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Fungicides phytotonic action on the development of soybean

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Some studies has shown that some fungicides may promote phytotonic effect on plants, resulting in gain on the growth and productivity. The objective here was to evaluate the phytotonic action of different fungicides, when applied on the seed and aerial part treatments on the soybean culture. Experimental design chosen was a randomized-block type with nine treatments consisting of eight groups of fungicides applied as seed treatment and aerial part of the plant with four replications. Pyraclostrobin + Methyl thiophanate favored root development on plantlets, plant height and grain yield, when it was applied on the soybean seeds. Fungicides applied on the plant aerial part showed positive effects on control of foliar diseases in soybean. The weight of one thousand grains was higher, when Pyraclostrobin + Methyl thiophanate was used for seeds treatment.

Key words: Pyraclostrobin, azoxystrobin, strobilurin, nutrients and productivity.

INTRODUCTION

The area destined to the grains harvest at Piauí state, Brazil, in crop year 2011/2012 was 1,173.9 thousands of hectares (ha), an increase of 2.4% compared to the harvest of grains at 2010/2011 and also it was higher than national average (2%). Plant species highlighted on this region are soybeans, corn, bean, rice and cotton which has the area for cultivation of 444.600, 351.600, 230.500, 117.400 and 21.300 ha, respectively (Conab, 2012). Moreover, preliminary surveys indicate that the Piauí state has the potential to expand its acreage.

Soybeans productive potential are determined by genetic factors, and withal, by extrinsic production factors as climate conditions, farm management, weeds, pests and diseases control. Huge losses are arising due to diseases incidence, mainly the fungal diseases. It can occur during all culture cycle or only on the later stages.

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The last one is known as late season diseases. It may causes losses that reach until 21% of the production, by decreases on the mass of 1000 grains (Fagan, 2007).

Beyond the effect on the disease control (Godoy and Henning, 2008), some studies show that some fungicide also can promote phytotonic effects on plants, resulting in gains on the growth and productivity (Glaab and Kaiser, 1999; Soares et al., 2011). Positive effects of fungicides are also shown by Fagan (2007) who noted that the use of some fungicides on the soybean culture resulted in increments on the carbon and nitrogen assimilation, photoassimilates partition and grains quality.

Dimmock and Gooding (2002) and Beck et al. (2002) emphasize that the strobilurin increases the period of photosynthetic activity in the leaves of wheat, providing quality and nitrogen content of the grain. Thus, improving the quality of grains is beneficial to the process of seed germination. Among the fungicides available and recommended to the soybean crop in Brazil, named sterol synthesis inhibitors, a systemic fungicide group popularly known as triazole and strobilurin group that act on the mitochondrial respiration process are highlighted. Both of them are used by singly or prefabricated mixtures (Koehle et al., 1997; Alessio, 2008). Because it is a very competitive market, there are several studies that have evaluated the effects of main fungicides registered for soybean culture. However, most existing studies are derived from tests conducted by the manufactures themselves.

Considering all information above, we have objective to evaluate the performance of nine fungicides groups applied on seed treatment and over aerial parts considering its phytotonic effect on the development and productivity of soybean crop.

MATERIALS AND METHODS

The experiment was performed in a Fazenda União, located at Serra da Laranjeira, municipality of Currais (Brazilian State of Piauí), latitude 16' 78"S, longitude 44° 44' 25"W and altitude of 628 m, during the crop year 2011/2012, from November 27 of 2011 (sowing) to April 6 of 2012 (harvest). The soil was classified as distrophic yellow Latossol. Textural analysis of soil on the layer of 0 to 20 cm had 190 g x kg^{-1} of clay, 50 g x kg^{-1} of silt and 760 g x kg^{-1} of sand. Chemical characteristics of the experimental area (0 to 20cm) were as follow: pH ($CaCl_2$): 4.7 mg/dm^{-3} (ppm), K: 47 mg/dm^{-3} (ppm), P (Mehlich): 37.4 mg/dm^{-3}, Ca: 1.9 cmol.c/dm^{-3} (mE/100 ml), Mg: 0.6 cmol.c/dm^{-3} (mE/100 ml), Al: 0.2 cmol.c/dm^{-3} (mE/100 ml), H + Al: 2.2 cmol.c/dm^{-3} (mE/100 ml), V: 54.42%, m: 7.09% and organic matter: 11 g x kg^{-1}.

