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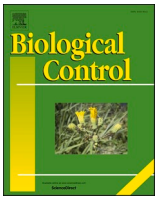


Seven years of white mold biocontrol product's performance efficacy on Sclerotinia sclerotiorum carpogenic germination in Brazil: A *meta*-analysis

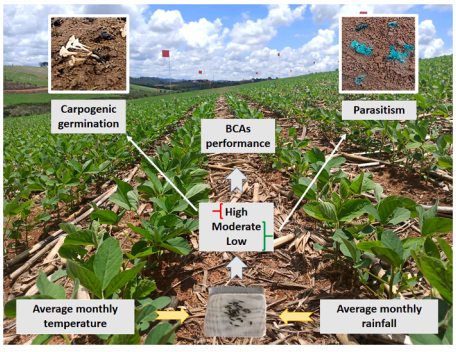
Amanda Flausino de Faria [a], Pablo Schulman [b], Maurício Conrado Meyer [c], Hercules Diniz Campos [d], Valter Cruz-Magalhães [a], Claudia Vieira Godoy [c], Rafaela Araújo Guimarães [a], Luis Henrique Carregal Pereira da Silva [e], Marcio Marcos Goussain [f], Monica Cagnin Martins [g], José Nunes Junior [h], Wilson Story Venancio [i], Lucas Fantin [j], Ricardo Brustolin [l], David de Souza Jaccoud Filho [m], Luciana Celeste Carneiro [n], Fernando Cezar Juliatti [o], Flávio H.V. Medeiros [a,*]

[a] *Laboratório de Controle Biológico, Departamento de Fitopatologia, Universidade Federal de Lavras, Lavras, 37200-900, Brazil*
[b] *Empresa de Assistência Técnica e Extensão Rural do Estado de Minas Gerais, Belo Horizonte, 30440-452, Brazil*
[c] *Embrapa Soja, 86001-970, Londrina, Brazil*
[d] *Universidade de Rio Verde, Faculdade de Agronomia, Rio Verde, 75901-970, Brazil*
[e] *Agro Carregal Pesquisa e Proteção de Plantas Eireli, Rio Verde, 75901-551, Brazil*
[f] *Assist Consultoria e Experimentação Agronômica, 78840-000 Campo Verde, Brazil*
[g] *Círculo Verde Assessoria Agronômica & Pesquisa, Rua Burle Marx, 565, sala 21, Jardim Paraíso, Luís Eduardo Magalhães, 47850-000, Brazil*
[h] *Centro Tecnológico de Pesquisa Agropecuária (CTPA), Av Assis Chateaubriand, qd-R30 lt-24, Setor Oeste, 74130-010, Goiânia, GO, Brazil*
[i] *Estação Experimental Agrícola Campos Gerais (EEACG), Rodovia BR 277 Altura do km 172,5, Estrada Velha da Vilinha km 1,5, 84130-000, Papagaios Novos, PR, Brazil*
[j] *Fundação Chapadão, Rodovia BR 060, km 011, C.P. 039, 79560-000, Chapadão do Sul, MS, Brazil*
[l] *RB Consultoria, Rua Henrique Dias, 162, Casa 10, Petropolis, Passo Fundo, RS, Brazil*
[m] *Departamento de Fitotecnia e Fitossanidade, Universidade Estadual de Ponta Grossa, Praça Santos Andrade, 01, Centro, 84030-900, Ponta Grossa, PR, Brazil*
[n] *Universidade Federal de Jataí, Campus Jataí, Rua Riachuelo, 1530, Setor Samuel Grahan, 75800-000, Jataí, GO, Brazil*
[o] *Universidade Federal de Uberlândia, Campus Umuarama, Av. João Naves de Ávila, 2121, Santa Mônica, 38408-100, Uberlândia, MG, Brazil*

HIGHLIGHTS

- Temperature and rainfall regulate *Sclerotinia sclerotiorum* sclerotia parasitism.
- Temperature (27 °C and up) and rainfall (250 mm and up) reduce biocontrol activity.
- Formulations of biological control agents reduce carpogenic germination in ca. 9%.

GRAPHICAL ABSTRACT



* Corresponding author.
E-mail addresses: amanda.faria@estudante.ufla.br (A.F. de Faria), schulman.pablo@gmail.com (P. Schulman), meyer@cnpso.embrapa.br (M.C. Meyer), campos@unirv.edu.br (H.D. Campos), valter.magalhaes@ufla.br (V. Cruz-Magalhães), claudia.godoy@embrapa.br (C.V. Godoy), rafaela.guimaraes3@ufla.br (R.A. Guimarães), carregal@fesurv.br (L.H.C.P. da Silva), flaviomedeiros@ufla.br (M.M. Goussain), flaviomedeiros@ufla.br (M.C. Martins), nunes@ctpa.com.br (J.N. Junior), flaviomedeiros@ufla.br (W.S. Venancio), fantinagro@gmail.com (L. Fantin), flaviomedeiros@ufla.br (R. Brustolin), dj1002@uepg.br (D. de Souza Jaccoud Filho), luciana.celeste.carneiro@gmail.com (L.C. Carneiro), juliatti@ufu.br (F.C. Juliatti), flaviomedeiros@ufla.br (F.H.V. Medeiros).

