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Phosphate Fertilization Reduces the Severity of Asian Soybean Rust Under High Disease Pressure

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Abstract

Mineral nutrition of plants is a strategy that can be used in the management of plant diseases. Therefore, the objective of this work is to determine which phosphorus dose reduces the severity of Asian soybean rust (*Phakopsora pachyrhizi*) with or without chemical control. Two trials were conducted under field conditions with six P doses (0, 25, 50, 100, 200 and 400 mg/dm³), and two trials in 100 L pots at P doses 0, 100, 200 and 400 mg/dm^3. The inoculation of border rows and inoculation directly on plants in pots was performed with 10^5/mL of fungus urediniospores 15 days before the application of fungicide to increase the disease pressure. The application of fungicide (azoxystrobin + ciproconazole) was carried out at the R1 stage, and afterwards the mixture was reapplied three times in chemical control treatments. The results showed that the application of triazol + strobilurin fungicides in the presence of P decreased the severity of the disease (area under disease progress curve and disease infection rate) greater than in the absence of the fungicides. The productivity and levels of chlorophyll *a*, *b* and total also increased with chemical control in the presence of P. The dose 400 mg/dm^3 of P was the most efficient in a soil with a low fertility, and 200 mg/dm³ was efficient in a soil with a high fertility. In conclusion the application of the fungicides triazol + strobilurin was very important to get good control of Asian soybean rust; phosphate fertilization contributed to the amelioration of Asian soybean rust.

Keywords: nutrient, *Glycine max*, chlorophyll, *Phakopsora pachyrhizi*, cultural control

1. Introduction

Brazil stands out in the world scenario of soy production, being the second largest producer and exporter in the world. However, production may be limited due to losses caused by diseases such as Asian soybean rust (ASR) caused by the fungus *Phakopsora pachyrhizi* H. Sydow and Sydow. In Brazil, ASR was reported for the first time in 2001 in the southern region of the country (Yorinori et al., 2005). In the following years, it disseminated throughout most of the Brazilian territory, generating losses of up to 80% in crops (Yorinori et al., 2005).

The management of ASR is mainly performed by using fungicides from the chemical group of triazoles, strobilurins and carboxamides isolated or in mixture (Xavier et al., 2015). More recently, mancozeb has been associated with systemic protective fungicides aiming to increase the efficiency of disease control and delaying the emergence of resistant isolates in the fungus population. In addition to the chemical control, crop measures such as sanitary emptiness, planting of early varieties, anticipation of planting time (Twizeyimana et al., 2011), adjustment in plant density by area (Roese et al., 2012) and balanced fertilization are cultural measures extremely important in the management of this disease (Balardini et al., 2006).

Mineral nutrition of plants can be easily manipulated and is part of an Integrated Disease Management (Zambolim et al., 2012). Among the essential elements for plant nutrition, phosphorus (P) stands out as a primordial macronutrient to guarantee high yields. In phosphate form, this nutrient is involved with metabolic processes of vital importance to plants, such as transfer and storage of energy via ATP and NADPH, synthesis of nucleic acids

(DNA and RNA), activation and deactivation of enzymes, carbohydrate metabolism, redox reactions, photosynthesis, nitrogen fixation, besides being a part of membrane phospholipids (Vance et al., 2003).

Despite the importance of phosphate fertilization, its effects on disease resistance are variable, and in some cases not very apparent (Datnoff et al., 2007). There are reports of a decrease in disease in some pathosystems, and in others there is an increase in severity (Zambolim et al., 2012).

There is a decrease in the incidence and severity of mildew (*Sclerospora graminicola*) in millet (Deshmukh et al., 1978), and wheat leaf rust (*Puccinia triticina*) in wheat (Sweeney et al., 2000) by the application of P. Phosphate fertilization of corn can reduce pytium root rot, especially when it is grown on soils deficient in P, and in other studies it can reduce the incidence of smut in corn (Huber & Graham, 1999). A number of other studies have shown that P application can reduce bacterial leaf blight in rice, downy mildew, blue mold, leaf curl virus disease in tobacco, pod and stem blight in soybean, yellow dwarf virus disease in barley, brown stripe disease in sugarcane and blast disease in rice (Huber & Graham, 1999; Kirkegaard et al., 1999; Reuveni et al., 1998, 2000). In a study by Nam et al. (2006), P had no effect on anthracnose (*Colletotrichum gloeosporioides*) on strawberry; on soybean, the application of P did not influence the severity of the coal rot (*Macrophomina phaseolina*) (Mengistu et al. 2016). Contrasting with the beneficial effects of the application of P, Sharma et al. (1996) observed that the purple spot (*Cercospora kikuchii*) in soybean seeds was more severe due to the increase of phosphorus.

The vast majority of studies addressing the effects of P on the incidence and severity of diseases have been carried out under greenhouse conditions, and the results are contradictory. Thus, since P does not have a defined pattern regarding effects on diseases, a more detailed study of the pathogen-host relationship is necessary in different environmental conditions (Datnoff et al., 2007). Due to the lack of clarity regarding the effect of P on diseases, notably on Asian soybean rust, the objective is to evaluate phosphate fertilization in the presence and absence of fungicides on Asian soybean rust control.

