

Addition of Mancozeb to DMI + QoI, and SDHI + QoI Co-formulations Improving Control of Asian Soybean Rust

Erlei Melo Reis¹, Mateus Zanatta¹ & Andrea Camargo Reis¹

¹ Instituto AGRIS, Passo Fundo, RS, Brazil

Correspondence: Instituto AGRIS, Rua Miguel Vargas, 291 Passo Fundo, CEP: 99025-380, RS, Brazil. E-mail: erleireis@upf.br

Received: August 12, 2020

Accepted: October 2, 2020

Online Published: December 15, 2020

doi:10.5539/jas.v13n1p195

URL: <https://doi.org/10.5539/jas.v13n1p195>

Abstract

The reduced sensitivity of *Phakopsora pachyrhizi* to site-specific fungicides used to control Asian soybean rust by the current co-formulations needs investigation. To improve the rust control the performance of cyproconazole + picoxystrobin, tebuconazole + picoxystrobin, cyproconazole + azoxystrobin, epoxyconazole + pyraclostrobin, fluxapyroxade + pyraclostrobin, benzovindiflupyr + azoxystrobin, prothioconazole + trifloxystrobin and cyproconazole + trifloxystrobin mixtures added by five doses of the multisite mancozeb were evaluated. The fungicides were sprayed at four growth stages the first performed at R1 growth stage and the others with 15-18 days intervals. The rust severity was quantified, the control was calculated, the percentage of chlorophyll and the yield of soybean were determined. The mean of rust control by the mixtures without addition of the multi-site fungicide was 46% (21 to 71%). There was an increase in control efficiency due to addition and mancozeb doses in all treatments. Control over 80% was obtained with tebuconazole + picoxystrobin, fluxapyroxade + pyraclostrobin, benzovindiflupyr + azoxystrobin, and prothioconazole + trifloxystrobin added at least of 2.0 kg/ha mancozeb. In unsprayed plots the maximum 78% severity corresponded to 59% damage. There was an increase in chlorophyll content and soybean yield as a function of the mancozebe increased doses: 2,019 kg/ha in the unsprayed control and in the best treatment 5,132 kg/ha. Actual control reduction due to fungal decrease in sensitivity can be improved by the multi-site fungicide addition.

Keywords: manganese ethylene bisdithiocarbamate, *Phakopsora pachyrhizi*, carboxamides, strobilurins, triazoles

1. Introduction

Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi* Sydow & Sydow, (*Pp*) (Sydow & Sydow, 1914) was first reported in Paraguay and Brazil in 2001/02 growing season (Morel, 2001; Yorinori et al., 2005). Damage cause by SAR to soybean can be appraised in commercial farms with equations reported by Danelli et al. (2015).

In Brazil, ASR chemical control started in the 2002/03 season. The first applied chemicals were triazoles or demethylation inhibitors (DMI) mainly flutriafol and tebuconazole solo. Difenoconazole, myclobutanil and tetraconazole with the same single-site mode of action were also used in a lesser extent. Later, cyproconazole, epoxiconazole and tebuconazole were used only in mixtures with QoIs azoxystrobin, pyraclostrobin, and trifloxystrobin (Godoy & Palaver, 2010).

Almost the total cultivated area of 36.9 million hectares in 2019/20 season (CONAB, 2020) has been sprayed for rust control with DMIs, QoIs or SDHIs in double or triple co-formulations (Reis et al., 2017).

After five growing seasons from the 2002/2003 of DMI used alone, Silva et al. (2008) reported the rust control failure in Goiás state in 2006/07 season, in farms and trials where flutriafol and tebuconazole had been sprayed alone. Moreover, until then the flutriafol was very effective and used as standard control and making it the market leader.

