Short title: Meta-analysis of soybean white mold relationships

Meta-analytic modeling of the incidence-yield and incidence-sclerotial production relationships in soybean white mold epidemics

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White mold (*Sclerotinia sclerotiorum*) is a destructive disease of soybean worldwide. However, little is known on its impact on soybean production in Brazil. A meta-analytic approach was used to assess the relationship between disease incidence and soybean yield (35 trials) and between incidence and sclerotia production (29 trials) in experiments conducted in 14 locations across four seasons. Region, site elevation and season included as moderators in random-effects and random-coefficients models did not significantly explain the variability in the slopes of the incidence-yield relationship. The Pearson's *r* obtained from back-transforming the Fisher's *Z* estimated by an overall random-effects model showed that incidence of white mold was moderately and negatively correlated with yield ($r = -0.76$, $P < 0.0001$). A random-coefficients model estimated a slope of - 17.2 kg ha^{-1} %$^{-1}$, for a mean attainable yield of 3,455 kg ha^{-1}, indicating that a 10% increase in white mold incidence would result in a mean yield reduction of 172 kg ha^{-1}. White mold incidence and production of sclerotia were strongly and positively correlated ($r = 0.85$, $P < 0.0001$). For every 10 % increase in white mold incidence, 1 kg of sclerotia ha^{-1} was

produced. The relationship between disease incidence and production of sclerotia was stronger in southern regions and at higher elevation. In the absence of management, economic losses associated with white mold epidemics, assuming 43% incidence in 22% of soybean area, were estimated at approximately $1.47 billion USD annually within Brazil.

Keywords: *Glycine max*, *Sclerotinia sclerotiorum*, Sclerotinia stem rot, meta-analysis

Introduction

White mold is one the most damaging diseases of soybean worldwide (Grau & Hartman, 1999). In Brazil, the disease causes significant crop losses with highly prevalent and severe epidemics in production regions at high (> 600 m) elevations (Meyer *et al*., 2014). In the USA, annual economic loss estimates due to white mold increased from 10 to 560 million dollars between 1996 and 2009, and was considered the second most important disease of soybean (Peltier *et al*., 2012).

White mold is caused by *Sclerotinia sclerotiorum*, an ascomycete fungus with broad host range including crops such as beans, canola, sunflower, tomato, potato and cotton (Boland & Hall 1994). The fungus is able to survive as sclerotia, which are long-term survival structures with an outer black rind containing melanin (Bolton et al., 2006). Sclerotia are abundantly produced on diseased soybean tissue and can remain viable for up to eight years in soil (Adams & Ayers, 1979). Sclerotia can germinate myceliogenically and grow as vegetative hyphae and directly infect some plants such as stem rot in canola. Alternatively, sclerotia can germinate carpogenically resulting in the production of apothecia and liberation of ascospores. These spores can be dispersed via air currents

48 resulting in infection of blossom tissues, which are used as a nutrient source to enable
49 growth and infection of other green tissues (Abawi & Grogan 1979). Infected petals
50 deposited on leaves serve as inoculum sources for mycelia that can directly infect healthy
51 tissues. However, the disease typically has a monocyclic progression because of the rare or
52 minimal contribution of secondary inoculum (mycelia) to plant-to-plant spread (Grau &
53 Hartman 1999).

54 The intensity of white mold is usually quantified as incidence, or the proportion of
55 symptomatic plants. White mold intensity can also be quantified as severity, or the
56 proportion of tissue area affected by the disease (Madden et al., 2007). The latter is
57 commonly estimated using an ordinal rating scale (Hall & Phillips 1996), or a severity
58 index calculated based on the frequency of the ordinal scores (Kolkman & Kelly 2002). A
59 standard area diagram has also been developed to aid visual assessment of white mold
60 severity in soybean (Garcia & Juliatti 2012). Other disease-related variables such as the
61 number of sclerotia per seed weight and the number of apothecia per m^2 on the soil surface
62 have also been used as an indirect measure of white mold intensity (Huang *et al*., 2000;
63 Zeng *et al*., 2012). Nevertheless, incidence remains a less subjective and rapid method of
64 describing white mold intensity for field assessments with utility for comparing
65 management tactics, such as fungicide efficacy or evaluating resistance (Hoffman *et al*.,
66 1998; Yang *et al*., 1999; del Rio *et al*., 2007).

67 The empirical relationship between white mold incidence and yield has been described
68 for common bean (del Rio *et al*., 2004), soybean (Chun *et al*., 1987, Hoffman *et al*., 1998,
69 Yang *et al*., 1999, Danielson *et al*., 2004), and canola (del Rio *et al*., 2007). These studies
70 were conducted in the mid-western USA with markedly different microclimates, cropping
71 systems and soybean genotypes compared to Brazil. The analysis of the coefficients of the

72 linear regression models fitted to soybean field data shows relative reductions ranging from
73 4.6 to 8.4 % in attainable yield for each 10 % increase in white mold incidence across nine
74 independent experiments (Chun *et al*., 1987; Hoffmann *et al*., 1998; Yang *et al*., 1999). It
75 is not known whether these models have predictive value for soybean production in Brazil
76 where soybean is grown in subtropical and tropical climates and white mold is intensively
77 managed with fungicides. Soybean production in tropical regions is increasing annually,
78 accelerating the need to model crop-loss relationships in these environments to fully
79 understand the impact of white mold on actual yield.

