In vitro sensitivity of *Corynespora cassiicola* isolated from soybean to fungicides and field chemical control of target spot

Adriana Teramoto[1]; Maurício Conrado Meyer[2]; Nelson Dias Suassuna[3]; Marcos Gomes da Cunha[1]

[1] Setor Fitossanitário, Escola de Agronomia, Universidade Federal de Goiás, Caixa Postal 131, 74001-970, Goiânia, Goiás, Brasil; [2] Embrapa Soja, Londrina, Paraná, Brasil; [3] Embrapa Algodão, Santo Antônio de Goiás, Goiás, Brasil
Autor para correspondência: Adriana Teramoto (adritera@terra.com.br)
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ABSTRACT

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Soybean target spot (*Corynespora cassiicola*) has become an important disease in most soybean growing regions in Brazil. The sensitivity of 34 isolates of *C. cassiicola* to 11 fungicides was evaluated based on mycelial growth inhibition (boscalid, carbendazim, cyproconazole, fluopyram, fluxapyroxad, prothioconazole and thiophanate-methyl) or spore germination inhibition (azoxystrobin, picoxystrobin, pyraclostrobin and trifloxystrobin). In addition, the efficacy of five fungicides to control target spot was tested in four field trials carried out during three crop seasons: 2011/2012, 2012/2013 and 2013/2014. Fungal isolates were collected from soybean plants in several soybean growing areas in Brazil. The effective concentration of each fungicide to inhibit fungal growth or spore germination by 50% (EC_{50}) was calculated for all isolates. Fluxapyroxad and prothioconazole provided the greatest mycelial growth inhibition and pyraclostrobin led to the lowest spore germination percentage, with the lowest EC_{50} values. At field experiments, cyproconazole and carbendazim showed target spot control ranging from 26% to 29%. On the other hand, fluxapyroxad and prothioconazole prevented an epidemic of the disease by 45% to 55%, respectively. In general, the efficacy of fungicides in the field reflected the *in vitro* sensitivity averages. Large sensitivity reduction was detected to benzimidazoles (MBC), indicating that this group of fungicides should no longer be used for target spot control. There was a negative and significant correlation (*-0.265)* between target spot severity and soybean yield. The pathogen showed variability in sensitivity to the fungicide groups carboxamides (SDHI), triazoles (DMI) and strobilurins (QoI), which denotes a high risk of selection for resistance.

Keywords: fungicide sensitivity reduction; effective concentration; *Glycine max*; disease management.

RESUMO

Teramoto, A.; Meyer, M.C.; Suassuna, N.D.; Cunha, M.G. Sensibilidade de *Corynespora cassiicola* isolado de soja a fungicidas ***in vitro*** e controle químico de mancha-alvo da soja no campo. ***Summa Phytopathologica,*** v.43, n.4, p.281-289, 2017.

A mancha-alvo da soja (*Corynespora cassiicola*) tornou-se uma importante doença na maioria das regiões produtoras de soja no Brasil. A sensibilidade de 34 isolados de *Corynespora cassiicola* a 11 fungicidas foi avaliada pela inibição do crescimento micelial (boscalida, carbendazim, ciproconazol, fluopiram, fluxapiroxada, protioconazol e tiofanato-metílico) ou inibição da germinação de esporos (azoxistrobina, picoxistrobina, piraclostrobina e trifloxistrobina). Além disso, a eficácia de cinco fungicidas para controlar mancha-alvo foi testada em quatro ensaios no campo, conduzidos durante três safras: 2011/2012, 2012/2013 e 2013/2014. Os isolados do fungo foram coletados de plantas de soja em diversas regiões produtoras de soja do Brasil. A concentração efetiva para inibir o crescimento micelial ou a germinação dos esporos em 50% (EC_{50}), para cada fungicida, foi calculada para todos os isolados. Fluxapiroxada e protioconazol proporcionaram as maiores inibições de crescimento micelial do patógeno e piraclostrobina a menor porcentagem de germinação de esporos, com os menores valores de $EC_{50.}$ Nos ensaios de campo, ciproconazol e carbendazim apresentaram percentuais de controle de mancha-alvo variando de 26% a 29%. Por outro lado, fluxapiroxada e protioconazol preveniram a epidemia da doença na ordem de 45% e 55%, respectivamente. No geral, a eficácia dos fungicidas no campo refletiu as médias de sensibilidade *in vitro*. Foi detectada ampla redução da sensibilidade aos benzimidazóis (MBC) indicando que esse grupo de fungicidas não deve ser mais usado no controle de mancha-alvo. Houve uma correlação negativa e significativa (-0,265) entre severidade da doença e produção da soja. O patógeno mostrou variabilidade na sensibilidade aos grupos fungicidas carboxamidas (SDHI), triazóis (DMI) e estrobilurinas (QoI), o que demonstra alto risco de seleção para resistência.

Palavras-chave: redução da sensibilidade a fungicidas; concentração efetiva; *Glycine max*; manejo de doença.

Brazil is one of the world's major soybean producers [*Glycine max* (L.) Merr.], with 30.03 million hectares grown in 2014 season and a yield average of 2,882 kg ha^{-1} (5). The country also has much arable land available for crop expansion, which has grown by 6.3% per year over the last five seasons (6). More than 50 diseases affect soybean crops in Brazil and they represent a major restrictive factor to increase productivity (30).

Target spot, caused by the fungus *Corynespora cassiicola* Berk. & M. A. Curtis (Wei, 1950), was first detected in São Paulo State (2) and currently occurs in almost all soybean producing regions in Brazil (30). The pathogen infects both the upper part of the plant and the root system. Severe outbreaks occur both in regions of cool temperatures and in the warm highlands of the Brazilian savannah (30). Typical target spot symptoms are roughly circular necrotic leaf lesions ranging from little brown to 11mm-diameter spots, but they are typically large, circular, dark brown spots of approximately 4 to 5 mm in diameter, with a yellow margin. Large lesions occasionally exhibit a zonate pattern often associated with this disease, and the spots have a dark point in

the center surrounded by darker concentric rings (19, 16).

The pathogen occurs in Brazilian soybean fields and affects a wide range of hosts, infecting a large number of native and cultivated plants (9). The fungus spreads mainly by means of infected seeds and survives in crop debris on the soil surface (1). Favorable environmental conditions for target spot outbreaks, i.e. temperatures above 25°C and relative humidity above 80% (16), can occur throughout the crop season in tropical and subtropical areas.

Although target spot is present in most soybean growing countries, economic damage is not frequently reported (28). In Brazil, target spot outbreaks have occurred in the main soybean growing areas and yield reduction of up to 21% has been reported (15). In the USA, incidence of target spot has increased in the southeast region, probably due to changes in weather patterns, pathogen virulence, and/or introduction of more susceptible host genotypes (19).