Since 2006/07, season after season a ASR continuous control reduction has been reported (Reis et al., 2017). Thus, in 2012/13 growing season control reduction for tebuconazole reach 15%, for azoxystrobin 16%, and for the three most common and used mixtures 37%, means of the National Fungicide Cooperative Trials conducted in several sites in Brazil and coordinated by Embrapa Soja (Reis et al., 2017). The maximum soybean yield is achieved with

control higher than 80%, thus, with 37% mean control losses due to control failure can threaten soybean economical sustainability (Godoy & Palaver, 2011; Godoy et al., 2015; Reis et al., 2015, 2017).

ASR control reduction has been reported in Brazil and fungus sensitivity reduction towards DMI, QoI and their mixtures has been shown (Fundação, 2008; Silva et al., 2008; Blum, 2009; Godoy et al., 2015). Mutation Cyp 51 towards DMI, and F129L to QoI have been reported (Schmitz et al., 2014; Klosowski et al., 2016), and on August 7th, 2017 the international Fungicide Resistance Action Committee (FRAC) warned on the presence of I86F mutation towards SDHI fungicides in sub unit C of SDH enzyme (Simões et al., 2018).

The threat that concerns soybean producers is the evolution season-after-season of the *P. pachyrhizi* reduced sensitivity to site-specific fungicides (Reis et al., 2017). The effectiveness of fungicide control of ASR and which results in maximum profit for the producer is over 80%, but currently it is less than 50% (Reis et al., 2017). When control is reduced by the development of resistance producers profits are also restricted (Main, 1977).

We hypothesized that the control failure and the evolution of control reduction, season after season is due to the cross and multiple resistance of *P. pachyrhizi* towards DMIs, QoIs and SDHIs fungicides can be partially reversed by the addition of multi-site fungicide.

The objective of this work was to compare the performance of commercial site-specific co-formulations with and without the addition of mancozeb a multisite mode of action fungicide in an attempt to recover ASR control efficacy.

2. Materials and Methods

Work was carried out in Água Santa county, Rio Grande do Sul state, Brazil, 52°07'26.49"W, 28°14'38.45"S, 752 m a.s.l.

Soybean Brasmax Ativa RR cultivar seeded on December 9th, 2015, 30 seed/m², fertilized with 300 kg/ha, 02-20-20 (N-P₂O₅-K₂O) in the seeding row were used.

The following fungicide to control ASR were tested: tebuconazole (20%) + picoxystrobin (12%) (Horos—500 mL/ha), cyproconazole (8%) + picoxystrobin (20%) (Approach Prima—300 mL/ha), benzovindiflupyr (15%) + azoxystrobin (30%) (Elatus—0.2 Kg/ha), prothioconazole (17.5%) + trifloxystrobin (15%) (Fox SC—400 mL/ha), epoxyconazole (5%) + pyraclostrobin (13.3%) (Opera—500 mL/ha), fluxapyroxade (16.7%) + pyraclostrobin (33.3%) (Orkestra SC—300 mL/ha), cyproconazole (8%) + azoxystrobin (20%) (Priori Xtra SC—300 mL/ha), cyproconazole (16.5%) + trifloxystrobin (37.5%) (Sphere Max—200 mL/ha) each one added of 0.0, 1.5, 2.0, 2.5, and 3.0 kg/ha mancozebe (Unizeb Gold 75 WG).

Spraying were performed with a backpack sprayer pressurized by CO₂ delivering 150 L/ha. Boom, with four Hypro DB015F120 nozzles 0.5 m apart was kept 40 cm above plant canopy. Four sprayings were programmed the first at growth stage (GS) R1 with zero leaflet incidence and the other with 15 to 18 days interval.

Experimental design was a factorial with four mancozeb rates × eight co-formulations (DMI + QoI and QoI + SDHI) in 2.25 × 6.0 m plots, in a randomized design with four repetitions. Four extra treatments with 0.0, 1.0, 1.5, 2.0, 2.5 and 3.0 kg mancozeb/ha were added where 0.0 mancozeb as the unsprayed treatment.

Two different methods for disease assessment were performed. To detect ASR onset the leaflet incidence was used as the most sensitive method. For severity central leaflet of leaves inserted in the main stem of 10 random plants per plot were taken weekly and analyzed under a stereomicroscope. Leaflet intensity was appraised according to Godoy et al. (2006) severity scale.

