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# Influence of row spacing and plant population density on management of "white mould" in soybean in southern Brazil

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## Abstract

White mould is a disease caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary and it has become a major problem for soybean in Brazil, mainly due to the use of contaminated seeds and machinery, monoculture, and the use of susceptible species as crop rotation. This study aimed to evaluate the influence of different row spacing and plant population densities on soybean crop in relation to the levels of incidence and the severity of *S. sclerotiorum*. Field trials were carried out during 2010-2012 crop seasons. Row spacings of 0.35, 0.45, 0.60 and 0.75 metres, and plant population densities of 150,000, 200,000, 250,000 and 300,000 plants ha<sup>-1</sup> were used. The incidence and severity of white mould, the yield, and the thousand grain weight were evaluated. Spacing at 0.35 metres increased yield but it caused greater incidence of the disease. A reduced number of plants in the crop rows reduced the severity of the disease. Farmers with a history of problems with *S. sclerotiorum* should avoid narrow row spacings and high plant population densities.

**Keywords:** *Glycine max; Sclerotinia sclerotiorum*; incidence; severity; yield. **Abbreviation:** TWG\_thousand grain weight.

# Introduction

White mould (*Sclerotinia sclerotiorum* (Lib.) de Bary) is one of the most important plant diseases nowadays because it attacks several crops, including soybean. This disease, which is difficult to control, is aggravated by the production of resistance structures (sclerotia) inside and outside the plants, which return to the soil at the end of the disease cycle. The period of viability of these sclerotia is still uncertain, but there have been reports of more than eight years (Adams and Ayers, 1979). Another important fact that can complicate the management of the disease is that it has populations that are resistant to fungicides (Gossen et al., 2001), which limits one of the main ways of managing the pathogen.

White mould causes an estimated loss of 83.2 to 229 kg.ha<sup>-1</sup> for every 10% incidence of the disease in soybean, with average losses of 136 kg ha<sup>-1</sup> (Danielson et al., 2004). Among the environmental factors that can lead to carpogenic germination of sclerotia, the ambient temperature and the depth at which the sclerotia are in the ground stand out (Sun and Yang, 2000; Wu and Subbarao, 2008). Sun & Yang (2000) found that low and high light intensities may influence the optimum temperature for the germination of apothecia, and these were produced more rapidly when exposed to high light intensity treatments. The apothecia result from the carpogenic germination of the sclerotia and they are the largest source of inoculum of the disease because they produce a lot of ascospores, which are easily transported by

the wind and can infect plants in a range of 50-100 m from source (Steadman, 1983). Apothecia can germinate well in moist soils as well as dry soils, depending on the temperatures of the soil, according to results found by Matheron and Porchas (2005) in laboratory tests. There are a number of factors related to the time of viability, the potential for infection, and the spatial distribution of sclerotia in the soil (Clarkson et al., 2003; Sun and Yang, 2000; Wu and Subbarao, 2008).

Plant canopy management been studied because it is a good option for managing diseases like white mould in various crops. Soybean varieties with reduced height and lodging, and early cycle showed a reduction of 74% in the appearance of *Sclerotinia scletotiorum* apothecia and an 88% reduction in incidence (McDonald et al., 2013). In Canada, crop management of the disease has been part of the control of white mould for over 25 years; short cycle crops (which require less heat units) are recommended for areas with a history of the disease (McDonald et al., 2013).

The reduction of moisture in the soil surface interferes with the formation of apothecia and ascospores (Saindon et al., 1995; Schwartz and Steadman, 1978). In crops such as beans, air circulation between the rows hampers the development of *S. sclerotiorum* because it prevents the development of moisture, reducing the levels of incidence and severity of the disease (Tu, 1988). Plant population density and growth habit (in the case of beans) has a direct effect on the moisture between the rows. Plants with an upright growth habit allow greater air circulation and greater penetration of sunlight, when compared to semi-erect or prostrate plants, which results in a rapid drop in humidity. Upright cultivars have straighter lines of plants and, depending on the row spacing, plants in adjacent lines do not touch each other (Napoleão et al., 2006). A denser canopy provides ideal moisture and temperature conditions for the development of *S. sclerotiorum* (Blad et al., 1978; Boland and Hall, 1988).

