

Original research papers

Resistance of Soybean to Fungal Diseases Using Copper-based Protectors

ABSTRACT: Fungal diseases that affect soy crop are one of the main causes of low productivity, it is estimated that annual losses may reach 21% of total production. In this context, the objective was to evaluate the efficiency of fungicides associated with copper-based protectors for the control of diseases *Phakopsora pachyrhizi*, *Corynespora cassicola*, *Septoria glycines*, *Cercospora kikuchii* and *Cercospora sojina*, as well as the impact on grain yield in soybean crop, in the region of Aparecida do Rio Negro – TO. Treatments was applied with the application of the protector associated with fungicides Azimut[®], Orkestra[®], Ativum[®] and Horos[®] + adjuvant Assist[®]. The evaluations were performed using LICOR portable meter (LI-3000) to determine the injured areas of each soybean leaflet and at the end the grain yield was evaluated. The application of Unizeb Gold[®], Difere[®], and the application of NHT Copper Super[®] is effective for the control of CFD in soybean crop. Associated applications of the fungicide + NHT Copper Super[®] fungicide reduced the severity of *Phakopsora pachyrhizi*, *Corynespora cassicola* and CFD and showed a greater increase in grain yield.

KEYWORDS: Induction of resistance, *Glycine max*, phytosanitary control, productivity, phytopathology.

1. INTRODUCTION

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

However, there are several diseases that affect and difficult to obtain high levels of crop productivity. Among the factors responsible for the low productivity of soybeans, special attention has been given to the lack of phytosanitary care, especially those caused by fungi, which may occur during the whole cycle or only at the end of the crop cycle [2].

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

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40 The use of commercial products that activate plant defence mechanisms or that benefit the
41 action of the fungicide are commonly found in the literature and several results can be observed on
42 the use of micronutrients [4]. However, new alternatives must be found to assist the traditionally used
43 practices of disease control, and the induction of plant resistance is an alternative that can be
44 integrated to the management.

45 In this scenario, the fungicides associated with copper-based protectors (Cu) have been
46 shown to be an effective and economical alternative in crop management, due to the fact that they
47 promote additive or synergistic effects when these chemicals are used together [5]. In the plant,
48 copper has structural function in enzymes, and several proteins containing Cu are important in the
49 processes of photosynthesis, respiration, detoxification of free radicals of superoxides and lignification,
50 the latter gives greater resistance to plants from the attack of pathogens [6].

51 However, the discussion on the use of micronutrients or resistance inducers in combination
52 with fungicides in the soybean crop should be evaluated regionally. Thus, the objective of this work
53 was to evaluate the efficiency of fungicides associated with copper-based protectors for the control of
54 *Phakopsora pachyrhizi*, *Corynespora cassiicola*, *Septoria glycines*, *Cercospora sojina* and *Cercospora*
55 *kikuchii* diseases, as well as the impact on soybean crop productivity, in the region of Aparecida do
56 Rio Negro – TO.

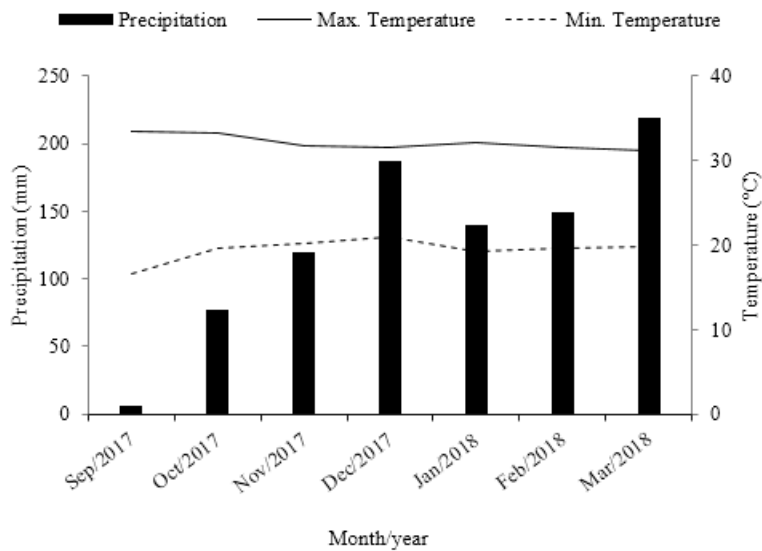
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58 2. MATERIAL AND METHODS

