Management of the Common Bacterial Blight of the Bean by *Rhodotorula glutinis* and *Sporidiobolus johnsonii*

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Abstract

Bean common bacterial blight reduces crop productivity and is difficult to control. However, biological control by yeast can be an efficient complementary measure in management. The objective was to evaluate the ability of *Rhodotorula glutinis* and *Sporidiobolus johnsonii* to reduce the severity of bean common bacterial blight. The cultivar used was IAPAR Tuiuiú. The first experiment was sown in March and repeated in October, in a 4×3 factorial scheme (zero, one, two and three applications and three treatments *R. glutinis*, *S. johnsonii* and Acibenzolar-S-Methyl (ASM)). For this purpose were evaluated the area under the disease progress curve (AACPD), number of pods per plant (NVP), number of grains per pod (NGV), thousand grain mass (MMG) and productivity. For the results of the March cultivation, due to the low temperature, the maximum severity of bean common bacterial blight was 8% and the applications of yeasts were not significant for AACPD. The isolate *R. glutinis* showed the highest average of productivity with two applications, being 1006.44 kg ha^{-1}. For October cultivation, *R. glutinis* and *S. johnsonii* isolates reduced AACPD by 66.84 and 58.42%, respectively with three applications. For productivity, *R. glutinis* and *S. johnsonii* showed no difference between the number of applications. The ASM showed a productivity of 4418.56 kg ha^{-1} with three applications. The results indicate that the yeasts *R. glutinis* and *S. johnsonii* reduce the severity of bean common bacterial blight and the most appropriate number of applications are two for both isolates.

Keywords: biological control, Fabaceae, *Phaseolus vulgaris* L., production, *Xanthomonas axonopodis* pv. *phaseoli*

1. Introduction

Beans (*Phaseolus vulgaris* L.) is one of the most produced and consumed legumes worldwide, occupying an important place in human nutrition, as it is rich in proteins, vitamins and minerals, such as calcium, phosphorus, iron and zinc (Hailu et al., 2017). However, numerous diseases affect the cultivation of this culture, reducing its productivity (Leta, Lamessa, & Ayana, 2017).

Bacterial blight diffusion by the bacteria *Xanthomonas axonopodis* pv. *phaseoli* is one of the main limitations in the production of beans in regions of medium altitude and high temperatures and relative humidity (Corrêa et al., 2017). The pathogen can survive in the straw, on seed coat, in host plants and in soil, for a long period of time (Darrasse et al., 2018), however, a main day of spread is the seed. Infested seeds internally and/or externally are important sources of primary inoculum (Leta, Lamessa, & Ayana, 2017).

The main control measures are made through the integrated management of diseases, using various methods that aim to produce an unfavorable environment to the pathogen, through crop rotation, use of healthy seeds (Torres & Maringoni, 2010), resistant cultivars, resistance induction by Acibenzolar-S-Methyl (ASM) (MAPA, 2020), prevention with cuprics (Wendland et al., 2016) and biological control of diseases (Fancelli & Dourado Neto, 2007).

The biological control of diseases consists of the use of antagonistic microorganisms with great adaptability, which compete in some way with phytopathogens (Fancelli & Dourado Neto, 2007). In addition to these, there are other mechanisms such as antibiosis, which is the ability of one microorganism to inhibit the growth of another, competition through the interaction between microorganisms with the environment and parasitism in which there is the production of enzymes for the attack, resulting in the death of one of those involved (Machado & Bettiol, 2010).

The induction of plant resistance to the pathogen may be another form of control (Lorito et al., 2010). Yeasts have great potential to act in biological control through several mechanisms, such as competition, antagonism or induction of plant resistance to pathogens.

Research using yeasts for biological control has been increasing in recent years, as is the case of research conducted by Hoffmann et al. (2012) who used *Saccharomyces cerevisiae* and *S. boulardii* to control bean common bacterial blight where there was a reduction in severity of up to 20% and an increase in production by up to 27.19%.

