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RESEARCH ARTICLE

Foliar fortification of Copper (Cu) in *Glycine max* L. for the protection against Asian Soybean Rust (*Phakopsora pachyrhizi* Syd. & P.Syd.)

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ABSTRACT

The Asian Soybean Rust caused by the fungus *Phakopsora pachyrhizi* is one of the serious phytosanitary problems faced by soybean [*Glycine max* (L.) Merrill], which cause up to 80% yield loss. An alternative for the integrated management of the disease is the use of mineral nutrition together with phytosanitary treatments. Thus, the objective of this study is to understand the effect of foliar fortification with copper (Cu) along with phytosanitary treatments in the soybean reaction to Rust by lignin content variation in leaf tissues, and how it reflects the yield. The experimental design was a randomized block with four replicates. Four concentrations Cu (30, 60, 90, 120 g Cu ha^{-1}) were tested in two distinct sources (cuprous oxide and copper carbonate) together with phytosanitary treatments. Evaluations were made to determine the progression of Asian Rust severity, micronutrient content in leaves and grains, as well as lignin content in leaves. The grain yield components and productivity were also evaluated. The Cu contents in the soybean leaves and grains were influenced by foliar spraying. Foliar spray with Cu retarded the disease progression, reducing the severity of Asian Rust and positively impacting grain yield. The amount of lignin present in the leaves was altered considerably with the application of the Cu associated with phytosanitary treatments. The results suggest that the leaf nutrition with copper together with phytosanitary treatments, may reduce the rust severity and improvement the plant performance. Future research with Cu application and analysis of specific enzymes, secondary metabolites and cell wall thickness may further contribute to the understanding of the role of Cu in defence against Asian Soybean Rust.

Introduction

Soybean [*Glycine max* (L.) Merrill] is one of major oilseed crops, with wide versatility of uses as a source of oil and protein, raw material in the food industry, cosmetics, human and animal feed and biodiesel production (1–3). However, several phytosanitary problems hinder the natural development and production of this crop. Among them, Asian Soybean Rust (*Phakopsora pachyrhizi* Syd. & P. Syd.) is considered as the most destructive fungal disease of the plant (1, 2). The conventional method for controlling the disease is the chemical treatment with fungicide (2, 3); however, this causes environmental degradation (7). Moreover, the resistance of fungus to some classes of fungicides is also a problem for disease control (5).

Therefore, more sustainable alternatives are needed for disease control. The micro and macronutrients have an impact on disease resistance and control (7), alongside its importance for growth and development. Micronutrients are the main elements associated with the induction of resistance. They are involved in many physiological and biochemical processes of the production of defence compounds (4, 5). They also play a vital role in inhibiting the pathogen from penetrating by affecting the cell wall rigidity and even the physical integrity of the membrane structure (7).

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Copper (Cu), a micronutrient, plays a primal role in the physicochemical processes of plants like gas exchange, metabolism and conduction of nutrients. It also participates in the metabolic transport and formation of resistance compounds, such as lignin, that imparts strength and rigidity of the cell wall (4, 6). Thus, copper has the potential to control diseases. Copper, even though required in small quantities by soybean plants, is considered essential for the crop to complete its vegetative cycle (10). The foliar fortification of Cu interferes with the reaction of Asian Soybean Rust. Hence, the foliar spraying of this micronutrient is capable of retarding the progression of Asian Rust severity. According to studies (11–13), Cu increases the rigidity of the cell wall by an increase of the lignin deposition. So, the foliar application of copper can inhibit the penetration of the fungus. Thus, the objective of this study was to identify how the foliar fortification with copper, along with phytosanitary treatments, interferes in the soybean reaction to Asian Rust, by lignin content variation in leaf tissues and change in the soybean yield components.

Materials and Methods

The BMX Ativa RR (BRASMAX Genética), genetically modified glyphosate-resistant soybean cultivar, with a maturation group 5.6, small size, determined growth habit and susceptibility to diseases was used in this experiment. Soybean was sown on December 6, 2016, in Passo Fundo (28°12´S, 52° 23´W; ± 667 m), Rio Grande do Sul, Brazil. The climate in the study area is Cfa subtropical, as classified by Köppen, with temperatures in the hottest month > 26 ± 3 °C and temperatures in the coldest month > 15 ± 4 °C. The rainfall and temperature occurred and normal during the crop cycle are shown in Fig. 1.

