

RESEARCH ARTICLE

Application of pyraclostrobin as an alternative to reduce phytotoxification of fomesafen in common bean

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ABSTRACT

The herbicide fomesafen is one of the few broadleaf herbicides for the post-emergence application in common bean. However, this application can cause phytointoxication to this crop, therefore the objective was to evaluate the application of the fungicide pyraclostrobin for reducing herbicide phytotoxification in common bean. The experimental design was a randomized block design, with ten treatments and four replicates. The treatments resulted from the combination of isolated or associated applications of fomesafen and pyraclostrobin at different stages of common bean development (V₂, V₃ and V₄). We evaluated herbicide phytotoxicity, photosynthetic rate, weight of pods per plant, weight of 1,000 grams and grain yield. The injuries caused by fomesafen were low. The treatments did not affect both the photosynthetic rates and the yield components of bean. In general, the treatments that pyraclostrobin were applied had higher production. The application of pyraclostrobin favored the selectivity of herbicide fomesafen to common bean cultivar IPR Tangará.

Highlighted Conclusion

The application of pyraclostrobin favors the selectivity of herbicide fomesafen to common bean 'IPR Tangará'.

INTRODUCTION

One of the main difficulties for common bean production is due to weed interference (Galon et al. 2018), since this shows a low competitive capacity, because it is a plant with reduced ability to shade the soil in the first stages of development. Moreover, its root system is poorly developed and the crop presents a short cycle, making the weed infestation privileged, interfering in the crop and impeding the maximum productive potential from being reached (Cury et al. 2011; Manabe et al. 2014; Galon et al. 2016).

According to Kozłowski et al. (2002), the critical period of prevention of weed interference for common bean crop occurs between the V₄ to R₆ stages. That is, in order to achieve maximum productivity, it is necessary that the crop be free of weeds during this period. The main method of weed management is chemical control, since it is efficient, practical and economical, being the most used in Brazil. However, the bean crop is sensitive to some herbicides and the level of phytotoxification depends on several factors, among which the following stand out: the cultivar, the herbicide, the climate, the dose and the stage of development of the crop at the moment of application (Takano et al. 2015).

The herbicide fomesafen is one of the few latifolicides registered for common bean applied in post emergence (Silva et al. 2013). Characterized as susceptible an inhibitor of the protoporphyrinogen oxidase (PROTOX) enzyme, take the susceptible plants to death due to oxidative stress, resulting from the formation of reactive oxygen species (ROS) (Alves et al. 2018). Studies show that the application of this herbicide alone or associated with other pesticides can cause phytotoxification to the crop. Having as a consequence of this injuries such as chlorosis and necrosis foliar, flowering delay, enlargement of the cycle period, and productivity reduction (Linhares et al. 2014; Takano et al. 2015).

In the last years, beneficial physiological effects have been studied for plants that are treated with strobilurin fungicides. In this group of fungicides there is a broad-spectrum molecule, which controls the major fungal diseases

of bean culture, the pyraclostrobin. This molecule increases the photosynthetic rate and activity of the enzyme nitrate reductase, decreases the mitochondrial respiratory rate and reduces oxidative stress in plants. In addition, there is an increase in the assimilation rate of CO₂, number of pods, mass of 1000 grains, protein content and the final yield (Fagan et al. 2010; Machado et al. 2018).

Knowing that one of the effects promoted by pyraclostrobin in the plant is to decrease oxidative stress, and that the mechanism of action of fomesafen results in an increase in the formation of ROS, it is assumed that the application of pyraclostrobin could prevent or reduce the formation of ROS after application of fomesafen, avoiding the occurrence of chlorosis and necrosis symptoms, besides deleterious effects on growth and productivity.

The hypothesis of the work was that the damages caused by the herbicide fomesafen in the common bean could be reduced with the application of the fungicide pyraclostrobin. Thus, the objective of the present work was to evaluate if the application of the fungicide pyraclostrobin would be beneficial to the selectivity of fomesafen to the common bean.

