Meta-analysis of fungicide efficacy on soybean target spot and cost–benefit assessment


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Target spot of soybean has spread in Brazil, the southeastern United States and Argentina in the last decade. A collaborative network of field Uniform Fungicide Trials (UFT) in Brazil was created in 2011 to study the target spot control efficacy of fungicides, including azoxystrobin + benzoindiflupyr (AZ_BF), carbendazim (CZM), fluxapyroxad + pyraclostrobin (FLUX_PYRA), epoxiconazole + FLUX_PYRA (EPO_FLUX_PYRA), mancozeb (MZB) and prothioconazole + trifloxystrobin (PROT_TRIF). Network meta-analysis was used to conduct a quantitative synthesis of UFT data collected from 2012 to 2016 and to evaluate the effects of disease pressure (DP, low ≤35% target spot severity in the nontreated control < high) and year of experiment on the overall mean efficacy and yield response to each of the tested fungicides. Based on mean percentage control of target spot severity, the tested fungicides fall into three efficacy groups (EG): high EG, FLUX_PYRA (76.2% control relative to the nontreated control) and EPO_FLUX_PYRA (75.7% control); intermediate EG, PROT_TRIF (66.5% control) and low EG, MZB (49.6% control), AZ_BF (46.7% control) and CZM (32.4% control). DP had a significant effect on yield response. At DPLow, the highest response was due to PROT_TRIF (+342 kg ha⁻¹, +12.8%) and EPO_FLUX_PYRA (+295.5 kg ha⁻¹, +11.2%), whereas at DPHigh, EPO_FLUX_PYRA and FLUX_PYRA outperformed the other treatments, with yield responses of 503 kg ha⁻¹ (+20.2%) and 469 kg ha⁻¹ (+19.1%), respectively. The probability of a positive return on fungicide investment ranged from 0.26 to 0.56 at DPHigh and from 0.34 to 0.66 at DPLow.

Keywords: Corynespora cassiicola, Glycine max, network meta-analysis, profitability, yield

Introduction

Target spot, caused by Corynespora cassiicola, was first reported on soybean (Glycine max) in the United States in 1945 (Olive et al., 1945), and can now be found in most soybean-growing countries. Since its first report in 1976 in Brazil (Almeida et al., 1976), target spot has been considered a disease of limited importance. However, due to the widespread adoption of no-till cultivation practices, sowing of susceptible cultivars and a decreased sensitivity of the pathogen to commonly used fungicides (Xavier et al., 2013), this disease has now established itself as endemic in Brazilian soybean-growing regions, Paraguay, Bolivia and northern Argentina (Ploper et al., 2013).

The reddish-brown leaf lesions, initially observed in the lower to middle part of the canopy, are round to irregular, varying from specks to mature spots of 1 cm or more in diameter (Snow & Berggren, 1989). A yellow halo commonly surrounds the lesions, which often develops concentric rings at maturity (hence the name target spot). Symptoms may develop during all growth stages and susceptible cultivars can suffer intense defoliation. For instance, defoliation of up to 50% was observed in 2016 in mid-southern states (Arkansas, Mississippi) of the USA, coinciding with wet weather conditions (AgWeb, 2017).

Significant yield losses in soybean due to target spot often occur in years with higher than normal rainfall or extended periods of rainfall during certain critical growth stages. Under such conditions, fungicide sprays...
may be adopted to minimize yield losses. In addition, Edwards Molina et al. (authors’ unpublished data) observed that some soybean cultivars were tolerant to target spot, making this a sustainable option for managing target spot. However, tolerance also could represent a source of variability in yield response to fungicides used to manage the disease.

Since 2011, a considerable amount of resources has been dedicated by public and private Brazilian research institutes to the study of fungicide efficacy against target spot. Through a collaborative network of field Uniform Fungicide Trials (UFTs), the control efficacy of current labelled and prelabel fungicides against target spot and the impact of target spot on yield have been evaluated annually in the soybean-growing regions of Brazil (Godoy et al., 2012, 2013, 2014, 2015, 2016; links to web file locations are listed in Text S1).

However, in most published individual studies on fungicide efficacy and yield response, only means and statistical significance of treatment effects are reported. A significant yield response may not provide enough information to guide farmers’ decision-making regarding pest management. In order to maximize growers’ profit, while minimizing unnecessary, wasteful and environmentally damaging fungicide sprays, technical reports should also include cost–benefit analyses. One approach would be to provide estimates of the probability of a profitable return on a fungicide investment. This could aid growers’ decision to spray or not, or even to select the most suitable fungicide for each particular situation, as reported by Paul et al. (2011) and Machado et al. (2017).

Every year, technical reports are published summarizing the results from target spot UFTs. However, these types of summaries do not model or quantify the effects of study-specific factors (moderator variables) on the control efficacy or yield response to the tested fungicides. Meta-analysis provides a suitable alternative for integrating and interpreting results from multiple individual studies (Madden et al., 2016). Through meta-analysis, the magnitude and significance of treatment effects in terms of disease control and yield response can be estimated, along with the effects of moderator variables on efficacy.