Rainfall, temperature and monthly mean of relative humidity are described in Figure 1. Data were obtained on the meteorological station of Sabiá farm. It was installed 5 km far away from experimental area. Soybean was sown at November, 27 of 2012 using a pneumatic sowing. Row between lines were 0.5 m, inserted 12 seeds per meter. Pioneer® P98Y70 that have semi-indeterminate growth and phenological cycle around 125 days was chosen to performed the experiment. Fertilization at planting was performed applying 400 kg of 00-24-12 held in the row and topdressing with 100 Kg of KCl spread around.

The experimental design was randomized block, consisted by eight groups of fungicides plus one control and four replicates, as described in Table 1, totaling 36 treatments, each one are set to 4.00 m wide (8 rows) by 8.0 m long, with an area of 32.0 m^2. From each end were eliminated 0.5 m, considering the remaining area of 21.0 m^2.

Fungicides were sprayed on the aerial part as a preventive management, considering, local climate conditions, inoculum pressure and residual range of the fungicides. According to this features spraying it was performed with interval from 15 to 20 between applications. Backpack sprayer with CO_2 injection, tip XR 11002 and a flat fan with spray volume of 150 L x ha^{-1} was used. Pulverizations with Opera or Opera Ultra fungicides were performed with mineral oil assist at the dosage of 0.5 L x ha^{-1}. For PrioriXtra fungicide was used the Nimbus oil in a dosage of 0.6 L x ha^{-1} and for Fox fungicide was used Aureus mineral oil at the dosage of 0.3 L x ha^{-1}.

Plant height at flowering was measured with a measure tape taking nine plants per plot in the usable area by random choice. Plant population was evaluate as initial stand (7 DAE) and final stand (112 DAE) counting the plants along three meters considering three points per plot. Root length was measured at 14 DAE choosing randomly 3 plants per point considering 3 points per plot. Destructive method by soil excavation and removal of the plant was employed to assess the underground part. The same plants used to measure the root length was also used to count the number of branches. At the begin of flowering was collected the third leaf from the apex of plants for chemical analysis of N, P, K, Ca, Mg, Cu, Zn and Mn levels (30 per plot). Drying was carried out in the oven at 60°C during 72 h. The samples were ground and sent for laboratory analysis (Embrapa, 2009). Root and aerial part biomass were also dried in the oven at 60°C during 72 h. They were weighed after drying. It was represented by three growth phases: an initial phase with low accumulation of biomass (0 to 45 days), followed by a significant accumulation stage (45 to 90 days) and the stabilization phase.

Number of string beans with 1, 2 and 3 grains were counted by random collection of 9 plants per plot. Trifoliate branches were also considered at previous sampling. The weight of one thousand seeds was performed with 8 sub-samples containing 100 seeds each separated in counter tray. Once we have these data was possible calculate the mean, variance, standard deviation and coefficient of variation to obtain the weight of 1000 seeds, according to the RAS-Rule Analysis of Seed (Brazil, 2009). The severity of phytopathogens *Corynespora cassiicola* and *Colletotrichum dematium* var. *Truncate* on soybean plants were evaluated according to the diagrammatic scale proposed by Soares et al. (2009) and Finoto et al. (2011), respectively.

Productivity was calculated as yield (kg/ha) by harvest the four square meter in each experimental plots. The moisture of seeds was measured with portable moisture meter (Multigran tool). Qualitative data were subjected to variance analysis. When the differences among means were significant they were compared by the Tukey and F tests at 5%. SISVAR 4.2. software was used to perform those analysis. Quantitative data were adjusted in equations with SIGMA PLOT 10.0. software.

