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RESEARCH ARTICLE

Yield and economic losses caused by *Corynespora cassiicola* on soybean under chemical control

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ABSTRACT

Target Spot is an important disease in soybean caused by the necrotrophic fungus *Corynespora cassiicola*. Recently, this fungus has gained importance in large crops such as soybeans and for its insensitivity fungicides reports. In this study, three assays were carried out with different application treatments in order to evaluate fungicide applications to control Target Spot and their interference on soybean productivity. Different application treatments were tested including fungicide products, doses, mixtures and number of applications, as well as estimate the application's economic viability. Cultivar TMG 2378 IPRO was used and the applications were performed with a CO_2 sprayer pressurized in a randomized block design with four replications. The harvest of 2.7 m^2 useful area in the plot center was carried out with a stationary plot harvester and the yield was estimated for kg ha^{-1}. For an economic analysis of the application treatments, the soybeans sale was considered at a fixed price of US$ 264 t^{-1}, while the operating cost at a value of US$ 8. The fungicides spraying cost was estimated in kg of grains ha^{-1} based on the average of two budgets made. The fungicide mixtures bixafen+prothioconazole+trifloxystrobin+mancozeb and trifloxystrobin+ prothioconazole+mancozeb presented both yield increase and economic viability compared to control.

Highlighted Conclusion

Mixtures of bixafen+prothioconazole+trifloxystrobin+mancozeb and trifloxystrobin+ prothioconazole+mancozeb have yield and economic viability to control *Corynespora cassiicola*.

INTRODUCTION

Ascomycete *Corynespora cassiicola* (Berk. & M. A. Curtis) C. T. Wei is a cosmopolitan specie often found in the tropics that causes necrotic spots in more than 400 different plant hosts (Sinclair 1999; Farr and Rossman 2020). Many of these host species are plants of economic importance, such as soybean (Boosalis and Hamilton 1957), cotton (Jones 1961), tomato (Blazquez 1972), eucalyptus (Reis et al. 2014), cocoa (Duarte et al. 1978) and rubber tree (Gasparotto et al. 1988). The pathogen was first identified in soybean in the USA in 1945 under the name *Helminthosporium vignae* (Olive et al. 1945). On soybean, *C. cassiicola* causes the disease known as Target Spot and the first report in Brazil occurred in 1974 in the state of Mato Grosso and two years later in the state of Paraná (Almeida et al. 1976).

Corynespora cassiicola is a necrotrophic fungus able to survive in cultural remains; also survives in alternative hosts and soybean seed (Sinclair 1999). The most common symptoms are spots on the leaves, circular in shape with concentric rings, yellowish halo and dark punctuation in the center. The lesions grow up to two centimeters in diameter and due to the concentric rings resemble the shape of a target. Premature defoliation can occur in susceptible cultivars, as well as spots on the stems and pods, reaching the seeds. The fungus can infect the roots, mainly in no-till crops, causing root rot and intense sporulation (Almeida et al. 2005). Yield losses caused by the target stain on soybean vary among the two largest soybean producing countries. In Brazil, losses vary from 8% up to 40% (Molina et al. 2018) and in the United States, losses ranged from 18% to 40% (Koenning et al. 2006).

Several strategies are recommended for the control of target stain, such as the use of resistant cultivars, the treatment of seeds, the rotation of crops with grass species and spraying with fungicides (Almeida et al. 2005).

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Currently, the fungicides regulated and in the process of being regulated to control target spot belong to the benzimidazois, strobilurins, carboxamides and triazoles groups (Xavier et al. 2013).

Modern systemic fungicides block only one specific pathogen site. For example, benzimidazoles prevent the assembly of tubulin units inhibiting cell division; strobilurins and carboxamides act in cell respiration hindering ATP synthesis; triazoles blocks membrane lipids synthesis. Although fungicides generally have a specific site of action, fungi can quickly develop resistance to them if handled improperly (Baibakova et al. 2019).