The yeast species *Rhodotorula glutinis* is a natural inhabitant of the phylloplane and currently has its potential explored by the agribusiness, for the synthesis of lipids, carotenoids and enzymes, which are used in the pharmaceutical, cosmetic and food areas (Hernández-Almanza et al., 2013), also has the ability to synthesize phycocyanin and antimicrobial compounds (El-Sheekh et al., 2010). And it was studied as a biological control agent because it reduces the mycotoxin patulin synthesized by *Penicillium expansum* in apples, reducing its damage (Castoria et al., 2005).

The yeast *Sporidiobolus johnsonii* is mentioned in the literature as capable of producing coenzyme Q10 (Ranadive et al., 2011), which has an important function in electron transport in oxidative aerobic respiration (Choi et al., 2005).

Yeast fungi can have great potential for the biological control of diseases such as bean common bacterial blight. Heling et al., (2016) observed that the yeasts *R. glutinis* and *S. johnsonii* reduced the disease severity by 53.70 and 50.83%, respectively, demonstrating the potential of these antagonists.

This research aimed to evaluate the ability of the yeasts *R. glutinis* and *S. johnsonii* to reduce the severity of bean common bacterial blight and the number of applications most suitable for phytosanitary treatment.

2. Method and Methods

The yeast isolates (*R. glutinis* (AH 14-3) were isolated from rose flowers and *S. johnsonii* (AH 16-1) from leaf of *Impatiens parviflora*) were obtained from the yeast collection of the Phytopathology Laboratory of the State University of Western Paraná and cultured in liquid Yeast Extract, Peptone and Dextrose (YEPD), under agitation (150 rpm) for seven days and after this period, the cells were separated from the culture medium by centrifugation at 290 g, discarding the supernatant and obtaining cell mass to prepare the dose with the aid of a precision scale (Hoffmann et al., 2012).

The pathogen *Xanthomonas axonopodis* pv. *phaseoli*, was obtained from trifolios of infected beans, isolated in nutrient-agar culture medium, maintained at 25 °C for growth and stored by the freezing method at -20 °C. For inoculation, a bacterial suspension adjusted to 10^8 ufc mL^{-1} was prepared with the aid of an optical density spectrophotometer of 580 nm and a previously prepared bacterial concentration curve (Gonçalves et al., 2007).

The experiments were carried out in a randomized block design, in a Ferralsol (IUSS Working Group WRB, 2015)—*LATOSSOLO VERMELHO Eutroférrico* (Santos et al., 2018), conducted in a 3×4 factorial scheme, with three treatments (the yeast isolates *R. glutinis*, *S. johnsonii* (5 g L^{-1}) and ASM 0.25 g L^{-1}) and four applications in zero, one, two or three applications, with four repetitions each.

The first application was carried out at the stage of third trifoliate leaf fully expanded with 5 g L^{-1} of yeast cells and after three days *X. axonopodis* pv. *phaseoli* was inoculated (1×10^8), with the aid of a Jacto® "backpack sprayer" with a capacity of 20 L, this procedure was repeated every 15 days, until completing the three applications (second application occurred at stage of fourth trifoliate leaf fully expanded and third application

occurred at stage of pre-flowering), the inoculation was carried out at dusk so that the leaf wetness providing moisture on the trefoil, favoring the penetration of the phytopathogen in the plant (Toillier et al., 2010).

The beans were sown on March 7th, 2016 to conduct the first experiment in autumn-winter cultivation and repeated on October 7th to conduct the second experiment in winter-summer cultivation, sowing 10 seeds per linear meter, in seven lines of 5 m each and line spacing of 0.45 m, with total area of 15.75 m² and useful area of 5.4 m². From the result of soil analysis, the soil correction was carried out 30 days before sowing to raise the base saturation to 70% and fertilization carried out to produce 3000 kg ha^{-1}, according to Pauletti & Motta (2017), applying 683.3 kg ha^{-1} of the formulated 2-18-18 (N-P-K) for basic fertilization, plus cover fertilization of 74.96 kg ha^{-1} of urea (N) on stage of fourth trifoliate leaf fully expanded.

