

Original research papers

Resistance of Soybean to Fungal Diseases Using Copper-based Protectors

Comment [REV A1]: Do we can change this wordj

ABSTRACT: At level word fFungal diseases that affect soybean crop are one of the main causes of low productivity and annual losses may reach 21% of total production. In this context, the objective of this study was to evaluate the efficiency of copper-based protectors associated with fungicides for the control of soybean diseases as: asian soybean rust (*Phakopsora pachyrhizi*), target spot of soybean (*Corynespora cassiicola*) and cercospora leaf blight (*Cercospora kikuchii*) + frogeye leaf spot (*Cercospora sojina*) + brown spot (*Septoria glycines*). Asian soybean rust, target spot of soybean and cercospora leaf blight + frogeye leaf spot + brown spot, which together were considered end-of-cycle diseases, well as the impact on grain yield, in the region of Aparecida do Rio Negro – TO, Brazil. Treatments were composed by the combination of different products and doses application of copper-based protectors fungicides: Azimut® (first application), Orkestra® (second application), Ativum® (third application) and Horos® (fourth application), in soybean crop. Diseases were identified and evaluations were performed using LI-COR® portable meter to determine the injured areas of each soybean leaflet at 7 days after the fourth application and assigned scores according to the diagrammatic scales. At physiological maturethe end, the grain yield was evaluated and from the results, obtained Pearson correlation indices (r) were evaluated. Associated applications of the fungicides + Unizeb Gold® (1.5 kg ha⁻¹), Difere® (0.5 L ha⁻¹), and the application of NHT® Copper Super with a concentration higher than 0.109 L ha⁻¹, were effective for the control of end-of-cycle diseases in soybean crop. Associated applications of the fungicides + NHT® Copper Super (0.219 L ha⁻¹) reduced the severity of Asian soybean rust, target spot of soybean and end-of-cycle diseases and showed a greater increase in grain yield of 4.5 Mg ha⁻¹.

Comment [REV A2]: In others countries we dont know this diseases; we know the diseasas with others comun names

KEYWORDS: *Glycine max*, Asian soybean rust, *Phakopsora pachyrhizi*, induction of resistance, grain yield, yield loss.

1. INTRODUCTION

The soybean (*Glycine max* (L.) Merrill) is one of the most important economic segments of Brazilian agribusiness and one of the main crops used during the harvest period, in the Northern region of Brazil. Tocantins covers a soybean cultivation area of 956.1 thousand hectares, with an average yield of 2.9 Mg ha⁻¹ (harvest of 2016/17), falling below the national average of 3.4 Mg ha⁻¹ of soybeans [1].

However, several diseases affect the cultivation of this crop and make it difficult to obtain high levels of crop productivity (yield). Among the factors responsible for the low yield of soybeans, special

39 attention has been given to the lack of phytosanitary care, especially those caused by fungi, which
40 may occur during the whole cycle or only at the end of the crop cycle [2].

41 The diseases affecting in the final phase of the cycle (a complex of diseases represented by
42 *Cercospora kikuchii*, *Cercospora sojina* and *Septoria glycines*) cause losses in production by up to
43 21%, being in most cases a reduction of the weight of seeds [3]. The most common diseases during
44 the vegetative and reproductive cycle are the powdery mildew (*Microsphaera diffusa*), mildew
45 (*Peronospora manshurica*), anthracnose (*Colletotrichum truncatum*), target spot (*Corynespora*
46 *cassicola*), teleomorph (*Thanatephorus cucumis*) and especially the Asian soybean rust (*Phakopsora*
47 *pachyrhizi* Sydow & P. Sydow) [4].

48 The use of commercial products that activate plant defense mechanisms or that benefit the
49 action of the fungicide are commonly found in the literature and several results may be observed on
50 micronutrients use [5]. However, new alternatives must be found to assist the traditionally used
51 practices of disease control, and the induction of plant resistance is an alternative that can be
52 integrated to the management.

53 In this scenario, the fungicides associated with copper-based (Cu) protectors have been
54 shown to be an effective and economical alternative in crop management, because they promote
55 additive or synergistic effects when these chemicals are used together [6]. In the plant, Cu has
56 structural function in enzymes, and several proteins containing Cu are important in the processes of
57 photosynthesis, respiration, detoxification of free superoxides radicals and lignification, and the latter
58 gives greater resistance to plants from the attack of pathogens [7].