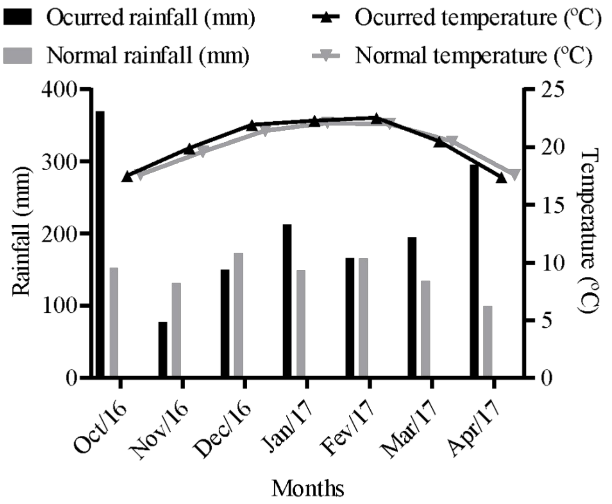

Fig. 1. Rainfall and average temperature occurred and normal during the crop cycle.
Source: Embrapa trigo 2016/2017.

Soil is classified as humid dystrophic red latosol (14). Soil samples were collected at two depths of 0–10 cm and 10–20 cm to characterize the soil chemical attributes of the experimental area (15).

Experimental design

A randomized block design with four replications was used, totalling 36 plots. Each experimental plot was composed of six five-meters long sowing lines spaced 0.50 m apart. The first and sixth row of sowing of each plot served as borders for the experiment, thus totalling 10 m² of useful area.

The experiment was conducted in an area naturally infested by *P. pachyrhizi*, under the no-tillage system, and on black oats. The *P. pachyrhizi* survival throughout the year relies on the continued production of uredospores on a suitable host, and more than 90 legume species serve as *P. pachyrhizi* hosts (16). Therefore, natural transmission suffices uniformity of inoculum in the experiment.

The herbicide glyphosate (720 g kg^{-1} i.a) (dose: 1.5 kg^{-1}) + saflufenacil (700 g kg^{-1} i.a) (dose: 0.08 kg ha^{-1}) was used for the management of weeds, which was applied at the phenological stage V3 (17). The seeds used for sowing had undergone pre-treatment with insecticides imidacloprid (150 g L^{-1} i.a.) + thiodicarb (450 g L^{-1} i.a.). Fertilization of sowing furrow was done with 6 kg ha^{-1} of N, 60 kg ha^{-1} of P_2O_5, and 60 kg ha^{-1} of K_2O.

Two sources of Cu—insoluble cuprous oxide (Cu_2O) and copper carbonate ($CuCO_3$) were used and divided into nine treatments as follows:

Treatment 1-control without application of foliar copper;

Treatment 2-application of 30 g ha^{-1} cuprous oxide;
Treatment 3-application of 60 g ha^{-1} cuprous oxide;
Treatment 4-application of 90 g ha^{-1} cuprous oxide;
Treatment 5-application of 120 g ha^{-1} cuprous oxide;
Treatment 6-application of 30 g ha^{-1} copper carbonate;
Treatment 7-application of 60 g ha^{-1} copper carbonate;
Treatment 8-application of 90 g ha^{-1} copper carbonate;

Treatment 9-application of 120 g ha^{-1} copper carbonate. As different doses were applied, the response may vary allowing to identify the most effective dose.

Four applications of phytosanitary treatments were done except in control (Table 1). A CO_2 pressurized spool was coupled to a spray bar provided with four tips, spaced 0.50 m apart was used for this. The tips used were Teejet® TT110015, with constant service pressure regulated to a volume of 150 L ha^{-1}. The phenological scale used was Fehr and Cavines (17). The applications were at the phenological stages of V7, V9, R2, and R6 (17).