The disease severity was evaluated in the upper and lower third of the bean plant, using a diagrammatic scale (Díaz et al., 2001), right after the appearance of the first disease symptoms in the culture, being repeated every four days to obtain the AACPD.

After harvesting, agronomic variables were evaluated: number of pods per plant (pod count of 10 plants randomly collected from each plot), number of grains per pod (grain count of the pods of the 10 plants randomly collected from each plot), thousand grain mass and productivity in kg ha^{-1}.

The results obtained were subjected to analysis of variance with the aid of the GENES program (Cruz, 2013), with regression analysis performed at 5% probability for the number of applications.

3. Results and Discussion

For the March cultivation due to the low temperature, the severity of common bacterial blight was 8% and the application of yeasts did not reduce the AACPD (Figure 1F).

In tests using these same isolates performed by Heling et al. (2016), there is a reduction in the severity of the common bacterial blight by 53.70 and 50.83% for the isolates *R. glutinis* and *S. johnsonii*, respectively, under conditions of average temperature of 22.9 to 24.4 °C. In this experiment it was not possible to verify the efficiency of the yeasts as a function of the temperatures varying from 3 to 7 °C, resulting in a low severity index.

There was an increase in the number of pods per plant with two applications of *R. glutinis*. The application of *S. johnsonii* reduced the number of pods with the increase in the number of applications and the treatment with ASM presented the highest number of pods per plant with three applications (Figure 1A).

The number of grains per pod showed no difference with the applications of the *R. glutinis* isolate and ASM, while the *S. johnsonii* isolate increased the number of grains per pod according to the increase in the number of applications (Figure 1B).

The thousand grain mass and productivity increased with two applications of *R. glutinis*. The application of ASM reduced the thousand grain mass with one application, although it did not interfere in productivity (Figures 1C and D).
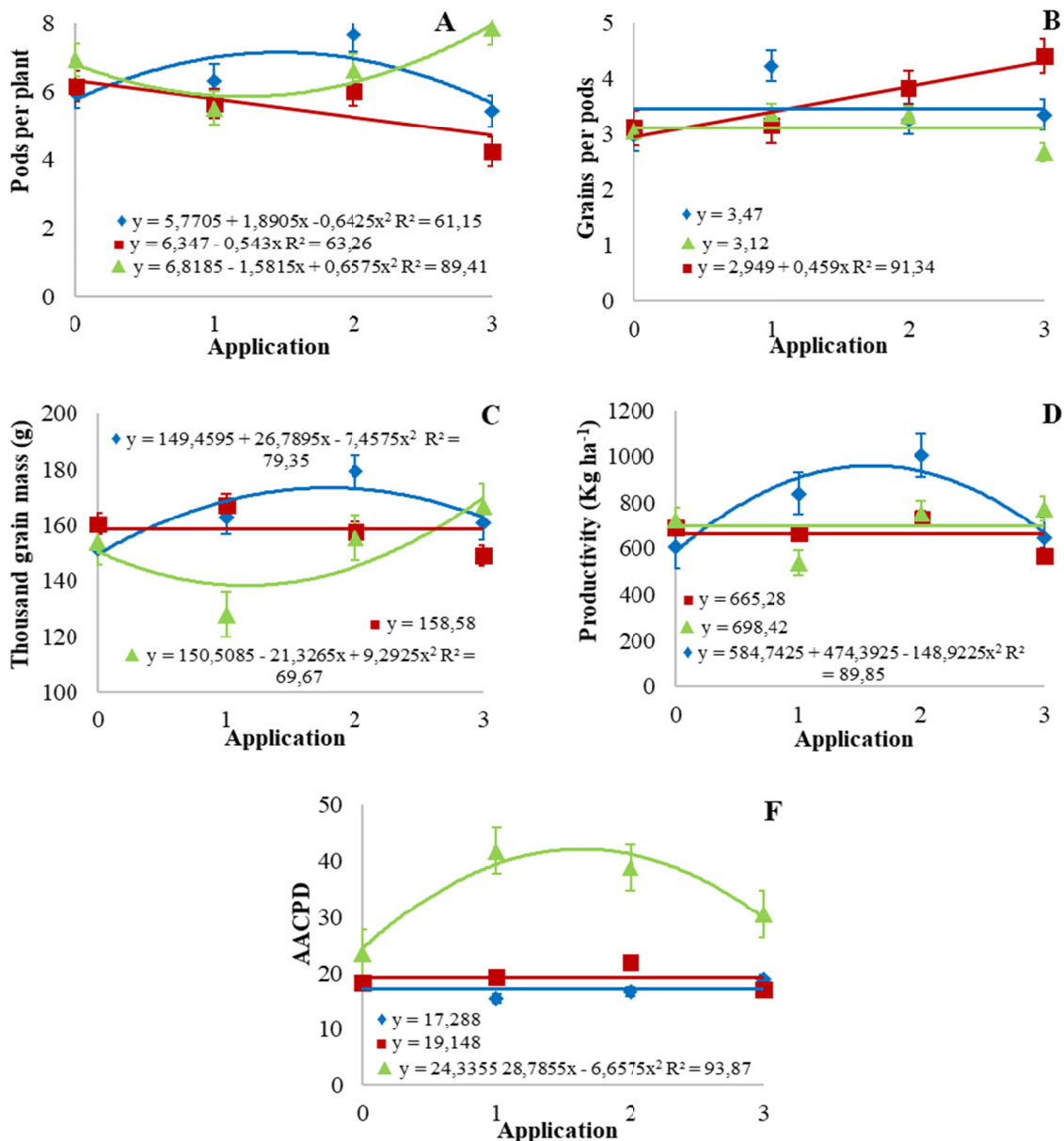
Figure 1. Number of pods per plant (A), grains per pods (B), thousand grain mass (C) and productivity (D), depending on the application of the yeast *Rhodotorula glutinis*—5 g L^{-1} (♦), *Sporidiobolus johnsonii*—5 g L^{-1} (■), Acibenzolar-S-methyl (ASM)—25 g L^{-1} (▲) and Area under the disease progress curve (AACPD) (F) in 0, 1, 2 or 3 applications, fortnightly in field conditions in the autumn-winter season. Bars indicate the average±the standard error