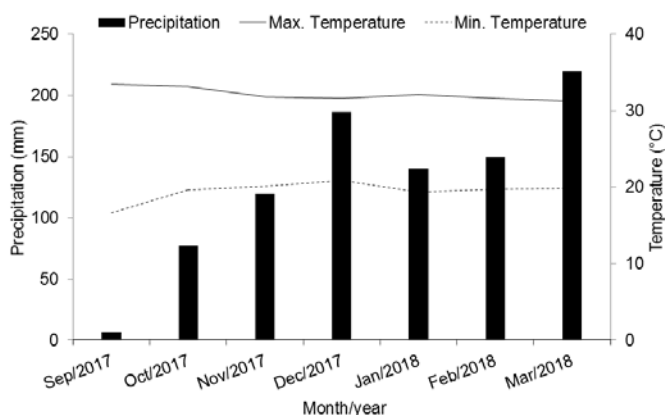
59 Therefore, the use of micronutrients or resistance inducers in combination with fungicides in
60 the soybean crop must be evaluated regionally. Thus, the objective of this work was to evaluate the
61 efficiency of copper-based protectors associated with fungicides for the control of soybean diseases
62 as: Asian soybean rust, target spot of soybean and cercospora leaf blight + frogeye leaf spot + brown
63 spot, which together were considered End-of-Cycle Diseases (ECD), well as the impact on grain yield,
64 in the region of Aparecida do Rio Negro – TO, Brazil.

65

66 2. MATERIAL AND METHODS

67 The experiment was conducted in the country of Aparecida do Rio Negro – TO, Brazil,
68 located at 9° 57' 17" South Latitude, 47° 58' 7" West Longitude and 262 m of altitude, in an
69 experimental area belonging to Ímpar Consultoria, located on the farm Santos Agropecuária. The
70 climate of the region is tropical humid with two well-defined periods: rainy season, from November to
71 March, with higher rainfall in December and January; and dry season, from April to October. The
72 average annual rainfall is 1,240 mm.

73 Rainfall in the experimental area and temperature variation during the period of conduction
74 of the experiment are shown in Fig. 1 [8].



75
76 **Figure- 1. Rainfall (mm) and monthly temperature observed in the experimental area in**
77 **Aparecida do Rio Negro, TO, Brazil**

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78
79 The soil of the experimental area was a clayey Oxisol from Cerrado [9]. Soil analysis made
80 before the implantation of the experiment showed, for the layers 0-0.2, 0.2-0.4 and 0.4-0.6 m,
81 respectively: clay content = 67, 62 and 55%, pH CaCl₂ = 5.5, 5.3 and 5.2; P (Mehlich 1) = 3.5, 2.0 and
82 1.9 mg dm⁻³; K (Mehlich 1) = 74.0, 27.0 and 19.0 mg dm⁻³; Ca = 2.95, 2.02 and 2.39 cmol_c dm⁻³; Mg =
83 1.54, 1.46 and 2.01 cmol_c dm⁻³; Al = 0.17, 0.13 and 0.12 cmol_c dm⁻³; H + Al = 3.8, 3.3 and 3.2 cmol_c
84 dm⁻³; CTC = 8.5, 6.8 and 7.7 cmol_c dm⁻³ and 36.6, 17.4 and 12.6 g kg⁻¹ of organic matter. The
85 determinations followed the methodologies proposed by Embrapa [10].

86 The experimental design was in randomized blocks, with four replications. The plots were
87 composed of six lines with spacing of 0.5 m and 6.0 m in length, totaling 18 m².

88 Distribution of the treatments involved the application of the protector associated with
89 fungicides: Azimut[®] 0.5 L ha⁻¹ (first application), Orkestra[®] 0.3 L ha⁻¹ (second application), Ativum[®] 0.8
90 L ha⁻¹ (third application) and Horos[®] 0.5 L/ha⁻¹ (fourth application) + adjuvant Assist[®], with applications
91 volume of 200 L ha⁻¹, as described in Table 1.

