ALTERNATIVE PRODUCTS TO CONTROL *Sclerotinia sclerotiorum* IN SOYBEAN

PRODUTOS ALTERNATIVOS NO CONTROLE DE Sclerotinia sclerotiorum *NA CULTURA DA SOJA*

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ABSTRACT: The aim of this study was to evaluate different alternative products, foliar sprayed either alone or in combination with fungicide fluazinam, to control white mold in soybean at three locations: Arapoti, Mauá-da-Serra, and Pinhão, in Paraná state, southern Brazil. The following chemical products were used: sodium dichloroisocyanurate, benzalkonium chloride, pyroligneous extract, sodium hypochlorite, cobalt + molybdenum, sucrose, sodium bicarbonate and acibenzolar-S-methyl. Incidence, severity, yield, and number of sclerotia produced were analyzed. The majority of the variables did not follow the normal distribution of the data according to the Kolmogorov-Smirnov test; therefore, the use of non-parametric analysis was necessary. The analysis of all the treatments individually revealed no significant effects so it was decided to separate the analysis into the following groups of treatments: 'control', treatments using alternative products ('alternatives'), treatment only with fungicide ('fungicide') and treatments with alternative products and fungicide ('alternatives + fungicide'). In Arapoti, no significant differences for any variables were observed. In Mauá-da-Serra, the 'fungicide' and 'alternatives + fungicide' had the lowest incidence and number of sclerotia, with higher yield when compared to 'control' and 'alternatives'. No differences were observed for severity. In Pinhão, the 'fungicide' and 'alternatives + fungicide' were superior for incidence, number of sclerotia produced, and yield when compared to the 'control' and 'alternatives'. The 'alternatives + fungicide' showed significantly lower severity when compared to the control, 'alternatives' and 'fungicide' in Pinhão experiment. In conclusion, the alternative products applied in combination with fluazinam resulted in lower severity of white mold in soybean in the experiment conducted in Pinhão, and the application of fluazinam alone or in combination with alternative products, was efficient to control white mold in soybean in locations with high disease incidence.

KEYWORDS: White mold. *Glycine max* (L.) Merrill. Household cleaning products. Chlorine. Induced resistance.

INTRODUCTION

Soybean (*Glycine max* (L.) Merril) is considered one of the most important Fabaceae in the world; it is used as a source of protein for human and animal consumption and also as raw material for oil extraction for food. It is also used in biodiesel production and in countless processed and semi-processed products. Due to these nutritional and industrial characteristics, and its great adaptability to different climates and soils, soybean is one of the major plants cultivated worldwide. Brazil is the second largest producer on a global scale, with an average yield of 2.882 kg ha^{-1} (CONAB, 2014). Several factors may limit productivity, such as the presence of diseases that can cause significant economic losses. One of these is white mold, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary. This soilborne pathogen can infect more than 400 plant species (BOLAND; HALL, 1994), and it can be responsible for severe losses when conditions are conducible for its development. Under these conditions, soybean crops have suffered losses up to 70%, as reported by Jaccoud Filho et al. (2010) in the 2008-2009 crop season in the southern Brazilian state of Paraná.

In order to minimize the damage of white mold in soybean, a set of preventive measures should be adopted, such as the use of pathogen-free seeds, seed treatment, the correct application timing for products, density and spacing of seeding, mulching, crop rotation, the use of biological control agents, alternative products and fungicides (GÖRGEN et al., 2009; HENNEBERG et al., 2012; JACCOUD FILHO et al, 2010; SILVA et al, 2010; ZENG et al., 2012). The use of fungicides have the potential to cause environmental and food contamination. In order to minimize the environmental impact of the chemical products usage, more research is required regarding the use

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of products with low toxicity to humans and lower potential of environmental impact (SMILANICK et al., 1999; WALTER et al., 2009) as it is the case of the alternative products used in this study.

The aim of this study was to assess the effectiveness of different alternative products, applied either alone or in combination with fungicide fluazinam, in the management of white mold in soybean under field conditions.

MATERIAL AND METHODS

Three experiments were conducted in areas naturally infested by white mold in the 2011-12 crop season in the municipalities of Arapoti, Mauá-da-Serra and Pinhão, all located in the southern state of Paraná, Brazil, and conducted under no-tillage system.

