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RESEARCH ARTICLE / *ARTIGO*

# Chemical control and responses of susceptible and resistant soybean cultivars to the progress of soybean rust

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**ABSTRACT**

This work compared development of soybean rust (SR) on the susceptible cultivar BRS 133 and on the resistant line CB06-953/963 (*Rpp4* gene) following different fungicide treatments applied in different developmental stages. The assessed variables were percent plant defoliation, coefficient of damage (CD) and economic threshold level (ETL), the latter two calculated from disease severity ratings and yield. Experiments were conducted in the field during the 2006-2007 growing season with one sowing date (Experiment I) and in 2007-2008 with two sowing dates (Experiments II and III). The experimental design was a randomized complete block with ten treatments and four replications. Overall, the *Rpp4* gene in the resistant line was effective in reducing development of SR in all three experiments. The values of CD and ETL were higher for the resistant line than for the susceptible cultivar. The resistant line needed 13.3 days longer than the susceptible cultivar to reach the ETL in Experiment II. Fungicide applications were more effective in protecting yield and minimizing defoliation in the susceptible cultivar than in the resistant line. Under high inoculum pressure (Experiment III), three and four fungicide sprays applied during the season resulted in significantly higher yields ($P<0.05$) in both soybean genotypes compared with the untreated controls. Late season fungicide applications reduced rust severity and increased the yield of the resistant cultivar.
**Key words:** *Glycine max*, *Phakopsora pachyrhizi*, economic threshold level, fungicide, vertical resistance.

**RESUMO**

**Controle químico e respostas de cultivares suscetíveis e resistentes de soja ao progresso da ferrugem asiática**

Este trabalho comparou o progresso da ferrugem asiática da soja na cultivar suscetível BRS 133 e na linhagem resistente CB06-953/963, com o gene de resistência *Rpp4*, sob tratamentos fungicidas em diferentes estádios de desenvolvimento. Foram medidas as variáveis porcentagem de desfolha, coeficiente de dano (CD) e limiar de dano econômico (ETL), as duas últimas calculadas a partir da severidade da doença e produtividade de grãos. Os ensaios foram realizados em condições de campo nos anos 2006-2007 em uma época de semeadura (Experimento I) e em 2007-2008 em duas épocas de semeadura (Experimentos II e III). O delineamento experimental utilizado foi blocos ao acaso, com dez tratamentos e quatro repetições. Houve redução do progresso da doença em CB06-953/963 nos três experimentos realizados. Os valores de CD e ETL foram superiores na linhagem resistente em comparação à suscetível. No experimento II, o ETL para CB06-953/963 foi atingido 13,3 dias após o registrado para BRS 133. As aplicações fungicidas apresentaram maiores benefícios na produtividade e redução da desfolha na cultivar suscetível. Sob condições de alta pressão de inóculo (Experimento III), três e quatro aplicações de fungicidas resultaram em ganho na produtividade ($P<0.05$) nos dois genótipos de soja quando comparados aos controles sem aplicação de fungicidas. As aplicações de fungicidas, mesmo quando realizadas mais tardiamente nas cultivares resistentes, auxiliaram no controle da SR e na manutenção da alta produtividade.
**Palavras-chave:** *Glycine max*, *Phakopsora pachyrhizi*, limiar de dano econômico, fungicida, resistência vertical.

## INTRODUCTION

Brazil is a major producer of soybean [*Glycine max* (L.) Merrill], with the highest yields per hectare (ABIOVE, 2008). Highly successful development of soybean cultivars adapted to the low latitude regions of Brazil by government and private breeders led to rapid expansion in the “Cerrado” region of the country (França Neto, 2004). However, diseases still limit soybean production and profitability. Over 100 diseases are known to affect this crop (Hartman et al., 1999) and 46 have been identified in Brazil (Tecnologia de produção de soja, 2008). Currently, the asian soybean rust (SR) caused by *Phakopsora pachyrhizi* Syd. & P. Syd is one of the main fungal diseases in tropical and subtropical areas (Sinclair & Hartman, 1999), and can cause total crop loss (Yorinori, 2006).

The control and management of SR is comprised of several measures, and research has focused on the use of fungicides and the development of resistant cultivars. Five dominant genes, *Rpp1* (McLean & Byth, 1980), *Rpp2* (Bromfield & Hartwig, 1980), *Rpp3* (Bromfield & Melching, 1982), *Rpp4* (Hartwig, 1986) and *Rpp5* (Garcia et al., 2008) have been reported in the literature. Soybean lines with these resistance genes are currently in advanced field tests to verify their efficacy prior to commercialization, so fungicide applications are still the main tool to control SR (Yorinori & Wilfrido, 2002; Tecnologia de produção de soja, 2005).

