Tropical Plant Pathology (2023) 48:236–240
https://doi.org/10.1007/s40858-023-00557-w

SHORT COMMUNICATION

Optimizing action thresholds for improved control of soybean powdery mildew with fungicides

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Received: 14 October 2022 / Accepted: 21 January 2023 / Published online: 10 February 2023

Abstract
Powdery mildew, caused by the fungus *Erysiphe diffusa*, is a common soybean disease that appears during periods of mild temperatures and reduced rainfall. The disease can be controlled with resistant cultivars and fungicide applications after the onset of symptoms. Control failures in the field may be related to reduced fungicide efficacy and/or high severity during the first application. This study aimed to evaluate the efficacy of 10 different fungicides sprayed when inoculated plants exhibited incremental levels of disease severity in greenhouse conditions. Five action thresholds, being each tested in a separate trial, for disease severity at first spray were used: 11%, 15%, 27%, 55%, and 92%. Disease severity was assessed visually at 2, 4, 7, and 14 days after application. The greatest control efficacy, as well a better differentiation among the fungicides, were observed for applications with 11% and 15% severity as action thresold. Applications with severities > 55% showed reduced control efficacy, with seven of 10 fungicides showing efficacy similar to the untreated check. Carbendazim and chlorothalonil showed the lowest control efficacies even when applied at the lowest action threshold.

Keywords Chemical control · Disease control · Disease severity · *Erysiphe diffusa*

Powdery mildew, caused by the fungus *Erysiphe diffusa* (Cooke & Peck) U. Braun & S. Takam [syn. *Microsphaera diffusa* (Cooke & Peck)], is a common disease in soybeans. The fungus is an obligate parasite and infects several legume hosts. The primary symptom is the formation of white, powder-like patches composed of mycelia and conidia on all above-ground plant parts, especially the leaves (Hartman 2015). Infection can significantly disrupt critical plant physiological processes including photosynthesis and transpiration (Mignucci and Boyer 1979). As the disease develops, fungal growth can cover the entire leaf surface and cause necrosis and defoliation. Yield losses as high as 35% have been recorded in susceptible cultivars (Phillips 1984).

Control of powdery mildew is based on the use of resistant cultivars and fungicide applications. In the Brazilian soybean crop, the disease was the first to be recommended for control with fungicides in the 1996–1997 season, when outbreaks were observed in the Midwest during winter sowing for seed production, as well as in the southern region. The first recommended fungicides were methyl benzimidazole carbamate (MBC), benomyl and carbendazim, and a demethylation inhibitor (DMI), difenoconazole. Fungicide application was recommended after the onset of symptoms, with a severity threshold of 40–50%, that is, when half of the leaf area of the plant had symptoms of the disease, with a repeated application after 10–15 days until the R6 stage (Recomendações 1997). A greenhouse experiment with tebuconazole (100 g a.i./ha) and sulfur (2 kg a.i./ha) applied at 20%, 30%, 40%, and 50% severity supported this recommendation, showing no difference in control when applied with different severities (Yorinori et al. 2004).

In the southern region of Brazil, the threshold for fungicides application is 20% of severity in the lower third of the plants, on an average of 20 plants across the field (Oliveira and Rosa 2014).

In Brazil, 14 fungicides are registered for *E. diffusa* and 206 for *Microsphaera diffusa* (syn.), including sulfur, DMI, MBC, quinone outside inhibitors (QoIs), succinate dehydrogenase inhibitors (SDHIs), and isophthalonitrile (chlorothalonil) (MAPA n.d.). In 2004–2005, fungicides were evaluated in a network fungicide trial in Brazil with

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an efficacy of ≥ 90% for azoxystrobin + cyproconazole, trifloxystrobin + tebuconazole, cyproconazole + propiconazole, flutriafol + thiophanate methyl, tetraconazole, flutriafol, and trifloxystrobin + cyproconazole and ≤ 90% for fenarimol (Godoy 2005).

In network fungicide trials conducted with different fungicides in 2020–2021 and 2021–2022, when fungicides were applied with severities < 12%, the efficacies were ≥ 70% in 2020–2021 (Godoy et al. 2021) and ≥ 61% in 2021–2022 (Godoy et al. 2022).