80 In Brazil trials have been conducted since 2008 using a standardized research protocol
81 to evaluate fungicide efficacy against white mold across several years and regions. Results
82 of these studies have been summarized for each trial and published as summaries of
83 fungicide efficacy for white mold control in soybean (Meyer *et al*., 2014). Available
84 summary statistics at the trial level can be selected using defined criteria, to extract and
85 combined data to address specific questions using meta-analysis. The latter is a
86 quantitative method of combining and summarizing results of individual studies using a
87 statistically robust framework (Madden & Paul 2011; Scherm *et al*., 2014). There are
88 multiple examples across a range of pathosystems focusing on quantitative summaries of
89 treatments effects on disease reduction and increases in yield (Paul *et al*., 2011; Ojiambo *et*
90 *al*., 2010; Ngugi *et al*., 2011) and on relationships between disease and agronomic-related
91 variables such as yield, disease severity and mycotoxin concentration in other
92 pathosystems (Shah & Dillard 2006; Paul *et al*., 2005; Paul *et al*., 2006; Madden & Paul
93 2009; Dalla Lana *et al*., 2015). Traditionally, meta-analysis is usually preceded by a
94 systematic review of multiple literature sources (Shah & Dillard 2006; Scherm *et al*.,
95 2014). However, results may also be obtained directly from researchers or extracted from
96 summaries in non-peer reviewed literature and analyzed using data at the individual level

97 (plots) or aggregated (treatment means) (Paul *et al*., 2006; Dalla Lana *et al*., 2015).

98 In this study, we synthesized aggregated data (means across replicated plots) from
99 multiple field trials designed to evaluate fungicide efficacy for white mold control over
100 four years in Brazil (Meyer *et al*., 2014). The primary objective of this study was to
101 summarize and quantify the heterogeneity of the relationships between mean estimates of
102 white mold incidence and soybean yield or production of sclerotia. Quantitative knowledge
103 of the disease-yield relationship and contribution of inoculum to future growing seasons
104 can assist in crop loss assessment studies and in practical disease management decisions
105 for white mold (del Río *et al*., 2004).

106

107 **Materials and Methods**

108 *White mold incidence, soybean yield and sclerotia production*

109 Data on the estimated mean white mold incidence, soybean yield and weight of sclerotia
110 were obtained from the tables of published reports of 36 trials conducted in Brazil during
111 four growing seasons (2008-09 to 2011-12) (Meyer *et al*., 2014). The trials were conducted
112 in 14 municipalities located at some regions of six states where white mold was previously
113 recorded in years previous to the experiments (Fig. 1). Together, these states comprise
114 approximately 70 % of the soybean production in Brazil (CONAB 2016). Field-specific
115 information for the trials selected for this study are described in Table S1.

116 Trials were conducted following a standard protocol as described by Meyer *et al*.,
117 (2014). Briefly, fungicides were applied two to four times during the season using a
118 backpack sprayer pressurized by CO_2. The spray volume ranged from 200 to 300 L ha^{-1}.
119 The first fungicide application occurred between the R1 and R2 growth stages and

subsequent applications were made at approximately 10-day intervals. In some trials, a mixture of azoxystrobin and cyproconazole (60 + 24 g a. i. ha^{-1}) was used to control soybean rust across the entire trial area. In all trials, the experimental design was a randomized complete block with four replications, including a nontreated control. Each plot was 6 m long × 4 rows wide (12 m^2).

White mold incidence was quantified between the R5 and R6 growth stage as the percentage of diseased plants within the two central rows of each plot. Sclerotia produced during the white mold epidemics were collected and weighed after threshing all plants from a single plot and normalized to g ha^{-1}. Crop yield was calculated and expressed as kilograms per hectare at 13 % seed moisture content.

Establishment of exclusion criteria for studies

Data from each of the 36 studies constituted an independent study in the meta-analysis. The data were explored and one study was excluded because of a too narrow range (5 %) between the minimum and maximum incidence, which was considered insufficient to reliably quantify the relationship. Six studies did not provide information on sclerotial weight. Hence, data from 35 and 29 studies were analyzed for the study of the yield-incidence and sclerotia-incidence relationships, respectively.

Disease and yield variables

Three dependent variables were obtained from each study: white mold incidence (%), crop yield (kg ha^{-1}) and weight of sclerotia (g ha^{-1}), all available as the mean across four

142 replicated plots for each trial (Meyer *et al*., 2014). Two relationships, the yield-incidence
143 and sclerotia-incidence were explored and the estimated coefficients and statistics
144 constituted the effect-size within each study. Three effect-sizes per study were summarized
145 for each relationship, including the Fisher's Z (Z_r) transformation of the Pearson
146 correlation coefficient (r) that summarizes the strength of the relationship (Paul *et al*.,
147 2005; Dalla Lana *et al*., 2015). Briefly, Pearson's r was calculated for each study using the
148 *cor.test* function within R. The Fisher's Z_r was calculated from r and number of pairs of the
149 relationship (Paul *et al*., 2005) using the *escalc* function of the metafor package of R. The
150 two other effect-sizes tested were the intercept and slopes of the relationship estimated by
151 two different approaches as described below.

152

153 *Meta-analytic models: Correlation coefficients*

154 For the Fisher's Z_r and respective sampling variances calculated (Paul *et al*., 2005; Dalla
155 Lana *et al*., 2015), a standard univariate random-effect meta-analysis was performed using
156 the *rma* function of the *metafor* package of R with parameters estimated via maximum
157 likelihood. Overall means and 95 % confidence interval (95 % CI) and prediction intervals
158 (95 % PI) were calculated (Dalla Lana *et al*., 2015). Heterogeneity among the true effect-
159 sizes was evaluated based on significance of the Cochran Q test and the I^2 index that
160 measures the extent of heterogeneity of the true effect-sizes (Higgins & Thompson 2002).

161 The inclusion of a moderator variable that could account for at least part of the
162 heterogeneity in the true effects (Borenstein *et al*., 2009) as a fixed effect expanded the
163 model from a random to a mixed effects model (Madden & Paul 2009). Moderator
164 variables were mostly categorical e.g., region (north or south), altitude (< 985 m or ≥ 985
165 m), incidence class (< 40 % or ≥ 40 %) and yield class (< 2600 kg ha^{-1} or ≥ 2600 kg ha^{-1}).