Soil was collected to quantify the number of sclerotia in four points of 0.25 m^{-2} at a depth of 5 cm, following the methodology proposed by Jaccoud Filho et al. (2010). In Arapoti, 55 sclerotia m^{-2} were found; in Mauá-da-Serra, 452 sclerotia m^{-2}, and in Pinhão, 149 sclerotia m^{-2}. In Arapoti the soybean cultivar used was BMX Turbo RR, sown on 11/27/2011 with 16 seeds m^{-1}. In Mauá-da-Serra, NA5909 RR, sown on 10/30/2011 with 14 seeds m^{-1}, and in Pinhão, BMX Apollo RR, sown on 11/10/2011 with 12 seeds m^{-1}. The seeds were treated with pyraclostrobin, thiophanate methyl and fipronil at the dosage of 5, 45 and 50 grams of active ingredient per 100 kg of seeds, respectively. In all three sites the spacing used was 45 cm between rows. The experimental design was a randomized block design with 18 treatments and four replications (Table 1). The plots were 3.0 m wide and 5.0 m long, with a total area of 15.0 m².

Table 1. Treatments, phenological stages of applications, and doses used in the experiments of the alternative control of white mold in soybean, conducted in Arapoti, Mauá-da-Serra and Pinhão, PR. 2011-12 crop season.

Treatments		Applications				Dosage ha^{-1}
		1st	2nd	3rd	4th	
1	control	-	-	-	-	-
2	cobalt (1%) + molybdenum (5%)	V4[2]	V9[3]	R1[4]	10 DAA[5]	0.3 L c.p.[6]
3	sucrose (98%)	V4	V9	R1	10 DAA	2 kg c.p.
4	bicarbonate of soda	V4	V9	R1	10 DAA	2 kg c.p.
5	sodium dichloroisocyanurate (70%)	V4	V9	R1	10 DAA	66 g c.p.
6	benzalkonium chloride (12.5%)	V4	V9	R1	10 DAA	1 L c.p.
7	pyroligneous extract	V4	V9	R1	10 DAA	1 L c.p.
8	sodium hypochlorite (2.5%)	V4	V9	R1	10 DAA	2 L c.p.
9	acibenzolar-S-methyl (50%)	V4	V9	R1	10 DAA	25 g c.p.
10	fluazinam	-	-	R1	10 DAA	0.5 kg g.a.i. [7]
11	cobalt (1%) + molybdenum (5%)	V4	V9	-	-	0.3 L c.p.
	fluazinam	-	-	R1	10 DAA	0.5 kg g.a.i.
12	sucrose (98%)	V4	V9	-	-	2 kg c.p.
	fluazinam	-	-	R1	10 DAA	0.5 kg g.a.i.
13	bicarbonate of soda	V4	V9	-	-	2 kg c.p.
	fluazinam	-	-	R1	10 DAA	0.5 kg g.a.i.
14	sodium dichloroisocyanurate (70%)	V4	V9	-	-	66 g c.p.
	fluazinam	-	-	R1	10 DAA	0.5 kg g.a.i.
15	benzalkonium chloride (12.5%)	V4	V9	-	-	1 L c.p.
	fluazinam	-	-	R1	10 DAA	0.5 kg g.a.i.

16	pyroligneous extract	V4	V9	-	-	1 L c.p.
	fluazinam	-	-	R1	10 DAA	0.5 kg g.a.i.
17	sodium hypochlorite (2.5%)	V4	V9	-	-	2 L c.p.
	fluazinam	-	-	R1	10 DAA	0.5 kg g.a.i.
18	acibenzolar-S-methyl (50%)	V4	V9	-	-	25 g c.p.
	fluazinam	-	-	R1	10 DAA	0.5 kg g.a.i.

-: not applicable; [1]ha: hectare [2]V4: 4 open trifoliates; [3]V9: 9 open trifoliates; [4]R1: first open flower; [5]10 DAA: 10 days after R1; [6]c.p.: commercial product; [7]g.a.i.: grams of the active ingredient.

The applications were conducted using a pressurized CO_2 backpack sprayer, with a pressure of 3 bars and a 6 nozzle spray boom (3.0 m length) using Teejet® XR 110 02 fan type spray nozzles and a volume of liquid of 200 L ha^{-1}.