Although there are different fungicides registered for powdery mildew control, complaints of control failures are frequent in the field. These failures may be due to the high action threshold or low efficacy of fungicides. This study aimed to evaluate the efficacy of different fungicides to control powdery mildew in soybeans with different initial severities in greenhouse conditions.

The experiment was performed in the greenhouse in Londrina, Paraná, from April to August 2021 using the soybean cultivar BRS 316 RR, which is susceptible to powdery mildew. The experimental design was a randomized block with five replicates (pots). Ten seeds were sown per pot and thinned to three plants per pot at the V2 stage. The average temperature during the period was 22 °C. Leaves were inoculated with *E. diffusa* by dusting conidia over the plants and heavily infected plants were kept in the same greenhouse during the entire period (Mignucci and Boyer 1979).

Six trials were conducted, each with a different severity during the single fungicide application: 11% at the V2 growing stage, 15% at V2, 27% (two experiments) at V3, 55% at V4, and 92% at V5. The experiments were performed at different times in the same environment. Ten fungicides composed of premix or solo active ingredient of the groups inorganic (sulfur), DMI (cyproconazole, difenoconazole, prothioconazole, and tebuconazole), MBC (carbendazim), QoI (trifloxystrobin and pyraclostrobin), SDHI (fluxapyroxad, fluindapyr, and bixafen), and chloronitrile (chlorothalonil) were tested (Table 1). The plants were sprayed using a backpack CO_2 sprayer with a TeeJet XR8002 nozzle and a pressure of 40 PSI. The disease severity in percentage was assessed at 2, 4, 7, and 14 days after application on all treated leaves with the aid of a standard area diagram set (Mattiazi 2003).

The average severity of the treated leaves across three plants in an individual pot was calculated and used in the analysis. The area under the disease progress curve (AUDPC) was also determined. For each trial, an individual analysis of the effect of the treatments was carried out. Statistical analyses were performed using the ExpDes.pt and Agro R packages of the R Core Team Program. The data were evaluated for normality using the Shapiro–Wilk test and for homogeneity of variance using Bartlett's test. The data were subjected to the Tukey test ($p = 0.05$) for comparison of the mean values among treatments.

The dry environment in the greenhouse and mild temperatures during winter (average of 22 °C) favored the growth and dissemination of the pathogen, which reached severities of 100% in some trials at 14 days after inoculation. As soon as the plants showed the first symptoms of the disease, the first experiments were installed at severities of 11% and 15%. The mean severity in the untreated check at 14 days after application increased to 95.5% and 100%, respectively (Table 1). In the two experiments installed with a severity of 27%, the final mean severity in the untreated check was approximately 60%. The decrease in final severity compared to those in experiments performed at 11% and 15%

Table 1 Severity (%) of powdery mildew (*Erysiphe diffusa*) 14 days after application in experiments with initial severities (IS) of 11%, 15%, 27% (2 experiments), 55%, and 92%

Active ingredient (a.i.)	Dose g a.i./ha	IS 11%		IS 15%		IS 27[1]%		IS 27[2]%		IS 55%		IS 92%	
Untreated		95.5	a	100	a	61.5	a	57.5	a	100	a	100	a
Cyproconazole	30	46.8	bc	65.8	b	31.9	cd	31.3	bc	96.0	ab	92.9	ab
Carbendazim	250	85.9	a	92.4	a	54.4	a	43.5	abc	100	a	100	a
Difenoconazole + cyproconazole	75 + 45	27.9	cd	31.0	de	31.9	cd	29.3	c	86.7	ab	84.5	b
Trifloxystrobin + prothioconazole[x]	60 + 70	33.4	bcd	39.0	cde	33.1	cd	32.9	bc	81.3	b	87.3	ab
Tebuconazole + carbendazim[y]	125 + 250	29.1	cd	39.2	cde	31.7	cd	31.1	bc	91.7	ab	64.9	c
Pyraclostrobin + fluxapyroxad[z]	116.5 + 58.4	41.9	bc	46.8	bcd	33.0	cd	35.5	bc	88.1	ab	88.9	ab
Prothioconazole + fluindapyr[y]	84 + 84	18.1	d	20.7	e	20.5	d	25.3	c	80.2	b	84.2	b
Bixafen + prothioconazole + trifloxystrobin[x]	62.5 + 87.5 + 75	35.1	bcd	35.5	cde	25.5	d	22.9	c	80.0	b	87.6	ab
Chlorothalonil	720	92.8	a	90.0	a	49.3	abc	52.5	ab	100	a	100	a
Sulfur	1600	50.4	b	54.8	bc	37.3	bcd	41.7	abc	92.0	ab	93.5	ab
CV %		16.9		16.6		26.0		27.1		9.47		7.54	

