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Contribution of soybean seed treatment with Fluquinconazole to manage yield losses caused by Phakospora pachyrhizi using meta-analysis

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The reduction in efficiency of triazole and strobilurin fungicides sprayed on soybean crops has stimulated investigations into alternative ways of controlling Asian rust (Phakopsora pachyrhizi). Treating seeds with fluquinconazole may provide additional benefits in terms of disease management. Results obtained through research are inconclusive, however, with studies presenting both positive and negative outcomes. In cases such as these, meta-analysis is recommended to systematically summarize and quantify the effects of treatment. Data collection brought together 74 different results obtained between 2004 and 2016 on the efficiency of this product in seed treatment. Analysis demonstrated high heterogeneity, indicating variability between study results and therefore the random-effect model was used. This also enabled data to be analysed using moderator variables. Across the datasets, the soybean yield gain with fluquinconazole treated seed was 120.4 kg ha\(^{-1}\) with a 95% confidence varying from 66 to 174 kg ha\(^{-1}\). There was a 69.4% probability of an increase of 60 kg ha\(^{-1}\), a 53.3% probability of an increase of 120 kg ha\(^{-1}\), a 36.7% probability of an increase of 180 kg ha\(^{-1}\) and a 22.3% probability of an increase of 240 kg ha\(^{-1}\). Therefore, this is another tool that can contribute to the integrated management of Asian rust.

Key words: Systematic review, effect size, software R, Phakopsora pachyrhizi.

INTRODUCTION

Soybean (Glycine max (L.) Merrill) is affected by various diseases, including Asian rust, caused by Phakopsora pachyrhizi Syd. & P. Syd. considered obligate pathogen. In Brazil, this disease was first recorded in 2001 (Yorinori et al., 2004) and is the most devastating soybean disease in tropical and subtropical regions (Kawuki et al., 2004) – in Brazil alone, it causes annual losses of approximately US$ 2 billion (Godoy et al., 2015). Chemical control is the

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main method used, and the triazole and strobilurin fungicides groups, whether used alone or mixed together were efficient in the control of this disease (Godoy and Canteri, 2004).

Changes observed in the behaviour of this fungus and the lower efficiency of triazoles (Godoy, 2012) and a significant reduction in strobilurins efficacy (Godoy, 2011; Barbosa et al., 2013; Reis et al., 2015) has stimulated reviews and the testing of alternative methods of control in recent harvests. Seed treatment (ST) with fungicides was then tested as a way of managing P. Pachyrhizzi in 2004 (Araujo et al., 2012). This type of practice aims not just to control pathogens in the seed, but also in the shoots, as they attack the crop during its initial stages of its development.

Fluquinconazole is a systemic fungicide in the triazole group. It was recommended in 2007 as treatment of soybean seeds and has been adopted in different soybean producing regions across the country (Tecnologias, 2013). However, research results such as those presented by Godoy and Henning (2008) question the efficacy of this method in controlling disease and subsequently increasing yields. Salam et al. (2013) cited that individual experiments provided vital information regarding the specific experimental conditions and do not provide information relating to the general response. Some statistical techniques can provide a more detailed analysis of the effects of ST and its influence on disease severity and yield, and one of these is meta-analysis.

The technique involves the statistical synthesis of results from a series of independent studies (Borenstein et al., 2009) and the objective according to Viechtbauer (2010) is to add together and contrast results from various related studies. Ramalho (2005) summarizes meta-analysis as an observational study of evidence based on statistical methods. This increases statistical power and the ability to examine the variability between studies, which is not possible by simply calculating the average of the published results (Fagard et al., 1996).

The results provide a general quantitative conclusion that a single experiment cannot provide (Adams et al., 1997).

Therefore, the aim of this study is elucidate the effect of ST with fluquinconazole to control Asian rust and subsequent yield losses using meta-analysis.