Measures for the management of target spot involve the use of partially resistant cultivars, seed treatment, crop rotation or succession with corn and other grass species, and chemical control by foliar fungicide spraying (29).

There are no resistant soybean cultivars to target spot (32). In the short term, the use of fungicides is required to control target spot in Brazilian soybean fields. Fungicide spraying to control foliar soybean diseases started with the emergence of Asian soybean rust caused by *Phakopsora pachyrhizi* Syd. & P. Syd. (1914) in 2001, subsequently becoming one of the main strategies for the management of this disease and indispensable to maintain soybean economic production (30). In the 2011/2012 crop season, the cost of using foliar fungicides on soybeans amounted to US$ 1.5 billion. Of this, 79% was spent on quinone outside inhibitors (QoI) and demethylation inhibitors (DMI), recommended for the control of Asian soybean rust, and 6% was spent on methyl benzimidazole carbamates (MBC), recommended for the control of late season diseases, target spot and anthracnose (18, 30).

More recently, succinate dehydrogenase inhibitors (SDHI) fungicides have been used to control white mold [*Sclerotinia sclerotiorum* (Lib) de Bary], Asian soybean rust and target spot in Brazilian soybean fields (21).

Evidence of resistance of *C. cassiicola* to fungicides and reports of inconsistency of the efficiency of some fungicides to control soybean diseases (4, 31, 32) seem to predict a threat to the stability of chemical control.

This study aimed to evaluate the *in vitro* sensitivity of 34 *C. cassiicola* isolates from different Brazilian growing areas to 11 fungicides and to measure the efficacy of five fungicides in controlling target spot in field assays.

MATERIALS AND METHODS

Fungicide treatments

Technical grade fluxapyroxad (BAS 700 AC F) was provided by BASF, while fluopyram (BCS 1015) and prothioconazole (Proline®) were provided by Bayer CropScience. Commercial formulations of picoxystrobin (Oranis®, DuPont), pyraclostrobin (Comet®, BASF), azoxystrobin (Priori®, Syngenta), trifloxystrobin (Flint®, Bayer CropScience), carbendazim (Carbendazim NTX®, Nortox), thiophanate-methyl (Cercobin®, Iharabras), boscalid (Cantus®, BASF) and cyproconazole (Alto 100®, Syngenta) were purchased (Table 1).

Table 1. Commercial names, active ingredients (a.i.), names and concentrations, and mode of action (MOA) group of the fungicides used in this study.

Commercial Name	Active Ingredient (a.i.)	a.i. Concentration	MOA Group[1]
Carbendazim NTX	Carbendazim	50%	MBC
Cercobin	thiophanate-methyl	50%	MBC
Alto 100	Cyproconazole	10%	DMI
Proline	Prothioconazole	25%	DMI
Cantus	Boscalid	50%	SDHI
Verango	Fluopyram	50%	SDHI
BAS 700	Fluxapyroxad	30%	SDHI
Priori	Azoxystrobin	25%	QoI
Oranis	Picoxystrobin	25%	QoI
Comet	Pyraclostrobin	25%	QoI
Flint	Trifloxystrobin	50%	QoI

[1]- Mode of action fungicide group [(FRAC (12)]; MBC = Methyl Benzimidazole Carbamates; DMI = Demethylation Inhibitors; SDHI = Succinate Dehydrogenase Inhibitors and QoI = Quinone outside Inhibitors.

Sampling, isolation and maintenance of *C. cassiicola* isolates

Isolates were collected from soybean growing areas in the states of Goiás, Mato Grosso, Mato Grosso do Sul, Maranhão, Minas Gerais, Tocantins, Paraná and Pará (Table 2). Isolates were identified based on morphological characters. Additionally, pathogenicity tests were carried out with two soybean cultivars to confirm Koch's postulates. From each sample, leaf fragments with pathogen structures were placed on a water-agar medium (WA) in Petri dishes (9 cm in diameter). After 3 to 5 days, mycelial disks (5 mm) from colony borders were cut from each colony and transferred to a potato dextrose agar medium (PDA). Petri dishes were maintained at 25ºC and 12h-day length. Several 6mm-diameter mycelial disks were transferred to glass vials with sterilized distilled water (SDW) for long-term preservation, as described by Figueiredo (11). For each experiment, an isolate was subcultured through a maximum of five transfers. Six isolates were generously provided by Embrapa Soybean.

In vitro mycelial growth inhibition of *C. cassiicola*

Sensitivity of 34 isolates to carbendazim, thiophanate-methyl, fluxapyroxad, fluopyram, boscalid, prothioconazole and cyproconazole was estimated based on the colony growth inhibition. Fungicides were dissolved in SDW to obtain stock solutions of 1000 µg mL^{-1} (for carbendazim and thiophanate-methyl) or 100 µg mL^{-1} (for the remaining fungicides). Based on preliminary tests, the stock solutions were added to a PDA medium after sterilization to produce final concentrations of 0, 1.6, 8, 40, 200 and 1000 µg active ingredient (a.i.) of carbendazim or thiophanate-methyl mL^{-1}, or 0, 0.16, 0.8, 4, 20 and 100 µg a.i. of fluxapyroxad, fluopyram, boscalid, prothioconazole and cyproconazole mL^{-1}.

For each isolate, a 6mm-diameter mycelial plug was cut from the edge of a 10-day-old colony grown on PDA medium and placed in the center of a Petri dish with PDA added of each fungicide concentration. Each concentration (for each fungicide) was replicated five times. The plates were kept at 25ºC in the dark. After 10 days, the colony diameter was measured in two perpendicular directions, and the diameter of the mycelial plug was subtracted before calculating the mean diameter of the colony (MD). For each concentration/fungicide, mycelial growth inhibition (MGI) of an isolate, *i*, was calculated by MGI = ((MD*c* - MD*i*) / MD*c*) x 100, where MD*c* = mean colony diameter for the control (no fungicide added), and MD*i* = mean colony diameter of the isolate grown on medium added of fungicide. For each replicate of

Table 2. Origin of *Corynespora cassiicola* isolates and the number of isolates per Brazilian state.