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ABSTRACT

Biocontrol is a plausible strategy to be considered on the management of white mold but the efficacy is not always the same. Therefore, the identification of the sources of such variable performance fosters a fine-tune product recommendation to achieve the highest performance. Based on seven soybean crop seasons, 59 uniform field trials were conducted throughout Brazil to assess the performance of *Sclerotinia sclerotiorum* sclerotia parasitism under different temperature and rainfall regimes. Hence, we carried out a *meta*-analysis to evaluate the effect of three treatment classes (*Trichoderma*-, *Bacillus*-, and a Mixed combination of both organisms) on the reduction of *S. sclerotiorum* sclerotia carpogenic germination (mean difference; *MD*) according to a multilevel network model (59 trials, $k = 340$). Throughout trials included in the *meta*-analysis, biocontrol reduced carpogenic germination ca. 70 % of times, with fitted $\overline{MD}$ values of − 10.6 for *Bacillus*, −8.6 for *Trichoderma*, and − 7.0 for Mixed *Bacillus* and *Trichoderma* groups. Separate network models were then fitted to determine the influence of average monthly temperature (AMT) and average monthly rainfall (AMR) under three classes (low, moderate, and high) for each variable on the carpogenic germination. Overall, interactions of treatments and low or moderate AMT and AMR were significant ($p < 0.1$). Temperatures above 27 °C and precipitation higher than 250 mm have not contributed to the reduction in carpogenic germination regardless of the considered active ingredient. Hence, biocontrol producís reduce carpogenic germination in ca. 9 % and dominant weather conditions. These relationships are important factors involved in sclerotia colonization and therefore, high temperatures and rainfall should not be indicated for the product's application for best BCAs performance.

1. Introduction

Soybean is the most important commodities in Brazil, with 38.9 million hectares sown in 2021 (Conab, 2021). It is estimated that *Sclerotinia sclerotiorum* (Lib.) Bary, the causal agent of white mold is present in 27 % of sown area with soybean in Brazil (Meyer et al., 2019). It is a monocyclic pathogen that survives from one crop season to the other by producing sclerotia, a structure that is also the source for disease development. The amount of sclerotia is a determining factor for the epidemiology of the disease, and it increases with each soybean crop season. Also, sclerotia are still viable on the field after up to ten years (Abawi and Grogan, 1979; Brustolin et al., 2016; Lehner et al., 2017a). Under high humidity and low temperatures, they germinate carpogenically producing apothecia (Adams and Ayers, 1979; Mueller and Hartman, 1999), causing a more severe epidemic.

Disease management is based on the integration of cultural, chemical, and biological management practices (Juliatti et al., 2015). Proper row spacing and plant density can reduce the favorable conditions to disease development (De Souza Jaccoud-Filho et al., 2016), while cultivating non-host species such as wheat can reduce the inoculum, physically inhibit apothecium germination, and improve the conditions for biological control agents (BCAs) (Görgen et al., 2009). Chemical fungicides are sprayed when plants reach the reproductive growth stage to protect the flowers during the critical period for ascospore infection (Meyer et al., 2014), but the widespread use of some compounds can select resistant pathogen population (Zhou et al., 2014; Lehner et al., 2015; Lehner et al., 2017b).

A new trend for white mold control has been the use of BCAs (Meyer et al., 2019; Medeiros et al., 2018). In tropical conditions, most products are based on *Trichoderma* spp. and *Bacillus* spp. (Cawoy et al., 2011; Juliatti et al., 2019). *Trichoderma* spp. penetrate the sclerotia via haustoria and secrete lytic enzymes that cause cell death (Vázquez-Garcidueñas et al., 1998; Geraldine et al. 2013). *Bacillus* spp. have been shown to control several soil pathogens (Hou et al., 2006; Li et al., 2014). They also produce endospores, which give them a larger shelf life (Angelo et al., 2010). *Bacillus* spp. Produce several antimicrobial metabolites in addition to antibiotics such as surfactins and iturin (Arguelles et al., 2009).

While these organisms show promising results in controlled conditions, field efficiency is variable (Zhang and Xue, 2010; Zeng et al., 2012). The effect of environmental conditions on some of the biological processes of microorganisms has already been established. For example, temperature and water availability influence the dynamics of *Trichoderma* spp. spore germination, mycelial growth, mechanisms of action, and metabolite production (Magan, 1988; Kredics et al., 2000; Kredics et al., 2003). The same has been shown to affect *Bacillus* spp. growth and colony formation (Satapute et al., 2012; Mezanges et al., 2012; Ke et al, 2015).

Since 2013, uniform field trials (UFTs) have been carried out in Brazil to assess the effectiveness of biological control products in the colonization of *S. sclerotiorum* sclerotia (Meyer et al., 2014; Meyer et al., 2016), with variable results. *meta*-analysis is a statistical analysis that allows researchers to combine the results of multiple independent studies to derive conclusions about a research question (Sutton and Higgins 2008) and establish statistical significance with studies that have conflicting results. It also allows researchers to evaluate the influence of different predictor variables in product performance.

Thus, the goal was to perform a multivariate *meta*-analysis to assess the overall effect of different treatment classes of biocontrol agents on the carpogenic germination of sclerotia of *S. sclerotiorum*, as well as to identify possible environmental factors that affect biocontrol of carpogenic germination.

