Original research papers

Use of Copper-Based Pesticides to Control Fungal Diseases of Soybean in Eastern Brazil

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 the study was to evaluate the efficiency of copper-based protectors associated with fungicides to control soybean diseases such 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 as late-crop cycle diseases, with impact on grain yield, in the region of Aparecida do Rio Negro – TO, Brazil. Treatments were composed of different rates of copper-based pesticides associated with fungicides like Azimut® (first application), Orkestra® (second application), Ativum® (third application) and Horos® (fourth application) in soybean. Diseases were identified and crop damage evaluations on leaves were performed using LI-COR® portable meter 7 days after the fourth application. At physiological maturity, grain yield was evaluated. Combined rates of fungicides + Unizeb Gold® (1.5 kg ha^{-1}), Difere® (0.5 L ha^{-1}), and NHT® Copper Super at a rate higher than 0.109 L ha^{-1}, were effective to control late crop-cycle diseases in soybean. Associated applications of fungicides + 0.219 L/ha of NHT® Copper Super reduced the severity of Asian soybean rust, target spot of soybean and late crop-cycle diseases with a greater increase in grain yield (4.5 Mg ha^{-1}).

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

1. INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the most important economic segments of Brazilian agribusiness and one of main crops used during the harvest period, in the Northern region of Brazil [1,2]. Tocantins covers a soybean cultivated area of 956 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, this crop is prone to lower yields due to its infestations by several diseases. Among factors affecting soybean yields, lies the lack of any crop protection strategy against fungus species which may cause damage over the whole crop cycle or only at late-crop cycle [4].

A complex of diseases affecting the final phase of crop cycle, namely *Cercospora kikuchii, Cercospora sojina* and *Septoria glycines)* causes 21% of production losses as well as reduction in seed weight [5]. Most common diseases affecting crop vegetative and flowering stages are 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 to activate plant defense mechanisms or to benefit from fungicide effects are often reported in the literature and several findings on micronutrients use are available [7]. However, new alternatives need to be developed to challenge traditional practices of disease control. The induction of plant resistance is also an alternative to be integrated in the crop protection strategy.

In this scenario, fungicides associated with copper-based (Cu) pesticides were shown as an effective and economical alternative in crop management, as they enhance additive or synergistic effects when used in combination with chemicals [8]. In the plant, Cu has a structural function in enzymes, and several proteins containing Cu are important in a number of biological processes such as photosynthesis, respiration, detoxification of free superoxides radicals, lignification, and plant resistance to pathogens [9].

Therefore, the use of micronutrients or resistance inducers in combination with fungicides in soybean needs to be evaluated on regional basis in Brazil. The objective of this work was to evaluate the efficiency of copper-based protectors associated with fungicides to control soybean diseases.

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 on Santos Agropecuária farm. The climate of the region is tropical wet 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 study are shown in Fig. 1 [10].

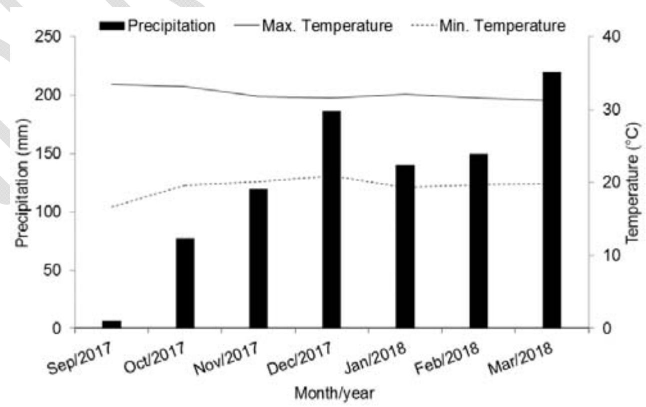

Figure 1. Monthly rainfall and temperature observed over the period of study in Aparecida do Rio Negro-TO, Brazil.

Soil type in the experimental area was a clayey Oxisol from Cerrado [11]. Results of soil analyses made before setting up the experiment determined following Embrapa methods [12] are show in Table 1.

The experimental design was a randomized complete block with four replications. Each plot was composed of six lines of 6 m in length with 0.5 m of inter-row spacing which give 18 m² of land surface.

All different treatments used are defined in Table 2.

Table 2. Description of pesticides and their rates applied in soybean.

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 variety of soybean used was M 8644 IPRO which seeds were treated and inoculated with Carbendazim + Tiram + Fipronil and sowed at a density of 530 plants per hectare. Direct sowing achieved on November 25, 2017, using a pneumatic tractor-seeder. At sowing rates of KCl were applied in combination of 250 kg ha^{-1} of Monoammonium phosphate (MAP). Crop protection was carried out according to Fundação Chapadão protocol [13].

Seven days after the fourth application, the severity of the following diseases was evaluated: 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].