2. Material and Methods

The tests were conducted at the Universidade Federal de Viçosa in the municipality of Viçosa, located in the state of Minas Gerais, at 20º45′14″ S and 42º52′53″ W. Four experiments were conducted to study the effect of phosphate fertilizer and or chemical control of Asian soybean rust being two in the field (FT1 and FT2), and two inside plastic house (PT1 and PT2). Before the preparation of the experimental areas and the installation of experiments, the soils were subjected to chemical analysis at the 0-20 cm layer The chemical analyses of the two soils (field and plastic house) used in the experiments are on the Table 1. The field area (FT 1 and FT2) had already been cultivated with soybean and corn in rotation, therefore with better physical and chemical characteristics. The tests in plastic house (PT1 and PT2) were done in pots of 100 L capacity, measuring 55 cm in diameter by 80 cm of height, poor in nutrient, aiming to prove the effect of P and chemical control on the severity of Asian soybean rust. Aplication of dolomitic limestone (PRNT = 96%) was done in both field and plastic house soils aiming to increase soil saturation to 70%, 15 days before planting. Both field and plastic house tests, were implanted in 2016 and 2017, respectively.

Table 1. Soil chemical analyses used in the field* and pot trials**

pH	**P**	**K**	**Ca**	**H + Al**
water 1:2.5	-------------------- mg dm^3 -------------------		---------------------- cmol dm^3 ---------------------	
6.0* (5.5)**	3.2 (3.9)	53 (24)	2.6 (1.0)	1.98 (1.2)
Mg	**ECEC**	**CEC**	**Base saturation**	**Remaining phosphorus**
-------------------- cmol dm^3 ------------------		pH 7.0	--------- % --------	----------- mg/L -----------
1.1 (0.1)	3.8 (1.2)	5.8 (2.4)	68 (49)	21.8 (16.6)

Note. P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; H + Al: hydrogen + aluminum; ECEC: effective cationic exchange capacity; CEC: cation exchange capacity.

The two field experiments were installed in a completely randomized block design with a 6 × 2 factorial, being six P rates (0, 25, 50, 100, 200 and 400 mg/dm³) with or without fungicide application. Each replication consisted of rows 6 m in length, being four rows of plants, spaced 0.50 m, and two side edges and two centers considered useful for a total of 200,000 plants/ha.

The two plastic house experiments were installed in a completely randomized experimental design with three replicates with 10 plants each pot. The 4 × 2 split plot design was adopted being four doses of P (0, 100, 200 and

400 mg/dm³) with or without fungicide application. The substrate used in the pots was a horizon B from a Red Yellow Latosol from Viçosa, state of Minas Gerais.

The soybean variety used was the transgenic "TMG 135". In the soybean sowing, phosphate, potassium and nitrogen fertilization was made in furrows using triple superphosphate (42% P_2O_5), potassium chloride (60% K_2O) and urea (44% N). Applications of potassium chloride and urea were divided into three times, the first application in planting, the second before flowering, and the last during flowering.

To guarantee the high pressure of the disease, the inoculations were done with uredospores suspension of *P. pachyrhizi* at the vegetative stage, 15 days before the application of fungicides. The inoculations were done on plants of the two border rows, of the field tests, and on all the 10 potted plants inside the plastic house. The inoculation was done with a manual costal sprayer with a fan-tipped nozzle at 200 L/ha of inoculum suspension.

The uredospores produced for inoculation were multiplied in a soybean susceptible variety (transgenic "TMG 135") in a greenhouse using an isolate of *P. pachyrhizi* of the Plant Protection Laboratoty of the Universidade Federal de Viçosa. The suspension of uredospores was composed of water and Tween 80 (0.1 µg/mL) at a concentration of 1×10^5/mL, produced in a greenhouse with germination higher than 90%.

In the treatments with chemical control, the fungicide azoxystrobin + ciproconazole (Priori XTRA®) was applied at the dose 300 mL/ha at the R1 stage (beginning flowering), and afterwards the mixture was reapplied three times, once every 15 days. The application of the fungicide was done with a pressurized atomizer with CO_2, at 80 psi of pressure, using a conical jet nozzle expending 150 L/ha.

The evaluation of the severity of soybean rust in FT1 and FT2 was made by counting the number of lesions per cm^2 on 6 leaves of the middle third of the plants of rows using a stereoscope microscope (80×) at the stages R1, R3 and R5. In plastic house experiments, the evaluations were performed at the stages V8, R1, R3 and R5. Six leaflets of the middle third of the plants of each pot were digitalized with a resolution of 600 dpi and, from the scanned images of the abaxial face of leaves, the percentage of injured area was determined using the software QUANT® (Vale et al., 2003). With severity data of the different evaluations, the area under the disease progress curve (AUDPC) was calculated by the trapezoidal integralization method (Kranz & Rotem, 1988). The rate of disease progression (r) and the percentage of control was calculated based on the difference between the control (with application of fungicide) and treatments (without fungicide application) at each dose evaluated.

The total chlorophyll content was obtained from five disks with 0.5 cm diameter collected from different leaves of the middle third at the R1 stage using a hollowed metal punch. The disks were placed in test tubes wrapped in foil containing 5 mL of dimethylsulfoxide (DMSO) reagent previously saturated with calcium carbonate ($CaCO_3$) and incubated at room temperature (25 °C) for 12 hours. To read the pigments, the BIO-RAD spectrophotometer, SmartSpec 3000, was used at the wavelengths of 665 and 649 nm. Values for each wavelength were used for the Wellburn's equation (1994).

The soybean productivity from the trials was obtained from the collection of 10 plants per row and 10 plants per pot at each replicate. Afterwards, the pods were harvested and the grains were weighed using a digital scale. The production data on 10 plants were converted into kg/ha, considering a population of 200,000/ha.