Evaluation of the severity of Asian Soybean Rust

The severity (percentage of leaf area with symptoms of the disease) was evaluated through visual observation with the aid of a diagrammatic scale (18). The evaluations were carried out at 75, 103 and 113 days after sowing (DAS), and six random trifoliate were collected in the evaluation line from each plot (two from the lower third, two from the middle third and two from the upper third of the plant). The area below the soybean rust severity progression curve (ABSRSPC) was determined by the formula:

$$\text{ABSRSPC} = \Sigma \times \left(t2 - t1\right) / D$$

Table 1. Phytosanitary treatments with copper association used in experiment.

Treatments phytosanitary							Phenological stage
P^1	Fungicide	(L ha^{-1})	Insecticide	(L ha^{-1})	Oil	(L ha^{-1})	
1^a	Orkestra®	0.35	Nomolt® Connect®	0.25 1.0	Assist®	0.50	V7
2^a	Fox®	0.40	Nomolt® Connect®	0.25 1.0	Áureo®	0.25	V9
3^a	Ativum®	1.0	Nomolt® Fastac Duo®	0.25 0.35	Assist®	0.50	R2
4^a	Fox®	0.40	Pirate® Perito®	1.0 1.0	Áureo®	0.25	R6

Spraying; ®: Trademark; Phenological stage (17).

where y1 and y2 are two consecutive evaluations performed at times t1 and t2, respectively, and D is the number of days of the cultivar cycle (19). The percentage of disease control was calculated using the formula:

$$PC(\%)=(T\,t)\times 100/T$$

where T is the severity of the control and t is the severity found in treatment (20).

Yield components

At the R6 stage (characterized by the presence of a pod containing green grain filling the cavity of the pod) (17), was performed the count of plants per line and determined the final group of plants. The yield were evaluated by plant stratification at physiological maturation stage. The number of pods (NP) and the number of grains (NG) were determined. Harvesting was completed using a plot harvester (Wintersteiger Classic, AT). The samples were weighed and corrected for 13% moisture and the grain yield (kg ha^{-1}) and thousand-seed weight (TSW) were defined.

Micronutrients in soybean leaves and seeds

At the R6 stage, six random trifoliate of each plot (two from the lower third, two from the middle third and two from the upper third of the plant) were collected to analyze the foliar micronutrients. The leaf blade was washed in distilled water and then dried in the drying oven for two days at a temperature of 50 ºC. For the analysis of micronutrients in seeds, after the harvest, a sample of 100 grams was collected from each plot. The seeds were dried until they stabilized at 13% moisture and stored. The perchloric nitric acid digestion of the leaves and seeds was carried out and the Fe, Zn, Mn and Cu contents determined using the atomic absorption spectrophotometry (PerkinElmer, NexlON 2000B ICP Mass Spectrometer). All analysis followed the standard methodology (21).

Leaf lignin contents

Leaf lignin contents were quantified in the R6 stage of soybean. Six trifoliate were collected of each plot, as mentioned previously. The leaves were dried in a drying oven for two days at a temperature of 50 °C. The lignin contents were evaluated following Sindirações method (22).

Statistical analysis

The data were subjected to analysis of variance (ANOVA), and the means were compared by the Scott Knott test at 5% probability of error using the statistical program CoStat (23).

Results

Preliminary observation on the physical and chemical attributes of the soil showed high level of clay and organic matter, providing adequate water retention for the crops. In the tangent of soil chemistry, high aluminium contents, low base saturation and low pH H_2O are observed, leading to the need for liming for soil correction (24). The sulphur is the most limiting nutrient, as the values were below that recommended for the soybean crop of 10 mg / dm³, whereas the other nutrients are present in levels adequate for the production of soybean (24) (Table 2). The average values of pluviometric precipitation (mm) occurred during the crop cycle were higher than the regional normals in most months, except in November and December (Fig. 1). In this sense, the crop cycle was affected by water restriction in the initial period and by high water availability in the cycle final. The average temperature remained close to the regional normal, not affecting the culture.