The aim of the present study was to evaluate how different row spacings and plant population densities of soybean may influence the incidence and severity of white mould.

# **Results and Discussion**

In the 2010-2011 crop season, disease was only observed in the experiment in the two last assessments, at the R5.4 and R5.5 phenological stages (Fehr et al., 1971). In the 2011-2012 crop season, the disease was observed from the R5.1 phenological stage (Fehr et al., 1971) onwards during the experiment. Results from R5.5 phenological stage (Fehr et al., 1971) are showed.

## Incidence and severity levels of white mould

The two crops seasons showed large differences between each other regarding the values for the incidence and severity of the disease. Unfavourable climate conditions for the development of the disease ( $T_{max}=26$  °C;  $T_{ave}=21$  °C;  $T_{min}=17$  °C) during the period of susceptibility of the crop (R1 and R2) (Fehr et al., 1971) resulted in low values for incidence and severity in the 2010-2011 crop season, which led to large variation in the data and distortion in the surface plot (see Fig 1 and 3). For variables (incidence and severity), smaller row spacings and plant population densities presented the highest levels, although they were lower than 1% (Fig 1 and 3).

There was no interaction between row spacing and plant population density for incidence and severity in the 2011-2012 crop season (table 1). Unlike the 2010-2011 crop season, all treatments showed incidence of the disease (Fig 2) and averages of severity above 40% (Fig 4). Differences in incidence were only observed between the different row spacings, where 0.45 metres and 0.75 metres differed significantly between each other; the 0.45 metres row spacing had the highest values and the 0.75 metres row spacing had the lowest values. Differences in severity were observed between row spacings and plant population densities. For the former, only row spacings of 0.35 metres to 0.45 metres differed significantly, where the first had lower level of severity than the second (Table 1). Regarding plant population density, 150,000 plants.ha<sup>-1</sup> differed significantly from all others, presenting lower severity level (Table 1).

Row spacing of 0.45 metres are most commonly used in soybean in Brazil. However, this size resulted in the highest incidence and severity levels of white mould during the 2011-2012 crop seasons. Significantly lower incidence than 0.45 metres treatments was only found for row spacing of 0.75 metros (Table 1). This may have been related to the fact that larger row spacings provide increased air circulation, light penetration, drier soil, fewer apothecia and shorter leaf wetness duration (McDonald et al., 2013), thereby resulting in a reduction in the incidence of the disease. In soybean field trials carried out in the USA by Grau and Radke (1984), smaller row spacings led to high levels of white mould. The authors explained that this fact was due to the microclimate, which was favourable to the disease and which was created by the denser canopy in relation to larger row spacings. The authors also mentioned that, according to Adams (1975), sclerotia on the soil surface deteriorate with alternating moisture and drought in the soil, a situation that occurs with larger row spacings because smaller row spacings retain more moisture in the soil. Severity significantly lower than that found in row spacings of 0.45 metres was observed in the row spacings of 0.35 metres (Table 1). A different result was observed by Macena et al. (2011) in beans, where increased row spacing led to a reduction in the severity of white mould. Plant population density only influenced the severity of white mould during the 2011-2012 crop season. 150,000 plants.ha<sup>-1</sup> showed significantly lower levels of severity than the other plant population densities (Table 1). In experiments with beans, Vieira et al. (2005) and Paula Junior et al. (2009) observed reductions in the severity of the disease in low plant population densities. Vieira et al. (2010) observed that a decrease in plant population density from 240,000 plants.ha<sup>-1</sup> to 80,000 plants.ha<sup>-1</sup> was effective in reducing white mould in beans, also increasing crop yield in areas with high pressure of the disease. According to Heiffig et al. (2006) and Herbert and Litchfield (1982), low plant population densities have lower rates of leaf area in the same space, which may lead to increased air circulation and light penetration between the plants and, hence, a lower severity of the disease due to moisture reduction and increased temperature. Vieira et al. (2012) observed similar results in beans, where broad row spacing and low plant population density showed to a promisor spatial arrangement for manage white mould when fungicide is not used.