Data were submitted to analysis of variance (ANOVA) by the Sisvar software; after evaluation of normality and homoscedasticity, by the Shapiro-Wilk and Bartlett tests, respectively. The effects of the quantitative treatments were calculated through regression analyses.

3. Results

For all treatments of the experiments conducted in the field (FT_1 and FT_2) and in pots (PT_1 and PT_2), there was no significant difference in the interaction between sprayed and non-sprayed treatments in pots. No significant differences was also obtained among P doses added to the soil, for all variables evaluated, except for chlorophyll *a* in PT_1, and productivity and chlorophyll *b* in PT_2 (Table 2). However, the unfolding was performed by regression of quantitative variables to obtain a more representative model.

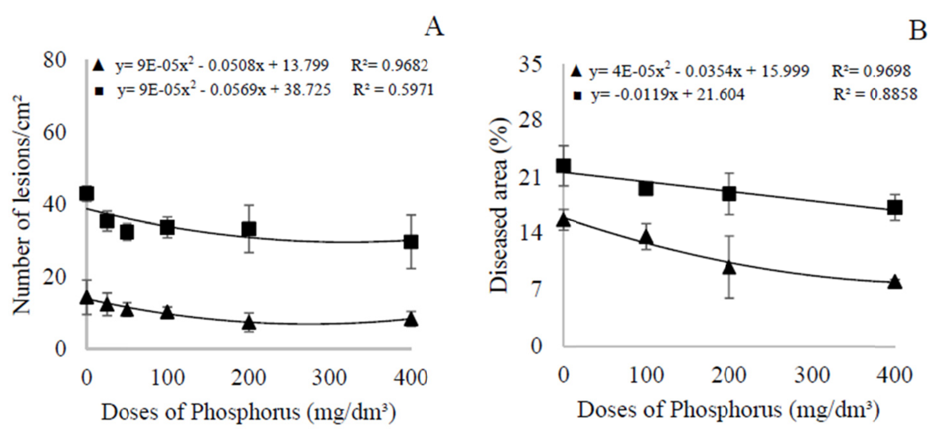
Table 2. Analysis of Variance of the effects of phosphate fertilization on severity (SEV), Area Under the Disease Progression Curve (AUDPC), production (PRO), chlorophyll a (CHLa), chlorophyll b (CHL b) and total chlorophyll of the field test 1 (FT_1), field test 2 (FT_2), pot test 1 (PT_1) and pot test 2 (PT_2) using the fungicide (azoxystrobin + cyproconazole)

Assay	Variables	S	P	S × P
FT_1	SEV	***	**	ns
	AUDPC	***	ns	ns
	PRO	***	**	ns
FT_2	SEV	***	**	ns
	AUDPC	***	ns	ns
	PRO	***	**	ns
PT_1	SEV	***	**	ns
	AUDPC	***	***	ns
	PRO	ns	***	ns
	CHL *a*	***	***	*
	CHL *b*	***	***	ns
	CHL t	***	***	ns
PT_2	SEV	***	***	ns
	AUDPC	***	***	ns
	PRO	***	***	**
	CHL *a*	ns	***	ns
	CHL *b*	***	***	*
	CHL t	***	***	ns

Note. The results are not significant (ns), significant to $P \leq 0.05$ (*), significant to $P \leq 0.01$ (**) or $P \leq 0.001$ (***); P: Doses of Phosphorus; S: sprayed with fungicide (azoxystrobin + cyproconazole).

3.1 Severity Assessment

Figure 1A and 1C (number of lesions/cm^2), 1B and 1D (diseased area %) showed ASR severity data evaluated at stage R_3 in field and pot trials. Based on the results, there was a decreased of the ASR severity with the increase of the dose of P. Plants submitted to the chemical control of the disease presented a lower severity in relation to plants in the absence of control. There was a significant difference between the sprayed and not-sprayed treatments and between the P doses incorporated into the soil (Table 2).



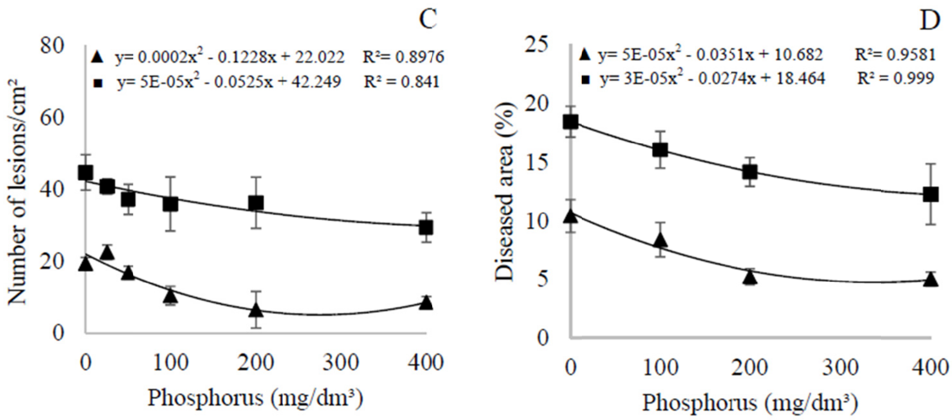


Figure 1. Severity of Asian soybean rust at the stage R_3, in FT_1 (A), FT_2 (B) and PT_1 (C), PT_2 (D) subjected to different doses of P with the application of fungicide (azoxystrobin + cyproconazole) (▲) or without application (■). FT1: field test 1; FT2: field test 2; PT1: pot test 1 and PT2: pot test 2

The disease severity of FT_1 and FT_2 (6.5 and 7.2 diseased area (%), respectively) was lower at 200 mg/dm³, in the chemically controlled treatment (Table 3). In PT_1 and PT_2 the lower disease severity (8.0% and 5.0%, respectively) was obtained in the treatment with 400 mg/dm³ of P, in treatments submitted to ASR chemical control. The non sprayed treatments was three times higher in field test trials and two times higher in pot test trials than the sprayed treatments, in almost all P doses evaluated (Table 3).