MATERIALS AND METHODS

Data collection

Initially, a systematic review of the Brazilian literature was carried out and data was also obtained from other collaborators to identify studies evaluating the treatment of soybean seeds with fluquinconazole in order to control Asian rust and resultant yield responses. The studies selected to make up the database met the following criteria: they were carried out in Brazil between 2004 and 2016 and treated seeds with fluquinconazole to control Asian rust; they were quantitative studies set up with a completely randomized design (CRD) or a randomized block design (RBD); they evaluated yield as a variable in response to seed treatment control of Asian rust; and they studied yield effect size and included a measure of statistical dispersion, such as the coefficient of variation (CV) or mean squared error (MSE).

Distribution and frequency of the data

The use of meta-analysis is based on the basic statistical assumption that data should present normality and independence between combined estimates (Hedges and Olkin, 1985). The normality was analysed using the Shapiro-Wilk test (Shapiro and Wilk, 1965), adopting a 5% level of significance. Outliers may be excluded where necessary to achieve normality of data through analysis of the residuals in a simple linear regression (Rodrigues and Ziegelmann, 2010). In this study, box-plot graphs, data distribution, data frequency distribution and a Forest plot were created in order to visualize the behaviour of the treatments and the standard error for each parameter.

Effect size

Effect size or productivity response, called D (kg ha⁻¹), was estimated for the difference between the group with ST with fluquinconazole (treatment) and the group without ST (control). The value of D gives an indication of the effect that treating seeds has on grain yield and as such is suitable for the purposes of this investigation (Madden and Paul, 2011). As part of the meta-analysis, each study was given an initial weighting that was inversely proportional to the sample variance (within each study), calculated as follows:

\[ S_i^2 = \frac{2 \times V}{r} \]  

where \( S_i^2 \) refers to the study, \( r \) is the number of repetitions in the study, \( V \) is the mean squared error (total variance given by the residual mean square – RMS) of the analysis of variance (ANOVA). RMS was deduced by transforming the coefficient of variation (CV), using the methodology proposed by Ngugi, Eser and Scherm (2010).

\[ \text{RMS} = \frac{(CV \times \bar{y})}{100} \]  

\( \bar{y} \) is the treatment mean.

Heterogeneity

To evaluate heterogeneity, the Q statistic test was used which estimated using the method of moments. This was calculated from the deviation of each effect size from the average, squaring this value and weighting it according to the inverse of the variance of the relevant study. The sum of the values for all of the studies is the weighted sum of the squared deviations, or Q:

\[ Q = \sum \left[ \frac{W_i (Y_i - \delta)^2}{r} \right] \]  

where \( W_i \) is the inverse of the variance for the effect size for the \( j \)th study, \( Y_i \) is the effect size for each study and \( k \) is the number of studies. The Q test indicates the total heterogeneity of the effect sizes, but this is not effective when a small number of studies is used. This led to Higgins and Thompson proposing \( H^2 \) and \( I^2 \) indices to measure the extent of the real heterogeneity. The \( H^2 \) index is calculated as follows:

\[ H^2 = \frac{Q}{K - 1} \]
The I² index (Higgins and Thompson, 2002) measures to what extent the variance proportion is true, or what proportion of the dispersion is a result of the heterogeneity. The inconsistency of the studies included in the meta-analysis is evaluated using the I² index. The I² index is calculated as follows:

\[
I^2 = \frac{Q - df'}{Q} \times 100
\]

where \(df'\) is degree of freedom. In addition to the indices provided by the heterogeneity between the treatments, the p-value was analysed in order to reject the null hypothesis, meaning that if p<0.05, the hypothesis should be rejected.

### Random-effect model

The random-effect model takes into consideration not just the sampling error, but also the variability between studies (Borenstein et al., 2009; Madden and Paul, 2011). Using the random-effect model, weighting factors for the effect sizes (ESs) are used to consider the variability between studies, as well as the sampling error (Viechtbauer, 2007; Borenstein et al., 2009; Madden and Paul, 2011).

\[
Y_i = \mu + \zeta_i + \epsilon_i
\]

where \(Y_i\) is the effect size observed in study, \(\mu\) is the measurement of the meta-analysis, \(\zeta_i\) is the random effect of each study and \(\epsilon_i\) is the random error.