RESULTS AND DISCUSSION

The initial and final stand does not show significant differences compared to the control treatment (Table 3). These results can be explained by the low incidence of pathogens which occur early in the development of soybean, since the number of seedlings observed were close to the number of seeds deposited in the sowing

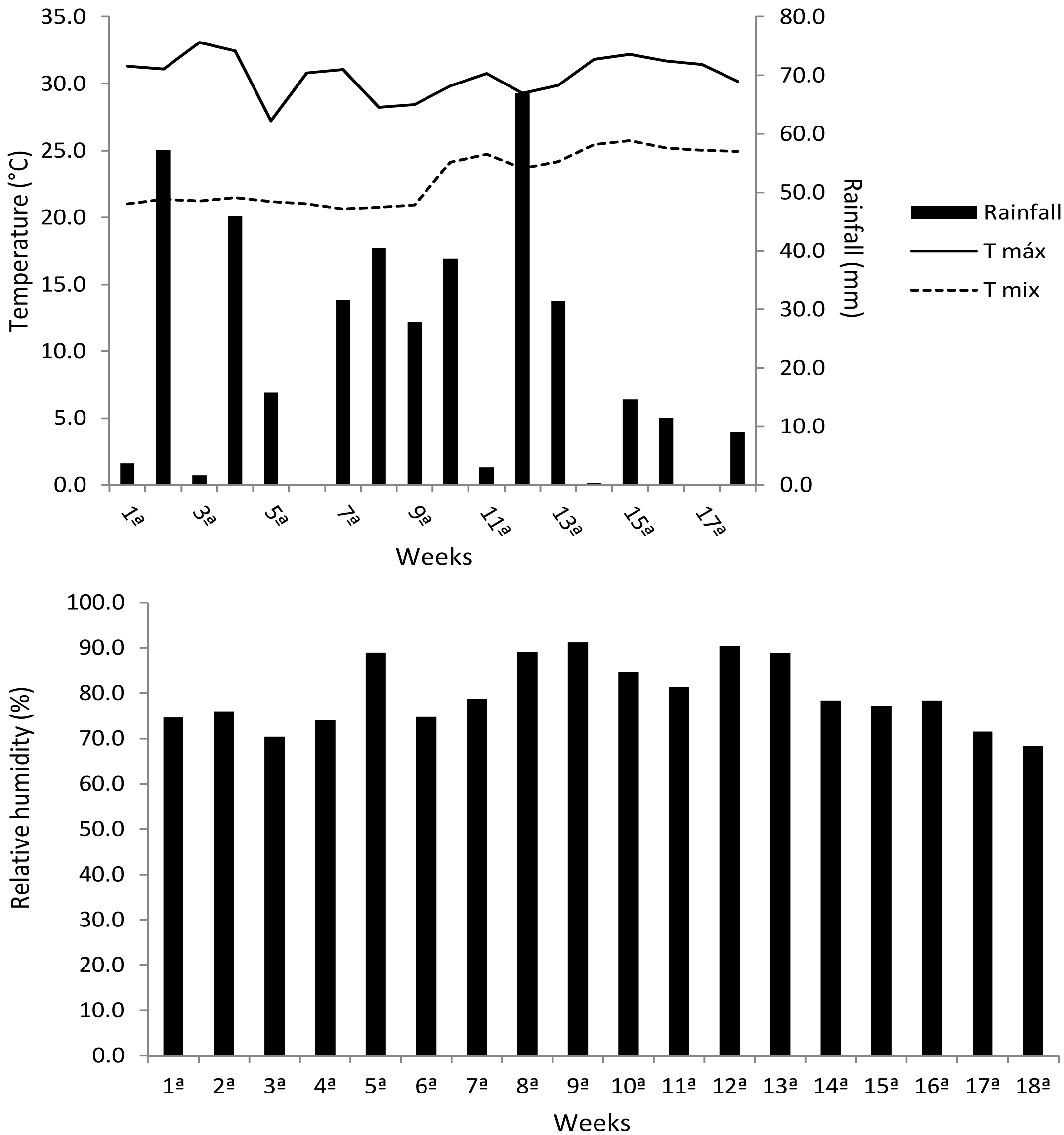

Figure 1. Precipitation, average temperatures (maximum and minimum) and relative humidity of air occurred during the experiment. Currais, PI.

furrows (germination and emergence above 80%). Moreover, climatic conditions may not have favored the occurrence of diseases that affect the initial stand. However, the stand can be severely affected under conditions of high disease incidence in soybean seedlings. As noted by Pereira (2002) the low percentage of germination due to high incidence of *Colletrotrichum truncatum* can be observed when seeds were not treated with fungicides, which reinforces the importance of fungicide to control diseases.