Although sowing occurred within the agroclimatic zoning, the low temperature (Figure 2) affected the agronomic variables of the crop. The minimum temperature from April until the cycle end in July varied from 3 to 7 °C, affecting the culture mainly through the occurrence of the abortion of flowers and pods, as the temperature for the adequate development of the culture is 21 to 29.5 °C (Fancelli & Dourado Neto, 2007).
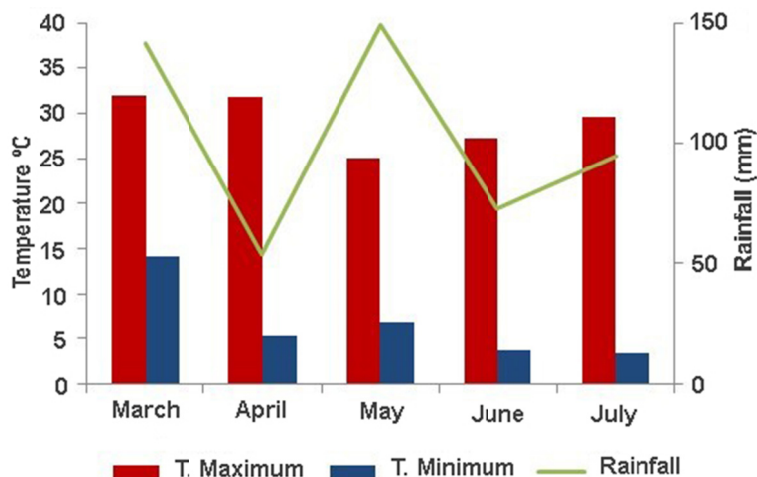
Figure 2. Meteorological data indicating the period of conduction of the experiment in the cultivation of drought with maximum temperature (T. Maximum, °C), minimum temperature (T. Minimum, °C) and rainfall (mm). Source: INMET (2020)

In fieldwork, Hoffmann et al. (2012), did not observe differences between the agronomic variables applying the yeasts *S. cerevisae*, *S. boulardii* and ASM, however in the experiment three applications were used for each treatment during the cycle, not testing a smaller number of applications.