Accurate quantification of damage caused by SR in field plots or individual plants is important in comparing the effectiveness of different disease management options. Statistical models such as critical point, multiple points, integrals, and surface-response are mathematical tools used to relate disease severity with plant damage or yield (Bergamin Filho et al., 1995; Bergamin Filho & Amorim, 1996) and have been applied in many pathosystems aimed at improving disease management. Critical point modeling has allowed researchers to identify a specific stage of host development in which the intensity of disease is correlated with grain yield or damage (Casa et al., 2010; Hikishima et al., 2010; Canteri et al., 1999). Relationships that quantify losses in production revenue based on disease development (Kropff & Spitters, 1991), given in this study by the coefficient of damage (CD), are essential to any economic analysis. With CD, it is also possible to calculate the economic threshold level (ETL), which indicates the optimum time to apply a fungicide to maximize profit. The ETL is a simple concept that integrates biological and economic factors with the aim to assist farmers in determining the most cost-effective time to apply a fungicide (Fleck et al., 2002; Weaver, 1991).

The objective of this work was to compare SR development in the susceptible cultivar BRS 133 and in the resistant line CB06-953/963 with different fungicide treatments in different developmental stages, and to calculate the CD and ETL for each soybean genotype.

## MATERIAL AND METHODS

Experiments were conducted in the field at the Tropical Melhoramento & Genética Ltda (TMG) research center, located in the county of Cambé, Paraná State, 23º16'33" latitude south and 51º16'42" longitude west, 650 m.a.s.l. The soil classification is Rhodic Eutrustox (Sistema brasileiro de classificação de solos, 2006).

Cultivar BRS 133, susceptible to SR, and the line CB06-953/963, bred from TMG which carries the resistance gene *Rpp4*, both from the 7.3 maturity group (Embrapa Soja, 2009), were used in all the experiments as soybean rust hosts. SR infection occurred spontaneously. Meteorological data were collected at the TMG automated station located approximately 2 km from the field plots.

Experiment I was conducted during the 2006-2007 growing season and had a single sowing date (Table 1). Experiments II and III were conducted during 2007-2008 and had two sowing dates. Seeds were treated with carbendazim+thiram (200 mL of commercial product 100 $kg^{-1}$ seeds) before sowing. Additionally, 300 kg $ha^{-1}$ of 0-20-20 NPK fertilizer was applied prior to planting.

The experiments were set in randomized complete block design with 10 treatments and four replications. The experimental plots consisted of four 5 m-long rows, spaced 0.5 m. The experimental unit had an area of 4.0 $m^2$, which corresponded to the central 4.0 m of the two inner rows of each plot. Pests and weeds were controlled as needed to keep plants free from these interfering factors. Treatment T1 was used as a control with no fungicide sprays, treatment T2 had carbendazin (250 g a.i $ha^{-1}$) sprayed to control late-season soybean disease complex (LSDC). Treatments T3 to T10 were comprised of pyraclostrobin + epoxiconazole (66.5 g + 25.0 g a.i $ha^{-1}$) for the control of SR starting in

**TABLE 1 -** Treatments (T) with one sowing time in 2006/2007 and two sowing times in 2007/2008. Phenological stages (Fehr & Caviness, 1977) when the fungicides were applied and days after sowing (DAS) until the first application

| 2006/2007 Sowing Dec. 11, 2006 | | 2007/2008 Sowing Nov. 5, 2007 | | 2007/2008 Sowing Dec 7, 2007 | |
|---|---|---|---|---|---|
| **Treatments/ stages of sprays** | **DAS until the first spray** | **Treatments/ stages of sprays** | **DAS until the first spray** | **Treatments/ stages of sprays** | **DAS until the first spray** |
| T1 – Control(1) | 0 | T1 – Control(1) | 0 | T1 – Control(1) | 0 |
| T2 – R1, R4, R5.3(2) (control LSCD†) | 52 | T2 – Vn, R2, R5.1, R5.4(2) (control LSCD†) | 51 | T2 – Vn, R2, R5.1, R5.4(2) (control LSCD†) | 44 |
| T3 – R1, R3, R5.1(3) (SR* control) | 52 | T3 – Vn, R1, R3, R5.1(3) (SR* control) | 51 | T3 – Vn, R1, R3, R5.1(3) (SR* control) | 44 |
| T4 – R2, R5.1(2) | 59 | T4 – R1, R4, R5.3(2) | 65 | T4 – R1, R4, R5.3(2) | 60 |
| T5 – R2 | 59 | T5 – R2, R5.1(2) | 72 | T5 – R2, R5.1(2) | 66 |
| T6 – R3 | 65 | T6 – R3 | 79 | T6 – R3 | 73 |
| T7 – R4 | 74 | T7 – R4 | 86 | T7 – R4 | 80 |
| T8 – R5.1 | 80 | T8 – R5.1 | 95 | T8 – R5.1 | 87 |
| T9 – R5.2 | 86 | T9 – R5.2 | 101 | T9 – R5.2 | 94 |
| T10 – R5.3 | 94 | T10 – R5.3 | 109 | T10 – R5.3 | 100 |