States	Population municipalities
Goiás (GO)	6 isolates (Morrinhos, Rio Verde, and Montividiu)
Mato Grosso (MT)	11 isolates (Sinop, Sorriso, Querência, Nova Mutum, Campo Novo Parecis, Itiquira, Barra do Garças, and Nova Xavantina)
Tocantins (TO)	3 isolates (Pedro Afonso)
Paraná (PR)	3 isolates (Palotina, Nova Ventura de São Roque, and Londrina)
Maranhão (MA)	3 isolates (Tasso Fragoso)
Minas Gerais (MG)	3 isolates (Tupaciguara)
Mato Grosso do Sul (MS)	1 isolate (Maracaju)
Pará (PA)	4 isolates (Dom Eliseu and Paragominas)
Total	34 isolates

each isolate-fungicide concentration combination, MGI*i* values were linearly regressed on the logarithm (log_{10}) of fungicide concentration to estimate the dose that inhibited mycelial growth by 50% (EC_{50} value). For each fungicide group, the sensitivity of each isolate was established according to criteria defined by Edgington et al. (8) for MBC. For DMI and SDHI fungicides, as there were no previous references to *C. cassiicola*: EC_{50} values lower than the lowest tested concentration (0.16 µg mL^{-1}) were considered "Sensitive" (S); EC_{50} values ranging from 0.16 to 1.0 µg mL^{-1} were considered "Moderately Sensitive" (MS); and EC_{50} values above 1.0 µg mL^{-1} were considered "Non-Sensitive" (NS). The sensitivity factor (SF) to fungicides was calculated as the highest EC_{50} value divided by the lowest EC_{50} value, in order to evaluate the extent of variability in fungicide sensitivity among the populations.

***In vitro* inhibition of *C. cassiicola* spore germination**

Previously, four *Corynespora cassiicola* isolates were tested for i) influence of salicylhydroxamic acid (SHAM) on spore germination and ii) evidence of the capability of *C. cassiicola* to overcome the toxicity of QoI fungicides through an alternative oxidative pathway. For the first test, water agar medium was used with 100 µg.mL^{-1} SHAM, as well as a control without SHAM. In addition, the influence of SHAM on in vitro spore germination was tested when added of QoI fungicides (azoxystrobin, picoxystrobin, pyraclostrobin, and trifloxystrobin) at 100 µg.mL^{-1}. The germination percentage values of the first assay were subjected to ANOVA to determine the effects of isolates and SHAM addition on the sensitivity of conidial germination. Chi-square tests were carried out for each combination (isolate x fungicide) to verify whether spore germination occurs at the same frequency with or without SHAM.

Sensitivity of the 34 isolates to the QoI fungicides picoxystrobin, pyraclostrobin, azoxystrobin and trifloxystrobin was estimated based on the spore germination inhibition. Fungicides were dissolved in SDW to obtain stock solutions of 100 µg a.i. mL^{-1}. Mycelium from 10-day-old colonies grown in Petri dishes containing PDA medium was gently agitated with 20 mL SDW to obtain a spore suspension. Suspensions were adjusted to a concentration of 10^4 spores mL^{-1}. Petri dishes (5 cm diameter) containing 5 mL of the water-agar medium (1.6%) were added of stock solutions to produce the final concentrations 0, 0.16, 0.8, 4, 20, and 100 µg mL^{-1}. Then, 150 µl of the spore suspension was deposited on the medium surface. The plates were kept in an incubator at 25ºC, in the dark, for 8 h. Conidial germination (presence of a germ tube greater than the spore diameter) was determined visually by using a microscope at 100X magnification. In each replicate, 100 spores were counted per concentration. Results were expressed as the relative germinated spores (RGS) when compared with the control. For each concentration/fungicide, RGS of an isolate i was calculated by RGS = ((GS*c* - GS*i*) / GS*c*) x 100, where GS*c* = germinated spores for the control (no fungicide added), and GS*i* = germinated spores of the isolate grown on medium added of fungicide. For each replicate of each isolate-fungicide concentration combination, RGS*i* values were linearly regressed on the logarithm (log_{10}) of fungicide concentration to estimate the dose that inhibited spore germination by 50% (EC_{50} value). The criteria used to establish the sensitivity level of the isolates to fungicides [as "Sensitive" (S), "Moderately Sensitive" (MS) or "Non-Sensitive" (NS)] were based on those proposed by Leroux et al. (20) with modifications: sensitive (EC_{50} < 0.16 µg mL^{-1}), weakly resistant (EC_{50} = 0.16–1.0 µg mL^{-1}), and highly resistant (EC_{50} > 1.0 µg mL^{-1}).

The sensitivity factor (SF) to fungicides was calculated as the highest EC_{50} value divided by the lowest EC_{50} value, in order to evaluate the extent of variability in fungicide sensitivity.

Effect of fungicides on target spot control under field conditions

Four field trials were conducted in Mato Grosso State to evaluate the efficacy of boscalid, carbendazim, cyproconazole, fluxapyroxad and prothioconazole in controlling soybean target spot. In the 2011/2012 crop season, one assay was conducted in Nova Xavantina Municipality with soybean cultivar TMG 132RR, which is susceptible to target spot. In 2012/2013, one assay was conducted in Nova Xavantina with the soybean cultivar TMG 1188RR. Planting was carried out on December 2, 2012. In 2013/2014, two assays were conducted in Nova Xavantina and Querência Municipalities with the soybean cultivars ST 820RR and BRSGO 8661RR, respectively. Sowing was carried out on December 5, 2013, in Nova Xavantina, and on November 30, 2013, in Querência. Rows were spaced 0.5 m apart, and plant density in each row was adjusted to 12 plants m^{-1}. Experimental design was in randomized complete blocks with four replicates, and each plot was composed of four rows of 5 m each.

An isolate of *C. cassiicola*, sampled from soybean fields near the trials in Mato Grosso State, was grown in a PDA medium in Petri dishes. After 10 days of growing at 25º C and 12h day length, 20 mL SDW was added to each plate, and suspensions (5 x 10^3 to 1 x 10^4 conidia mL^{-1}) were prepared to inoculate soybean plants in the field. Inoculations were carried out in soybean vegetative stages V6–V8 (5–7 trifoliate leaves, 6–8 nodes) (10) by spraying conidial suspension over the whole plant.

The fungicides boscalid (75 g a.i. ha^{-1}), carbendazim (500 g a.i. ha^{-1}), cyproconazole (30 g a.i. ha^{-1}), fluxapyroxad (50 g a.i. ha^{-1}) and prothioconazole (70 g a.i. ha^{-1}), as well as an unsprayed control, were evaluated. Fungicides were sprayed three times. In the 2011/2012 season, inoculations were performed on December 30, 2011, and fungicides were sprayed on plants in the V8, R2, and R5.1 stages. In the 2012/2013 season, inoculations were performed on January 4, 2013, and fungicide treatments were sprayed on plants in R1, R4, and R5.3 stages. In the 2013/2104 crop season, inoculations were performed on January 17, 2014, in Querência, and on January 16, 2014, in Nova

Xavantina. Fungicide treatments were sprayed on plants in R1, R4, and R5.2 stages in Querência, and in R1, R4, and R5.2 stages in Nova Xavantina.

