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Use of pyraclostrobin on the nitrate assimilation and agronomic traits of the Pérola common bean cultivar

Uso de piraclostrobina na assimilação do nitrato e nos caracteres agronômicos de feijoeiro Pérola

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Abstract

Fungicides of the strobilurin chemical group are efficient in controlling pathogens, but can also act on plants to affect their development and increase grain yield. In this context, the aim of the present work was to verify the physiological effect of fungicides and their application time regarding the action of pyraclostrobin in the Pérola common bean cultivar. For this purpose, the experiment was carried out in an incomplete factorial arrangement (3x2x2+1), with four replications, being three seed treatments combined with the application of pyraclostrobin before or after fertilization, plus one application or non-application of another fungicide with pyraclostrobin, and an additional treatment without fungicide application. The following were evaluated: nitrate reductase activity, chlorophyll index, first pod insertion height and plant height, incidence of diseases, number of pods per plant and number of grains per pod, thousand-grain weight and grain yield. The application of pyraclostrobin before cover fertilization resulted in higher nitrate reductase activity with positive effects on the number of grains per pod, grain yield and plant height of the Pérola bean cultivar.

Additional keywords: fungicide; *Phaseolus vulgaris*; grain yield.

Resumo

Fungicidas do grupo químico das estrobilurinas são eficientes no controle de patógenos, mas também podem agir nas plantas de modo a afetar seu desenvolvimento e aumentar a produtividade. Neste contexto, objetivou-se com o presente trabalho verificar o efeito fisiológico de fungicidas e sua época de aplicação quanto à ação da piraclostrobina na cultivar de feijão Pérola. Para tal, foi instalado o experimento em arranjo fatorial incompleto (3x2x2+1), com quatro repetições, sendo três tratamentos de sementes combinados com a aplicação de piraclostrobina antes ou após adubação de cobertura, mais uma aplicação ou não de outro fungicida com piraclostrobina, e um tratamento adicional sem aplicação de fungicida. Foram avaliadas, atividade da redutase do nitrato, índice de clorofila, altura de inserção de primeira vagem e altura de plantas, incidência de doenças, número de vagens por planta e de grãos por vagens, massa de mil grãos e produtividade. A aplicação de piraclostrobina antes da adubação de cobertura resultou em maior atividade da redutase do nitrato com efeitos positivos sobre no número de grãos por vagem, produtividade de grãos e altura de plantas de feijoeiro cultivar Pérola.

Palavras-chave adicionais: fungicida; *Phaseolus vulgaris*; produtividade

Introduction

The common bean (*Phaseolus vulgaris* L.) crop has great economic and social importance for Brazil, due to the tradition of its consumption, nutritional quality, cultivation in large areas and mainly the production from family farmers who are responsible for 70% of the national production (Borém & Carneiro, 2006; IBGE, 2006).

In the 2015/2016 harvest, the crop area was about 2.83 million hectares, with production of 2.51 million tons and consumption in Brazil of 2.8 million tons. It is estimated that in the 2016/2017 harvest, 3.00 million hectares will be cultivated and 3.11 million tons produced, with cultivar BRS Pérola being one of the most cultivated. The average grain yield for Brazil in the 2015/2016 harvest was 886 kg ha^{-1}, and indicates the need for improvements in cultivation techniques in some regions of the country, despite the estimation of a 16.7% increase in grain yield in the 2016/2017 harvest, since it is known that in some states more technically advanced producers can obtain grain yield averages above 3,000 kg ha^{-1} (CONAB, 2016; Borém & Carneiro, 2006).

One of the main causes of low grain yield of common bean are diseases. Among the most important are the angular leaf spot (*Phaeoisariopsis griseola*), which causes damage mainly in the vegetative period due to foliar lesions, and the anthracnose (*Colletotrichum lindemuthianum*), which causes lesions on the stem, petiole, pods, and seeds, when transmitted through them (Paula Júnior & Zambolim, 2006).

Fungicides of the strobilurin chemical group act as pathogen control agents, which inhibit the mitochondrial respiration of phytopathogens by binding to the cytochrome Qo site of the enzymatic complex bc1 (complex III), blocking the transfer of electrons in the respiratory tract, leading thus to an energy deficiency due to a lack of ATP. These fungicides became known as Qo inhibitors (QoIs) (Gisi et al., 2002). Since the bc1 complex exists in all eukaryotes, at least some partial inhibition in electron transport must also be expected in plant cells after absorption of the fungicide (Venâncio et al., 2003).