166 The field trials conducted in locations situated below 20° south were classified as southern
167 and the remaining locations as northern trials. For altitude, incidence and yield classes,
168 categories were defined based on the median of the data. Year was tested as both a
169 continuous and categorical variable. In both the random-effects and mixed models, the
170 among-study variance was estimated using maximum likelihood (Viechtbauer, 2010) and
171 the mean effect was estimated using weights based on the among-study variance and
172 within-study variance, the latter being held fixed for each study (Borenstein *et al*., 2009;
173 Madden & Paul 2011). Wald-type tests and 95 % CIs were obtained using an assumption
174 of normality.

175

176 *Meta-analytic models: Regression coefficients*

177 The intercepts and slopes of the relationships were estimated and synthesized using two
178 approaches. In the first approach, the estimated intercepts and slopes, together with the
179 standard error of the parameter estimates from the linear regression models (the sampling
180 variances), were summarized using a two-stage process (Diggle *et al*., 2002). This
181 consisted of fitting of a linear regression model for each trial, then aggregating the data
182 using a multivariate meta-analytic model with corresponding random effects. Therefore,
183 the averages of the effect sizes were estimated assuming the true effect varied between
184 studies, and the studies were a random sample of the population. The distribution of the
185 linear coefficients estimated independently for each trial was summarized by the
186 calculation of the inter-decile (ID) range (90 to 10 %), or 80 % of the estimated intercepts
187 and slope values, as a robust measure of the spread of the parameter estimates (Madden &
188 Paul 2009). These analyses were run using *rma.mv* function of the *metafor* package of R
189 with parameters estimated via maximum likelihood.

190 In the second, and more complex, approach, a random coefficients model, a type of
191 mixed or multi-level model, was fitted to the data from all experiments (Madden & Paul
192 2009). This model estimated a population-average effect and provided study-specific
193 predictions of the intercept and slope coefficients following the procedures described
194 suitable for data with a relatively large number of subjects (studies) and small number of
195 observations for each study (Madden & Paul 2009; Leeuw & Kreft 1995). The *lmer*
196 function of the *lme4* package of R was used as an equivalent procedure to that used by
197 Madden & Paul (2009) based on SAS. This approach allowed focusing on direct overall
198 (i.e. averaged population results) and individual study results to characterize relationships
199 and explicitly account for the random effect of study.

200 *Relative yield loss estimation*

201 The scale of the estimated slopes was kg ha^{-1} per unitary increase in white mold incidence.
202 Since damage functions are commonly reported in relative terms (% increase in yield loss
203 or % yield reduction), and also for the purpose of comparison with other studies, the
204 percent yield loss was calculated by dividing the estimated slope (kg ha^{-1} $\%^{-1}$) with the
205 estimated intercept (kg ha^{-1}), both derived from the fit of random-effects model, and
206 multiplying by 100 (Madden & Paul 2009; Dalla Lana *et al*., 2015).

207

208 **Results**

209 *Study-level variables and relationships*

210 There was substantial variation in white mold incidence (15.4 to 90.3%), maximum
211 soybean yield (1,451 to 4,056 kg ha^{-1}) and sclerotial weights (461 to 9480 g ha^{-1}) in the
212 non-treated check treatments among the studies (Fig. 2, Table S1). The fit of the linear

213 regression at the study level demonstrated that, in general, yield decreased as white mold
214 incidence increased (Fig. 3C, Fig. S1). The gradients in white mold incidence were due to
215 variability in the efficacy of the fungicide treatments in reducing disease intensity. In some
216 cases (e.g. trials 5 and 32, Fig. S1), the most effective treatment (lower incidence) did not
217 result in the highest yield within the study. In other cases, fungicides with a similar level of
218 disease control led to variable yield (e.g. trials 13 and 27, Fig S1). The amount of sclerotia
219 produced generally increased with the increase in white mold incidence across all trials,
220 but the slopes of these relationships within individual trials were highly variable (15.3 to
221 406.9 k ha^{-1} %) (Fig. 3C, Table S3). There were always some sclerotia present within the
222 plots even in the treatments with lowest incidence, since the fungicides were not 100 %
223 effective. The maximum amount of sclerotia produced varied greatly across the trials. In
224 some cases (e.g. trials 12 and 32, Fig. S2), maximum sclerotial production did not occur in
225 the treatments with highest incidence within the study. In general there was a gradient in
226 sclerotial production with a few exceptions (e.g. trials 21 and 23, Fig. S2). In these cases,
227 the fungicides reduced the sclerotial production similarly and at lower levels than in the
228 non-treated check treatment (Fig. S2).

229 *Yield-incidence relationship: Correlation coefficients*

230 Pearson's correlation coefficients (r) varied from - 0.96 to 0.11 (Fig. 3A). However, the
231 null hypothesis of homogeneity in the estimated mean Fisher's Z ($\bar{Z}_r$) among studies was
232 rejected ($P = 0.0029$). The estimated $\bar{Z}_r$ by the random-effects model varied from – 0.95 to
233 0.11 (Fig. 3B) and the mean was - 1.01 (95 % CI = - 0.85 to - 1.16), corresponding to a
234 mean back-transformed correlation coefficient across all studies of - 0.76 (95 % CI = -0.36
235 to - 0.92). The between-study variability (τ^2) estimated using maximum likelihood was
236 high ($\tau^2 = 0.095$, SE = 0.0531) and confirmed by the significance of the Q test ($Q = 61.17$,

df = 34, p = 0.002), and high values of the I^2 (42.97 %) and H^2 (1.75) statistics. In the mixed-model, none of the categorical or continuous (year) moderator variables tested individually as fixed effects significantly affected $\bar{Z}_r$ ($P > 0.3$).