The data was introduced using R Statistical Software (R Development Core Team, 2011) and processed using the Metafor Package (Viechtbauer, 2010), using the RMA function, applying the fixed, random and mixed-effect methods and using a restricted maximum likelihood (REML) estimator. The REML estimator is recommended by Viechtbauer (2010) for conducting meta-analyses with a low number of studies due to its sound statistical properties.

### Subgroups

The studies focused on the control of Asian rust (\(P. pachyrhizi\)) using ST and demonstrated the effect of this control through yield. However, many of these studies included spraying fungicides onto the shoots of the plants after ST. These treatments were applied during one, two or three different time periods and were considered to be subgroups for the purposes of the meta-analysis. Another subgroup was obtained by separating the studies into two groups: onset of epidemic during the vegetative period and onset of epidemic during the reproductive stage.

In order to study these subgroups, the mixed-effect model was used on the following moderator variables: number of applications (0, 1, 2 and 3) and time of onset of the disease (vegetative period and reproductive period). Analysis of moderator variables was carried out using the I index, tau, tau² and the p-value.

### Performance of the analysis

To perform the meta-analysis, the R program used the effect size (D) and the previously calculated estimated standard error and residual standard error, or standard error of regression, \(S_{D}(D)\). From these results, the total variance and the variance between and within the studies were presented as tau².

### Probability of occurrence

The effect size (D) and the estimate for the variance between the studies (\(\sigma^2\)) were used to estimate the probability of achieving preset levels of return per effect size, in this case for yield. The pre-established levels chosen were 60, 120, 180 and 240 kg/ha. The probability was estimated as follows:

\[
p = \Phi((C - D)/\hat{\sigma})
\]

where \(\Phi\) is the standard normal cumulative function and \(\hat{\sigma}\) is the estimated standard deviation between the studies. These estimates allow the probability of economic return as a result of ST to be calculated. This probability is expressed in terms of risk probability (the risk of not increasing yield through the use of fluquinconazole).

### RESULTS AND DISCUSSION

#### Data collection

The selection of study data is key to obtaining reliable results from meta-analyses. Borenstein et al. (2009), Rodrigues and Ziegelmann (2010) and Madden and Paul (2011) reported that data selection criteria must be precise so that results that truly reflect the real behaviour of the evaluated effect can be obtained. Twenty studies were selected originally and twelve of these were eventually used. Exclusion criteria included no measure of dispersion, no effect sizes, no control without ST and no treatments using fluquinconazole.

Although some authors disagree with the use of more than one input per study, the papers of Ojiambo and Scherm (2006), Paul et al. (2006, 2008), Ojiambo et al. (2010) and Calvo et al. (2016) used more than one input per study, and thus, that criteria was adopted.

### Pre-analysis

Before obtaining the statistical inferences, the data was visualized using an effect size histogram (Figure 1). The data was concentrated above the zero axis, suggesting that treatment with fluquinconazole has a positive yield effect.

According to Madden and Paul (2011), histograms should not be used alone as they may be misleading due to the fact that they do not present the measures of precision or variation attributed to effect sizes. The authors also state that models that present a standard error for effect size are frequently used for meta-analyses. These have a confidence interval of 95% and are called forest plots. The forest plot for this study is presented in Figure 2 and also indicates that most of the data is concentrated above zero yield effect.

Another graph was created for this study to give an idea of the effect size. Figure 3 presents the effect sizes of all the studies before the meta-analysis was carried
Figure 1. Frequency distribution of the difference between means for seed treatment with fluquinconazole and an untreated control for Asian rust in soybean, taken from studies conducted between 2004 and 2016. Values above zero show a positive yield effect.

Figure 2. Mean yield difference between seed treatment of fluquinconazole and an untreated control, sorted from lowest to highest. Each bar represents the yield difference averaged across two to six replicates and the vertical lines extending from each bar represent the standard errors.

It was observed that despite fluquinconazole treatment having a larger median, it could not be statistically confirmed that ST increases yield. In other words, this type of graphical analysis was inconclusive.

Data normality

The Shapiro-Wilk test indicated the normality of the data used. According to Rodrigues and Ziegelmann (2010), data presenting discrepancies in meta-analysis can be excluded or disconsidered through residual analysis of regression. Analysis did not present any outliers, meaning data with standard error values larger or smaller than 3 and -3, therefore all 74 pieces of data were considered.