Field trials have revealed that strobilurin-treated cereals have increased their grain yield, since in addition to pathogen control there is a gain in grain yield due to the additional effects on plant physiology, which is most evident when there is no incidence of pathogens (Khöle et al., 2002). In maize, Barbosa et al. (2011) found that the application of strobilurin (pyraclostrobin) provided an increase in grain yield of 8 bags ha^{-1} for every 30 kg of N applied. Marafon & Simonneti (2012) verified a reduction in the incidence of maize rust and an increase in grain yield with the use of pyraclostrobin.

In soybean, three hours after application of strobilurin (pyraclostrobin) at phenological stage R1, there is a 3% increase in the photosynthetic rate. After seven days of application, the increase is of 56% compared to plants without application of fungicides. In addition to the increase in the photosynthetic rate, when the application is carried out at phenological stage R5.1, a decrease in the respiratory rate is also observed (Fagan et al., 2010).

Thus, the aim of this work was to verify the physiological effect of fungicides and their application time regarding the action of pyraclostrobin in the Pérola bean cultivar.

Material and methods

The experiment was conducted in the municipality of Jataí-GO, at 17º 55' South latitude, 51º 42' West longitude and with 668 meters of altitude, in a soil classified as Dystroferric Red Latosol (Embrapa, 2013). According to the Köppen classification, the climate is Cw, mesothermal, with dry and rainy seasons defined by the months of March to September and October to April, respectively. The climatological data of the experimental period were collected by the INMET (National Meteorological Institute) meteorological station (Figure 1).

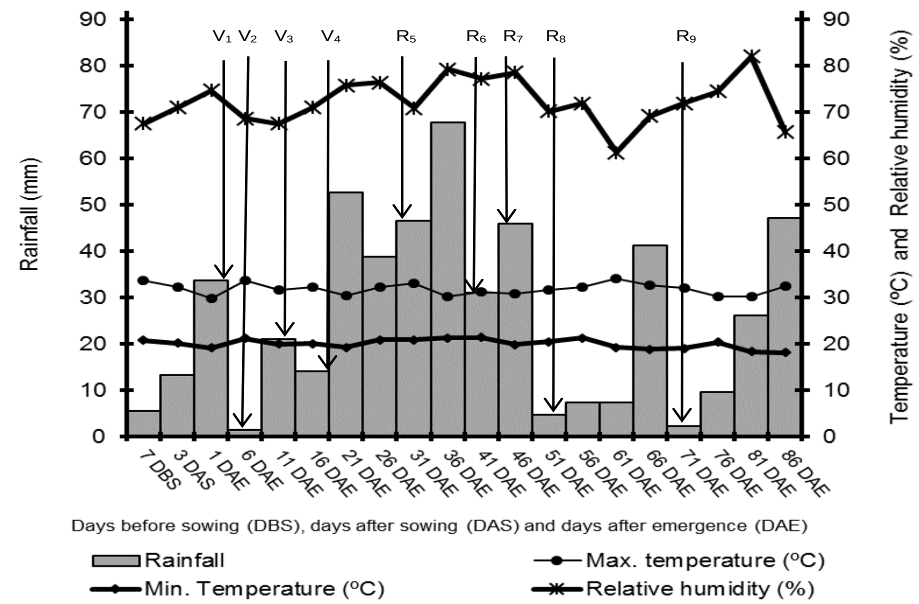

Figure 1 - Rainfall, relative humidity, maximum and minimum air temperature in periods of five days during the experiment (where the arrows above the bars indicate the beginning of each phenological stage of bean plant).

Before the installation of the experiment, soil samples were collected in the 0-20 cm depth layer for chemical analysis (Table 1), following the methodology described by Raij et al. (2001), except for Mehlich-1 P, which was measured following the methodology of Silva et al. (2009).

Table 1. Chemical properties of Red Latosol in the 0-20 cm layer of the experimental area.

pH ($CaCl_2$)	O. M. (g dm^{-3})	P (Mehlich I) ---- (mg dm^{-3}) ----	K	Ca ---- ($cmol_c$ dm^{-3}) ----	Mg	Al	H+Al
5.3	34.2	1.9	52	1.22	0.68	0.07	5,3
CEC ($cmol_c$ dm^{-3})	Bases saturation (V%)	Al saturation (m%)		Zn ---- (mg dm^{-3}) ----	Fe	Mn	Cu
7.3	27.8	3.3		0.4	28	34.4	9.6

According to the results, application of dolomitic limestone (86% TNRP) in the amount of 2.3 Mg ha^{-1} was recommended based on the saturation limits established for bean (Souza & Lobato, 2004) to raise the base saturation to 55%.