59 The experiment was conducted in the municipality of Aparecida do Rio Negro – TO, located
60 at 9° 57' 1" South Latitude, 47° 58' 7" West Longitude and 262 m of altitude, in an experimental area
61 belonging to Ímpar Consultoria, located on the farm Santos Agropecuária. The climate of the region is
62 classified as tropical humid and an average annual rainfall of 1,240 mm, with two well-defined periods,
63 rainy season, from November to March, with higher rainfall in December and January, and dry season,
64 from April to October.

65 Rainfall in the experimental area and temperature variation during the period of conduction
66 of the experiment are shown in Figure 1 [7].

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Figure 1. Rainfall (mm) and monthly temperature observed in the experimental area in Aparecida do Rio Negro, TO.

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The soil of the experimental area is classified as a clayey Oxisol from Cerrado [8]. The soil analysis before the implantation of the experiment showed clay content = 67, 62 and 55%, pH CaCl₂ = 5.5, 5.3 and 5.2; P (Mehlich 1) = 3.5, 2.0 and 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 = 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 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 (M.O), respectively for the layers 0-0.2, 0.2-0.4 and 0.4-0.6 m. The determinations followed the methodologies proposed by Embrapa [9].

The experimental design was in randomized blocks, with four replications. The plots were composed of 6 lines with spacing of 0.5 m and 6.0 m in length, totalling 18 m².

Distribution of the treatments involved the application of the protector associated with fungicides: Azimut[®] 0.5 L/ha⁻¹ (1st application), Orkestra[®] 0.3 L/ha⁻¹ (2nd application), Ativum[®] 0.8 L/ha⁻¹ (3rd application) and Horos[®] 0.5 L/ha⁻¹ (4th application) + adjuvant Assist[®], as described in Table 1.

Table 1. Description of the treatments (protectors and doses) to be applied in the soybean crop.

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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	-	-

93 *1 = Application with Azimut[®] at 39 Days After Emergency (DAE); 2 = Application with Orkestra[®] at 43
 94 DAE; 3 = Application with Ativum[®] at 55 DAE and 4 = Application with Horos[®] at 67 DAE.

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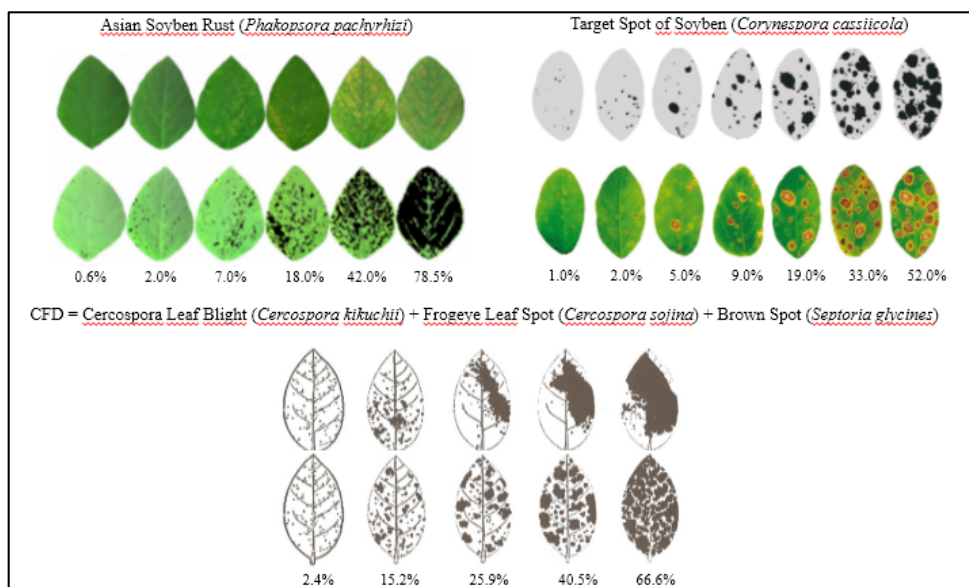
96 The soybean used was the M 8644 IPRO of indeterminate growth, treated and inoculated with
 97 Carbendazim + Tiram + Fipronil, with a population of 530 thousand plants per hectare. The planting
 98 was carried out on November 25, 2017 using direct sowing system with pneumatic seeder and traction
 99 with tractor. In the sowing fertilization, 250 kg ha⁻¹ of MAP and potassium provided by variable rate in
 100 the form of KCl were used. Phytosanitary management in pest control was carried out according to the
 101 protocol of the Fundação Chapadão [10].

