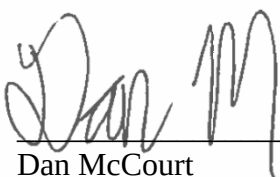


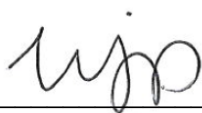


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Protective and systemic fungicides in the management of *Corynespora Cassiicola* in soybeans

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RESUMO

A mancha alvo da soja vem ganhando notoriedade no Brasil, sendo poucos os estudos que visaram identificar controles químicos eficientes. O objetivo deste trabalho foi avaliar o efeito de fungicidas protetores, sistêmicos e associação entre ambos no controle desta doença, em casa de vegetação. O experimento foi conduzido em delineamento inteiramente casualizado, com sete repetições, utilizando plantas de soja da cultivar M6410 IPRO. O fungicida Oxicleto de cobre SC foi previamente aplicado nas plantas no estágio V4 e, os fungicidas Trifloxistrobina + Prothioconazol, Azoxistrobina + Ciproconazol e Fluxapiroxade + Piraclostrobina, isolados e em associação, foram aplicados após a inoculação dos conídios. As avaliações foram realizadas semanalmente, baseadas em escala de notas de severidade da doença. Os dados de severidade foram utilizados para o cálculo da área abaixo da curva de progresso da doença (AACPD). Todos os tratamentos foram eficientes em reduzir a severidade da mancha alvo em soja ao final das avaliações. O tratamento Oxicleto de cobre SC + Azoxistrobina + Ciproconazol foi o que mais suprimiu o desenvolvimento da doença ao longo do experimento, evidenciando que a associação de fungicidas protetores e sistêmicos pode ser utilizada como prática viável para controle desta doença.

Palavras-chave: controle químico, *Glycine max*, mancha alvo da soja.

Protectives and systemic fungicides in the *Corynespora cassiicola* control in greenhouse conditions

ABSTRACT

The soybean target spot is gaining notoriety in Brazil, with few studies aimed at identifying efficient chemical controls. The objective was to evaluate the effect of protective fungicides, systemic and protective associated with systemic in control of soybean target spot in the greenhouse. The experiment was conducted in a completely randomized design, with seven replications, using soybean plants of the M6410 IPRO variety. The fungicide copper oxychloride, was previously applied to plants in V4 stage and the Trifloxystrobin + Prothioconazole fungicide, Azoxystrobin + Cyproconazole and fluxapyroxad + pyraclostrobin alone and in combination were applied after the inoculation of conidia. The evaluations were performed weekly, based on a scale of disease severity notes. The severity data were used to calculate the area under the disease progress curve (AUDPC). All treatments were efficient in reducing the severity of the soybean target spot at the end of the evaluations. The copper + SC + Azoxystrobin + Ciproconazole treatment was the most suppressing the development of the disease throughout the experiment, showing that the association of protective and systemic fungicides can be used as a viable practice to control this disease.

Keywords: Chemical control; *Glycine max*; Soybean target spot.

1. Introduction

The soybean [*Glycine max* (L.) Merril.] is one of the most economically important crops for Brazil, and it is the most produced grain in the country. According to CONAB (Cia. Nacional de Abastecimento [National Food Supply Company]) (2017), total grain production in the country was 186.6 million tons in the 2015/2016 season, with soybeans accounting from more than half this volume, at 95.4 million tons.

Among the foliar diseases that affect this crop species, the leaf spot, caused by the *Corynespora Cassiicola* fungus, has been gaining in notoriety. Its incidence has grown in recent seasons as a result of the increase in production of susceptible crop species, and it is now found in practically all crop-producing regions of the country (Godoy *et al.*, 2012). Godoy *et al.* (2016) mention reductions in productivity of up to 35% among susceptible crop species, while Silva *et al.* (2008) report frequent losses of 10 to 20% caused by the pathogen in the state of Tocantins.

This is a necrotrophic fungus that destroys leaf cells in order to obtain nutrients (Lamotte *et al.*, 2007) and it benefits from high humidity and high temperatures (Fortunato *et al.*, 2015). According to Godoy *et al.* (2016), symptoms begin in the leaves with brown spots with a yellowish halo, evolving to large circular, nun-colored stains, which normally feature spots in the center and darker-colored concentric circles. The fungus survives on crop waste and infected seeds, and it can colonize a wide range of residues in the soil. Crop species that are more sensitive to this disease may suffer intense defoliation and also present stains on their stems and seed pods.