The relative chlorophyll content was measured with a chlorophyll meter (SPAD-502, Minolta, and Osaka, Japan) (Minolta, 1989) which measures the percentage of chlorophyll in the leaf blade. The readings were performed in five leaflets per plot of the upper leaflets and taking four readings per leaflet.

Plots were mechanical harvested with a Wintersteiger plot combine in 13.5 m²/plot.

Data were submitted to analysis of variance and the treatments means compared by Tukey's test ($p < 0.05$) for severity and for grain yield. Regression analysis between grain yield and mancozebe rate was also performed. The extra treatments were not included in the complete statistical factorial analysis.

3. Results and Discussion

To detect the ASR onset and plots progress curve the disease was measured by leaflet incidence a sensitive method. First spraying was performed on January 12th, 2016 at R1 GS. Rust was first found on January 28th with 49% leaflet incidence coincided with the time of the second spraying, 16 days after rust onset. In 41 days, rust epidemics increased from zero to 100% leaflet incidence (Figure 1).

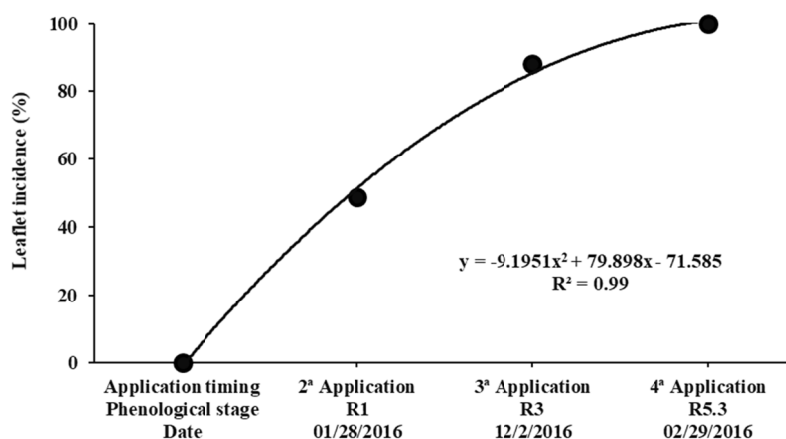


Figure 1. Progress curve of rust incidence in soybean leaflets in unsprayed plots, soybean growth stages, and spraying times

For rust severity the interaction between fungicide co-formulations and mancozebe doses was significant ($p = 0.05$).

The highest ASR leaflet severity in unsprayed control plots was 78%, and considering the four rates of mancozeb (1.5, 2.0, 2.5, and 3.0 kg/ha) solo were 57, 50, 41, 41% respectively.

The lowest rust severity considering mixtures alone (without adding the multisite) were: azox + benz (21%), for 1.5 kg/ha mancozeb—azox + benz (18%), for 2.0 kg/ha mancozeb—azox + benz (13%), and for 3.0 kg/ha mancozeb—azox + benz (6%) (Table 1).

Table 1. Effect of treatments on Asian soybean rust leaflet severity (%)

Fungicide	Addition of mancozebe (Kg/ha)															
	0.0		1.5		2.0		2.5		3.0		Mean					
Cypr + pico 0.3 L/ha	A	38	c	A	32	c	A	32	b	B	26	b	B	22	bc	30
Tebu + pico 0.5 L/ha	A	37	c	B	25	de	C	17	c	CD	11	cd	D	8	d	19
Cypr + azox 0.3 L/ha	A	59	a	B	45	ab	C	35	ab	D	24	b	D	20	c	36
Epoxy + pyra 0.5 L/ha	A	59	a	B	44	a	C	35	ab	C	34	a	C	35	a	41
Flux + pyra 0.3 L/ha	A	38	c	B	29	cd	C	18	c	CD	16	c	D	12	d	22
Benz + azox 0.2 Kg/ha	A	21	c	B	18	e	BC	13	c	C	8	d	C	6	d	13
Prot + trif 0.4 L/ha	A	32	d	AB	28	e	B	19	c	B	18	cd	C	10	d	21
Cypr + trif 0.2 L/ha	A	42	b	B	39	b	B	41	a	B	35	a	C	28	b	37
Mean		41		32		26		21		18						
C.V. (%): 11.30																