Evaluation of heterogeneity

Measuring heterogeneity is useful in order to isolate and identify the true variance of the studies effects and this
can be inferred using the Cochran’s Q test, which comprises the weighted sum of the squared deviations and reflects the total dispersion of the data. The Q value for the test was 161.48 (Table 1), and using the p-value to test the null hypothesis of homogeneity between effect sizes in the studies, the desired heterogeneity was verified for the dispersion of the study data (p<0.001).

Borenstein et al. (2009) report that the Q value is not a good measurement to use in studies. It represents the sum of the dispersion and is dependent on the number of degrees of freedom, as well as the fact that the p-value can be extremely significant in some cases. However, Higgins and Thompson (2002) propose indices such as H-squared ($H^2$), R-squared ($R^2$) and I-squared ($I^2$), which are not dependent on the number of studies considered, and these help measure the real extent of the heterogeneity. These indices are not associated with determining the existence of significant heterogeneity either. $H^2$ is the ratio between the total quantity of variability in the observed results and the sampling variability. $I^2$ estimates as a percentage how much of the total variability can be attributed to the heterogeneity between the effects. Higgins and Thompson (2002) suggest bands of 0-25, 25-50, 50-75 and 75-100% for low, moderate, average and high heterogeneity, respectively. The value of $I^2$ for this study was 77.87%, demonstrating high heterogeneity (Table 1). In addition, the hypothesis test presented p<0.001 (Table 1), demonstrating statistically that there is significant heterogeneity between the observed effect studies, meaning a better investigation into the cause of this is required. The observed effect may be causing by difference between ingredient active using on foliar sprays and number of foliar sprays.

In order to do this, moderator variables, or covariables, were defined in order to isolate groups of studies with similar characteristics, reducing the heterogeneity and increasing knowledge about this phenomenon. These results are explored further in item 3.5.

### Effect of seed treatment on effect size

The meta-analytic model considered the number of data pairs (treatment-test) in each study to estimate the precision of the effect size, defining its contribution to the

**Figure 3.** Distribution of yield effect size (kg ha$^{-1}$) for the data collected with systematic revision for ST with fluquinconazole (treatment) for the control of *P. pachyrhizi*, compared to yields for the control, without the use of ST with fluquinconazole.

**Table 1.** Heterogeneity measurements ($Q$, $\tau^2$, $I^2$, $H^2$) for the meta-analysis using a random effects model for the treatment of soybean seeds with fluquinconazole to control Asian rust.

<table>
<thead>
<tr>
<th>P value</th>
<th>Q</th>
<th>Hetergenity</th>
<th>$I^2$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.001</td>
<td>161.48</td>
<td>4.52</td>
<td>132.02</td>
</tr>
</tbody>
</table>
Table 2. Effect size summary statistics for the meta-analysis of seed treatment with fluquinconazole and an untreated control to combat Asian rust in soybean and subsequent soybean yield loss, taken from studies conducted between 2004 and 2016.

<table>
<thead>
<tr>
<th>Effect model</th>
<th>Study</th>
<th>K</th>
<th>Effect size (C%)</th>
<th>Confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Upper limit</td>
</tr>
<tr>
<td>Random</td>
<td>12</td>
<td>74</td>
<td>120.43</td>
<td>27.37</td>
<td>174.07</td>
</tr>
<tr>
<td>Fixed</td>
<td>12</td>
<td>74</td>
<td>67.44</td>
<td>7.84</td>
<td>82.81</td>
</tr>
</tbody>
</table>

K: Number of studies; SE: Standard deviation.

mean estimate (Borenstein et al., 2009). Therefore, two effect models can be considered, fixed and random. The fixed-effect model, which considers the variation between the studies to be the same and the differences observed to be caused by experimental error (variability within each study), is described by Rodrigues and Ziegelmann (2010). With the random-effect model, the real effect of the treatments can vary from study to study. It was assumed that the behaviour of the variable in question was different for different sampling environments (Borenstein et al. 2009). Therefore, despite not being considered equal, they are connected (Rodrigues and Ziegelmann, 2010). In this study, due to the different methodologies and additional data of the studies included, the random-effect model was considered more suitable. For this reason, variability between the studies and sampling variability were used as the weighting factor for the effect sizes (Viechtbauer, 2007; Borenstein et al., 2009; Madden and Paul, 2011).