[1]No fungicide application; [2]reapplication every 21 days; [3]reapplication every 15 days; †late season soybean disease complex; *soybean rust.

varying phenological stages to obtain an SR intensity gradient (Table 1). T3 was the control treatment with the applications started at the appearance of the first SR symptoms. The phenological stages (Fehr & Caviness, 1977) were followed in this treatment, as the plants would be the least affected by SR. SR severity (Godoy et al., 2006) was assessed by taking trifoliolates from the lower, medium, and upper thirds of the plants (one per plant region) from four random points in each of the experimental units. Assessments were performed until the complete defoliation of the untreated plots (T1). The percent of the severity values for each treatment were used to calculate the area under the disease progress curve (AUDPC) (Campbell & Madden, 1990). AUDPC values were corrected according to the duration of the epidemic (days) to enable comparison among the three experiments. Corrected AUDPC values (AUDPCc) are presented as the average disease percentage throughout epidemic duration. Defoliation was evaluated with a scale developed by Canteri et al. (2006) in the two experiments conducted during the 2007-2008 season.

The fungicides were applied with a $CO_2$-pressurized backpack sprayer with a 2.0 m wide spray bar with four nozzles 0.5 m apart. Four Teejet XR 11002 nozzles (Spraying Systems Co., Wheaton, IL), calibrated to deliver 300 L ha$^{-1}$, were used. The plots were harvested and the soybean grain was mechanically threshed and weighed, and moisture level was determined. The yield was calculated in kg ha$^{-1}$ and corrected for 13% seed moisture (Brazil, 1992).

All response variables were used in exploratory data analysis (EDA) followed by analysis of variance (ANOVA). This EDA consisted of the evaluation of pre-requirements such as normal distribution of the experimental errors by the Shapiro & Wilk (1965) method, the homogeneity of the variance of the treatments by the Burr & Foster (1972) method, additivity of the experimental design model by Tukey's method (1949), and the analysis of the residues according to Parente (1984). Besides ANOVA, the means were compared with the Scott-Knott test at significance level of $\alpha = 0.05$ (Cochran, 1957). The statistical software applications SANEST (Zonta et al., 1982), SAS (SAS Institute, 2001) and SASM-Agri (Canteri et al., 2001) were used.

Correlations between severity and AUDPCc with yield and defoliation were analyzed by linear regression using Microsoft Excel. The intercept was obtained with the linear regression equation y=a+bx, (potential yield mathematically given by the equation), and the CD, which is the percent difference in yield between treatments with different levels of disease (Bergamin Filho & Amorim, 1996). The CD was used to estimate ETL, which is calculated as a function of the potential crop yield (intercept), the rust control cost (US $73.00 ha$^{-1}$), the commercial value of soybean during the experimental period (US $29.00/60 kg), and the CD caused by SR (Reis et al., 2001; Consórcio Anti-Ferrugem, 2008)

## RESULTS AND DISCUSSION

### Experiment I – 2006-2007 cropping season

The mean temperature and relative humidity data recorded from December 11, 2006 to April 2, 2007 were 24.6°C and 77.9%, respectively, and the accumulated rainfall was 794.8 mm. The grain yield and defoliation data were not collected in this experiment due to the lack of uniformity of the plant stands in several plots, which was caused by runoff after plant emergence. Furthermore, in addition to the SR symptoms, a large presence of bacterial pustule (*Xanthomonas axonopodis pv. glycines*) and bacterial blight (*Pseudomonas savastanoi pv. glycinea*) were also observed on the resistant line.

