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 cassiicola*, *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).

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

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

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

2. MATERIAL AND METHODS

The experiment was conducted in the municipality of Aparecida do Rio Negro – TO, located at 9º 571’ 7’’ South Latitude, 47º 58’ 7’’ West Longitude and 262 m of altitude, in an experimental area belonging to Ímpar Consultoria, located on the farm Santos Agropecuária. The climate of the region is classified as tropical humid and an average annual rainfall of 1,240 mm, with two well-defined periods, rainy season, from November to March, with higher rainfall in December and January, and dry season, from April to October.

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

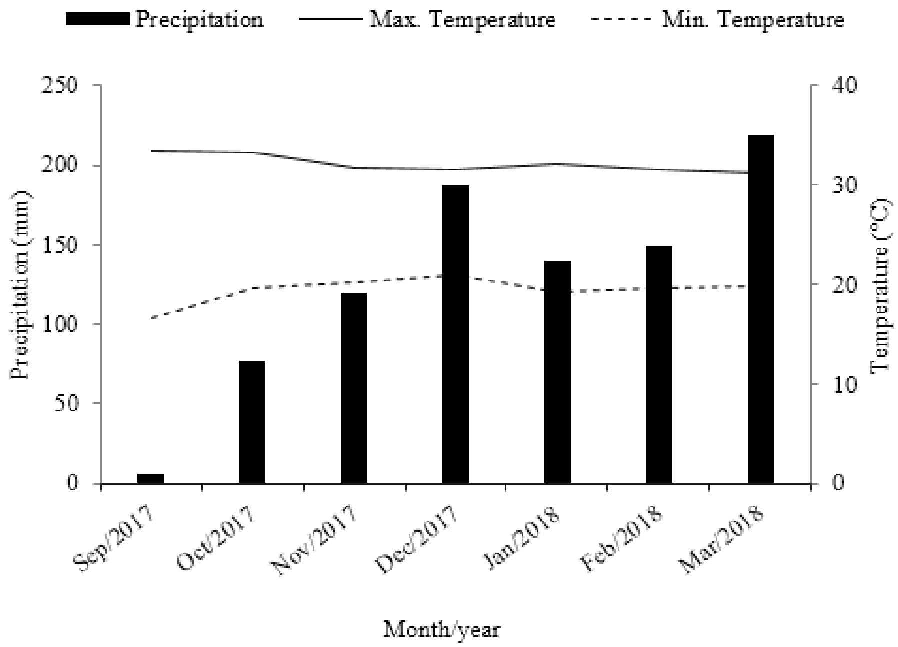


Figure 1. Rainfall (mm) and monthly temperature observed in the experimental area in Aparecida do Rio Negro, TO.

The soil of the experimental area is classified as in 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 Metter (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, totaling 18 m^2.

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.

