

*Full Length Research Paper*

# **Chemical and biological management of white mold (*Sclerotinia sclerotiorum*) disease in irrigated common beans (*Phaseolus vulgaris*) cultivation**

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The aim of this work was to study the effect of fungicides and biological agents on the control of white mold (*Sclerotinia sclerotiorum*) in common beans (cv. Pérola). Nine treatments were applied in six blocks (54 experimental units) using a randomized block design (RBD). The treatments were: T1 (control); T2, *Bacillus subtilis* strain QST 713 (4 L / ha); T3, *B. subtilis* strain QST 713 (4L / ha); T4, *B. subtilis* strain QST 713 (2 L / h); T5, *B. subtilis* strain QST 713 trifloxystrobin + prothioconazole (4 L / ha, 0.5 L / ha); T6, *B. subtilis* strain QST 713 trifloxystrobin + prothioconazole (2 L / ha, 0.5 L / ha); T7, *B. subtilis* strain QST 713 trifloxystrobin + prothioconazole, fluazinam (2 L / ha, 0.5 L / ha, 1 L / ha); T8- trifloxystrobin + prothioconazole, fluazinam (0.5 L / ha, 1L / ha); T9- *Trichoderma harzianum*, difenoconazole and azoxystrobin fluazinam + (1.5 L / ha, 0.5 L / ha, 1 L / ha). White mold (WM) incidence was evaluated at 39 days after planting (DAP), with subsequent evaluations at 39, 46, 53, 60, 67 and 74 DAP. Average yield from T5, T6, T7 and T8 was statistically higher than in the other treatments and consequently, treatments T7, T8 and T9 had the lowest mean area under disease progress curve values. The combined chemical and biological treatment was an effective white mold management strategy that increased yield and decreased disease incidence in common beans.

**Key words:** Active ingredient, white mold, *Bacillus subtilis*, *Trichoderma harzianum*, trifloxystrobin, prothioconazole.

## **INTRODUCTION**

The common bean [*Phaseolus vulgaris* L. (Fabaceae)] is one of 55 species in the genus *Phaseolus* sp. It is one of

the most important, oldest and most cultivated crops worldwide. It is extremely important in Brazil where, along

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with rice, it is a dietary staple (Santos and Gavilanes, 1998).

White mold (WM) in common beans is caused by the soil fungus, *Sclerotinia sclerotiorum* (Lib.) De Bary (1884) and can trigger epidemics with annual losses exceeding 50%. WM mainly occurs in crops irrigated by central pivot (Oliveira, 2005; Soule et al., 2011). The disease thrives in cool temperatures and / or micro-climatic conditions and during a period of intense bean planting in Brazil called the third growing season (especially in the Southeast and Center-West regions of Brazil). Other conditions that favor the disease include the presence of various fungi hosts, scleroids in the soil and transmission by seeds (Faria et al., 2011).

Initial symptoms of WM include sparse plants with wilted upper leaves and cottony structures in the stems, leaves and pods formed by fungus mycelium. This last characteristic has led to the name "white-mold" (Paula et al., 2015).

Plant pathogens such as *S. sclerotiorum* can also colonize seed endosperm. The pathogens can then be transported over long distances by these propagules, proliferate and provide a source of inoculum in new fields. Resistant structures from previous crops or infested soil can also be transported with seeds, machines and agricultural implements, such as tractors, seeders and harvesters when not properly cleaned. Irrigation water, floods and wind can also disseminate the plant pathogen. Infections that begin in the myceliogenic cycle, followed by the ascogenous cycle, can multiply during the crop cycle (secondary cycle) and cause reinfestations, which lead to new resistant structures and increased inocula in the soil (Paula et al., 2015).

The fungus, *S. sclerotiorum*, identified in 1884, has been studied ever since. It is present throughout the world. It can infect more than 408 species of plants, monocots and dicots (Görge, 2009). Changes in pigment, leaf wrinkling, wilt, chlorosis, atrophy, necrosis or abscission of parts of the plant are signs of this pathogen-host interaction (Prabhakar et al., 2013).

Solarization is an alternative method for reducing inoculum and controlling fungal plant pathogens in the soil. While this practice has shown promise in small crop areas it may not be practical in larger ones (Ferraz et al., 2003).

Fungicide application is the most common control method because it can be easily adapted to crop management plans and because it effectively prevents, controls and reduces disease severity (Mueller et al., 2002).

WM can be controlled by physiological resistance and escape mechanisms, a consequence of the growth habit of the plant, which provides favorable soil aeration and climatic conditions. Neither of these mechanisms provide adequate control of the disease (Kim et al., 2000;

Kolkman and Kelly, 2002; Huang et al., 2003; Soule et al., 2011). These mechanisms are mainly found in sources of genetic resistance and could be incorporated in commercial cultivars mainly by retro-crossings (Görge et al., 2003).