102 During the experiment were evaluated the severity of the diseases *Phakopsora pachyrhizi*,
 103 *Corynespora cassicola* and *Cercospora kikuchii* + *Cercospora sojina* + *Septoria glycines*, which
 104 together were considered End-of-Cycle Diseases (CFD). The leaves were analyzed and assigned
 105 scores according to the diagrammatic scales [11,12], as shown in Figure 2.

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

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

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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 collection of each leaflet, the injured area (necrotic tissue and yellowish halo) was drawn in transparent plastic and subsequently subjected to leaf area measurement using the LICOR portable meter (LI-3000) to determine the injured area and the total area. Thus, it was possible to determine the soybean leaflet with the lowest number of injuries, intermediate injuries and the one with the most leaf injuries, thus establishing the lower, intermediate and upper limits in the diagrammatic scale, respectively.

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Grain productivity, expressed in Mg ha^{-1} , was estimated from the mass of grain, corrected to 13% moisture [13], with area for analysis of production of 3 m^{-1} , collected in two central lines of each portion, discarding 1.5 m of border at each end. The Pearson correlation (r) between grain yield and severity of fungal diseases were also evaluated in soybean plants.

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The results were submitted to analysis of variance and the means of the treatments submitted to the Scott-Knott test at 5% of probability, using the statistical program SIRVAR® [14].

3. RESULTS AND DISCUSSION

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In the region of Aparecida do Rio Negro - TO, adverse climatic conditions are found in each agricultural year, not following the same temperature pattern, relative air humidity and rainfall. Due to the factors mentioned above, the 2017/18 harvest was considered out of standard when compared to previous harvests due to the good climatic conditions observed and regular rainfall distribution (Figure

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132 1). The disease severity data show that only CFD and *Corynespora cassicola* showed some degree
 133 of infestation. *Phakopsora pachyrhizi* pustules were not observed in any of the treatments (Table 2).

134

135 **Table 2.** Severity of *Phakopsora pachyrhizi* (Pha), *Corynespora cassicola* (Cor) and CFD at 7 days
 136 after the 4th application in the lower and middle third of the soybean crop, in the region of Aparecida
 137 do Rio Negro – TO.

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Treat.	Pha (%)	*CFD (%)	Cor (%)	Pha (%)	*CFD (%)	Cor (%)
T1	0	40.5 e	5.0 c	0	25.9 d	2.0 c
T2	0	1.00 a	0.5 a	0	1.00 a	0.5 a
T3	0	1.00 a	0.5 a	0	1.00 a	0.5 a
T4	0	15.2 c	0.5 a	0	2.40 b	0.5 a
T5	0	2.40 b	1.0 b	0	1.00 a	0.5 a
T6	0	25.9 d	1.0 b	0	15.2 c	0.5 a
T7	0	25.9 d	1.0 b	0	15.2 c	0.5 a
T8	0	1.00 a	0.5 a	0	1.00 a	0.5 a
T9	0	1.00 a	1.0 b	0	1.00 a	1.0 b
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

138 **Cercospora kikuchii + Cercospora sojina + Septoria glycines*

139 Averages followed by the same letter in the column do not differ in the Scott-Knott test at 5%
 140 probability

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142 At the 7th day after the 4th application, in the lower third of the plants, severe symptoms of
 143 CFD and *Corynespora cassicola* were observed in a higher percentage (40.5% and 5.0%,
 144 respectively) when the fungicide was applied in isolation and in the treatment without application
 145 (control), with significantly higher occurrences when compared to the treatments that contained the
 146 mixture with the protectors. The high severity observed in treatments without application (T12) and
 147 with isolated application (T1) of resistance inducers can be attributed to the great virulence of CFD
 148 and reduced latency period.