After performing the meta-analysis (Table 2), the estimate reached using the random-effect model confirmed that there was a significant positive yield effect for ST (p<0.001), demonstrating an average increase of 120 kg ha⁻¹ with a confidence interval varying from 66 to 174 kg ha⁻¹. Note that these results are from all studies included in the meta-analysis, without considering conditions that could influence the effect of ST as the beginning of the epidemic, environmental conditions, number of foliar sprays, active ingredient.

Fluquinconazole and yield

This significant increase in yield (Table 3) differs from the results presented by Togni et al. (2007), Goulart et al. (2011), Pimenta et al. (2011), Araujo et al. (2012), and Goulart et al. (2015) who did not demonstrate a statistically significant difference in yield between treatments with and without fluquinconazole (p<0.05). On the other hand, the authors of these studies cited that ST with fluquinconazole delayed the spread of disease, compared to plants with untreated seeds, even with foliar applications to control the disease. Unfortunately, the present study was not able to use meta-analysis to measure disease severity, because only few papers presented such information.

Fluquinconazole and the reduction of the severity of Asian rust

Other studies, such as that by Goulart et al. (2011, 2015) described that the main advantage of ST is a lower incidence of the disease during the initial phase of an epidemic. Rezende and Juliatti (2010) also report that the use of fluquinconazole for ST slowed the onset of epidemics and the progress of Asian soybean rust, as well as the defoliation of the plants, although again without these results being significant in terms of yield. The delay in the onset and progress of the epidemic contributes to maintenance of the low population of *P. pachyrhizi*. The delay in the progress of the disease can also be affected by nutritional balance. Gaspar et al. (2015) demonstrated that the relationship between nutritional Ca, Mg and K influence the severity and area under the Asian rust progress curve. In a study conducted in a greenhouse, Furlan et al. (2005) observed that ST with fluquinconazole significantly reduced disease severity in treated plots up to 61 days. Togni et al. (2007) verified that ST significantly reduced the number of pustules up to 75 days after sowing; however, once again without any significant difference to yield.

General observations

The evidence presented by Goulart et al. (2015) indicate that ST with fluquinconazole has a positive effect to control the initial phases of this epidemic. However, due to the use of the traditional statistical method, in the majority of these cases it was not possible to prove that there was a significant difference between the treatments (p<0.05). One study published by Godoy and Henning (2008) aimed to be more comprehensive, testing ST for three different sowing periods in combinations with and without ST with fluquinconazole. The authors used a randomized block design with a factorial arrangement and did not observe any significant difference between the treatments. The onset of the disease was noted just after the R3 reproductive stage. This led to the
Table 3. Effect size summary statistics for meta-analysis of seed treatment with fluquinconazole and number of foliar applications for the control of Asian rust in soybean, and soybean yield, taken from studies conducted between 2004 and 2016.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>k</th>
<th>Mean</th>
<th>Se</th>
<th>Confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower limit</td>
<td>Upper limit</td>
</tr>
<tr>
<td>No spray</td>
<td>10</td>
<td>83.99</td>
<td>68.46</td>
<td>-50.19</td>
<td>218.16</td>
</tr>
<tr>
<td>1 spray</td>
<td>23</td>
<td>203.89</td>
<td>82.18</td>
<td>81.34</td>
<td>403.50</td>
</tr>
<tr>
<td>2 sprays</td>
<td>23</td>
<td>131.11</td>
<td>35.58</td>
<td>38.51</td>
<td>177.98</td>
</tr>
<tr>
<td>3 sprays</td>
<td>18</td>
<td>211.4</td>
<td>110.12</td>
<td>-4.12</td>
<td>224.35</td>
</tr>
</tbody>
</table>

K: Number of studies; SE: Standard deviation.

collection that ST was not effective in delaying the progression of the disease and did not increase yield. This conclusion was correct for the analysis used in that study, which considered only the mean tests and significant results (p<0.05). In order to be used in meta-analysis, studies must present mean data and the study variability, normally expressed as the coefficient of variation. Therefore, the study published by Godoy and Henning (2008) could not be in this study as it did not contain this data.