Fungicides were sprayed by using a CO_2-powered backpack sprayer, at 300 kPa and approximately 150 L ha^{-1}, with a flat fan-type nozzle (AVI 11002). Target spot severity was assessed in the two central rows of each plot. Disease severity was assessed three times (seven days before the first fungicide application, seven days after the second fungicide application, and seven days after the third fungicide application) with a diagrammatic scale (29). Disease progress curves were plotted, and each area under the disease progress curve (AUDPC) was determined (13). The two central lines of each plot were manually harvested, and the grain yield was calculated. Yield values were estimated for one hectare (kg ha^{-1}). To estimate the fungicide efficacy on disease control and the yield reduction, the overall mean for AUDPC and soybean grain yield of all field assays were calculated and compared with those for the untreated control.

Statistical analysis

For *in vitro* tests, F statistic was used to check the goodness of fit ($P < 0.05$). Each experiment (for each fungicide) was conducted

Table 3. Identification code, year of isolation and Brazilian state of origin of the *Corynespora cassiicola* isolates, and effective concentrations of the fungicides carbendazim, thiophanate-methyl, cyproconazole and prothioconazole to reduce 50% mycelial growth - EC_{50} (µg mL^{-1}) and sensitivity level to MBC and DMI fungicides.

Code	Year of isolation	State	MBC fungicides				DMI fungicides			
			Carbendazim		Thiophanate-methyl		Cyproconazole		Prothiconazole	
			EC_{50}	Sensitivity	EC_{50}	Sensitivity	EC_{50}	Sensitivity	EC_{50}	Sensitivity
GO-12-2-4	2012	GO	>1000 ns	HNS	>1000 ns	HNS	29.98 *	MS	8.20 *	MS
GO-12-2-11	2012	GO	>1000 ns	HNS	>1000 ns	HNS	31.36 *	MS	<0.16 *	S
GO-12-2-13	2012	GO	>1000 ns	HNS	>1000 ns	HNS	46.11 *	MS	4.25 **	MS
GO-12-4-1	2013	GO	>1000 ns	HNS	>1000 ns	HNS	23.53 *	MS	0.28 *	S
GO-12-4-5	2012	GO	>1000 ns	HNS	924.25 *	HNS	11.32 **	MS	0.82 *	S
GO-12-4-2	2013	GO	>1000 *	HNS	>1000 ns	HNS	21.08 *	MS	1.75 **	MS
MT-12-5-1	2012	MT	556.7 *	HNS	>1000 ns	HNS	8.62 *	MS	<0.16 *	S
MT-12-5-2	2012	MT	1.60 ns	MS	449.02 ns	HNS	7.29 **	MS	<0.16 *	S
MT-12-4-1	2012	MT	>1000 ns	HNS	294.76 *	HNS	12.34 **	MS	7.43 *	MS
MT-12-4-4	2012	MT	>1000 ns	HNS	393.42 *	HNS	13.43 **	MS	0.41 *	S
MT-12-7-1	2012	MT	>1000 *	HNS	>1000 ns	HNS	40.30 **	MS	1.51 ns	MS
MT-12-7-2	2012	MT	0.55 *	S	>1000 ns	HNS	40.63 **	MS	0.82 *	S
MT-12-1-3	2013	MT	>1000 ns	HNS	>1000 ns	HNS	8.41 **	MS	8.01 **	MS
MT-12-3-2	2012	MT	>1000 ns	HNS	>1000 ns	HNS	5.21 **	MS	2.01 **	MS
MES 312	1997	MT	<0.16 ns	S	>1000 ns	HNS	16.40 **	MS	<0.16 ns	S
MES 313	1998	MT	<0.16 ns	S	<0.16 ns	S	<0.16 ns	S	<0.16 ns	S
MES 318	1999	MT	<0.16 ns	S	<0.16 *	S	38.33 *	MS	0.20 ns	S
MS-12-1-1	2012	MS	>1000 ns	HNS	>1000 ns	HNS >	100 *	HNS	46.44 *	MS
MA-12-3-2	2012	MA	>1000 *	HNS	>1000 ns	HNS	8.31 *	MS	0.67 **	S
MA-12-3-1	2012	MA	>1000 ns	HNS	>1000 *	HNS	8.73 **	MS	0.47 **	S
MA-12-3-5	2012	MA	>1000 *	HNS	>1000 *	HNS	17.71 *	MS	0.60 **	S
MG-12-1-1	2012	MG	>1000 *	HNS	>1000 *	HNS	9.99 **	MS	0.56 *	S
MG-12-1-2	2012	MG	>1000 ns	HNS	>1000 *	HNS	8.38 **	MS	0.66 *	S
MG-12-1-3	2012	MG	>1000 ns	HNS	>1000 **	HNS	6.87 **	MS	0.29 *	S
TO-12-1-1	2012	TO	>1000 ns	HNS	>1000 ns	HNS	6.66 **	MS	<0.16 *	S
TO-12-1-2	2012	TO	>1000 ns	HNS	66.47 *	NS	8.17 **	MS	0.25 *	S
TO-12-1-3	2012	TO	>1000 ns	HNS	<0.16 ns	S	6.94 **	MS	<0.16 *	S
MES 317	1999	PR	>1000 ns	HNS	<0.16 ns	S	2.37 *	MS	<0.16 ns	S
MES 322	2001	PR	>1000 ns	HNS	<0.16 ns	S	<0.16 *	S	<0.16 ns	S
MES 928	2011	PR	>1000 ns	HNS	>1000 *	HNS	29.98 *	MS	<0.16 ns	S
PA-12-1-4	2012	PA	>1000 ns	HNS	>1000 *	HNS	9.53 **	MS	<0.16 *	S
PA-12-1-2	2012	PA	>1000 ns	HNS	>1000 ns	HNS	14.01 **	MS	<0.16 *	S
PA-12-1-1	2012	PA	>1000 ns	HNS	>1000 *	HNS	8.66 *	MS	<0.16 *	S
PA-12-1-3	2012	PA	>1000 ns	HNS	>1000 *	HNS	7.09 *	MS	<0.16 **	S
S % (nr.)			12% (4)		15% (5)		6% (2)		76% (26)	
MS % (nr.)			3% (1)		-- (0)		91% (31)		24% (8)	
NS % (nr.)			-- (0)		3% (1)		-- (0)		-- (0)	
HNS % (nr.)			85% (29)		82% (28)		3% (1)		-- (0)	

S= sensitive to fungicide (EC_{50} <1 µg mL^{-1}); MS= moderately sensitive to fungicide (EC_{50} 1-50 µg mL^{-1}); NS= non-sensitive to fungicide (EC_{50} 50-100 µg mL^{1}); HNS= highly non-sensitive to fungicide (EC_{50} >100 µg mL^{-1}) [adapted from Avozani et al. (4)] * = Statistical significance at 5% probability; ** = statistical significance at 1% probability; ns= not statistically significant.

five times. Data were pooled only when the hypothesis of equal variances was not rejected. All statistical analyses were performed using the SAS version 9.0 routine (SAS Institute, Cary, NC).