2. Material and methods

2.1. Database and soybean field trials

It was used the database consisted of raw data and reports from 74 UFT conducted during seven soybean crop seasons (from 2012/13 to 2016/17 and 2018/19 to 2019/20) as part of a nationwide white mold biological control trial network (Meyer et al., 2020). The soybean variety that cultived were different regarding of place and the choice of according of edaphoclimatic conditions of each region of the assay (Supplementary Table 1). It were used sclerotia produced in the laboratory at Embrapa Soja from a *S. sclerotiorum* strain obtained in an infested field in Campos Novos (SC, Brazil) and provided to carry out all field trials. A total of 30 sclerotia (within average length ranged from 3.24 to 8.17 mm to a width of 2.16–3–31 mm) were placed in a screened nylon enveloped (10x10cm), on styrofoam tray and covered through the remains of previous crops. Four replicates were adopted for each field trial. The data used in the summarization was the average of carpogenic germination for the four replicated of each experiment. Colonization essays were performed following the methodology of Meyer et al., (2019). In short, polystyrene trays containing sclerotia were deposited in the center of each experimental plot and covered with straw from the previous cultivated crop (either corn or wheat). Biocontrol products were applied twice to the plots following the manufacturer's instructions when soybean plants reached the V2 and V4 phelogical stages

Table 1
Mean difference (effect size) and corresponding statistics for the effect of biocontrol treatments on the carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*.

Treatment	k[a]	Effect Size[b]				
		$\overline{MD}$	$se(\overline{MD})$	CI_{LB}	CI_{UB}	p-value
Bacillus	85	−10.6270	1.5058	−13.5782	−7.6757	< 0.0001
Trichoderma	235	−8.5928	1.3289	−11.1973	−5.9883	< 0.0001
Mixed	20	−7.0177	2.7039	−12.3172	−1.7182	0.0094

[a] Total number of entries used in each analysis.
[b] $\overline{MD}$ = mean difference of germination, calculated by subtracting mean germination of check treatment from mean germination of biocontrol treatment; $se(\overline{MD})$= standard error of $\overline{MD}$; CI_{LB} and CI_{UB} = limits of the 95 % confidence interval around $\overline{MD}$; p value = probability value (significance level).

(two and four fully expanded trifoliate leaves, respectively). Twenty days after the second application, the sclerotia samples were removed from the field and sent to a laboratory, where they were placed in transparent plastic boxes containing 200 g of 3x sterilized sand and kept at 17 ± 2 °C and 90 % field capacity under a 12:12 L/D photoperiod until carpogenic germination evaluations were stable, which occurred between 20 and 30 days of incubation. After carpogenic germination, sclerotia were quantified for the number of germinated and BCA-colonized sclerotia one. Sclerotia were considered colonized whenever they were covered with *Trichderma* mycelial matt or slimy *Bacillus* biofilm on the melanized surface of sclerotia was considered as such.

2.2. Studies and treatment selection criteria

Out of 74-reported UFT, eight were missing information regarding carpogenic germination, therefore have been excluded from the analysis. Seven additional UFT were excluded since had mean germination (near or exactly) 0 % on the check treatment, assuming the sclerotia sent to those UFTs were non-viable. Fifty-nine UFT conducted in thirteen research institutions across seven Brazilian states (Universidade Federal de Lavras, Lavras, MG, Brazil; Embrapa Soja, Londrina, PR, Brazil; Universidade de Rio Verde, Rio Verde, GO, Brazil; Agro Carregal Pesquisa e Proteção de Plantas Eireli, Rio Verde, GO, Brazil; Assist Consultoria e Experimentação Agronômica, Campo Verde, MT, Brazil; Círculo Verde Assessoria Agronômica & Pesquisa, Luís Eduardo Magalhães, BA, Brazil; Centro Tecnológico de Pesquisa Agropecuária (CTPA), Goiânia, GO, Brazil; Estação Experimental Agrícola Campos Gerais (EEACG), Papagaios Novos, PR, Brazil; Fundação Chapadão, Chapadão do Sul, MS, Brazil; RB Consultoria, Passo Fundo, RS, Brazil; Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil; Universidade Federal de Jataí, Jataí, GO, Brazil; Universidade Federal de Uberlândia, Uberlândia, MG, Brazil) Fig. 1A). From the remaining studies, only treatments that had formulations with live microorganisms as active ingredients were included (14 out of 17 tested products). The included products were grouped based on the formulated biocontrol treatment (Supplementary Table 1), resulting in the following classes: Bacillus (4 formulations; 59 trials), Trichoderma (8 formulations; 59 trials), and Mixed (Bacillus + Trichoderma; 2 formulations; 9 trials) (Supplementary Table 1). For all treatments, the total volume sprayed was 150 L/ha using a CO_2 compressor cylinder at 3PSI and coupled to a 4-cone nozzle bar. The central plot was considering of four the central lines with 5 m of size and 0.5 m distance between rows.

Temperature and rainfall were adopted as moderators (Fall et al., 2018). Information regarding both variables was obtained from each each scientist carrying out the experiment. One data was generated per field trial. The average monthly temperature and rainfall were determined based on the whole soybean season. When necessary, the tools Graphreader (https://www.graphreader.com) (Tolmeijer et al., 2020) and GetData DigitizeIt® (version 2.26) (Perez et al., 2021) were used to access the trials data.