Note. Rust severity on control plots 78%. Cypr + pico = cyproconazole + picoxystrobin; Tebu + pico = tebuconazole + picoxystrobin; Cypr + azox = cyproconazole + azoxystrobin; Epoxy + pyra = epoxyconazole + pyraclostrobin; Flux + pyra = fluxapyroxade + pyraclostrobin; Benz + azox = benzovindiflupyr + azoxystrobin; Prot + trif = prothioconazole + trifloxystrobin; Cypr + trif = cyproconazole + trifloxystrobin. Means followed by the same letter do not differ according to Tukey at 0.05. Capital letters compare means in the lines and lower case in the columns. Coefficient of variation: 11.3.

Regarding the control of ASR severity in the extra treatments with mancozeb rates solo control were 14, 24, 29 and 30 respectively. The interaction between fungicide co-formulations and mancozebe doses was significant ($p = 0.05$). Thus, the best control for co-formulations alone was prot + trif (71%) and benz + azox (63%); with the addition of mancozeb (1.5 kg/ha) benz + azox (77%) and prot + trif (76%); for 2.0 kg/ha mancozeb, benz + azox (83%), prot + trif (82%), and flux + pyra (77%); 2.5 kg/ha mancozeb benz + azox (89%); for 3.0 kg/ha mancozeb prot + trif (92%), benz + azo (90%) and fuxa + pyra (85%). As mancozeb dose was increased control also was incremented (Table 2).

In 2018/19 season control for prothioconazole + trifloxistrobin was 41%.

Control of benzo + azoxi (63%) shows reduction in relation to previous season.

Table 2. Effect of treatments on Asian soybean rust severity control (%)

Treatment	Addition of mancozeb (Kg/ha)					Mean
	0.0	1.5	2.0	2.5	3.0	
Cypr + pico 0.3 L/ha	52	59	59	67	72	62
Tebu + pico 0.5 L/ha	53	69	78	86	88	75
Cypr + azox 0.3 L/ha	22	43	55	67	73	52
Epox + pyra 0.5 L/ha	21	40	55	57	55	46
Flux + pyra 0.3 L/ha	52	63	77	80	85	71
Benz + azox 0.2 Kg/ha	63	77	83	89	90	81
Prot + trif 0.4 L/ha	71	76	82	83	92	81
Cypr + trif. 0.2 L/ha	37	50	48	56	64	51
Mean	46	60	67	73	77	-

Note. Cypr + pico = cyproconazole + picoxystrobin; Tebu + pico = tebuconazole + picoxystrobin; Cypr + azox = cyproconazole + azoxystrobin; Epox + pyra = epoxyconazole + pyraclostrobin; Flux + pyra = fluxapyroxade + pyraclostrobin; Benz + azox = benzovindiflupyr + azoxystrobin; Prot + trif = prothioconazole + trifloxystrobin; Cypr + trif = cyproconazole + trifloxystrobin. Coefficient of variance: 11.3.

The overall rust control mean by the eight most used mixtures solo due to *P. pachyrhizi* sensitivity reduction to the DMIs, QoIs, and SDHIs, was 46% (21 to 71%) (Table 2) considered very low. It should be reinforced that > 80% control is required to equalize the costs of fungicide application (Boller, 2010).