The soil was conventionally prepared, on October 31, 2013, with harrowing and fertilization. 400 kg ha^{-1} of the formulation 04-30-16 was applied in the sowing furrow, and at 31 days after emergence (DAE), cover fertilization with N and K was performed (420 kg ha^{-1} ammonium sulfate + 60 kg ha^{-1} potassium chloride).

The design was randomized blocks in an incomplete factorial scheme 3x2x2+1, resulting in 13 treatments, with three replications. Each plot had 6.75 m^2 of useful area consisting of five rows of six meters, spaced 0.45 m apart, totaling 52 experimental units, where each unit had 13.5 m^2, with a total experimental area of 972 m^2.

Therefore, the three levels of the first factor were: treatment of seeds with the application of fipronil (250 g L^{-1}) at a dose of 200 ml of the commercial product (c.p.) to 100 kg^{-1} of seeds, pyraclostrobin (25 g L^{-1}) + + methyl thiophanate (225 g L^{-1}) + fipronil (250 g L^{-1}) at a dose of 200 ml 100 kg^{-1} seeds, and seeds not treated with phytosanitary products. The two levels of the second factor were given by the application of pyraclostrobin (250 g L^{-1}) at a dose of 0.3 L ha^{-1} before or after nitrogen fertilization. Finally, the two levels of the third factor consisted of the application or non-application of pyraclostrobin (130 g L^{-1}) + + metconazole (80 g L^{-1}) at a dose of 0.5 L ha^{-1}. The additional treatment was represented by the absence of application of any type of fungicide.

The common bean was sown without inoculation of *Rhizobium tropici* on November 01, 2013, using seeds of the cultivar Pérola, at the density of 10 seeds per linear meter. Pest control was carried out in the vegetative period and during the pod formation period. The insecticides chlorfenapyr (240 g L^{-1}), at a dose of 0.8 L ha^{-1} of the c.p., imidacloprid (100 g L^{-1}) + beta--cyfluthrin (12.5 g L^{-1}), at a dose of 0.75 L ha^{-1}, gamma--cyhalothrin (150 g L^{-1}), at a dose of 0.3 L ha^{-1}, and acetamiprid (200 g kg^{-1}), at a dose of 300 g ha^{-1}, were used when necessary. At the beginning of the reproductive period R5, manual weeding was performed. Applications were carried out with CO2 pressurized costal sprayer with constant pressure of 200 kPa, equipped with a bar with four flat jet nozzles (DG 11002), spaced 0.5 m, and with a flow rate of 200 L ha^{-1}.

At 27 DAE, application of pyraclostrobin (250 g L^{-1}) was performed in the morning, prior to cover fertilization, with average temperature of 26.5 °C and relative air humidity of 88%. At 36 DAE, application of pyraclostrobin (250 g L^{-1}) was carried out in the afternoon, after cover fertilization. The average air temperature at the time of application was 29 °C and the relative air humidity was 60%. Nitrate reductase enzyme activity was analyzed six days after application.

At 46 DAE, application of pyraclostrobin + metconazole was performed in the morning, with average air temperature of 27 °C and relative air humidity of 80%. Nitrate reductase enzyme activity was analyzed four days after application.

For the in vivo determination of the nitrate reductase activity, the methodology cited by Meguro & Magalhães (1982) was used, with the addition of propanol, according to Jaworski's (1971) methodology. Three trifolia were collected from the upper third of the common bean plants after three hours of solar irradiation, being taken to the laboratory, kept at room temperature, and immediately after the removal of central veins, rectangular cuts were performed of approximately one millimeter thickness. 0.2 g of the plant sample was weighed and transferred to test tubes containing 5 ml of incubation solution (0.1 mol L^{-1} KH_2PO_4 with pH adjusted to 7.5 with 0.2 mol L^{-1} KOH, KNO_3 and added with 1% n-propanol), then the samples immersed in the incubation medium were placed in a vacuum desiccator for 30 seconds for three times, at 6 mm Hg.