Yield-incidence relationship: Intercept and slopes

The random-coefficients model successfully fitted the data across the 35 studies. The estimated mean for the slope and intercept was highly similar between the two modeling approaches (*data not shown*). Estimates of the population-average intercept and slope were $\hat{\beta}_0$= 3,455.7 kg ha^{-1} (SE = 132.93) and $\hat{\beta}_1$= 17.24 kg ha^{-1} %$^{-1}$ (SE = 1.45), respectively. Moreover, both estimated parameters differed significantly from zero ($P < 0.001$). The estimates of the random effects variances of the intercept and slope were $\sigma^2_{\mu_0}$= 602,192.89 and $\sigma^2_{\mu_0}$= 37.60, respectively. The estimated covariance was $\hat{\sigma}_{\mu_0,\mu_1}$= - 1,477.49. The among-study correlation of the effects of study on the intercept and slope was - 0.31, estimated based on the between-study variance components. The estimated best linear unbiased predictions (EBLUPs) for the intercepts ($\hat{b}_0$) ranged from 1,821 to 4,888.9 kg ha^{-1} (Table S2), and the inter-decile (ID) range was 1,900 kg ha^{-1}. The ID was similar between estimates by the simple linear model and random-coefficients model, depicted by similar density curves (Fig. S3). The EBLUPs for the slopes ($\hat{b}_1$) ranged from 7.57 to 28.02 kg ha^{-1} %$^{-1}$ (Table S2), and the ID was 10.41. This range was smaller than for the slopes from fitting the simple linear model to the data, for which the distribution was wider than the EBLUPs (Fig. S2B). The observations and the fit of the linear model for 35 individual regressions are shown in Fig. 3C (individual plots in Fig S1). The study-specific prediction lines and population-average predictions of yield and respective 95 % CI are shown in Fig. 3D. The conditional (pseudo) R^2 of the entire model and the Akaike Information Criterion (AIC) was 0.94 and 5,319.29, respectively. Similar to Fisher's *Z*, none of the moderator

261 variables affected significantly the population-average predictions of yield. Based on the
262 overall mean (95 % CI) of the intercept and slope estimated by the random-coefficients
263 model, the overall relative damage coefficient was estimated to be 0.49 $\%^{-1}$ (0.45 to 0.54).

264

265 *Sclerotia-white mold incidence relationship: correlation coefficients*

266 The Pearson's correlation coefficients varied from 0.37 to 0.99 (Fig. 4A). The null
267 hypothesis of homogeneity in the estimated mean Fisher's Z ($\bar{Z}_r$) among studies was
268 rejected. The estimated $\bar{Z}_r$ by the random-effects model varied from 0.39 to 2.94 (Fig. 4B)
269 and the mean was 1.25 (95 % CI = 0.085 to 14.75). This value corresponded to a mean
270 back-transformed correlation coefficient across all studies of 0.85 (95% CI = 0.79 to 0.89,
271 95% PI = 0.58 to 0.95). The between-study variability (τ^2) estimated using maximum
272 likelihood was high (τ^2 = 0.0819, SE = 0.0549) confirmed by the significance of the Q test
273 (Q= 48.68, df = 28, P = 0.009), and high values of the I^2 (39.24%) and H^2 (1.65) statistics.
274 In the mixed-model, the inclusion of region, significantly affected $\bar{Z}_r$ (P < 0.003) and
275 accounted for 62 % of the heterogeneity. Stronger associations were estimated in trials
276 within the southern region (r_Z = 0.93, 95 % CI = 0.87 to 0.96, 95 % PI = 0.83 to 0.97)
277 compared to the trials in the northern region (r_Z = 0.81, 95 % CI = 0.75 to 0.86, 95 % PI =
278 0.64 to 0.90). Elevation was marginally significant (P = 0.06) and accounted for 26.98 %
279 of the heterogeneity of $\bar{Z}_r$. Stronger associations were found in fields at higher elevation (r_Z
280 = 0.88, 95 % CI = 0.82 to 0.92, 95 % PI = 0.70 to 0.95) than the alternative (r_Z = 0.80, 95
281 % CI = 0.70 to 0.86, 95 % PI = 0.51 to 0.92). Year, incidence and yield classifications did
282 not significantly affect $\bar{Z}_r$ (P > 0.5).

283

Sclerotia-white mold incidence relationship: Intercept and slopes

Estimates of the population-average intercept and slope were $\hat{\beta}_0$= 280.5 g ha^{-1} (SE = 163) and $\hat{\beta}_1$= 98.59 g ha^{-1} %$^{-1}$ (SE = 13.3), respectively, and both estimated parameters differed significantly from zero ($P < 0.001$). The estimates of the random effects variances of the intercept and slope were $\sigma^2_{\mu_0}$= 431,433 and $\sigma^2_{\mu_0}$= 4,201, respectively. The estimated covariance was $\hat{\sigma}_{\mu_0,\mu_1}$= 40,115. The among-study correlation of the effects of study on the intercept and slope was 0.94, estimated based on the between-study variance components. The EBLUPs for the intercepts ranged from - 498.7 to 2,312.8 g ha^{-1} (Table S3), and the ID was 1,420.5. The ID of the predictions for the intercepts by random-coefficients model was lower than the estimates at the study level with the simple linear model (ID = 1,793.5), but the density curves were similar (Fig. S4A). The EBLUPs for the slopes ranged from 18.4 to 307.8 g ha^{-1} %$^{-1}$ (Table S3), and the ID was 145.87, which was slightly smaller from fitting the simple linear model to the data (ID = 163.71) (Fig. S4B). Observations and the fit of linear model for the 29 individual linear regressions and the study-specific prediction lines are show in Fig. 4C (individual plots in Fig S2). The population-average predictions of yield and respective 95 % CI are presented in Fig. 4D. The conditional (pseudo) R^2 of the entire model and the AIC was 0.90 and 5,379, respectively. None of the moderator variables affected significantly the population-average intercept and slope.