MATERIAL AND METHODS

The experiment was implemented on November 21, 2016, in the city of Curitibaanos, State of Santa Catarina, Brazil. The soil of the area is classified as Cambissolo Háplico with a slightly wavy topography and good drainage (Embrapa 2018). It consists of 760 g kg⁻¹ clay, 110 g kg⁻¹ silt, 130 g kg⁻¹ sand, 24.00 g dm⁻³ organic matter and pH (CaCl₂) of 4.94 (layer analysis of 0-20 cm of depth). The climate of the region is classified according to Köppen (1948) as cfb - humid subtropical climate. The climatic conditions (precipitation, radiation, minimum, maximum and average temperature) during the period of conduction of the experiment are presented in Figure 1.

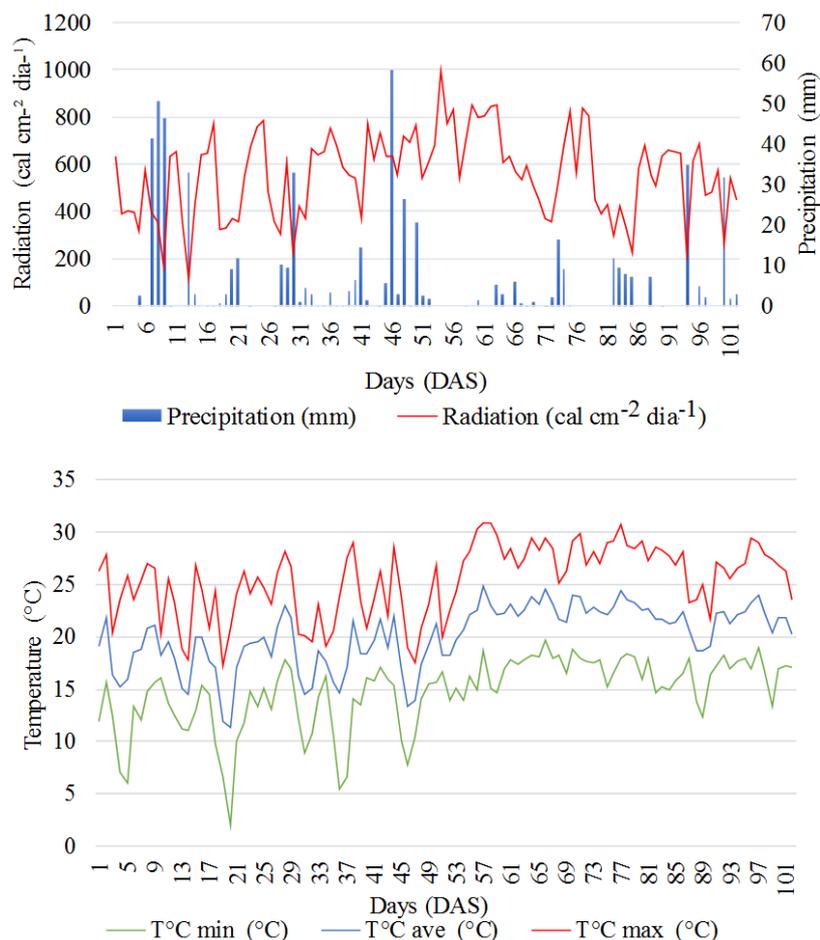


Figure 1. Precipitation, average daily radiation and minimum, average and maximum temperature during the cycle of the bean.

The cultivation was in no-tillage system, with the anticipated burndown, at 20 days before sowing, using the herbicides, tepraloxymid 100 g ha⁻¹ (Aramo 20®), 2,4-D 806 g ha⁻¹ (DMA 806 BR®) and glyphosate 720 g ha⁻¹ (Roundup Original®). A John Deere® tractor model 5085E and a Vence Tudo® seeder-fertilizer model AS 11500 were used for sowing.

The experiment was conducted free of weeds, being realized in pre-emergence one application of paraquat 400 g ha⁻¹ of active ingredient (Gramoxone 200®) with the Agral® adjuvant (0.2% v v⁻¹) in total area after bean sowing, weeding was performed, after new weed emergence flows (when weeds had four leaves) and before the canopy closure of the crop. The free weed experiment was conducted to isolate the effect of the treatments of possible weed interference.

The fertilization was made first in the base with 200 kg ha⁻¹ of 09-33-12 and the complement with a coverage fertilization with 70 kg ha⁻¹ of potassium chloride (60% K) and 150 kg ha⁻¹ of urea (45% N), realized on V2 stage.