[1]Added Áureo 0.25% v/v. [2]Added Agris 0.25% v/v. [3]Added Assist 0.25% v/v. Numbers in the same column followed by different letters are statistically different according to the Tukey test ($P = 0.05$)

was related to the environment, which coincided with the increase in temperature in late winter, with lower AUDPC in the experiments (Table 2).

At 14 days after application, greater differentiation of treatments was observed when fungicides were applied with low severity (Table 1). In the application with severities of 11% and 15%, the severity of treatments with carbendazim and chlorothalonil did not differ from the untreated check. The lowest severities were observed for the dual and triple premixes: prothioconazole + fluindapyr, difenoconazole + cyproconazole, tebuconazole + carbendazim, trifloxystrobin + prothioconazole, and bixafen + prothioconazole + trifloxystrobin. In the application with a severity of 27%, carbendazim and chlorothalonil also did not differ from untreated check; however, treatments with fungicide showed less differentiation in severity. In applications with higher severities (55% and 92%), a low curative effect of fungicides was observed. With application at 55%, seven of 10 fungicides did not show a difference from the untreated check, with final severities ≥ 80%. A similar pattern was observed for application with a severity of 92%, where, of the 10 fungicides, seven did not differ from the untreated check in the final severity. In applications with severities of 55% and 92%, the final severity for the treatments with carbendazim and chlorothalonil was 100%.

The results of this study were different from those of Yorinori et al. (2004), who tested tebuconazole and sulfur applied with severity from 20 to 50%, and observed 100% severity in 44 days. In Yorinori et al. (2004) study, the levels of severity did not affect the level of powdery mildew control. In our study, the disease pressure was higher, with 100% of severity after 14 days, differing from Yorinori et al. (2004).

For AUDPC, when fungicide was applied at severity of 11%, only disease severity resulting from carbendazim application was similar to that of the untreated check (Table 2). In trails when fungicides were applied with disease severity from 15 to 27%, all treatments differed from the untreated check, indicating that they presented an initial control. For the application at severity of 55%, the AUDPC of the treatments with carbendazim and chlorothalonil were similar to that of the untreated check. With severity of 92%, in addition to these two fungicides, cyproconazole and sulfur were also similar to that of the untreated check.

Since the experiments were conducted at different times, it was not possible to compare the products within each application severity due to the different disease progression. The percentage of AUDPC control reduced as the threshold application increased, with the exception of carbendazim and chlorothalonil (Fig. 1). Based on this pattern, the fungicide delayed disease progress more effectively when applied with disease severity lower than 27%.

Although the experiments were performed under conditions of high disease pressure, the results indicate that field control failure complaints may be related to high-severity applications, such as those in the Recomendações (1997). Applications with severities of 40–50% can lead to reduced control efficacy as shown in experiments with different fungicides. In the network fungicide trials that evaluated powdery mildew control in the 2020–2021 crop season (Godoy et al. 2021), the efficacy of nine fungicides evaluated averaged ≥ 70%. In these trials, the maximum initial severity for fungicide application was 11%, and the favorability for disease development was low. In the analysis of seven trials conducted during 2021–2022, the fungicides showed efficacy ranging from 61% (tetraconazole) to 93%

Table 2 Area under the disease progress curve (AUDPC) after treatments with different initial severities (IS) of 11%, 15%, 27% (two experiments), 55%, and 92%