Discussion

The strength and heterogeneity of relationships between white mold incidence and soybean yield or sclerotial production was summarized using multi-level and random-effects meta-analytic models, following approaches previously used to address related questions in plant

pathology (Madden & Paul 2009; Dalla Lana *et al*., 2015). To the best of our knowledge, this is the first description of these relationships for soybean grown in Brazil, and provides a useful approach to explore these relationships in other subtropical and tropical regions of soybean production. The wide range of microclimates where the trials were conducted spanning four seasons and several growing regions may be one factor contributing to the wide variation in mean white mold incidence, production of sclerotia and soybean yield in non-treated check plots across the locations.

As expected, while soybean yield tended to decrease, sclerotial production tended to be positively associated with white mold incidence. Similar negative relationships between yield and white mold incidence have been reported for soybean (Chun *et al*., 1987; Hoffman *et al*., 1998; Yang *et al*., 1999; Danielson *et al*., 2004), dry bean (del Rio *et al*., 2004) and canola (del Rio *et al*., 2007), using correlation and regression analyses. In soybean, Pearson's correlation coefficients reported for nine trials conducted in the USA varied from 0.67 to - 0.94, with a mean *r* of - 0.84 across three studies (Chun *et al*., 1987; Hoffman *et al*., 1998; Yang et al., 1999). This is higher than the mean estimated correlation coefficient obtained in the present study (r = - 0.76). Differences among studies are expected and may be due to field-specific conditions but also the approach used to generate variation in disease incidence gradients. In this study, disease gradients were generated using fungicides with various efficacy levels for white mold control. In these previous studies, disease gradients were obtained by three different approaches: i) selecting sub-areas within a larger plot representing variable white mold incidence (Hoffman *et al*., 1998); ii) using 16 or 20 cultivars with variable white mold susceptibility, and artificial inoculation of fields (Chun *et al*., 1987); iii) using 63 to 64 cultivars in three small plot, replicated trials relying upon natural soilborne inoculum (Yang *et al*., 1999). Hence, in addition to the differential effect of fungicides it is likely that the different cultivars used in

the Brazilian trials may have contributed to the variability associated with the incidence-yield relationships.

Results reported here corroborate previous reports claiming white mold as one of the most damaging diseases of soybean (Peltier *et al*., 2012). The mean estimate of potential maximum relative crop loss of 50 % is only slightly lower than similar estimations made for soybean rust (60 % crop loss) (Dalla Lana *et al*., 2015). For a mean attainable yield of 3,455 kg ha^{-1} (estimated intercept in this study), an average of 172.4 kg ha^{-1} (about to 3 bags ha^{-1} or 2.67 bushels $acre^{-1}$) would be lost for every 10 % increase in white mold incidence. In relative terms, this translates into a decrease of 4.9 % in attainable yield for each 10 % unitary increase in white mold incidence. This relative loss estimate is lower than findings reported in studies conducted in the mid-western USA, where the same relative loss (10 % incidence) across nine trials varied from 4.5 to 8.4 % and the mean estimated yield reduction was of 6.3 % (Chun *et al*., 1987; Hoffman *et al*., 1998; Yang *et al*., 1999). Although a range of edaphic factors adds stochasticity to direct comparisons, in general, white mold epidemics appear less damaging to soybean in Brazil compared to the mid-western production region of the USA.

None of the moderator variables tested (region, elevation, year, incidence and yield) significantly affected the strength and magnitude of the relationship between white mold incidence and yield. Other epidemiological factors that were unable to be qualified in our study that may be potentially affecting these relationships include primary inoculum densities, environmental conditions, ascosporic infection timing and disease severity (Grau & Hartman 1999). For the latter, the strength of the disease intensity – yield loss relationships may be increased because severity encapsulates the cumulative effect of epidemic progression in infected plants (Savary *et al*., 2006), such as the within-plant

spread of mycelia in white mold. Analogous observations have been as reported for similar diseases such as Fusarium head blight in wheat (Spolti *et al*., 2015), where secondary inoculum has a limited contribution to disease increase.

Studies on the relationships between white mold incidence and sclerotial production under field conditions are scarce. Studies conducted *in vitro* for evaluating isolate variability in sclerotial production (Li *et al*., 2008; Lehner *et al*., 2014) may be of limited value to extrapolate to field conditions. Hoffman *et al*., (1998) estimated the number of sclerotia per 300 g of seed across five soybean cultivars, but the disease incidence-sclerotial production relationship was not investigated. In our study, we found that sclerotial production was strongly associated with white mold incidence and there was high heterogeneity in the estimated slopes. Some potential contributors to the high variability in these relationships may be the variation across geographical locations and microclimatic conditions (19 locations across six states), and genetic differences of the *S. sclerotiorum* populations at these locations.

The mean estimated slope allowed for the determination that approximately 1 kg ha^{-1} of sclerotia were produced for every 10 % increase in white mold incidence. The estimated weight of an individual sclerotium produced under field conditions is unknown. However, studies conducted *in vitro* have reported average weights of a sclerotium ranging between 0.006 g (Irani *et al*., 2011) and 0.01 g (Kuang *et al*., 2011). Based on the mean estimated value in these studies (0.008 g each sclerotium), 10 % incidence of white mold may result in 125,000 sclerotia ha^{-1} or 12.5 sclerotia m^{-2}. One sclerotium may produce several apothecia (Bolton *et al*., 2006) and each apothecia may release up to 7.6×10^5 ascospores over 20 days (Clarkson *et al*., 2003). This data highlights the potential for substantial increases in inoculum densities following the introduction of white mold primary inoculum

into a field.