Recommended management strategies to control his disease are use of resistant crop species, seed treatment, alternation/succession of crop species with corn or other gramineae and use of fungicides (Godoy *et al.*, 2016; Ribeiro *et al.*, 2017).

The first recommendations for use of fungicides on crops in Brazil were made in 1996 and 1997, for the management of *Oidium* (*Erysiphe difusa*). However, the use of these products really intensified after the appearance of Asian soybean rust (*Phakopsora pachyrhizi* Syd. & P. Syd) in 2001. As the efficiency of triazoles for the management of the rust diminished, from 2007, it was recommended that only the mixture of triazoles and strobilurins were to be used to control this pest (Godoy *et al.*, 2012).

Even with frequent applications of fungicides in the crop, leaf spot is still commonly observed in fields of susceptible crop species (Xavier *et al.*, 2013), and management failures with use of benzimidazoles have been reported (Xavier *et al.*, 2013; Avozani *et al.*, 2014).

Given the difficulty in managing *C. cassiicola*, the purpose of this work was to evaluate the efficiency of

protective and systemic fungicides and the joint use of both in order to manage this phytopathogen.

2. Material and Methods

The experiment was conducted in a greenhouse in the municipality of Jaboticabal – SP, located at 21°16'29"S and 48°20'08"W, from January to March 2015. Plants from the M6410 IPRO crop were used, sewn in plastic vases containing 2 kg of sand and substrate mixture (1:1), with one plant per vase. The experimental design was totally randomized, with seven repetitions, where each repetition comprised one vase.

The *C. cassiicola* conidia were isolated from symptomatic soybean leaves in a water agar medium, and they were subsequently multiplied in PDA (potato-dextrose-agar) culture medium, in Petri dishes. These were placed in a growth chamber at 25°C for 15 days, by which time abundant sporulation had occurred.

All fungicides were applied using an electric sprayer set at 30 psi of pressure and equipped with a double-plane jet nozzle model TJ 60-11004, calibrated for a volume mixture of 100 liters/ha, until the point of run-off had been achieved. The treatments are described in Table 1.

Table 1. Treatments and doses tested in the experiment to manage leaf spot on soybeans in a greenhouse. Jaboticabal, SP, 2015.

Treatments	Dose (L/ha)
1. Control	–
2. Copper oxychloride SC	0.5
3. Mancozeb	1.5
4. Trifloxystrobin	0.3
Prothioconazole	
5. Copper oxychloride SC and Trifloxystrobin + Prothioconazole	0.5 + 0.3
6. Azoxystrobin + Ciproconazole	0.5
7. Copper oxychloride SC and Azoxystrobin + Ciproconazole	0.5 + 0.5
8. Fluxapyroxad + Pyraclostrobin	0.3
9. Copper oxychloride SC and Fluxapyroxad + Pyraclostrobin	0.5 + 0.3

In order to assess the protective and curative effect, the protective fungicides were applied to plants in stage V4 (Fehr & Craviness, 1977) during the morning. Subsequently, in the afternoon, the plants were inoculated with a concentration of 3×10^4 conidia mL⁻¹ to the point of run-off, with the aid of a manual sprayer. After inoculation, the plants were treated with systemic fungicides and kept in a humid chamber for 48 hours to

favor the infectious process. In order to guarantee the presence of free water on the leaves, the nebulization system was activated for 15 minutes every six hours throughout the crop cycle.

Five weekly assessments were conducted, at the onset of the first symptoms until the senescence of the leaves. These were based on the mean severity of the disease in leaflets on the top and lower thirds of the plants, using the diagrammatic scale of disease severity proposed by Soares *et al.* (2009), assigning grades of 1 to 7, according to Figure 1.

The severity data were used to calculate the area under the curve of disease progression, based on the model proposed by Campbell & Madden (1990):

$$AACDP = \Sigma ((X_i + X_{i+1})(t_{i+1} - t_i))/2$$

where n equals the number of severity assessments, X_i represents disease severity and $(t_{i+1} - t_i)$ is the number of days between assessments.