Active ingredient (a.i.)	IS 11%		IS 15%		IS 27[1]%		IS 27[2]%		IS 55%		IS 92%	
Untreated	979	a	977	a	788	a	794	a	1254	a	1364	a
Cyproconazole	471	c	499	c	468	cde	418	cd	1150	bc	1260	abcd
Carbendazim	836	ab	753	b	626	b	591	bc	1191	ab	1353	ab
Difenoconazole + cyproconazole	302	cd	380	de	436	de	445	bcd	1032	d	1211	bcde
Trifloxystrobin + prothioconazole[x]	359	cd	409	cde	514	bcd	501	bcd	977	de	1131	de
Tebuconazole + carbendazim[y]	356	cd	413	cde	468	cde	443	bcd	1102	c	1068	e
Pyraclostrobin + fluxapyroxad[z]	386	c	386	cde	424	de	478	bcd	1012	d	1148	cde
Prothioconazole + fluindapyr[y]	190	d	320	e	350	e	396	d	991	d	1158	cde
Bixafen + prothioconazole + trifloxystrobin[x]	338	cd	397	cde	383	de	360	d	911	e	1157	cde
Chlorothalonil	742	b	785	b	577	bc	611	b	1201	ab	1340	ab
Sulfur	435	c	469	cde	499	bcd	520	bcd	1184	b	1290	abc
CV %	18.1		9.97		10.43		16.83		2.98		2.66	

[1]Added Áureo 0.25% v/v. [2]Added Agris 0.25% v/v. [3]Added Assist 0.25% v/v. Numbers in the same column followed by different letters are statistically different according to the Tukey test ($P = 0.05$)