92
93 **Table 1. Description of the treatments (protectors and doses) applied in the soybean crop**

Treat.	Protector (Product/Dose)	Adjuvant (L/ha ⁻¹)	*Seasons of application
T1	Fungicides	0.5	1.2.3.4
T2	Fungicides + Unizeb Gold [®] -1.5 kg ha ⁻¹	0.5	1.2.3.4
T3	Fungicides + Difere [®] - 0.5 L ha ⁻¹	0.5	1.2.3.4
T4	Fungicides + Fertilis Phitopress Copper [®] - 0.5 L ha ⁻¹	0.5	1.2.3.4
T5	Fungicides + Fertilis Phitopress Copper [®] - 1.0 L ha ⁻¹	0.5	1.2.3.4
T6	Fungicides + Fertilis Phitopress Copper [®] - 1.5 L ha ⁻¹	0.5	1.2.3.4
T7	Fungicides + NHT [®] Copper Super - 0.055 L ha ⁻¹	0.5	1.2.3.4
T8	Fungicides + NHT [®] Copper Super - 0.109 L ha ⁻¹	0.5	1.2.3.4

T9	Fungicides + NHT [®] Copper Super - 0.219 L ha ⁻¹	0.5	1.2.3.4
T10	Fungicides + NHT [®] Copper Super - 0.4375 L ha ⁻¹	0.5	1.2.3.4
T11	Fungicides + NHT [®] Copper Super - 0.875 L ha ⁻¹	0.5	1.2.3.4
T12	Control	-	-

*1 = Application with Azimut[®] at 39 Days After Emergency (DAE); 2 = Application with Orkestra[®] at 43 DAE; 3 = Application with Ativum[®] at 55 DAE and 4 = Application with Horos[®] at 67 DAE.

The soybean used was the M 8644 IPRO of indeterminate growth, treated and inoculated with Carbendazim + Tiram + Fipronil, with population of 530 thousand plants per hectare. Planting was carried out on November 25, 2017, using direct sowing system with pneumatic seeder of tractor traction. On sowing fertilization, 250 kg ha⁻¹ of Monoammonium phosphate (MAP) and potassium provided by variable rate in the form of KCl were used. Phytosanitary management in pest control was carried out according to the protocol of the Fundação Chapadão [11].

At 7 days after the fourth application, it were evaluated the severity of the following diseases: asian soybean rust (*Phakopsora pachyrhizi*), target spot of soybean (*Corynespora cassicola*) and cercospora leaf blight (*Cercospora kikuchii*) + frogeye leaf spot (*Cercospora sojina*) + brown spot (*Septoria glycines*), which together were considered End-of-Cycle Diseases (ECD) [4]. Based on the foliar analysis, notes were assigned according to the diagrammatic scales [12, 13] (Fig. 2).

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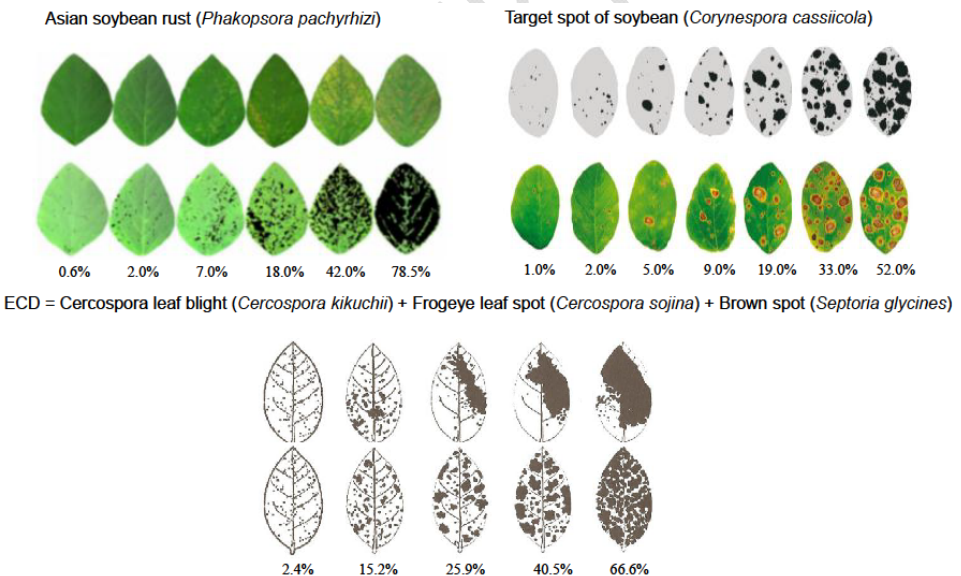


Figure. 2. Diagrammatic Scales for evaluations of fungal diseases in soybean

Top panel: Aggregated symptoms. Bottom panel: Randomly distributed symptoms.