The following formula was used to calculate the control percentage (CP):

$$CP = \frac{\text{Disease in the Control} - \text{Disease in the Treatment}}{\text{Disease in the Control}} \times 100$$

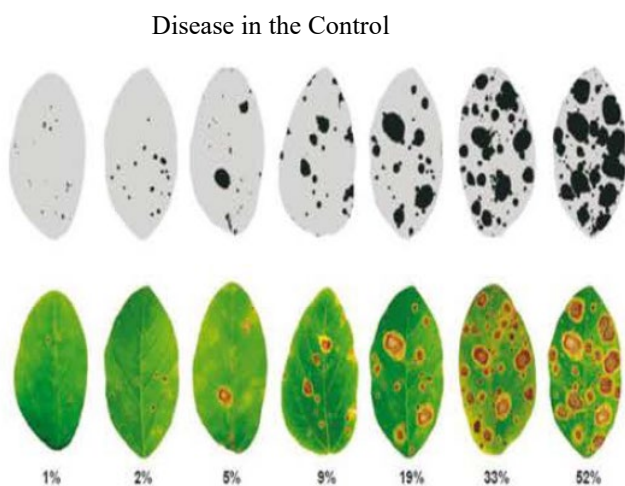


Figure 1. Diagrammatic scale to assess severity of Leaf Spot in soybeans.

The disease data were subjected to variance analysis and the means were compared using the Tukey test at the 5% level of probability, using the SASM-Agri program (Canteri *et al.*, 2001).

3. Results and Discussion

The first symptoms of leaf spot occurred 20 days after inoculation of *C. cassicola*. The severity results can be found in Table 2. At the end of the assessments, an increase in disease severity was observed for all treatments, especially in the control plants. In the first assessment, only the Azoxystrobin + Ciproconazole and Copper oxychloride SC and Azoxystrobin +

Ciproconazole treatments differed from the other treatments, while the other treatments resulted in intermediate values between these and the control. In the second assessment, however, only the Azoxystrobin + Ciproconazole with the protective fungicide Copper oxychloride SC maintained this difference from the other treatments. These treatments again differ from the others, together with Copper oxychloride SC, in the third assessment.

In the fourth and fifth assessments, with the exception of Copper oxychloride SC, Trifloxystrobin + Prothioconazole and Copper oxychloride SC and Trifloxystrobin + Prothioconazole, the other treatments differed from the control; in the final assessment, all the treatments involving application of fungicides differed from the control, although they did not differ among themselves.

With regard to efficiency of management, values varying from 57.9% for the application of systemic fungicide Fluxapyroxad + Pyraclostrobin, up to 78.8% for the Copper oxychloride SC and Azoxystrobin + Ciproconazole combination of fungicides (Figure 2). The lowest value for AACDP was obtained for the Copper oxychloride SC and Azoxystrobin + Ciproconazole treatment, indicating that, throughout the assessments, this was the treatment that most effectively suppressed the disease. Mixtures of triazoles and strobilurins are commonly used in soybean crops to manage Asian rust; thus, the addition of a protective fungicide alone would result in more efficient control of leaf spot.

The Azoxystrobin + Ciproconazole mixture has been reported to have poor efficiency in the management of leaf spot in soybeans, and it has only been used to manage Asian rust in experiments conducted to assess management of leaf spot by Godoy *et al.* (2012). In the experiments conducted by these authors, the best results were obtained with the mixture of Pyraclostrobin + Fluxapyroxad, which was also ascertained in the work by Belufi *et al.* (2015). These discrepancies may also be explained by methodological differences, considering that these works were conducted under field conditions, were several other factors may have had an influence.

Avozani (2014) also cited the low fungitoxicity of ciproconazole for the management of leaf spot. However, no loss of sensitivity for strobilurins in leaf spot has been reported in Brazil. Thus, this group continues to have a major role in the management of this pathogen.

Ribeiro *et al.* (2016), when assessing fungicides used alone or in combination with protective fungicides, also found higher rates of control of leaf spot in soybeans when increasing the number of associations of mancozeb (protective fungicide) with systemic fungicides. The authors deduced that these results were due to the fact that

mancozeb is a multi-site fungicide, with protective and residual effect.

These results show that use of protective fungicides in association with systemic fungicides is of great value in the management of this phytopathogen.

Table 2. Comparison of percentage means of severity of *Corynespora Cassiicola* in soybeans. Jaboticabal, 2015.