Due to the late sowing date (December), the initial symptoms of SR were detected just after the R2 stage. For this reason and because of the plot stand flaws, the AUDPCc value was low (Table 2). Differences among treatments T3, T4, T5 and T6 for both BRS 133 and CB06-953/963 were non-significant ($P>0.05$) (Table 1), indicating that early season fungicide applications starting at 52 and 65 days after sowing (DAS) provided significant SR control compared with the untreated control (T1). The AUDPCc was lower for CB06-953/963 than BRS 133 for all treatments, indicating the strong effect of the SR-resistance gene *Rpp4* present in CB06-953/963 in limiting SR development (Figure 1 A and Table 2).

### Experiment II – 2007-2008 cropping season (Time 1)

In Experiment II, the mean temperature and relative humidity recorded were 22.8°C and 71.7%, respectively (December 7, 2007 to April 4, 2008). The accumulated rainfall for the growing season (November 5, 2007 to April 9, 2008) was 788.2 mm.

The determination coefficient ($R^2$) for the regression of grain yield vs. AUDPCc ranged from 0.82 for CB06-953/963 planted on the later date to 0.93 for BRS 133 planted on the later date (Table 3). Grain yield reduced with the increase in SR intensity. The CD (reduction in grain yield for every 1% AUDPCc) ranged from 34.55 kg ha$^{-1}$ for BRS 133 planted on the early date to 163.81 kg ha$^{-1}$ for CB06-953/963 planted on the later date (Table 3). ETL estimates ranged from 0.45% for CB06-953/963 planted on the earlier date to 2.11% for BSR 133 planted on the later date (Table 3).

Although the CD of the resistant line CB06-953/963 was over three times higher than that of the susceptible cultivar BRS 133 (Table 3), SR development on CB06-953/963 was over three times less than BRS 133 for T1 (Table 2). Regression results indicated a negative relationship between SR development in stage R5.5 and grain yield. For every 1% increase in SR developmentthere was a corresponding reduction in grain yield of 41.6 kg ha$^{-1}$ and 72.4 kg ha$^{1}$ for BRS 133 and CB06-953/963, respectively, planted on the early date (Table 4). The relationship was similar for the genotypes