Fungicide applications are recommended when flowering begins and again after 10 to 14 days if the disease progresses. Applications via boom sprayers may be hindered by canopy closure between rows and the consequent need for higher volume applications (Tu, 1989). Therefore, to achieve economically viable and effective disease control, growers must pay careful attention to application timing and positioning. The spray should create a uniform layer over the plant surface and act as a barrier to the host-pathogen. Systemic fungicide applications can also provide protection from contact (Oliveira, 2005).

Spraying should be uniformly diffused over the entire plant and soil surface where apothecia develops. Initial spraying should be carried out preventively at the opening of the first flowers. Subsequent applications should occur when apothecia appears and when the crop presents other favorable conditions for disease (Oliveira, 2005). Integrated disease management can lower costs and reduce diverse production risks (Ferreira et al., 2013). Azoxystrobin (estrobirulin chemical group) has been registered for disease control in 32 crops, including beans, and is the active ingredient (ai) in 23 registered commercial products. Another fungicide, trifloxystrobin (strobilulins) has been registered to control diseases in 24 crops, including beans, and is the main in six commercial products; while prothioconazole (triazolitione) has been registered to control diseases in 3 crops, in addition to beans, and is the main in two commercial products (Paula et al., 2009).

*B. subtilis* QST strain 713 is an organic fungicide but is the active ingredient in only one commercial product. While it is not intended for any specific crop it can be used on various (Silva et al., 2015).

Diphenconazole (triazole group) has been registered in Brazil for WM control in 37 crops, including beans, and 10 commercial products. Diphenconazole (phenylpyridinylamine group) has been registered for disease control in eight crops, whereas the biological agent *Trichoderma harzianum* has been registered for disease control in beans and is the main in three commercial products (Agrofit, 2016).

The two main WM control practices in beans involve conventional fungicide use, which is expensive and has strong environmental impacts related to toxic waste (Rocha and Oliveira, 1998). Another practice involves the use of various species of *Trichoderma* spp. to control not only *S. sclerotiorum* but various soil pathogens (Lobo and Abreu, 2000).

Our objective is to evaluate the use of chemical and biological management on *Sclerotinia sclerotiorum* in

**Table 1.** Chemical and biological agents for WM control applied on different days after planting and water volume control aiming bean cv. Perola cultivated in condition by Central Pivot in the crop 2015.

| Treatments | Active ingredients and commercial fungicides  | Dosages (L ha <sup>1</sup> ) | Day after planting   | Volume of spray (L ha <sup>1</sup> ) |
|------------|---|------------------------------|--|--------------------------------------|
| T1         | Negative control  | empty                        | empty  | empty                                |
| T2         | <i>Bacillus subtilis</i> lineage QST 713-Serenade <sup>®</sup> (CB)   | 4                            | 1st. spray 18 (CB)   | 200                                  |
| T3         | <i>B. subtilis</i> lineage QST 713- Serenade <sup>®</sup> (BC)  | 4                            | 1st. spray 26 (CB)   | 200                                  |
| T4         | <i>B. subtilis</i> lineage QST 713- Serenade <sup>®</sup> (BC)  | 2                            | 1st. spray 18 (CB); 2nd spray 26(CB)   | 200                                  |
| T5         | <i>B. subtilis</i> lineage QST 713- Serenade <sup>®</sup> (BC) and Trifloxistrobina + prothioconazol- Fox <sup>®</sup> (CC)   | 4 and 0,5                    | 1st. spray 18(CB); 2nd spray 26 (CQ); 3rd spray 34 (CQ); 4th spray 46 (CQ)                   | 200                                  |
| T6         | <i>B. subtilis</i> lineage QST 713- Serenade <sup>®</sup> (BC) and Trifloxistrobina + prothioconazol- Fox <sup>®</sup> (CC)   | 2 and 0,5                    | 1st. Aplic. 18(CB); 2nd spray 26 (CB-CQ); 3rd spray 34 (CQ) and 4th spray 46 (CQ)            | 200                                  |
| T7         | <i>B. subtilis</i> lineage QST 713- Serenade <sup>®</sup> Trifloxistrobina + prothioconazol - Fox <sup>®</sup> (CC1) and fluazinam- Frowcide (CC2)                              | 2, 0,5 and 1                 | 1st. Aplic. 18(CB); 2nd spray 26 (CB-CQ1-CQ2); 3rd spray 34 (CQ1-CQ2) and 4th spray 46 (CQ1) | 200                                  |
| T8         | Trifloxistrobina + prothioconazol- Fox <sup>®</sup> (CC1) and fluazinam- Frowcide <sup>®</sup> (CC2)  | 0,5 and 1                    | 1st. spray 26(CQ1-CQ2); 2nd spray 34 (CQ1-CQ2); 3rd spray 46 (CQ1-CQ2)                       | 200                                  |
| T9         | <i>Trichoderma harzianum</i> - Trchodermil SC 1306 <sup>®</sup> (BC), azaxistrobina + difenoconazol- Amistar Top <sup>®</sup> (CC1) and fluazinam- Frowncide <sup>®</sup> (CC2) | 1,5; 0,5 and 1               | 1st. spray 18(CB); 2nd spray 26 (CB-CQ1-CQ2); 3rd spray 34 (CQ1-CQ2) and 4th spray 46 (CQ1)  | 200                                  |