The objective of this study was to conduct a quantitative synthesis of target spot UFT data to: (i) determine target spot control efficiency and yield response to several fungicides evaluated in the main soybean-growing regions of Brazil; (ii) identify factors affecting the efficacy of the tested products; and (iii) estimate the probability of economic benefit for applying a fungicide under a wide range of grain price–application cost scenarios.

### Materials and methods

#### Uniform fungicide trials

Data from a total of 56 UFTs carried out during five growing seasons (from 2012 to 2016, years of harvest) across six Brazilian states (Bahia, BA; Paraná, PR; Mato Grosso do Sul, MS; Mato Grosso, MT; Goiás, GO; and Tocantins, TO) were used for the present analysis (Fig. 1). Trials were established using soybean cultivars that were known to be susceptible to C. cassiciola, and all UFTs were managed following typical commercial production practices. Four of the 23 cultivars planted over the 5 years were used in at least five trials: BMX Potência RR, M9144RR, TMG803 and NA5909 RG.

Treatments consisted of three or four applications of labelled fungicides for soybean diseases by the Ministry of Agriculture, Livestock, and Food Supply in Brazil (Agrofit, 2017) (Table 1). The fungicides included in this investigation were evaluated in at least 20 UFTs from 2012 to 2016. They belonged to the methyl benzimidazole carbamate (MBC), demethylation inhibitors (DMI), quinone-outside inhibitors (QoI), succinate dehydrogenase inhibitors (SDH) single-site mode of action groups and one multisite dithiocarbamate (Table 1). Over the 5 years, four different combinations of the six fungicides were evaluated, i.e. they were not tested simultaneously in all the studies, but in four different sets. A nontreated control was used as the control and reference against which all treatments were compared. A CO2-pressurized backpack sprayer, calibrated to deliver 150–200 L ha

#### Meta-analytic synthesis of fungicide effect on target spot and yield

In meta-analysis, an effect size is any statistic (means, ratio of means, difference between treatments and its control, etc.) that can be used to evaluate the overall effect of some treatment or the strength of a relationship between variables (Borenstein et al., 2010). When estimating an overall effect size, random-effects meta-analytical techniques handle the two sources of variability that are common in a multi-environment study by giving a weight to each experiment that is an inverse function of the within-study variance (the higher the variance, the lower the precision) and the between-study variance (inherent differences among trials) (Madden & Paul, 2011).

### Fungicide control efficiency

The natural log-transformed response ratio of target spot severity ($\ln L^{sev}$) was estimated for each fungicide treatment as a measure of efficacy (Paul et al., 2008). This is given by:

$$L^{sev} = \ln \left( \frac{\text{Sev}_{Trt}}{\text{Sev}_{Check}} \right) = \ln(\text{Sev}_{Trt}) - \ln(\text{Sev}_{Check})$$

where $\text{Sev}_{Trt}$ is the mean disease severity for a fungicide treatment and $\text{Sev}_{Check}$ is the mean disease severity for the nontreated control. The right hand side of the equation is the equivalent form of the log response ratio as the difference between log means is equal to the log ratio. For an easier interpretation of the results, $L^{sev}$ (estimated after fitting the meta-analytical
Figure 1 Brazilian states and locations of the Uniform Fungicide Trial sites. Numbered points inside each state (dark grey) correspond to a different experimental site detailed as follows (n ~ number of trials): Bahia (BA): 1-Luís Eduardo Magalhães (1), Goiás state (GO): 1-Brasília (1), 2-Planaltina (1), 3-Porangatu (4), 4-Rio Verde (7); Mato Grosso do Sul state (MS): 1-Campo Grande (1), 2-Chapadão do Sul (4), 3-Maracaju (3), 4-São Gabriel do Oeste (3); Mato Grosso state (MT): 1-Campo Novo do Parecis (1), 2-Campo Verde (5), 3-Decíliândia (4), 4-Lucas do Rio Verde (2), 5-Nova Mutum (1), 6-Nova Xavantina (2), 7-Pedra Preta (1), 8-Primavera do Leste (4), 9-Querência (1), 10-Sorriso (1); Paraná state (PR): 1-Londrina (1), 2-Campo Mourão (1), 3-Palmeira (3); Tocantins state (TO): 1-Porto Nacional (4). Refer to Table S1 for detailed field-specific information.

Table 1 Description of fungicide treatments included in the meta-analysis.