Table 3. Severity of soybean rust at the stage R3 in sprayed (S) and not-sprayed (NS) treatments with fungicide (azoxystrobin + cyproconazole) for FT_1^a, FT_2^b, PT_1^a and PT_2^b trials

P (mg/dm³)	Severity(%)							
	FT_1^a		FT_2^b		PT_1^a		PT_2^b	
	S[c]	NS[d]	S	NS	S	NS	S	NS
0	19.4(1.7)[c]	44.6(3.2)	14.2(1.4)	42.9(3.0)	15.7(1.6)	23.0(1.4)	10.4(1.2)	18.4(1.5)
100	10.4(1.2)	35.9(2.8)	10.1(1.2)	33.6(2.5)	13.6(1.0)	19.5(1.7)	8.4(1.0)	16.0(1.2)
200	6.5(0.6)	36.2(2.9)	7.2(0.8)	33.2(2.3)	9.8(1.1)	18.9(1.5)	5.2(0.4)	14.1(1.0)
400	8.5(1.0)	29.4(1.8)	8.2(0.9)	29.6(1.7)	8.0(0.9)	17.2(1.3)	5.0(0.3)	12.2(0.8)
$\bar{x}$	14.0	37.3	10.5	34.5	11.8	19.4	7.2	15.2

Note. FT_1^a: field test 1; FT_2^b: field test 2; PT_1^a: pot test 1; PT_2^b: pot test 2. [c]S: sprayed; [d]NS: not sprayed; [c]Number in parentheses means standard deviation.

Table 4 shows the results concerning the percentage of ASR control in FT_1 and PT_2 obtained by calculating the difference between the treatment with fungicide application and without application at each P dose evaluated. In FT_1 and PT_2, the highest values were 82.0 and 63.0%, respectively, at the dose of 200 mg/dm³ of P. The mean percentage of disease control in FT1 was 63.0%, and 53.0% in PT2. When compared the percentage of control of the highest dose of P of plants without phosphate fertilization with the percentage of control, the difference between these treatments ranged from 14.2 to 45.0%, considering all field experiments and pots evaluated. In the case of PT_1, the increase in the productivity obtained with the chemical control of ASR at the dose of 400 mg/dm³ of P was 65.0%. In the PT_2, the highest increase was 34.0% at the dose of 200 mg/dm³ of P. In general, the mean increases of these assays were 54.0% in FT_1 and 25.0% in PT_2 (Table 4).

In the FT_1, the lowest values for disease progression rate ("r") (0.69 lesions/day) were at the dose of 200 mg/dm³ of P for treatments receiving fungicide; in the treatments without application of fungicide at the dose 400 mg/dm³ of P, the lowest value was 1.8 lesions/day. In PT_2, the lowest values were 0.26 and 0.71 in the sprayed and non-sprayed treatments, respectively, both at the dose of 400 mg/dm³ P (Table 4).

Table 4. Percentage of control of Asian soybean rust and disease progression rate ("r") in sprayed (S) and not-sprayed (NS) treatments with fungicide (azoxystrobin + cyproconazole) and increase in productivity (IP) for FT_1 and PT_2

P (mg/dm^3)	FT_1[a]				PT_2[b]			
	Control (%)	r (S)[c]	r (NS)[d]	IP (%)	Control (%)	r (S)	r (NS)	IP (%)
0	56(1.5)[e]	1.0	2.3	52(1.2)	43(1.3)	0.60	1.1	5(0.9)
25	44(1.4)	0.91	2.0	52(1.2)	-[f]	-	-	-
50	54(1.6)	0.80	2.0	53(1.3)	-	-	-	-
100	70(1.8)	0.81	1.9	58(1.5)	47(1.5)	0.40	1.0	33(1.0)
200	82(1.9)	0.69	1.9	47(1.1)	63(1.6)	0.30	0.80	34(0.9)
400	70(1.8)	0.80	1.8	65(1.6)	59(1.4)	0.26	0.71	27(1.0)
$\bar{x}$	63	0.83	2.0	54	53	0.39	0.88	25

Note. [a]FT_1: field trial 1; [b]PT_2: pot trial 2; [c] means Sprayed and [d] Not Sprayed. [e]Number in parentheses means standard deviation; [f]Treatments not incorporated into the test.

The variation of the doses of P did not differ significantly in AUPDC in FT_1 and FT_2 (Table 2); however, a decreased in AUPDC was observed according to the increase in phosphate fertilization. The treatments with chemical control of ASR, presented lower values when compared to the treatments in which the control was not performed. This tendency was observed in all trials developed (Figure 2).

