Meta-analytic model in the evaluation of yield increase with the use of fungicides to control *Sclerotinia sclerotiorum*

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ABSTRACT

Among the soybean diseases, the white mold caused by *Sclerotinia sclerotiorum* is considered one of the most important and can reduce the yield up to 60%. However, there are questions about efficiency of the chemical control of the disease applied on the shoot system. The meta-analysis allows statically significant conclusions for variables that, under the traditional experimentation, in individual tests, there were no level of significance. Thus the purpose of the work was to evaluate the connection between the use of chemical control of the white mold and the soybean yield. It was a systematic review of bibliographical studies through the CAPES website. The selection criteria were: National papers published between 2004 and 2012, containing the use of chemical control of white mold caused by *Sclerotinia sclerotiorum*. Data dispersion measurements and papers containing chemical control of the shoot system, active ingredient and dose. It was selected 42 papers of which 18 papers were selected following the criteria, totaling 126 entries. The statistical template was created with the Software R using the Metafor Package. On the results found, the meta-analysis measure presented a estimate grow of 396 kg.ha⁻¹ with the use of fungicides as chemical control of the white mold. The inferior and superior confidence interval varied from 341.8 to 451.9 kg.ha⁻¹, respectively. In conclusion, the chemical control of the white mold contributes positively to increase the soybean production.

Key words: Agro-informatics, yield, meta-analysis, systematic review, white mold.

INTRODUCTION

Diseases in soybean crops, such as the white rot, also known as white mold, are one of the main limiting factors, and can lead to losses of up to 60% of the soybean crops (Almeida et al., 2005). The fungus that causes the disease is the *Sclerotinia sclerotiorum* (Lib.) de Bary, considered one of the most destructive (Massola and Krugner 2011). Among the control measures, the use of fungicides can be applied both on the aerial part of the plant as well as during seeds treatment (Neergard 1979; Machado 1988; Zambolim 2005; Somda et al., 2008). However, the aggressiveness of the pathogen, associated with survival and proliferation mechanisms, requires the control to be associated with a set of other measures to be efficient.

Another important factor is the variation in the fungus behavior. Meyer et al. (2014) working with network trials in several Brazilian regions found inconsistent results, in some cases showed significant yield reduction (p < 0.05) and others not (p > 0.05).

The difference in results can be explained by the use of statistical techniques such as meta-analysis, which will contribute to a careful analysis, generating evidences, which under traditional experimentation and isolated trials would be impossible.

Meta-analysis is an analysis of results of several studies with the objective to synthetize evidences from different sources into a single observation (Madden and Paul 2011). It was developed originally by Smith and Glass (1977) in social sciences, based on previous statistical studies carried out by Fisher (1932), Yates and Cochran (1938) and Cochran (1954). The technique is considered standard in the medical area, and up to that time, few articles had been published on plant pathology, but a considerable progress may be expected in the next couple of years, since many plant pathologists are starting to use meta-analysis methodologies (Madden and Paul 2011).

By using meta-analysis, it is possible to provide a grouped summary of the test performance through different summarizing forms or combination methods that can be divided in methods with fixed effects and methods with randomized effects (Souza and Ribeiro 2009). Results obtained with meta-analysis can guide actions and help to define areas that need more research efforts, contributing to the sustainability of the agricultural chain. The aim of the present work was to quantify the effect of the chemical control of the white mold caused by the fungus *Sclerotinia sclerotiorum* on soybean crops yield, through meta-analysis.

MATERIAL AND METHODS

Systematic revision

The study adopted the methodology proposed by Borestein et al. (2009), and, after having defined the

objectives of the meta-analysis and the variable reaction, it conducted a systematic review of the literature, including published articles on the chemical control of *Sclerotinia sclerotiorum* in soybean. Studies were surveyed via CAPES portal of journals and network assays/tests, according to selection criteria.

Selection criteria

Selection criteria: Studies published in Brazilian journals between 2004 and 2012 on the use of fungicides on the aerial part to control white mold caused by *Sclerotinia sclerotiorum* and its effect on yield (Kg.ha⁻¹), active ingredient and dosage, plus data dispersion measurements such as the coefficient of variation (CV) and the residual mean square (RMS).

Data distribution and frequency

For meta-analysis studies, data must have a normal distribution. Thus, the Shapiro-Wilk (Shapiro and Wilk 1965) test was carried out at 5% of significance. To verify the treatments effect behavior visually, box-plot and frequency distribution graphs were developed, adopting the effect measurement (D).

Effect measurement (D)

The effect measurement was obtained from the yield difference (kg.ha⁻¹) between the treatment with the application of fungicides and the control without the application of fungicides. The measurement provides an informed summary of the chemical control on yield and, as such, evidences for the questions raised by this investigation, as suggested by Madden and Paul (2011).

Effect model

During a meta-analysis, the effect attributed to each study is given proportionally to the inverse of its sampling variance (variance within the study), calculated as:

 $Si^2 = 2 \times V/r$

Where Si^2 indicates the study variance, **r** is the number of replications within a study and **V** is the residual mean square (RMS). The RMS of the studies used for the calculation was inferred through the coefficient variation (CV) proposed by Ngugi et al. (2011).