Even though the environmental conditions during the conduct of the present experiment are not ideal for the development of bean, it was observed that the isolates *R. glutinis* and *S. johnsonii* increased some of the analyzed production components.

There was an increase in the number of pods per plant (NVP) with two applications of *R. glutinis* showing an average of 7.67 pods per plant. However, the application of *S. johnsonii* reduced the NVP with the increase in the number of applications ranging from 6.18 (without the application) to 4.25 pods per plant with three applications. On the other hand, with the application of ASM, the NVP decreased with one application and increased after two applications, reaching the highest value (7.80) with three applications (Figure 1A).

Even the unfavorable climatic conditions impairing the fixation of pods, it was noted that the presence of *R. glutinis* favored the fixation of pods (Figure 2). For the number of grains per pod (NGV), the isolate *R. glutinis* and ASM showed no differences between the number of applications, resulting in an average of 3.47 and 3.12 grains per pod, respectively. The application of *S. johnsonii* increased the number of grains per pod as the number of applications increased, reaching a maximum of 4.42 grains per pod with three applications (Figure 1B).

Sun et al. (2014) indicates that yeasts can produce growth regulator indole-3-acetic acid (AIA), thus being able to act as promoters of plant growth, so an interaction between the common bean and the yeast *S. johnsonii* may have occurred, resulting in maintenance the number of grains per pod.

The thousand grain mass (MMG) increased with two applications of the isolate *R. glutinis*, presenting 179.33 g. The environmental conditions contributed to reduce the thousand grain mass in the culture, since the thousand grain mass in normal conditions for this cultivar is 227 g (IAPAR, 2020).

The production results also indicate that the *R. glutinis* isolate promoted an increase in productivity with two applications reaching 1006.44 kg ha^{-1}, presenting an increase of 39.63% (which is equivalent to 398.85 kg), even in unfavorable climatic conditions for the culture development.

This indicates that the isolate may be acting as a growth promoter with two applications during the cycle, but with three applications, the plant probably has an imbalance in maintaining the distribution of metabolites, reflecting in productivity (Kuhn & Pascholati, 2010; Malavolta, 2006).

For treatments with isolate *S. johnsonii* and ASM the productivity did not change regardless of the dose. Hoffmann et al. (2012) also found no difference between the application of yeast and the control (H_2O) for productivity, however the authors observed a reduction in productivity with the application of ASM, in which the authors indicate this reduction in the metabolic cost of resistance induction. .