Chlorophyll content in unsprayed treatment was 15% and for mancozeb solo rates in extra treatments were 17, 18, 20, and 20. Considering the chlorophyll content the interaction between co-formulations alone and mancozeb doses was significant ($p = 0.05$). The overall chlorophyll content for the co-formulations alone was 27% while for the addition of 3.0 kg/ha mancozeb increased to up 33% (23 to 42%). Considering the minimum and the maximum content there was an increase from 19% (epox + pyra) up to 42% (prot + trif + mancozeb). The chlorophyll content can also be increased by strobilurin effect on soybean plant physiology (Fagan et al., 2015) as well for manganese nutritional effect as shown in wheat plants (Reis & Floss, 1980). It is likely that the effect on turning the plants greener can have a reflection on the grain yield.

Table 3. Effect of treatments on chlorophyll content (%) in soybean leaves

Treatment	Addition of mancozeb (Kg/ha)															
	0.0		1.5		2.0		2.5		3.0		Mean					
Cypr + pico 0.3 L/ha	B	25	de	B	27	c	A	31	b	A	33	cd	A	31	c	30
Tebu + pico 0.5 L/ha	B	34	ab	B	34	ab	AB	36	a	A	38	ab	AB	38	ab	36
Cypr + azox 0.3 L/ha	B	22	ef	B	22	d	A	27	c	A	29	d	A	30	c	26
Epox + pyra 0.5 L/ha	B	19	f	B	19	d	AB	21	d	AB	21	e	A	24	d	21
Flux + pyra 0.3 L/ha	B	29	cd	A	35	ab	A	35	ab	A	36	bc	A	37	b	34
Benz + azox 0.2 Kg/ha	C	31	bc	BC	33	b	AB	36	a	AB	36	bc	A	37	ab	35
Prot + trif 0.4 L/ha	A	37	ab	AB	38	ab	AB	38	a	A	41	a	A	42	a	39
Cypr + trif. 0.2 L/ha	A	20	de	A	22	d	A	23	cd	A	24	e	A	23	d	22
Mean		27			29			31			32			33		

Note. Chlorophyll content (%) in the control treatment—15%.

Cypr + pico = cyproconazole + picoxystrobin; Tebu + pico = tebuconazole + picoxystrobin; Cypr + azox = cyproconazole + azoxystrobin; Epox + pyra = epoxyconazole + pyraclostrobin; Flux + pyra = fluxapyroxade + pyraclostrobin; Benz + azox = benzovindiflupyr + azoxystrobin; Prot + trif = prothioconazole + trifloxystrobin; Cypr + trif = cyproconazole + trifloxystrobin. Means followed by the same letter do not differ according to Tukey at 0.05. Capital letters compare means in the lines and lower case in the columns. Coefficient of variation: 6.5.

Soybean yield for mancozeb solo rates were (unsprayed 2,019), 2,396, 2,432, 2,617, 2,771 respectively. The interaction between co-formulations and mancozeb doses was not significant for soybean grain yield. Thus, the highest yield was for prot + trif (4,920 kg/ha) and the lowest for epox + pyra (3,236 kg/ha). As far as the response in yield the highest (4,146 and 4,257 kg/ha) were for the addition of 2.5 and 3.0 kg/ha mancozeb, respectively and the lowest (3,417 kg/ha) for co-formulations alone (Table 4).

Regression between soybean grain yield (kg/ha) and rust severity (%) showed the relationship $y = 5.103, 4 \text{ kg/ha} - 41.46\% \text{ rust severity}$, with $r^2 = 0.88$. Therefore, each 1% rust severity reduced 41.46 kg of soybean grains/ha for a 5103.4 kg/ha maximum yield.

Chlorophyll content and rust control were increased by the addition of mancozeb.

Table 4. Effect of treatments on soybean grain yield (kg/ha)

Treatment	Addition of mancozeb (Kg/ha)					Mean	
	0.0	1.5	2.0	2.5	3.0		
Cypr + pico 0.3 L/ha	3140	3438	3429	3640	3705	3471	de
Tebu + pico 0.5 L/ha	3538	4075	4353	4337	4648	4190	c
Cypr + azox 0.3 L/ha	2887	3511	3671	3632	3926	3525	de
Epox + pyra 0.5 L/ha	2693	3290	3268	3465	3464	3236	e
Flux + pyra 0.3 L/ha	3352	4267	4268	4538	4667	4218	c
Benz + azox 0.2 Kg/ha	3949	4390	4738	4722	4729	4505	b
Prot + trif 0.4 L/ha	4475	4809	5132	5078	5106	4920	a
Cypr + trif 0.2 L/ha	3297	3733	3801	3760	3814	3681	de
Mean	C 3417	B 3939	AB 4082	A 4146	A 4257	-	-
C.V. (%): 7.23							