RMS = [{*CV*/100} \bar{y}] = *V*, where \bar{y} indicates the treatment average effect.

Heterogeneity

Heterogeneity in the meta-analysis process was estimated by the H^2 and I^2 indexes. The I^2 index shows how much variance is attributed to heterogeneity among the studies. The H^2 , on the other hand, indicates how much the difference among studies such as location, application stadia, product and dosage used, influenced the effect attributed to the studies. Values above 2 can signal the need to consider the variables that caused this effect (co-variables or moderators) on the model.

Meta-analysis execution

The effect measurement considered was the difference between the treated and untreated (control) areas, using the methodology proposed by Paul et al. (2010). Data from the selected works were tabulated and tested for normality, generating a frequency graph to verify the effect behavior of the treatments. A meta-analysis was carried out by the software R (R Development Core Tem 2016) through the Metafor package (Viechtbauer 2010).

RESULTS AND DISCUSSION

Data showed normal distribution (p>0.05) by the Shapiro-wilk test. Histogram and frequency distribution graph, given by the yield difference between the control without the application and the treatment, showed that in most surveyed studies there was an increase in yield (Figures 1 e 2).

Meta-analytical measurement estimated an increase by 396 kg.ha⁻¹ at confidence intervals below and over 340.81 kg.ha⁻¹ and 451.93kg.ha⁻¹, respectively. According to Table 1, the analysis p-value was lower than 0.0001, i.e., results were significant at minus 1%.

The results obtained by this study corroborates with those of Tupich (2015) who through a meta-analysis study, presented a 77% probability of an increase in yield of any magnitude in plants treated with Fluazinam. In similar studies using metaanalysis, Paul et al. (2011) and Silva (2015), working with the application of fungicides on corn, also verified positive effects of the control. Fantin et al. (2016) showed contribution of seed treatment with fluquinconazole in management of Asian soybean disease using meta-analysis. The authors also mention the importance of meta-analysis studies. Dalla Lana et al. (2015), working with the severity correlation between soybean rust and yield through meta-analysis, reported on the negative linear relation between these two factors. They also comment on the possibility of using meta-analytical models

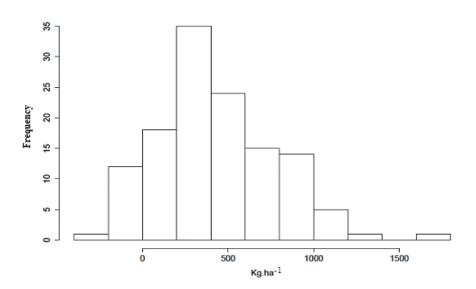


Figure 1. Yield distribution frequency data in kg.ha⁻¹ used for the meta-analysis of the fungicides effect on soybean white mold control; total of 126 entries with the difference between fungicide treatment yield and control without application.

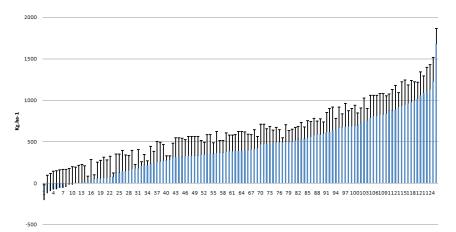


Figure 2. Forest Plot – Graph of the differences and standard errors for the analyzed trials. The bars show data dispersion, representing standard errors.

Table1. Variance, Standard error and limits for fixed and random effects, Higgins and Thompson index and Tau Squared calculated for the set of meta-analysis data from the white mold chemical control. K (number of entries analyzed), yield mean, SE (standard error), confidence interval superior and inferior limit of the confidence interval for the yield difference (kg ha⁻¹) data analysis estimate, P-value (level of significance of the study).

		Effect n	Effect measurement		Confidence Interval		
				Superior	Inferior		
Model	Studies	K	Mean	SE	limit	limit	P value
Random							
effects	18	126	396.37	28.34	451.93	340.81	< 0.0001
Fixed effects	18	126	305.55	17.73	340.31	270.78	< 0.0001

to provide yield losses estimates based on composed diseases severity assessments, considering the different disease latent and pressure situations.

Results on studies heterogeneity (Table 2) showed H^2 of 1.78. According to Madden and Paul (2009), these results indicate that the differences among works, either methodological, environmental or others, did not have any substantial influence on the global effect measurement. The I² presented by the test showed heterogeneity of 43.96%. According to the Higgins & Thompson index, heterogeneity was moderate, showing the importance of the criteria in selecting the works. According to the results, no co-variables study or effect moderators were realized.

-	Heterogeneity							
Model	P value	Q	H2	Tau	I2(%)			
Random effects Fixed effects	<0.0001 <0.0001	233.04	1.78	178.81	43.96			

Table 2. P value values for the Q test (heterogeneity among studies), H^2 (heterogeneity index proposed by Higgins and Thompson), Tau (variance among studies), I^2 (relationship between explained unexplained heterogeneity).