<table>
<thead>
<tr>
<th>Fungicide code</th>
<th>Sprays (L ha⁻¹)</th>
<th>Product dose (g a.i. ha⁻¹)</th>
<th>Active ingredient (a.i.)</th>
<th>US$ per doseᵇ</th>
<th>Trade name (company)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZ_BF</td>
<td>3</td>
<td>0.2</td>
<td>Azoxystrobin</td>
<td>Elatus (Syngenta)</td>
<td>32.8</td>
</tr>
<tr>
<td>CZM</td>
<td>3</td>
<td>1</td>
<td>Benzovindiflupyr</td>
<td>Carbendazim (Nortox)</td>
<td>5.6</td>
</tr>
<tr>
<td>FLUX_PYRA</td>
<td>3</td>
<td>0.35</td>
<td>Fluxapyroxad</td>
<td>Orkestra (BASF)</td>
<td>31.5</td>
</tr>
<tr>
<td>EPO_FLUX_PYRA</td>
<td>3</td>
<td>0.8</td>
<td>Epoxiconazole</td>
<td>Ativum (BASF)</td>
<td>31.2</td>
</tr>
<tr>
<td>MZB</td>
<td>4</td>
<td>1.5</td>
<td>Mancozeb</td>
<td>Unizeb Gold (UPL)</td>
<td>12.1</td>
</tr>
<tr>
<td>PROT_TRIF</td>
<td>3</td>
<td>0.4</td>
<td>Prothioconazole</td>
<td>Fox (Bayer)</td>
<td>31.3</td>
</tr>
</tbody>
</table>

ᵃQoI, quinone-outside inhibitors; SDHI, succinate dehydrogenase inhibitors; MBC, methyl benzimidazole carbamates; DMI, demethylation inhibitors. ᵇPrice in July 2017 for the state of Sao Paulo, Brazil.
model) were back-transformed to obtain estimates of percentage control efficiency (\%) (Paul et al., 2008), calculated as:

$$\hat{C} = (1 - \exp(L_{\text{lev}})) \times 100$$

(2)

By definition, a large negative $L_{\text{lev}}$ value corresponds to a large positive $\hat{C}$, i.e. higher efficacy. Only those trials in which mean target spot severity in the nontreated plots was higher than 15% were kept for assessing control efficacy.

**Yield response**

Two effect sizes were estimated as measures of yield response to the tested fungicides: the absolute difference in yield (D, estimated as the difference between the means for each fungicide treatment and the nontreated control) (Paul et al., 2010, 2011) and the relative yield response ($L_{\text{lid}}$). The latter was estimated by following the same form of Eqn 1, then $L_{\text{lid}}$ was back-transformed to obtain estimates of yield response as percentage (R, %) (Paul et al., 2008) as:

$$L_{\text{lid}} = \ln \left( \frac{\bar{Y}_{\text{lid}}}{\bar{Y}_{\text{Check}}} \right) = \ln(\bar{Y}_{\text{lid}}) - \ln(\bar{Y}_{\text{Check}})$$

where $\bar{Y}_{\text{lid}}$ is the mean soybean yield for a fungicide treatment and $\bar{Y}_{\text{Check}}$ is the mean yield for the nontreated control (Paul et al., 2008). Percentage yield response (R) was then estimated from $L_{\text{lid}}$ as:

$$R = (\exp(L_{\text{lid}}) - 1) \times 100$$

where $L_{\text{lid}}$ is the mean log response ratio for yield (estimated by meta-analysis) for a fungicide treatment. Trials with presence of soybean rust were removed for the analysis of yield response (three trials removed).

**Quantitative synthesis**

Multitreatment (or network) meta-analysis was used to estimate $L_{\text{lid}}$, $L_{\text{lev}}$ and D because the six fungicide treatments of interest were tested in different combinations in the different trials. In this situation, researchers often perform a separate univariate meta-analysis for each effect size of interest (i.e. for each pair of treatment means); however, by doing so, they ignore the correlation of effect size estimates within studies, which could lead to biased estimates (Paul et al., 2008; Madden & Paul, 2011). The multivariate meta-analytical approach also allows for comparisons between all pairs of treatments across trials. Two different forms of effect sizes can be estimated in network meta-analysis: the most frequent is the response vector is given by the vector

$$\hat{Y}_i \sim N(\mu, \Sigma + S_i)$$

where $Y_i$ is the vector of responses for the $i$th study, and was assumed to have a multivariate normal distribution, with a mean vector $\mu$ and a variance-covariance matrix $\Sigma + S_i$, where $\Sigma$ is a $7 \times 7$ between-study variance-covariance matrix (Higgins et al., 2012).

An unstructured $\Sigma$ matrix was used and the models were fitted to the data with a maximum-likelihood parameter. R metafor package (Viechtbauer, 2010) was used to fit all the meta-analytical models.