Note. Yield in unsprayed treatment kg/ha. Cypr + pico = cyproconazole + picoxystrobin; Tebu + pico = tebuconazole + picoxystrobin; Cypr + azox = cyproconazole + azoxystrobin; Epox + pyra = epoxyconazole + pyraclostrobin; Flux + pyra = fluxapyroxade + pyraclostrobin; Benz + azox = benzovindiflupyr + azoxystrobin; Prot + trif = prothioconazole + trifloxystrobin; Cypr + trif = cyproconazole + trifloxystrobin. Grain yield in the plots without fungicide application was 2,019 kg/ha. Means followed by the same letter do not differ according to Tukey at 0.05%. Capital letters compare means in the lines and lower case in the columns. Coefficient of variation: 7.23.

After six seasons of the DMIs use alone in the control of soybean rust, growers complained on the control failure. At that moment, Silva et al. (2008) reported the control reduction in experiments, and later was confirmed by Reis and Deuner (Reis et al., 2015) in the *P. pachyrhizi* sensitivity reduction measured by the IC_{50} . Later, Xavier et al. (2015) demonstrated the presence of cross-resistance among DMIs.

Regression analysis between grain yield and mancozeb rates showed $y = 220.09x + 3,798.3$, $R^2 = 0.83$ [where 'y' is soybean grain yield (kg/ha), and 'x' = kg/ha mancozeb, R^2 coefficient of determination] showing that each 0.5 kg/ha mancozeb increment increased 220.09 kg/ha soybean grain yield. In our experiment the damage (sensu Nutter et al., 1993) in the soybean yield was 59% considering the productivity in the unsprayed plots (rust severity 78%) of 2,019 kg/ha and 4,920 kg/ha the highest overall average (21% control).

On March 8th, 2017, FRAC released in internet a note on the *P. pachyrhizi* resistance to carboxamides and that reduction in sensitivity could be crossed to members of this group. In our study, the control resulting from the application of benzovindiflupyr + azoxystrobin was only 63% showing the sensitivity of *P. pachyrhizi* to SDHI since it was previously 82% (Godoy et al., 2015). This co-formulation was released in the market in 2014 growing season, therefore, being exposed to fungal directional selection for just three seasons. Therefore, SDHIs can be considered a high-risk chemical group and *P. pachyrhizi* a high risk fungus for resistance development.

Overall mean for rust control by the eight most used mixtures due to *P. pachyrhizi* sensitivity reduction to the DMIs, QoIs and SDHIs, was just 46%. It is accepted that for economic control 80% efficiency is required (Boller, 2010).

It should be noted that there is evidence that mancozeb has no potential to recover control of mixtures that have less than 50% efficacy when sprayed solo (Table 3). There is evidence that some co-formulation reaching < 52% control will no longer be effective, even by adding 3.0 kg / ha of mancozeb (Tables 3 and 4).

In view of the widespread reduction of soybean rust control by DMI + QoI, SDHI + QoI the mixtures it is suggested to use only ready mixtures containing DMI + QoI + multi-site, or SDHI + QoI + multi-site to reduce/paralyze directional selection.

Phakopsora pachyrhizi sensitivity reduction has been reported for DMIs, QoIs and SDHIs alones and the respective mutations evolved identified (Klosowski et al., 2016; Schmitz et al., 2014; Simões et al., 2018).

Phakopsora pachyrhizi cross resistance towards DMIs, QoIs, and SDHIs alone and multiple resistance reach all of them solo or in mixtures (Xavier et al., 2015). Therefore, the triple co-formulations (DMI + QoIs + SDHIs), now most used in Brazil, would show a better performance in the rust control than their isolated components?

