%.

The data from measurements carried out with the chlorophyll meter also showed a reduction in the SPAD readings at silking. This was based on 3 points with the increment of the variation coefficient of plant spatial distribution ranging from 0 to 100 in both growing seasons (Figure 6A and B). Thus, the decrease in relative chlorophyll content, which was associated with the reduction of crop leaf area (Figure 5), probably accounted for the reduction in the number of grains produced per ear (Figure 3) and the grain yield (Figure 2) when within-row variability of plant spatial distribution was enhanced.



**Figure 6.** SPAD readings of the relative chlorophyll content of the maize index leaf as affected by the variation coefficient of plant spatial distribution at the sowing row (A, B) and nitrogen side-dress rates (C, D). Lages, Santa Catarina, Brazil. <sup>1</sup>Each point represents the average of four nitrogen rates (A, B) or three variation coefficients (C, D). <sup>2</sup>Vertical bars indicate each average standard error. <sup>3</sup>\*Equation is significant at the significance level of 5%. <sup>4</sup>\*Equation is significant at the significance level of 1%.

The main hypothesis that prompted this work stated that an increment in nitrogen side-dress rate can mitigate damages to maize grain yield caused by irregularities in plant spatial distribution at the sowing row. This management strategy was efficient in terms of improving the maize leaf area (Figure 5C and D) and the relative chlorophyll content of the index leaf at silking (Figure 6C and D). Nonetheless, such effects were not able to avoid yield losses caused by uneven plant spatial variability (Figure 2A and B). Table 2 presents the average values of each combination of variation coefficient and nitrogen rate evaluated in the experiment. Averaging both growing seasons, there was a decrease of 1,620 kg ha<sup>-1</sup> in grain yield when the VC was enhanced from 0 to 100% and no nitrogen was side-dressed to the canopy. On the other hand, when 375 kg ha<sup>-1</sup> of N was side-dressed, the average yield presented by the crop was 1,800 kg ha<sup>-1</sup> smaller in the treatments with 100% of spatial variation coefficient than in the plots where plants were evenly distributed at the sowing row.

Collectively, the information gathered in the present work indicates that only elevating the amount of nitrogen fertilizer used during the side-dress operation is not an efficient management strategy to compensate for the spatial irregularity of plant distribution at the sowing row. Therefore, the best way to minimize problems caused by poor plant spatial distribution is to avoid its occurrence by taking appropriate caution during the sowing process.

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**Table 2.** Grain yield values of maize for each combination of spatial variation coefficient at the sowing row and nitrogen side-dress rate evaluated in the experiment. Lages, Santa Catarina, Brazil.

		Variation Coefficient (%)				
		0	50	100		
		Grain Yield (kg ha <sup>-1</sup> )				
		Growing Season 2012/13				
Nitrogen rates (kg ha <sup>-1</sup> )	0	7062	6361	5414		
	125	11051	10946	10172		
	250	14370	14630	12679		
	375	16064	13989	13685		
	Growing Season 2013/14					
Nitrogen rates (kg ha <sup>-1</sup> )	0	9393	8438	7801		
	125	12244	12009	11121		
	250	14405	14757	13282		
	375	15321	15046	14088		

#### Conclusion

The increase in the variation coefficient of plant spatial distribution at the sowing row from 0 to 100% decreased maize grain yield, regardless of the nitrogen side-dress rate.

The increase in nitrogen side-dress rate from 0 to 375 kg ha-1 does not prevent maize yield losses caused by the uneven spatial distribution of plants at the sowing row.

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