**Moderator variables**

The between-study variance ($\sigma^2$) reflects the heterogeneity of treatment effects across studies. There can be multiple causes of this heterogeneity, such as the diversity in the ways the studies were conducted and other characteristics of the studies (Borenstein et al., 2010). Study-specific factor effects can be accounted for by incorporating ‘moderator variables’ in the meta-analytical model (van Houwelingen et al., 2002). The effect of the moderator variable for the $i$th study on the response vector is given by the vector $\delta_i$, with seven rows (for the six treatments, plus the control). The model can now be rewritten as:

$$Y_i \sim N(\mu + \delta_i, \Sigma + S_i)$$

Here the expected log means are now a function of the moderator variable ($\mu + \delta$) and not just a constant vector.

For the purposes of this study, the effects of target spot pressure (DP), year of the trial, and cultivar were tested as moderator variables. For DP, target spot severity (TSs) in the nontreated control was used to classify trials into two groups based on severity thresholds of 20%, 25%, 30%, 35% or 40%. Trials were classified as low DP (DP$_{\text{low}} \leq$ threshold) or high DP (threshold < DP$_{\text{high}}$). Year was tested as a factor or continuous moderator variable to determine whether there was a trend of decreasing control efficacy over time. This could represent, for example, reduced sensitivity to the fungicide active ingredients in the C. cassicola population.

In an attempt to evaluate the effect of cultivar on yield response to the tested fungicides, the original data set was reduced by removing those trials with cultivars used fewer than five times and fungicides tested in fewer than 30 trials. This selection criterion resulted in cultivars BMX Potência RR, M9144RR, NAS909 RG, TMG803, and fungicides CZM, EPOFLICT_PYRA and PROT_TRIF being retained in the reduced data set. The network meta-analytical model was then refitted to the yield data with ‘cultivar’ as a categorical
moderating variable. Based on slopes from linear regression analyses of relationships between soybean yield and target spot severity, Edwards Molina et al. (authors’ unpublished data) classified BMX Potência RR as being highly tolerant to target spot, M9144RR as having low tolerance, and TMG803 as being of intermediate tolerance. NAS909 RG was not classified.

Economic analysis

Estimated yield responses and between-study variances ($D$, $\sigma^2$, respectively) from the meta-analysis were used to calculate the probability ($P$) of the expected yield response being high enough to offset a given fungicide application cost ($C$, product + operational costs, $\$ \text{ ha}^{-1}$) at a given soybean grain market price ($Sp$, $\$ \text{ t}^{-1}$). The lower the $C:Sp$ ratio, the more favourable it would be for growers to obtain a profit from spraying a fungicide at a fixed level of yield response, because a smaller yield gain would be needed to pay for the fungicide application. This probability was estimated as: $P = \Phi(D - \frac{C}{Sp})$, where $\Phi(*)$ is the cumulative standard-normal function and $\sigma$ is the estimated between-study standard deviation (Paul et al., 2011; Machado et al., 2017). For the current $C:Sp$ ratio, the operational costs were fixed at $8 \text{ ha}^{-1}$; fungicide costs were calculated as the average of three market prices paid for each product in the state of Sao Paulo, Brazil, in May 2017 (Table 1); and an exchange rate of BRL3.3 = $1 was used. The mean price paid for soybean for the period from 2012 to 2016 was $330 \text{ t}^{-1}$. Probability of breaking-even ($P$) was calculated for different $C:Sp$ combinations considering both reference prices ± 10%.

Results

Based on the nontreated plot, mean target spot severity ranged from 6.8% to 75%, with a median value of 29% (Fig. 2a). As expected, the median level of severity was lower in fungicide-treated plots than in the nontreated control: 10–20% for AZ_BF, CZM and MZB and lower than 10% for EPO_FLUX_PYRA, FLUX_PYRA and PROT_TRIF. Soybean yield in the nontreated control ranged from 1160 to 4252 kg ha$^{-1}$, with a median value of 3174 kg ha$^{-1}$ (Fig. 2b). The six fungicide treatments had higher yield median values than the nontreated control.

Fungicide efficacy against target spot

For all fungicides, $L^{sev}$ differed significantly from zero, based on the standard normal test from the meta-analysis ($P = 0.009$ for CZM and $P < 0.001$ for the other fungicides; Table 2). Estimated $L^{sev}$ values ranged from $-1.433$ to $-0.392$, corresponding to $C$ ranging from 76.2% (FLUX_PYRA) to 32.4% (CZM). Based on percentage control relative to the nontreated control ($C$), three groups of efficacy were determined: the most effective fungicides against target spot were FLUX_PYRA (76.2%) and EPO_FLUX_PYRA (75.7%); PROT_TRIF had intermediate control efficiency (66.5%); and the lowest levels of efficiency were observed for MZB (49.6%), AZ_BF (46.7%) and CZM (32.4%).

The tested moderator variables did not have a significant effect on fungicide control efficacy ($P > 0.05$). Treating the effects of disease pressure or year as categorical and continuous moderator variables, respectively, did not lead to a substantial reduction in the among-study variability (data not shown). Based on the Wald test statistic, lack of inconsistency was observed in the present network (a nonsignificant design-by-treatment interaction was found, $P > 0.05$).