The use of multi-site fungicides to fight resistance is not a new strategy (Gullino et al., 2010; FRAC, 2015, 2020). It has been used with success in the control of potato and tomato late blight and of downy mildew in grapes (FRAC, 2020; Gulino et al., 2010; Muchiri et al., 2009). Our results confirm the potential of mancozeb to improve ASR control as it was performed with *Phytophthora infestans* (Mont.) de Bary. Multisite fungicides remain essential for management of fungicide resistance in and *Plasmopara viticola* (Berk. & Curtis) Berl. & De Tonia (Corio-Costet, 2012).

Our results are promising to improve ASR control but how many soybean seasons will be needed to growers be aware and treat the whole area (39.6 million ha) with the site-specific double or triple co-formulations added by a multi-site fungicide to reduce directional selection?

Finally, to reduce directional selection the whole soybean grown area and all sprayings should be performed with co-formulations containing site-specific + multi-site mix fungicide (chlorothalonil, mancozeb, copper oxychloride).

References

- Blum, M. M. C. (2009). *Sensibilidade de Phakopsora pachyrhizi a fungicidas* (Tese (Doutorado), Universidade de Passo Fundo, Brazil).
- Boller, W. (2010). Análise econômica da aplicação de fungicidas em órgãos aéreos da soja e do trigo. In E. M. Reis (Ed.), *Critérios indicadores do momento para a primeira e intervalo de aplicações de fungicidas nas culturas de soja e trigo* (pp. 53-65). Passo Fundo, Aldeia Norte Editora.
- CONAB. (2020). *Acompanhamento da safra brasileira de grãos, V. 7—Safra 2019/2020*. Retrieved from file:///C:/Users/User/Downloads/GrosZjulhoZresumo%20(1).pdf
- Corio-Costet, M. F. (2012). Fungicide Resistance in *Plasmopara viticola* in France and anti-resistance measures. In T. S. Thind (Ed.), *Fungicide resistance in crop protection. Risk and management* (pp. 157-171). CABI, Oxfordshire.
- Danelli, A. L. D., Reis, E. M., & Boaretto, C. (2015). Critical-point model to estimate yield loss caused by Asian soybean rust. *Summa Phytopathologica*, 41(4), 262-269.
- Fagan, E. B., Dourado Neto, D., Vivian, R., Franco, R. B., Yeda, M. P., Massignam, L. F., ... Martins, K. V. (2010). Efeito da aplicação de piraclostrobina na taxa fotossintética, respiração, atividade da enzima nitrato redutase e produtividade de grãos de soja. *Bragantia*, 69(4), 771-777.
- FRAC-UK. (2015). *Fungicide resistance management in potato late blight*.
- FRAC-UK. (2020). *Recommendations for fungicide mixtures designed to delay resistance evolution*.
- Fundação MT. (2008). *Boletim Informativo*. Fundação MT.
- Godoy, C. V., & Palaver, L. (2011). *Ensaio cooperativo para avaliação da eficiência de fungicida no controle da ferrugem da soja*. Reunião de Pesquisa de Soja da Região Central do Brasil, XXXII, São Pedro, SP, Brazil.
- Godoy, C. V., Koga, L. J., & Canteri, M. G. (2006). Diagrammatic scale for assessment of soybean rust severity. *Fitopatologia Brasileira*, 31, p.63-68.
- Godoy, C. V., Utiamada, C. M., Meyer, M. C., Campos, H. D., Forcelini, C. A., Pimenta, C. B., ... Venâncio, W. S. (2015). *Eficiência de fungicidas multissítios e fertilizantes no controle da ferrugem asiática da soja*,