Pyraclostrobin + Methyl thiophanate on seed treatment (Treatments 1, 2 and 8) favored the root development of seedlings, once significant increases in the length and initial root biomass were observed compared to control treatment. Root development is important, because prolonged periods of water stress is common in the Brazilian savannah of Piauí. Practices with phytotonic effect can facilitate the rooting of the seedlings reducing the time of exposure to a soil, which has fungi that cause seed deterioration or seedlings death (Henning, 2005).

These results are reinforced by Rodrigues (2009), who observed that Pyraclostrobin in the treatment of soybean seeds can favor nitrogen assimilation. It happens due to increases in the activity of the enzyme nitrate reductase. Once nodulation is not efficient at this stage, the use of this fungicide may guarantee plantlet vitality.

All treatments which use fungicides at the soybean flowering (R1 and R2) showed increases in plant height

Table 1. Treatments with products, active ingredient, dose and time of spraying.

Treat.*	Commercial name	Active ingredient	C.p.dose [(6)]	Pulverization
			L or kg/ha	Stage
1	Standak Top	Fipronil + Pyraclostrobin + Methyl thiophanate	0.1	S.T.[(1)]
	Comet	Pyraclostrobin	0.3	V6[(2)]
	Opera	Pyraclostrobin + Epoxiconazole + assist	0.5	R1[(3)]
	Opera	Pyraclostrobin + Epoxiconazole + assist	0.5	R3[(4)]
	Opera	Pyraclostrobin + Epoxiconazole + assist	0.5	R5.3[(5)]
2	Standak Top	Fipronil + Pyraclostrobin + Methyl thiophanate	0.1	S.T.
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R1
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R3
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R5.3
3	Standak	Fipronil	0.1	S.T.
	Protreat	Carbendazim + Thiram	0.1	S.T.
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R1
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R3
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R5.3
4	Standak	Fipronil	0.1	S.T.
	Maxin	Fludioxonil + Metalaxil-M	0.1L/100Kg	S.T.
	PrioriXtra	Azoxystrobin + Cyproconazole	0.3	R1
	PrioriXtra	Azoxystrobin + Cyproconazole	0.3	R3
	PrioriXtra	Azoxystrobin + Cyproconazole	0.3	R5.3
5	Standak	Fipronil	0.1	S.T.
	Maxin	Fludioxonil + Metalaxil-M	0.1L/100Kg	S.T.
	Priori	Azoxystrobin	0.2	V6
	PrioriXtra	Azoxystrobin + Cyproconazole	0.3	R1
	PrioriXtra	Azoxystrobin + Cyproconazole	0.3	R3
	PrioriXtra	Azoxystrobin + Cyproconazole	0.3	R5.3
6	Standak	Fipronil	0.1	S.T.
	Derosal Plus	Carbendazim + Thiram	0.2L/100kg	S.T.
	Fox	Prothioconazole + Trifloxystrobin	0.4	R1
	Fox	Prothioconazole + Trifloxystrobin	0.4	R3
	Sphere Max	Cyproconazole + Trifloxystrobin	0.15	R5.3
7	Standak Top	Fipronil + Pyraclostrobin + Methyl thiophanate	0.1	S.T.
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R1
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R3
	Opera Ultra	Pyraclostrobin + Metconazole	0.5	R5.3
8	Standak Top	Fipronil + Pyraclostrobin + Methyl thiophanate	0.1	S.T.
	Comet	Pyraclostrobin	0.3	V6
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R1
	Opera	Pyraclostrobin + Epoxiconazole	0.5	R3
	Opera Ultra	Pyraclostrobin + Metconazole	0.5	R5.3
9	Standak	Fipronil	0.1	S.T.