Yield response

The coefficient of variation for mean yield was 23.9%, considering only the nontreated control means, and 20.7% when including all treatments ($n = 238$ entries). This is indicative of relatively low variability in baseline yield among trials, and suggests that $D$ (mean difference) would be informative as an effect size for quantifying the effect of fungicide treatments on yield (Madden & Paul, 2011). $D$ values ranged from $-294$ to 1024 kg ha$^{-1}$ with a mean value of 296.2 kg ha$^{-1}$. All the treatments had at least three $D$ values <0 (11–17% of all the entries, depending on the treatment; Fig. 3). The overall estimated $D$ was

![Figure 2](image-url)
Model fitted to data of selected studies from the Uniform Fungicide Trials conducted in the Brazilian soybean-growing region from 2012 to 2016.

A summary of the overall fungicide control efficiency (C) and relative yield response (Δ) (the latter described with the include DP) is shown in Figure 4. Correlation analysis and simple linear regression were fitted using the disease control efficacy and yield response estimates as the independent and dependent variables, respectively. For both DP classes, a significant positive correlation was observed between C and R at DP\textsubscript{Low} \([r = 0.81 (P = 0.049); R^2: 0.66]\) and DP\textsubscript{High} \([r = 0.98 (P = 0.001); R^2: 0.97]\), with a stronger relationship between yield response and disease control efficacy at DP\textsubscript{High} context than at DP\textsubscript{Low}.

Economic analysis

At a fixed grain price of $330 t\textsuperscript{-1} and operational costs of $8 ha\textsuperscript{-1}, the cost of spraying the fungicides (expressed in kg of soybean grains) was estimated to be 123 kg for CZM, 240 kg for MZB, 350 kg for FLUX\_PYRA, 350 kg for EPO\_FLUX\_PYRA, and 373 kg for AZ\_BF. CZM was the only fungicide for which the yield response was high enough to offset the application cost at both DP levels: +89 kg ha\textsuperscript{-1} at DP\textsubscript{Low} and +108 kg ha\textsuperscript{-1} at DP\textsubscript{High} (Fig. 5). On the other hand, the yield response to AZ\_BF was not sufficient to pay for this treatment at either DP level: −190 kg ha\textsuperscript{-1} at DP\textsubscript{Low} and −53 kg ha\textsuperscript{-1} at DP\textsubscript{High}. For the other fungicides, the estimated profits at DP\textsubscript{High} were 151, 120, 60 and 38 kg ha\textsuperscript{-1} for EPO\_FLUX\_PYRA, FLUX\_PYRA, MZB and PROT\_TRIF, respectively.

The probability of offsetting the fungicide investment (P) for the range of reference C\textsubscript{Sp} values ranged from 0.26 to 0.56 at DP\textsubscript{Low} and from 0.34 to 0.66 at DP\textsubscript{High}, across the tested fungicides (Fig. 6a,b). At DP\textsubscript{Low} and the highest C\textsubscript{Sp} (most pessimistic economic simulated situation, bottom right plot region) CZM had a P value of 0.52, whereas for the other fungicides, P values were less than or equal to 0.4, with the lowest value of 0.26 for AZ\_BF. For the most optimistic economic situation (lowest C\textsubscript{Sp}, top left plot region) P was higher than 0.5 for all the fungicides except for AZ\_BF (0.44; Fig. 6a). At

<table>
<thead>
<tr>
<th>Fungicide(^a)</th>
<th>Effect size(^b)</th>
<th>Control efficacy (%)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungicide (\alpha) &amp; (L^{\text{inv}}) &amp; SE &amp; 95% CI &amp; Z &amp; (P) &amp; C &amp; 95% CI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZ_BF &amp; 20 &amp; −0.628 &amp; 0.197 &amp; −0.98; −0.28 &amp; −3.50 &amp; &lt;0.001 &amp; 46.7 &amp; 24.2; 62.4</td>
<td></td>
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</tr>
<tr>
<td>CZM &amp; 35 &amp; −0.392 &amp; 0.151 &amp; −0.68; −0.09 &amp; −2.59 &amp; 0.009 &amp; 32.4 &amp; 9.1; 49.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPO_FLUX_PYRA &amp; 44 &amp; −1.416 &amp; 0.156 &amp; −1.72; −1.11 &amp; −9.09 &amp; &lt;0.001 &amp; 75.7 &amp; 67.1; 82.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLUX_PYRA &amp; 37 &amp; −1.433 &amp; 0.164 &amp; −1.75; −1.11 &amp; −8.76 &amp; &lt;0.001 &amp; 76.2 &amp; 67.1; 82.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZB &amp; 20 &amp; −0.684 &amp; 0.181 &amp; −1.04; −0.33 &amp; −3.78 &amp; &lt;0.001 &amp; 49.6 &amp; 28.1; 64.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROT_TRIF &amp; 44 &amp; −0.192 &amp; 0.149 &amp; −1.38; −0.79 &amp; −7.31 &amp; &lt;0.001 &amp; 66.5 &amp; 55.0; 75.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Active ingredients: AZ\_BF: azoxystrobin + benzoindiflupyr; CZM: carbendazim; EPO\_FLUX\_PYRA: epoxiconazole + fluxapyroxad + pyraclostrobin; FLUX\_PYRA: fluxapyroxad + pyraclostrobin; MZB: mancozeb; PROT\_TRIF: prothioconazole + trifloxystrobin.

