Reduction of soybean rust control by fungicides from 2003 to 2014 growing seasons in field experiments

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RESUMO

Reis, E. M.; Zanatta M.; da Silva, L. H. C. P. Redução do controle da ferrugem da soja por fungicidas nas safras de 2003 a 2014, em experimentos de campo. **Summa Phytopathologica**, v. 00, p. 00-00, 2015.

Os dados do Ensaio Nacional Cooperativo de Fungicidas, coordenados pela Embrapa Soja e disponibilizados na Internet, foram analisados e apresentados em gráficos anuais do controle da ferrugem da soja. Foi feita a análise de regressão entre a percentagem de controle e as safras de uso dos fungicidas. Houve uma redução do controle da ferrugem da soja pelos fungicidas isolados, tanto os inibidores da desmetilação, como os inibidores da quinona externa e ou de suas misturas ao longo do período de 2003 a 2014. A redução do controle para o tebuconazol foi de 90 para 15%, do tebuconazol + picoxistrobina de 80 para 68%; do ciproconazol + picoxistrobina de 81 para 61%; do ciproconazol + azoxistrobina de 79 para 41%, do epoxiconazol + piraclostrobina de 79 para 23% e da azoxistrobina de 90 para 16%. O coeficiente de redução anual do controle variou de 3.7 para o tebuconazol + picoxistrobina a 13.8% para a azoxistrobina. A diminuição do controle da ferrugem pode ser atribuída a redução da sensibilidade de *Phakopsora pachyrhizi* aos fungicidas triazóis, estrobilurinas e as suas misturas, portanto um caso de resistência cruzada e múltipla.

Palavras-chave: estrobilurinas, falha de controle, *Glycine max*, *Phakopsora pachyrhizi*, resistência fungicidas, triazóis.

ABSTRACT

Reis, E. M.; Zanatta M,.; da Silva, L. H. C. P. Reduction of soybean rust control by fungicides from 2003 to 2014 growing seasons, in field experiments. **Summa Phytopathologica**, v. 00, p. 00-00, 2015.

Data from the National Cooperative Fungicide trials, coordinated by Embrapa Soja and available on the Internet, were analysed and shown in yearly graphs of soybean rust control. Regression analysis of the control percentage x growing seasons of fungicide use, coefficients of determination and of annual control reduction coefficient were performed. There was a reduction in fungicide control either for fungicides triazoles and estrobilurinas alone or in co-formulation over the period from 2003 to 2014. The rust control reduction for tebuconazole alone was from 90 to 15%, for tebuconazole + picoxystrobin from 80 to 68%; for cyproconazole + picoxystrobin from 81 to 61%; for cyproconazole + azoxystrobin from 79 to 41%, for pyraclostrobin + epoxyconazole from 79 to 23%, and for azoxystrobin alone from 90 to 16%. The annual control reduction coefficient ranged from 3.7 for tebuconazole + picoxystrobin to 13.8% for azoxystrobin. The control decrease may be due to reduced sensitivity of *Phakopsora pachyrhizi* to triazoles and strobilurins fungicides alone or to their mixtures therefore, a case of cross and multiple resistance.

Keywords: control failure, fungicide resistance, *Glycine max*, *Phakopsora pachyrhizi,* strobilurins, triazoles.

Asian soybean rust, caused by *Phakopsora pachyrhizi* Syd. & Syd (*Pp*) was reported in Brazil in 2001/02 and in the next season started its control with fungicides (5, 36).

Chemical control of Asian soybean rust (ASR) has been the most efficient tactic for the epidemic management, maintaining soybean yield, and keeping crop economic sustainability. Currently 30 million hectares of soybean has been annually treated with fungicide with on the average of three sprayings/ha especially with demethylation inhibitor (DMI) + quinone outside inhibitor (QoI) alone or in their co-formulations (Kleffmann, 2013). In some regions, where there is no winter, soybean is cultivated in a second annually crop, and receiving up to 11 sprayings/ha (4).

DMIs have been used alone in 24% of the area and the QoIs alone area has increased, in 2012, reaching 3.66%. Therefore, this situation favors the selection of *Pp* mutants with reduced sensitivity towards fungicides (9, 11, 21).