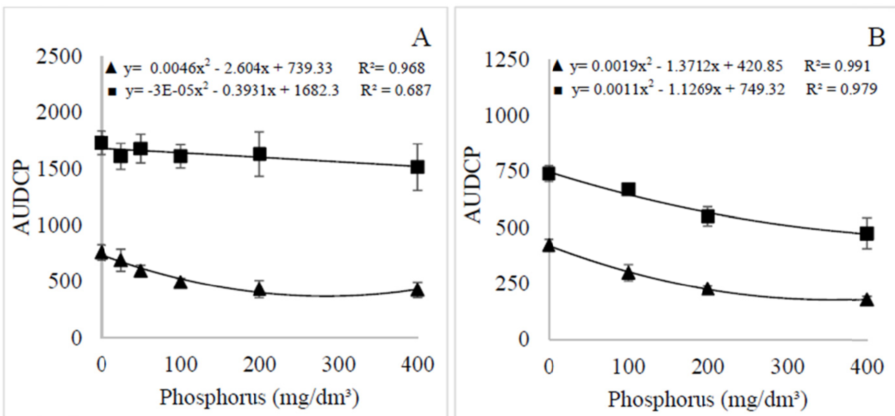


Figure 2. Area under the disease progress curve (AUDPC) in FT_1 (A) and PT_2 (B) using different doses of phosphorus with the application of fungicide (azoxystrobin + cyproconazole) (▲) or without application (■). FT_1: field trial 1; PT_2: pot trial 2

The lowest values of AUPDC in FT_1 were 425.2 at the P dose of 400 mg/dm^3 in the sprayed treatment and 1,515.5 at the same dose, in the non-sprayed treatment (Table 5). In PT2, the best AUDPC values were 180.0 in the sprayed treatment, and 475.0 in the non-sprayed treatment with chemical control at a dose of 400 mg/dm^3 of P for both treatments (Table 5).

Table 5. Area Under Disease Progress Curve (AUDPC) in sprayed (S) and not sprayed (NS) treatments with fungicide (azoxystrobin + cyproconazole) in diferent phosphorus dose (P) in FT_1, FT_2, PT_1 and PT_2 trials

P (mg/dm³)	AUDPC							
	FT_1[a]		FT_2[b]		PT_1[a]		PT_2[b]	
	S	NS	S	NS	S	NS	S	NS
0	756.5(28.9)[c]	1731.8(56.5)	565.1(24.6)	1264.3(54.3)	635.1(28.5)	907.2(38.5)	422.4(16.5)	740.7(26.5)
100	494.0(18.5)	1612.6(52.3)	417.0(12.5)	1036.1(43.5)	453.2(17.2)	745.5(26.7)	298.8(13.0)	670.2(22.0)
200	432.7(16.8)	1631.3(58.8)	417.5(12.0)	985.0(40.1)	309.7(13.2)	732.4(25.1)	226.7(9.5)	550.2(19.5)
400	425.2(13.5)	1515.5(54.1)	447.1(18.7)	1127.3(48.9)	249.8(10.5)	587.7(19.9)	180.0(6.4)	475.0(24.7)
$\bar{x}$	565.7	1647.2	471.7	1134.2	412.0	743.2	282.0	609.0

Note. FT_1[a]: field test 1; FT_2[b]: field test 2; PT_1[a]: pot test 1; PT_2[b]: pot test 1. [c]Number in parentheses means standard deviation.

3.2 Productivity Assessment

Soybean productivity in the trials FT_1 and PT_2 increased as the phosphate fertilizer increased. However, in the FT_1, at the dose of 400 mg/dm³ of P in the treatment without fungicide aplication, a lower productivity was obtained in relation to the dose of 200 mg/dm³ of P (Figure 3). Treatments with fungicide application provided a higher productivity in relation to the treatments in which rust control was not performed.

The optimum phosphorus levels based on the models obtained in this study for FT_1 was 275 mg/dm³ of P for the sprayed treatment and, 214 mg/dm³ of P for the not-sprayed treatment; for PT_2 trials the optimum was 318 and 373 mg/dm³ of P, for the sprayed and not-sprayed treatments, respectively (Figure 3).

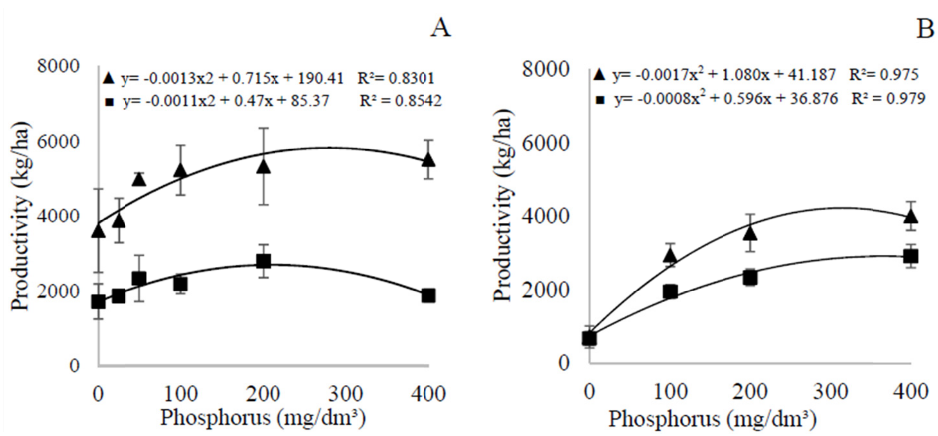


Figure 3. Productivity of soybean (kg/ha) in FT_1 (A) and PT_2 (B) using different doses of phosphorus with the application of fungicide (azoxystrobin + cyproconazole) (▲) or without application (■).
FT_1: field trial 1; PT_2: pot trial 2

The highest yields in FT_1 were observed at the doses 400 mg/dm³ of P in the sprayed treatment (5,500 kg/ha) and 200 mg/dm³ of P in the non-sprayed treatment (2,793 kg/ha). The treatments submitted to chemical control of ASR produced an average of 4,747 kg/ha, and in the treatments in which the control was not performed, the average productivity was 679 kg/ha (Table 6). In PT_2, the highest productivity per plot was obtained at the dose of 400 mg/dm³ of P, both in the test with chemical control (4,006 kg/ha) and in the test without control (2,913 kg/ha) (Table 6).