The Fungicide Resistance Action Committee (FRAC) classifies *C. cassiicola* as a high-risk pathogen for the development of fungicide resistance (FRAC 2019). Therefore, the knowledge of the most effective fungicides or combinations for controlling target spot in soybeans and data regarding productivity losses caused by this pathogen can provide useful information in combating this emerging disease in soybean culture.

The main objective of this study was to evaluate soybean yield under fungicides applications for controlling *C. cassiicola*. Different application treatments were tested including fungicide products, doses, number of applications, mixtures, as well as economic viability estimation of the applications.

MATERIAL AND METHODS

Genetic material and experimental design. The cultivar TMG 2378 IPRO was sown on 10/16/2019 using a precision seeder spacing of 0.45 m between rows and 15 seeds per linear meter density, in order to obtain a final stand of 300 thousand plants ha^{-1}. Three assays were conducted in the 2019/20 crop season in the experimental area of Fazenda Chapada II in Sapezal-MT, Brazil (-13º 39' 11” S, -58º 34' 0” W) to assess the interference of application treatments on crop yield. The experimental design used was randomized blocks, with four replications. Each plot had dimensions of 1.8 m wide by 5.0 m long, totaling 9.0 m², and it was considered a useful area of 2.7 m^2 in the plot center.

Fungicides assays. Before sowing, the seeds were previously treated with 250 mL of a mixture containing carboxine (200 g L^{-1}) + tiram (200 g L^{-1}) fungicides, 50 mL of the product containing chlorantraniliprole insecticide (625 g L^{-1}), for each 100 kg of seeds and 50 mL ha $^{-1}$ of Co-Mo was added. After chemical treatment, the seeds were inoculated with the strains SEMIA 5079 (CPAC 15) and SEMIA 5080 (CPAC 7), components of the BIOMAX® product in the recommended concentration of 250 g 100 kg^{-1} of seeds. Fertilization was also carried out with Super Triple mineral fertilizer (41% P_2O_5 and 7 to 12% Ca), in the proportion of 130 kg ha^{-1}. Area history counts on pasture until the 2018/19 crop season. In 2019/19, the Cowpea beans culture was introduced and after the bean harvest this experiment was installed.

The treatments are described in Table 1. In Assay I, four applications were performed, the first in R1 stage and the others in 14-days intervals, evaluating isolated products and mixtures. In the second assay, applications were performed with 14-days intervals with mancozeb applications between application intervals (7-days). In the third assay, the multisite chlorothalonil and mancozeb were evaluated in mixtures with two applications, the first in R5 (in the presence of symptoms) and the second with an interval of 14 days. Fungicide applications were carried out using a pressurized CO_2 sprayer, with a pressure of 30 psi, equipped with a 2 m long bar and a conical tip ATR 2.0, with a 0.5 m spacing between tips regulated for a flow of 120 L ha^{-1}.

After harvesting, the plants present in the useful area of each plot were harvested with the stationary plot harvester aid to estimate the productivity (kg ha^{-1}) of each treatment. And then continuing with thousand kernel weight (TKW) and the samples humidity determination (Brasil 2009), the humidity was corrected to 13%.

Statistical analysis. The data was subjected to analysis of residues normality (Shapiro-Wilk, $p \leq 0.05$) and variance homogeneity (Neill and Mathews, $p \leq 0.05$). Subsequently, the variance analysis was performed by the F test ($p \leq 0.05$) and the means when significant were compared using the Tukey’s test ($p \leq 0.05$), using the statistical software R, version 3.6.1 and the ExpDes.pt data package (R Core Team 2017).

Economic analysis. For economic analysis applications, fixed operating prices were set at US $ 8 ha^{-1}; fungicide costs were calculated as the average of the two market prices paid for each product in the states of Rio Grande do Sul and Mato Grosso, Brazil, in April 2020, converted to kg ha $^{-1}$ (Table 1); and the exchange rate of BRL 5.16 = US$ 1 was used. The price considered for soybeans sale was US$ 264 t^{-1}, the average price in March 2020 for Brazil (CONAB 2020).