Subsequently, the tubes were vortexed and isolated from light with aluminum foil. They were incubated for 1 hour at 30 °C. After this time, the solution was filtered and 0.2 ml of the incubation medium was transferred to test tubes where 2.0 ml of reagent was added (1% sulfanilamide in 1.5 molar HCl and 0.02% n-naphthyl ethylene diamine [m/v]). After being vortexed, 30 minutes were taken to read absorbance at 540 nm in a MICRONAL spectrophotometer (model B572).

Before starting the readings, a calibration solution was made with 0.2 ml H_2O + sulfanilamide + n--naphthyl in the same proportions as previously described. The data were expressed in µmol

NO_2 g ^{1} h ^{1} fresh plant tissue, and nitrite concentration was obtained by means of the equation generated by a standard curve with known nitrite concentration.

The chlorophyll content was determined indirectly at 69 DAE, in ten plants per plot, where a reading per plant was performed in the ventral part of a fully expanded leaflet of the upper trifolia of the bean plant, using an electronic portable chlorophyll meter (clorofiLOG®, model CFL1030). The reading data were expressed in Falker Chlorophyll Index (FCI), being considered the levels of chlorophyll A and B for its calculation, made possible by the combination of the light wavelengths analyzed by the equipment (FALKER Automação Agrícola Ltda, 2008).

The percentage of leaf area affected by pathogens was evaluated at 70 DAE for *Colletotrichum lindemuthianum* (anthracnose) and *Phaeoisariopsis griseola* (angular leaf spot) with the aid of a diagrammatic scale for common bean proposed by Godoy et al. (1996) (Figure 2), with 15 leaflets per experimental unit being randomly evaluated.

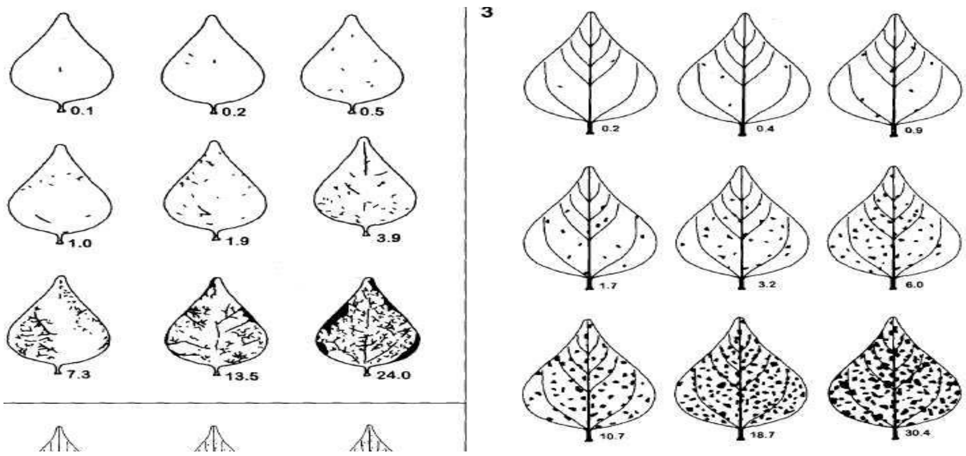

Figure 2 - Diagrammatic scale of severity (percentage of diseased leaf area) of anthracnose (*Colletotrichum lindemuthianum*) and angular leaf spot (*Griseola phaeoisariopsis*)

At 84 DAE, the first pod insertion height was evaluated in a sample of ten plants from each plot, measuring from the base of the soil until the first pod insertion; and from the base of the soil to the plant apex for plant height.

Harvest was performed at 86 DAE, when a sample of 10 plants was collected from each plot to count the number of pods per plant and the number of seeds per pod. For grain yield, the three central rows were collected, eliminating 0.5 m from the borders at the beginning and end of each plot, also counting the final population of plants. The plants were mechanically harvested and then the total grain weight of the plot was measured to account for the production, with the weight being corrected to 13% moisture (wet basis), and the result of grain yield expressed in kg ha^{-1}. Thousand-grain weight was also verified, according to the Rules for Seed Analysis (Brazil, 2009).

The values obtained were submitted to the Shapiro-Wilk test for normality and analysis of variance was performed, applying the Tukey test at 5% probability when significant, through the software Assistat 7.7 beta.

Results and discussions

For nitrate reductase activity, no significant effect was observed between treatments pyraclostrobin + methyl thiophanate + fipronil and pyraclostrobin + metconazole (Table 2). The application of pyraclostrobin before cover fertilization (BCF) provided an increase of 23.36% in the nitrate reductase enzyme activity compared to application after cover fertilization (ACF) (Table 2).