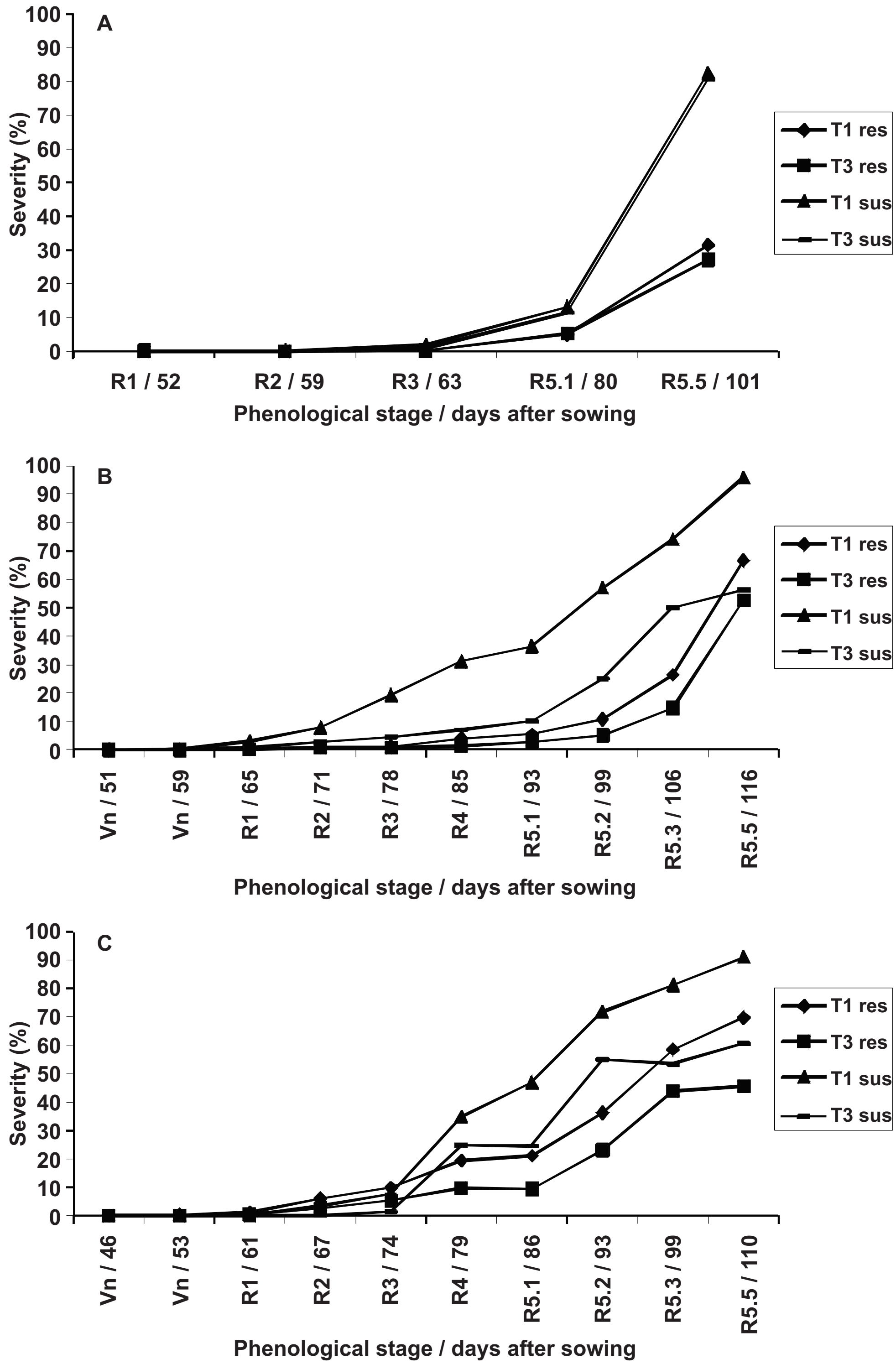


**FIGURE 1 -** Disease progress curves for T1 (without fungicide applications) and T3 (with reapplication every 15 days after the onset of first symptoms) in the resistant line CB06-953/963 and the susceptible cultivar BRS 133. **A.** 2006/2007; **B.** 2007/2008 - Nov. 5, 2007 and **C.** 2007/2008 - Dec. 7, 2007).

**TABLE 2 -** Corrected area under the disease progress curve (AUDPCc) of the susceptible cultivar BRS 133 and the resistant line CB06-953/963 for *Phakopsora pachyrhizi* with the application of fungicides at different stages of development, 2006/2007 and 2007/2008 cropping seasons

| | 2006/2007 Crop | | 2007/2008 Sowing Nov. 5, 2007 | | 2007/2008 Sowing Dec 7, 2007 | |
|---|---|---|---|---|---|---|
| **Treat.**(2) | **BRS 133** | **CB06-953/963** | **BRS 133** | **CB06-953/963** | **BRS 133** | **CB06-953/963** |
| T1 | 18.17 a | 6.78 a | 29.14 a | 7.94 a | 33.38 a | 20.23 a |
| T2 | 17.09 a | 6.18 a | 22.69 b | 6.63 a | 31.41 a | 20.02 a |
| T3 | 11.07 c | 3.31 b | 13.02 d | 4.90 c | 15.06 c | 14.23 b |
| T4 | 11.53 c | 4.68 b | 16.59 c | 5.39 b | 21.82 b | 16.31 b |
| T5 | 12.18 c | 3.91 b | 18.06 c | 5.34 b | 24.76 b | 16.17 b |
| T6 | 11.70 c | 3.44 b | 22.94 b | 5.71 b | 25.49 b | 18.51 a |
| T7 | 13.79 b | 5.64 a | 25.70 a | 6.42 a | 24.97 b | 19.53 a |
| T8 | 16.91 a | 5.92 a | 28.49 a | 5.86 b | 30.41 a | 20.82 a |
| T9 | 15.75 a | 6.08 a | 29.21 a | 7.88 a | 32.27 a | 19.94 a |
| T10 | 17.39 a | 6.62 a | 28.15 a | 7.92 a | 31.58 a | 19.05 a |
| C.V. | 1.62% | 11.45% | 1.16% | 2.74% | 10.49% | 11.33% |

[1]Treatments - see Table 1. [2]Means followed by the same letter in the column are not significantly different at 5% probability by the Scott-Knott test.

**TABLE 3 -** Potential yield (intercept), coefficient of damage (CD), determination coefficient ($R^2$) of AUDPC vs. yield, and economic threshold level (ETL) for the two sowing times of the 2007/2008 cropping season

| | **Genotype** | **Intercept kg ha$^{-1}$** | **CD kg ha$^{-1}$** | **$R^2$** | **ETL %** |
|---|---|---|---|---|---|
| Time 1 | BRS 133 | 3357.25 | 48.71 | 0.91 | 1.50 |
| Time 1 | CB06-953/963 | 4042.91 | 163.81 | 0.88 | 0.45 |
| Time 2 | BRS 133 | 2486.40 | 34.55 | 0.93 | 2.11 |
| Time 2 | CB06-953/963 | 4885.52 | 80.79 | 0.82 | 0.90 |