Table 6. Soybean productivity (Kg/ha) in sprayed (S) and not sprayed (NS) treatments with fungicide (azoxystrobin + cyproconazole) in different phosphorus dose (P) in FT_1, FT_2, PT_1 and PT_2 trials

P (mg/dm³)	Soybean productivity (Kg/ha)							
	FT_1[a]		FT_2[b]		PT_1[a]		PT_2[b]	
	S	NS	S	NS	S	NS	S	NS
0	3606.6(56.7)[c]	1713.3(25.0)	3440.0(50.0)	1380.0(16.0)	40.2(6.8)	32.2(6.0)	710.2(13.0)	669.3(10.3)
100	5213.3(87.0)	2186.6(29.8.0)	4933.3(79.0.0)	3346.6(53.0)	1422.6(25.8)	1016.5(16.0)	2943.7(35.2)	1949.2(36.0)
200	5313.3(89.1)	2793.3(30.2.0)	5140.0(76.9)	3493.3(59.8)	1793.2(30.0)	1571.2(27.6)	3543.7(53.0)	2333.0(26.0)
400	5500.0(92.8)	1873.3(27.1)	4420.0(66.0)	3220.0(55.5)	2025.0(28.7.0)	1939.3(31.0)	4006.3(58.0)	2913.2(32.9)
x̄	4747.7	679.5	4395.5	2721.1	1320.3	1139.8	2801.0	1966.2

Note. FT_1[a]: field test 1; FT_2[b]: field test 2; PT_1[a]: pot test 1; PT_2[b]: pot test 1.

3.3 Chlorophyll Content

Based on the obtained models, it was possible to verify that the increase in the dose of phosphorus provided an increase in chlorophyll *a*, *b* and total content levels in PT_2 (Figures 4A and 4B) and Figure 5. The highest levels of chlorophyll *a*, *b* and total were found at 400 mg/dm³ of P, in treatments sprayed or not with fungicide, and the lowest values were found in plants that did not receive phosphate fertilization (Table 7). It was possible to verify that the sprayed treatments had higher levels of chlorophyll *a*, *b* and total in relation to the treatments in which the disease was not controlled by fungicides.

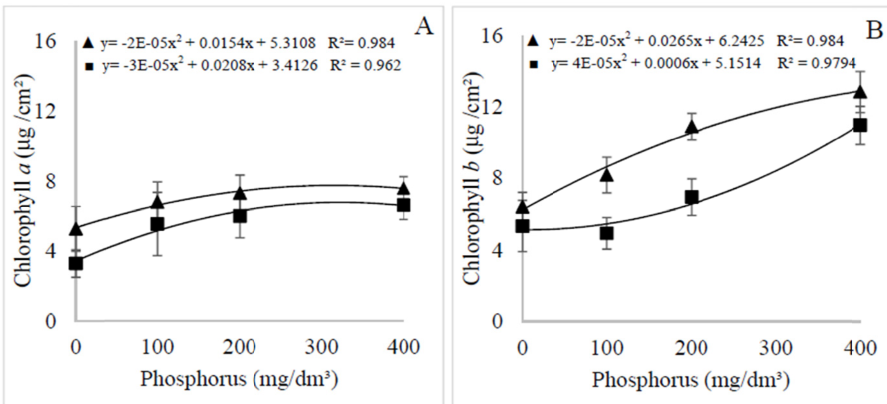


Figure 4. Chlorophyll a and b contents (µg/cm²) in soybean leaves subjected to different doses of phosphorus with the application of fungicide (azoxystrobin + cyproconazole) (▲) or without the application of fungicide (s) (■) in the pot trial 2 (PT_2)

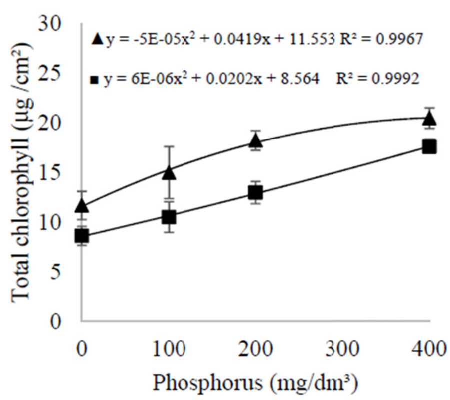


Figure 5. Total chlorophyll content (µg/cm²) in soybean leaves subjected to different doses of phosphorus with the application of fungicide (azoxystrobin + cyproconazole) (▲) or without the application of fungicide (s) (■) in the pot trial 2 (PT_2)

The mean increase in the concentration of chlorophyll *a*, *b* and total was obtained by calculating the difference between the treatment with fungicide application and without application at each dose tested. The application of fungicide provided an increase of, on average, 21.5% of chlorophyll *a*, 26.7% of chlorophyll *b* and 24.6% of total chlorophyll. The ratio of chlorophyll content *a/b* was higher in plants that did not receive fungicide application (Table 7).