In the present experiment, the soil presented average levels of organic matter, and showed positive effect of the use of pyraclostrobin, since, according to Kozoloski et al. (2009), there is no need for pyraclostrobin to increase nitrate reductase activity in common bean when it is cultivated in soil with high organic matter content, where nitrogen supply from the soil is sufficient to maintain nitrate assimilation.

In the maize crop, the application of pyraclostrobin associated with soil nitrogen fertilization increases the nitrate reductase activity by, on average, 56% in relation to the control (Barbosa et al., 2011), and the nitrate reductase enzyme activity is the higher the closer to the application of nitrogen cover fertilization (Machado et al., 2013).

In soybean, seven days after the application of pyraclostrobin, there is an increase in nitrate reductase activity of up to 250% in relation to the control, and if a second application is subsequently carried out, the increase is of 84% (Soares et al. 2011).

In the present work, there was no additive effect of pyraclostrobin throughout the applications, once for seed treatment the application of pyraclostrobin + methyl thiophanate + fipronil followed by application of pyraclostrobin and then application of pyraclostrobin + metconazole did not result in significant interaction between these treatments for nitrate reductase activity (Table 2).

Table 2 - Effect of seed treatment (ST), pyraclostrobin application (C) and pyraclotrobin + metconazol (P+M), the nitrate reductase activity (NRA) of Pérola common bean cultivar, Jataí-GO, 2013-2014.

Treatments	NRA[1] after ST	NRA after C application	NRA after P+M application
		[#] Seed treatment	
Fipronil (FI)	3.19 a	2.30 a	2.25 a
C + Methyl thiophanate + FI	2.97 a	2.36 a	1.93 a
Without ST	3.08 a	2.24 a	1.86 a
F test	1.05^{ns}	0.20^{ns}	1.88^{ns}
MSD[4]	0.46	0.46	0.53
		[#] Pyraclostrobin (C)	
BCF[2]	-	2.48 a	1.91 a
ACF[3]	-	2.12 b	2.11 a
F test	-	6.03*	1.30^{ns}
MSD	-	0.31	0.36
		[#] Pyraclostrobin + metconazol (P+M)	
With P+M	-	-	2.04 a
Without P+M	-	-	1.98 a
F test	-	-	0.12^{ns}
MSD	-	-	0.36
ST x C	-	0.39^{ns}	0.43^{ns}
ST x (P+M)	-	-	0.26^{ns}
C x (P+M)	-	-	0.05^{ns}
ST x C x (P+M)	-	-	0.68^{ns}
CV[5]	6.90%	16.10%	26.16%
W[6] (p-value)	0.84N	0.31N	0.26N

^{ns} Not significant by the F test. (*) Significant at 5% probability level by the F test. (#) Means in the same column followed by different letters are statistically different (P ≤ 0.05) according to Tukey's test. (1) Expressed in µmol NO_2^- g^{-1} h^{-1} fresh mass. (2) BCF-Pyraclostrobin before fertilization of covering. (3) ACF-Pyraclostrobin after fertilization of covering. (4) MSD - Minimum significant difference. (5) CV - Coefficient of variation. (6) Shapiro-Wilk: N-Normal e NN- Not-normal.

The application period in soybean is determinant because, when applied at R1 (flowering), there is an increase in nitrate reductase activity in relation to the control, however at R5.1 (grain filling), this is not observed (Fagan et al., 2010).

There was interaction between the time of application of pyraclostrobin and pyraclostrobin + + metconazole for plant height. Hence, according to the results presented in Table 3, there was a positive effect of pyraclostrobin, applied before cover fertilization without application of pyraclostrobin + metconazole, resulting in higher plant height, possibly also due to the contribution of higher nitrate assimilation.

Table 3 - Effect of pyraclostrobin for interaction between pyraclostrobin application (C) and pyraclotrobin + metconazole (P+M) in height of plants Pérola common bean cultivar, Jataí-GO, 2013-2014.