2.3. Quantitative synthesis of biocontrol agents' effect across trials

Data from each of the 59 UFT constituted an independent study in the meta-analysis. Mean germination from biocontrol and check treatments were collected from each study and used to find the effect size, estimated as the mean difference (MD), by subtracting the mean

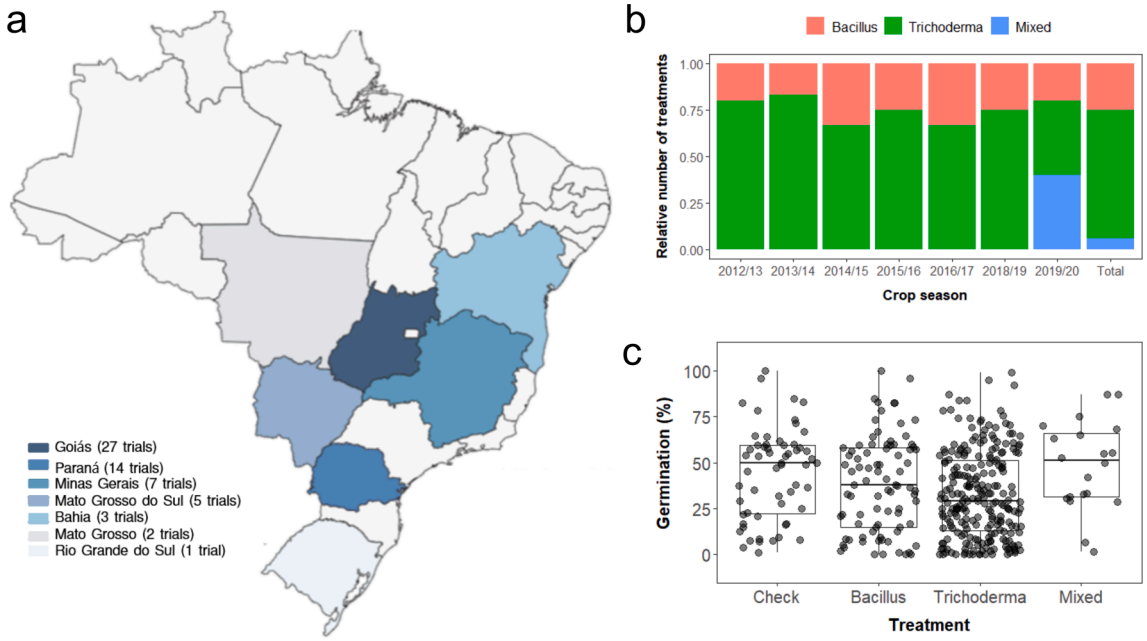

Fig. 1. a) State distribution and number of trials of *Sclerotinia sclerotiorum* biocontrol throughout Brazil. b) Proportion of products by biocontrol treatment class used at each crop season. c) Carpogenic germination distribution of sclerotia of *Sclerotinia sclerotiorum* under different treatments. Check = water treatment; Mixed = *Bacillus* + *Trichoderma*.

germination of check treatment at the specific study from the mean germination of the biocontrol product using the escalc function of the metafor package (Viechtbauer, 2010) of R.

2.4. meta-analytic models

A multilevel network model was fitted using the rma.mv function of the metaphor package (Viechtbauer, 2010). The random components were considered the treatment and trial, with correlated random effects for the different treatments within trials. The amount of heterogeneity (τ^2 and ρ) was estimated using the restricted maximum-likelihood estimator (Viechtbauer, 2005) and evaluated based on the significance of the Cochran's Q test. The within-study variance (V) was estimated from the coefficient of variation (CV) of an analysis of variance of the effects of treatment by first estimating the standard deviation (*SD*). Studies were weighted in inverse proportion to their sampling variances (within-study variances). Wald-type tests and 95 % CIs were obtained using an assumption of normality.

2.5. Effect of temperature and rainfall on carpogenic germination

Average monthly temperature (AMT) and average monthly rainfall (AMR) were included as moderator variables to account for at least part of the heterogeneity in the true effects (Borenstein et al., 2009). A separate multilevel network model was fitted for each moderator variable, with three categorical classes each. For average monthly temperature, classes were UFTs were grouped in low (AMT $\leq$ 23 °C), moderate (23 °C < AMT $\leq$ 27 °C), and high (AMT > 27 °C). For average monthly rainfall, classes were low (AMR < 150 mm), moderate (150mm $\leq$ AMR $\leq$ 200 mm), and high (AMR > 250mm).

Parameters classes were calculated according to data amplitude.

3. Results

3.1. Distribution of BCA and germination across trials

Trichoderma was the most frequently used treatment (8 formulations across 235 entries; 69.12 % of all BCA entries), followed by *Bacillus* (4 formulations across 85 entries; 25 %) and Mixed (2 formulations across 20 entries; 5.88 %) (Fig. 1B). Germination in check entries ranged from 0.83 to 100 % (median: 49.99 %), while germination in BCA-treated entries ranged from 0 to 100 % (median: 37.74 %), 0 to 99.17 % (median: 29.17 %), and 1.33 to 87.38 % (median: 51.11 %) for *Bacillus*, *Trichoderma*, and Mixed, respectively (Fig. 1C). Mean difference values for *Bacillus* varied from − 52.840 to 25.800, with 77.65 % being negative (i.e., reduced germination), while for *Trichoderma* varied from − 55.340 to 52.500 (71.91 % negative) and for Mixed from − 42.660 to 19.430 (75 % negative) (Fig. 2).