Table 7. Chlorophyll a, b and total (μg/cm²) contents, chlorophyll a/b ratio and increase of chlorophyll (CI) in soybean leaves, in sprayed (S) and not-sprayed (NS) treatments, with fungicide (azoxystrobin + cyproconazole) in PT_2^a test

P (mg/dm³)	CHL *a*[b]		CHL *b*[c]		CHL t[d]		CHL *a/b*		IC *a*	IC *b*	IC t
	S	NS	S	NS	S	NS	S	NS			
	---------- μg/cm² ----------								---- % ----		
0	5.2	3.2	6.4	5.3	11.6	8.6	0.80	0.61	37.7	16.6	26.1
100	6.7	5.5	8.1	4.9	14.9	10.5	0.81	1.12	18.1	39.4	29.8
200	7.2	6.0	10.9	6.9	18.1	12.9	0.63	0.86	17.5	36.2	28.7
400	7.5	6.6	12.8	10.9	20.4	17.6	0.54	0.60	12.6	14.4	13.8
$\bar{x}$	6.7	5.3	9.5	7.0	16.3	12.4	0.69	0.80	21.5	26.7	24.6

Note. [a]PT_2: pot trial 2; [b]CHL *a*: Chlorophyll *a*; [c]CHL *b*: Chlorophyll *b*; [d]CHL t: Total Chlorophyll.

It was possible to verify that productivity correlated strongly and positively with chlorophyll *a*, *b* and total in the not-sprayed treatment of PT_1, and with the chlorophyll parameter *b* in PT_2. The correlation showed the same tendency to be positive; however, it was not as strong as in sprayed treatments. The severity of ASR correlated negatively with productivity in all trials (Table 8).

Table 8. Pearson's simple correlation coefficients of the parameter productivity of soybeans of sprayed (S) or not-sprayed treatments (NS) with fungicide (azoxystrobin + cyproconazole) in relation to the chlorophyll a (Chl *a*), chlorophyll b, (Chl *b*) and total chlorophyll (Chl *t*) contents and severity of Asian soybean rust in FT_1^a, FT_2^b, PT_1^c and PT_2^d trials

			Chl *a*	Chl *b*	Chl *t*	Sev
Productivity	FT_1^a	S		-	-	-0.88^{ns}
		NS	-	-	-	-0.84^{ns}
	FT_2^b	S	-	-	-	-0.87^{ns}
		NS	-	-	-	-0.84^{ns}
	PT_1^c	S	0.99^*	0.98^*	0.99^*	-0.98^*
		NS	0.76^{ns}	0.71^{ns}	0.74^{ns}	-0.90^*
	PT_2^d	S	0.99^*	0.90^*	0.94^*	-0.93^*
		NS	0.99^*	0.75^{ns}	0.90^*	-0.97^*

Note. * Significant at 5% probability of error, by t test; [ns] Not significant. [a]FT_1: field trial 1; [b]FT_2: field trial 2; [c]PT_1: pot trial 1; [d]PT_2 pot trial 2.

4. Discussion

The results obtained in the field and in pots experiments showed that increasing phosphate fertilization and fungicide application (azoxystrobin + cyproconazole) reduced the severity of ASR. Phosphorus is one of the main macronutrients in agriculture, being a constituent of many organic molecules of the cell, mainly as phosphate. This nutrient is constitutive of the deoxyribonucleic acid (DNA), ribonucleic acid (RNA), adenosine triphosphate (ATP) and phospholipids. In addition, it is involved with several metabolic processes in the plant and in the pathogen (Dordas, 2008). Phosphate fertilization may have a variable response in plant resistance to disease, depending on the interaction of plant species and pathogen (Datnoff et al., 2007). Similar results were obtained when different phosphorus and potassium doses were combined, on the control of ASR (Balardin et al., 2006). However, this study was carried out in a greenhouse, under controlled conditions, unlike the present study, which was conducted in the field (FT1 and FT2) and plastic house (PT1 and PT2) for two years. In addition, severity reduction of ASR as a result of phosphate fertilization has been reported for several crops such as bacterial leaf blight in rice, downy mildew, blue mold, leaf curl virus disease in tobacco, pod and stem blight in soybean, yellow dwarf virus disease in barley, brown stripe disease in sugarcane and blast disease in rice (Huber & Graham, 1999; Kirkegaard et al., 1999). However, as other nutrients, P does not have a defined pattern regarding its effect on diseases. A more detailed study of the pathogen-host relationship is necessary in different environmental conditions (Datnoff et al., 2007).

Our hypothesis for reduction on ASR as phosphorus doses increased is due to the formation of more vigorous plants, resulting in higher yields as obtained in all the experiments. Phosphate fertilization has the characteristic of favoring a vigorous development of the root system and accelerating the process of maturation of tissues, reducing the infectious period of rust and other leaf pathogens, causing the plant to avoid infection by pathogens, which attack mainly younger leaves (Huber & Graham 1999; Amtmann et al., 2008). In addition, the application of superphosphate may produce biochemical changes such as an increase in protein synthesis, polyphenols, ammonium peroxidase and an increase in cellular activity in leaf tissues, creating an environment unfavorable to pathogens (Zambolim et al., 2012).

