Comparison of the efficacy of QoI fungicides, alone or in mixture with triazoles, in Asian soybean rust control, 2016/17 growing season

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ABSTRACT

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Evolution has been reported for the reduction in *Phakopsora pachyrhizi* sensitivity to the mixtures of the three site-specific mechanisms of action (DMIs, QoIs and SDHIs) used in its control. The aim of this study was to quantify the current contribution of QoIs, alone or in mixture with triazoles, in the control of Asian soybean rust. In an experiment carried out in the field, the efficacy of quinone outside inhibitors (QoIs), or strobilurins, was compared for Asian soybean rust control. The isolated effects of four doses of azoxystrobin, picoxystrobin, pyraclostrobin and trifloxystrobin were evaluated, as well as of four applications during the crop cycle. Control by commercial mixtures of these QoIs with triazoles was also compared. Severity was evaluated in four phenological stages, and control was calculated based on final severity data, area under rust progress curve, defoliation, one-thousand-grain mass, grain yield and damage caused by the disease. The lowest mean of rust control efficacy resulted from applications of azoxystrobin (15.8 and 11.19%) and pyraclostrobin (15.4 and 16.76%) and the highest mean was found for picoxystrobin (54.1 and 54.35 %) and trifloxystrobin (69.7 and 64.46%), calculated based on final severity and AUDPC, respectively. Such efficacy is not enough to cover the fungicide application cost, although there was a positive effect of treatments on disease control and grain yield.

Keywords: quinone outside inhibitors, sensitivity reduction, *Phakopsora pachyrhizi,* resistance to fungicides.

RESUMO

Reis, E.M.; Carregal, L.H.; Zanatta, M. Comparação da eficácia de fungicidas IQes, isolados ou em mistura com triazóis, no controle da ferrugem asiática da soja, safra 2016/17. ***Summa Phytopathologica,*** v.45, n.2, p.28-32, 2019.

Tem sido relatada a evolução da redução da sensibilidade de *Phakopsora pachyrhizi* às misturas dos três mecanismos de ação sítio-específicos (IDMs, IQes e ISDHs) usadas no seu controle. O objetivo desse trabalho foi quantificar a contribuição atual dos IQes, isolados ou em mistura com triazpois, no controle da ferrugem asiática da soja. Em experimento conduzido no campo comparou-se a eficácia dos inibidores da quinona externa (IQes), ou estrobilurinas, no controle da ferrugem da soja. Foram avaliados os efeitos isolados de quatro doses da azoxistrobina, picoxistrobina, piraclostrobina e trifloxistrobina e de quatro aplicações durante o ciclo da cultura. Comparou-se também o controle das misturas comerciais desses IQes com triazóis. Avaliou-se a severidade em quatro estádios fenológicos, calculou-se o controle com dados da severidade final, com dados da área abaixo da curva de progresso da ferrugem, a desfolha, a massa de mil grãos, o rendimento de grãos e o dano causado pela doença. A menor eficácia média no controle da ferrugem resultou das aplicações da azoxistrobina (15,8 e 11,19%) e da piraclostrobina (15,4 e 16,76%) e a maior para a picoxistrobina (54,1 e 54,35%) e trifloxistrobina (69,7 e 64,46%) calculada respectivamente com dados da severidade final e da AACPD. Essa eficácia não é suficiente para cobrir o custo da aplicação dos fungicidas, embora tenha sido observado reflexo positivo dos tratamentos no controle e no rendimento de grãos.

Palavras-chave: inibidores da quinona externa, redução da sensibilidade, *Phakopsora pachyrhizi*, resistência a fungicidas

The soybean [*Glycine max* (L.) Merr.] area cultivated in Brazil has been increasing every growing season. In 2016/17, it was 33.4 million hectares and in 2017/18 it was estimated at 35.33 million hectares (1).

Considering the diseases that affect soybean, the greatest damage is due to Asian soybean rust (ASR), caused by the Basidiomycete fungus *Phakopsora pachyrhizi* Sydow & Sydow (16), which was detected in South America in 2001 (7). Damage to soybeans can be scientifically estimated based on the damage function y = 1,000 - 6.21 LI, where 'y' is the yield normalized to 1,000 kg/ha and LI is the incidence in the central leaflets inserted in the main plant stem (2).