Pyraclostrobin (C)	[#] Height of plants (cm)	
	With P+M	Without P+M
BCF[1]	115.22 a A	120,73 a A
ACF[2]	117.63 a A	114,64 b A
F test	4.63*	
MSD[3] Column	5.76	
MSD Line	5.76	
CV[4]	5.3%	
W[5] (p-value)	0.41N	

(*) Significant at 5% probability level by the F test. (#) Means in the same row followed by different capital letters or averages in the same column followed by different lowercase letters are statistically different (p ≤ 0.05) according to Tukey's test. (1) BCF-Pyraclostrobin before fertilization of covering. (2) ACF- Pyraclostrobin after fertilization of covering. (3) MSD - Minimum significant difference. (4) CV - Coefficient of variation. (5) Shapiro-Wilk: N-Normal e NN- Not-normal

In soybean, 42 days after the emergence, plant height increased due to the application of pyraclostrobin + methyl thiophanate + fipronil in the treatment of seeds (Balardin et al., 2011). Notwithstanding, this product did not interfere with the height of bean plants in the present work. Still in soybean, Roese & Lima Filho (2010) did not observe interference in plant height resulting in additive effects with the use of pyraclostrobin.

In the evaluation of the first pod insertion height, Falker Chlorophyll Index, angular leaf spot severity and number of pods per plant, there was no significant difference for seed treatment with the application of pyraclostrobin and pyraclostrobin + metconazole (Table 4).

In the evaluation of the percentage of leaf area affected by anthracnose (AN), number of grains per pod and grain yield, there were differences only between the time of application of pyraclostrobin, indicating that the application before cover fertilization reduced the severity of anthracnose with a consequent increase in the number of grains per pod (Table 4).

Table 4 - Effect of pyraclostrobin (C) at the height of insertion of the first pod (FPI), Falker chlorophyll index (FCI), leaf area affected (severity of illness) for anthracnose (A) and angular leaf spot (ALS), number of pods per plant (NP), seeds per pod (SP) and grain yield (Yield) of Pérola common bean cultivar, Jataí-GO, 2013-2014.

Treatments	FPI (cm)	FCI	A (%)	ALS (%)	NP	SP	Yield (kg ha^{-1})
			(#)Treatment of seeds				
Fipronil (FI)	23.69 a	40.12 a	0.0470 a	0.5375 a	14.65 a	4.67 a	1951.64 a
C + Methyl thiophanate+ FI	25.70 a	38.59 a	0.0233 a	0.5641 a	15.03 a	4.74 a	1896.42 a
Without ST	24.46 a	39.78 a	0.0291 a	0.5308 a	17.82 a	4.71 a	2000.10 a
F test	1.07^{ns}	1.74^{ns}	1.33^{ns}	0.13^{ns}	2.72^{ns}	0.19^{ns}	0.53^{ns}
MSD(3)	3.44	2.10	0.03	0.16	3.62	0.28	244.32
			(#)Pyraclostrobin (C)				
BCF(1)	24.06 a	40.00 a	0.0205 b	0.5511 a	16.08 a	4.82 a	2064.42 a
ACF(2)	25.17 a	38.99 a	0.0458 a	0.5372 a	15.59 a	4.59 b	1834.34 b
F test	0.98^{ns}	2.04^{ns}	4.18*	0.06^{ns}	0.15^{ns}	5.43*	7.93*
MSD	2.32	1.42	0.02	0.11	2.46	0.19	165.79
			(#)Pyraclostrobin + metconazol (P+M)				
With P+M	23.97 a	39.41 a	0.0369 a	0.5363 a	15.57 a	4.64 a	1898.21 a
Without P+M	25.26 a	39.58 a	0.0294 a	0.5519 a	16.10 a	4.77 a	2000.56 a
F test	1.32^{ns}	0.05^{ns}	0.36^{ns}	0.08^{ns}	0.18^{ns}	1.55^{ns}	1.56^{ns}
MSD	2.32	1.42	0.02	0.11	2.46	0.19	165.79
CV(4)	13.58%	6.16%	4.14%	12.37%	26.07%	7.13%	14.67%
W(5) (p-value)	0.09N	0.78N	0.00NN	0.04NN	0.001NN	0.17N	0.88N

^{ns} Not significant by the F test. (*) Significant at 5% probability level by the F test. (#) Means in the same column followed by different letters are statistically different (P ≤ 0.05) according to Tukey's test. (1) BCF-Pyraclostrobin before fertilization of covering. (2) ACF- Pyraclostrobin after fertilization of covering. (3) MSD - Minimum significant difference. (4) CV - Coefficient of variation. (5) Shapiro-Wilk: N-Normal e NN- Not-normal.

