Original research papers

Use of Copper-Based Protectors to Control Fungal Diseases of Soybean

ABSTRACT: At level word fungal 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*), 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 application by of different products and doses of copper-based protectors associated with fungicides as 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 mature, 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}.

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 [1,2]. 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 [3].

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 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 [4].

The diseases affecting in 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 [5]. The most common diseases during the vegetative and reproductive cycle are the powdery mildew (*Microsphaera diffusa*), mildew (*Peronospora manshurica*), anthracnose (*Colletotrichum truncatum*), target spot (*Corynespora cassicola*), teleomorph (*Thanatephorus cucumis*) and especially the Asian soybean rust (*Phakopsora pachyrhizi* Sydow & P. Sydow) [6].

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 may be observed on micronutrients use [7]. 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 (Cu) protectors have been shown to be an effective and economical alternative in crop management, because they promote additive or synergistic effects when these chemicals are used together [8]. In the plant, Cu has structural function in enzymes, and several proteins containing Cu are important in the processes of photosynthesis, respiration, detoxification of free superoxides radicals and lignification, and the latter gives greater resistance to plants from the attack of pathogens [9].

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

2. MATERIAL AND METHODS

The experiment was conducted in the country of Aparecida do Rio Negro – TO, Brazil, 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 tropical humid 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. The average annual rainfall is 1,240 mm.

Rainfall in the experimental area and temperature variation during the period of conduction of the experiment are shown in Fig. 1 [10].

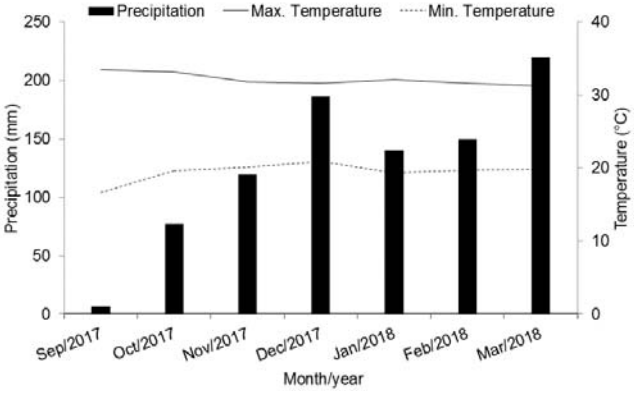

Figure 1. Rainfall (mm) and monthly temperature observed in the experimental area in Aparecida do Rio Negro, TO, Brazil

The soil of the experimental area was a clayey Oxisol from Cerrado [11]. Soil analysis made before the implantation of the experiment showed, for the layers 0-0.2, 0.2-0.4 and 0.4-0.6 m, respectively: clay content = 67, 62 and 55%, pH $CaCl_2$ = 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. The determinations followed the methodologies proposed by Embrapa [12].

The experimental design was in randomized blocks, with four replications. The plots were composed of six 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} (first application), Orkestra® 0.3 L ha^{-1} (second application), Ativum® 0.8 L ha^{-1} (third application) and Horos® 0.5 L/ha^{-1} (fourth application) + adjuvant Assist®, with applications volume of 200 L ha^{-1}, as described in Table 1.

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 [13].

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 cassiicola*) and cercospora leaf blight (*Cercospora kikuchii*) + frogeye leaf spot (*Cercospora sojina*) + brown spot (*Septoria glycines*), which together were considered End-of-Cycle Diseases (ECD) [6].

For the assessment/quantification of disease severity accurately, diagrammatic scales were used, which are illustrated representations of a series of leaves with symptoms at different levels of severity [14,15] (Fig. 2). Thus, in its elaboration, it is necessary to consider aspects such as the upper and lower limits, which must correspond, respectively, to the maximum and minimum amount of the disease found in the field; the representation of the symptoms, and should be as close as possible to those observed in the plant; and the intermediate levels of disease severity, considering the limitations of acuity of human vision, as defined by the " Weber-Fechner's Law of Stimulation " [16,17].