ASR control measures include sowing early maturing cultivars at the beginning of the recommended season, growing cultivars with partial resistance to rust, observing the soybean-free period, eliminating volunteer plants and applying fungicides in the foliage when the disease is detected in the region (13).

Chemical control of ASR began in the 2002/03 season (4). Differently from FRAC's recommendations (3), site-specific DMI fungicides (difenoconazole, flutriafol, tebuconazole) have been used alone, resulting in over 80% control (8).

From the 2005/06 season, based on producers' complaints of control failure and on scientific reports (8, 15), double mixtures of site-specific fungicides DMIs + QoIs (azoxystrobin + cyproconazole and pyraclostrobin + epoxiconazole), endorsed by FRAC (3) as an anti-resistance strategy, started to be used. However, in the 20012/13 season, producers complained again of control failure regarding these site-specific co-formulations (9).

In the 2014/15 soybean crop, ASR control was done by spraying fungicide mixture containing SDHI (benzovindiflupyr + azoxystrobin), which had an efficiency greater than 90%. After three seasons of use, data from experimental fields showed reduced control by this mixture (9). Therefore, in 16 years, the three site-specific fungicides became inefficient in controlling ASR in Brazil. Moreover, DMIs and QoIs alone or in co-formulation with each other or with SDHIs showed a reduction in ASR control efficacy, indicating that double or triple mixtures do not work as an anti-resistance strategy.

In parallel to the reduction in *P. pachyrhizi* sensitivity to the three active ingredients (DMI, QoI and SDHI), the respective fungal mutations that confer cross and multiple resistance to the three mechanisms of action (MOA) were identified (4, 6, 14).

We hypothesized that the fungitoxicity of the four QoIs currently in use against *P. pachyrhizi*, with the same mechanism of action, has differently reduced, resulting in differences in efficacy among them.

The aim of this study was to quantify the current contribution of QoIs, alone or in mixture with triazoles, to Asian soybean rust control.

MATERIAL AND METHODS

The experiment was carried out at Agrocarregal Experimental Station - AES, Rio Verde, Goiás State, Brazil, coordinates: longitude 17º47'1.4''S, latitude 51º00'37''W, at 790 m a.s.l. The effects of treatments were evaluated on soybean cultivar NS 7209, seeded on 12/14/2016, 16 seeds/m, under natural inoculation.

Four QoIs were compared in the commercial formulations: (i) azoxystrobin (Priori 250 SC) 240, 360 and 480 mL/ha; (ii) pyraclostrobin (Comet 250 CE) 240, 360 and 480 mL/ha, (iii) picoxystrobin (Oranis 250 SC) 240, 360 and 480 ml/ha, and (iv) trifloxystrobin (Flint 500 WG) 180, 240 and 400 g/ha. As additional treatments, the performance of the mixtures azoxystrobin + cyproconazole (Priori Xtra 300 mL/ha), pyraclostrobin + fluxapyroxad (Orkestra 300 ml/ha), picoxystrobin + cyproconazole (Aproach Prima 300 mL/ha), and trifloxystrobin + prothioconazole (Fox 400 mL/ha) were compared. The recommended oil was added to each fungicide.

The fungicides were sprayed four times with a CO_2 pressurized precision sprayer and 150L/ha volume: (i) on 01/30/2017, at V6 growing stage; (ii) on 02/14/2017, at R5.1; (iii) on 02/28/2017, at R5.4, and (iv) on 13/03/2017 at R5.5.

Experimental design was in completely randomized blocks with four replicates and experimental units of 2.25 x 6.00 m long; data were analyzed according to Scott-Knott test.

ASR severity, based on the percentage of leaf area covered with symptoms/signs, was evaluated on 3/6/2017 (GS R5.3), 03/11/2017 (R5.4), 03/19/2017 (R6) and 03/23/2017 (R6), which was used to calculate the area under disease progress curve (AUDPC). Defoliation (%), one-thousand-grain mass (g) and grain yield were also evaluated.

RESULTS AND DISCUSSION

Table 1. Effect of treatments on rust severity, area under the disease progress curve (AUDPC), and disease control.