CONCLUSIONS

The application of fungicides for the control of white mold in soybean contributed positively to yield, increasing it by 396 kg.ha⁻¹ in treatments with the application of fungicides compared to treatments without the application, with a confidence interval below and over 340.8 kg.ha⁻¹ and 451.9 kg.ha⁻¹.

Besides this increase in yield, fungus control can also contribute positively to the reduction in number of formed sclerotia.

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REFERENCES

Almeida AMR, Ferreira LP, Yorinori JT, Silva JFV, Henning AA, Godoy CV, Costmilan LM and Meyer MC (2005) Doenças da Soja. In: Kimati H. Manual de Fitopatologia. Agronômica Ceres, São Paulo, p. 569-588.

Borenstein M, Hedges LV, Higgins JPT and Rothstein HR (2009) Introduction to Meta-analysis. Wiley and sons, United Kingdom, 421 p.

Cochran WG (1954) The combination of estimates from different experiments. Biometrics, 10: 101-129.

Dalla Lana F, Ziegelmann PK, Maia AHN, Godoy CV and Del Ponte EM (2015) Meta-analysis of the relationship between crop yield and soybean rust severity. Phytopathology 105: 307-315.

Fantin LH, Canteri MG, Silva ALda, Tupich FLB and Madden LV (2016) Contribution of soybean seed treatment with Fluquinconazole to manage yield losses caused byPhakopsora pachyrhizi using meta-analysis. 11:3880-3888.

Fisher RA (1932) Statistical Methods for Research Workers. Oliver & Boyd, London.

Machado JC (1988) Patologia de sementes: fundamentos e aplicações. ESAL/FAEPE, Lavras.

Madden LV and Paul PA (2009) Assessing heterogeneity in the relationship between wheat yield and Fusarium head blight intensity using random-coefficient mixed models. Phytopathology 99: 850-860.

Madden LV and Paul PA (2011) Meta-analysis for evidence synthesis in plant pathology: An overview. Phytopathology 101: 16-30.

Massola NS and Krugner TL (2011) Fungos Fitopatogênicos. In: Amorim L, Rezende JAM and Bergamim Filho A. Manual de Fitopatologia: princípios e conceitos. Agronômica Ceres, São Paulo: v.1, p. 149-206.

Meyer MC, Campos HD, Godoy CV and Utiamada CM (2014) Ensaios cooperativos de controle químico de mofo branco na cultura da soja: safra 2009 a 2012. Londrina: Embrapa Soja.

Neergaard P (1979) Seed pathology. The Macmillan, London.

Ngugi HK, Lehman BL and Madden LV (2011) Multiple treatment meta-analysis of products evaluated for control of fire blight in the eastern United States. Phytopathology, 101: 512-522.

Paul PA, Hershman DE, Mcmullen MP and Madden LV (2010) Meta-analysis of the effects of triazole-based fungicides on wheat yield and test weight as influenced by Fusarium head blight intensity. Phytopathology 100: 160-171.

Paul PA, Madden LV, Bradley CA, Robertson AE, Munkvold GP, Shaner G, Wise KA, Malvick DK, Allen TW, Grybauskas A, Vincelli P and Esker P (2011) Meta-analysis of yield response of hybrid field corn to foliar fungicides in the U.S. Corn Belt. Phytopathology 101:1122-1132.

R Development Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed 26 november 2016.

Shapiro SS and Wilk MB (1965) An analysis of variance test for normality (complete samples). Biometrika 52: 3-4. Silva AL (2015) Meta-análise do ganho em produtividade com aplicação de fungicidas foliares em milho no Brasil. Dissertação de Mestrado em Agronomia, Universidade Estadual de Londrina.

Smith ML and Glass GV (1977) Meta-analysis of psychotherapy outcome studies. American Psychologist 32: 752-760.

Souza MR and Ribeiro ALP (2009) Revisão sistemática e meta-análise de estudos de diagnóstico e prognóstico: um tutorial. Arquivos Brasileiros de Cardiologia 92: 229-238.

Somda I, Sanou J and Sanon P (2008). Seed-borne infectin of farmer-saved maize seeds by pathogenic fungi and therir transmission to seedlings. Plant Pathology Journal 7:98-103.

Tupich FLB (2015) Meta-análise do efeito de Fluazinam na produtividade influenciada pela intensidade de *Sclerotinia sclerotiorum* (Lib de Bary). Dissertação de Mestrado em Agronomia, Universidade Estadual de Londrina.

Viechtbauer W (2010). Conducting meta-analyses in R with the metafor package. Journal of Statistical Software 36(3):1-48. http://www.jstatsoft.org/v36/i03/ . Accessed 7 June 2015.

Yates F and Cochran WG (1938) The analysis of groups of experiments. Journal Agricultural Science 28: 556-580. Zambolim L (2005) Sementes: qualidade fitossanitária. UFV- DFP, Viçosa.

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