Asian Soybean Rust severity

The area under the curve of the progression of Asian Rust severity was changed by Cu leaf spraying along with phytosanitary treatments (p> 0.05). Treatments 3 (60 g ha^{-1} cuprous oxide), 4 (90 g ha^{-1} cuprous oxide), 5 (120 g ha^{-1} cuprous oxide), 6 (30 g ha^{-1} copper carbonate), 7 (60 g ha^{-1} copper carbonate), 8 (90 g ha^{-1} copper carbonate), and 9 (120 g ha^{-1} copper

Table 2. Physical and chemical atributes the macronutrient and micronutrient contents of the soil of the experimental area.

	Clay	pH	SMP	P	K	MO	Al	Ca	Mg	H+Al	CTC	Saturation (%)			S	B	Cu	Zn	Mn
Layer	%	H_2O	index	....mg dm³...		%	......................cmol$_c$dm^3.......................					Bases	Al	K	............mg/dm³............				
0-10	40	4.9	5.3	29.2	224	2.7	1.0	4.4	2.0	9.7	16.84	42.1	12.8	3.4	3.2	0.38	2.86	4.61	37.8
10-20	50	4.8	5.26	22.4	170	1.9	1.4	4.1	2.1	10.2	16.91	39.7	17.2	2.5	5.7	0.63	1.83	1.00	14.1

Methodology described in Tedesco *et al.* (2004). SMP (Shoemaker, Mac Lean and Pratt) index; P: phosphorus; K: potassium; MO: organic matter; Al: aluminum; Ca: calcium; Mg: magnesium; H: hydrogen; S: sulfur; B: boron; Cu: copper; Zn: zinc; Mn: manganese.

carbonate) delayed progressions of the disease (Table 3).

Yield components

The number of pods per plant, number of seeds per pods and 1000-seed weight presented no differences. However, for seed yield, the application of 120 g ha^{-1} cuprous oxide and 60 g ha^{-1} Cu carbonate showed the best results, differing statistically from the other treatments (Table 4).

Table 3. Area below the soybean rust severity progression curve as a function of leaf copper treatments.

	Treatments	[1]Total ABSRSPC Rust-Asian	C (%)
1	Control	310.45 c	0
2	30 g ha-1 de cuprous oxide	253.37 b	23
3	60 g ha-1 de cuprous oxide	215.71 a	44
4	90 g ha-1 de cuprous oxide	232.44 a	34
5	120 g ha-1 de cuprous oxide	195.56 a	59
6	30 g ha-1 de Cu carbonate	190.13 a	63
7	60 g ha-1 de Cu carbonate	179.85 a	73
8	90 g ha-1 de Cu carbonate	187.64 a	65
9	120 g ha-1 de Cu carbonate	182.08 a	71
	Mean ± SD	216.36 ± 43.21	48.00 ± 24.82
	CV (%)	14.05	-

Means followed by different letters in the columns differ between treatments, according to the Scott Knott Test ($p \leq 0.05$); Cu: copper; [1]ABSRSPC: area below the soybean rust severity progression curve; C.V. (%): coefficient of variation; C (%): Control in relation to the control.

Micronutrients content in soybean leaves and husked seed

For the analysis of the micronutrients in soybean leaves, the treatments had no significant effects on the accumulation of Fe and Zn ($p > 0.05$). But there was a significant change for the micronutrient Mn, in treatments 2, 3, 4, 5 and 9 where the most significant accumulations were achieved (367.50, 350.25, 386.75, 365.50 and 318.75 respectively). For the Cu micronutrient, treatment 9 (120 g ha^{-1} copper carbonate) had the highest accumulation in the leaves (61.00), varying from other treatments ($p > 0.05$) (Table 5).

The analysis of the micronutrients in the soybean husked seeds showed that the treatments had no significant effects on the accumulation of Fe, Zn and Mn ($p > 0.05$). The treatments differed statistically only for the Cu micronutrient. The best results were obtained in treatments 5, 8 and 9 with values 16.00, 15.15, 15.30 respectively (Table 5).