Treatment	Severity (%)	Control (%) in relation to severity	AUDPC	Control (%) in relation to AUDPC
1- Unsprayed	75.00 i	-	1216.37 h	-
2- Azoxystrobin	66.25 h	11.7	1033.62 g	15.02
3- Azoxystrobin	66.25 h	11.7	1043.75 g	14.19
4- Azoxystrobin	57.00 f	24.0	992.87 f	18.37
Mean azoxystrobin	63.17	15.8	**1023.41**	**11.19**
5- Azoxystrobin + cyproconazole	41.75 e	44.3	656.00 e	46.07
6- Pyraclostrobin	63.00 g	16.0	1023.37 g	15.87
7- Pyraclostrobin	66.75 h	11.0	1035.25 g	14.89
8- Pyraclostrobin	60.50 g	19.3	976.62 f	19.71
Mean pyraclostrobin	63.41	15.4	704.74	16.76
9- Pyraclostrobin + fluxapyroxad	21.37 b	71.5	381.62 b	68.63
10- Picoxystrobin	39.25 e	47.7	671.75 e	44.77
11- Picoxystrobin	31.00 d	58.7	481.87 d	60.38
12- Picoxystrobin	33.00 d	56.0	512.12 d	57.9
Mean picoxystrobin	34.41	54.1	**552.24**	**54.35**
13- Picoxystrobin + cyproconazole	24.75 c	67.0	370.50 b	69.54
14- Trifloxystrobin	23.25 c	69.0	448.50 c	63.13
15- Trifloxystrobin	20.25 b	73.0	387.37 b	68.15
16- Trifloxystrobin	24.50 c	67.3	424.25 c	65.12
Mean trifloxystrobin	22.66	69.7	**420.04**	**64.46**
17- Trifloxystrobin + prothioconazole	15.50 a	**79.3**	291.12 a	76.07
C.V.	4.95	-	3.14	-

Means followed by the same letter in each column did not differ statistically from one another according to Scott-Knott test at 5%.

ASR was detected in the experimental area on March 7th, 2017, and within 41 days the severity progressed from 0 to 100% (data not shown).

Severity was 75% in unsprayed plots, and 63.1% for azoxystrobin, 63.4% for pyraclostrobin, 34.4% for picoxystrobin, 22.6% for trifloxystrobin and 15.50% for trifloxystrobin + prothioconazole (Table 1).

ASR control was calculated based on the data from the third severity assessment and from AUDPC. As to severity, the efficacy of treatments ranged from 11.0% (pyraclostrobin 360 mL/ha) to 73% (trifloxystrobin 240 mL/ha). When calculated by using AUDPC, the control efficacy varied from 14.8% (pyraclostrobin 360 mL/ha) to 68.1% (trifloxystrobin 240 mL/ha). Considering QoI mixed with DMIs, when calculated based on severity, the efficacy ranged from 44.3 for azoxystrobin + cyproconazole, 71.5 for pyraclostrobin + fluxapyroxad to 79.3 for trifloxystrobin + prothioconazole, respectively. Similarly to the calculation based on AUDPC data, the control efficacy was 46.0, 68.3 and 76.0%, respectively (Table 1).

If not transformed into efficacy, the values for AUDPC make comparisons difficult among treatments. In the unsprayed plots, the highest value was 1216.37 and the lowest one was 291.1 units, only allowing the identification of the best treatment. However, when expressed as control, such difficulty was eliminated. For example, the maximum control of 76.0%, close to 80%, is considered the minimum to match the fungicide spraying cost in order to control ASR (11).

Defoliation of soybean plants ranged from 77.25% to 100% and was a function of ASR intensity (Table 2).

Regarding one-thousand-grain mass, the lowest value was 129.7g, determined for grains of the unsprayed plots, while the highest value was 162.9 g for grains from plots that were sprayed with pyraclostrobin + fluxapyroxad (Table 2). Data regarding one-thousand-grain mass, as well as AUDPC, have little importance in the selection of treatments with greater efficacy for disease control.