Comment [REV A5]: the images of the leaves at the bottom; belong to ECD = Cercosporaleafblight (Cercosporakikuchii) + Frogeyeleafspot (Cercosporasojina) + Brown spot (Septoriaglicinas) ????????????????

is confuse

Thus, the severity of the diseases of soybean was elaborated from the collection of five trefoils totally open by repetition in the second, fourth and sixth reproductive node of the plants, counted from the apex to the base, thus simulating the upper, middle and lower thirds, respectively. After the

116 collection of each leaflet, the injured area (necrotic tissue and yellowish halo) was drawn in
 117 transparent plastic and subsequently it was subjected to leaf area measurement using the LI-COR®
 118 portable meter (LI-3000) to determine the injured area and the total area. [SoTherefore](#), it was possible
 119 to determine the soybean leaflet with the lowest number of injuries, intermediate injuries and the one
 120 with the most leaf injuries, thus establishing the lower, intermediate and upper limits in the
 121 diagrammatic scale, respectively.

122 Grain yield (in Mg ha⁻¹), was estimated from the mass of grain, corrected to 13% moisture
 123 [14], with area for analysis of production of 3 m⁻¹, collected in two central lines of each plot, discarding
 124 1.5 m of border at each end. The Pearson correlation (r) between grain yield and severity of fungal
 125 diseases were also evaluated in soybean plants.

126 The data were submitted to analysis of variance (p < 0.05) and when significant differences
 127 were found the means of the treatments were submitted to the Scott-Knott test at 0.05 of probability,
 128 using the statistical program SIRVAR® [15].

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130 3. RESULTS AND DISCUSSION

131 In the region of Aparecida do Rio Negro - TO, adverse climatic conditions are found in each
 132 agricultural year, not following the same temperature pattern, relative air humidity and rainfall. Due to
 133 the aforementioned fact, the 2017/18 crop harvest was considered out of standard when compared to
 134 previous harvests, due to the good climatic conditions observed and regular rainfall distribution (Fig.
 135 1). Disease severity data show that only ECD and target spot of soybean presented some degree of
 136 infestation. Asian soybean rust pustules were not observed in any of the treatments (Table 2).

137

138 [Table 2. Severity of Asian Soybean Rust \(ASR\), End-of-Cycle Diseases \(ECD\) and Target Spot](#)
 139 [of Soybean \(TSS\) at 7 days after the fourth application in the lower and middle third of the](#)
 140 [soybean crop, in the region of Aparecida do Rio Negro – TO, Brazil](#)

<u>Treat.</u>	<u>ASR (%)</u>	<u>*ECD (%)</u>	<u>TSS (%)</u>	<u>ASR (%)</u>	<u>*ECD (%)</u>	<u>TSS (%)</u>
	<u>----- Lower third -----</u>			<u>----- Middle third -----</u>		
<u>T1</u>	<u>0</u>	<u>40.5 e</u>	<u>5.0 c</u>	<u>0</u>	<u>25.9 d</u>	<u>2.0 c</u>
<u>T2</u>	<u>0</u>	<u>1.00 a</u>	<u>0.5 a</u>	<u>0</u>	<u>1.00 a</u>	<u>0.5 a</u>
<u>T3</u>	<u>0</u>	<u>1.00 a</u>	<u>0.5 a</u>	<u>0</u>	<u>1.00 a</u>	<u>0.5 a</u>
<u>T4</u>	<u>0</u>	<u>15.2 c</u>	<u>0.5 a</u>	<u>0</u>	<u>2.40 b</u>	<u>0.5 a</u>
<u>T5</u>	<u>0</u>	<u>2.40 b</u>	<u>1.0 b</u>	<u>0</u>	<u>1.00 a</u>	<u>0.5 a</u>
<u>T6</u>	<u>0</u>	<u>25.9 d</u>	<u>1.0 b</u>	<u>0</u>	<u>15.2 c</u>	<u>0.5 a</u>
<u>T7</u>	<u>0</u>	<u>25.9 d</u>	<u>1.0 b</u>	<u>0</u>	<u>15.2 c</u>	<u>0.5 a</u>
<u>T8</u>	<u>0</u>	<u>1.00 a</u>	<u>0.5 a</u>	<u>0</u>	<u>1.00 a</u>	<u>0.5 a</u>
<u>T9</u>	<u>0</u>	<u>1.00 a</u>	<u>1.0 b</u>	<u>0</u>	<u>1.00 a</u>	<u>1.0 b</u>