The cultivar used was IPR Tangará, cultivar of the carioca commercial group (White bean), undetermined, type II and with a mean cycle of 87 days (IAPAR 2019). Seeds were treated before sowing with pyraclostrobin + methyl thiophanate + fipronil (Standak Top® at a dose of 200 mL 100 kg of seed⁻¹).

The experimental design was a randomized block with ten treatments and four replications. Each experimental unit consisted of 5 lines of 3.00 m of length, spaced at 0.40 m between rows and seeding density of 12 seeds per linear meter. The plot area was composed of the three central lines, and the 0.50 m border was removed from the ends of each line.

The treatments resulted from the combination of isolated or associated applications (sequential applications or tank mix) of fomesafen and pyraclostrobin at different stages of common bean development (V2, V3 and V4), according to Table 1.

Table 1. Treatments used to evaluate the selectivity of fomesafen and pyraclostrobin combinations in the crop of IPR Tangará.

Treatments	Product - Application Phenological Stages
1	Control
2	Fomesafen (FOM) -V ₃ *
3	Pyraclostrobin (PIR) -V ₃
4	PIR/PIR - V ₂ /V ₄
5	PIR/FOM - V ₂ /V ₃ *
6	PIR + FOM - V ₃ *
7	PIR/FOM - V ₃ *
8	FOM/PIR - V ₃ */V ₄
9	PIR/FOM/PIR- V ₂ /V ₃ */V ₄
10	PIR/PIR/FOM/PIR - V ₂ /V ₃ /V ₃ */V ₄

“*” represents the treatments that had the addition of Agral® adjuvant at the dose of 0,5% v v⁻¹.

“+” represents the application of fomesafen and pyraclostrobin in tank mix.

Phenological stages based on Fernandez et al. (1982).

The commercial product used with the active ingredient fomesafen was Flex®, recommended for post-emergence application of common bean, between stages V2 and V4 at doses of 0.9 to 1.0 L ha⁻¹ of c.p. (225 to 250 g ha⁻¹ of a.i.), the highest recommended dose was used for the experiment. In relation to the fungicide, the commercial product Comet® was used, having as active ingredient pyraclostrobin, recommended from the appearance of the fourth bean trifolium, or in the first symptoms of the diseases in the culture, using a dose of 0.3 L ha⁻¹ from c.p. (75 g ha⁻¹ of a.i.), the same dose used in the experiment. These pesticides were chosen because they had only the desirable active ingredients. In the herbicide treatments the Agral® adjuvant was used at a dose of 0.5% v v⁻¹.

The first application of the treatments occurred when the plants were in V2 stage (10 days after sowing – DAS), where more than 50% of the plants had two primary leaves, the second application was carried out in V3 (23 DAS), where more than 50% of the plants had the first fully expanded trifolium and the last application in the V4 stage (29 DAS), where more than 50% of the plants already had the third fully expanded trifolium, according to Fernandez et al. (1982).

For the application of the treatments was used a CO₂ pressurized sprayer with a working pressure of 275 kPa, equipped with a 2.0 m bar, containing four spray tips type AVI 11002, flat jet, spaced in 0.5m displacement speed of 1.0 m s⁻¹, using application rate equivalent to 200 L ha⁻¹.

Phytotoxicity evaluations were initially performed at 7, 15 and 30 days after application of the treatments in V3 (DAAV₃). For this evaluation scores were attributed from 0 to 100%, where 0 represents no poisoning damage and 100 death of all plants (SBCPD 1995).

The evaluation of gas exchange was performed at 06 DAAV₃. Spot measurements were performed on the central leaflet of the last fully expanded trifolium, with simulated light intensity in the range of 1200 μmol m⁻² s⁻¹. A portable photosynthesis apparatus, the Infra-Red Gas Analyzer (IRGA, Li-6400, Licor Ltda., Lincoln, NE) was used.

The gas exchange measurements were performed in the morning between 9:00 am and 11:00 am, where the values of CO₂ (A) assimilation, stomatal conductance (Gs), CO₂ internal concentration (Ci), transpiration (E) and water use efficiency (WUE).

At the end of the bean cycle the desiccation of the culture was carried out with the application of paraquat 400 g ha⁻¹ (Gramoxone 200®) with the Agral® adjuvant (0.2% v v⁻¹) in total area. After grace period of herbicide, manual harvesting of the plot area was realized.

The stand and number of pods per plant were evaluated at the end of the cycle and after harvesting it was determined the mass of 1,000 grains and productivity, with moisture correction to 130 g kg⁻¹.