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

154 According to Embrapa [9], the soybean plants infected by the CFD decrease the
 155 photosynthetic rates due to necrosis or early senescence of the leaves. This premature fall of the

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

158 The treatments that showed statistically the best results in the control of CFD in the soybean
159 crop were T2 = Unizeb Gold[®] (1.5 kg/ha⁻¹), T3 = Difere[®] (0.5 L/ha⁻¹) and the application of NHT
160 Copper Super[®] in a concentration higher than 0.109 L/ha⁻¹, according to Table 2. It is observed that,
161 as the doses of NHT Copper Super[®] were increased, the lower the evolution of the CFD was in the
162 lower third of the plant, however, there were no good results of these treatments in the severity of
163 *Corynespora cassiicola* in the lower third, except for T8.

164 Regarding the severity of *Corynespora cassiicola*, it was observed that there were different
165 responses to those found in the control of CFD. The application of NHT Copper Super[®] with a
166 concentration of 0.055 L/ha⁻¹ (T7) and higher than 0.219 L/ha⁻¹ (T9, T10 e T11), were not able to
167 minimize the presence of the disease in the lower third of the plant, and did not differ statistically from
168 the treatments with the application of the fungicide + Fertilis Phitopress Copper[®] with a dosage of 1.0
169 and 1.5 L/ha⁻¹ (T5 and T6).

170 Significant differences in the control of fungal diseases were observed in T2 = Unizeb Gold[®]
171 (1.5 kg/ha⁻¹), T3 = Difere[®] (0.5 L/ha⁻¹), T4 = Fertilis Phitopress Copper[®] (0.5 L/ha⁻¹) and NHT Copper
172 Super[®] - 0.109 L/ha⁻¹ (T8). The associated application of the abovementioned fungicide + protectors
173 promoted a greater reduction in the number of *Corynespora cassiicola* in the lower third of the plant
174 and, consequently, there was less progress of the disease.

175 A targeted study to phytosanitary control considers that the mixture in the tank of protectors
176 based on micronutrients associated to the fungicides can be an important strategy in the control of
177 fungal diseases [4]. It is observed that in this work it was possible to verify the effectiveness of three
178 protectors able to combat CFD and *Corynespora cassiicola* in the lower third of soybean, T2 = Unizeb
179 Gold[®] (1.5 kg/ha⁻¹), T3 = Difere[®] (0.5 L/ha⁻¹) and T8 = NHT Copper Super[®] - 0.109 L/ha⁻¹. This simple
180 decision-making may ultimately result in a more competitive product in the domestic/external market,
181 greater efficiency in controlling fungal diseases; minimize land use restrictions and compaction.

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

186 Concomitantly to the results found in the lower third of the soybean crop, the middle third also
187 showed to be sensitive to the isolated application of the fungicide (T1) and the treatment without
188 application (T12), with more severe symptoms of CFD and *Corynespora cassiicola*, respectively. It is
189 also observed a tendency in the control of the CFD in the middle third with those of the lower third,
190 that is, the most effective protectors in the control of the CFD of the middle third were, respectively, the
191 most efficient in the lower third of the soybean plant, except Fertilis Phitopress Copper[®] (1.0 L/ha⁻¹),
192 which also obtained satisfactory results in controlling the disease in the middle third of the plant. This
193 fact can be explained by the uniform and homogeneous application of the fungicide in contact with the
194 entire canopy of the plant.

195 In controlling the severity of *Corynespora cassiicola* in the middle third of the plant, it is
 196 observed that the best results, i.e. the protectors that best control the disease are T2 = Unizeb Gold[®]
 197 (1.5 kg/ha⁻¹), T3 = Difere[®] (0.5 L/ha⁻¹), T4 = Fertilis Phitopress Copper[®] (0.5 L/ha⁻¹), T5 = Fertilis
 198 Phitopress Copper[®] (1.0 L/ha⁻¹), T6 = Fertilis Phitopress Copper[®] (1.5 L/ha⁻¹), T7 = NHT Copper
 199 Super[®] (0.055 L/ha⁻¹) and T8 = NHT Copper Super[®] (0.109 L/ha⁻¹). These results show higher criteria
 200 in the selection of these protectors for control of CFD and *Corynespora cassiicola* in the soybean crop,
 201 giving the producer more options for application and more economically viable products.