The use of meta-analysis increases statistical power and enables the real effect of seed treatment to be identified with greater precision. This method involves weighting the values according to the level of reliability of the study, meaning that studies with less statistical variability are given a bigger weighting.

Effect of subgroups on effect size

Due to the results obtained for heterogeneity, the subgroup model was used as recommended (Borenstein et al., 2009). This involves separating the original data into groups with similar effects. Meta-analysis using moderator variables, or covariables, enables the factors that have a significant influence on global effect size estimates to be identified (Paul, Lipps and Madden, 2006; Paul et al., 2010; Madden and Paul, 2011).

The moderator variable used was number of foliar applications, and this had four levels of effect (0, 1, 2 and 3 applications). It is important to emphasize that the effect measure was obtained from the difference between the control (without ST) and treatment (with ST). For example, for the sub-group with two leaf sprays the data inputs were given by the difference between “treatment with ST + two foliar sprays” minus “treatment with two foliar spray”. Thus, treatments that make up each input data have the same inoculum pressure characteristics, environmental conditions of disease development, genotype, fertilization, cultural practices. The only difference between them is the treatment of seeds with fluquinconazole.

Due to a lack of information in the studies, the moderator “onset of disease” was not used. Table 3 shows that the results for ST in three foliar applications and ST without foliar applications have higher p-values, giving them a significance level of just 10%.

The moderator variable presented a significant difference (p<0.01) for 1 and 2 applications, with effect size intervals for increased yield varying from 81.34 to 403.50 and 38.51 to 177.98 kg ha⁻¹ respectively. According to changes observed in the behaviour of the <i>P. pachyrhizi</i> and the lower efficiency of triazoles cited by Godoy (2012) and a significant reduction in strobilurins efficacy cited by Godoy (2011), Barbosa et al. (2013) and Reis et al. (2015) the integration of foliar spray and seed treatment may be benefits in management of soybean rust, through reducing the action of initial inoculum and delaying development, as presented by Goulart et al. (2011, 2015) using only fluquinconazole in TS was observed delay in the progression in severity and defoliation caused by rust.

Probability of occurrence

Analysis of the probability of occurrence, which enables growers to estimate the probability of return on their investment, indicated a probability of 82.4% of there being an increase in yield, using ST with fluquinconazole (Figure 4). The probability of an increase of 60 kg ha⁻¹ was 69.4% and the probability of an increase of 120 kg ha⁻¹ was 53.3%, indicating a positive relationship with the use of this treatment. As yield increase rises, the probability for the control without ST decreases by 36.7% for increases of 180 kg ha⁻¹ and by 22.3% for increases of 240 kg ha⁻¹.

Madden and Paul (2011) cite that even if the global effect size (D) indicates an advantage, this alone cannot reveal the probability of this occurring. Therefore, Figure 4 presents results that are important for growers, helping them to know what to expect from a determined treatment or crop management method and being able to use it for a future growth season. In addition, this probability analysis may also be used to estimate the risk of financial loss associated with increases in production.
costs.

Conclusion

The epidemic is influenced by many variables as amount of primary inoculum, genotypes, environmental conditions and agricultural practices. The metanalysis has the advantage to summarize over the average of tested conditions (Fagard et al., 1996). So, the papers analysed allow concluding that the treatment of seeds with fluquinconazole increased at 120.4 kg.ha\(^{-1}\) the soybean yield (\(P<0.001\)), with a confidence interval from 66 to 174 kg.ha\(^{-1}\)). Nevertheless, it would be interesting if there were more studies using ST with this active ingredient, thus the effect of other variables could be analysed. If there was more data entry it will be possible to measure the effect of ST with fluquinconazole on subgroups as: epidemics beginning during the vegetative stage compared to those starting during the reproductive stage, or considering others variables as genotypes, environmental conditions and agricultural practices.

Conflict of Interests

The authors have not declared any conflict of interests.

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