\(^b\)Total number of studies used for each specific fungicide treatment and their respective control.

\(^c\)Mean log response ratio \((L^{\text{inv}})\) for the mean effect of each fungicide treatment on target spot severity relative to non-treated control; standard error (SE) of \(L^{\text{inv}}\) and 95% confidence interval (CI) containing \(L^{\text{inv}}\); Z (standard normal) statistic from the meta-analysis model. \(P\) = probability value (significance level).

\(^d\)Mean percentage control (\(\bar{C}\)) and 95% CI containing \(\bar{C}\).

significantly different from 0 for all the treatments, based on the standard normal test \((Z)\) from the meta-analysis \((P < 0.001)\). In other words, spraying the fungicides significantly increased the mean yield relative to nontreated plots. For the six fungicides, the estimated \(\bar{D}\) values ranged from 200 to 400 kg ha\textsuperscript{-1}: 365 kg ha\textsuperscript{-1} for EPO\_FLUX\_PYRA; 348 kg ha\textsuperscript{-1} for PROT\_TRIF; 330 kg ha\textsuperscript{-1} for FLUX\_PYRA; 267 kg ha\textsuperscript{-1} for AZ\_BF; and 209 kg ha\textsuperscript{-1} for CZM. Based on the Wald test statistic, lack of inconsistency was observed in the present network (a nonsignificant design-by-treatment interaction was found, \(P > 0.05\)).

Influence of moderator variables on yield response to fungicides

The inclusion of moderator variable ‘disease pressure’ (DP) with a target spot severity threshold of 35% (DP\textsubscript{Low} ≤ 35% TSs < DP\textsubscript{High}) was statistically significant for both \(\bar{D}\) \((P = 0.0252)\) and \(L^{\text{yield}}\) \((P = 0.037)\). The highest yield response at DP\textsubscript{Low} was observed with PROT\_TRIF \((342 \text{ kg ha}^{-1}, 12.8\%\) higher yield than the nontreated control) and EPO\_FLUX\_PYRA \((295.5 \text{ kg ha}^{-1}, 11.2\%)\) higher yield) (Fig. 4 for \(R\) values and Fig. 5 for \(D\) estimates; Table 3). AZ\_BF had the lowest estimated \(\bar{D}\) value: 182.6 kg ha\textsuperscript{-1} (7% yield increase). On the other hand, at DP\textsubscript{High}, the highest \(\bar{D}\) values were observed for EPO\_FLUX\_PYRA \((503.5 \text{ kg ha}^{-1})\) and FLUX\_PYRA \((469.5 \text{ kg ha}^{-1})\), corresponding to 20.2% and 19.1% higher yields, respectively, than the control. The two latter fungicides had significantly higher \(\bar{D}\) values in comparison to the same fungicides at DP\textsubscript{Low} \((P = 0.025\) and \(P = 0.011\)). For AZ\_BF, yield response was marginally significantly higher at DP\textsubscript{High} than at DP\textsubscript{Low} \((P = 0.052)\). Yield response of CZM, MZB or PROT\_TRIF was not affected by DP.

A summary of the overall fungicide control efficiency (\(\bar{C}\)) and relative yield response (\(\bar{R}\)) (the latter described with the
DP_{High} and the highest C:Sp (bottom right plot region) \( P \) ranged from 0.34 (AZ_BF) to 0.54 (CZM), and at the lowest C:Sp (top left plot region), EPO_FLUX_PYRA and FLUX_PYRA had the highest probability values, 0.66 and 0.64, respectively (Fig. 6b).

**Cultivar effect on yield response to fungicides**

The cultivar-by-fungicide interaction had a significant effect (\( P < 0.001 \)) on mean yield response (\( D \)) to fungicide treatments. For cultivars BMX Potencia RR, NA5909 RG and TMG803, the effect of fungicides on \( D \) was not statistically significant (\( P > 0.05 \); Fig. 7). On the other hand, for cultivar M9144RR, fungicide treatment did have a statistically significant effect on the yield response, with both PROT_TRIF (647 kg ha\(^{-1}\)) and EPO_FLUX_PYRA (573 kg ha\(^{-1}\)) outperforming CZM (248 kg ha\(^{-1}\)).