Data were pooled from three field trials for grain yield (hypothesis of equal variances was not rejected) and from all trials for AUDPC. Variables were subjected to ANOVA and the means were subjected to multiple comparisons. Pearson correlation was performed between yield and AUDPC. All statistical analyses were performed by using SAS version 9.0 routine (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

In vitro mycelial growth inhibition of *C. cassiicola*

Most of the *C. cassiicola* isolates tested with MBC fungicides showed sensitivity reduction (EC_{50} values were greater than 100 µg mL^{1}); non-sensitivity was 85% for carbendazim and 82% for thiophanate-methyl (Table 3). None of the tested isolates were non-sensitive to the SDHI fungicide fluopyram. In addition, 3% isolates were non-sensitive and highly non-sensitive to boscalid, another SDHI fungicide. On the other hand, fluxapyroxad had the highest

Table 4. Identification code, year of isolation and Brazilian state of origin of the *Corynespora cassiicola* isolates, and effective concentrations of the fungicides fluxapyroxad, boscalid and fluopyram to reduce 50% mycelial growth - EC_{50} (µg mL^{-1}) and sensitivity level to SDHI fungicides.

Code	Year of isolation	State	SDHI fungicides					
			Fluxapyroxad		Boscalid		Fluopyram	
			EC_{50}	Sensitivity	EC_{50}	Sensitivity	EC_{50}	Sensitivity
GO-12-2-4	2012	GO	0.17 *	S	2.95 **	MS	2.27 **	MS
GO-12-2-11	2012	GO	0.47 **	S	2.46 *	MS	2.97 **	MS
GO-12-2-13	2012	GO	0.19 *	S	3.43 **	MS	2.10 **	MS
GO-12-4-1	2013	GO	<0.16 *	S	12.22 *	NS	5.87 *	MS
GO-12-4-5	2012	GO	<0.16 **	S	2.10 *	MS	0.86 *	S
GO-12-4-2	2013	GO	<0.16 *	S	4.65 *	MS	0.52 *	S
MT-12-5-1	2012	MT	<0.16 *	S	0.86 *	S	0.18 **	S
MT-12-5-2	2012	MT	<0.16 **	S	1.38 *	MS	0.33 *	S
MT-12-4-1	2012	MT	<0.16 *	S	2.62 *	MS	1.53 **	MS
MT-12-4-4	2012	MT	<0.16 *	S	4.85 *	MS	1.67 **	MS
MT-12-7-1	2012	MT	<0.16 *	S	7.53 **	MS	5.48 **	MS
MT-12-7-2	2012	MT	<0.16 *	S	3.82 *	MS	4.81 **	MS
MT-12-1-3	2013	MT	0.56 *	S	2.71 *	MS	0.38 *	S
MT-12-3-2	2012	MT	0.63 *	S	2.47 *	MS	<0.16 *	S
MES 312	1997	MT	>100 ns	HNS	1.52 *	MS	1.44 *	MS
MES 313	1998	MT	<0.16 ns	S	0.61 *	S	0.51 *	S
MES 318	1999	MT	<0.16 *	S	0.80 *	S	0.23 *	S
MS-12-1-1	2012	MS	<0.16 *	S	8.00*	MS	7.18*	MS
MA-12-3-2	2012	MA	<0.16 *	S	1.49 *	MS	0.64 **	S
MA-12-3-1	2012	MA	<0.16 *	S	2.57 *	MS	1.55 **	MS
MA-12-3-5	2012	MA	<0.16 *	S	1.77 *	MS	0.22 *	S
MG-12-1-1	2012	MG	<0.16 *	S	0.69 ns	S	0.69 *	S
MG-12-1-2	2012	MG	<0.16 *	S	2.67 *	MS	0.55 *	S
MG-12-1-3	2012	MG	<0.16 *	S	1.25 *	MS	0.75 *	S
TO-12-1-1	2012	TO	1.42 **	MS	0.99 *	S	2.68 **	MS
TO-12-1-2	2012	TO	<0.16 *	S	0.45 ns	S	0.53 *	S
TO-12-1-3	2012	TO	1.76 **	MS	4.07 *	MS	6.36 **	MS
MES 317	1999	PR	>100 **	HNS	>100 ns	HNS	1.33 **	MS
MES 322	2001	PR	91.43 ns	HNS	8.02 ns	MS	9.84 *	MS
MES 928	2011	PR	<0.16 ns	S	0.46 ns	S	0.84 *	S
PA-12-1-4	2012	PA	<0.16 ns	S	5.48 **	MS	1.25 *	MS
PA-12-1-2	2012	PA	<0.16 **	S	6.21 **	MS	1.75 *	MS
PA-12-1-1	2012	PA	0.32 *	S	6.22 **	MS	0.51 *	S
PA-12-1-3	2012	PA	<0.16 ns	S	3.94 **	MS	0.71 *	S
S % (nr.)			85% (29)		21% (7)		50% (17)	
MS % (nr.)			6% (2)		73% (25)		50% (17)	
NS % (nr.)			-- (0)		3% (1)		-- (0)	
HNS % (nr.)			9% (3)		3% (1)		-- (0)	

S= sensitive to fungicide (EC_{50} <1 µg mL^{-1}); MS= moderately sensitive to fungicide (EC_{50} 1-10 µg mL^{-1}); NS= non-sensitive to fungicide (EC_{50} 10-25 µg mL^{-1}); HNS= highly non-sensitive to fungicide (EC_{50} >25 µg mL^{-1}) [adapted from Miyamoto et al. (23)]. * = Statistical significance at 5% probability; ** = statistical significance at 1% probability; ns= not statistically significant.

Table 5. Percentage of *in vitro* spore germination (SG) of four *Corynespora cassiicola* isolates to fungicides with or without *salicylhydroxamic acid (SHAM)*.