T10	0	1.00 a	1.0 b	0	1.00 a	1.0 b
T11	0	1.00 a	1.0 b	0	1.00 a	1.0 b
T12	0	40.5 e	5.0 c	0	40.5 e	5.0 d
C.V.(%)	0.0	9.46	12.71	0.0	14.28	8.02

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Table 2. Severity of Asian Soybean Rust (ASR), End-of-Cycle Diseases (ECD) and Target Spot of Soybean (TSS) at 7 days after the fourth application in the lower and middle third of the soybean crop, in the region of Aparecida do Rio Negro – TO, Brazil

Treat.	Lower third			Middle third		
	ASR (%)	*ECD (%)	TSS (%)	ASR (%)	*ECD (%)	TSS (%)
T1	0	40.5 e	5.0 e	0	25.0 d	2.0 e
T2	0	4.00 a	0.5 a	0	4.00 a	0.5 a
T3	0	4.00 a	0.5 a	0	4.00 a	0.5 a
T4	0	45.2 e	0.5 a	0	2.40 b	0.5 a
T5	0	2.40 b	4.0 b	0	4.00 a	0.5 a
T6	0	25.0 d	4.0 b	0	45.2 e	0.5 a
T7	0	25.0 d	4.0 b	0	45.2 e	0.5 a
T8	0	4.00 a	0.5 a	0	4.00 a	0.5 a
T9	0	4.00 a	4.0 b	0	4.00 a	4.0 b
T10	0	4.00 a	4.0 b	0	4.00 a	4.0 b
T11	0	4.00 a	4.0 b	0	4.00 a	4.0 b
T12	0	40.5 e	5.0 e	0	40.5 e	5.0 d
C.V.(%)	0.0	9.46	12.71	0.0	14.28	8.02

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*Cercospora leaf blight + Frogeye leaf spot + Brown Spot; T: treatments; averages followed by the same letter in the column do not differ in the Scott-Knott test at 0.05 of probability

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At 7 days after the fourth application, in the lower third of the plants, severe symptoms of ECD and target spot of soybean were observed in a higher percentage (40.5% and 5.0%, respectively), when the fungicide was applied in isolation and in the treatment without application (control), with significantly higher occurrences compared to the treatments that contained the mixture of protectors. The high severity observed in treatments without application (T12) and with isolated application (T1) of resistance inducers may be attributed to the great virulence of ECD and reduced latency period.

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For treatments that worked synergistically, it was observed that the application of Fertilis Phitopress Copper® (T4, T5 and T6), independently of the concentration, showed high progression in the attack intensity of ECD in the lower third of the plant, which shows low efficiency of the protector in association with fungicides. This effect was also verified in the treatment with application of NHT® Copper Super in the minimum concentration.

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According to Embrapa [10], the soybean plants infected by the ECD decrease the photosynthetic rates due to necrosis or early senescence of the leaves. This premature fall of the

161 leaves prevents the full grains formation, and earlier the defoliation occurs, the smaller the grain size
162 and, consequently, a greater loss of yield and seed quality.

163 The treatments that presented, statistically, the best results in the control of ECD in the
164 soybean crop were T2 = Unizeb Gold[®] (1.5 kg ha⁻¹), T3 = Difere[®] (0.5 L ha⁻¹) and the application of
165 NHT[®] Copper Super in a concentration higher than 0.109 L ha⁻¹ (Table 2). As the doses of NHT[®]
166 Copper Super were increased, the lower the evolution of the ECD was in the lower third of the plant,
167 however, there were no good results of these treatments in the severity of target spot of soybean in
168 the lower third, except for T8.