**Discussion**

Since the re-emergence of target spot as a disease of concern in major soybean-growing countries such as the
USA, Brazil and Argentina, the use of fungicides as an approach for minimizing yield losses due to defoliation has been the subject of debate. The present study synthesizes the results from a multi-environment fungicide network of fungicide trials (2012–2016 in six states) conducted in the Brazilian soybean-growing region, bringing new basic insights about the chemical management of target spot.

Considerable variability was detected in fungicide efficacy against target spot, with the tested fungicides ranging from very low efficacy for carbendazim to high efficacy for products containing fluxapyroxad (SDHI) and pyraclostrobin (QoI). For the latter group, control levels were higher than 75%. These results confirm previously reported findings from other studies in Brazil (Godoy et al., 2012, 2013, 2014, 2015) and other parts of the world (Ploper et al., 2013). Based on results from fungicide experiments performed in the state of Mato Grosso, Brazil, Belufi et al. (2015) reported that the greatest control efficiency against target spot was observed when plots were treated sequentially with mixtures of FLUX_PYRA and PROT_TRIF. They used a similar set of four sequential sprayings to those tested in the present study, which reduced the area under the target spot progress curve by 75%.

Also in Brazil, two applications of FLUX_PYRA (at R1 and 15 days after R1) reduced target spot severity by 47% and defoliation by 22% relative to the nontreated control, and increased yield by 13% (283 kg ha⁻¹) in the state of Tocantins (Ribeiro et al., 2017). However, in Mato Grosso, this fungicide treatment reduced target spot by 50% without a significant yield increase (Basso et al., 2015). In field experiments conducted in northern Argentina in 2016 under more subtropical-type conditions, EPO_FLUX_PYRA had the best performance in terms of yield response compared to several tested fungicides (De Lisi, 2016). However, in that particular study brown spot (Septoria glycines) was present together with
target spot. The nontreated control plots had a mean brown spot and target spot severity of 40% and 25%, respectively, which were reduced by 77% and 50%, respectively, with two applications of EPO_Flux_Pyra, the first at R3 followed by a second at R5.

The relatively low efficacy of CZM observed here could be attributed to the low fungitoxicity of the active ingredient itself or to the reported increased frequency of C. cassiicola isolates with resistance to this active ingredient in local populations of the pathogen (Teramoto et al., 2017; Xavier et al., 2013).

In field experiments conducted in Mississippi (USA) in 2016, several fungicide active ingredients did not reduce the severity of target spot relative to the nontreated control. The tested treatments consisted of either stand-alone strobilurin (QoI) or triazole (DMI) fungicides or a premix of the two (QoI + DMI), and in one case, a three-way premix of active ingredients (QoI + DMI + MBC – thiophanate-methyl), all applied at R4 (Allen, 2017). This reported lack of significant treatment effects could have been due to two primary factors: the use of ineffective active ingredients or the fact that applications were made relatively late (probably after canopy closing). Early applications (close to R1) similar to those made in the present study are not commonly recommended in the mid-southern states of the USA. This is largely because data from more than 12 years of fungicide trials showed that in the absence of disease, the greater yield benefit from using a fungicide occurred when applications were made between R3 and R4 (T. Allen, Mississippi State University, Stoneville, USA, personal communication).

However, because target spot is commonly first detected in the lower canopy, attention should be given to identifying and managing early disease onset, before the canopy closes. Effective target spot control will probably require an application schedule that is different from those commonly recommended for other foliar diseases such as brown spot and frogeye spot or leaf blight. However, further research would be needed to test this latter hypothesis to avoid unwarranted, ineffective and harmful fungicide applications.

Results from fungicide trials conducted in the state of Louisiana (USA) to control target spot of cotton, also caused by C. cassiicola, showed that compared to other treatments, the application of FLUX_Pyra resulted in the greatest reduction in the rate of disease development (Price, 2017). This indicated that the efficacy of this treatment is fairly consistent across pathosystems such as Phakopsora pachyrhizi–soybean (Asian soybean rust), the overall efficacy of fungicides against target spot in Brazil is considerably lower. For instance, for soybean rust, efficacy as high as 90–100% relative to unsprayed controls has been reported (Scher et al., 2009). These authors concluded that two well-timed applications are optimal for maintaining yield.

At relatively low disease pressure (target spot severity at R5–R6 ≤ 35%), the highest yield response was observed with the application of PROT_TRIF; however, the only fungicide that paid the application costs (based on the costs and prices considered here) was CZM, the
cheapest of the tested products. A similar trend was observed in a set of fungicide experiments in growers’ fields in Texas (USA) in the absence of disease pressure (Grichar, 2013). Of the fungicide programmes tested, the mixture PROT_TRIF (sprayed at R3 + R5) was the only treatment to result in significant yield increments of 23% and 14% in 2010 or 2011, relative to the nontreated control. However, net increase in dollars per hectare over the nontreated control was only observed in two out of eight experiments (Grichar, 2013). In another trial under low disease pressure, no changes in leaf area index, dry matter, respiration, transpiration, stomatal conductance, leaf temperature, number of pods or weight of 1000 seeds were observed in response to the application of fluxapyroxad alone or in combination with pyraclostrobin (Carrijo, 2014).