# Yield and Thousand grain weight (TGW)

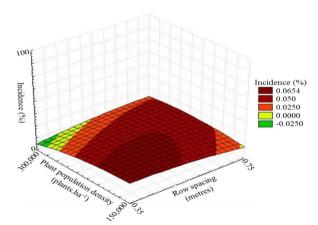
There was no interaction between row spacings and plant population densities for yield in any of the two field trials on both crop seasons. All treatments showed average yields higher than 4,000 kg.ha<sup>-1</sup> (Fig 5 and 6). Differences were only observed between row spacings for both crop seasons. Row spacing of 0.35 metres resulted in improved yield in both crop seasons, differing significantly from row spacings of 0.60 and 0.75 metres in the first crop season and from all others in the second crop season (Table 1). A reduction in row spacing resulted in increased yield in both crop seasons (Table 1). However, there was no influence of plant population density on the yield in either of the years (Table 1). According to Knebel et al. (2006) and Rambo et al. (2003), the factor that has the greatest impact on yield is row spacing. Nakagawa et al. (1986) did not observe an increase in yield due to increased plant population density. The higher yield where reduced row spacings were used may have been due to greater interception of solar radiation during the growing season (Board and Harville, 1992; Taylor, 1980).

Regarding TGW, all treatments showed weights higher than 160 grams in both crop seasons (Fig 7 and 8). In the 2010-2011 crop season there was no interaction between row spacings and plant population densities. Differences were only observed between the different row spacings, where 0.75 metres presented higher weight and were significantly different from the others row spacings (Table 1). For the 2011-2012 crop season there was interaction between the factors; the lowest TGW was obtained for the row spacing of 0.45 metres associated with a plant population density of 250,000 plants ha<sup>-1</sup> (Table 1). In a similar way to yield, the different row spacings also influenced the TGW values. Row spacing of 0.75 metres resulted in the largest values during

**Table 1.** Tukey's multiple-comparison analysis at 5% of: yield 2010/2011 (Yld 10/11), thousand grain weight 2010-2011 (TGW 10/11), incidence 2011-2012 (Inc 11/12), severity 2011-2012 (Sev 11/12), sclerotia.plants<sup>-1</sup> 2011-2012 (Scl.plt<sup>-1</sup>), yield 2011-2012 (Yld 11/12) and thousand grain weight 2011-2012 (TGW 11/12). Values of incidence and severity represent the last assessment of the disease. Values marked with "\*"represent the coefficient of variation for each analysis.