169 Regarding the severity of target spot of soybean, there were different responses to those
170 found in the control of ECD. The application of NHT[®] Copper Super with a concentration of 0.055 L ha⁻¹
171 (T7) and higher than 0.219 L ha⁻¹ (T9, T10 e T11), were not able to minimize the presence of the
172 disease in the lower third of the plant, and it did not differ statistically from the treatments with the
173 application of the fungicide + Fertilis Phitopress Copper[®] with a dosage of 1.0 and 1.5 L ha⁻¹ (T5 and
174 T6).

175 Significant differences in the control of fungal diseases were observed in T2 = Unizeb Gold[®]
176 (1.5 kg ha⁻¹), T3 = Difere[®] (0.5 L ha⁻¹), T4 = Fertilis Phitopress Copper[®] (0.5 L ha⁻¹) and NHT[®] Copper
177 Super - 0.109 L ha⁻¹ (T8). The associated application of the abovementioned fungicide + protectors
178 promoted a greater reduction in the number of target spot of soybean in the lower third of the plant
179 and, consequently, there was less progress of the disease.

180 A targeted study to phytosanitary control considers that the mixture in the tank of protectors
181 based on micronutrients associated to the fungicides can be an important strategy in the control of
182 fungal diseases [6]. In this work, it was possible to verify the effectiveness of three protectors able to
183 combat ECD and target spot of soybean in the lower third of soybean, T2 = Unizeb Gold[®] (1.5 kg ha⁻¹),
184 T3 = Difere[®] (0.5 L ha⁻¹) and T8 = NHT[®] Copper Super - 0.109 L ha⁻¹. This simple decision-making
185 may ultimately result in a more competitive product in the domestic/external market, greater efficiency
186 in controlling fungal diseases; minimize land use restrictions and compaction.

187 In spite of these control results and the increasing importance of these diseases in soybean
188 crop, this information on the efficiency of Cu-based protectors associated with fungicides will certainly
189 contribute to the progress of research in the area, increase the use of these protectors, increase the
190 productivity and, especially, increase the productive efficiency.

191 Concomitantly to the results found in the lower third of the soybean crop, the middle third **was**
192 also **observed** to be sensitive to the isolated application of the fungicide (T1) and the treatment without
193 application (T12), with more severe symptoms of ECD and target spot of soybean, respectively. A
194 tendency in the control of the ECD in the middle third with those of the lower third were also noted,
195 that is, the most effective protectors in the control of the ECD of the middle third were, respectively,
196 the most efficient in the lower third of the soybean plant, except Fertilis Phitopress Copper[®] (1.0 L ha⁻¹
197 ¹), which also obtained satisfactory results in controlling the disease in the middle third of the plant.
198 This fact can be explained by the uniform and homogeneous application of the fungicide in contact
199 with the entire canopy of the plant.

200 In controlling the severity of target spot of soybean in the middle third of the plant, the best
 201 results, i.e. the protectors that best control the disease are T2 = Unizeb Gold[®] (1.5 kg ha⁻¹), T3 =
 202 Difere[®] (0.5 L ha⁻¹), T4 = Fertilis Phitopress Copper[®] (0.5 L ha⁻¹), T5 = Fertilis Phitopress Copper[®] (1.0
 203 L ha⁻¹), T6 = Fertilis Phitopress Copper[®] (1.5 L ha⁻¹), T7 = NHT[®] Copper Super (0.055 L ha⁻¹) and T8 =
 204 NHT[®] Copper Super (0.109 L ha⁻¹). These results show higher criteria in these protectors selection for
 205 control of ECD and target spot of soybean in the soybean crop, giving the producer more options for
 206 application and more economically viable products.

207 In the upper third of the soybean, no possible disease was found within the complex of
 208 diseases after the fourth application of the fungicide + protector.

209 The use of the protectors in soybean crops has shown a significant improvement in the
 210 efficiency of the systemic fungicides to combat the complex of diseases of the culture. The protectors
 211 come with the objective of reducing the incidence and resistance of fungi to products with old active
 212 principles already on the market (triazoles and strobilurinss) and newer active principles, as in the
 213 case of carboxamides [5].

214 This introduction of protective fungicides in soybean crop has created a new market within the
 215 protection of plants. In this study, noted that there are differences between the market protectors and
 216 their greater efficiency is associated to the adjustment of doses and times of application. In addition to
 217 its multisite action, which acts at various points in the metabolism of the pathogen, the protectors are
 218 composed of micronutrients such as Cu, which also collaborates to raise the potential of curative
 219 products [16].