\*BC, biological control, CC chemical control.

irrigated common bean crops (*Phaseolus vulgaris*).

## MATERIALS AND METHODS

We set up our experiment during the dry season of 2015 on an irrigated (central pivot) crop of cv. Pearl at a farm called Fazenda São José in Cristalina, GO, Brazil. The field was situated at 17 ° 5'56 "S and 47 ° 38'44" W (GPS) and at an altitude of 861 m.

The soil was prepared using the no-tillage system and was preceded by soybean and corn crops. The crop was fertilized after planting by broadcast fertilization using 270 kg ha<sup>-1</sup> of formulated 05-37-00 potassium chloride (KCl, Triton<sup>®</sup>). The crop was managed according to Carneiro et al. (2015).

The beans were sown in the first week of October. Nine types of treatments were applied from 1 to 4, times during the crop cycle. Some of these treatments were biological and chemical combinations (Table 1). A randomized block design was used with six replicates, totaling 54 experimental units or plots (Table 2).

Each plot measured 6x6 m (36 m<sup>2</sup>), spaced at 0.5 m between rows and 0.2 m between plants. The last 0.5 m from the ends of the two central rows was discarded (9 m<sup>2</sup> total). The evaluations were carried out on the ten centermost rows (useful area). There were 30 plants per row and a total of 300 plants per plot.

White mold incidence (% WM) was evaluated at 39 days after planting (DAP) and again at 46, 53, 60, 67 and 74 DAP (Table 3). The numbers of symptomatic plants (white mold symptoms) were counted five times divided by the total number of evaluated plants (10 plants).

The area under white mold progress curve (AUDPC) was

calculated by integrating the disease progress curve for each plot (% white mold incidence at x days), using the formula:

$$AUDPC = \sum_{i=1}^{n-1} \frac{(X_i + X_{i+1})(t_{i+1} - t_i)}{2}$$

Where, n is the number of the severity ratings, Xi is the severity of the disease and (t<sub>i+1</sub> - t<sub>i</sub>) is the number of days between consecutive evaluations (Campbell and Madden, 1990). The value of AUDPC synthesizes all the WM impact assessments into a single value representing the crop-cycle epidemic.

The yield (kg / ha) of the plots was evaluated at 87 DAP (desiccation was carried out 2 days before harvest, affecting 70% of the leaves). The number of plants in 4 rows (2.5 m each) was counted; it was divided by the line spacing used and multiplied by 10, giving the number of plants per ha. Next, the number of pods in 10 consecutive plants in a row was counted and divided by 10, yielding the mean number of pods per plant. Fifty pods were collected, and the number of beans counted. This value was then divided by 50 to find the average number of beans per pod. Next, 1000 beans were weighed. Then, yield was estimated as the mathematical product of the number of plants per ha, the number of pods per plant, the average number of seeds per pod and weight of 1000 beans, divided by 60,000 (Koss and Lewis, 1993).

Control efficiency (CE) is the percent reduction in AUDPC due to a treatment application, relative to the AUDPC values of the control treatment (without applications). Yield efficiency (YE) represents the relationship between yield increases relative to the yield of the control treatments (without application of chemical and biological

**Table 2.** Biological and chemical treatments (T) for the control of white mold a bean cv Pérola crop under central pivot during the winter crop.