202 In the upper third of the soybean, no possible disease was found within the complex of
 203 diseases after the 4th application of the fungicide + protector.

204 The use of the protectors in soybean crops has shown a significant improvement in the
 205 efficiency of the systemic fungicides to combat the complex of diseases of the culture. The protectors
 206 come with the objective of reducing the incidence and resistance of fungi to products with old active
 207 principles already on the market (triazoles and strobilurinss) and newer active principles, as in the
 208 case of carboxamides.

209 This introduction of protective fungicides in soybean crop has created a new market within the
 210 protection of plants. In this study it was possible to observe that there are differences between the
 211 market protectors and their greater efficiency is associated to the adjustment of doses and times of
 212 application. In addition to its multisite action, which acts at various points in the metabolism of the
 213 pathogen, the protectors are composed of micronutrients such as Cu, which also collaborates to raise
 214 the potential of curative products [15].

215 In soybean yield, significant differences were found by the F test. The control treatment (T12)
 216 showed the lowest average yield of 3.4 Mg ha⁻¹ and the highest increment under soybean yield was
 217 obtained when the crop presented mild severity to the pathogen attack, observed in the treatment with
 218 fungicide associated to NHT Copper Super[®] (0.109 L/ha⁻¹), with productivity of 4.5 Mg ha⁻¹ (Table 3).
 219

220 **Table 3.** Soybean productivity depending on the application of protectors in the region of Aparecida do
 221 Rio Negro - TO, harvest 2017/18.

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

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C.V (%)

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224 Averages followed by the same letter in the column do not differ in the Scott-Knott test at 5%
225 probability.]

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228 The increase in productivity observed in the treatment with the associated application of the
229 fungicide + protector NHT Copper Super[®] (0.109 L/ha⁻¹), may be attributed to increased
230 photosynthetic activity in the leaves during the grain filling stage (R1), mainly due to the lower
231 occurrence of fungal diseases. The larger photosynthetic active leaf surface at the beginning of the
232 reproductive stage of soybean may have aided in crop establishment and consequently, an increase
233 in production, since the development of the plant depends on the interception of solar radiation for
greater production of photo-assimilates [16].

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235 In addition, Cu is an important micronutrient related to plant growth and development. Its
236 function in the plant is linked to enzymes that participate in redox reactions, such as plastocyanin,
237 which is involved in the transport of electrons in photosynthesis [17]. It also acts as an activator of
238 enzymes that participate in the terminal electronic transport of respiration.

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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. [18] 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. As a consequence, the photosynthetic capacity of soybean plants was
245 significantly reduced. In 2012 [19], using the same dose, the aforementioned authors verified a
246 reduction in the weight and the number of soybean nodules, in addition to a reduction in the N content
247 in the plant.

248

249 A study by Bernal et al. [20] reveals that the mode of absorption of Cu by the plant can show
250 different results. When the nutrient absorption occurred by the roots, in hydroponic medium, there was
251 reduction in the biomass, the chlorophyll content and the oxygen release activity in the thylakoids of
252 the leaves. On the other hand, when absorption occurred on the leaf, Cu promoted an increase in
253 chlorophyll content and a stimulus in the photosynthetic activity of soybean plants, results that validate
254 the use of protectors via foliar application.