The *Pp* isolates reduction in sensitivity to triazoles has been previously reported (6, 15, 30, 33, 34) and recently for QoI (2, 3).

The hypothesis for the control failure by co-formulations may be due to multiple resistance by the fungus towards triazoles and strobilurins.

The aim of the study was to quantify the ASR annually control reduction to triazoles, co-formulations, and azoxystrobin from the 2003 to 2014 growing seasons.

MATERIAL E METHODS

Data published by Soybean Antirust Consortium, a research group that conducts National Fungicides Cooperative Trials, at several sites in Brazil and coordinated by Embrapa Soja, were analyzed for fungicides efficiency (15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25). The analysed period covers seasons from the 2003/04 to 2013/14 totaling 86 trials.

Fungicides trials had the objectives to compare the most potent fungicides to control soybean rust started in 2003/04 growing season.

Graphs of control were drawn and data subjected to regression analysis. The trend line, coefficient of determination and the annual control reduction coefficient were calculated.

RESULTS

There was a reduction in rust control for fungicide, either alone as tebuconazole and azoxystrobin or their co-formulations.

Tebuconazole. In eleven growing seasons of tebuconazole spraying, one of the most used fungicides alone; there was a control reduction from 90% (2003/04 season) to 15% (2013/14 season). The reduction in the control was represented by the line y = -7,2x

+ 94 018, where y is the control (%) x time (growing seasons). The coefficient of determination was 0.83 and the ACRC 7.2% (Fig 1. A).

(Insert Fig. 1)

Data analysis showed not only the control reduction by tebuconazole but also for the co-formulations of strobilurins with triazoles

Tebuconazole + picoxystrobin. From 2010/11 to 2013/14 seasons, a 12% control reduction from 80 (2010/11 season) to 68% (2013/14 season) was found. The control reduction was represented by the line y = -3.7x + 83 where y is the control (%) and x time (growing seasons). The coefficient of determination was 0.94 and the ACRC of 3.7% (Fig. 1 B). The lowest ACRC was determined for this co-formulation.

This mixture was introduced in the market in the 2011/12 season showing 80% control and control reduction in just four season of exposure to *Pp* has been reduced 1n 12%.

Cyproconazole + picoxystrobin. In five crop seasons (2009 – 2014) of use, there were a 19% control reduction from 81% (2009/10 season) to 61% (2013/14 season). The control reduction was represented by the line y = -4.5x + 89 where y is the control (%) and x time (growing seasons). The coefficient of the determination was 0.941 and the ACRC 4.5%, higher than the previous mixture (Fig. 1 C).

Cyproconazole + azoxystrobin. This mixture was introduced in the market in the 2003/04 season showing high efficiency. In the analyzed period (seven seasons), there was a 38% control reduction from 79% (2008/09 growing season) to 41% (2013/14 season). The control reduction was represented by the line y = -6.5429x 90,067 + where y is the control (%) and x time (growing seasons). The coefficient of determination was 0.7701 and ACRC 6.5429% (Fig. 1 D).

Epoxyconazole + pyraclostrobin. This mixture was introduced in the market in the 2002/03). In five analyzed seasons there was a 56% control reduction from 79 (2009/10 season) to 23% (2013/14 season). The control reduction was represented by the line *y* = -12.6x + 99 where y is the control (percentage) and x time (growing seasons). The coefficient of determination was 0.7888 and the ACRC 12.6%, the fastest among the analyzed co-formulations (Fig. 1 E).

Azoxystrobin. This fungicide alone was introduced in the market in the 2002/03. In five analyzed seasons, there was a 63% reduction in rust control by azoxystrobin alone from 90% (31) to 16% (2013/14 season). The control reduction was represented by the line y = -13.8x + 92.8, where y is the control (%) and x time (growing seasons). The coefficient of determination was 0.9012 and the annual control reduction coefficient (ACRC) 13.8% (Fig. 1 F).

The ACRC over time ranged from 73.7 to 13.8% for tebuconazole + picoxystrobin and azoxystrobin alone respectively. All of them showed a slow control reduction along the time and not abrupt.