The data were submitted to analysis of variance by the F test and the means were compared by the Scott-Knott group test, both at 5% probability. Statistical analysis was performed using the SISVAR statistical software.

RESULTS AND DISCUSSION

In general, there was a low intensity of injuries after the treatments. The symptoms were characterized by mild chlorosis in the trefoil bean plants. A significant difference was observed only in the first evaluation (7 DAAV₃), where the use of fomesafen and pyraclostrobin (V₃) by tank mix and the three applications of pyraclostrobin (V₂, V₃ and V₄) and one of fomesafen (V₃) resulted in higher phytotoxicity, with 9.75 and 11.25%, respectively (Table 2). The other treatments presented symptoms of phytotoxicification with intensity varying from 4 to 7%, being superior to the control. In the subsequent evaluations, the phytotoxication symptoms were reduced, and at 30 DAAV₃ no more symptoms were seen in any of the treatments (data not shown), indicating the complete recovery of the injuries observed in the first evaluation. This is due to the low mobility of fomesafen, which is typical of Protox inhibitors (Silva et al., 2018), where trefoils emitted after the application show no symptoms of intoxication.

Table 2. Percentage of phytointoxication of IPR Tangará bean plants treated with fomesafen e pyraclostrobin at 7 e 15 days after application of V₃ treatments (DAAV₃).

Treatments	Phytointoxication (%)	
	7 DAAV ₃	15 DAAV ₃
Control	0.00 a	0.00 ^{ns}
Fomesafen (FOM) -V ₃ *	7.00 b	0.75
Pyraclostrobin (PIR) -V ₃	4.50 b	1.25
PIR/PIR - V ₂ /V ₄	5.50 b	0.50
PIR/FOM - V ₂ /V ₃ *	4.00 b	0.75
PIR + FOM - V ₃ *	9.75 c	1.75
PIR/FOM - V ₃ *	6.25 b	0.50
FOM/PIR - V ₃ */V ₄	5.25 b	1.00
PIR/FOM/PIR- V ₂ /V ₃ */V ₄	5.75 b	1.00
PIR/PIR/FOM/PIR - V ₂ /V ₃ /V ₃ */V ₄	11.25 c	1.00
CV (%)	31.00	125.86

/ treatments application on different tanks. + treatment application on tank mix.

^{ns} not significant at 5% probability.

Marchioretto et al. (2017) verified low phytotoxicity levels for common bean cultivars ANfc 9, IPR Uirapuru and BRS style at 14 DAA fomesafen (250 g ha⁻¹) at the V₂ stage. The same was observed by Galon et al. (2018) for common bean IPR Tuiuiu with application of fluazifop-p-butyl (250 g ha⁻¹) + fomesafen (250 g ha⁻¹). The results also corroborate with Cieslik et al. (2014) who have recovered phytotoxicity symptoms in common bean plants, cultivars IPR Tiziu and IPR-81 at 21 fomesafen DAA.

Research by Mancuso et al. (2016) with cowpea bean cultivar BRS Guariba, found low levels of phytointoxication after the application of 250 g ha⁻¹ of fomesafen at the stage of eight to ten pairs of leaves. The highest phytotoxicity (17.0%) was observed at 7 DAA, decreasing to 10.2 and 4.0%, at 15 and 30 DAA, respectively. In another work using the same cowpea bean cultivar, the authors observed a percentage of phytotoxicification of 34 and 40%, at 14 and 28 DAA of 225 g ha⁻¹ of fomesafen at the V₄-V₅ stage, respectively, for this experiment (Fontes et al. 2013).

The low phytotoxicity observed in this work may be related to seed treatment, since one of the active ingredients of the commercial product used was pyraclostrobin, this fungicide remains active in the plant for a minimum period of 14 days according to the commercial product leaflet. Thus, even plants that did not receive pyraclostrobin after emergence had the physiological effect of pyraclostrobin from seed treatment, thus not suffering severe injuries from the application of fomesafen.

Another factor that may have interfered in the low levels of phytointoxication is the climate of the region where the experiment was implanted. In the period of conduction of the experiment the conditions were optimal for the growth and development of the culture. The volume of precipitation was greater than 300 mm, its distribution being homogeneous throughout the crop cycle. The daytime temperature rarely reached 30 °C and the nights had mild temperatures (Figure 1). These conditions are extremely favorable to liquid photosynthesis of plant C3, such as common bean, this favorable energy condition certainly contributed to the low intensity of symptoms and to the rapid recovery of plants.