The assessment of incidence and severity of plants infected by *S. sclerotiorum* was performed in 100 plants in the two central rows of each plot when the soybean was at R5.5 phenological stage (pods with 75-100% graining). For the assessment of severity, the diagrammatic scale proposed by Juliatti and Juliatti (2010) was used.

Prior to harvest, eight infected plants were marked in the central rows of each plot and these were subsequently collected using plastic bags to quantify the number of sclerotia produced in laboratory. From these data, and the incidence of the disease in the plots, it was possible to estimate the production of sclerotia ha^{-1} for each treatment.

For yield evaluation, four meters in length were harvested by hand, using the four central rows of each plot, (7.2 m² total). The harvested area was threshed with a motorized grain mixer and moisture was adjusted to 13% v/v. All the data were tested for normality using the Kolmogorov-Smirnov test and submitted to the nonparametric Kruskal-Wallis and Mann-Whitney-U tests, using Statistica 10.0® (Statsoft®) software.

Meteorological data were provided by the stations of SIMEPAR (Meteorological System of Paraná) the ones that have the nearest location to the experiments.

RESULTS AND DISCUSSION

The statistical analysis showed that most of the variables did not follow the normal distribution of the data, according to the Kolmogorov-Smirnov test; consequently, the use of non-parametric tests was necessary. The analysis of all the treatments individually revealed no significant effects so it was decided to separate the analysis into the following four treatment groups: one treatment without the application of products ('control'); treatments 2 to 9, which included all the alternative products applied individually ('alternatives' group); treatment 10, which consisted of the isolated application of the fungicide fluazinam ('fungicide' group); and treatments 11 to 18, which consisted of fungicide applied together with alternative products ('alternatives + fungicide' group). No differences were found between the treatments within the same group.

Arapoti

In the experiment conducted in Arapoti, a lower incidence (12.5% in the 'control' - Figure 1A) and intermediate severity (69.1% in the 'control' - Figure 1B) of *S. sclerotiorum* were detected, compared with the severity detected in the experiments conducted in Mauá-da-Serra (65.3% in the control - Figure 2) and Pinhão (84.3% in the control - Figure 3). This was probably due to the low rainfall during flowering of the crop in the region of Arapoti. In the 20 days after the appearance of the first flowers there was a total of 20 mm rainfall, a decrease in relative humidity to approximately 76%, and an air average temperature of 20°C (Appendix A).

On average, the cultivars used in the three trials had flowering periods of approximately 20 days. Meteorological data for this period were monitored because flowering is the period of greatest susceptibility to infection by *S. sclerotiorum*. The climatic conditions during the flowering period are crucial for the occurrence of white mold in soybean because the flowers are used as primary energy source for the germination of the ascospore. Free water on the plants surface is necessary for the infection to occur (ABAWI; GROGAN, 1979; NAPOLEÃO et al., 2005).

In Arapoti no difference was found in any of the analyzed variables (Figure 1), occur possibly due to the low recorded incidence of white mold (Figure 1A). This fact generated a large variation in the data, as can be seen by the error bars (Figure 1). Because of this, non-parametric statistical analysis on the data set still resulted in limitations in the sensitivity of the analysis (Figure 1A and 1C). The 'alternatives', 'fungicide' and 'alternatives + fungicide' groups showed a reduction of 34.4, 44.0 and 53.6% in the incidence, and 48.7; 53.7 and 52.7% in the production of sclerotia, respectively. These absolute values can be significant in a long term management of this disease, because it is a pathogen that is difficult to control and it produces resistant structures (sclerotia) that remain viable in the soil for many years.