Grain yield in the unsprayed plots was 2508 kg/ha and, in the best treatment (pyraclostrobin + fluxapyroxad), it was 3579 kg/ha, corresponding to a maximum damage of 1071.6 kg/ha or 29.9%. The average yield of sprayed plots was 2528.9 kg/ha, 2613.8 kg/ha for pyraclostrobin, 2991.6 kg/ha for picoxystrobin, and 3110.4 kg/ha for trifloxystrobin.

Defoliation of soybean plants was a positive and linear function of ASR severity expressed as the function $y = 0.4729x + 68.815$, with coefficient of correlation, $R^2 = 0.8316$, where 'y' is plant defoliation and 'x' is rust severity (Fig. 1). The equation indicates that every 1% rust severity caused 0.4729% defoliation. The report by Reis et al.

Table 2. Effect of treatments on the defoliation of soybean plants, one-thousand-grain weight (GW), and grain yield.

Treatment	Defoliation (%)	GW (g)	Grain Yield (kg/ha)
1- Unsprayed	100.00 h	129.76 e	2508.13 c
2- Azoxystrobin	98.75 h	131.52 e	2561.63 c
3- Azoxystrobin	99.50 h	134.93 e	2486.43 c
4- Azoxystrobin	100.00 h	133.08 e	2538.67 c
Mean azoxystrobin	-	-	2528.91
5- Azoxystrobin + cyproconazole	90.25 g	144.45 c	2957.42 b
6- Pyraclostrobin	100.00 h	132.81 e	2554.18 c
7- Pyraclostrobin	100.00 h	136.34 e	2640.59 c
8- Pyraclostrobin	99.50 h	131.19 e	2646.77 c
Mean pyraclostrobin	**-**	**-**	**2613.85**
9- Pyraclostrobin + fluxapyroxad	66.75 a	162.96 a	3579.80 a
10- Picoxystrobin	90.50 g	141.51 d	2965.04 b
11- Picoxystrobin	83.00 e	141.46 d	2976.29 b
12- Picoxystrobin	80.75 d	145.19 c	3032.92 b
Mean picoxystrobin	**-**	**-**	**2991.62**
13- Picoxystrobin + cyproconazole	79.25 c	154.42 b	3368.95 a
14- Trifloxystrobin	88.50 g	145.57 c	3003.86 b
15- Trifloxystrobin	80.00 d	147.95 c	3132.40 b
16- Trifloxystrobin	80.75 d	148.75 c	3195.23 b
Mean trifloxystrobin	**-**	**-**	**3110.49**
17- Trifloxystrobin + prothioconazole	77.25 b	156.55 b	3489.27 a
C.V. (%)	1.23	2.83	6.55

Means followed by the same letter in each column did not differ statistically from one another according to Scott-Knott test at 5%.

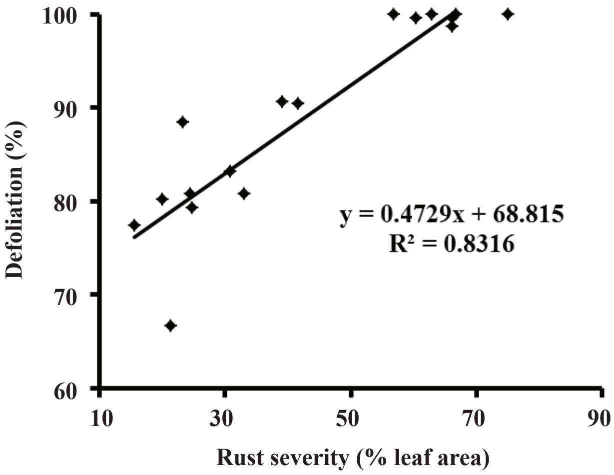

Figure 1. Positive relationship between foliar rust severity (x) and soybean plant defoliation (y).

(10) confirms that defoliation is a function of ASR severity and that its evaluation is not necessary since severity data alone are sufficient to explain the effect of disease intensity on soybean yield, a reflex of the efficacy of treatments.