| Line | Block 1  | Block 2  | Block 3  | Block 4  | Block 5  | Block 6   |
|------|--|--|--|--|--|---|
| L1   | T2 - Serenade® (4 L/ha)  | T 3 - Serenade® (4 L/ha)   | T 3 - Serenade® (4 L/ha)   | T2 - Serenade® (4 L/ha)  | T 5 - Serenade® (4 L/ha) + Fox® (500 mL/ha)                                      | T 9 - Tricodermil (1,5 kg/ha) + Amistar Top® (500 mL/ha) + Frownicide® (1 L/ha) |
| L2   | T 5 - Serenade® (4 L/ha) + Fox® (500 mL/ha)                                      | T 6 - Serenade® (2 L/ha) + Fox® (500 mL/ha)                                    | T 6 - Serenade® (2 L/ha) + Fox® (500 mL/ha)                                      | T 8 - Fox® (500 mL/ha) + Frownicide® (1 L/ha)                                    | T 7 - Serenade (2 L/ha) + Fox® (500 mL/ha) + Frownicide® (1 L/ha)                | T 3 - Serenade® (4 L/ha)  |
| L3   | T 4 - Serenade® (2 L/ha)   | T 4 - Serenade® (2 L/ha)   | T 1 - Control  | T 3 - Serenade® (4 L/ha)   | T 8 - Fox® (500 mL/ha) + Frownicide® (1 L/ha)                                    | T2 - Serenade® (4 L/ha)   |
| L4   | T 9 - Tricodermil® (1,5 kg/ha) + Amistar Top® (500 mL/ha) + Frownicide® (1 L/ha) | T 5 - Serenade® (4 L/ha) + Fox® (500 mL/ha)                                    | T 5 - Serenade® (4 L/ha) + Fox® (500 mL/ha)                                      | T 1 - Control  | T 1 - Control  | T 4 - Serenade® (2 L/ha)  |
| L5   | T 8 - Fox® (500 mL/ha) + Frownicide® (1 L/ha)                                    | T2 - Serenade® (4 L/ha)  | T 8 - Fox® (500 mL/ha) + Frownicide® (1 L/ha)                                    | T 6 - Serenade® (2 L/ha) + Fox® (500 mL/ha)                                      | T 4 - Serenade® (2 L/ha)   | T 5 - Serenade® (4 L/ha) + Fox® (500 mL/ha)                                     |
| L6   | T 7 - Serenade® (2 l/ha) + Foxv (500 ml/ha) + Frownicide® (1 l/ha)               | T 8 - Fox® (500 mL/ha) + Frownicide® (1 L/ha)                                  | T2 - Serenade® (4 L/ha)  | T 5 - Serenade® (4 L/ha) + Fox® (500 mL/ha)                                      | T 6 - Serenade® (2 L/ha) + Fox® (500 mL/ha)                                      | T 1 - Testemunha  |
| L7   | T 3 - Serenade® (4 L/ha)   | T 1 - Control  | T 4 - Serenade® (2 L/ha)   | T 4 - Serenade® (2 L/ha)   | T 3 - Serenade® (4 L/ha)   | T 7 - Serenade® (2 L/ha) + Fox® (500 mL/ha) + Frownicide® (1 L/ha)              |
| L8   | T 1 - Control  | T 7 - Serenade® (2 L/ha) + Fox® (500 mL/ha) + Frownicide® (1 L/ha)             | T 9 - Tricodermil® (1,5 kg/ha) + Amistar Top® (500 mL/ha) + Frownicide® (1 L/ha) | T 9 - Tricodermil® (1,5 kg/ha) + Amistar Top® (500 mL/ha) + Frownicide® (1 L/ha) | T 9 - Tricodermil® (1,5 kg/ha) + Amistar Top® (500 mL/ha) + Frownicide® (1 L/ha) | T 8 - Fox® (500 mL/ha) + Frownicide® (1 L/ha)                                   |
| L9   | T 6 - Serenade® (2 L/ha) + Fox (500 mL/ha)                                       | T 9 Tricodermil® (1,5 kg/ha) + Amistar Top® (500 mL/ha) + Frownicide® (1 L/ha) | T 7 - Serenade® (2 l/ha) + Fox (500 ml/ha) + Frownicide® (1 l/ha)                | T 7 - Serenade® (2 L/ha) + Fox (500 mL/ha) + Frownicide® (1 L/ha)                | T2 - Serenade® (4 L/ha)  | T 6 - Serenade® (2 L/ha) + Fox (500 mL/ha)                                      |

combinations) (Silva, 2018).

The crop health and yield variables were subjected to analysis of variance and the means compared by the Tukey test at 5% probability (Assistat® version 7.7 Beta).

## RESULTS AND DISCUSSION

No symptoms of white mold were observed during

the first evaluation (39 DAP); however, symptoms of fusarium wilt (*Fusarium oxysporum* f.sp. *phaseoli*) were observed. Furthermore, mean incidence values did not differ significantly among the various treatments. Similarly, Boechat et al. (2014), using spectral analysis, also did not detect white mold within the same DAP range and suggested that crop phase or the residual effects

of previous crop management practices could explain the lack of white mold.