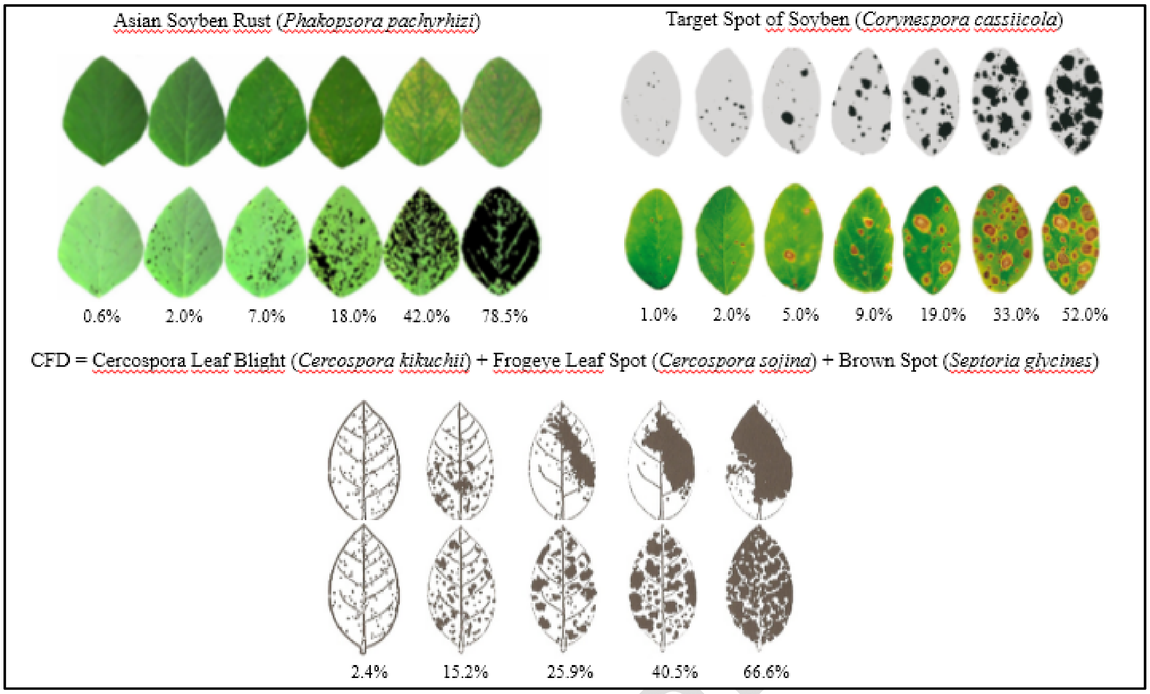
Treat.	Protector (Product/Dose)	Adjuvant (L/ha^{-1})	*Seasons of application
T1	Fungicides	0.5	1.2.3.4
T2	Fungicides + Unizeb Gold® -1.5 kg/ha^{-1}	0.5	1.2.3.4
T3	Fungicides + Difere® - 0.5 L/ ha^{-1}	0.5	1.2.3.4
T4	Fungicides + Fertilis Phitopress Copper® - 0.5 L/ ha^{-1}	0.5	1.2.3.4
T5	Fungicides + 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 a population of 530 thousand plants per hectare. The planting was carried out on November 25, 2017 using direct sowing system with pneumatic seeder and traction with tractor. In the sowing fertilization, 250 kg ha^{-1} of 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 [10].

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

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



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

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.

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.

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

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

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

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

Treat.	***Pha* (%)**	***CFD (%)**	***Cor* (%)**	***Pha* (%)**	***CFD (%)**	***Cor* (%)**
	----------- Lower third ---------			--------- Middle third ---------		
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

**Cercospora kikuchii* + *Cercospora sojina* + *Septoria glycines*
Averages followed by the same letter in the column do not differ in the Scott-Knott test at 5% probability

At the 7th day after the 4th application, in the lower third of the plants, severe symptoms of CFD and *Corynespora cassiicola* 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 when compared to the treatments that contained the mixture with the protectors. The high severity observed in treatments without application (T12) and with isolated application (T1) of resistance inducers can be attributed to the great virulence of CFD and reduced latency period.

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

According to Embrapa [9], the soybean plants infected by the CFD decrease the photosynthetic rates due to necrosis or early senescence of the leaves. This premature fall of the leaves prevents the full formation of the grains, and earlier the defoliation occurs, the smaller the grain size and, consequently, a greater loss of yield and seed quality.

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

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

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

A targeted study to phytosanitary control considers that the mixture in the tank of protectors based on micronutrients associated to the fungicides can be an important strategy in the control of fungal diseases [4]. It is observed that in this work it was possible to verify the effectiveness of three protectors able to combat CFD and *Corynespora cassiicola* in the lower third of soybean, T2 = Unizeb 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 decision-making may ultimately result in a more competitive product in the domestic/external market, greater efficiency in controlling fungal diseases; minimize land use restrictions and compaction.

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

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

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

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

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

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

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

Table 3. Soybean productivity depending on the application of protectors in the region of Aparecida do 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

C.V (%)	7.59

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

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

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

In this way, an important aspect to be considered in fertilization with Cu also refers to the amount of this nutrient to which the plant is exposed. Sánchez-Pardo et al. [18] concluded that the application of a high dose – 192 µM de $CuSO_4$ – in soybean plants provided changes in thylakoid structure, loss of chloroplast membrane integrity and stromal degradation, as well as reduction of leaf area and leaf thickness. As a consequence, the photosynthetic capacity of soybean plants was significantly reduced. In 2012 [19], using the same dose, the aforementioned authors verified a reduction in the weight and the number of soybean nodules, in addition to a reduction in the N content in the plant.

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

Particularly, the averages of the yields obtained in this study remained above the region average of 3.4 Mg ha^{-1}, which means that the application of the fungicide + Cu-based protector tends to provide plants with greater resistance to stress factors, such as attack of fungal diseases. More studies should be conducted on this issue to validate this important management strategy of the phytosanitary control. Also mentions that the treatment of seeds to control soybean Asian rust may 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].