**TABLE 4 -** Potential yield (intercept), reduction for every 1% of severity, determination coefficient ($R^2$) between severity (%) in R5.5 stage vs. yield, and economic threshold level (ETL) for the two sowing times of the 2007/2008 cropping season

| | **Genotype** | **Intercept kg ha$^{-1}$** | **Reduction at every 1% severity kg ha$^{-1}$** | **$R^2$** | **ETL %** |
|---|---|---|---|---|---|
| Time 1 | BRS 133 | 4410.15 | 41.62 | 0.97 | 3.63 |
| Time 1 | CB06-953/963 | 6136.33 | 72.40 | 0.93 | 2.09 |
| Time 2 | BRS 133 | 3004.84 | 31.14 | 0.87 | 4.85 |
| Time 2 | CB06-953/963 | 5177.75 | 55.55 | 0.87 | 2.72 |

planted on the later date, although weaker (Table 4). These results indicate that controlling SR with fungicide applied at R5-R6 would likely be economically viable when SR severity is at least 2.1% for a resistant cultivar and 3.6% for a susceptible cultivar (Table 4), as long as agronomical practices and environmental conditions are similar to those observed on Experiment II in this study.

Overall, the ETL for CB06-953/963 was lower than that for BRS 133, which suggests that chemical control of SR on CB06-953/963 would be economically beneficial at an earlier growth stage than on BRS 133. Furthermore, the grain yield of CB06-953/963 decreased nearly twice as much as the yield of BRS 133 for every 1% of SR development. Nevertheless, it took over 13 days more for CB06-953/963 to reach ETL compared to BRS 133. Therefore, the resistant line CB06-953/963 had a longer window of opportunity to fungicides to control SR than the susceptible cultivar BRS 133.

The percent reduction in the yield from treatments T4-T10 compared with the control treatment (T3) of BRS 133 ranged from about 4% (80 kg ha$^{-1}$) to nearly 78% (1,630 kg ha$^{-1}$) in the first sowing date (Table 5). For the resistant line CB06-953/963, yield losses were from 2% (53.6 kg ha$^{-1}$) to 43% (1,033.8 kg ha$^{-1}$) in the first sowing date. In this sowing date, the grain yield of cultivar BRS 133 ranged from 460.2 kg ha$^{-1}$ for T1 (control) to 2,089.8

kg ha$^{-1}$ for T3. The grain yield of the resistant line CB06-953/963 ranged from 1,384.6 kg ha$^{-1}$ for T1 to 2,393.1 kg ha$^{-1}$ for T3 (Table 5), which was also higher than that of the susceptible cultivar BRS 133.

There was a significant difference in defoliation between the treatments (Table 6). BRS 133 was severely defoliated when compared to CB06-953/963 in growth stage R5.5. There was 96.5% defoliation of BRS 133 compared with 30% of CB06-953/963 in treatment T1. Even with maximum rust control in treatment T3, there was 60% defoliation of BRS 133 compared with 17.5% for CB06-953/963. Similar results were obtained by Kelley (2001) in a study of wheat cultivars with different degrees of resistance to diseases. Treatment T3 resulted in significantly ($P<0.05$) lower defoliation of BRS 133 than treatments T5-T10. The effects of the application of fungicides in treatments T4-T8 were non-significant ($P>0.05$) for the percent defoliation of CB06-953/963

**Experiment III – 2007/2008 cropping season (Time 2)**

The mean temperature and relative humidity recorded during experiment III were 22.9°C and 70.1%, respectively, and the accumulated rainfall was 710.4 mm. The rainfall in each of the three experiments was within the range of total water requirements for maximum soybean yield, 480 to 800 mm/season, depending on the

**TABLE 5 -** Yield (kg ha$^{-1}$) and yield reduction (%) of the susceptible cultivar BRS 133 and the resistant line CB06-953/963 against *Phakopsora pachyrhizi* with fungicide application at different stages (treatments) in the two sowing times of 2007/2008 cropping season