*Treat.: Treatments; (1) S.T.: Seed treatment; (2) V6: Fifth Trifoliate leaf fully developed; (3) R1.: Beginning of flowering stage; (4) R3.: Early formation of string beans; (5) R5.3.: 26 to 50% of the grain filling; (6) c.p.: Commercial product

Table 2. Initial and final stand, length and biomass root and plant height at flowering soybean under different fungicide treatments applied as seed treatment and over the aerial part. Brazilian savannah of Piauí. Crop year 2011/2012.

Treat.*	Stand		Initial root length	Initial root biomass	Height
	Initial(14DAE)	Final(112DAE)	14 DAE	14 DAE	56 DAE
	---plants x m^{-2}---		----cm----	----g x m^{-2}----	----cm----
1	19.92^{ns}	17.61^{ns}	18.41^{A}	4.80^{A}	80.42^{A}
2	19.15	17.34	17.71^{A}	4.83^{A}	81.66^{A}
3	18.15	17.11	15.29 ^{Bc}	4.39^{AB}	70.66^{B}
4	17.95	16.08	15.54^{BC}	4.25^{AB}	72.67^{B}
5	18.45	16.28	15.08^{C}	3.53^{B}	76.75^{AB}
6	18.55	16.22	15.71^{BC}	3.67^{AB}	75.58^{AB}
7	19.85	17.28	16.92^{AB}	4.42^{AB}	75.17^{AB}
8	19.10	15.72	18.33^{A}	4.79^{A}	79.50^{A}
9	18.10	15.89	15.83^{BC}	3.39^{B}	66.25^{C}
C.V.(%)	4.94	8.55	4.64	10.74	6.29

Means followed by the same lowercase letters in the column are not significant by Tukey test.*Treat: Treatments.

compared to control (Table 2), especially for Treatments 1, 2 and 8. The results showed that the increases in root growth attributed to the use of Pyraclostrobin + Methyl thiophanate in the seed treatment also have favored the growth of shoots of soybean plants. Similar results were observed by Koslowski et al. (2009), who shows that plant height was influenced by Pyraclostrobin via seed treatment.

The benefits of using Pyraclostrobin + Methyl thiophanate via seed treatment were also observed in dry matter accumulation of aerial part from 45 DAE, as can be seen in Figure 2. At the first phase of soybean growth (0 to 45 days) there were no significant differences among treatments. However, at the second phase of growth (45 to 90 days) Treatments 1, 2 and 8 (with use of Pyraclostrobin + Methyl thiophanate) were the most promising in promoting the biomass accumulation during soybean development.

According to our results the effects of the fungicides are most evident, when it was applied via seed treatment than the spraying over aerial part, since all treatments with the use of Pyraclostrobin + Methyl thiophanate had increases in plant growth-related variables, independent of fungicides applied over aerial part. These results corroborate with studies published by Beck et al. (2002), which observed the high photosynthetic activity in wheat cultivars treated with strobilurin when applied via seed treatments under field conditions. The vegetative stage had plants producing photoassimilates to use it at the growth. At the reproductive stage, plants had a reduction in breathing rate, which ensured greater amount of photoassimilates to be directed to the grain filling.

Once at the control treatment presented low infestation of plant diseases, it can be inferred that in the present study was observed the direct fungicides effects on the growth of soybean plants (Table 3). Rodrigues (2009) demonstrated that only a few fungicides have phytotonic effects on soybean plants, especially Pyraclostrobin on seed treatment. It is possible by providing enhancements in carbon assimilation (reduction of compensation point for CO_2) and nitrogen (activity of reductase enzyme over nitrate). Regarding nutritional status of soybean plants at the flowering stage (R2), we observed no statistical differences among treatments. The mean values of the nutrients found in the present study are within the ideal range: N, P, K, Ca, Mg, Cu, Fe, Mn and Zn with values of 4.70 dag x kg^{-1}; 0.25 dag x kg^{-1}; 1.70 dag x kg^{-1}; 1 dag x kg^{-1}; 0.40 dag x kg^{-1}; 10 mg x kg^{-1}; 50 mg x kg^{-1}; 20 mg x kg^{-1} and 20 mg x kg^{-1}, respectively. One exception must to be made for copper, iron and potassium that had values below the recommended. The ideal values was proposed by Ribeiro et al. (1999). According to our results the foliar nutritional analysis is not a tool for predicting the effect of fungicides on the growth of soybean plants. It may be explained due the soil fertility, soil water availability and cultivar, which can interfere on the nutrient levels present (Alcântara Neto et al., 2010). Wells (1993) and Board and Modali (2005), demonstrated that soybean foliar nutrition and its grains yield depends on the plants ability to maximize the radiation interception and/or accumulate a certain levels of dry matter depends of several factors as climatic conditions, date of sowing, plant genotype, population and spacing between plants. However, studies are needed in an attempt to evaluate the fungicides effects on the efficiency of absorption and nutrients redistribution and also photoassimilates produced via photosynthesis for soybeans plants.