RESULTS AND DISCUSSION

Applications interference in crop yield. To the detriment of the experiment sowing time in the state of Mato Grosso, Asian rust no incidence was found in the assay plots, with no interference of this disease in the results obtained. The climatic conditions were favorable to the Target spot occurrence (Figure 1), severity percentages of 3 to 30% were observed in the plots, reaching levels considered to be of high severity (33%) (Molina et al. 2019).

Due to higher severity percentages, premature defoliation was observed and this parameter was not evaluated in these assays.

In the first assay, fungicidal treatments that obtained statistical difference in yield regarding the control were: EI.T14 (trifloxystrobin + prothioconazole + mancozeb) and EI.T16 (bixafen + prothioconazole + trifloxystrobin + mancozeb), with 5,381.6 and 5,234.5 kg ha^{-1}, respectively. Regarding TKW, again the treatments EI.T14 (174.2 g) and EI.T16 (172.6 g) showed higher values, followed by EI.T10 (fluxpyroxade + pyraclostrobin - 168.3 g), EI.T8 (trifloxystrobin + prothioconazole - 0.7 - 167.9 g) and EI.T7 (trifloxystrobin + prothioconazole - 0.4 - 167.7 g) (Table 2).

Table 1. Description of the treatments applied in each assay.

Code	Fungicide a.i ha^{-1} **	g active ingrediente (a.i.) ha^{-1}	Treatment cost (kg ha^{-1})*
	Assay I		
EI.T1	Control	-	-
EI.T2	mancozeb -1.5	1125	112.7
EI.T3	mancozeb -2.5	1875	167.7
EI.T4	copper oxychloride-0.7	411	126.4
EI.T5	copper oxychloride-1.17	688	191.1
EI.T6	Carbendazim	500	101.9
EI.T7	trifloxystrobin + prothioconazole-0.4	60 + 70	311.1
EI.T8	trifloxystrobin + prothioconazole-0.7	105 + 122,5	521.9
EI.T9	bixafen + prothioconazole + trifloxystrobin	62.5 + 87.5 + 75	467.7
EI.T10	fluxpyroxade + pyraclostrobin	50 + 81	327.2
EI.T11	Diphenoconazole	75	132.9
EI.T12	diphenoconazole + cyproconazole	75 + 45	146.9
EI.T13	propiconazole + diphenoconazole	37.5 + 37.5	118.0
EI.T14	trifloxystrobin + prothioconazole + mancozeb	60 + 70 + 1125	393.7
EI.T15	fluxpyroxade + pyraclostrobin+ copper oxychloride	50 + 81 + 688	423.5
EI.T16	bixafen + prothioconazole + trifloxystrobin + mancozeb	62.5 + 87.5 + 75 + 1125	550.2
EI.T17	mancozeb + carbendazim	1125 + 500	239.4
EI.T18	copper oxychloride + carbendazim	688 + 500	198.1
EI.T19	copper oxychloride + mancozeb + carbendazim	688 + 1125 + 500	280.7
EI.T20	copper oxychloride + mancozeb + diphenoconazole	688 + 1125 + 75	287.1
EI.T21	copper oxychloride + diphenoconazole + carbendazim	688 + 75 + 500	300.9
EI.T22	fluxpyroxade + pyraclostrobin + diphenoconazole	50 + 81 + 75	430.0
	Assay II		
EII.T1	Control	-	-
EII.T2	mancozeb -1.5 (14 day intervals)	1125	112.7
EII.T3	mancozeb -1.5 + tebuconazole (14 day intervals)	1125 +100	207.8
EII.T4	mancozeb -1.25 (7 day intervals)	937	180.6[1]
EII.T5	mancozeb -1.25 + tebuconazole (14 day intervals) + aplicação de mancozeb - 1.25 entre as aplicações de mancozeb -1.25 + tebuconazole.	937 +100 + 937	276.7[1]
EII.T6	Application treatment: (14 day intervals) 1st-mancozeb -1.5 + tebuconazole 2nd- copper oxychloride + mancozeb + tebuconazole 3rd- chlorothalonil + tebuconazole 4th- chlorothalonil + tebuconazole	 1125 +100 688 + 1125 + 100 1080 +100 1080 +100	322.7
	Assay III [2]		
EIII.T1	Control	-	-
EIII.T2	trifloxystrobin + prothioconazole-0.7	105 + 122.5	261.0
EIII.T3	bixafen + prothioconazole + trifloxystrobin	62.5 + 87.5 + 75	233.9
EIII.T4	trifloxystrobin + prothioconazole + mancozeb	60 + 70 + 1125	196.9
EIII.T5	bixafen + prothioconazole + trifloxystrobin + mancozeb	62.5 + 87.5 + 75 + 1125	275.2
EIII.T6	chlorothalonil + diphenoconazole	1080 +75	116.2
EIII.T7	chlorothalonil + tebuconazole	1080 +100	112.4
EIII.T8	chlorothalonil + carbendazim + diphenoconazole	1080 + 500 + 75	152.0
EIII.T9	chlorothalonil + carbendazim + tebuconazole	1080 + 500 + 100	148.2
EII.T10	chlorothalonil + diphenoconazole + tebuconazole	1080 + 75 + 100	163.9