Two moderator variables, region and elevation, significantly explained the heterogeneity of the correlation coefficients for relationships between white mold incidence and sclerotia production. Stronger associations were found in trials conducted in the southern regions and at high elevation areas. It is difficult to explain this result, but it might be related to climatic conditions. The trials classified as Southern were those conducted in the Paraná State, Brazil. In this region, temperatures are typically milder than those in the northern region. It is likely that more favorable and uniform weather conditions favoring the disease might have occurred across the trials conducted in the Southern region and higher elevations. For the latter, the longer dew period is another factor that favor would disease development.

The results of present study may be useful for additional studies on risk assessments and economic analyses as a foundation for strategic and tactical disease management decisions. For example, considering a hypothetical scenario of 43 % white mold incidence (mean incidence in the non-treated plots across the trials), 651 kg ha^{-1} (or $217.00 USD assuming $ 20.00/60 kg USD) would be lost at harvest in the absence of fungicides with an expected production of 3,000 kg ha^{-1} (in the absence of white mold). In Brazil, white mold is estimated to occur on approximately 22 % of the total soybean area, or 6.8 million hectares (Meyer *et al*., 2015). In the above scenario, white mold could potentially result in an economic loss of $ 1.47 billion USD in soybean production in Brazil. To manage the disease, two applications of the fluazinam fungicide can effectively manage the disease leading to 73 % reduction of white mold incidence (Meyer *et al*., 2014). Therefore, a reduction from 43 % to 11.6 % in white mold incidence from fungicide application would still result in a reduction of 170.4 kg ha^{-1}, or $ 56.8 ha^{-1} USD. Thus, even when using the most effective fungicides currently available, $ 386.24 M USD would still be lost due to

white mold. When added this to the total fungicide cost of US$ 408 M (US$ 30.00 per application), a total economic loss of $ 794.24 USD would be expected, which is approximately 50 % of the total economic loss without fungicides. These results highlight the potential impact of white mold in Brazilian agriculture if not well managed and the need to combine other strategies such as host resistance, biological control and cultural practices that help avoid or reduce the damage caused by the disease.

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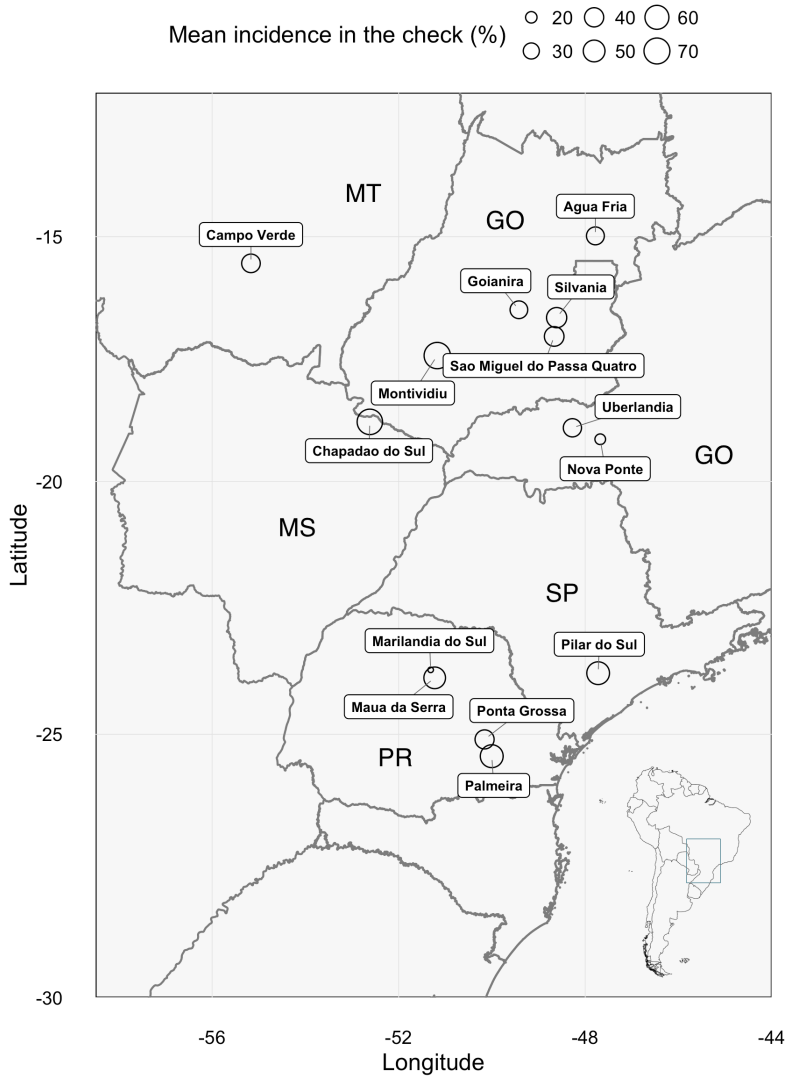
Figure 1 Location and names of the municipalities where 35 uniform fungicide trials were conducted and white mold incidence was recorded and related to sclerotia weight and soybean yield. The size of the circle is proportional to the mean incidence in the untreated check plot across trials and years. Refer to Table S1 for other field-specific information.

Figure 2 Histograms for the observations of white mold and soybean variables in 35 independent uniform trials conducted in Brazil from 2008-09 to 2011-12. White mold mean incidence in the check treatment of the study (A); Maximum mean soybean yield in one fungicide treatment of the study (B); Maximum mean *Sclerotinia sclerotiorum* sclerotia weight in one treatment of the study (C).