Very similar trends to those observed in the present study in terms of yield response to fungicides under low disease pressure were observed for other necrotrophic foliar diseases of soybean and other crops. For instance, based on trials conducted in Illinois, Bradley (2009) observed a mean yield difference of 200 kg ha\(^{-1}\) under low frogeye leaf spot (Cercospora sojina) pressure compared to 600 kg ha\(^{-1}\) at moderate to high disease pressure on susceptible varieties. Similarly, based on a quantitative synthesis of data from maize fungicide efficacy trials, Paul et al. (2011) concluded that unless the maize crop is at risk from fungal disease development, farmers would be advised against applying fungicide treatments to increase yield. They further concluded that fungicides were most economically beneficial when used in fields where conditions were optimal for fungal disease development.

As expected, the most efficacious fungicides in terms of disease control, EPO_FLUX_PYRA or FLUX_PYRA, also had the highest mean yield differences (503.5 and 469.5 kg ha\(^{-1}\), respectively [c. 19–20%]) relative to the nontreated control under high disease pressure (>35%). This disease severity is comparable to the 33% previously reported as the level of defoliation above which yield
reduction occurred (Begum & Eden, 1965). However, despite the high level of disease control efficacy of EPO_FLUX_PYRA and FLUX_PYRA, the probability of break-even was only 0.65 \( /C6 \) under high disease pressure at the highest soybean price:fungicide application cost scenario evaluated in this study. Conversely, despite its relatively lower disease control efficacy, carbendazim ended up being a profitable option under both high and low disease pressure for the same economic scenario. This was largely due to the low cost of this fungicide, which resulted in a yield gain of only 123 kg being sufficient to offset the cost of three applications.

There is a great need for more applied research to better understand how to manage soybean target spot; however, based on the findings here, some basic principles of target spot management can be established. One of the main factors than can influence the fungicide yield response is cultivar selection. For instance, results from a previous analysis of the data set used in this study showed that tolerance to target spot varied considerably among the tested cultivars (authors’ unpublished data). For M9144 RR, a cultivar with low tolerance to target spot, yield response was highly dependent on the use of an effective fungicide, without which yield loss may occur as a consequence of a reduction in healthy leaf area caused by the disease. On the other hand, for cultivars such as BMX Potência RR, with a high level of tolerance to target spot, chemical management of target spot appears to be unwarranted.

However, the fact that Brazilian soybean breeding programmes have traditionally focused on breeding for resistance to nematodes, such as the soybean cyst nematode \((Heterodera glycines)\), widespread across the midwestern region of the country, may have resulted in the development of cultivars with low tolerance and high susceptibility to \(C. cassiicola\) (and other important pathogens). Growers should avoid planting such cultivars in fields with a history of target spot or close to fields planted with an alternative host crop such as cotton (a common situation in the southern states of the USA).

The results from this study should be interpreted with caution because sequential applications of the same fungicide are not recommended as this may lead to the development of fungicide-resistant isolates in \(C. cassiicola\) populations. However, findings from this study may serve as a guide for estimating and comparing the range of disease control with the tested fungicides, their effect on yield, and the likelihood of a return on fungicide investment as a strategy for managing soybean target spot. Therefore, it would be of great relevance to conduct further experiments to test different fungicide programmes, such as different combinations of active ingredients, number of sprays and application timing. Reducing the number of sprays by one, from three (used in the UTFs) to two, would reduce application costs and thus increase the probability of recovering those costs. Priority should also be given to studying interactions between cultivar and fungicides, as well as meteorological conditions that are conducive to epidemics of target spot. Interestingly, in both field experiments conducted in 2016 in Mississippi (Allen, 2017) and in northern
Argentina (De Lisi, 2016), target spot occurred simultaneously with brown spot, suggesting that soybean necrotrophic foliar diseases often occur as a complex. As such, rarely should a single disease be the focus of a fungicide application program. This important aspect of soybean foliar disease epidemics may be taken into account by evaluating fungicide efficacy for the whole complex of diseases.

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s website.

Table S1. Summary description of the 56 uniform fungicide trials conducted from 2012 to 2016 (harvest year) in the six soybean-producing states of Brazil (BA = Bahia, GO = Goiás, MS = Mato Grosso do Sul, MT = Mato Grosso, PR = Paraná, TO = Tocantins, BA = Bahia). Target spot severity at R5–R6 means at the unsprayed treatment (TS_check) and maximum mean soybean yield (YLD_max).

Text S1. A summary of the Uniform Fungicide Network annual reports and web file links.