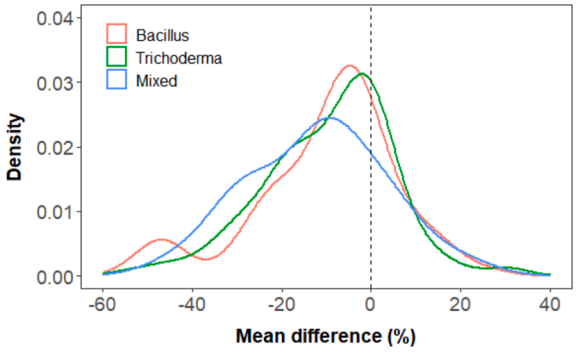

Fig. 2. Distribution of mean difference values of the carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* between biocontrol treatment classes and check (water) treatment. Mixed = *Bacillus* + *Trichoderma*. All values to the left of the dashed line indicate biocontrol treatment reduced carpogenic germination compared to check treatment.

3.2. Effect of biocontrol treatments on carpogenic germination

A total of k = 340 entries was included in the carpogenic germination *meta*-analysis. BCA treatment as a whole affected carpogenic germination (<0.0001). The overall mean difference ($\overline{MD}$) was − 10.6270 (95 % CI: −13.5782 to − 7.6757) for *Bacillus*, −8.5928 (95 % CI: −11.1973 to − 5.9883) for *Trichoderma*, and − 7.0177 (95 % CI: −12.3172 to − 1.7182), for Mixed; all values were different from zero with probability levels of < 0.0001, < 0.0001, and 0.0094, respectively (Table 1). All pairwise $\overline{MD}$ comparisons but one (*Bacillus* and *Trichoderma*) had *p* values > 0.1 (Fig. 3A). According to Cochran's test of heterogeneity, the true outcomes appear to be heterogeneous (QE = 3985.4536, p < 0.0001).

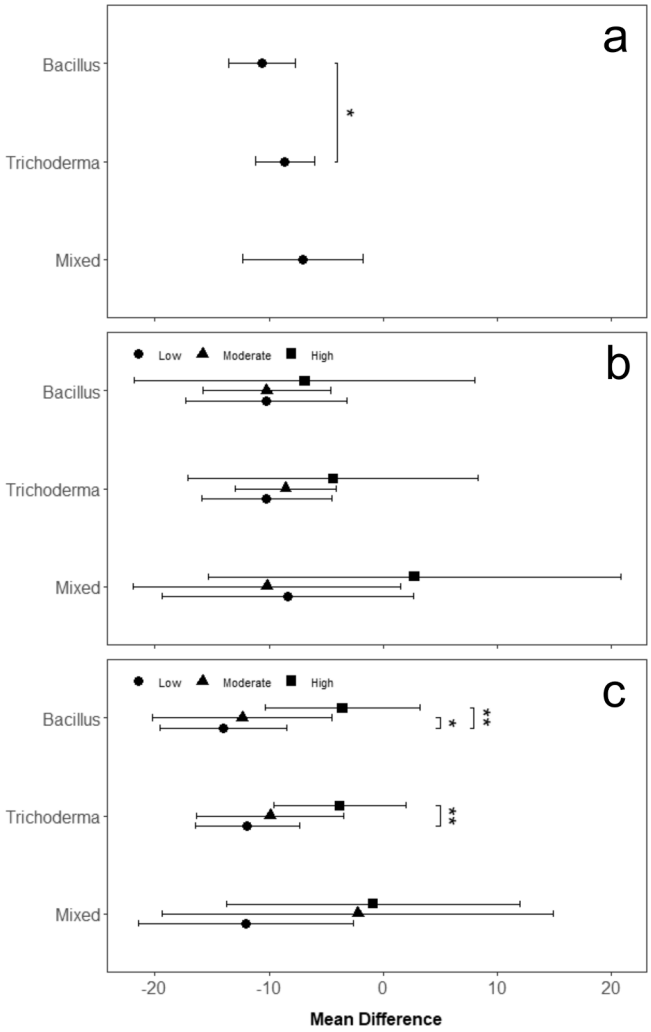

Fig. 3. Mean difference (effect size) and 95 % confidence interval for the effect of A) biocontrol treatment class and the interaction of treatments and three classes of average monthly temperature (AMT; B) and average monthly rainfall (AMR; C) on the carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*. Mixed = *Bacillus* + *Trichoderma*. AMT = Low: AMT $\leq$ 23 °C; Moderate: 23 °C < AMT $\leq$ 27 °C; High: AMT > 27 °C. AMR = Low: AMR < 150 mm; Moderate: 150 mm $\leq$ AMR $\leq$ 200 mm; High: AMR > 250 mm. Pairwise comparisons linked by brackets are statistically significant with p = 0.05 (**) and p = 0.10 (*).