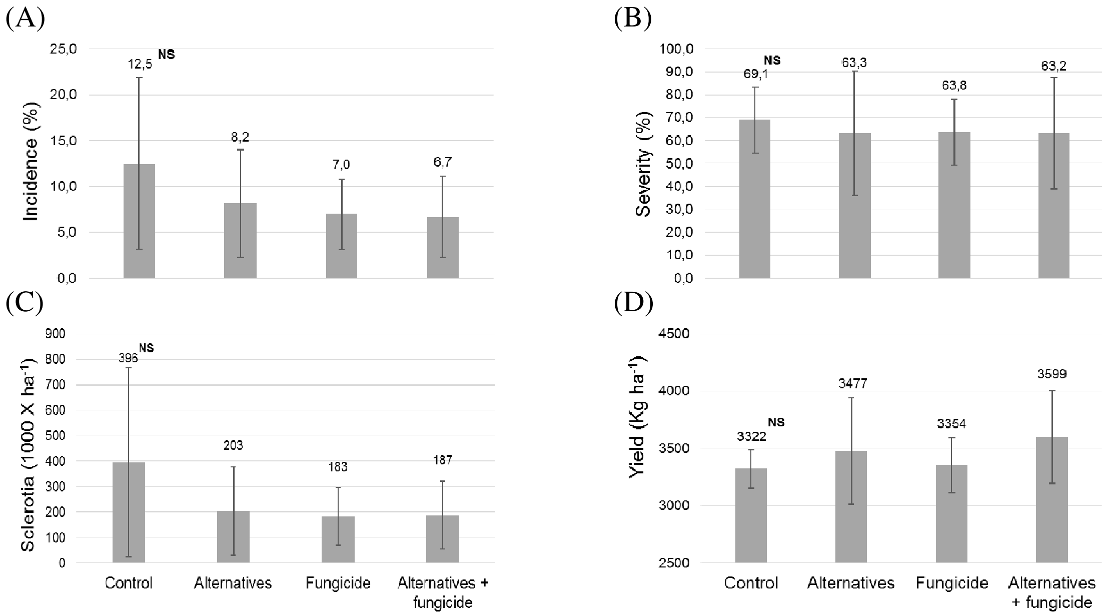

Figure 1. Data from the experiment conducted in Arapoti, PR in the 2011-12 crop. A - average incidence of S. *sclerotiorum* (NS: no statistically significant difference - Kruskal-Wallis p = 0.241); B - Average severity of plants infected by *S. sclerotiorum* (Kruskal-Wallis p = 0.241); C - Number of sclerotia ha^{-1} (Kruskal-Wallis, p = 0.427); D – yield in kg ha^{-1} (Kruskal-Wallis, p = 0.371). The error bar represents the standard deviation.

Mauá-da-Serra

In Mauá-da-Serra the highest incidence of *S. sclerotiorum* (27% in the 'control' - Figure 2A) and the lowest values for severity (65.3% in the control – Figure 2B) were observed, when compared with Arapoti (12.5% incidence and 69.1% severity in the control - Figure 1) and Pinhão (20.5% incidence and 84.3% and severity in the control - Figure 3). This may be explained by the high level of contamination in the area (452 sclerotia m^{-2}), high rainfall (107 mm), average air humidity of 82% and an average temperature of 21.6°C during the 20 days after flowering (Appendix B).

The 'fungicide' and 'alternatives + fungicide' groups showed lower incidence of white mold (Figure 2A), with a control of 81.5 and 77.7%, respectively, when compared to the 'control' group. However, no statistically significant differences were observed for severity (Figure 2B).

The low incidence values (Figure 2A) observed for 'fungicides' and 'alternatives + fungicide' reflected in a low production of sclerotia (Figure 2C) and in a higher crop yield (Figure 2D), which significantly differed from the 'control' and 'alternatives'.

These results demonstrate that in areas with history occurrence, and high contamination of *S. sclerotiorum* it is necessary to use fungicide treatment to decrease the production of survival structures, which will affect subsequent crops. This use will also result in crop yield increase, which will cover the costs of acquisition and application of fungicide, thereby maintaining profitability for growers. It is noteworthy that the control of white mold in soybean should be performed considering a combination of several strategies and not based exclusively on the fungicides application.

Pinhão

In the experiment conducted in Pinhão (Figure 3), the incidence was intermediate (20.3% in the control - Figure 3A) but with high severity (84.3% in the control - Figure 3B) compared to Mauá-da-Serra (27% incidence and 65.3% severity in the control - Figure 2) and Arapoti (12.5% incidence and 69.1% severity in the control - Figure 1).