DISCUSSION

Chemical control has been the main method for ASR control since its report in Brazil in 2002/03 season (15). Thus, *Pp* has been facing selection against the fungicides over 13 growing seasons.

Control reduction by DMI fungicides. After five growing seasons (from the 2002/03) of DMI used alone, Silva et al. (34) reported the rust control failure in Goiás in 2006/07 season, for cyproconazole, flutriafol and tebuconazole. Until then, the flutriafol was very effective and used by the researchers as standard control. From 2005/06 onwards, there was an effectiveness reduction of flutriafol, in Mato Grosso state, assigning first, the control failure to formulation quality (30).

After the flutriafol failure, although as a DMI, tebuconazole became widely used and with high efficiency, and taken as a reference fungicide to control ASR. This is an evidence that DMIs are not equal in their mode of action.

At the University of Rio Verde, and in other institutes, was confirmed the reduction of control efficiency. Was clearly shown the control effectiveness reduction, when one compares the DMI performance in 2005/06 season with the 2012/13. In 2005/06 season, the rust average control by DMIs was 90.3%. After only eight seasons, corresponding to the 2012/13, the alone DMI control was 52.0, with 42% control efficacy reduction (30, 31, 33).

Phakopsora pachyrhizi sensitivity reduction to tebuconazole and cyproconazole with only 18% and 38% control, respectively, was also shown by Godoy and Palaver (15). In this season, the DMI + QoI co-formulations did not show efficiency reduction at that timet; cyproconazole + azoxystrobin, 72% and pyraclostrobin + epoxyconazole 88% control. At this time, the mean control for co-formulations was 80%. It is likely that at this time the efficiency was ensured by QoIs, as the control mean for the DMI alone was only 40% (3, 31).

Control failure towards the co-formulations was first noticed in the 2012/13 growing season.

Control reduction by DMI + QoI co-formulations. It has been scientifically documented the reduction of rust control by triazole + strobilurin co-formulations (3, 6). An intriguing question is whether the low control showed by mixtures can be attributed to a sharp reduction in sensitivity of the fungus towards the DMIs. Or, the low mixtures control can be attributed now to the reduced sensitivity of *Pp* to QoIs? Data from the fungicides cooperative trials has brought the answer to these questions. The Figure 1 (A, B, C, D, F) showed an annually slow control reduction by the traditionally fungicide used in soybean in the onward seasons.

The control reduction of soybean rust either by triazole (Fig. 1-A) or strobilurin (Fig 1-F) alone or in co-formulations is a worrying fact having the possibility to be a multiple resistance case.

Cross-resistance in a pathogen occurs when exposure to one fungicide from a group confers resistance to other fungicides in the same group. Multiple resistance occurs when a pathogen is resistant to a number of fungicides from more than one group as a result of multiple exposures to different fungicides (1, 7, 8, 14).

Control reduction by QoIs. The control reduction by co-formulations can be attributed to *Pp* reduced sensitivity towards QoI. From the 2010/11 season was detected the early control reduction by azoxystrobin, reaching only 16% control in the 2013/14 season (Fig. 1-F).

The *Pp* sensitivity shift towards QoI has been shown by Blum & Reis in 2013/14 growing season (3). Work conducted *in vivo*, with two *Pp* strains suspected of reduced control showed reduction in sensitivity to QoIs. There was an IC_{50} increase from 1.4 to 95.9 mg/L and the sensitivity reduction factor from 10.2 to 700.0 (3).

Although the FRAC (10,11) anti-resistance strategies proposal, do not recommend the sole use of fungicides with specific site mode of action, the QoIs were used alone, in the 2008 season in 0.45% of the soybean area, and in the 2012 season, reached 3.66%

(over 27 million ha) (Kleffmann, 2015). Contrary to the FRAC statement their use alone in a short time, in a wide area probably increased selection pressure on the fungus to the directional selection of mutants with reduced sensitivity to these fungicides. Furthermore, in Paraguay strobilurins have been used, both alone and in the off-season crop, which contributes to further increase the selection pressure for resistance of the fungus towards QoIs.