220 In soybean yield, significant differences were found by the F test. The control treatment (T12)
 221 showed the lowest average yield of 3.4 Mg ha⁻¹ and the highest increment under soybean yield was
 222 obtained when the crop presented mild severity to the pathogen attack, observed in the treatment with
 223 fungicide associated to NHT[®] Copper Super (0.109 L ha⁻¹), with productivity of 4.5 Mg ha⁻¹ (Table 3).
 224

225 **Table 3. Soybean yield depending on the application of protectors in the region of Aparecida**
 226 **do Rio Negro - TO, Brazil**

Treatments	Productivity (Mg ha ⁻¹)
T1	3.7 C
T2	3.9 B
T3	4.0 B
T4	4.0 B
T5	4.0 B
T6	3.8 C
T7	3.9 B
T8	4.5 A
T9	4.1 B
T10	3.7 C
T11	3.9 B
T12	3.4 C

Comment [REV A6]: 200 kg of difference???

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227 *T: treatments; averages followed by the same letter in the column do not differ in the Scott-Knott test at 0.05 of probability.*

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229 The yield increase in the treatment with the associated application of the fungicide + protector
230 NHT[®] Copper Super (0.109 L ha⁻¹), may be attributed to increased photosynthetic activity in the leaves
231 during the grain filling stage (R1), mainly due to the lower occurrence of fungal diseases. The larger
232 photosynthetic active leaf surface at the beginning of the reproductive stage of soybean may have
233 aided in crop establishment and consequently, an increase in production, since the development of
234 the plant depends on the interception of solar radiation for greater production of photo-assimilates
235 [17].

236 In addition, Cu is an important micronutrient related to plant growth and development. Its
237 function in the plant is linked to enzymes that participate in redox reactions, such as plastocyanin,
238 which is involved in the transport of electrons in photosynthesis [18]. It also acts as an activator of
239 enzymes that participate in the terminal electronic transport of respiration.

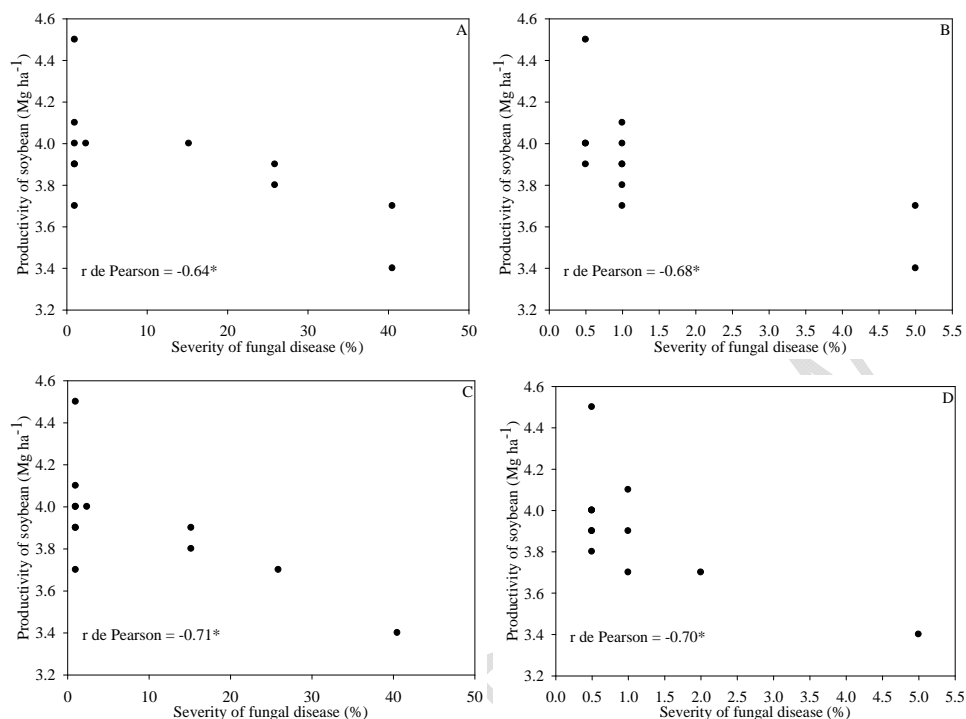
240 In this way, an important aspect to be considered in fertilization with Cu also refers to the
241 amount of this nutrient to which the plant is exposed. Sánchez-Pardo et al. [19] concluded that the
242 application of a high dose – 192 µM de CuSO₄ – in soybean plants provided changes in thylakoid
243 structure, loss of chloroplast membrane integrity and stromal degradation, as well as reduction of leaf
244 area and leaf thickness. Consequently, the photosynthetic capacity of soybean plants was significantly
245 reduced. In 2012 [20], using the same dose, the aforementioned authors verified a reduction in the
246 weight and the number of soybean nodules, in addition to a reduction in the N content in the plant.