*Average price quoted for products, plus operating cost, converted to kg ha^{-1}. **Oil was added to the mixture based on the manufacturer's indication. [1] It was added to the 2x operating cost. [2] Both applications were considered half operating cost (US $ 4).

In the second assay, there was no statistical difference between the yields obtained in the different treatments. Like as for TKW, the treatments EII.T5 (mancozeb - 1.25 + tebuconazole (14 day intervals) + application of mancozeb - 1.25 between applications); EII.T4 (mancozeb - 1.25 - 7-day interval) and EII.T3 (mancozeb - 1.5 + tebuconazole) presented values different from those obtained in plots with no application, with an increase of 9 to 11.3 g compared to the control (Table 3).

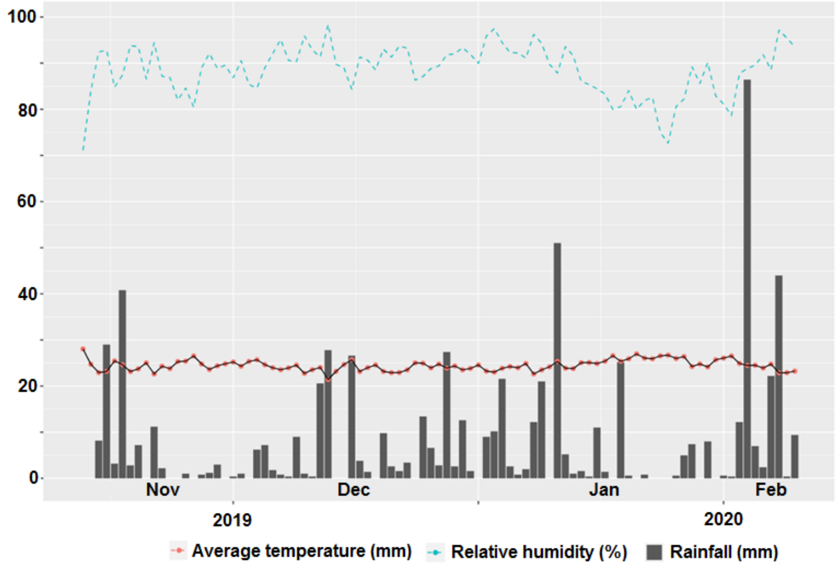

Figure 1. Average daily temperature (°C), rainfall (mm) and relative humidity (%) between November 12, 2019, and February 18, 2020, the period between the R1 stage and the crop harvest in the plots in Sapezal-MT. Source: weather station in the area.