It should be stressed that the DMIs, the first fungicide used to control soybean rust, were also used alone, with several sprayings per season, in a large area has contributed for the selection of strains with reduced sensitivity *Pp* in just four seasons of use.

It is likely that the *Pp* gradual reduction in sensitivity to the co-formulations components (DMI and QoI), started with DMIs and more recently with QoIs. A similar fact rarely occurs with fungicides mixtures for other fungi. *P, pachyrhizi* won the strategy of using two different site-specific chemicals mixture to fight resistance.

DMI and QoI fungicides, act in only a specific site in the fungal cell. Therefore, they are vulnerable to fungi strain selection towards reduction or lost in sensitivity. FRAC (11) classifies DMI and QoI fungicides at high risk for the development of resistance and, therefore, they should not be used alone.

Published reports have shown that, by the known mechanism, *Pp* could not develop resistance to QoI fungicides (26, 27, 29) by which the mutation that would enable resistance is lethal eliminating the mutants from the selection process. In most cases, resistance resulted from modification of the cytochrome *b* target site.

However, an increasing amount of experimental evidence has suggested that QoI resistance can occur via other mechanisms (1, 7, 8, 35).

Contrary to what was expected the DMI and QoI mixture did not avoid the *Pp* sensitivity reduction towards IQes. The same that has happened with DMIs, now *Pp* showed reduced sensitivity towards QoIs. The *Pp* sensitivity shift to azoxystrobin was not sharp but slow taking several seasons (Fig. 1-F)

It has been reported that reduction in the sensitivity of fungi to QoIs (8, 14, 26) can be crossed among strobilurins, which makes the situation most worrisome.

A chemical group, also site-specific, was recently introduced in the market for rust control, the carboxamidas, with a similar QoI mechanism of action also linked to the fungi respiration, or the succinate dehydrogenase inhibitors enzyme (SDHI) (respiratory complex II process) (1).

The fungal selection towards fungicide resistance takes time. For DMI was four seasons and for co-formulations eight seasons. The longer the co-formulation exposure to the fungal selection the greatest was the control reduction detected in 2013/14 season.

Some actions should have been taken to avoid the fastest sensitivity reduction of *Pp* towards DMI, QoI, SDHIs and their co-formulation fungicides.

The Cooperative Fungicidal Trials data, here analyzed, points out two distinct situations:

(i) mixtures that were longer exposed to directional selection toward reducing the control, in the 2013/14 season, showed the greatest reductions, and therefore, there was little improvement in trying to reverse control from low to high (> 80%) by the addition of multisite protector fungicide. This happened to the oldest co-formulations that suffered the highest reductions as cyproconazole + azoxystrobin and epoxyconazole + pyraclostrobin (Fig. 1 B, C, D, E).

These mixtures could be withdrawn from the market for some seasons, exploiting the directional selection, and reintroduced into the market but now added to the multisite protector as was done with *Phytophthora infestans* Mont. De By resistance to metalaxyl M in Ireland and in The Netherlands (11, 13).

ii) mixtures that were less time exposed to selection toward reducing control, but still with high control performance, such as azoxystrobin + benzovindiflupyr, epoxyconazole + pyraclostrobin + SDHI, prothioconazole + trifloxystrobin + bixafen, pyraclostrobin + SDHI and tebuconazole + picoxystrobin, should no longer be exposed to directional selection without the multisite protector addition to make their lives longer.

The addition of multisite protector to site-specific fungicides before the control reduction aims to preserve them without experiencing directional selection for reduced control (10, 28). It is likely that the addition of multisite protector before reduction control can avoid this inconvenience. Moreover, considering the shift in sensitivity of *Pp* towards IDM, QoI ant their co-formulations, site-specific fungicides, they should reach the market already added to a multisite protector.

It is likely the short exposure time of the new DMI + QoI + SDHI co-formulations and added by a multisite protector, has the potential to preserve the high performance of site-specific fungicides as happened with the very specific used to control late blight of potato and tomato.