| | 2007/2008, sowing Nov. 5, 2007 | | | | 2007/2008, sowing Dec. 7, 2007 | | | |
|---|---|---|---|---|---|---|---|---|
| | Yield (kg ha$^{-1}$) | | Yield Reduction (%) | | Yield (kg ha$^{-1}$) | | Yield Reduction (%) | |
| Treat. (2) | BRS 133 | CB06-953/963 | BRS 133 | CB06-953/963 | BRS 133 | CB06-953/963 | BRS 133 | CB06-953/963 |
| T1 | 460.23 c | 1384.59 b | 77.98 | 42.14 | 194.25 c | 1223.16 d | 85.81 | 50.55 |
| T2 | 985.15 b | 1477.40 b | 52.86 | 38.26 | 269.80 c | 1650.49 c | 80.29 | 33.27 |
| T3 | 2089.77 a | 2393.08 a | 0.00 | 0.00 | 1369.10 a | 2473.48 a | 0.00 | 0.00 |
| T4 | 2009.19 a | 2305.07 a | 3.86 | 3.68 | 1185.97 a | 2445.26 a | 13.38 | 1.14 |
| T5 | 1154.48 b | 2339.46 a | 44.76 | 2.24 | 676.76 b | 2032.51 b | 50.57 | 17.83 |
| T6 | 1123.05 b | 2165.41 a | 46.26 | 9.51 | 632.35 b | 1760.59 c | 53.81 | 28.82 |
| T7 | 618.83 c | 1959.01 a | 70.39 | 18.14 | 530.90 b | 1658.99 c | 61.22 | 32.93 |
| T8 | 601.04 c | 1866.05 a | 71.24 | 22.02 | 235.40 c | 1613.12 c | 82.81 | 34.78 |
| T9 | 459.12 c | 1515.50 b | 78.03 | 36.67 | 192.25 c | 1565.44 c | 85.96 | 36.71 |
| T10 | 492.13 c | 1355.44 b | 76.45 | 43.20 | 194.93 c | 1538.46 c | 85.76 | 37.80 |
| C.V. | 7.36% | 7.91% | - | - | 8.56% | 10.09% | - | - |

[1]Treatments - see Table 1.[2]Means followed by the same letter in the column are not significantly different at 5% probability by the Scott-Knott test.

**TABLE 6 -** Percent defoliation at stage R5.5 (2007/2008 cropping season) after treatments in time 1 (116 DAS) and time 2 (110 DAS) of the susceptible cultivar BRS 133 and the resistant line CB06-953/963 against *Phakopsora pachyrhizi*

| | BRS 133(2) | | CB06-953/963(2) | |
|---|---|---|---|---|
| Treat.(1) | Time 1 2007/2008 | Time 2 2007/2008 | Time 1 2007/2008 | Time 2 2007/2008 |
| T1 | 96.5 a | 94.5 a | 30.0 a | 36.3 a |
| T2 | 96.0 a | 86.3 b | 26.3 a | 31.3 a |
| T3 | 60.0 d | 61.3 d | 17.5 b | 16.3 b |
| T4 | 65.7 d | 63.8 d | 18.8 b | 15.0 b |
| T5 | 72.5 c | 76.3 c | 16.3 b | 23.8 b |
| T6 | 77.5 c | 83.8 b | 21.3 b | 26.3 b |
| T7 | 88.8 b | 92.5 a | 25.0 b | 31.0 a |
| T8 | 95.3 a | 93.8 a | 25.0 b | 33.8 a |
| T9 | 95.8 a | 95.8 a | 38.8 a | 37.5 a |
| T10 | 97.8 a | 94.0 a | 35.0 a | 33.8 a |
| C.V. | 5.1 % | 7.4 % | 7.9 % | 25.8% |

[1]Treatments - see Table 1. [2]Means followed by the same letter in the same column are not significantly different at 5% probability by the Scott-Knott test.

climate, cultural practices, and cycle duration (Embrapa Soja, 2005).

Studies done in Thailand by Kawuki et al. (2003) reported 10-15% reductions in yield due to SR during the dry season, as compared to 100% loss during the rainy season. This information has been used to develop SR risk evaluation and epidemic forecasts (Del Ponte et al., 2006).

The $R^2$ of the regression of grain yield vs. AUDPCc in experiment III were 0.93 with CD of 34.6 kg $ha^{-1}$ for BRS 133 and 0.82 with 80.8 kg $ha^{-1}$ for CB06-953/963 (Table 3). The ETL values were 2.1% for BRS 133 and 0.9% for CB06-953/963.

Similar to experiment II, regarding the relationship between SR severity at R5.5 and grain yield, the ETL was 2.7% for CB06-953/963 and 4.9% for BRS 133 (Table 4). Differences in the ETL values found for the two sowing dates of each cultivar during the 2007-2008 season suggest that ETL can vary depending on planting date, and that this variability should be considered when developing an integrated management plan for SR (Zadoks, 1985; Bergamin Filho, 1996).