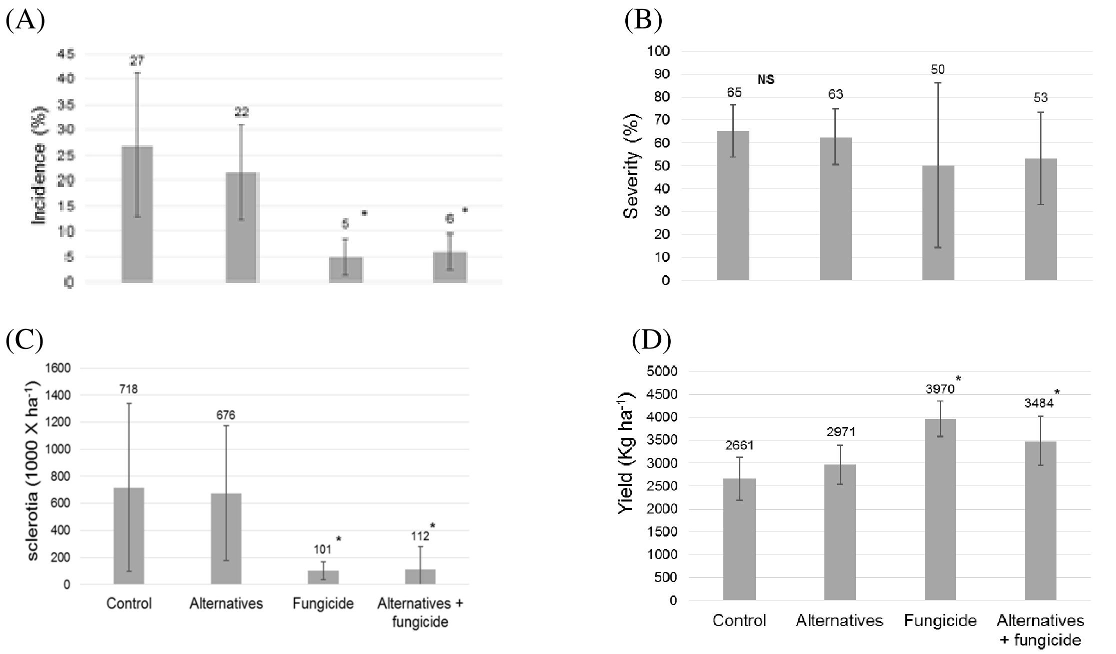

Figure 2. Data from the experiment conducted in Mauá-da-Serra,PR 2011-12 crop. A - Average incidence of *S. sclerotiorum* (* statistically significant difference with control and 'alternatives' groups - Mann-Whitney-U, $p < 0.05$); B - Average severity of plants infected by *S. sclerotiorum* (NS: no statistically significant difference (Kruskal-Wallis $p = 0.973$), C - number of sclerotia ha^{-1} Mann-Whitney-U, $p < 0.05$);. D – yield in kg ha^{-1} Mann-Whitney-U, $p < 0.05$). The error bar represents the standard deviation.