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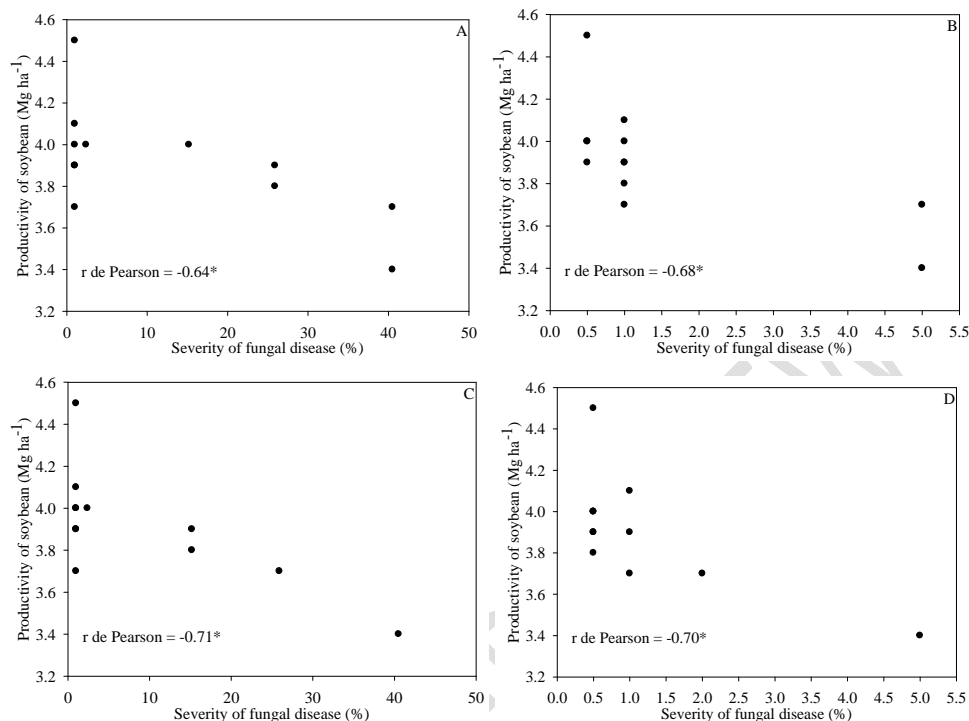
256 Particularly, the averages of the yields obtained in this study remained above the region
257 average of 3.4 Mg ha⁻¹, which means that the application of the fungicide + Cu-based protector tends
258 to provide plants with greater resistance to stress factors, such as attack of fungal diseases. More
259 studies should be conducted on this issue to validate this important management strategy of the
260 phytosanitary control. Also mentions that the treatment of seeds to control soybean Asian rust may
261 have conferred a greater initial protection to the plants, delaying the entry of disease into the area,
reducing the initial inoculum potential and even improving the efficiency of foliar sprays [21].

262

263 The correlation analysis between soybean yield and the severity of fungal diseases showed a
264 negative and significant correlation for all evaluated parameters: CFD and *Cercospora kikuchii* in the
265 lower third and CFD and *Cercospora kikuchii* in the middle third of soybean plants (Figure 3).

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265 **Figure 3.** Data dispersion and Pearson (r) correlation between soybean yield and severity of CFD (A)
266 and *Corcospora kikuchii* (B) in the lower third and severity of CFD (C) and *Corcospora kikuchii* (D) in
267 the middle third in soy leaflets.
268 *significant correlation at 5%.

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These results demonstrate that soybean yield is strongly influenced by the degree of disease severity during the early stage of grain filling (R1), particularly for CFD and *Corcospora kikuchii* in the middle third, which presented strong correlation (>0.70), in soybean plants. In this context, the data obtained in the present work are innovative and certainly can compose a database for calibration of the use of multisite action protectors, products and doses, associated with the application of fungicides in soybean

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Agricultural experimentation guides management actions by adding benefits that, besides presenting an efficient control of pathogens, propitiates the optimization of plant defense and metabolism mechanisms, allowing the production of higher yields and better quality products.

279

280 4. Conclusions

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The application of 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, are effective in controlling CFD in soybean crop.

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284 To control the severity of *Corynespora cassiicola* the application of Unizeb Gold[®] (1.5 kg/ha⁻¹)
285 ¹), Difere[®] (0.5 L/ha⁻¹), Fertilis Phitopress Copper[®] (0.5 L/ha⁻¹) and NHT Copper Super[®] (0.109 L/ha⁻¹),
286 showed higher efficiency in the latency stage of the pathogen with greater control of the disease.

287 Associated applications of fungicide + NHT Copper Super[®] (0.219 L/ha⁻¹) reduced the severity
288 of *Phakopsora pachyrhizi*, *Corynespora cassiicola* and CFD and showed a greater increase in grain
289 yield.

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