Leaf lignin content

In the lignin analysis, treatments 2 (30 g ha^{-1} cuprous oxide), 3 (60 g ha^{-1} cuprous oxide), 4 (90 g ha^{-1} cuprous oxide), 5 (120 g ha^{-1} cuprous oxide) and 9 (120 g ha^{-1} copper carbonate) obtained the highest accumulations of lignin (162.85, 161.37, 178.97, 170.85 and 158.52, respectively) (Table 6).

Discussion

The soybean was sowed late to obtain a higher disease severity in the experimental field. However, in the year 2015–16, there was a severe winter that eliminated host plants from the disease. The first outbreak of the disease in the state occurred only on January 4, 2017 (25). This resulted in low initial inoculum in the field, even under favourable environmental conditions. The temperature was within usual means, for this period of the year, oscillating between 17.4 and 22.5 °C; which were favourable for the development of the pathogen. The accumulated rainfall during the period, from December 2016 to April 2017, was 1023.1 mm. These continuous periods of leaf wetting aid in the infection and expansion of the inoculum in the crop (26). Even under these conditions, Cu foliar application, together with phytosanitary treatments delayed the soybean Asian-rust severity, varying

Table 4. Number of pods per plant, grains per plant, thousand-seed weight, productivity and percentage increase of soybean yield under variations of applications of copper via foliar

	Treatments	NPP	NGP	TSW (g)	Yield (kg ha^{-1})	PY (%)
1.	Control	35^{ns}	90^{ns}	155.9^{ns}	4275.08 b	0
2.	30 g ha^{-1} de Cuprous oxide	38	98	164.5	4615.09 b	7
3.	60 g ha^{-1} de Cuprous oxide	38	93	167.1	4503.79 b	5
4.	90 g ha^{-1} de Cuprous oxide	40	105	163.9	4520.37 b	5
5.	120 g ha^{-1} de Cuprous oxide	39	102	168.2	4815.55 a	11
6.	30 g ha^{-1} de Cu Carbonate	38	99	166.4	4381.06 b	2
7.	60 g ha^{-1} de Cu Carbonate	37	94	167.3	4896.64 a	13
8.	90 g ha^{-1} de Cu Carbonate	39	103	169.4	4595.04 b	7
9.	120 g ha^{-1} de Cu Carbonate	40	102	166.8	4461.97 b	4
	Mean ± SD	38.22 ± 1.56	98.44 ± 5.13	165.50 ± 3.98	4562.73 ± 196.97	6.00 ± 4.09
	C.V. (%)	13.37	15.11	3.96	5.11	5.11

Means followed by different letters in the columns differ between treatments, according to the Scott Knott Test ($p \leq 0.05$); ns - not significant; C.V. (%): coefficient of variation; NPP: number of pods per plant; NGP: number of grains per plant; TSW: thousand-seed weight. PY: Percentage increase in yield compared to the most productive treatment.

Table 5. Micronutrients in soybean leaves and seeds under variations of copper applications via foliar.

Treatments		Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})
1.	Control	114.00^{ns}	35.10^{ns}	201.75 b	11.50 d	61.20 ns	38.10 ns	36.90 ns	13.45 b
2.	30 g ha^{-1} de Cuprous oxide	133.50	35.55	367.50 a	16.75 d	65.85	38.85	41.70	13.55 b
3.	60 g ha^{-1} de Cuprous oxide	106.50	35.35	350.25 a	28.75 c	67.65	37.95	39.75	14.20 b
4.	90 g ha^{-1} de Cuprous oxide	109.50	37.70	386.75 a	22.00 c	66.60	39.75	44.65	13.95 b
5.	120 g ha^{-1} de Cuprous oxide	103.50	30.45	365.50 a	47.50 b	60.15	37.80	36.60	16.00 a
6.	30 g ha^{-1} de Cu Carbonate	109.50	36.30	277.75 b	17.85 d	70.00	40.35	37.05	14.40 b
7.	60 g ha^{-1} de Cu Carbonate	111.00	31.45	292.50 b	23.40 c	67.35	39.00	38.70	14.60 b
8.	90 g ha^{-1} de Cu Carbonate	102.00	32.85	242.25 b	40.00 b	63.30	39.60	37.95	15.15 a
9.	120 g ha^{-1} de Cu Carbonate	109.50	42.70	318.75 a	61.00 a	65.40	39.45	43.50	15.30 a
Mean ± SD		111.00± 9.22	35.27± 3.64	311.44± 62.86	29.86± 16.36	65.28± 3.18	38.98± 0.89	39.64± 2.99	14.51± 0.85
CV (%)		13.33	14.45	21.73	17.98	13.33	5.34	12.74	7.37

C.V. (%): coefficient of variation

Table 6. Total leaf lignin content of soybean under variations of copper applications via foliar.