Phosphorus deficiency also exerted an effect on ASR severity. Treatments that did not receive phosphate fertilization had the highest disease severity in relation to the treatments that received fertilization. High level of ASR severity in P-deficient plants was reported by Balardin et al. (2006). Phosphorus deficiency reduces the amount of phospholipids in the plasma membrane, changing its permeability, resulting in the extravasation of metabolites and favoring germination of fungus spores (Datnoff et al., 2007).

Soybean production increased according to the increase in phosphorus application and the chemical control. In field experiments, the best results were obtained with the application of 200 mg/dm³ of P; in pots, 400 mg/dm³ of P. This difference between experiments could have occurred due to the chemical differences of the soil. The soil used in the pots was poorer in nutrients than the soil used in field trials; thus, they required a greater fertilization so that the plants were able to express their productive potential.

In plants that did not receive phosphate fertilization, we verified that productivity was low or almost null. These results are in agreement with reports by Godoy et al. (2016); reduction of soybean productivity is related with less availability of soil P.

At higher doses of phosphorus, there was a reduction in productivity possibly due to a nutritional stress caused by high nutrient levels, which may affect production (Sing et al., 2013). In addition, high levels of phosphorus may decrease the availability of zinc to the plant (Kranz & Rotem 1988).

Asian rust is a very aggressive disease with a high potential to cause damage to the soybean crop. The latent period of the disease in the field ranges from 8 to 12 days. Thus, the application of the fungicide (ciproconazole + azoxystrpbine) was critical to slow the progression of the disease and to provide less productivity losses. According to Godoy et al. (2016), the application of the fungicide (ciproconazole + azoxystrobin) provided a better control of ASR, generating positive effects on grain yield.

The fungicides of the triazole chemical group (cyproconazole) inhibit the demethylation of C-14, forming methylated compounds by inhibiting the formation of ergosterol of the fungal membrane. Thus, there is an imbalance between membrane lipids, with the inhibition of phospholipids and accumulation of free fatty acids, reaching levels that are harmful to the fungus (FRAC, 2018). The fungicides of the strobilurin group (azoxystrobin) inhibit mitochondrial respiration by blocking electron transport between the cytochrome b and c_1, disrupting ATP production and being effective against spore germination (Bartlett et al., 2002). Thus, plants that received applications of cyproconazole + azoxystrobin obtained less losses caused by the fungus *P. pachyrhizi* because of the efficient control provided by the fungicide, ensuring a greater soybean production. Thus the application of this mixture (triazol + strobilurin) of fungicide with adequate level of phosphorus on the soil is very important integrated control measure to control ASR.

The increase in the phosphate fertilization in the soil increased chlorophyll *a*, *b* and total levels. Plants that received low or no phosphate fertilization presented lower values of chlorophyll. According to Plesniar et al. (1994), sunflower plants with a phosphorus deficiency have lower levels of chlorophyll. Phosphorus is not a constituent of chlorophyll (Datnoff et al., 2007); however, it plays an important role in plant nutrition, benefiting the active process of nitrogen absorption, which is an integrant to enzymes associated with chloroplasts that participates in the synthesis of chlorophyll molecules, reflected in the indexes of photosynthetic pigments (Kranz & Rotem 1988).

The levels of chlorophyll *a*, *b* and total in plants that received chemical control were higher than plants that were not treated with fungicide. Pathogens may affect several physiological processes in their hosts, both directly and indirectly (Owera et al., 1981). As a result of the infection caused by pathogens, the development of chlorotic and necrotic areas in the plant may occur due to the structural damage of chloroplasts, reducing chlorophyll and producing photosynthetic assimilates (Berger et al., 2007).

Another factor that may have contributed to a higher concentration of chlorophyll in plants that received the chemical treatment was because the fungicide applied in its composition, azoxystrobin, belongs to the group of strobilurin. This chemical group acts by preventing the germination of spores and presents an eradicating and curative action, inhibiting the development of pathogens at initial stages after germination and avoiding the formation of chlorotic or necrotic areas (McCartney et al., 2007). In addition, active ingredients belonging to this chemical group have the characteristic of promoting an increase in chlorophyll content due to an increase of nitrogen assimilation and a reduction of ethylene production, resulting in the "green effect" of the plants. These factors may contribute to the lower stress of the plants, resulting in a higher productivity (Bartlett el al., 2002).

The positive correlation of productivity with the photosynthetic pigment contents is in agreement with the results of other studies, which found positive correlations between leaf chlorophyll content and productivity of different crops (Ramesh et al., 2002; Boggs et al., 2003, Guler & Ozcelik, 2007). Thus, the measured chlorophyll can be used as an indication of productivity.

The results in the present work demonstrate that the increase in phosphorus doses in the presence of fungicide (ciproconazole + azoxystrobin) decreases severity (AUDPC, rate of progression) of Asian soybean rust. It provides a higher productivity and higher levels of chlorophyll *a*, *b* and total, being a strategy that can be implemented in the integrated management of the disease. In general, in field trials, the best responses for the evaluated variables were obtained at the dose of 200 mg/dm³ of P, and in pots, the best dose was 400 mg/dm³ of P.

5. Conclusion

Chemical control associated with an increase in phosphorus doses, decreased the severity and rate of progression of Asian soybean rust, increased the productivity and chlorophyll *a*, *b* and total. The best responses was obtained at the dose of 200 mg/dm³ of P in field trials and in plastic house 400 mg/dm³ of P. Phosphate fertilization contributed to the amelioration of Asian soybean rust.

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