3.3. Moderator analysis

To account for some of the residual heterogeneity found in the first germination *meta*-analysis, two separate *meta*-analyses were performed considering the interaction between biocontrol agent and average monthly temperature (k = 182) or average monthly rainfall (k = 199). Based on Wald-type chi-square tests, both interactions affected carpogenic germination ($p = 0.0003$ and $p < 0.0001$ for AMT and AMR, respectively). Both models reduced the amount of residual heterogeneity, but Cochran's Q test was still significant (QE = 1049.9133, $p < 0.0001$; and QE = 1121.8287, $p < 0.0001$ for AMT and AMR, respectively).

For AMT, only $\overline{MD}$ from interactions with low and moderate AMT were statistically significant from zero ($p < 0.1$) on all treatment classes except Mixed:Low ($\overline{MD}$ = -8.3712; $p = 0.1367$) (Table 2). By treatment classes, all pairwise comparisons had p-values > 0.1 (Fig. 3B). When AMT was high, Bacillus had the lowest $\overline{MD}$ (-6.8557), but it was not statistically different from check ($p = 0.3682$) (Table 2).

Similarly to AMT, only $\overline{MD}$ from interactions with low and moderate AMR were statistically significant from zero ($p < 0.1$), but results varied in that Mixed:Moderate (and not Mixed:Low) did not differ from check ($\overline{MD}$ = -2.2034; $p = 0.8013$) (Table 3). No treatment class performed well when rainfall levels were high: probability levels were 0.3082, 0.2026, and 0.8955 for Bacillus, Trichoderma, and Mixed, respectively. For Bacillus, pairwise comparisons between low and high, and moderate and high AMR had p values of 0.0202 and 0.0979, respectively. Regarding Trichoderma, the pairwise comparison between low and moderate had a p-value of 0.0315 (Fig. 3C).

4. Discussion

White mold (*Sclerotinia sclerotiorum*) can cause major yield losses in soybean, and its management is complicated given the pathogen's wide host range and ability to form resistance structures (Boland and Hall 1994; Abawi and Grogan, 1979). White mold control is achieved by integrating several practices, including biological control, a sustainable tool that acts by reducing the initial inoculum of *S. sclerotiorum* (Juliatti et al., 2015). Analyzing carpogenic germination due to BCA colonization in uniform field trials carried out in seven crop seasons in Brazil, we found that sclerotia sprayed with biocontrol products germinated significantly less than untreated sclerotia (Fig. 4). Throughout trials, average monthly rainfall and average monthly temperature, affected the efficacy of the genera in reducing carpogenic germination.

Biological control of *S. sclerotiorum* is largely used in Brazil, with the first commercial product, based on *Trichoderma harzianum*, being registered in 2007 (Bettiol et al., 2019). The number of biological products registered in Brazil has increased due to the lower developmental cost compared to chemical products and new legislation that made the registration process for biocontrol products based on biological targets (Guimarães et al., 2019; Bettiol et al., 2012), allowing their use in several crops (unlike chemical products, which require crop-specific efficacy trials). Nowadays, there are 33 biocontrol products

Table 2
Mean difference (effect size) and corresponding statistics for the effect of the interaction of biocontrol treatments and average monthly temperature on the carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*.

Treatment	Avg. Temp.[a]	k[b]	Effect Size[c]				
			$\overline{MD}$	$se(\overline{MD})$	CI_{LB}	CI_{UB}	p-value
Bacillus	Low	12	−10.2163	3.5983	−17.2688	−3.1639	0.0045
	Moderate	29	−10.2034	2.8766	−15.8414	−4.5654	0.0004
	High	3	−6.8557	7.6193	−21.7892	8.0779	0.3682
Trichoderma	Low	37	−10.1974	2.9122	−15.9053	−4.4896	0.0005
	Moderate	83	−8.5499	2.2536	−12.9667	−4.1330	0.0001
	High	6	−4.3518	6.4914	−17.0747	8.3711	0.5026
Mixed	Low	4	−8.3712	5.6251	−19.3962	2.6538	0.1367
	Moderate	6	−10.1605	5.9909	−21.9024	1.5814	0.0899
	High	2	2.7783	9.2532	−15.3576	20.9142	0.7640

[a] Average monthly temperature during trials. Low: Avg. Temp. $\leq 23^{\circ}$C; Moderate: 23°C < Avg. Temp. $\leq 27^{\circ}$C; High: Avg. Temp. > 27°C.
[b] Total number of entries used in each analysis.
[c] $\overline{MD}$ = mean difference of germination, calculated by subtracting mean germination of check treatment from mean germination of biocontrol treatment; $se(\overline{MD})$= standard error of $\overline{MD}$; CI_{LB} and CI_{UB} = limits of the 95 % confidence interval around $\overline{MD}$; p-value = probability value (significance level).

Table 3
Mean difference (effect size) and corresponding statistics for the effect of the interaction of biocontrol treatments and average monthly rainfall on the carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*.