Variables referring to the number of trifoliate leaves at flowering, number of branches and string beans at harvest did not differ among treatments (Table 4). These results are possible because these variables are related to the intrinsic characteristics of the cultivar used,

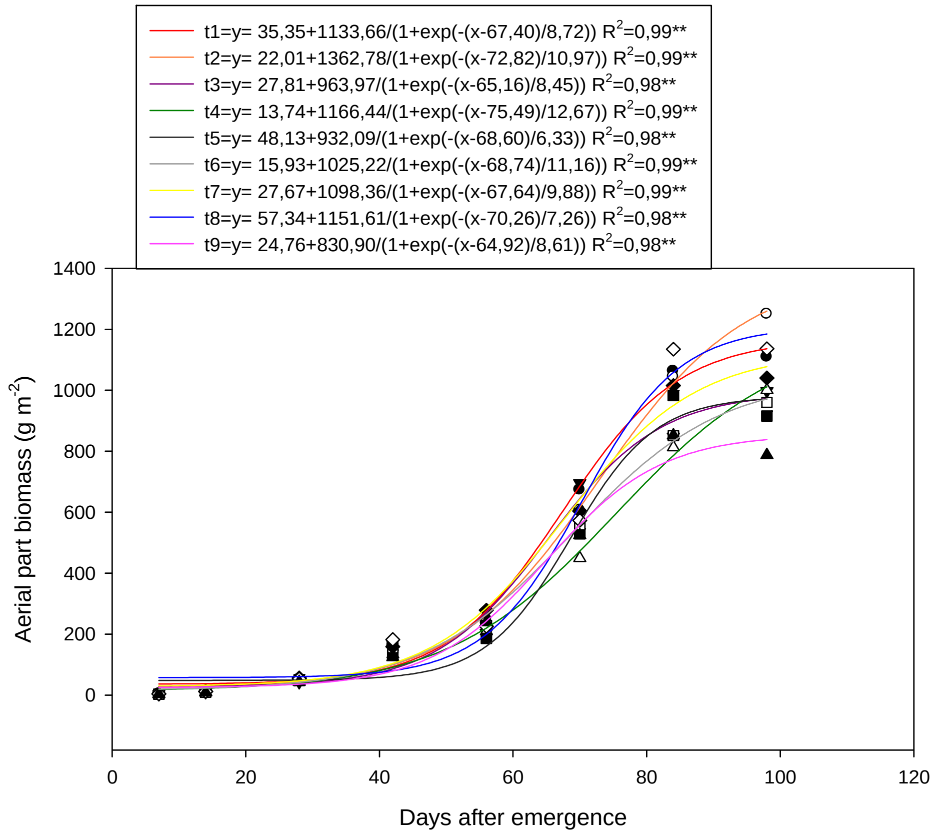

Figure 2. Aerial part biomass of soybean plants subjected to the fungicides via seed treatment and over aerial part. Brazilian savannah of Piauí. Crop year 2011/2012.

Table 3. Number of branches, trifoliate leaves and string beans of soybean plants subjected to different treatments with fungicides applied via seed treatment and over aerial part. Brazilian savannah of Piauí. Crop year 2011/12.