At 46 DAP, when the beans were in the R5 stage, the T5 treatment (*B. subtilis* strain QST 713 - Serenade® + prothioconazole and trifloxystrobin - Fox® - 4 L ha<sup>-1</sup> and 0.5 L ha<sup>-1</sup> - V3, V4, R5, R5 +10 days) showed statistically lower incidence of white mold than did the other treatments. Wutzki

**Table 3.** Area under below progress curve disease (AUDPC), control efficiency (CE), productivity (kg ha<sup>-1</sup> and sc ha<sup>-1</sup>) and yield efficiency (YE) in different combinations of biological and chemical treatments (T) applied to the bean cv. Pérola in the winter crop under central pivot irrigation (2015).

| Code | Treatments   | AUDPC               | CE (%) | Produc. kg / ha (sc / ha) | YE (%) |
|------|--|---------------------|--------|---------------------------|--------|
| T1   | Control  | 397.4 <sup>a</sup>  | Empty  | 2093 (34.8) <sup>b</sup>  | Empty  |
| T2   | <i>Bacillus subtilis</i> strain QST 713 - Serenade <sup>®</sup> (CB)   | 313.7 <sup>ab</sup> | 21.3   | 2371 (39.5) <sup>b</sup>  | 13.2   |
| T3   | <i>B. subtilis</i> QST 713 strain - Serenade <sup>®</sup> (CB)   | 303.2 <sup>ab</sup> | 23.9   | 2400 (40.0) <sup>b</sup>  | 14.6   |
| T4   | <i>B. subtilis</i> QST 713 strain - Serenade <sup>®</sup> (CB)   | 325.6 <sup>ab</sup> | 18.3   | 2244 (37.3) <sup>b</sup>  | 7.2    |
| T5   | <i>B. subtilis</i> QST 713 strain - Serenade <sup>®</sup> (CB) and trifloxystrobin + prothioconazole - FOX <sup>®</sup> (CQ)   | 208.0 <sup>b</sup>  | 48     | 2780 (46.3) <sup>ab</sup> | 32.8   |
| T6   | <i>B. subtilis</i> QST 713 strain - Serenade <sup>®</sup> (CB) and trifloxystrobin + prothioconazole - FOX <sup>®</sup> (CQ)   | 211.5 <sup>b</sup>  | 47     | 2725 (45.4) <sup>ab</sup> | 30.2   |
| T7   | <i>B. subtilis</i> QST 713 strain - Serenade <sup>®</sup> , trifloxystrobin + prothioconazole - FOX <sup>®</sup> (CQ1) and fluazinam - Frowncide <sup>®</sup> (CQ2)                | 87.6 <sup>d</sup>   | 77.9   | 2840 (47.3) <sup>ab</sup> | 35.7   |
| T8   | Trifloxystrobin + prothioconazole - FOX <sup>®</sup> (CQ1) and fluazinam - Frowncide <sup>®</sup> (CQ2)  | 109.0 <sup>c</sup>  | 72.4   | 3178 (53.0) <sup>in</sup> | 51.8   |
| T9   | <i>Trichoderma harzianum</i> - Trichodermil SC 1306 <sup>®</sup> (CB), azoxystrobin + difenoconazole - Amistar Top <sup>®</sup> (CQ1) and fluazinam - Frowncide <sup>®</sup> (CQ2) | 127.6 <sup>c</sup>  | 67.8   | 2683 (44.7) <sup>ab</sup> | 28.2   |

\*Means followed by same letter vertically to the test Tukey  $P \leq 0.05$ .

et al. (2016) found that chemical control applications at these phenological stages did not differ statistically from the control and were therefore not effective. Chromatography–mass spectrometry showed that the bioagent *Trichoderma longibrachiatum* T6 achieved the same antifungal potential as *Trichoderma* in the control of *Verticillium* sp. (Zhang et al., 2018).