Treatment	Isolates											
	MES 322			MG-05			Sinop 1			MES 313		
	SG*	χ^2	*P*	SG	χ^2	*P*	SG	χ^2	*P*	SG	χ^2	P
Azoxystrobin	2.0	0.11	0.737	89.2	0.02	0.886	88.4	0.02	0.888	4.8	0.35	0.553
Azoxystrobin + SHAM	1.6			88.8			88.8			6.0		
Pyraclostrobin	0.4	1.01	0.315	83.6	0.01	0.903	23.2	0.18	0.668	0.8	15.41	0.000
Pyraclostrobin + SHAM	1.2			84.0			21.6			8.0		
Picoxystrobin	1.2	1.02	0.313	88.4	12.87	0.000	88.0	1.77	0.184	9.6	0.00	1.000
Picoxystrobin + SHAM	2.4			96.8			91.6			9.6		
Trifloxystrobin	2.0	2.98	0.084	95.6	0.38	0.538	92.0	0.23	0.632	7.2	0.03	0.864
Trifloxystrobin + SHAM	4.8			94.4			90.8			7.6		

* SG = Spore germination.

Table 6. Identification code, year of isolation and Brazilian state of origin of the *Corynespora cassiicola* isolates, and effective concentrations of the fungicides pycoxystrobin, pyraclostrobin, azoxystrobin and trifloxystrobin to reduce 50% spore germination - EC_{50} (µg mL^{-1}) and sensitivity level to QoI fungicides.

Code	Year of isolation	State	QoI fungicides							
			Picoxystrobin		Pyraclostrobin		Azoxystrobin		Trifloxystrobin	
			EC_{50}	Sensitivity	EC_{50}	Sensitivity	EC_{50}	Sensitivity	EC_{50}	Sensitivity
GO-12-2-4	2012	GO	9.34 **	NS	< 0.16 **	S	73.37 **	HNS	>100 *	HNS
GO-12-2-11	2012	GO	4.75 *	NS	<0.16 *	S	1.69 **	NS	>100 *	HNS
GO-12-2-13	2012	GO	0.94 *	MS	0.67 *	MS	14.45 *	NS	>100 *	HNS
GO-12-4-1	2013	GO	>100 **	HNS	1.92 **	NS	>100 **	HNS	>100 **	HNS
GO-12-4-5	2012	GO	5.34 *	NS	0.48 **	MS	63.12 ns	HNS	8.92 **	NS
GO-12-4-2	2013	GO	1.96 **	NS	1.18 **	NS	5.85 **	NS	16.54 **	NS
MT-12-5-1	2012	MT >	100 ns	HNS	5.65 ns	NS	>100 *	HNS	>100 *	HNS
MT-12-5-2	2012	MT	6.02 ns	NS	1.18 ns	NS	27.73 *	HNS	>100 ns	HNS
MT-12-4-1	2012	MT	2.32 *	NS	<0.16 *	S	5.46 **	NS	17.78 *	NS
MT-12-4-4	2012	MT >	100 *	HNS	2.10 *	NS	>100 *	HNS	>100 ns	HNS
MT-12-7-1	2012	MT	0.11 **	S	0.77 **	MS	2.32 *	NS	0.14 *	S
MT-12-7-2	2012	MT	0.19 *	MS	6.06 *	NS	0.52 ns	MS	4.92 *	NS
MT-12-1-3	2013	MT	28.45 *	HNS	<0.16 *	S	7.37 *	NS	28.31 *	HNS
MT-12-3-2	2012	MT	2.04 **	NS	11.84 **	NS	2.68 **	NS	42.12 *	HNS
MES 312	1997	MT	12.63 **	NS	<0.16 **	S	<0.16 ns	S	3.99 ns	NS
MES 313	1998	MT	0.61 *	MS	<0.16 *	S	1.94 *	NS	2.4 *	NS
MES 318	1999	MT	<0.16 *	S	<0.16 *	S	<0.16 *	S	>100 ns	HNS
MS-12-1-1	2012	MS	4.46 *	NS	0.41 *	MS	>100 ns	HNS	>100 ns	HNS
MA-12-3-2	2012	MA	2.55 **	NS	0.43 **	MS	0.57 **	MS	>100 ns	HNS
MA-12-3-1	2012	MA	0.32 **	MS	1.12 **	NS	0.91 *	MS	8.08 **	NS
MA-12-3-5	2012	MA	37.36 **	HNS	1.55 **	NS	20.39 *	NS	>100 *	HNS
MG-12-1-1	2012	MG	17.52 **	NS	2.33 **	NS	1.80 **	NS	>100 *	HNS
MG-12-1-2	2012	MG >	100 **	HNS	8.19 **	NS	>100 *	HNS	>100 *	HNS
MG-12-1-3	2012	MG >	100 **	HNS	1.66 **	NS	>100 *	HNS	>100 *	HNS
TO-12-1-1	2012	TO	0.14 ns	S	<0.16 ns	S	0.57 *	MS	1.12 *	NS
TO-12-1-2	2012	TO	13.61 **	NS	1.31 **	NS	>100 ns	HNS	<0.16 ns	S
TO-12-1-3	2012	TO	1.75 *	NS	0.90 *	MS	>100 ns	HNS	>100 *	HNS
MES 317	1999	PR	0.94 ns	MS	<0.16 ns	S	<0.16 *	S	2.56 *	NS
MES 322	2001	PR	<0.16 ns	S	<0.16 *	S	<0.16 *	S	<0.16 ns	S
MES 928	2011	PR	0.97 **	MS	0.40 **	MS	3.65 **	NS	85.3*	HNS
PA-12-1-4	2012	PA	>100 ns	HNS	36.55 ns	HNS	>100 ns	HNS	>100 *	HNS
PA-12-1-2	2012	PA	0.5 *	MS	0.22 **	MS	2.28 ns	NS	>100 ns	HNS
PA-12-1-1	2012	PA	>100 *	HNS	2.44 *	NS	>100 ns	HNS	>100 ns	HNS
PA-12-1-3	2012	PA	>100 *	HNS	15.13 *	NS	>100 *	HNS	<0.16 ns	S
S % (nr.)			12% (4)		29% (10)		12% (4)		12% (4)	
MS % (nr.)			20% (7)		24% (8)		12% (4)		-- (0)	
NS % (nr.)			38% (13)		44% (15)		35% (12)		26% (9)	
HNS % (nr.)			29% (10)		3% (1)		41% (14)		62% (21)	

S= sensitive to fungicide (EC_{50} <0.16 µg mL^{-1}); MS= moderately sensitive to fungicide (EC_{50} 0.16-1 µg mL^{-1}); NS= non-sensitive to fungicide (EC_{50} 1-25 µg mL^{-1}); HNS= highly non-sensitive to fungicide (EC_{50} >25 µg mL^{-1}) [adapted from Leroux et al. (20)]. * = Statistical significance at 5% probability; ** = statistical significance at 1% probability; ns= not statistically significant.

frequency of sensitive isolates (85%) but also shows three isolates as highly non-sensitive (Table 4). The DMI fungicides prothioconazole and cyproconazole had 0 and 3% non-sensitive isolates, respectively (Table 3).