Table 2. Yield (kg ha^{-1}) and a Thousand Kernel Weight (TKW – g) according to different treatments with chemical fungicides to control the target spot on soybean, crop season 2019/2020; Assay I.

Treatment	Yield	TKW	Net Yield[1]
EI.T1	4,499.3 cd	152.1 d	4,499.3 b
EI.T2	4,859.9 abc	161.9 bcd	4,747.2 a
EI.T3	4,896.1 abc	161.1 bcd	4,728.4 a
EI.T4	4,478.6 cd	156.3 cd	4,352.2 b
EI.T5	4,585.4 cd	152.8 d	4,394.3 b
EI.T6	4,405.2 cd	154.3 d	4,303.4 b
EI.T7	4,903.5 abc	167.7 abc	4,592.4 a
EI.T8	4,736.7 bcd	167.9 abc	4,387.0 b
EI.T9	4,549.1 cd	155.5 d	4,269.0 b
EI.T10	4,908.8 abc	168.3 abc	4,221.9 b
EI.T11	4,560.9 cd	160.5 bcd	4,428.0 b
EI.T12	4,247.6 d	151.9 d	4,100.7 b
EI.T13	4,658.5 bcd	153.6 d	4,540.5 a
EI.T14	5,381.6 a	174.2 a	4,988.0 a
EI.T15	4,593.2 cd	158.2 cd	4,169.7 b
EI.T16	5,234.5 ab	172.6 ab	4,684.3 a
EI.T17	4,374.2 cd	160.8 bcd	4,134.7 b
EI.T18	4,331.0 cd	153.5 d	4,132.9 b
EI.T19	4,859.7 abc	159.6 cd	4,579.0 a
EI.T20	4,813.4 abcd	158.7 cd	4,526.3 a
EI.T21	4,728.3 bcd	150.7 d	4,427.4 b
EI.T22	4,806.3 abcd	159.6 cd	4,376.4 b
CV (%)	4.1	2.9	5.16
p value	10,200x10^{-8}	1,000x10^{-9}	82,431x10^{-6}

* Means followed by the same lower case letter in the column do not differ statistically by Tukey's test ($p \leq 0.05$). [1] The cost in kg ha^{-1} was subtracted from the estimated productivity for each treatment, compared by Scott-Knott's test ($p \leq 0.05$). Treatments: EI.T1: Control; EI.T2: mancozeb - 1.5; EI.T3: mancozeb - 2.5; EI.T4: copper oxychloride-0.7; EI.T5: copper oxychloride-1.17; EI.T6: carbendazim; EI.T7: trifloxystrobin + prothioconazole - 0.4; EI.T8: trifloxystrobin + prothioconazole-0.7; EI.T9: bixafen + prothioconazole + trifloxystrobin; EI.T10: fluxpyroxade + pyraclostrobin; EI.T11: diphenoconazole; EI.T12: diphenoconazole + cyproconazole; EI.T13: propiconazole + diphenoconazole; EI.T14: trifloxystrobin + prothioconazole + mancozeb; EI.T15: fluxpyroxade + pyraclostrobin + copper oxychloride; EI.T16: bixafen + prothioconazole + trifloxystrobin + mancozeb; EI.T17: mancozeb + carbendazim; EI.T18: copper oxychloride + carbendazim; EI.T19: copper oxychloride + mancozeb + carbendazim; EI.T20: copper oxychloride + mancozeb + diphenoconazole; EI.T21: copper oxychloride + diphenoconazole + carbendazim; EI.T22: fluxpyroxade + pyraclostrobin + diphenoconazole.

Table 3. Yield (kg ha^{-1}) and a Thousand kernel Weight (TKW – g) according to different treatments with chemical fungicides to control the target spot on soybean, crop season 2019/2020; Assay II.