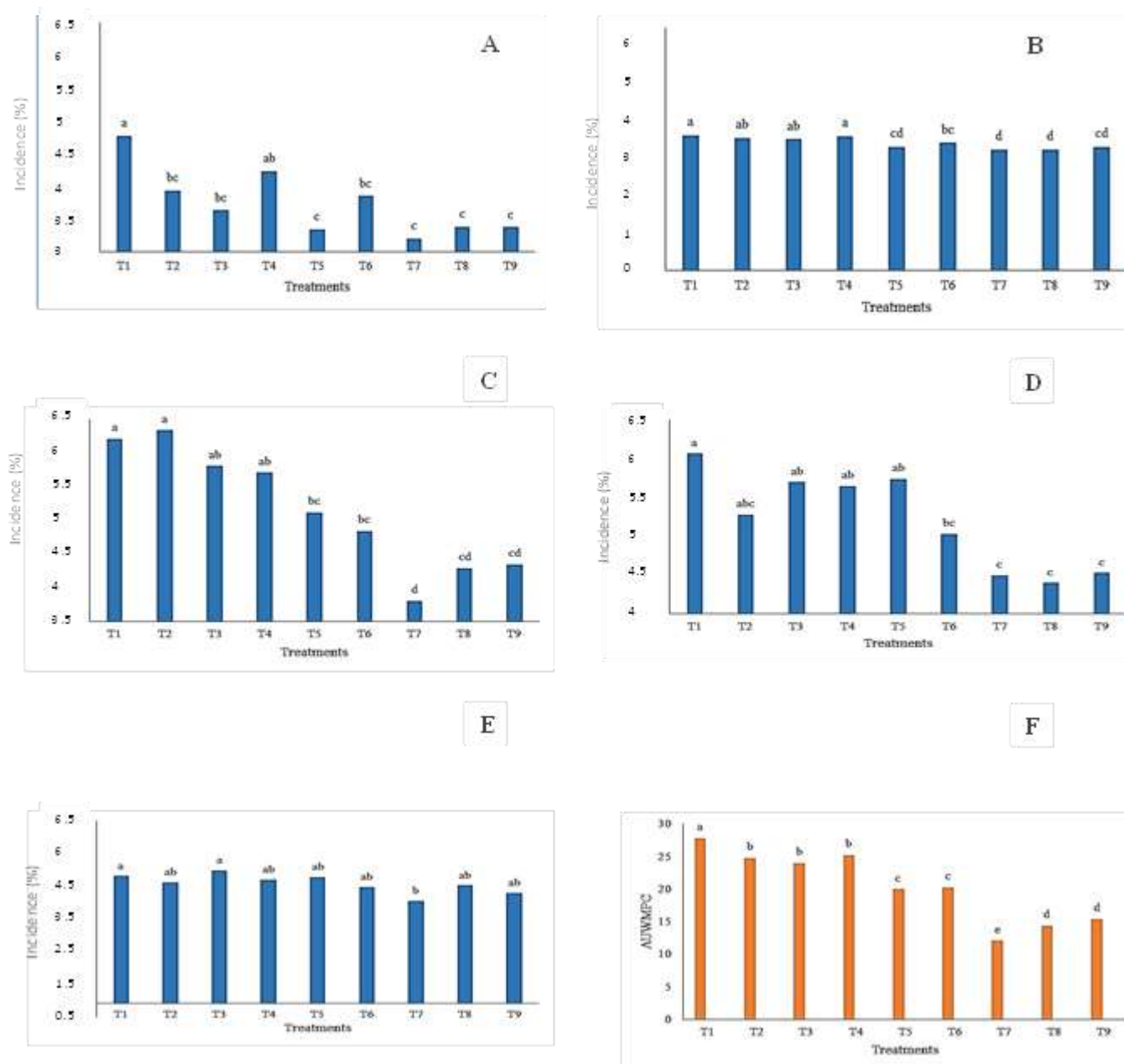
At 53 DAP, when the beans were in the R6 stage, the T5, T7, T8 and T9 treatments showed statistically lower incidence of white mold than the other treatments. As expected, the highest incidence occurred in the control (T1) and in the T2, T3 and T4 treatments (Figure 1A, B). Lower performance from the biological treatments is expected, given that this is the first time they had been used during this crop cycle. Continuous use of biological control achieves better results, whereas the first application is only the starting point for results that should continue to improve (Pomella and Ribeiro, 2009).

At 60 DAP, when the beans were in the R6 stage, the T7, T8 and T9 showed the lowest incidence of white mold relative to the other treatments. As expected, the highest incidence of white mold occurred in the control (T1) and in the T2, T3 and T4 treatments (Figure 1C). Meyer et al. (2014) showed that chemical control of WM in

soybean crops was efficient and that the active ingredient fluazinam was the most efficient. The chemical treatments in the present study also yielded the best results.

At 67 DAP, when the beans were in the R7 stage, the lowest, statistically different incidence of white mold was found in the T7, T8 and T9 treatments. Again, as expected, the highest incidence occurred in the control (T1) and in T2, T3, T4 and T5 (Figure 1D). Although the *T. harzianum* treatment showed statistically significant results at this stage we can not say that it was effective, given that it was used in concert with fungicides that were producing much better results. Contrary to Silva et al. (2015), who examined these two biological agents in the control of *S. sclerotiorum* in lettuce, we found that *T. harzianum* provided better control of WM (Silva et al., 2015). Not only was biological control (*Trichoderma* spp.) of WM studied, but also, edornaviruses, which are specific to fungi, were studied in *Vicia faba* (Khalifa and Pearson, 2014).