Grain yield for BRS 133 ranged from 192.3 kg $ha^{-1}$ to 1,369.1 kg $ha^{-1}$ for treatments T9 and T3, respectively. For CB06-953/963, grain yields ranged from 1,223.2 kg $ha^{-1}$ to 2,473.5 kg $ha^{-1}$ for T1 and T3. There was 85.9% yield loss for BRS 133 and 50.6% yield loss for CB06-953/963 in treatment T1 (Table 5). Treatment T4 had the largest effect in minimizing yield loss for both cultivars, and yield was not significantly different ($P$>0.05) between T3 and T4 treatments. The low yield for BRS 133 probably resulted from the high inoculum pressure and the sowing time (early December) later than the optimum in November (Embrapa Soja, 2007). Even the SR negative control (T3) had a yield limited to 1,369.1 kg $ha^{-1}$, suggesting that the pathogen had already infected the plants before the fungicides sprays had started.

The fungicides currently available, including the ones used in the experiments in this study, provide only prophylactic protection and not curative power. Therefore, even with application of the fungicide the pathogen continues to grow and reproduce, developing uredinia, continuing the disease cycle into the next rainy season (Bergamin Filho, 2006). These results emphasize the importance of early season monitoring of soybean rust development along with proper timing of the application of fungicides to manage the disease, especially in susceptible cultivars. Yield reductions for the untreated control treatment T1 in relation to T3 for BRS 133 were 78.0% and 85.8% in experiments II and III, respectively (Table 5), which provides evidence that maximum fungicide applications in T3 significantly increased yield of the susceptible cultivar. The yield of the resistant line CB06-953/963 during the 2007-2008 season for the untreated control T1 was 42.1% and 50.5% lower in experiments II and III than yield for maximum fungicide control treatment T3 (Table 5). Although the beneficial effect of fungicide application on the resistant line was lower than for the susceptible cultivar, fungicide applications did significantly increase yields in T3-T8, mainly for the December sowing time (Experiment III) under high inoculum pressure. In Experiment III, the yield in T3, with four, and T4, with three fungicide applications, was significantly higher ($P$<0.05) than in the other treatments (Table 4). These results provide evidence of the importance of fungicide application in the management of SR even for a rust resistant line. However, for the susceptible cultivar, fungicides sprays were most economically effective when the sowing date was optimum for achieving maximum yield potential, as found in a previous report (Miles et al., 2007).

Similar overall yields were attained for the two sowing times of the 2007-2008 season (experiments II and III). In some treatments, the yield was even higher in the December sowing date (experiment III) despite the high inoculum pressure, and the values of AUDPCc were about 35% higher (Table 2). This result was in agreement with results of a previous study (Koga et al., 2008) of the parents of the resistant line CB06-953/963. The parents were among a group of soybean genotypes that expressed resistance in the field during all growth stages. CB06-953/963 has the resistance gene *Rpp4,* which likely interacts with a pathogen avirulence gene in a gene-for-gene manner (Flor, 1971) signaling the activation of resistance, which was effective during the reproductive phase of the soybean, protecting grain yield in experiment III. Panthee et al. (2009) had already demonstrated this type of defense system in soybean against the SR fungus.

The results of this study demonstrate the importance to maintain SR integrated management strategies, because, although five dominant complete resistance genes have already been identified, only genes *Rpp2*, *Rpp4*, and *Rpp5* have been demonstrated to be effective in Brazil (Calvo et al., 2008; Garcia et al., 2008). Therefore, the use of complete resistance genes is not a long-lasting management solution. The combination of complete resistance with incomplete or partial resistance would improve resistance durability (Parlevliet et al., 1985; Parlevliet & Van Ommeren, 1988). Selection for tolerance may also contribute an additional tool for the management of SR, but its heritability is often low (Parlevliet, 1978) and has not yet been identified for SR on soybean (Oliveira et al., 2005; Carneiro, 2007).

In susceptible cultivars the pathogen does not find a barrier to start the infection process, and once the pathogen is established in the plant host, its control with fungicides is difficult. In resistant cultivars, in which establishment of pathogen infection is inhibited or prevented (Nürnberger et al., 2004; Flor, 1971), prophylactic fungicide applications can reduce the pathogen population to levels that decrease the frequency of pathotypes adapted to the resistance gene, slowing the selection for higher frequency of adapted pathotypes in the pathogen population. For these reasons, fungicide application, even if applied in later developmental stages and under high inoculum pressure, still contributes to control SR and to maintaining the yield of resistant cultivars.

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