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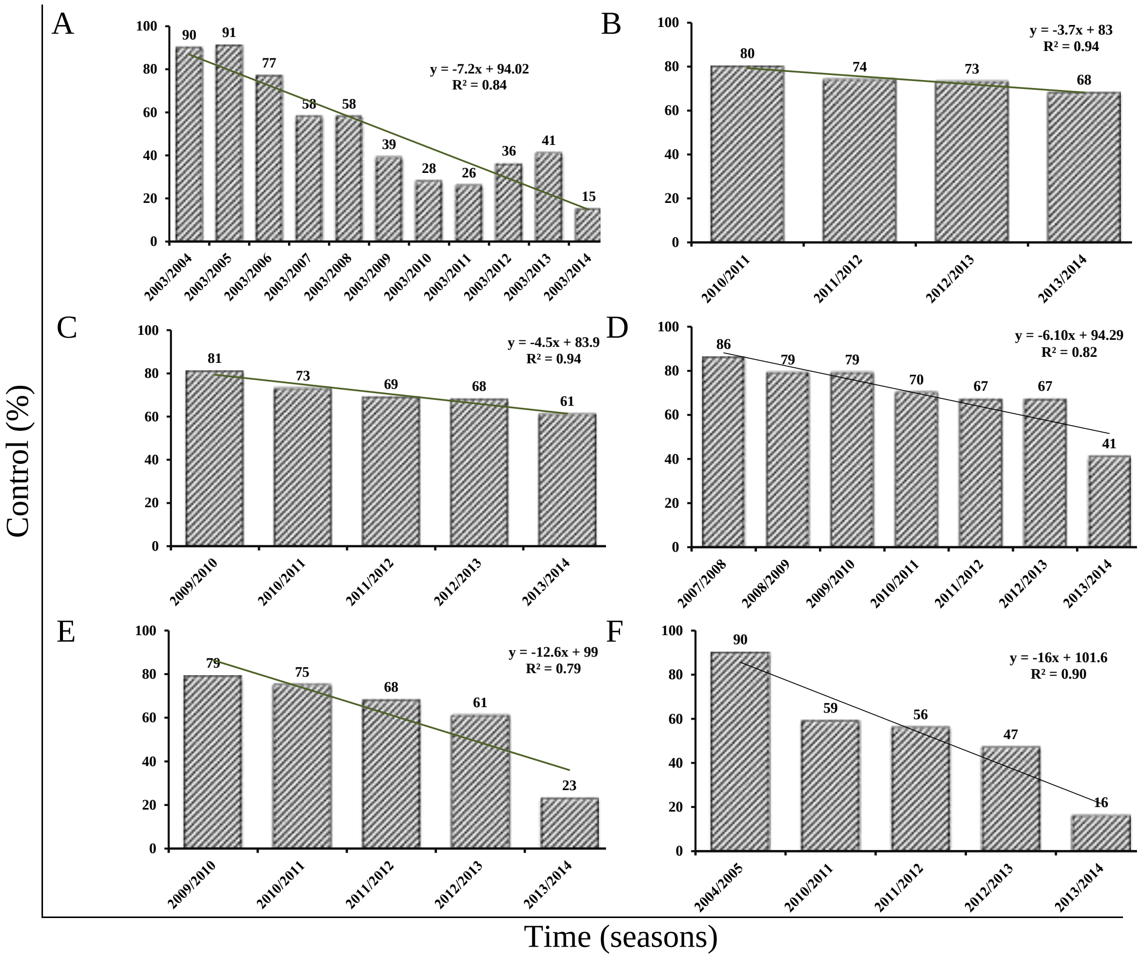

Figure 1. Relationship between control reduction of *Phakopsora pachyrhizi* by fungicides and the growing seasons. (A) – tebuconazole; (B) cyproconazole + picoxystrobin; (C) tebuconazole + cyproconazole; (D) cyproconazole + azoxystrobin; (E) epoxyconazole + pyraclostrobin; and (F) azoxystrobin (Data taken from 4, 5, 16, 17, 18, 19, 20, 21, 22, 23, 24, and 25; data for F, azoxystrobin, 2004-05 season, taken from 31).