At 74 DAP, when the beans were in the R8 stage, the lowest, statistically different incidence of white mold was found in T8. The highest incidence of white mold occurred in the control (T1) while statistically similar

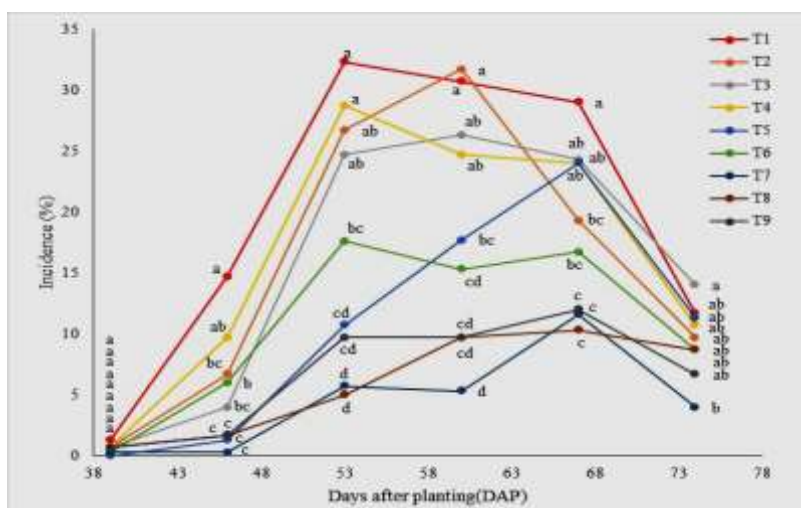


**Figure 1.** Mean of the transformed incidence  $\sqrt{(x + 10)}$  of white mold in the common bean cv. Pearl during the winter harvest under central pivot irrigation (2015), submitted to varioud biological and chemical control combinations. **A.** incidence at 46 days after planting DAP. **B.** incidence at 53 DAP, **C.** incidence of white mold at 60 DAP. **D.** incidence of white mold after 67 DAP. **E.** incidence of white mold after 74 DAP. **F.** Area under the white mold progress curve (AUDMPC).

results were found in T2, T3, T4, T5, T6, T8 and T9 (Figure 1E). This shows that biological control of WM was not as effective as chemical control in both seed treatment and in post-emergence applications (Moraes and Teixeira, 2008).

AUDMPC, which summarizes the extent of the white mold epidemic, had the lowest value in the T7 treatment,

followed in ascending order by T8 and T9, and shortly after by T5 and T6, T2, T3 and T4, which were statistically similar. Finally, the highest incidence of white mold was in the control (T1) (Figure 1F). A commercial product based on *Coniothyrium minitans* combined with low doses of fungicides was effective at managing white bean mold (Elsheshtawi et al., 2016).



**Figure 2.** Temporal progress curves of white mold incidence in beans cv. Pérola using different combinations of treatments during the 3rd. harvest under center pivot irrigation (2015) [Means followed by the same letter do not differ by Tukey test relative to the progress curve ( $P \leq 0.05$ )].

The progress curve expressed the critical limits of disease development in the different treatments, with the control treatment producing the upper limit of incidence (Figure 2). Thus, the best treatment (T7) reduced disease incidence by 0-13 % (control 0-33%) (Figure 2). When pyrisoxazole (rarely used in Brazil) was used to control 166 strains of *Sclerotinia sclerotiorum*, it provided excellent protection and reduced disease in oleaginous plants (Duan et al., 2018). The fungicides procymidone and fluazinam (commonly used in Brazil) combined with benzalkonium chloride were more efficient in controlling WM in soybeans (73.1 - 71.6%, 2010 crop; 75.7 - 77.6 %, 2011 crop) than isolated applications of *T. harzianum* (Sumida et al., 2015).

From 48 to 53 DAP, disease development was considered critical due to progressive growth in all the chemical treatments. In the T8 treatment, reductions in incidence began to decrease at 60 DAP (Figure 2). Single chemical applications are not effective over long crop periods; however, efficacy can be extended by combining treatments chemical and biological (Moraes et al., 2008; Mueller et al., 2002; Paula Junior et al., 2006). After harvesting, we compared bean yields from the various treatments. The statistically highest yields were in T8 (3590 kg / ha) and T5, T6, T7 and T9, which were statistically similar. The control (T1) and T2, T3 and T4 produced the lowest yields (<3490 kg / ha) (Figure 3). Chemical fungicides provided better WM control and consequently higher yields. Similar conclusions were drawn by Paula Junior et al. (2009).