	Treatments	Lignin g kg^{-1} DM
1	Control	153.55 b
2	30 g ha^{-1}Cuprous oxide	162.85 a
3	60 g ha^{-1}Cuprous oxide	161.37 a
4	90 g ha^{-1}Cuprous oxide	178.97 a
5	120 g ha^{-1}Cuprous oxide	170.85 a
6	30 g ha^{-1}Cu Carbonate	140.60 b
7	60 g ha^{-1}Cu Carbonate	137.12 b
8	90 g ha^{-1}Cu Carbonate	146.15 b
9	120 g ha^{-1}Cu Carbonate	158.52 a
	Mean ± SD	156.66 ± 13.80
	CV (%)	7.71

Means followed by different letters in the columns differ between treatments, according to the Scott Knott Test ($p \leq 0.05$); C.V. (%): coefficient of variation; DM: dry mass.

from 23–73% in the ABSRSPC of the treatments with the control (Table 3). Both sources were efficient in reducing the severity of Asian Rust. Still, treatment 7 (60 g ha^{-1} of Cu carbonate) was more productive with the lowest ABSRSPC (179.85), obtaining 73% of disease delay with the control. In soybean, Cu has both nutritional and plant health effects through secondary growth (lignin synthesis) and the production of phytoalexins, which are substances in the defence system.

The Asian Rust severity was lower for carbonate Cu in relation to the cuprous oxide, thus favouring the plant. There was a linear decrease in the severity of Asian Rust as a function of the increase in doses for both sources. These data are justified by the participation of Cu in the components of carbohydrate metabolism in the synthesis of lignin and chlorophyll (13, 19). Corroborating this study, in beans, a linear decrease of 35% of the area under the anthracnose severity progression curve was observed, with increasing Cu doses (28). These doses of Cu, applied via foliar, are very low to have direct fungi toxic effects through contact with the pathogen (28).

The yield component, number of pods per plant is the variable that can present the most significant variability and can be influenced by mineral fortification. The results show only a trend of increasing the number of pods per plant and the number of seeds per plant with the elevation of the Cu doses, but these variations did not differ statistically from the control. For productivity, the treatments 5 (120 g ha^{-1} cuprous oxide) and 7 (60 g ha^{-1} Cu carbonate) obtained a productivity percentage of 11 and 13, respectively higher than the control, which equals 540.47 kg ha^{-1} (9 bags ha^{-1}) and 621.56 kg ha^{-1} (10 bags ha^{-1}) of productivity. An explanation for the highest productivity in these treatments is the lower rates of Asian Rust severity presented by these treatments. A higher number of healthy leaves allows the plant to have a more significant photosynthetic activity to meet the demand for grain filling (29). The carbonate Cu is considered a partially soluble source and is absorbed more than cuprous oxide source. The productivity decreases with increasing carbonate Cu in treatment 8 and 9 are due to the excess Cu absorbed by the soybean, causing an imbalance in leaf tissue concentrations. This is a typical micronutrient response curve, where they reach the top and then decay. The Cu excess causes the soybean plants to generate reactive oxygen species, increasing the phytotoxic effect at the cellular level (30). Besides, excessive amounts of Cu in the leaves can lead to inhibition of growth, chlorosis and early defoliation (31)

The production and quality of crops are affected by the availability of micronutrients in the soil (32). The Fe micronutrient ranges from 125–150 mg kg^{-1} Fe in dry matter (26, 27), in the present study, the treatment 2, was above the minimum line of sufficiency, accumulating 133.5 mg kg^{-1} of dry matter. For Zn, the minimum range of sufficiency is 50–70 mg kg^{-1} in dry matter (26, 27). In this study, all the treatments were below this line, with the best treatment 9 accumulated 42.70 mg kg^{-1} Zn. In this case, leaf sprays of Cu caused antagonism in Zn uptake, because of the high initial concentration of Zn in the soil (Table 2).