Figure 3 Statistics and coefficients from fitting models for the relationship between mean values of soybean yield and white mold incidence across four plots of various fungicide treatments, and a non-treated check, evaluated in 35 independent uniform trials in Brazil during four years. Frequency of the Pearson's correlation coefficient (A) and their respective Fisher's transformation of r (Z) (B); observations (dots) and study-specific prediction regression lines (solid gray) of a simple linear model fit (C) and study-specific prediction lines (grey solid) and population-average predictions (thick solid black) of yield and respective 95% confidence interval (thick dashed black) (D).

Figure 4 A. Statistics and coefficients from fitting models for the relationship between mean values of sclerotial weight and white mold incidence across four plots of various fungicide treatments, and a non-treated check, evaluated in 29 independent uniform trials in Brazil during four years. Frequency of the Pearson's correlation coefficient (A) and (B) their respective Fisher's transformation of r (Z) (B); observations (dots) and study-specific prediction regression lines (solid gray) of a simple linear model fit (C); and study-specific prediction lines (grey solid) and population-average predictions (thick solid black) of yield and respective 95% confidence interval (thick dashed black) (D).


Mean incidence in the check (%)
20
30
40
50
60
70
MT
GO
MS
SP
PR
GO
Campo Verde
Agua Fria
Goianira
Silvania
Sao Miguel do Passa Quatro
Montividiu
Uberlandia
Chapadao do Sul
Nova Ponte
Marilandia do Sul
Pilar do Sul
Maua da Serra
Ponta Grossa
Palmeira
-15
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Latitude
-56
-52
-48
-44
Longitude

Figure 1

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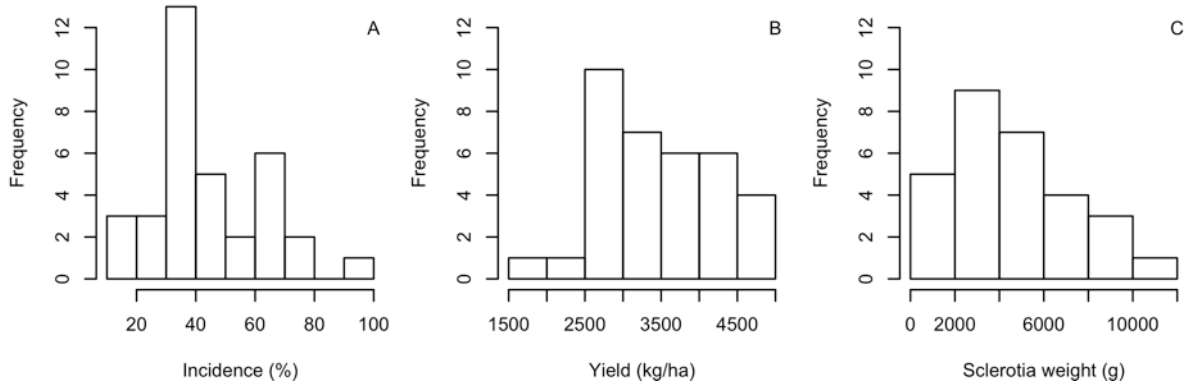