The period of pyraclostrobin application is extremely important (Fagan et al., 2010), thus, BCF application may have differed from ACF application, also because the prior was applied at the beginning of the reproductive period (R5), a phase in which the plant ends its vegetative growth and moves towards its production, and ACF application was performed already at flowering (R6). BCF application increased nitrate reductase activity, and this increase was accompanied by higher number of grains per pod and grain yield (Table 4).

In soybean, no significant effects were observed for first pod insertion height after three applications of pyraclostrobin (Roese & Lima Filho, 2010). This result agrees with that obtained in the present study, since even after the third application with pyraclostrobin + metconazole there was no significant difference compared to the absence of application for the first pod insertion height of common bean.

In the present study, no changes were observed in the chlorophyll index after 20 days of the last application of pyraclostrobin + metconazole at R7 (Table 4), corroborating with the results found by Kozlowski et al. (2009) in common bean, where there was no change in chlorophyll content one day before, on the day, and 7, 14 and 21 days after a second application of pyraclostrobin, which was also performed at R7.

In contrast to that verified for common bean in the present study, for soybean, a higher chlorophyll content was found in comparison to the control, with application in seed treatment with pyraclostrobin + + methyl thiophanate + fipronil (Balardin et al., 2011), and application after V8 with picoxystrobin + cyproconazole (Roese & Lima Filho, 2010). In coffee plants, Paulo Junior et al. (2013) also verified an increase in chlorophyll content with the use of pyraclostrobin, probably caused by the increase in cytokinin production, as these delay leaf senescence by preserving foliar proteins and chlorophyll, which consequently increases the green effect (Sampaio, 1998).

Regarding the severity of anthracnose, even with the finding of differences between treatments, the severity was low. These differences may have occurred because the application of pyraclostrobin before cover fertilization was more efficient since it controls the pathogen at the beginning, considering that anthracnose is more severe when it occurs at the beginning, after sowing (Paula Júnior & Zambolim, 2006). Furthermore, it is reported that pyraclostrobin is efficient in the control of anthracnose pathogens (Cobucci & Lobo Júnior 2006) and angular leaf spot (Lima et al., 2010). Therefore, the application of pyraclostrobin and its mixture with metiram (dithiocarbamate) or metconazole provide low levels of disease severity (around 7.5%), showing a control 60% higher than the control treatment (Lima et al., 2010).

In the present study, disease severity did not reach 0.5%, indicating a low level of severity, possibly due to the climatic conditions, since there was no excess moisture (Figure 1), a fact also confirmed because there were no significant differences between the treatments and the additional control, which did not receive fungicide.

Pyraclostrobin did not increase the number of pods per plant (Table 4), the same being verified in soybean (Roese & Lima Filho, 2010) and for the Pérola bean cultivar (Demant & Maringoni, 2012). This fact may have occurred because during the pod formation period there was neither water deficit nor influence of elevated nocturnal (above 24 ºC) and diurnal (above 35 ºC) temperatures, which, when occur, may lead to a reduction in the number of pods due to abortion (Fancelli, 2011).

Nonetheless, contrary results were found for the bean cultivar IPR Uirapuru, for which there was an increase in the number of pods per plant when two applications (V4 and R7) of pyraclostrobin were performed (Kozlowski et al., 2009).

Pyraclostrobin increased the number of grains per pod when applied prior to cover fertilization compared to the application after cover fertilization (Table 4). In other studies with common bean (Kozlowski et al., 2009) and also with the cultivar Pérola (Demant & Maringoni, 2012), no variation was observed in the number of grains per pod.

The increase in respiratory rate during pod filling may occur due to high temperatures (above 30 ºC) or excess nitrogen, because of the greater stimulus of leaf production, which can reduce the number of grains per pod (Fancelli, 2011). In this context, it can be seen in Table 4 that the treatment with pyraclostrobin applied before cover fertilization presented a higher number of grains per pod, possibly contributing to a decrease in the respiratory rate (Fagan et al., 2010).

As there was no effect between the different seed treatments and the application of pyraclostrobin + + metconazole, it is evident the positive effect of pyraclostrobin applied before cover fertilization on the grain yield of the Pérola bean cultivar, which was accompanied by a higher number of grains per pod (Table 4) and higher nitrate reductase activity (Table 2), compared to the application after cover fertilization.