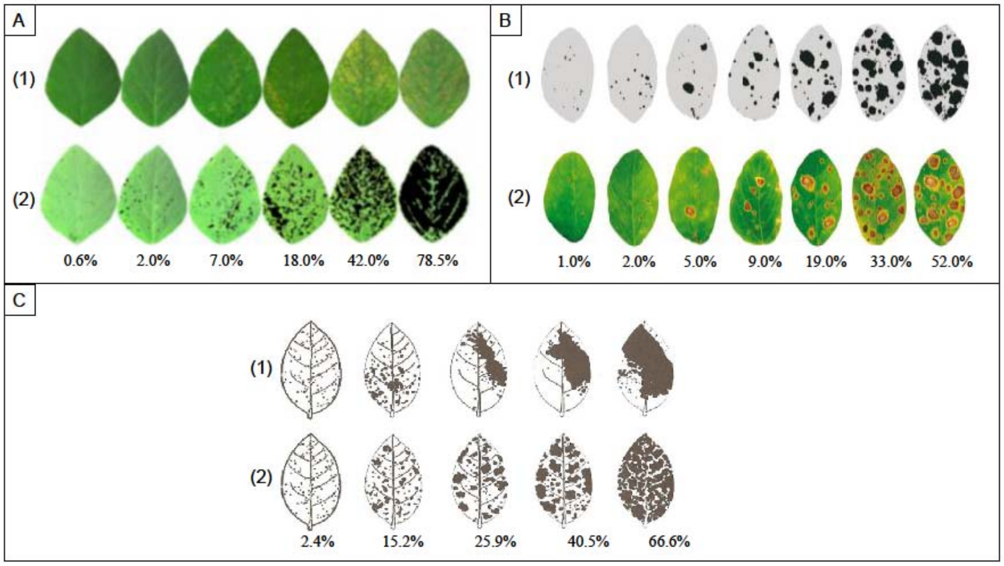

Figure 2. Diagrammatic scales of asian soybean rust (A), target spot of soybean (B) and End-Cycle Diseases (C) for evaluation the severity of fungal diseases in soybean

(1) Top panel: Aggregated symptoms. (2) 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 it was subjected to leaf area measurement using the LI-COR® portable meter (LI-3000) to determine the injured area and the total area. Therefore, 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 yield (in Mg ha^{-1}), was estimated from the mass of grain, corrected to 13% moisture [18], with area for analysis of production of 3 m^{-1}, collected in two central lines of each plot, 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 data were submitted to analysis of variance ($p < 0.05$) and when significant differences were found the means of the treatments were submitted to the Scott-Knott test at 0.05 of probability, using the statistical program SIRVAR® [19].

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 aforementioned fact, the 2017/18 crop harvest was considered out of standard when compared to previous harvests, due to the good climatic conditions observed and regular rainfall distribution (Fig. 1). Disease severity data show that only ECD and target spot of soybean presented some degree of infestation. Asian soybean rust pustules were not observed in any of the treatments (Table 2).

Treat.	**ASR (%)**	***ECD (%)**	**TSS (%)**	**ASR (%)**	***ECD (%)**	**TSS (%)**
	----------- 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

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

**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*

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.

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.

According to Embrapa [12], 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 leaves prevents the full grains formation, and earlier the defoliation occurs, the smaller the grain size and, consequently, a greater loss of yield and seed quality [20].

The treatments that presented, statistically, the best results in the control of ECD 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} (Table 2). As the doses of NHT® Copper Super were increased, the lower the evolution of the ECD was in the lower third of the plant, however, there were no good results of these treatments in the severity of target spot of soybean in the lower third, except for T8.

Regarding the severity of target spot of soybean, there were different responses to those found in the control of ECD. 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 it 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 target spot of soybean 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 [7]. In this work, it was possible to verify the effectiveness of three protectors able to combat ECD and target spot of soybean 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 was also observed to be sensitive to the isolated application of the fungicide (T1) and the treatment without application (T12), with more severe symptoms of ECD and target spot of soybean, respectively. A tendency in the control of the ECD in the middle third with those of the lower third were also noted, that is, the most effective protectors in the control of the ECD 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 target spot of soybean in the middle third of the plant, 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 these protectors selection for control of ECD and target spot of soybean 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 fourth 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 [7].

This introduction of protective fungicides in soybean crop has created a new market within the protection of plants. In this study, noted 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 [21].

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 yield depending on the application of protectors in the region of Aparecida do Rio Negro - TO, Brazil

Treatments	Productivity (Mg ha^{-1})	Standard deviation (%)
T1	3.7 c	0.19
T2	3.9 b	0.24
T3	4.0 b	0.26
T4	4.0 b	0.30
T5	4.0 b	0.28
T6	3.8 c	0.17
T7	3.9 b	0.24
T8	4.5 a	0.35
T9	4.1 b	0.27
T10	3.7 c	0.20
T11	3.9 b	0.25
T12	3.4 c	0.18
C.V (%)	7.59	-

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

The yield increase 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 [22].