Pop	ulation	1														
			150k			200k			250k			300k				
.ha		0.35	5111.4			5451.8			5281.3			5399.3			5311.0	а
(kg		0.45	4893.9			5337.1			4951.5			5197.9			5095.1	а
/11		0.60	4318.7			4717.2			4814.6			4515.3			4591.5	b
10		0.75	4317.9			4223.9			4284.4			4289.6			4278.9	b
Yld 10/11 (kg.ha <sup>-</sup> )		*10.6%	4660.5	a <sup>1</sup>		4932.5	a		4833.0	a		4850.5	a			
		0.35	165.5			165.1			165.2			165.6			165.4	с
1 (g		0.45	164.3			165.8			166.9			168.5			166.4	bc
0/1		0.60	167.3			167.7			169.5			169.7			168.6	b
W 1		0.75	171.2			172.2			172.5			173.4			172.3	а
TGW 10/11 (g)		*1.9%	167.1	а		167.7	а		168.5	а		169.3	а			
		0.35	10.1			4.7			13.1			10.7			9.6	ab
(%		0.45	18.2			19.7			10.3			11.8			15.0	а
12 (		0.60	7.5			12.6			10.3			9.5			10.0	ab
11/1		0.75	9.0			6.9			5.5			3.2			6.1	b
Inc 11/12 (%)		*-8.0%	11.2	а		11.0	а		9.8	а		11.3	а			
		0.35	42.9			78.0			81.2			84.6			71.7	b
(%		0.45	89.7			94.9			91.0			99.9			93.8	а
12 (		0.60	69.0			74.9			94.9			89.8			82.1	ab
11/		0.75	75.1			88.4			84.2			97.0			86.2	ab
Sev 11/12 (%)		*11.9%	69.2	b		84.0	а		87.8	а		92.8	а			
		0.35	20.4			17.4			19.3			21.1			19.5	а
Yld 11/12 (kg.ha <sup>-</sup> Scl.plt <sup>-1</sup> 11/12 <sup>1</sup> )		0.45	26.7			11.4			19.5			17.3			18.7	а
		0.60	18.8			14.3			20.6			17.0			17.7	а
		0.75	20.6			18.6			13.6			13.8			16.6	а
		*36.7%	21.6	а		15.4	а		18.2	а		17.3	а			
		0.35	5093.2			5124.3			5364.4			4775.5			5089.3	а
(kg		0.45	4314.2			3761.3			3695.4			4030.3			3950.3	bc
12		0.60	4556.9			4073.9			4166.4			4446.7			4311.0	b
11		0.75	3731.6			3540.1			3851.5			3832.1			3738.8	с
۲ ۲Iq		*12.0%	4423.9	а		4124.9	а		4269.4	а		4271.2	а			
		0.35	192.3	а	$A^2$	189.0	а	AB	187.2	а	AB	181.95	а	В		
2 (g)	50	0.45	188.8	а	Α	185.6	а	А	177.4	b	В	188.73	а	А		
TGW 11/12 (g)	acin	0.60	191.8	а	А	188.1	а	А	184.5	ab	А	185.90	а	А		
× 1	Row spacing	0.75	188.7	а	Α	189.3	а	А	187.9	а	А	184.43	а	А		
ſĠ	Sov	*2.6%														

☐ ☐ ☐ 2 [ \*2.6%
<sup>1</sup> Values with different letters significantly different by Tukey's multicomparison analyses at a confidence level of 95%.
<sup>2</sup> Lower case letters refer to row spacings; Upper case letters refer to plant population densities.



**Fig 1.** Response surface plot relating interactions between incidence of white mould of 2010-2011 crop season at R5.5 phenological stage. Different colours in the legend mean significantly different values in the chart.

Treatment	Spacing	Population
1	0.35 metres	150,000 plants.ha <sup>-1</sup>
2	0.35 metres	200,000 plants.ha <sup>-1</sup>
3	0.35 metres	250,000 plants.ha <sup>-1</sup>
4	0.35 metres	300,000 plants.ha <sup>-1</sup>
5	0.45 metres	150,000 plants.ha <sup>-1</sup>
б	0.45 metres	200,000 plants.ha <sup>-1</sup>
7	0.45 metres	250,000 plants.ha <sup>-1</sup>
8	0.45 metres	300,000 plants.ha <sup>-1</sup>
9	0.60 metres	150,000 plants.ha <sup>-1</sup>
10	0.60 metres	200,000 plants.ha <sup>-1</sup>
11	0.60 metres	250,000 plants.ha <sup>-1</sup>
12	0.60 metres	300,000 plants.ha <sup>-1</sup>
13	0.75 metres	150,000 plants.ha <sup>-1</sup>
14	0.75 metres	200,000 plants.ha <sup>-1</sup>
15	0.75 metres	250,000 plants.ha <sup>-1</sup>
16	0.75 metres	300,000 plants.ha <sup>-1</sup>

Table 2. Combinations of different row spacings and plant population densities, resulting in each of the sixteen treatments.

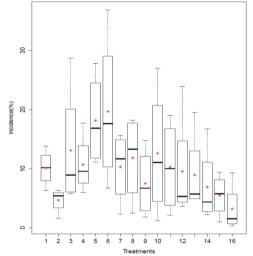


Fig 2. Boxplot relating incidence in 2011-2012 crop season at R5.5 phenological stage. Averages of treatments are represented by "\*".

the 2010-2011 crop season and they also resulted in one of the highest values during the 2011-2012 crop season (Table 1). Different plant population densities did not alter the TGW values during the 2010-2011 crop season (Table 1). However, there was interaction between this factor and row spacing in the second crop season. These results disagree with those found by Vazquez et al. (2008), where the variations in the spatial arrangement did not affect the thousand grain weight.