Regarding the photosynthetic evaluations performed at the 6 DAAV₃, no significant difference between the treatments was observed (Table 3). Different from that observed by Manabe et al. (2014) that verified a reduction in the photosynthetic rate of common bean plants cultivar BSR Supremo after application of the commercial mixture of fluazifop-p-butyl + fomesafen (180 + 225 g ha⁻¹).

Table 3. Photosynthetic evaluations (net assimilation rate of carbon - A (μmol CO₂ m⁻² s⁻¹), stomatal conductance - Gs (mol H₂O m⁻² s⁻¹), internal concentration of CO₂ - Ci (ppm), transpiration rate - E (mmol H₂O m⁻² s⁻¹) and photosynthetic efficiency in the use of water (WUE) of IPR Tangará bean plants at 06 DAAV₃.

Treatments	A	Gs	Ci	E	WUE
Control	14.44 ^{ns}	0.46 ^{ns}	192.80 ^{ns}	8.18 ^{ns}	1.76 ^{ns}
Fomesafen (FOM) -V ₃ *	16.23	0.46	178.28	8.16	1.99
Pyraclostrobin (PIR) -V ₃	16.25	0.44	174.15	7.73	2.12
PIR/PIR - V ₂ /V ₄	15.23	0.49	181.60	7.92	1.92
PIR/FOM - V ₂ /V ₃ *	15.18	0.47	180.81	7.62	1.98
PIR + FOM - V ₃ *	15.37	0.49	178.40	8.49	1.80
PIR/FOM - V ₃ *	15.48	0.44	169.16	8.11	1.91
FOM/PIR - V ₃ */V ₄	15.10	0.45	173.90	8.16	1.85
PIR/FOM/PIR - V ₂ /V ₃ */V ₄	14.08	0.39	168.17	7.84	1.77
PIR/PIR/FOM/PIR - V ₂ /V ₃ /V ₃ */V ₄	12.44	0.43	181.81	7.87	1.57
Mean	14.98	0.45	177.91	8.01	1.87
CV (%)	12.83	12.33	5.26	.82	11.45

/ treatments application on different tanks. + treatment application on tank mix.

^{ns} not significant at 5% probability.

According to Takano et al. (2015), which used the same carioca bean cultivar and with the same dose of pyraclostrobin and fomesafen, when two applications of pyraclostrobin (V₂ and V₄) and one of fomesafen (V₃) were performed, there was a significant increase in liquid assimilation of carbon in relation to the control at 7 DAA in contrast to the results obtained in this work. However, the mean net carbon assimilation rate observed by Takano et al. (2015) varied from 9.92 μmol m⁻² s⁻¹ on witness, to 10.01 e 11.92 μmol m⁻² s⁻¹ on treatments with one (V₂) and two applications (V₂ and V₄), respectively, presenting minor variations to those found in this work.

In the work performed by Takano et al. (2015) evaluated the rate of net carbon assimilation in post-emergence fungicide treatments of common bean, when using seed treatment and with the application of bentazon herbicide. These authors verified that when bentazon was applied in plants that received the seed treatment and in post emergence two applications of pyraclostrobin there was significant increase for this variable. When the seed treatment was not used, the reverse occurred. However, the rate of net assimilation of carbon did not exceed 14.03 μmol m⁻² s⁻¹.

Increases in the photosynthetic rate of wheat, soybean and tomato plants after application of pyraclostrobin are also described in the literature (Grossmann et al. 1997; Fagan et al. 2010; Marek et al. 2018).

The increase in liquid photosynthesis is linked to changes in the CO₂ compensation point, which favors CO₂ uptake as opposed to release by respiration. This increase may be explained by the transient inhibition of respiration when the plant is treated with pyraclostrobin (Grossmann et al. 1997). According to Köehle et al. (2002), pyraclostrobin acts directly on the electron transport chain and also acts as a moderator of biotic and abiotic stresses because it participates in the metabolism of citric acid and stress hormones, such as abscisic acid. Thus, the decrease in respiration in plants treated with this fungicide may be an important factor, since it reduces the energy wastage of the plant to the maintenance metabolism.