Leaf application of Cu together with phytosanitary treatments, significantly raise leaf concentrations of Mn (Table 5). The Mn sufficiency range was between 35–50 mg kg^{-1} in the dry matter (26, 27). The Mn micronutrient content was very high in leaves in the phenological stage R6. Treatment 4 had Mn levels at 386.75 mg kg^{-1}, which was the largest accumulation, almost eight times more than the sufficiency line. For Mn, there is a relation between its concentration in the plant tissue and the severity of the diseases. Higher concentrations of this micronutrient occur in healthy tissues irrespective of its lower concentration in tissues victimized by diseases (35). Thus, in tissues that most affected by Asian Rust, there is a lower deposition of Mn and resulted in a decrease in productivity, confirming the previous relationship. Collaborating with other studies where the application of Mn via foliar spraying, seed treatment or addition to the soil, helped reduce the severity of diseases (36). Also, the efficacy of fungicides on Asian Rust control is significantly high when they are mixed with Mn (37).

For Cu, an earlier study (33) indicated the sufficiency range was 12–15 mg kg^{-1} in dry matter. All treatments in the present study were above sufficiency range, except for the control, with an accumulation of 11.5 mg kg^{-1} Cu in the dry matter. This may have altered the production of resistance compounds, directly affecting the natural protection of the plants, consequently obtained the highest severity indexes of Asian Rust, the lower Cu contents in the seeds and the lower productivity (Table 5). The Cu deficiency impairs the lignification of cell walls and xylem vessels (8), making the plant more vulnerable to biotic and abiotic stresses. On the other hand, Cu excess is also highly damaging, causing cellular phytotoxicity. An earlier study (34) demonstrated increases in soybean yield with leaf Cu applications, reaching 4546 and 4513 kg ha^{-1} with increases of 3.1 and 2.4%, respectively, in the doses of 10 and 20 g ha^{-1}.

Soils with high limestones tend to raise pH, which may lead to the unavailability of Cu, Fe, Mn and Zn micronutrients (38). The deficiency of essential micronutrients results in metabolic disorders, affecting the structure and enzymatic activities (39). However, the use without a prescription and indiscriminately can result in phytotoxicity, increasing stress situations in plants (38). For the interpretation of micronutrient levels in soils to be considered high, they must contain> 0.4 mg dm^3 Cu; > 5.0 mg dm^3 Mn; and> 0.5 mg dm^3 Zn. <0.2 mg dm^3 Cu; <2.5 mg dm^3 Mn and <0,5 mg dm^3 Zn (27). According to this interpretation, the study site contained high concentrations of Cu, Mn and Zn (Table 2). Besides, soil analysis performed in the experimental area shows that the pH is low in both layers (0–10 cm - pH 4.9) and (10–20 cm - pH 4.8) (Table 2), which favours the availability of these micronutrients to plants.

The availability of the micronutrient Cu in the soil is affected by the phosphate fertilization, forming little soluble precipitates of H_2PO_4 with this metal cation (40). Cu is usually deficient in soils with a high organic matter content, forming stable complexes (41). In the present study, the amount of organic matter was of 2.7% in the layer of 0–10 cm and 1.9% in the layer of 10–20 cm, considerably low. In addition, the low pH also influences the availability of this micronutrient.