Treatment	Avg. Rain[a]	k[b]	Effect Size[c]				
			$\overline{MD}$	$se(\overline{MD})$	CI_{LB}	CI_{UB}	p value
Bacillus	Low	23	−14.0025	2.8558	−19.5998	−8.4052	<0.0001
	Moderate	12	−12.3375	4.0146	−20.2059	−4.4691	0.0021
	High	13	−3.5452	3.4792	−10.3643	3.2740	0.3082
Trichoderma	Low	64	−11.9008	2.3453	−16.4976	−7.3040	<0.0001
	Moderate	37	−9.9034	3.2896	−16.3509	−3.4558	0.0026
	High	38	−3.7736	2.9619	−9.5788	2.0316	0.2026
Mixed	Low	8	−12.0627	4.8142	−21.4982	−2.6271	0.0122
	Moderate	2	−2.2034	8.7574	−19.3677	14.9609	0.8013
	High	2	−0.8607	6.5553	−13.7089	11.9874	0.8955

[a] Average monthly rainfall during trials. Low: Avg. Rain. < 150 mm; Moderate: 150mm $\leq$ Avg. Rain. $\leq$ 200 mm; High: Avg. Rain. > 250mm.
[b] Total number of entries used in each analysis.
[c] $\overline{MD}$ = mean difference of germination, calculated by subtracting mean germination of check treatment from mean germination of biocontrol treatment; $se(\overline{MD})$= standard error of $\overline{MD}$; CI_{LB} and CI_{UB} = limits of the 95 % confidence interval around $\overline{MD}$; p value = probability value (significance level).

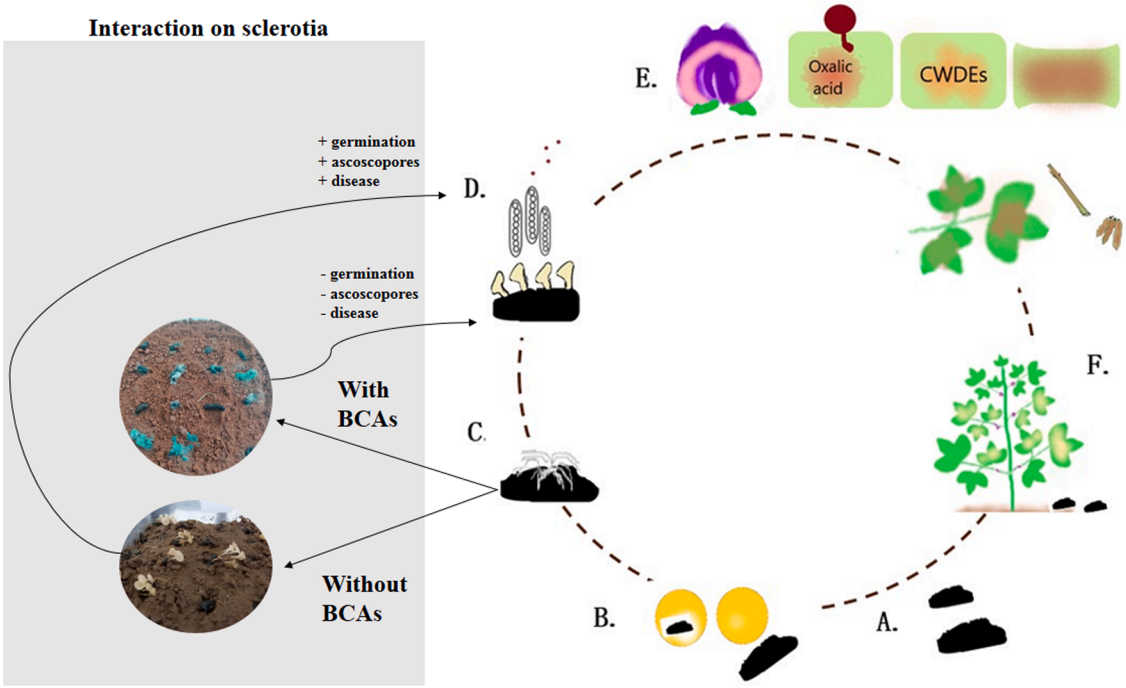

Fig. 4. *Sclerotinia sclerotiorum* disease cycle on soybean (Glycine max L.). (A) Survive structure. (B) Seeds infect by sclerotia mycelial or sclerotia presence on seed lots. (C) Asexual phase: Mycelial germination. (D) Sexual phase: sclerotia germination to produce apothecia and disperse ascospores into the air. (E) Ascospores colonize flowers, developed appressorium and secret Oxalic acid that support action of Cell well degraded enzymes (CWDEs). Brown lesions the initial symptoms, can be disseminate into the stem, leaves and pods. (F) Sclerotia from plant tissue can turn to the soil during harvest or disseminated by seeds. In the left or grey side are related the possible interaction on sclerotia, when spray BCAs and without BCAs sprays.

registered against *S. sclerotiorum*. All of the tested products were formulations of *Bacillus* and *Trichoderma* (Fig. 1B). Both genera have desirable traits for the biocontrol industry: they multiply fast, have a long shelf-life, and are known to have multiple modes of action (Jacobsen et al., 2004). All products registered for *S. sclerotiorum* control are based on *Trichoderma* and *Bacillus* (Agrofit, 2022), whereas outside of Brazil, *Coniothyrium minitans* is the most widely available and tested BCA for white mold control (Peltier et al., 2012).