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

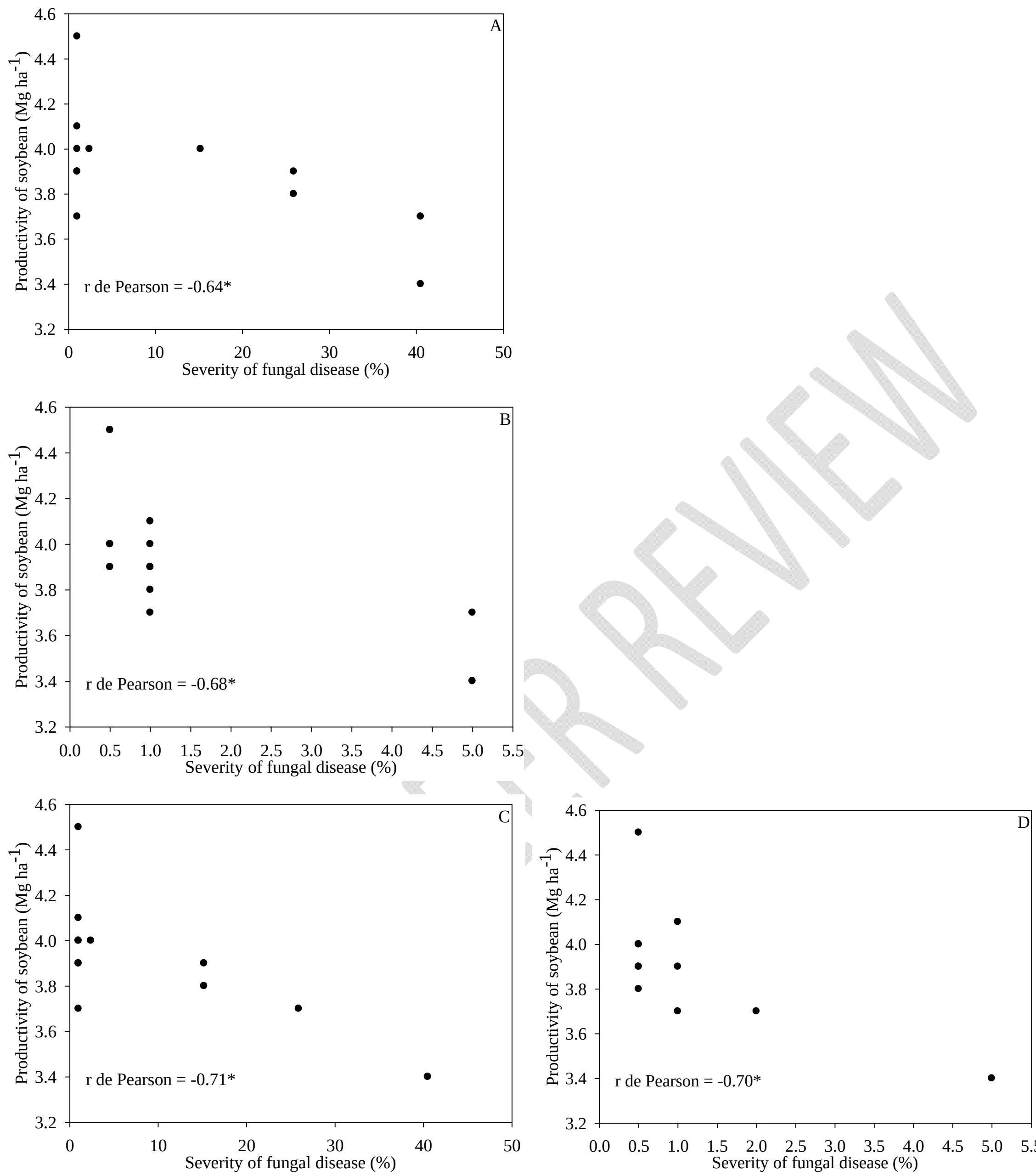


Figure 3. Data dispersion and Pearson (r) correlation between soybean yield and severity of CFD (A) and *Corcospora kikuchii* (B) in the lower third and severity of CFD (C) and *Corcospora kikuchii* (D) in the middle third in soy leaflets.
*significant correlation at 5%.

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

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.

4. Conclusions

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.

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

Associated applications of fungicide + NHT Copper Super® (0.219 L/ha^{-1}) reduced the severity of *Phakopsora pachyrhizi*, *Corynespora cassicola* and CFD and showed a greater increase in grain yield.

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