For the experiment sown in October (water cultivation) the application of *R. glutini* reduced the AACPD from 26.48 to 8.78 with three applications, a reduction of 66.84%. *S. johnsonii* reduced from 31.70 to 13.18 with three applications, a reduction of 58.42%. ASM presented a reduction in severity with two applications, decreasing from 83.84 to 11.51, which means a reduction of 86.27% in AACPD (Figure 3A).
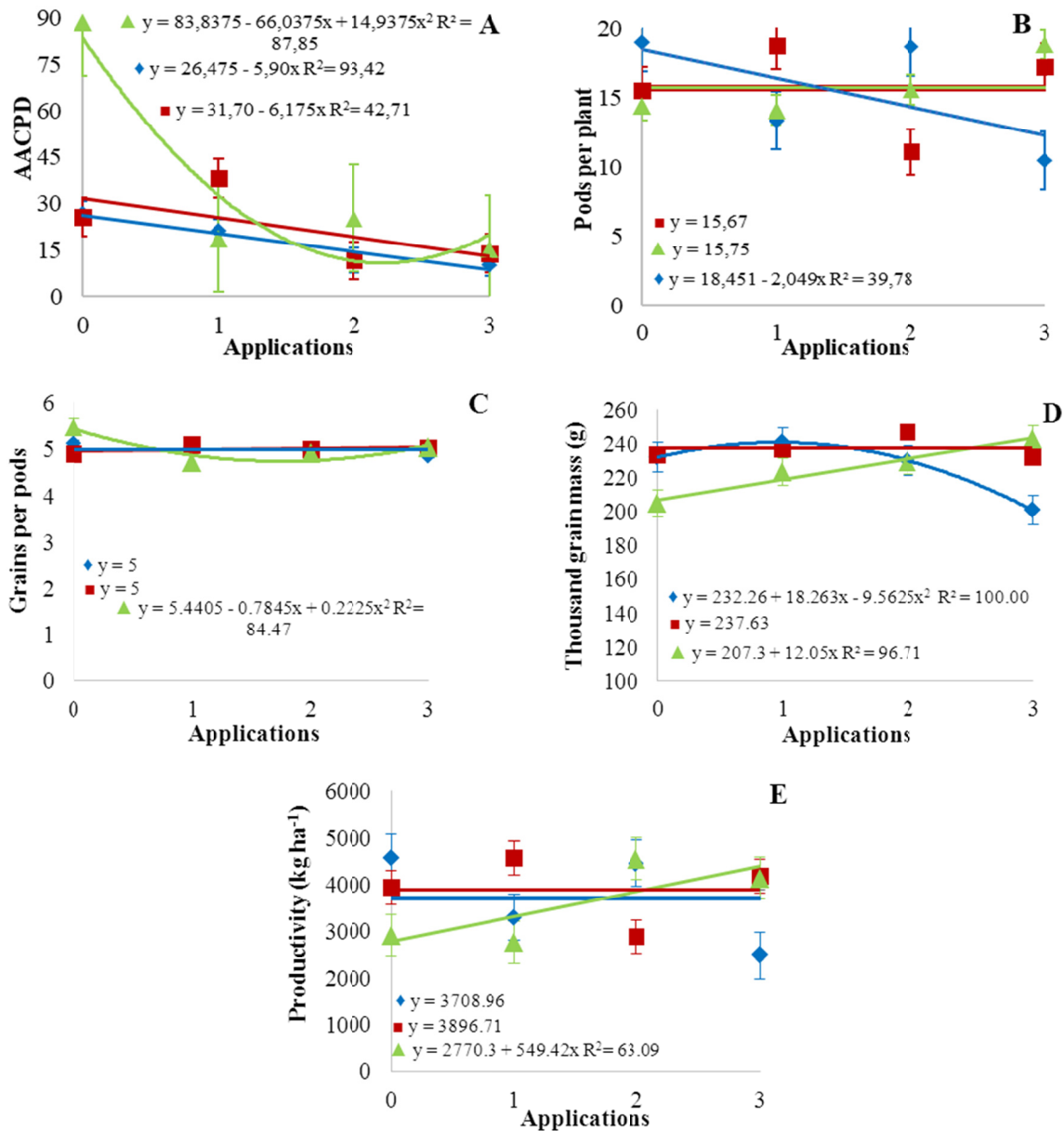

Figure 3. Area under the disease progress curve (AACPD) (A), pods per plant (B), grains per pods (C), thousand grain mass (D) and productivity (E), depending on the application of *Rhodotorula glutinis* yeast—5 g L^{-1} (♦), *Sporidiobolus johnsonii*—5 g L^{-1} (■) and Acibenzolar-S-methyl (ASM)—25 g L^{-1} (▲) in 0, 1, 2 or 3 applications, fortnightly in field conditions in the water harvest. Bars indicate the average±the standard error

The number of pods per plant was not affected by the application of *S. johnsonii* and ASM (15.67 and 15.75, respectively), however with the application of *R. glutinis* there was a reduction in this parameter with the increase in the number of applications, reducing from 18.45 without application to 12.31 with three applications (Figure 3B).

The NGV was not changed due to the number of applications of the yeasts *R. glutinis* and *S. johnsonii* presenting an average of 5.00 grains per pod for both isolates and the ASM, showed a reduction in the number of grains per pod with two applications, with 4.76 grains per pod (Figure 3C).