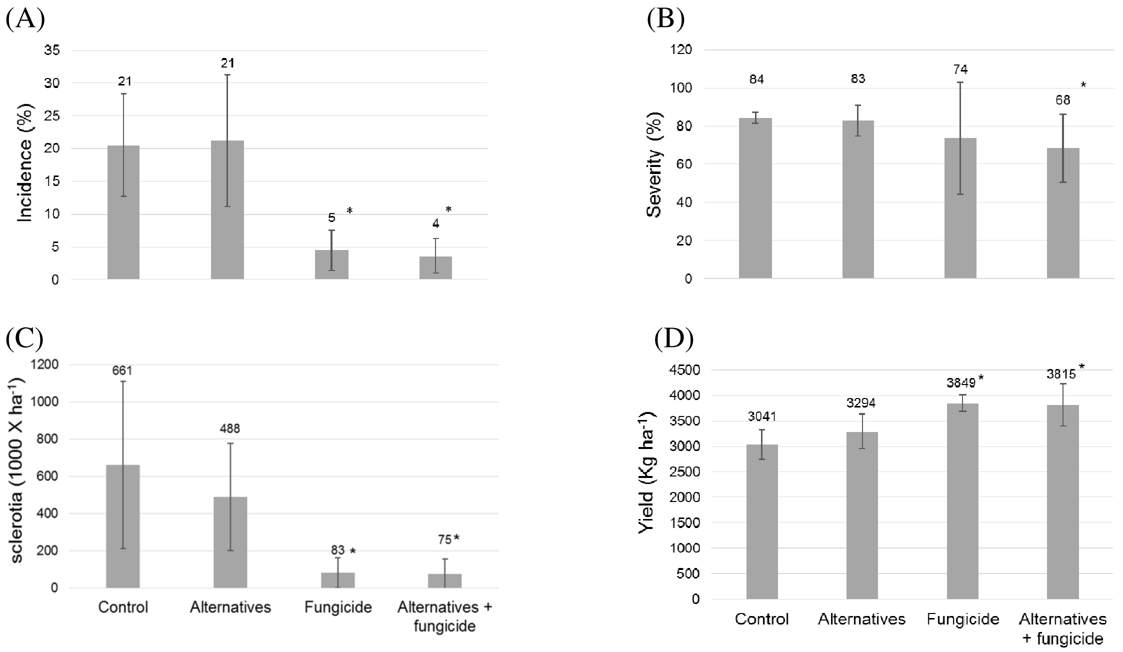

Figure 3. Data from the experiment conducted in Pinhão, PR, 2011-12 crop. A - Average incidence of *S. sclerotiorum* (* statistically significant difference with control and 'alternatives' groups - Mann-Whitney-U, $p < 0.05$); B - Average severity of plants infected by *S. sclerotiorum* Mann-Whitney-U, $p < 0{:}05$); C - number of sclerotia ha^{-1} Mann-Whitney-U, $p < 0.05$); D – Yield in kg ha^{-1} Mann-Whitney-U, $p < 0.05$). The error bar represents the standard deviation.

The lower incidence in the region of Pinhão may have possibly been due to the lower initial inoculum (149 sclerotia m^{-2}) compared to Mauá-da-Serra (452 sclerotia m^{-2}). However, the higher severity in Pinhão may be explained by the higher rainfall (132 mm) that occurred during the 20 days-flowering, average air humidity of 80% and average air temperature of 21.3°C, which provided a conducive environment for disease development (Appendix C) (AGRIOS, 2005; COLEY-SMITH; COOKE, 1971; MORAL, 1977).

Data show the effectiveness of the 'fungicide' and 'alternatives + fungicide' in reducing the incidence of white mold in soybean (78.1 and 82.4%, respectively) (Figure 3A). There was no difference between the 'control' and 'alternatives' groups. Regarding the severity of white mold, the 'alternatives + fungicide' group was statistically lower when compared to the 'control', 'alternatives' and 'fungicide' groups, with a reduction of 18.8, 17.4 and 7.2%, respectively (Figure 3B).

Similarly to the results observed in Mauá-da-Serra, control provided by 'fungicide' on its own or 'alternatives + fungicide' was observed for incidence. That reflected in a significant reduction in the production of sclerotia (Figure 3C), with a decrease of 87.5 and 88.6%, when compared to the 'control' group, respectively. These values are extremely important regarding the long term management of white mold in infested areas, due to the lengthy persistence of viable sclerotia in the soil and the limitation of crops for rotation. This represents another strategy to be used, as it provides incremental yield, which can be seen in Figure 3D where the 'fungicide' and 'alternatives + fungicide' were comparatively different from the control and 'alternatives', with 26.6 and 25.4% increment in yield when compared to the 'control', respectively.

These results emphasize the importance of the fungicide use, combined or not with alternative products, in areas with medium-high inoculum potential, for both increase on yields and decrease on production of sclerotia for subsequent crops. It is evident that the use of alternative products provided a significantly lower severity in the 'alternatives + fungicide' group when compared to the other groups in Pinhão, where the use of alternative product possibly had a negative effect on the development of the pathogen. This result is very important because the isolated use of the fungicide resulted in lower levels of incidence in Mauá-da-Serra (Figure 2A) and Pinhão (Figure 3A) but there were no differences for severity in these two locations.

There have been reports of the successful use of alternative products tested for several pathogens, such as the control of *Penicillium digitatum* in citrus fruits using sodium bicarbonate (SMILANICK et al., 1999.); the disinfestation of green coconuts using sodium hypochlorite (WALTER; NASCIMENTO; KUAYE, 2009); and the decontamination of plants for *in vitro* cultivation using sodium dichloroisocyanurate (PARKINSON; PRENDERGAST; SAYEGH, 1996) among others.