# Sclerotia per plant production

There was no measurement of this variable in the 2010-2011 crop season due to low incidence and severity of the disease. All treatments presented production of sclerotia per plant higher than 10 (Fig 9). There was no interaction between the factors or significant differences between the row spacings and plant population densities (Table 1). Neither row spacings nor plant population densities showed an influence on the variable of number of sclerotia per plant (Table 1), contrary to the findings of Macena et al. (2011), where production of sclerotia decreased as the row spacings increased.

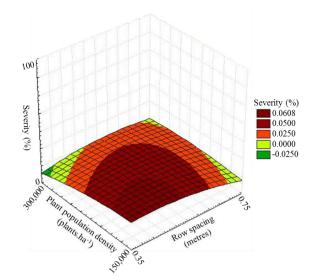
#### Materials and Methods

#### Localization

The experiments were carried out in the city of Arapoti, state of Parana, Brazil (Alfisol  $CEC_{0-10}$  cm = 8.98 cmol.dm<sup>-3</sup>; organic matter  $_{0-10}$  cm = 30.89 g.dm<sup>-3</sup>; altitude = 966 metres) in an area naturally infested by the disease. Prior to the installation of the experiments, the soil was sampled at4 points of 0.25 m<sup>2</sup> and 0.05 m depth (Jaccoud-Filho et al., 2010) in the area in order to determine the number of sclerotia.m<sup>-2</sup>; 55 sclerotia.m<sup>-2</sup> was found in the 2010-2011 crop season and 31 sclerotia.m<sup>-2</sup> in 2011-2012 crop season.

#### Sowing and plant material

In the first year of the experiment, sowing was performed on December 10<sup>th</sup>, 2010 and in the second year, on October 11<sup>st</sup>, 2011. The cultivar used in both years of the experiment was Apollo RR® (susceptible to white mould, of indeterminate growth, and maturation stage of 5.5) and crop management was performed according to the standards of the farm where the experiments were performed, except for the application of products whose target was the white mould.



**Fig 3.** Response surface plot relating interactions between severity of white mould of 2010-2011 crop season at R5.5 phenological stage. Different colours in the legend mean significantly different values in the chart.

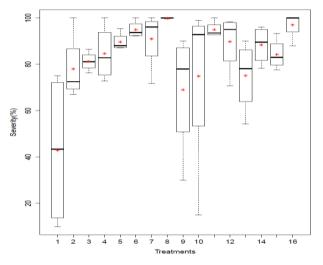


Fig 4. Boxplot relating severity in 2011-2012 crop season. Averages of treatments are represented by "\*".

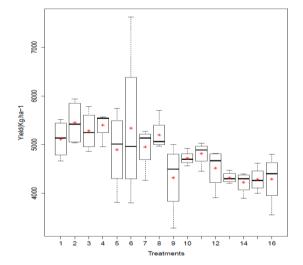


Fig 5. Boxplot relating yield in 2010-2011 crop season at R5.5 phenological stage. Averages of treatments are represented by "\*".

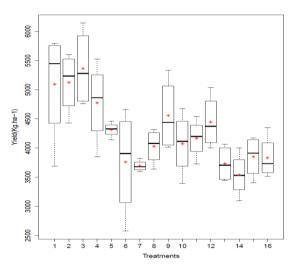


Fig 6. Boxplot of data relating to yield in 2011-2012 crop. Averages of treatments are represented by "\*".

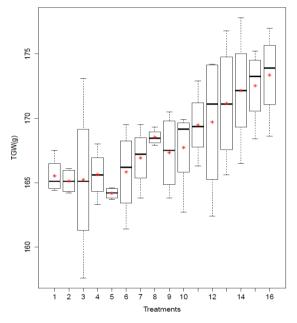


Fig 7. Boxplot of data relating to thousand grain weight in 2010-2011 crop. Averages of treatments are represented by "\*".