Treat.	Trifoliate leaves	Number of branches	Number of string beans			
			1 grain	2 grain	3 grain	Total
	--- m^{-2}--	No. of branches m^{-2}	------------------------ m^{-2}----------------------			
1	442^{ns}	273.89^{ns}	154.33^{ns}	719.21^{ns}	759.84^{ns}	1633.38^{ns}
2	413	279.07	192.34	705.88	629.90	1528.13
3	361	262.72	179.93	657.69	652.77	1490.39
4	373	248.80	202.91	611.45	607.80	1422.16
5	351	259.36	248.88	653.92	565.42	1468.22
6	372	257.15	253.94	623.45	515.22	1392.62
7	454	274.11	229.14	716.88	604.13	1550.15
8	430	260.65	127.15	739.40	736.06	1602.62
9	425	241.38	195.71	637.11	516.54	1349.37
C.V(%)	11.24	8.82	32.29	16.53	18.63	12.92

ns: not significant by F test ($p<0.05$).* Treat.: treatments

because of that; they are just a little influenced by fungicides.

The use of Pyraclostrobin + Methyl thiophanate in seed treatment also provided significant increments for grain yield. Effects on plant growth by the use of these fungicides are reflected in productivity gains up to 752 kg x ha^{-1} (Table 5). Increases at the productivity related to these treatments can be also observed at higher grain

Table 4. Severity of major diseases in soybean plants subjected to different treatments with fungicides applied via seed treatment and over aerial part. Brazilian savannah of Piauí. Crop year 2011/12.

Treat.	***Colletotrichum dematium* var. *truncata***		***Corynespora cassiicola***	
	56 DAE	**84 DAE**	**56 DAE**	**84 DAE**
	--------------------%	--------------------		
1	10^{A}	15^{A}	5^{A}	20^{A}
2	18^{A}	17^{A}	10^{A}	23^{A}
3	21^{A}	35^{A}	16^{A}	27^{A}
4	17^{A}	38^{A}	18^{A}	35^{A}
5	15^{A}	34^{A}	10^{A}	27^{A}
6	19^{A}	30^{A}	18^{A}	23^{A}
7	14^{A}	24^{A}	5^{A}	27^{A}
8	16^{A}	20^{A}	5^{A}	20^{A}
9	28^{B}	40^{B}	35^{B}	55^{B}
C.V(%)	37	38	39	37

Means followed by the same lowercase letters in the column are not significant by Tukey test.* Treat: Treatments. DAE: days after emergence of soybean.

Table 5. Weight of one thousand grains and soybean yield subjected to different fungicide treatments on seed treatment and over aerial part. Brazilian savannah of Piauí. Crop year 2011/12.

Treat.	Weight of one thousand grains	Soybean yield*
	----g----	----kg x ha^{-1}---
1	192.50^{A}	3100^{A}
2	192.50^{A}	2990^{A}
3	177.50^{AB}	2739^{C}
4	177.50^{AB}	2851^{B}
5	182.50^{AB}	2979^{AB}
6	182.50^{AB}	2933^{AB}
7	185.00^{AB}	2967^{AB}
8	192.50^{A}	3169^{A}
9	170.00^{B}	2417^{D}
C.V (%)	3.48	4.24

Means followed by the same lowercase letters in the column are not significant by Tukey ($p<0.05$). *The seed moisture applied for this calculation was 13%.

weight, which reinforces the positive influence of fungicides on the production and redistribution of photoassimilates by plants. Fagan et al (2010) noted that the use of Pyraclostrobin via seed treatment and over aerial parts of soybeans delayed the leaves senescence and increased the photosynthesis rate by reducing the activity of the enzyme ACC synthase and ethylene synthesis. Another important change observed by the author was elevation of endogenous indole acetic acid level. Therefore, plants might accumulate more energy and increase the efficiency in the use of assimilates to produce substances against pathogens directing more energy for grains production.

Conclusions

1) Pyraclostrobin + Methyl thiophanate via seed treatment favored root development of seedlings, plant height and grain yield on the soybean crop.
2) The plant height and dry weight of aerial part had greater increase with the use of Pyraclostrobin + Methyl thiophanate via seed treatment.
3) Treatments with fungicides did not affect the stand of

plant under low pathogen infestation.

4) The nutritional status by leaf analysis is not a useful tool in predicting phytotonic effects of fungicides on the soybeans plants.

5) Fungicides applied over aerial part seem do not provide any phytotonic effect on soybean plants.

Conflict of Interest

The authors have not declared any conflict of interest.

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