The results obtained at Pinhão trial (Figure 3 B), disagree with those obtained by Sumida (2012), who found no statistical differences between the applications of fungicide in isolation, or mixed with benzalkonium chloride, on the severity of white mold in soybean. However, Sumida did not observe statistically significant differences in the incidence of white mold in soybean using the fungicide fluazinam together (or not) with benzalkonium chloride, which were similar to the results observed for incidence in all three locations used in this experiment (Figure 1A, 2A and 3A).

There is little research on the use of alternative products to manage white mold in soybean that can be used as reference for discussion, which emphasizes the importance of this study in a field that still lacks information.

CONCLUSIONS

The alternative products that were applied in combination with the fungicide fluazinam resulted in reduced severity of white mold in soybean in the experiment conducted in Pinhão, PR.

The application of fluazinam, either alone or in combination with alternative products, was effective in reducing white mold incidence and sclerotia production in Mauá-da-Serra and Pinhão, with yield increase for both locations.

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RESUMO: O objetivo deste estudo foi avaliar diferentes produtos alternativos pulverizados via foliar, isoladamente ou em combinação com o fungicida fluazinam para o controle do mofo branco na cultura da soja, em três locais: Arapoti, PR, Mauá-da-Serra, PR e Pinhão, PR, Brasil. Os produtos utilizadas foram: dicloroisocianurato de sódio, cloreto de benzalcônio, extrato pirolenhoso, hipoclorito de sódio, cobalto + molibdenio, sacarose, bicarbonato de sódio e acibenzolar-S-metil. As variáveis analisadas foram incidência, severidade, rendimento e o número de escleródios produzidos. A maioria das variáveis não segue a distribuição normal dos dados conforme o teste Kolmogorov-Smirnov, assim sendo, o uso de testes não-paramétricos se fez necessário. A análise de todos os tratamentos individualmente não revelou efeitos significativos, onde optou-se por separar a análise em grupos de tratamentos, onde a testemunha foi denominada "controle"; os tratamentos com os produtos alternativos como "alternativos", o tratamento com o fungicida isolado como “fungicida” e os tratamentos com produtos alternativos e fungicida foi denominado "alternativos + fungicidas". Em Arapoti, não foram observadas diferenças estatisticamente significantes em nenhuma das variáveis analisadas. Em Mauá-da-Serra, os grupos ‘fungicida’ e ‘alternativos + fungicida’, apresentaram a menor incidência e número de escleródios, com maior rendimento quando comparados aos grupos ‘controle’ e ‘alternativos’. Não foram observadas diferenças na severidade. Em Pinhão, os grupos ‘fungicida’ e ‘alternativos + fungicida’ foram estatisticamente superiores para incidência, número de escleródios produzidos e rendimento, quando comparados aos grupos ‘controle’ e ‘alternativos’. O grupo ‘alternativo + fungicida’ apresentou severidade significativamente menor quando comparado aos grupos ‘controle’, ‘alternativos’ e ‘fungicida’ no experimento conduzido em Pinhão. Como conclusões, observou-se que os produtos alternativos aplicados em associação com o fungicida fluazinam proporcionaram menor severidade do mofo branco na cultura da soja no experimento conduzido em Pinhão, PR, e a aplicação do fungicida fluazinam isolado ou em associação com os produtos alternativos foi eficiente no controle do mofo branco na cultura da soja nos locais com alta incidência da doença.

PALAVRAS-CHAVE: Mofo-branco. *Glycine max* (L.) Merrill. Produtos domissanitários. Cloro. Indutor de resistência.