## Treatments and traits measured

The treatments consisted of four row spacings (0.35, 0.45, 0.60 and 0.75 metres) and four plant population densities (150,000, 200,000, 250,000 and 300,000 plants.ha<sup>-1</sup>) (Table 2). The incidence and severity assessments of the disease started at R1 phenological stage (Fehr et al., 1971) in four rows of four metres length in each plot and were made weekly until the crop reached the R5.5 phenological stage (Fehr et al., 1971) in both crop seasons. The measurement of the incidence values was made using the percentage of infected plants per metre, and in terms of severity, percentages of 1 to 100 were awarded, in accordance with Juliatti et al. (2013). Thus, the incidence values presented in this article refer to the percentage of diseased plants per metre. The severity values shown are averages of infected plants. During the evaluations in 2011-2012, five plants per plot that showed the presence of the disease were randomly marked with coloured tape to quantify the sclerotia.plant opening up the five plants which were marked with tape to count the resistance structures in the same day that harvest

was performed. To evaluate the yield, four lines of four metres in length per plot (the same that was assessed for incidence and severity of white mould) were harvested in both crop seasons. Assessments of yield and thousand grain weight (TGW) were made in the laboratory after correction of grain water content to 130g.kg<sup>-1</sup>.

#### Experimental design and statistical analyses:

The experimental design was a randomized complete block in a 4x4 factorial scheme (row spacings X plant population densities), totalling sixteen treatments (table 2) with four replications. Each plot had twelve lines, ten metres long, varying the width of the plot depending on the spacing used. Variance analyses, Shapiro-Wilk test, Bartlett test and Tukey's multicomparison test was performed on all variables using R® software (R Core Team, 2013). The Shapiro-Wilk and Bartlett tests indicated the necessity for data transformation for incidence and severity of both crop seasons. The 2010-2011 incidence and severity could not be

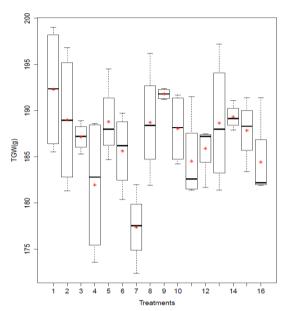


Fig 8. Boxplot relating TGW in 2011-2012 crop season. Averages of treatments are represented by "\*".

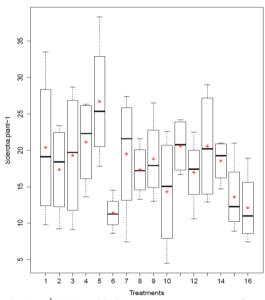


Fig 9. Boxplot relating sclerotia.plant<sup>-1</sup> in 2011-2012 crop season. Averages of treatments are represented by "\*".

transformed by any method. 2011-2012 incidence was transformed by Box-Cox transformation (Box and Cox, 1964) and 2011-2012 severity was transformed by the equation x=1/cos(x). As 2010-2011 incidence and severity could not be transformed, it was decided to perform response surface analyses, which consisted of variance analyses of the regression of row spacings and plant population densities to each variable. The response surface analysis showed a uniform covariance matrix, a necessary condition according to Huynh-Feldt (H-F), to carry out univariate statistical analysis for an assay in randomized blocks. Functions of the type  $Y = b_0 + b_1 X_1 + b_2 X_2 + b_{11} X_{12} + b_{22} X_{22} + b_{12} X_1 X_2$  were adopted, where Y was the dependent variable,  $b_0$  to  $b_{12}$  were the regression coefficients, X<sub>1</sub> corresponded to the spacing and X<sub>2</sub> corresponded to the populations of plants. The estimates were made with p≤.05 using STATISTICA 10® software (Stat-Soft, 2010).

## Conclusions

The use of row spacing of 0.75 metres was effective in reducing the incidence of disease. However, such row spacing led to a large reduction in the soybean yield. Farmers with a history of disease in their area should not adopt reduced row spacing to increase productivity, due to high risk of incidences of this disease. A reduction in plant population density is a good strategy to reduce the severity of *Sclerotinia sclerotiorum*.

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