Treatment	Yield	TKW	Net Yield[1]
EII.T1	4,387.2^{ns}	153.4 b	4,387.2^{ns}
EII.T2	4,758.2	158.8 ab	4,645.5
EII.T3	4,793.9	162.4 a	4,586.1
EII.T4	4,763.9	163.0 a	4,583.3
EII.T5	4,909.3	164.7 a	4,632.6
EII.T6	4,593.3	161.6 ab	4,270.2
CV (%)	7.5	2.3	7.8
p value	0.41	0.009	0.64

* Means followed by the same lower case letter in the column do not differ statistically by Tukey's test (p ≤ 0.05). ^{ns} Not significant. [1] The cost in kg ha^{-1} was subtracted from the estimated productivity for each treatment. Treatments: EII.T1: Control; EII.T2: mancozeb - 1.5 (14-day intervals); EII.T3: mancozeb -1.5 + tebuconazole (14-day intervals); EII.T4: mancozeb -1.25 (7-day intervals); EII.T5: mancozeb -1.25 + tebuconazole (14-day intervals) + mancozeb application - 1.25 between mancozeb applications -1.25 + tebuconazole; EII.T6: Application treatment with 14-day intervals: 1º-mancozeb -1.5 + tebuconazole, 2º- copper oxychloride + mancozeb + tebuconazole, 3º-chlorotalonil + tebuconazole and 4º- chlorotalonil + tebuconazole.

The third assay with a late application, positioned at R5, after symptoms visualization in the field, we intended to simulate spray application treatment performance and viability after the symptom presence. In this assay, only the treatment EIII.T5 (bixafen + prothioconazole + trifloxystrobin + mancozeb) showed a difference in the control plots for both crop yield and TKW, with 952 kg ha^{-1} and 16.4 g increase, respectively compared with the plots without application (Table 4).

Table 4. Yield (kg ha^{-1}) and a Thousand kernel Weight (TKW – g) according to different treatments with chemical fungicides to control the target spot on soybean, crop season 2019/2020; Assay III.

Treatment	Yield	TKW	Net yield[1]
EIII.T1	4,127.5 b	154.4 b	4,112.5 b
EIII.T2	4,771.6 ab	161.6 ab	4,480.6 a
EIII.T3	4,569.4 ab	161.3 ab	4,305.5 b
EIII.T4	4,353.3 ab	159.7 ab	4,126.4 b
EIII.T5	5,079.1 a	170.8 a	4,773.9 a
EIII.T6	4,187.5 b	158.7 ab	4,041.3 b
EIII.T7	4,331.5 b	155.9 b	4,189.1 b
EIII.T8	4,455.4 ab	163.2 ab	4,273.4 b
EIII.T9	4,237.6 b	157.7 ab	4,059.4 b
EIII.T10	4,098.8 b	164.4 ab	3,904.8 b
CV (%)	6.9	3.5	7.2
p value	0.002	0.018	0.02

* Means followed by the same lower case letter in the column do not differ statistically by Tukey's test (p ≤ 0.05). [1] The cost in kg ha^{-1} was subtracted from the estimated productivity for each treatment, compared by Scott-Knott's test (p ≤ 0.05). Treatments: EIII.T1: Control; EIII.T2: trifloxystrobin + prothioconazole-0.4; EIII.T3: bixafen + prothioconazole + trifloxystrobin; EIII.T4: trifloxystrobin + prothioconazole + mancozeb; EIII.T5: bixafen + prothioconazole + trifloxystrobin + mancozeb; EIII.T6: chlorothalonil + difeconazole; EIII.T7: chlorothalonil + tebuconazole; EIII.T8: chlorothalonil + carbendazim + diphenoconazole; EIII.T9: chlorothalonil + carbendazim + tebuconazole; EII.T10: chlorothalonil + diphenoconazole + tebuconazole

The treatments with products containing double mixture plus multisite (EI.T14: prothioconazole + trifloxystrobin + mancozeb) and triples mixtures plus multisite, mainly EI.T16 (bixafen + prothioconazole + trifloxystrobin + mancozeb), showed a higher average yield. In the first case due to it has active ingredients of a specific action, allied to the multisite; in the second situation to the detriment of having the same active ingredients added to another action mode (they are SDHI, DMI and QoI) plus a multisite, thus expanding the action spectrum (Godoy et al. 2018).