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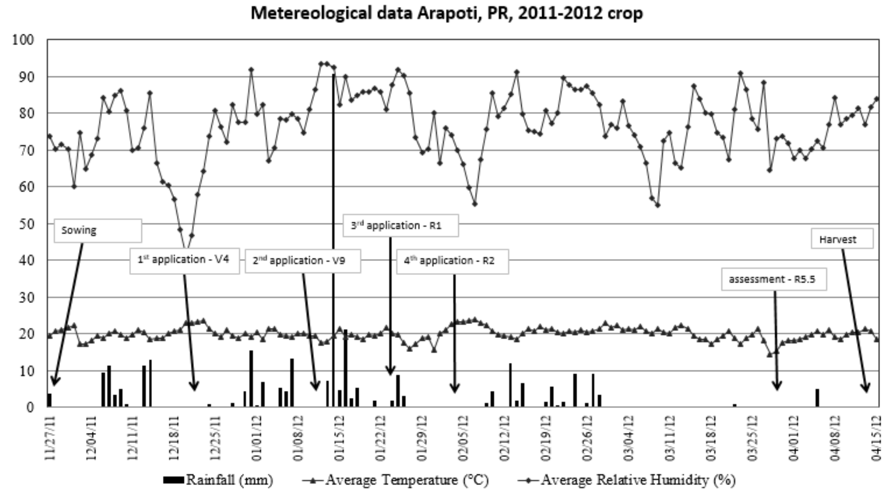
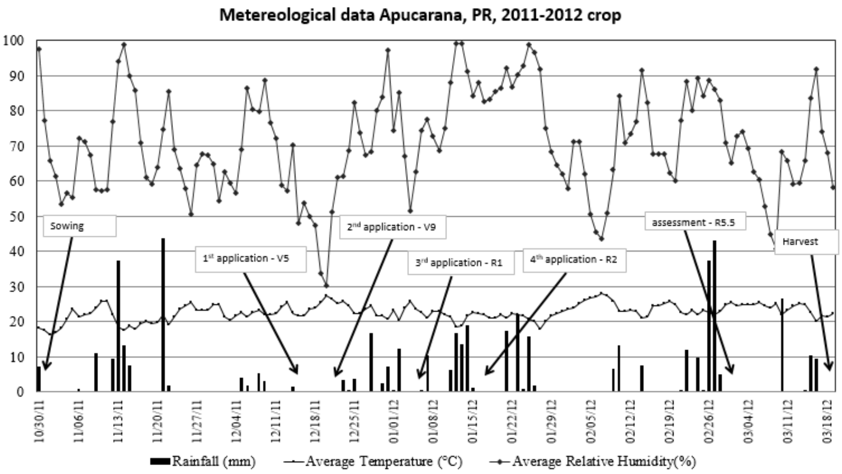
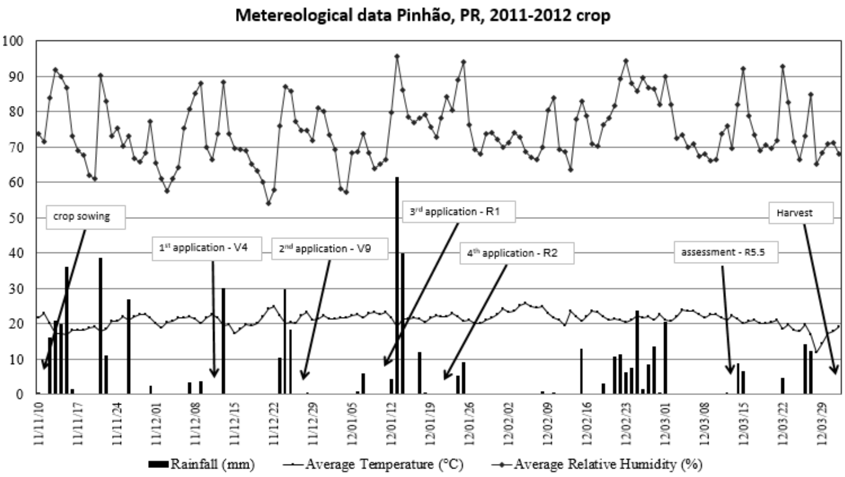
APPENDICES



Appendix A. Meteorological data from experiment conducted in Arapoti, PR in 2011-2012 crop. V4: 4 trifoliate leaves open; V9: 9 trifoliate leaves open; R1: first open flower; R2: full bloom.



Appendix B. Meteorological data from Apucarana,PR, 2011-2012 crop, used as a reference for experiment conducted in Mauá-da-Serra, PR. Source: SIMEPAR. V5: 5 trifoliate leaves open; V9: 9 trifoliate leaves open; R1: first open flower; R2: full bloom.



Appendix C. Meteorological data from the experiment conducted in Pinhão, PR, 2011-2012 crop. Source: SIMEPAR. V4: 4 trifoliate leaves open; V9: 9 trifoliate leaves open; R1: first open flower; R2: full bloom.