The relationship between grain yield and ASR severity was

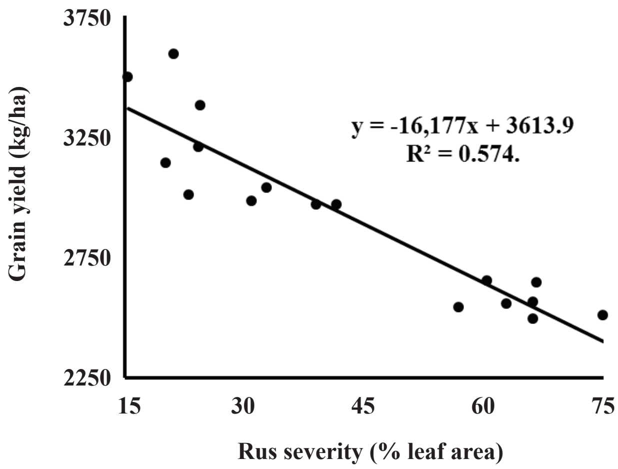

Figure 2. Negative relationship between grain yield (y) and Asian soybean rust severity (x).

expressed as the function y = -16.177x + 3613.9, **R٠,٥٧٤ = ², where 'y' is grain** yield, 'x' is ASR severity, and R is the coefficient of determination. According to the equation, every 1.0% severity reduces 16.177 kg/ha grains in 3613.9 kg/ha potential yield (Fig. 2).

Considering the AUDPC ratio of rust on grain yield, the obtained damage function was y = -1.061x + 3665.5, R2 0.875, where each unit of AUDPC reduced 1.061 kg/ha in 3666.5 kg/ha yield (Fig. 3). The AUDPC has been recommended especially for the evaluation of the reaction of cultivars to diseases, having no practical importance to estimate the damage caused by a disease in a crop (5). In this case, the determination and practical use of severity has an advantage over AUDPC, which is more time-consuming.

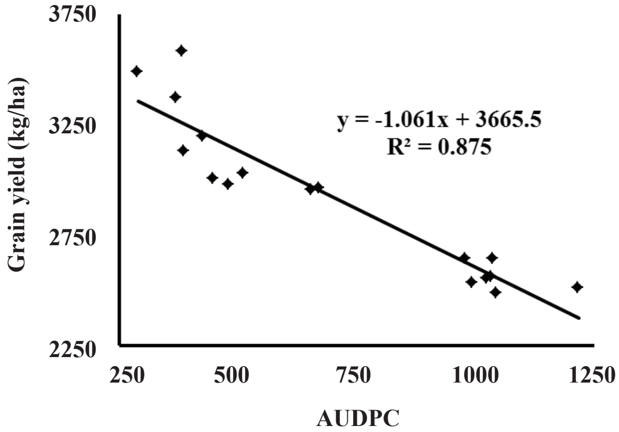

Figure 3. Negative relationship between soybean grain yield (y) and area under Asian soybean rust severity progress curve (AUDPC) (x).

Our results confirmed (6, 8, 9, 14) the low field performance of fungicides containing strobilurins in ASR control. After detection of the mutation at position F129L, which confers a reduction in *P. pachyrhizi* sensitivity to QoIs, studies have been carried out to quantify whether this mutation differently affected the fungitoxicity of the four strobilurins mostly used for ASR control. Although they have the same mechanism of action, inhibition of electron transfer in the respiratory chain of complex III in the mitochondria, their efficacy was different: one group showed 15.8% (azoxystrobin) and 15.4% (pyraclostrobin) control, and the other group had superior mean control, picoxystrobin 54.1% and trifloxystrobin (69.7%) (Table 1).

Azoxystrobin + cyproconazole co-formulation, used in the 2009/10 season, was sprayed on 10 million ha in the 2006/07 season, and pyraclostrobin + epoxiconazole mixture in 15 million ha. Therefore, exposure to the fungus for a long time may have determined the most pronounced reduction in fungitoxicity to *P. pachyrhizi*; in addition, differences in the intrinsic characteristics of the molecules of QoIs can determine differences in fungitoxicity among them.

Finally, it can be inferred that the greater efficacy of picoxystrobin and trifloxystrobin explains the better performance of commercial mixtures of picoxystrobin + cyproconazole and trifloxystrobin + prothioconazole in ASR control. In contrast, the reduced fungitoxicity of azoxystrobin and pyraclostrobin explains the inferior performance of the mixtures in which they are incorporated.

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