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 [23]. 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. [24] 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. Consequently, the photosynthetic capacity of soybean plants was significantly reduced. In 2012 [25], 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.

Bernal et al. [26] observed that the mode of absorption of Cu by the plant could 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. 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 [27].

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

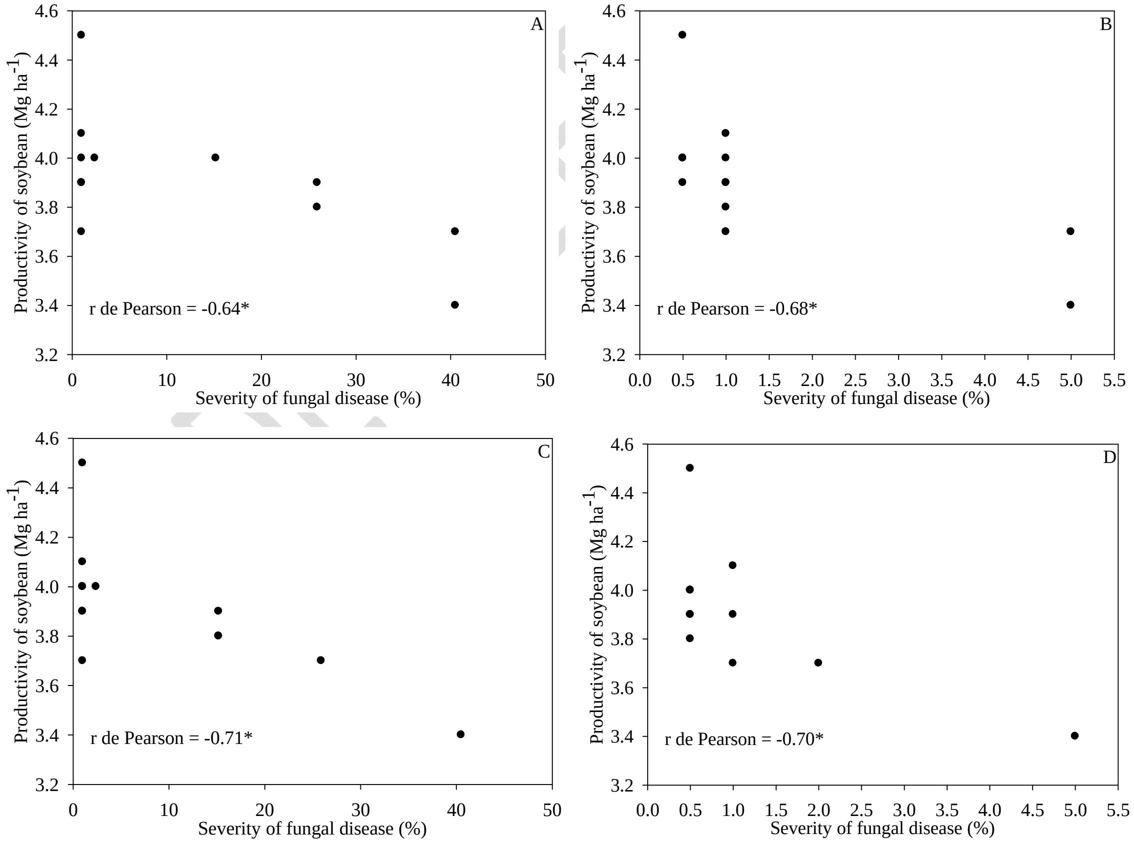

Figure 3. Data dispersion and Pearson (r) correlation between soybean yield and severity of End-of-Cycle Diseases (ECD) (A) and target spot of soybean (B) in the lower third and severity of End-Cycle Diseases (ECD) (C) and target spot of soybean (D) in the middle third in soybean leaflets.

**significant correlation at 0.05 of probability.*

These results demonstrated that soybean yield is strongly influenced by the degree of disease severity during the early stage of grain filling (R1), particularly for ECD and target spot of soybean 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

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 common diseases in the production system of soybean crop at Aparecida do Rio Negro – TO, Brazil.

To control the severity of target spot of soybean 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 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}.

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