575
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577 Figure 2
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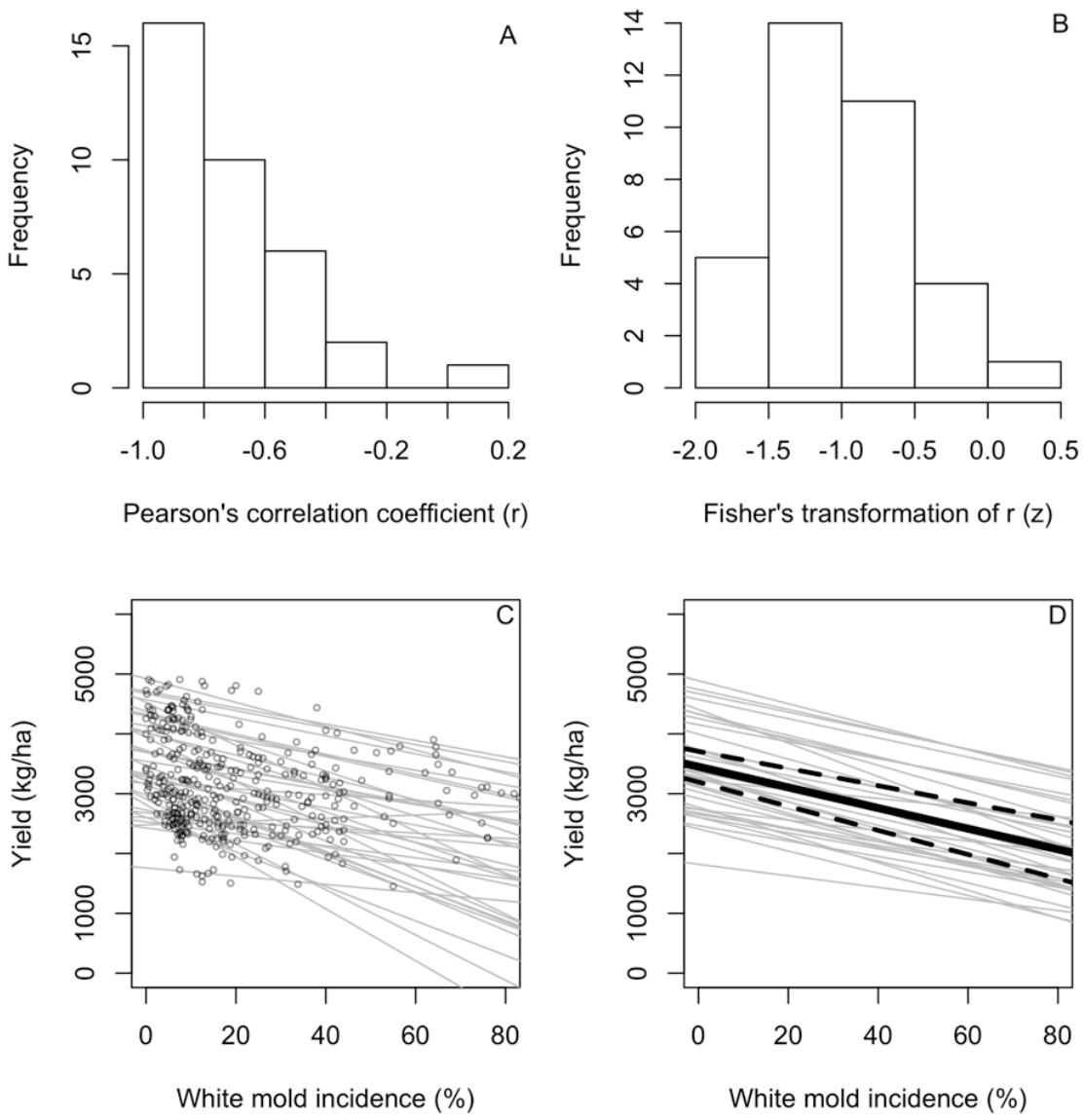
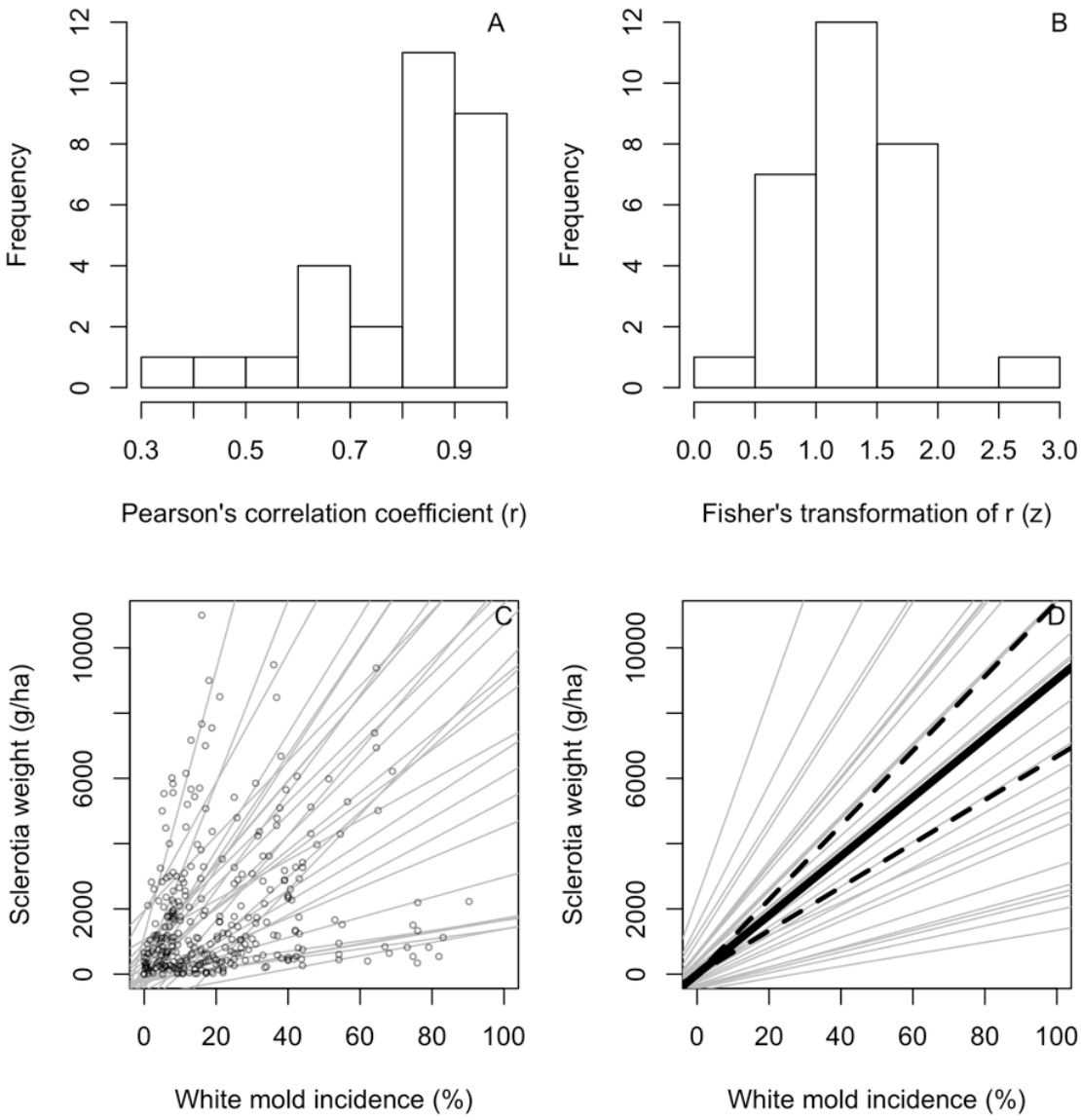

A
Frequency
0
5
10
15
-1.0
-0.6
-0.2
0.2
Pearson's correlation coefficient (r)
B
Frequency
0
2
4
6
8
10
12
14
-2.0
-1.5
-1.0
-0.5
0.0
0.5
Fisher's transformation of r (z)
C
Yield (kg/ha)
0
1000
3000
5000
0
20
40
60
80
White mold incidence (%)
D
Yield (kg/ha)
0
1000
3000
5000
0
20
40
60
80
White mold incidence (%)

Figure 3

588



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592 Figure 4

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