Studies that evaluated the application interference of prothioconazole + trifloxystrobin reported an increase in yield of 276 (2 applications); 546 (3 applications) and 647 (4 applications) kg ha^{-1} (Ribeiro et al. 2017; Godoy et al. 2018; Molina et al. 2018). The bixafen + prothioconazole + trifloxystrobin application the increments were on average 628.5 kg ha^{-1} (Godoy et al. 2018, 2019).

The prothioconazole + trifloxystrobin applications increased by 404 and 646 kg ha^{-1} in assay I and III, respectively, while bixafen + prothioconazole + trifloxystrobin application presented 50 (assay I) and 444 (assay III) kg ha^{-1} on crop yield increases. However, the application of only specific site fungicides showed a significant difference compared with mixtures containing specific and multisite fungicides because there is an expansion of action spectrum, reducing the pressure on the specific action mechanisms (Reis et al. 2016). The observed increments were 881.7 kg ha^{-1} for the prothioconazole + trifloxystrobin + mancozeb application (assay I) and 734.7 and 954 kg ha^{-1} with the bixafen + prothioconazole + trifloxystrobin + mancozeb application in assay I and III, respectively.

Economic analysis of application treatments. The soybean sale was considered at a fixed price of US $ 264 t^{-1} (CONAB 2020), while the operating cost at a value of US$ 8 (Molina et al. 2018). The fungicides spraying cost was estimated in kg of grains per ha (Table 1). In the economic evaluation, treatments that obtained a good productive response but had a high cost were not interesting, while others of lower-cost gained prominence, as occurred with EI.T13.

When comparing treatments when the cost in kg ha^{-1} was subtracted from the yield crop the first assay, treatment EI.T14 (prothioconazole + trifloxystrobin + mancozeb) showed higher net yield (4988.0 kg ha^{-1}), followed by EI.T2 treatments (mancozeb -1.5: 4747.2 kg ha^{-1}), EI.T3 (mancozeb -2.5: 4728.4 kg ha^{-1}), EI.T14 (bixafen + prothioconazole + trifloxystrobin + mancozeb: 4684.3 kg ha^{-1}), EI.7 (trifloxystrobin + prothioconazole-0.4: 4592.4 kg ha^{-1}), EI.T13 (propiconazole + diphenoconazole: 4540.5 kg ha^{-1}) and T20 (copper oxychloride + mancozeb + diphenoconazole: 4526.3 5 kg ha^{-1}).

In Assay III, the treatment EIII.T5 (bixafen + prothioconazole + trifloxystrobin + mancozeb) showed higher productivity, and when subtracted the application treatment cost remained with a higher net yield (4773.9 kg ha^{-1}), followed by the treatment EIII.T2 (trifloxystrobin + prothioconazole-0.4), with 4480.6 kg ha^{-1}.

The economic response under the application treatment is complex and takes into account many factors that may vary over the crop seasons, among these factors we can highlight the pathogen population constitution, which may present individuals with resistance to different chemical groups thus directly affecting control and consequently crop yield. Even considering the pathogen, environmental conditions interfere for a year with more or less pressure from the disease. Other factors like fungicide price and counting of soybean sales are also relevant factors that interfere in the final response.

This is in line with what was observed in a set of experiments with fungicides in producer fields in Texas (USA). From the fungicide treatments used, the application treatment with prothioconazole plus trifloxystrobin considering two applications (R3 + R5) was the only treatment resulting on significant yield increases between 14 and 23%. However, the amount increase over untreated control was observed only in two of the eight experiments (Grichar 2013, Molina et al. 2019).

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