The treatments 5 (120 g ha^{-1} cuprous oxide), 8 (90 g ha^{-1} copper carbonate) and 9 (120 g ha^{-1} copper carbonate) with the highest doses of Cu for both sources, presented statistical differences in relation to the control and other treatments. These same treatments, in the leaf Cu analysis, had values higher than 40 mg kg^{-1} Cu in the dry matter (Table 5). Also, the analysis showed that the control has least accumulated Cu in the seeds with 13.45 mg kg^{-1} Cu. This is due to a slight deficiency of Cu found in soybean leaves. When the treatments were compared, it was observed that treatment 5 (120 g ha^{-1} cuprous oxide) caused more accumulation in the seeds, which can be correlated with the higher accumulation in the leaves, in relation to the lower doses, also presenting lower rates of the severity of Asian Rust and higher productivity. This effect is mainly related to the form of micronutrient absorption (concentration gradient) and the lower solubility of the source. In the carbonate Cu source, the highest accumulations were obtained in treatments 8 (90 g ha^{-1} Cu carbonate) and 9 (120 g ha^{-1} Cu carbonate), which accumulated higher Cu contents in the seeds. This is due to the higher accumulations of Cu in the leaves, and translocate this micronutrient to the reproductive organs. For this source, this increase in leaf Cu contents and the reserve, organs were beneficial, although these increases did not increase the productivity of crop, it decreased the severity indexes of Asian Rust.

Lignin plays a vital role in the resistance of plants to attack by pathogens by promoting the strengthening of the cell wall, varying their precursors of biosynthesis according to cultivated species (42). The highest accumulations of lignin in the dry matter occurred in the treatments with the cuprous oxide source, and all the treatments produced more lignin than the control, varying from 5–14% increase. Because it is an insoluble source, it was probably absorbed more slowly by the soybean plants. These increases in foliar lignin contents are reflective of increases in Cu contents in leaves and seeds. In this way, reducing the severity of Asian Rust, but were useful only to increase productivity in treatment 5 (120 g ha^{-1} cuprous oxide), which differed from the control and other treatments. When compared only the severity of Asian Rust between the sources, the highest visual notes of the disease were in these treatments of Cu oxide. The treatment 4, (90 g ha^{-1} of cuprous oxide), obtained the highest percentage of lignin accumulation, with a 14% increase from the control. This increment resulted in a significant decrease in the progression of Asian Rust severity.

For the carbonate Cu source, only treatment 9, (120 g ha^{-1} Cu carbonate) accumulated higher lignin contents (3%) in the leaves compared to the control. The carbonate Cu is a source partially insoluble in relation to the cuprous oxide. It is more absorbed by the soybean plants, represented by the increases in the foliar contents and seeds. This source of Cu probably has no direct action on lignin biosynthesis, but rather on the production of phytoalexins. As it was more absorbed, it likely stimulated the higher production of phytoalexins. These substances were also responsible for the results in reducing the severity of Asian Rust. It is perceived a tendency of this source to have lower visual notes of the disease and consequently to increase productivity, even at the dose of 60 g ha^{-1} Cu carbonate (treatment 7). However, in the present study, the production of phytoalexins was not quantified.

Conclusion

The foliar application of copper in cuprous and carbonate forms is beneficial to reduce the progression curve of Asian Soybean Rust. The application of 120 g ha^{-1} Cu cuprous accumulated more Cu in the leaves and the seeds, enhancing the productivity to 11%. All treatments of the cuprous source positively affected the accumulation of manganese in the leaves. For leaf lignin content, all treatments were superior to control, however, these didn't influence the yield components. For the carbonate Cu source, all treatments decreased the severity of Asian Rust. Regarding the accumulation of manganese and copper in the leaves, only the application of 120 g ha^{-1} Cu carbonate showed positive values. In the seeds, the application of 90 and 120 g ha^{-1} Cu carbonate provided higher copper contents. The application of 120 g ha^{-1} Cu carbonate differed from the control in lignin content, but this didn't interfere with the yield components. Concerning grain yield, only the application of 60 g ha^{-1} Cu carbonate obtained an increase of 13%. The results suggest that the leaf fortification with copper together with phytosanitary treatments, may reduce the rust severity and improvement of the plant performance. Future research with Cu application and analysis of specific enzymes, secondary metabolites and cell wall thickness may further contribute to the understanding of the role of Cu in defence against Asian Soybean Rust.

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Authors' contributions

EB and GC conceived and designed the experiments; ED and AC performed the experiments, analyzed the data and wrote the paper. DCS helped in analysis of data. GC critically revised the manuscript.

Conflict of interests

The authors have declared that no competing interests exist.

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