A recent trend has been the use of mixtures of isolates, species and/or genera: according to AGROFIT (2020), there are six mixed products for *S. sclerotiorum* control, with the first being registered in 2018. In our *meta*-analysis, there were two formulations combining *Trichoderma* and *Bacillus* in the 2019/20 crop season. The combination of two or more biocontrol agents can improve the management of disease possibly by positive synergism and reduced risk of variability (Guetsky et al., 2001). Indeed, Guetsky et al., (2002) found that the combination of *Trichoderma*, and *Bacillus* was more efficient to control *Botrytis cinerea* in strawberry leaves. However, each combination should be thoroughly analyzed as there is evidence there are more antagonistic interactions than synergistic interactions among BCAs (Xu et al., 2011). While mixed formulations only accounted for about 7 % of all total entries (Supplementary Table S1), we expect to see more of those being tested in the next uniform field trials and a rationale for the combination of strains to be the higher plasticity in temperature and rainfall for sclerotia parasitism.

All treatment classes reduced germination compared to check treatment, with an average germination 10.63, 8.59, and 7.02 % lower than untreated plots for products based on *Bacillus*, *Trichoderma*, and *Bacillus* + *Trichoderma* (Mixed), respectively. The amount of germinated sclerotia is a critical factor in white mold epidemics, given that a single sclerotium can produce several apothecia (Bolton et al., 2006), with each apothecium releasing up to 7.6×10^5 ascospores over 20 days (Clarkson et al. 2003). Indeed, there is a significant correlation between the number of apothecia (and indirectly, the number of viable sclerotia) and white mold incidence in soybean (Boland and Hall, 1988).

The current chemical control strategy is to apply fungicides when plants reach the flowering stage, when plants are the most vulnerable (Meyer et al., 2018). At this moment, most sclerotia have already germinated and produced apothecia, under favorable conditions (Fall et al., 2018), so fungicide applications are mostly deployed to protect against infection and reduce ascospore germination, plant infection and tissue colonization rate. On the other hand, BCAs parasite sclerotia, thus decreasing the primary inoculum (Meyer et al., 2019); by implementing both practices, we affect two of the parameters that affect disease epidemics: the initial inoculum and the rate of disease progress (Campbell, 1998).

Environmental conditions can alter the dynamics of BCA interactions (Kredics et al., 2004). The effect of temperature on *Trichoderma* sp. varies from species and strains, affecting conidia germination, mycelium growth, antibiosis, and parasitism (Guigón-lópez et al. 2010). There is evidence that sclerotia parasitism by *Trichoderma* spp. is optimal around 17 °C and 30 °C (Trutmann and Keane, 1990; Domingues et al., 2016), temperatures that span all 3 AMT classes in the current study. The optimal temperature for *Bacillus* spp. growth and metabolite production is also strain-dependent, varying from 15 to 37 °C for *Bacillus subtilis*, for example, but most isolates prefer higher temperatures (Jiménez-Delgadillo et al. 2018; Sidorova et al. 2020). We found there was a significant effect of the interaction of AMT with biocontrol genera on carpogenic germination, but reduction varied between temperature classes inside each genus. Here, no BCA genus significantly reduced germination in high temperatures, even though both genera have been shown to grow in temperatures in this AMT class. Most trials included in this *meta*-analysis were carried out in regions with low and moderate AMT, resulting in low statistical power in high AMT, but we cannot discard the existence of other correlated variables that affected biocontrol performance. Mixed formulations had better results in moderate temperatures; moderate temperatures may benefit both species, resulting in better synergistic potential.

Water availability is one of the most important factors that affect both *Trichoderma* and *Bacillus*, with restrictive water conditions drastically affecting populational growth and interaction with other microorganisms (Magan, 1988; Luard and Griffin, 1981; West et al., 1985; Leuschner and Lillford, 1999; Kredics et al., 2004). The interaction between genus classes and high AMR did not affect carpogenic germination. The excessive rainfall might have washed away the products, especially if precipitation occurred just right after product application when populations were not yet established. It has been shown that different isolates react differently to a gradient of matric potential. Jones et al. (2015) found that depending on the isolate, not only drier soils but almost-saturated soils compromised *Trichoderma* ability to affect sclerotia viability of *S. sclerotiorum* in controlled conditions. Also, it is not known how different matric potentials or fluid flows affect *Trichoderma* conidia adhesion and substrate colonization. Environmental conditions deeply affect bacterial biofilm dynamics, with communities exposed to desiccation having a higher biofilm formation ability than communities exposed to saline or non-stressful conditions (Bogino et al., 2013). Although *B. subtilis* biofilm has been shown to have non-wetting characteristics (Arnaouteli et al., 2016), it is possible that *Bacillus* attachment to sclerotia substrate was compromised by high water availability, with bacteria being more likely to disperse actively (which depends on cell motility or extracellular polymeric substances degradation) and/or passively (due to physical factors such as liquid flow conditions) (Toyofuku et al., 2016).

5. Conclusion

As BCAs contributes to *Sclerotinia sclerotiorum* management through inoculum reduction from sclerotia parasitism and 70 % of the evaluated products and fields resulted in reduction of inoculum viability. The combined analyses of several uniform field trials revealed that reduced carpogenic germination ca. 9 % was mostly impaired by high levels of average monthly temperature (above 27 °C) and average monthly rainfall (250 mm). Therefore, cooler temperatures and drier environmental conditions should be considered for maximum sclerotia parasitism.

Data availability

Data will be available upon reasonable request.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2022.105080.

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