***In vitro* inhibition of *C. cassiicola* spore germination**

The interaction SHAM × isolate was not significant (P=0.7113). ANOVA indicated that SHAM had no effect on the sensitivity of *C. cassiicola* conidial germination (P=0.9462). Only the factor isolate had significant effects in the analysis (P=0.0001).

For the *in vitro* percentage of spore germination, with or without SHAM, there was no difference between the expected and the null hypothesis, based on Chi-square tests for all combinations QoI fungicide x SHAM (Table 5), except for isolate MES 313, for which addition of SHAM to the fungicide pyraclostrobin enhanced spore germination (c^2= 15.4; P=0.0009).

Pyraclostrobin had higher percentage of sensitive and moderately sensitive isolates of *C. cassiicola* (53%) than the other fungicides. Most of the isolates were non-sensitive to picoxystrobin (68%), azoxystrobin (76%), and trifloxystrobin (88%) (Table 6).

Effect of fungicides on target spot progress under field conditions

There was a significant interaction among fungicide treatment and field trials for AUDPC (P = 0.0001) and for soybean yield (P = 0.0002) (Table 7). This significance led us to carry out one-way ANOVAs for each field trial (Table 8).

In the trial conducted in Nova Xavantina, in 2011/2012 crop season, a significant effect of the treatments was detected for AUDPC (P = 0.001) but not for grain yield (P = 0.796). All fungicide treatments had significantly lower AUDPC means than the untreated control. The most effective treatments in preventing the progress of target spot were the fungicides fluxapyroxad and prothioconazole, showing 78% and 87% control, respectively (Table 8).

In the 2012/2013 trial, there were significant effects on both AUDPC and yield (P = 0.001 and 0.002, respectively). A lower AUPDC was achieved by spraying prothioconazole, which had 79% control. However, soybean grain yield did not differ among fungicide treatments (Table 8).

Trials conducted in the 2013/2014 crop season in Nova Xavantina and Querência Municipalities had an extended period without rain in January. These drier days were unfavorable for the development of target spot epidemics. Although AUDPC means were lower, differences could be detected among fungicide treatments. In both locations, a lower AUDPC was achieved with fluxapyroxad treatment, but low percentages of control (26% to 43%) were obtained (Table 8).

There was a negative and significant correlation between AUDPC and yield (-0.265, P = 0.010). The overall mean for AUDPC with the untreated control was 520, while the lowest AUDPC mean was 143 (prothioconazole), a reduction of 72%. Soybean treated with prothioconazole also had a higher grain yield (2152 kg ha^{-1}), 31% greater than that with the untreated control.

The means of target spot control in the four field trials varied from 26% to 55% and the mean of soybean yield reduction was 28% for the untreated control (Table 8).

There was no incidence of Asian soybean rust (*P. pachyrhizi*), and the incidence of other foliar diseases was not significant in all crop seasons.

Table 7. *Combined* analyses of variance of Area Under Disease Progress Curve (AUDPC) of soybean target spot and soybean grain yield (GY = kg ha^{-1}) of six fungicide treatments tested in 4 field trials (environments)

Sources of variation	df	F		*P* > F	
		AUDPC	GY	AUDPC	GY
Environment	3	314.12	51.61	0.0001	0.0001
Fungicide	5	115.79	5.69	0.0001	0.0003
Bloc (Environment)	12	0.53	1.25	0.8856	0.2755
Environment x Fungicide	15	43.22	3.72	0.0001	0.0002
Error	60				
Total	**95**				

Table 8. Area Under Disease Progress Curve (AUDPC), soybean yield and control percentage of target spot in four field trials with soybeans artificially inoculated with *Corynespora cassiicola* in Nova Xavantina (NX) and Querência (QR), Mato Grosso State, Brazil. Crop seasons were 2011/2012 (11–12), 2012/2013 (12–13) and 2013/2014 (13–14).

Fungicide	NX 11–12			NX 12–13				NX 13–14				QR 13–14				Mean	
	AUDPC	Disease Control (%)	Yield (kg/ha)	AUDPC	Disease Control (%)	Yield (kg/ha)	Yield Reduction (%)	AUDPC	Disease Control (%)	Yield (kg/ha)	Yield Reduction (%)	AUDPC	Disease Control (%)	Yield (kg/ha)	Yield Reduction (%)	Disease Control (%)	Yield Reduction (%)
Untreated	984 a	0	2009 ns	661 a	0	1274 b	39	265 a	0	1486 b	31	170 a	0	2152 a	14	0	28
Boscalid	495 b	50	2176	368 c	44	2084 a	0	174 bc	34	1518 b	29	134 ab	21	2388 ab	4	37	11
Carbendazim	483 b	51	1745	543 ab	18	1779 a	15	171 bc	35	1609 b	25	148 ab	13	2478 b	1	29	14
Cyproconazole	343 c	65	2029	682 a	0	1882 a	10	200 b	25	1525 b	29	149 ab	12	2402 ab	4	26	14
Fluxapyroxad	213 d	78	1965	452 bc	32	1749 ab	16	150 c	43	2071 a	4	126 b	26	2474 b	1	45	7
Prothioconazole	127 d	87	1847	141 d	79	1740 ab	17	158 bc	40	2153 a	0	147 ab	14	2494 b	0	55	6
Mean	441		1960	474		1751		186		1727		146		2398			
C.V. (%)	12.2		18.4	15.3		11.95		10.14		6.04		11.55		4.61			
P	0.001		0.796	0.001		0.002		0.001		0.001		0.034		0.005			

Means followed by the same letter in each column do not differ according to Tukey's test at 5% probability. ns= not statistically significant.

Due to the intensification of fungicide usage (two to four sprays per season) in Brazilian soybean production areas over the last 13 years in order to control Asian soybean rust and other foliar diseases, the selection pressure on soybean pathogen complexes has been high and may have caused resistant isolates to arise (14).