When analyzing MMG, it can be seen that the application of *R. glutinis* results in its reduction with three applications, which was not reflected in productivity. *S. johnsonii* showed no difference in productivity between the number of applications. On the other hand, ASM increased with the increase in the number of applications for both MMG and productivity (Figures 3D and 3E).

The yeast isolates (*R. glutinis* and *S. johnsonii*) showed a reduction in severity, presenting potential for its control, as well as the ASM that showed the highest efficiency point with two applications. Similar results to those presented were obtained by Heling et al. (2016), in this same growing season, who obtained a reduction in the severity of *X. axonopodis* pv. *phaseoli*, from 53.70% with the application of *R. glutinis* (reduced the AACPD from 15.66 to 7.25) and 50.83% with *S. johnsonii* (reduced the AACPD from 15.66 to 7.70).

With the analysis of the productive parameters we observed that with the increase in the number of applications of *R. glutinis* the result was negative for the number of pods per plant and the thousand grain mass. What can be linked to activation of metabolic defense routes induced by yeasts, because with successive applications the maintenance of alert status can present significant consumption of energy that would be destined for production (Kuhn & Pascholati 2010, Verhagen et al., 2004).

It is also considered that the increase in the number of applications of the *R. glutinis* isolate may have caused stress on the plant, causing the abortion of flowers and pods, consequently reducing the number of pods per plant (Fancelli, & Dourado Neto 2007). However Hoffmann et al. (2012) observed different results using *S. cerevisae*, *S. boulardii* and ASM with three applications for each treatment and what did not result in the variation in the number of pods, perhaps because they are different species of yeasts, there may be specificity in the microorganism plant interaction.

For NGV, both isolates do not differ in terms of the number of applications, indicating that this production component is more influenced by the climate as observed in the March cultivation, where a reduction of this component to an average of 3.4 grains per pod was observed when the temperature decreased (Fancelli, & Dourado Neto 2007).

For MMG, the bean plant showed maximum point with an application of the isolate *R. glutinis* (240.96 g), the treatment with ASM showed an increase in MMG with an increase in the number of applications, presenting 242.08 g with three applications and the isolate *S. johnsonii* showed no difference between the number of applications for this parameter, resulting in an average of 237.63 g, results that exceed those obtained by the Agronomic Institute of Paraná (IAPAR) in productivity tests for the cultivar IAPAR Tuiuiú, presenting an average of 227 g for MMG (IAPAR, 2020).

The isolates *R. glutinis* and *S. johnsonii* showed no difference for productivity with the increase in the number of applications during the crop cycle, presenting averages of 3708.96 and 3896.71 kg ha^{-1}, respectively, whereas ASM promoted an increase of productivity with the increase in the number of applications, presenting a productivity of 4418.56 kg ha^{-1} with three applications, an increase of 16.06 and 11.81% when compared with the isolates of *R. glutinis* and *S. johnsonii*, respectively.

The results indicate that with two applications there was a reduction in the disease severity and an increase in production components such as NVP, MMG and productivity for the yeast *R. glitinis* in the March cultivation, reducing the effects of stress due to low temperature, *S. johsonii* presented an increase for NGV, in October cultivation yeasts reduced the severity with up to three applications, indicating that with two applications it is the best level of applications for control, without interfering in the productive components.

Hoffmann et al. (2012) performed three applications of the yeasts *S. cerevisiae* and *S. boulardii*, reduced the severity of *X. axonopodis* pv. *phaseoli*, showing no significant difference for the productive components of NVP and NGV and obtained an increase in MMG and productivity under water stress conditions, indicating that yeasts alleviate the adverse effects on bean plants, as observed in the present research in March cultivation

4. Conclusions

The yeasts *R. glutinis* and *S. johnsonii* reduce the severity of bean common bacterial blight, with two applications not interfering in the production components. In conditions of cold stress, the application of *R. glutinis* reduces its effects and favors the maintenance of productivity.

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