Treatments	0 DAOS ¹	7 DAOS ¹	14 DAOS ¹	21 DAOS ¹	28 DAOS ¹	35 DAOS ¹
	Severity (%)					
Control	1.00 a	1.14 ab	1.29 ab	2.36 a	3.43 a	6.43 a
Occ	0.50 ab	0.50 bc	0.50 c	1.50 ab	1.57 ab	1.43 b
Mancozeb	0.50 ab	0.64 bc	0.64 bc	1.00 b	1.07 b	1.64 b
Triflox + Prothio	0.79 ab	1.57 a	1.57 a	1.60 ab	2.14 ab	2.50 b
Occ and Triflox + Prothio	0.50 ab	0.50 bc	0.57 bc	0.79 b	0.86 b	2.21 b
Azoxy + Cipro	0.29 b	0.50 bc	0.50 c	0.64 b	1.50 b	1.79 b
Occ and Azoxy + Cipro	0.21 b	0.36 c	0.43 c	0.64 b	0.71 b	1.36 b
Fluxa + Pyra	0.64 ab	0.66 bc	0.86 abc	0.93 b	1.43 b	2.71 b
Occ and Fluxa + Pyra	0.57 ab	0.57 bc	0.57 bc	1.43 ab	1.55 ab	2.21 b
CV(%)	15.31	17.64	15.65	16.15	20.71	14.19

Means followed by the same letter in the column do not differ statistically with each other by the Tukey test at 5% probability; ¹Data transformed into $(x + 0.5)^{0.5}$ for statistical analyses. Occ – Copper oxychloride; Triflox + Prothio – Trifloxystrobin + Prothioconazole; Azoxy + Cipro – Azoxystrobin + Ciproconazole; Fluxa + Pyra – Fluxapyroxad + Pyraclostrobin; DAOS – days after onset of symptoms.

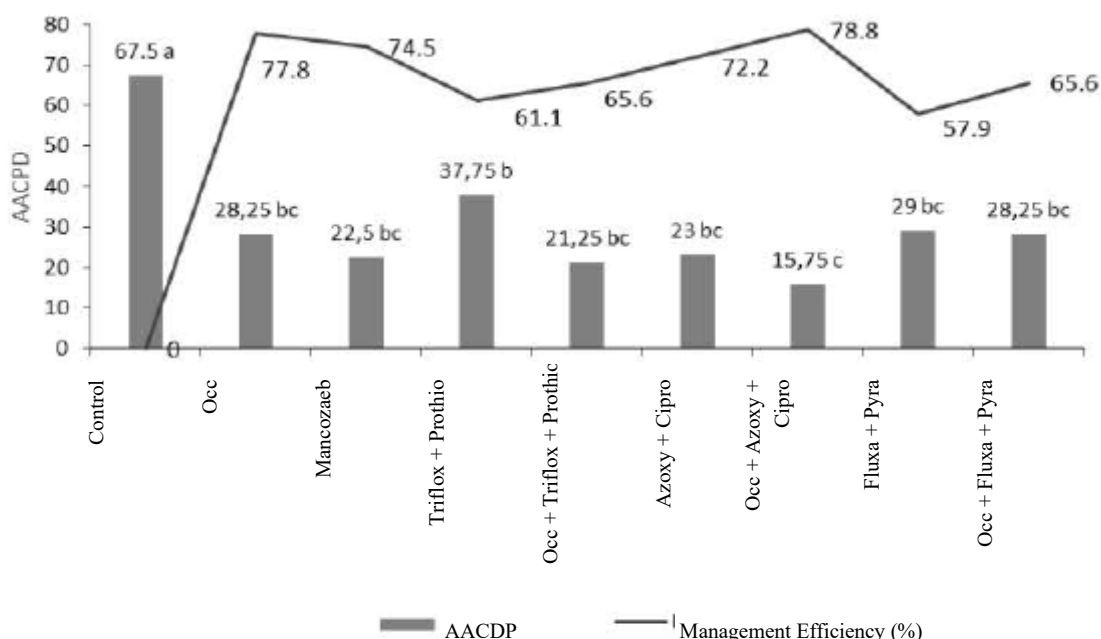


Figure 2. Means of the Area Under the Curve for Disease Progression (AACDP) in the different treatments. Jaboticabal, SP, 2015. Occ – Copper oxychloride; Triflox + Prothio – Trifloxystrobin + Prothioconazole; Azoxy + Cipro – Azoxystrobin + Ciproconazole; Fluxa + Pyra – Fluxapyroxad + Pyraclostrobin.

4. Conclusions

The treatments with chemical control suppressed leaf spot, wherein the treatment comprising Copper oxychloride SC and Azoxystrobin + Ciproconazole presented the lowest AACDP, proving that the application in conjunction with protective and systemic products is an important tool for disease management.

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