The MBC fungicides carbendazim and thiophanate-methyl have been used in the last decade to control target spot, anthracnose (*Colletotrichum truncatum* (Schw.) Andrus & Moore, 1935), Cercospora leaf blight (*Cercospora kikuchii* T. Matumoto & Tomoy, 1925), and Septoria brown spot (*Septoria glycines* Hemmi, 1915), but their efficacy has been decreasing year after year. In this study, 90% and 85%, respectively, of the sampled *C. cassiicola* isolates showed reduction in the sensitivity to these fungicides, according to the classification proposed by Edgington et al. (8). Most isolates had EC_{50} values above the highest tested concentration (1000 μg mL^{-1}), indicating resistance of *C. cassiicola* to carbendazim and thiophanate-methyl in Brazil. Similar results were obtained in other studies (26, 31, 33).

In a recent survey, there was evidence of sensitivity of *C. cassiicola* to carbendazim only in samples collected before 2007, and non-sensitivity in recent isolates obtained after 2008 (33). These authors considered isolates sensitive when $EC_{50} \leq 1.0$ μg mL^{-1}, and highly resistant when $EC_{50} \geq 50$ μg mL^{-1}.

The fungicide cyproconazole had the highest mean of EC_{50} in the test for mycelial growth inhibition. Most isolates (94%) were non-sensitive ($EC_{50} > 1.0$ μg mL^{-1}) to cyproconazole. Even though non-sensitivity occurred, the mean EC_{50} was higher for isolates from southern Brazil.

DMI fungicides were the first to be widely used after the initial Asian soybean rust epidemics in Brazil, followed by QoI solo or in mix formulations with DMI. Low sensitivity may be a result of the extensive use of cyproconazole in Brazil. This fungicide is the most common DMI found in the commercial mix formulations of DMI and QoI, widely sprayed in soybean fields. The difference in EC_{50} can be attributed to longer exposure to the fungicide by isolates from the southern population. In another survey carried out in 2011, five *C. cassiicola* isolates taken from soybean were considered moderately resistant to cyproconazole (4).

As for prothioconazole, another DMI fungicide, the percentage of non-sensitive isolates was 23%. This result demonstrates the existence of non-sensitive isolates, mainly collected in the northern part of Brazil, as EC_{50} values above 67 μg mL^{-1} were estimated. Variability in sensitivity to prothioconazole was also found by Xavier et al. (33), who tested 24 isolates of *C. cassiicola* with prothioconazole and obtained an EC_{50} ranging from 0.47 μg mL^{-1} to 26.44 μg mL^{-1}.

Sensitivity of *C. cassiicola* isolates to the SDHI fungicides fluopyram, fluxapyroxad and boscalid was not equal. Most isolates were sensitive to fluxapyroxad, while others were non-sensitive to fluopyram and boscalid. EC_{50} means for fluopyram and boscalid were lower than 5 μg mL^{-1}; however, there were isolates with an EC_{50} higher than 5 μg mL^{-1}. From the SDHI fungicides, boscalid had the highest EC_{50}. There are other reports of fungicide resistance to boscalid for *C. cassiicola* (23, 24).

This variability in sensitivity to SDHI fungicides by Brazilian *C. cassiicola* isolates represents a serious concern for long-term fungicide management and for preventing the emergence of resistance. Therefore, fungicides of this group must be used with extreme care in order to minimize directional selection on the pathogen and to avoid the premature inefficacy of fungicides to control target spot in soybean plants.

Conidial germination is directly affected by QoI fungicides (25). Assessment of spore germination is a suitable method to evaluate the sensitivity to this fungicide group. Isolates of *C. cassiicola* in this study ranged from sensitive to highly non-sensitive to all evaluated QoI fungicides. Most of the isolates were sensitive (29%) or moderately sensitive (24%) to pyraclostrobin, and only 3% isolates were highly non-sensitive. For the other QoIs, the frequency of highly non-sensitive isolates was much higher, reaching 62% to trifloxystrobin, 41% to azoxystrobin and 29% to picoxystrobin.

Higher spore germination EC_{50} values were also found for five *C. cassiicola* isolates from soybean to azoxystrobin (6.20 μg mL^{-1}), picoxystrobin (2.74 μg mL^{-1}), and pyraclostrobin (1.47 μg mL^{-1}) (4).

There are reports of resistance of *C. cassiicola* to QoI fungicides (7, 17). The variation in sensitivity reduction factor (from 100.6-fold to 8984.5-fold) found in this study indicates occurrence of resistance in *C. cassiicola* from soybean in Brazil. Crossing resistance appears to occur in some isolates. Seven isolates (GO 12-4-1, MT 12-5-1, MT 12-4-4, MG 12-1-2, MG 12-1-2, PA 12-1-4 and PA 12-1-1) were highly resistant to all QoI fungicides, except pyraclostrobin. This may suggest a diverse resistance mechanism for that compound.

Studies with plant pathogenic fungi, in which strobilurin resistance was examined at molecular level, revealed that resistance often correlates with a point mutation in target cytochrome *b* gene when replacing glycine with alanine at amino acid codon 143 makes an individual resistant to strobilurin (27). Nevertheless, in some cases, other mutations are still present (3). On the other hand, isolate MES 318 was sensitive to all tested QoI fungicides but not to trifloxystrobin. Only one isolate (MES 322) was sensitive to all fungicides.

Considering the *in vitro* assays, the site of action of QoI fungicides can in principle be bypassed by an alternative oxidase, which is inhibited by salicylhydroxamic acid (SHAM) or propyl gallate (22). In this study, there was no effect of SHAM in increasing the sensitivity of four *C. cassiicola* isolates to QoI fungicides, which made us disregard the use of SHAM for all evaluated isolates.

In four field experiments, carbendazim was not effective in controlling target spot in soybean. These results reinforce the *in vitro* tests. On the other hand, prothioconazole consistently prevented target spot epidemics in all field assays. However, this fungicide must be used with caution, especially in northern regions, where the frequency of non-sensitive isolates is higher. Among SDHI fungicides, fluxapyroxad had better results in the field, corroborating the *in vitro* results. In general, the efficacy of fungicides in the field reflected the *in vitro* sensitivity measures.

In the field trials of this study, the averages of target spot control and of soybean yield reduction had the same trend as the ones observed in the Brazilian network trials for soybean target spot (15), although the fungicides used in those network trials are formulated in mixtures of at least two different modes of action.

Prothioconazole and fluxapyroxad provided the highest *in vitro* inhibition of mycelial growth, and pyraclostrobin had the highest inhibition of *in vitro* spore germination, with the lowest EC_{50} values. Widespread non-sensitivity to MBC was detected, indicating that this group of fungicides should no longer be used for target spot control. The pathogen showed variability in sensitivity to SDHI, DMI and QoI fungicides, which denoted a high risk to selection for resistance and suggests that multiple resistance to fungicides can occur in *C. cassiicola*.

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