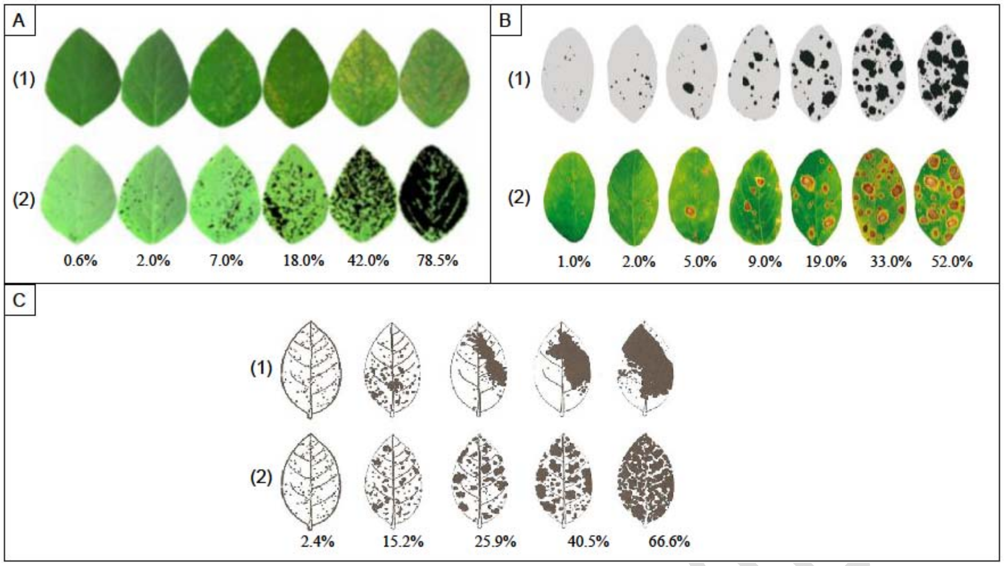

Figure 2. Diagrammatic scales of asian soybean rust (A), target spot of soybean (B) and End-Cycle Diseases (C) for evaluation the severity of fungal diseases in soybean

(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).

Table 3. 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.	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

**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 yields depending on different application rates of pesticides.

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).

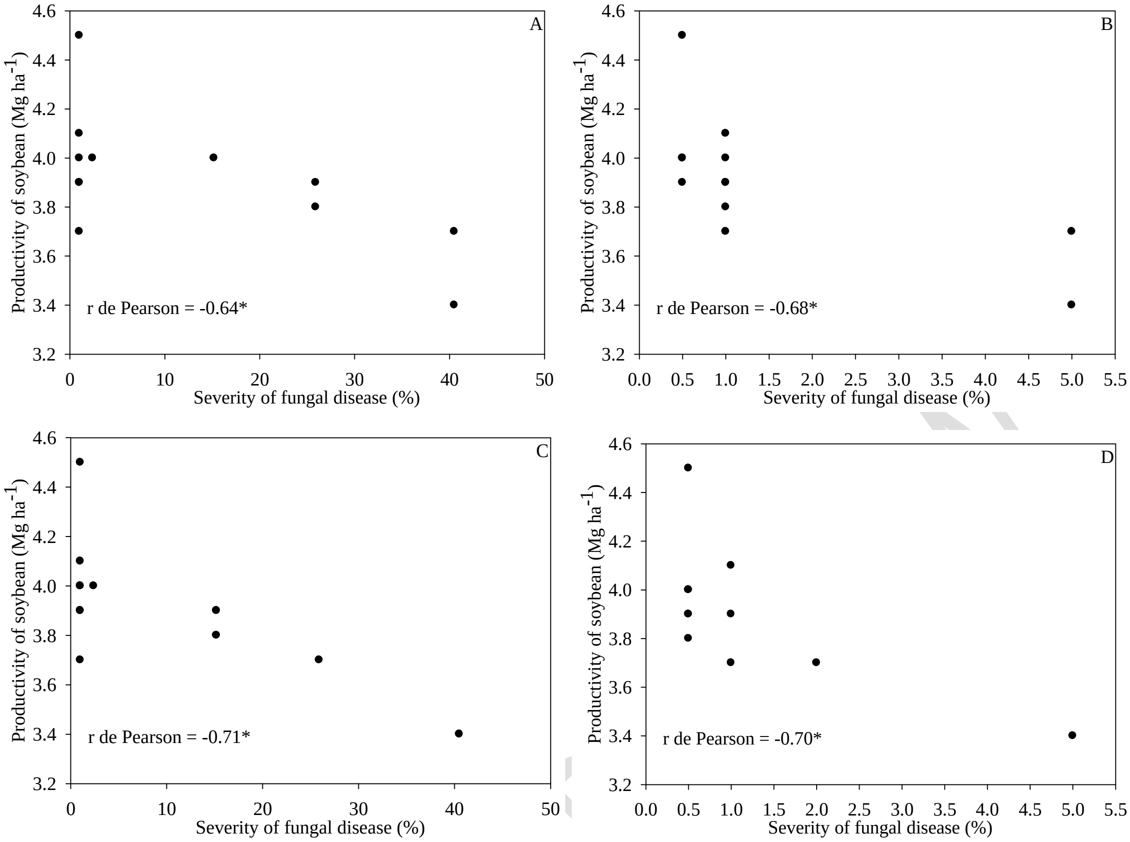

Figure 3. Data dispersion and Pearson (r) correlation between soybean yield and severity of End-of-Cycle Diseases (ECD) (A) and target spot of soybean (B) in the lower third and severity of End-Cycle Diseases (ECD) (C) and target spot of soybean (D) in the middle third in soybean leaflets.

**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

Application of fungicides in combination of Unizeb Gold® (1.5 kg ha^{-1}), Difere® (0.5 L ha^{-1}) as well as NHT® Copper Super with a rate higher than 0.109 L ha^{-1}, were effective in the control of common diseases in soybean.

To control the severity of soybean fungal infections, a combination of fungicides with 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}), was found highly effective.

Particularly, a combined application of fungicides with 0.219 L ha^{-1} NHT® Copper Super reduced the severity of Asian soybean rust and late crop-cycle diseases, and induced a grain yield increase of 4.5 Mg ha^{-1}.

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