247 Bernal et al. [21] observed that the mode of absorption of Cu by the plant could show different
248 results. When the nutrient absorption occurred by the roots, in hydroponic medium, there was
249 reduction in the biomass, the chlorophyll content and the oxygen release activity in the thylakoids of
250 the leaves. On the other hand, when absorption occurred on the leaf, Cu promoted an increase in
251 chlorophyll content and a stimulus in the photosynthetic activity of soybean plants, results that validate
252 the use of protectors via foliar application.

253 Particularly, the averages of the yields obtained in this study remained above the region
254 average of 3.4 Mg ha⁻¹, which means that the application of the fungicide + Cu-based protector tends
255 to provide plants with greater resistance to stress factors, such as attack of fungal diseases. More
256 studies should be conducted on this issue to validate this important management strategy of the
257 phytosanitary control. The treatment of seeds to control soybean Asian rust may have conferred a
258 greater initial protection to the plants, delaying the entry of disease into the area, reducing the initial
259 inoculum potential and even improving the efficiency of foliar sprays [22].

260 The correlation analysis between soybean yield and the severity of fungal diseases showed a
261 negative and significant correlation for all evaluated parameters: ECD and target spot of soybean in
262 the lower third and ECD and target spot of soybean in the middle third of soybean plants (Fig. 3).

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265

266 | **Figure. 3. Data dispersion and Pearson (r) correlation between soybean yield and severity of**
 267 **End-of-Cycle Diseases (ECD) (A) and target spot of soybean (B) in the lower third and severity**
 268 **of ECD (C) and target spot of soybean (D) in the middle third in soy leaflets.**

269 *significant correlation at 0.05 of probability.

270

271 These results demonstrated that soybean yield is strongly influenced by the degree of disease
 272 severity during the early stage of grain filling (R1), particularly for ECD and target spot of soybean in
 273 the middle third, which presented strong correlation (>0.70), in soybean plants.

274 In this context, the data obtained in the present work are innovative and certainly can
 275 compose a database for calibration of the use of multisite action protectors, products and doses,
 276 associated with the application of fungicides in soybean. Agricultural experimentation guides
 277 management actions by adding benefits that, besides presenting an efficient control of pathogens,
 278 propitiates the optimization of plant defense and metabolism mechanisms, allowing the production of
 279 higher yields and better quality products.

280

281 4. Conclusions

282 Associated applications of the fungicides + Unizeb Gold[®] (1.5 kg ha⁻¹), Difer[®] (0.5 L ha⁻¹),
 283 and the application of NHT[®] Copper Super with a concentration higher than 0.109 L ha⁻¹, were
 284 effective for the control of [common diseases in the production system of End-of-Cycle Diseases in](#)
 285 [soybean crop at Aparecida do Rio Negro – TO, Brazil.](#)

286 To control the severity of target spot of soybean the application of Unizeb Gold[®] (1.5 kg ha⁻¹),
287 Difere[®] (0.5 L ha⁻¹), Fertilis Phitopress Copper[®] (0.5 L ha⁻¹) and NHT[®] Copper Super (0.109 L ha⁻¹),
288 showed higher efficiency in the latency stage of the pathogen with greater control of the disease.

289 Associated applications of the fungicides + NHT[®] Copper Super (0.219 L ha⁻¹) reduced the
290 severity of Asian soybean rust, target spot of soybean and End-of-Cycle Diseases and showed a
291 greater increase in grain yield of 4.5 Mg ha⁻¹.

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