However, for the present experiment no difference was observed in this variable. This probably occurred because the climatic conditions during the conduction of the experiment favored the development of the crop. The nights with mild temperatures are directly associated with reduced respiration, thus leading to lower energy losses (higher net photosynthetic rate) that would be required for maintenance of the plant.

Daytime temperatures between 15 and 30 ° C may have contributed to the high rates of crude CO₂ assimilation, and therefore no difference was observed with the application of pyraclostrobin, since the plants were exposed to optimal conditions for their development. What probably did not happen in the work of Takano et al. (2015), due to the region (North Central Paranaense, Maringá-PR, Brazil) and the time of conduction of the experiment (February to July), which resulted in bean plants developing in less favorable environmental conditions than those observed in Curitiba-SC.

For the yield components: stand, number of pods per plant and mass of 1000 grains, there was no significant difference between the treatments (Table 4).

Table 4. Productivity components (stand, number of pods per plant and mass of 1000 grains) and productivity of IPR Tangará bean plants treated with fomesafen and pyraclostrobin.

Treatments	Stand	N ^o of pods	Mass of 1000 grains (g)	Productivity (kg ha ⁻¹)
Control	12.50 ^{ns}	8.50 ^{ns}	318.2 ^{ns}	2742.03 b
Fomesafen (FOM) -V ₃ *	13.81	7.00	328.5	2198.07 b
Pyraclostrobin (PIR) -V ₃	12.98	7.55	328.5	3592.24 a
PIR/PIR - V ₂ /V ₄	12.80	6.95	324.2	3425.87 a
PIR/FOM - V ₂ /V ₃ *	12.26	7.20	327.4	3571.95 a
PIR + FOM - V ₃ *	12.20	7.65	316.2	3480.76 a
PIR/FOM - V ₃ *	12.14	7.65	313.9	3498.70a
FOM/PIR - V ₃ */V ₄	10.89	8.10	320.9	3635.84 a
PIR/FOM/PIR- V ₂ /V ₃ */V ₄	13.69	8.20	329.3	2593.22 b
PIR/PIR/FOM/PIR- V ₂ /V ₃ /V ₃ */V ₄	10.95	8.40	317.4	3272.01 a
Mean	12.42	7.72	322.45	-
CV (%)	13.84	14.33	2.85	16.98

Means followed by the same letter in the column do not differ by Scott-knott test. p <0.05%.

^{ns} not significant at 5% probability.

For the variable productivity, a significant difference was observed, where the control (2742.03 kg ha⁻¹), treatment with fomesafen (V₃) only (2198.07 kg ha⁻¹) and treatment with two applications of pyraclostrobin (V₂ and V₄) and one of fomesafen (V₃) (2593.22 kg ha⁻¹), presented lower productivity than the others that ranged from 3272.01 to 3635.84 kg ha⁻¹. The treatments that did not receive the application of pyraclostrobin were grouped with the lowest levels of productivity. These results corroborate with those obtained by Takano et al. (2015), where there was reported the improvement in the productivity of the Tangará IPR with the use of pyraclostrobin in V₂ and V₄ compared to the application in V₃, and without the treatment of the fungicide seed. When seed treatment was used, the increase productivity was similar than that found in this study.

It can be noted that when the fungicide pyraclostrobin is not applied, productivity is affected, even with favorable conditions for the development of the crop. A study by Kozłowski et al. (2009) shows that the highest productivity of the IPR Uirapuru bean cultivar was achieved when two applications of pyraclostrobin were carried out, according to the authors, the increase was due to the higher number of pods per plant. This situation was not repeated in the present research.

Takano et al. (2015) pointed out that regardless of the treatment of the seeds with pyraclostrobin, the common bean plants that received two applications of pyraclostrobin presented productivity similar or superior to the other treatments. However, when the productivity was compared only in the treatments where seed treatment was not performed, this increase was more evident. This is probably due to the effect of pyraclostrobin used in seed treatment, where it would have promoted a physiological effect on the initial development of common bean plants. With the results obtained it can be observed that the herbicide fomesafen caused minor injuries in the common bean crop, however, these did not directly influence the productivity. However, new work should be performed to clarify the physiological effect of pyraclostrobin in this crop, as it was noted a positive effect of pyraclostrobin applications on the productivity of common bean cultivar IPR Tangará.

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