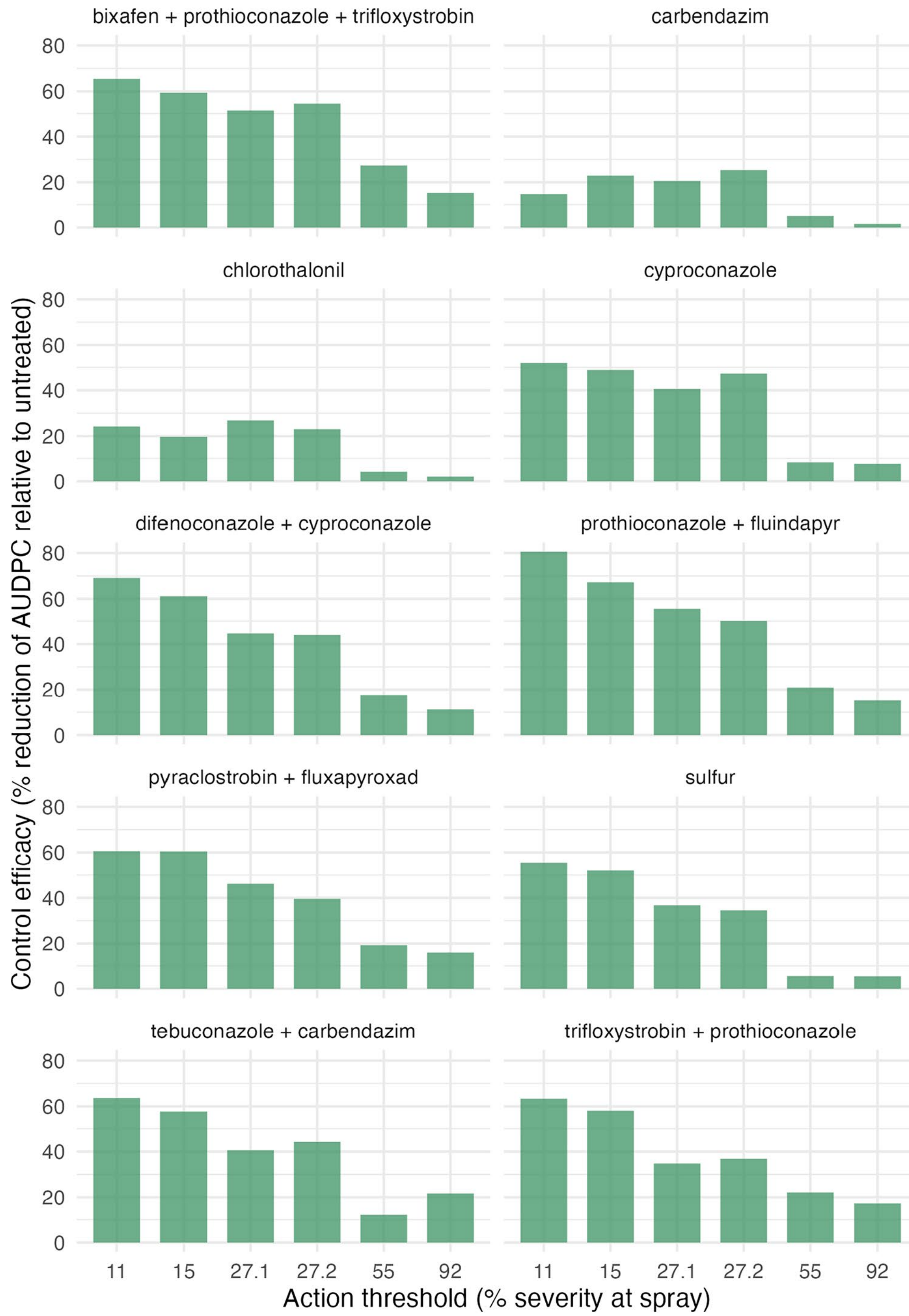

Fig. 1 Control efficacy, or the percent reduction of the area under the disease (powdery mildew) progress curve (AUDPC) relative to untreated plots, for ten fungicides applied at incremental action thresholds: 11%, 15%, 27% (two experiments), 55%, and 92% disease severity during the spray

(fluxapyroxad + prothioconazole) when applied with a severity of up to 11.8%. In the experiment performed in a greenhouse with a threshold of 28%, the same treatments showed lower control efficacy, ranging from 42% (tetraconazole) to 65% (fluxapyroxad + prothioconazole) (Godoy et al. 2022). As in the experiments performed in this study, control efficacy was reduced with applications at higher severity.

Despite the absence of *E. diffusa* sensitivity monitoring data for fungicide resistance in Brazil, there were reports of resistance to fungicides of fungal isolates that cause powdery mildew in other crops. For example, resistance or lower sensitivity to MBC, DMI morpholine, and QoI fungicides were reported for *Podosphaera xanthii* in cucumber (McGrath 2001). The lower efficacy of carbendazim may be related to the resistance of the fungus to this fungicide, which needs further investigation. The intensive use of fungicides in the soybean crop has led to an increase in the fungicide-resistant pathogen population (Godoy et al. 2020).

Populations of the fungus *Corynespora cassiicola* resistant to MBC and IQe (de Mello et al. 2022), *Cercospora* spp. resistant to MBC and QoI (de Mello et al. 2021), and *Phakopsora pachyrhizi* less sensitive to DMI, QoI, and SDHI have been reported (Schmitz et al. 2013; Klosowski et al. 2016; Simões et al. 2018).

Chlorothalonil did not show a curative effect in the experiments since it is a protective fungicide that does not penetrate the soybean leaf tissue, but rather stays on the leaf surface and prevents the penetration of fungi. However, even when applied with low severity in this experiment (11% and 15%), its control efficacy was low.

Trials conducted in the field sown with regular rainfall throughout the crop season does not favor disease progress. The final average severities in the untreated check in the field trials conducted on the network were 40% in 2021 (Godoy et al. 2021) and 36% in 2022 (Godoy et al. 2022) at 14 days after the second fungicide application. In our study, the experiments were performed in a greenhouse with environmental conditions that favored rapid disease development, with severity of 100% in 14 days, different from the disease progression and severities found in the field.

The sensitivity of *E. diffusa* to fungicides must be monitored to update the recommendations regarding the efficacy of fungicides, and the action threshold must be updated for higher efficacy control of powdery mildew. Applications with lower severities (11% and 15%) showed higher disease control efficacies compared to applications with severities of ≥ 27%. Among the evaluated products, carbendazim and chlorothalonil were not effective for powdery mildew control and should not be recommended for disease control.

Acknowledgements The first author thanks the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior –Brasil (CAPES) for the scholarship.

Author contribution All authors have been personally and actively involved in substantive work leading to the manuscript.

Data availability The datasets generated during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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