- Phakopsora pachyrhizi*, na safra 2014/15: Resultados sumarizados dos ensaios cooperativos, 2015 (Circular Técnica 113). Londrina, PR.
- Gullino, M. L., Tinivella, F., Garibaldi, A., Kemmitt, G. M., Bacci, L., & Sheppard, B. (2010). Mancozeb, past, present and future. *Plant Disease*, *94*(9), 1076-1087.
- Klosowski, A. C., Mai De Mio, L. L., Miessner, S., & Rodrigues, R. (2016). Detection of the F129L mutation in the cytochrome *b* in *Phakopsora pachyrhizi*. *Pest Management Science*, *72*(6), 1211-1215.
- Minolta. (1989). *Chlorophyll meter SPAD-502* (Instruction Manual, p. 22). Minolta Co., Osaka, Japan.
- Morel, P. W. (2001). *Roya de la soja*. Comunicado Técnico-Reporte Oficial (Série Fitopatologia, 1). Ministério de Agricultura y Ganaderia, Subsecretaria de Agricultura, Dirección de Investigación Agrícola, Centro Regional de Investigación Agrícola (CRIA), Capitán Miranda, Itapúa, Paraguay.
- Muchiri, F. N., Narla, R. D., Olanya, O. M., Nyankanga, R. O., & Ariga, E. S. A. (2009). Efficacy of fungicide mixtures for the management of *Phytophthora infestans* (US-1) on potato. *Phytoprotection*, *90*, 19-29.
- Nutter, F. W., Teng, S. P., & Royer, M. H. (1993). Terms and concepts for yield, crop, and disease threshold. *Plant Disease*, *77*, 211-215.
- Reis, E. M., & Floss, E. L. (1980). Efeito nutritivo de fungicidas carbamatos em trigo (*Triticum aestivum* L.). *Summa Phytopathologica*, *6*, 116-122.
- Reis, E. M., Deuner, E., & Zanatta, M. (2015). *In vivo* sensitivity of *Phakopsora pachyrhizi* to DMI and QoI fungicides. *Summa Phytopathologica*, *41*(1), 21-24.
- Reis, E. M., Reis, A. C., Zanatta, M., Silva, L. H. C. P., Siqueri, F. V., & Silva, J. R. C. (2017). Evolution of reduced *Phakopsora pachyrhizi* sensitivity to fungicides and strategy to recover control (3rd ed., p. 104). Passo Fundo: Berthier.
- Schmitz, H. K., Medeiros, C. A., Craig, I. R., & Stammler, G. (2014). Sensitivity of *Phakopsora pachyrhizi* towards quinone-oxidoreductase-inhibitors and demethylation-inhibitors, and corresponding resistance mechanisms. *Pest Management Science*, *70*(3), 378-88.
- Silva, L. H. C. P., Campos, H. D., Silva, J. R. C., Ribeiro, G. C., Rocha, R. R., & Moraes, D. G. (2008). Eficácia reduzida de triazóis no controle da ferrugem asiática. *Fitopatologia Brasileira*, *33*, 228.
- Simões, K., Hawlik, A., Rehfus, A., Gava, F., & Stammler, G. (2018). First detection of a SDH variant with reduced SDHI sensitivity in *Phakopsora pachyrhizi*. *Journal of Plant Diseases and Protection*, *125*, 21-26.
- Sydow, H., & Sydow, P. (1914). A contribution to knowledge of parasitic fungi of the island of Formosa. *Annales Mycologici*, *12*, 108.
- Xavier, S. A., Koga, L. J., Barros, D. C. M., Canteri, M. G., Lopes, I. O. N., & Godoy, C. V. (2015). Variação da sensibilidade de populações de *Phakopsora pachyrhizi* a fungicidas inibidores da desmetilação no Brasil. *Summa Phytopathologica*, *41*(3), 191-196.
- Yorinori, J. T., Paiva, W. M., Frederick, R. D., Costamilan, L. M., Bertagnolli, P. F., Hartman, G. E., ... Nunes Junior, J. (2005). Epidemics of soybean rust (*Phakopsora pachyrhizi*) in Brazil and Paraguay from 2001 to 2003. *Plant Disease*, *89*, 675-677.

Copyrights

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).