The physiological effect of pyraclostrobin on grain yield can be confirmed in part by the climatic conditions favorable to common bean cultivation, since precipitation occurred throughout the entire experiment and relative humidity was around 72% (Figure 1), which contributed to reduced levels of disease severity (Table 4). When diseases occur, the physiological effect of the fungicide is confounded with the higher grain yield due to pathogen control in relation to the control treatment (Oliveira et al., 2008). For grain yield, there were significant differences only with the application of pyraclostrobin, which provided a higher value for the application before cover fertilization (Table 4).

In soybean (Fagan et al., 2010; Soares et al., 2011) and maize (Barbosa et al., 2011), the use of pyraclostrobin increased the amount of photoassimilates and the nitrate reductase enzyme activity, which possibly caused an increase in grain yield due to the greater translocation of photoassimilates to the grains. According to Oliveira et al. (2008), the increase in common bean grain yield is positively correlated with the increase in nitrogen fertilization.

The literature describes an increase in bean grain yield of 23.5% when pyraclostrobin is applied (Kozlowski et al., 2009). Under water stress conditions, the application in common bean helps plants to overcome photosynthetic stress and oxidative stress, acting to maintain plant grain yield (Jadoski, 2012). In such conditions, soybean seeds treated with pyraclostrobin + + methyl thiophanate + fipronil have increases in grain yield of 57.8% in relation to the control, however under normal conditions of cultivation there are no additive effects (Balardin et al., 2011).

Regarding the thousand-grain weight, there was interaction between seed treatment and application of pyraclostrobin and seed treatment and application of pyraclostrobin + metconazole. Notwithstanding, the Tukey test did not show significant differences between averages (Table 5).

The fungicides applied did not change the thousand-grain weight (Table 5), the same being verified with the use of pyraclostrobin in the common bean cultivar IPR Uirapuru in comparison with other fungicides and the control (Kozlowski et al., 2009), soybean (Roese & Lima Filho, 2010) and soybean cultivar TMG--7161-RR 'Inox' (Vendramini & Braciforte, 2011).

However, in the soybean cultivar SYN 7059-RR 'VMAX RR' there was an increase in thousand-grain weight (TGW) with applications of pyraclostrobin + + epoxiconazole at flowering and grain-filling stages (Vendramini & Braciforte, 2011), being verified that pyraclostrobin may increase the TGW of soybean by 7% (Fagan et al., 2010). In addition, the increase in TGW is verified for the soybean cultivars BRS 245 RR, M-SOY 7878 RR, BRS Valiosa RR and BRS Conquista (Canedo et al., 2013) and RB L.8307 RR (Soares et al., 2011).

The application period of the trial may influence the results of the thousand-grain weight of different trials. An experiment conducted between March and May 2010 with the Pérola bean cultivar resulted in an increase in hundred-grain weight with the use of pyraclostrobin, however, in the same year, for cultivation between September and November, these differences were not verified (Demant & Maringoni, 2012).

Table 5 - Effect in the thousand-grain weight for interaction between treatment of seed and pyraclostrobin (C) application and seed treatment and application of pyraclotrobin + metconazol (P+M) in Pérola common bean cultivar, Jataí-GO, 2013-2014.

Seed Treatment (ST)	(#) Thousand-grain weight (g)		F test
	Pyraclostrobin		
	BCF(1)	ACF(2)	F
Fipronil (FI)	263.2 a A	258.4 a A	
C + Methyl thiophanate + FI	259.4 a A	255.4 a A	0.018*
Without ST	260.9 a A	257.3 a A	
	Pyraclostrobin + metconazol (P+M)		
	With P+M	Without P+M	F
Fipronil (FI)	265.4 a A	256.2 a A	
C + Methyl thiophanate + FI	255.4 a A	259.4 a A	3.59*
Without ST	254.9 a A	263.3 a A	
MSD(3) Column	11.87		
MSD line	9.87		
CV(4)	3.74%		
W(5) (p-value)	0.74N		

(*) Significant at 5% probability level by the F test. (#) Means in the same row followed by different capital letters or medium in the same column followed by different lowercase letters are statistically different (P ≤ 0.05) according to Tukey's test. (1) BCF-Pyraclostrobin before fertilization of covering. (2) ACF-Pyraclostrobin after coverage. (3) MSD - Minimum significant difference. (4) CV - Coefficient of variation. (5) Shapiro-Wilk: N-Normal e NN- Not-normal.

Conclusions

The application of fungicide containing pyraclostrobin (250 g L^{-1}) prior to nitrogen cover fertilization increased the nitrate reductase, plant height, number of grains and grain yield of the Pérola bean cultivar.

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