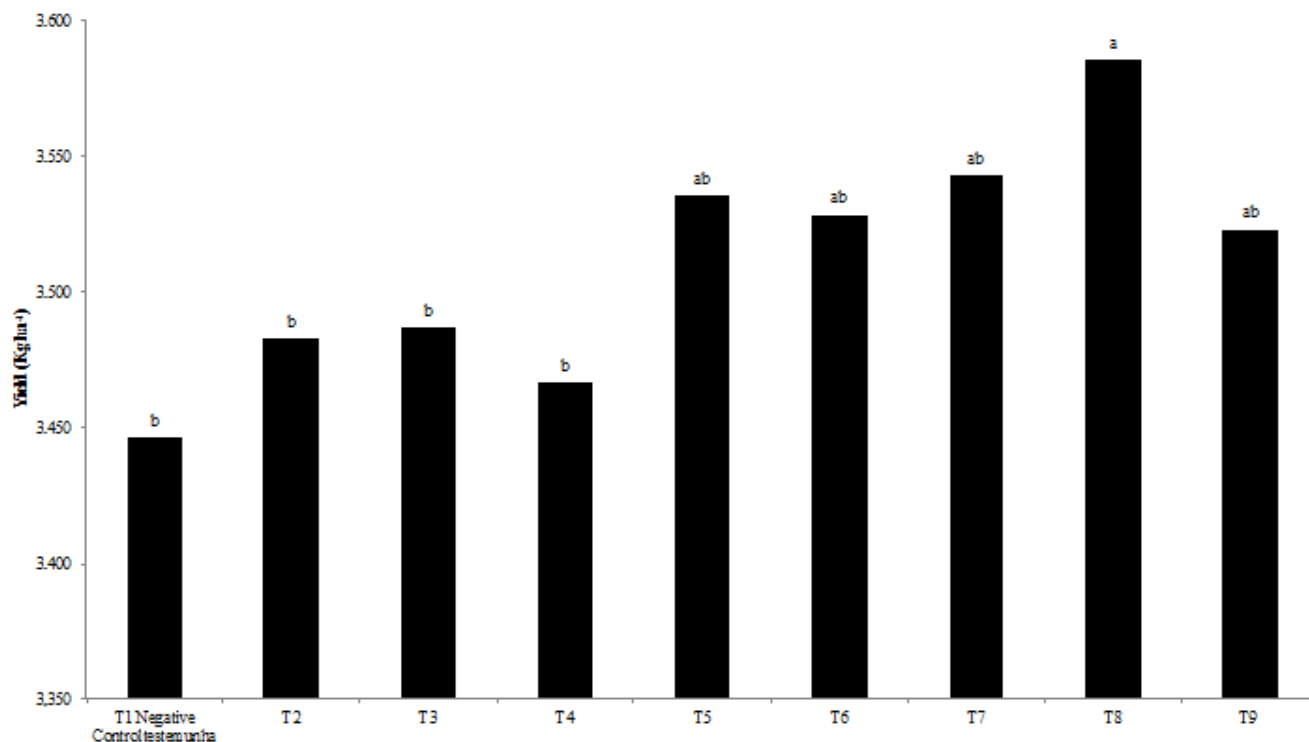
The highest control efficiency, as measured by AUDPC, was observed in T7 (77.9%), followed by T8 (72.4%) and then T9 (67.8 %), which demonstrates that

control efficiency greater than 50% was achieved in these treatments (Carneiro et al., 2015).

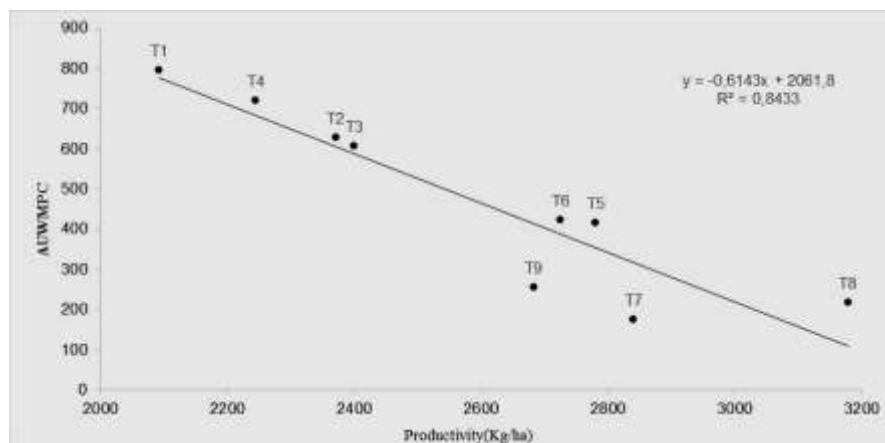
The highest yield efficiency was T8 (51.8%), followed by T7 (35.7 %), T5 (32.8 %), T6 (30.2 %), T9 (28.2 %), T3 (14.6 %), T2 (13.2 %) and T4 with the lowest percentage (7.2 %) (Table 1), showing that control efficiency is linked to yield (that is the lower the WM intensity, the higher the yield). The fact that this was the first time these biological controls were used to control this pathogen may partly explain why these treatments produced the lowest yield and control efficiencies (Vinale et al., 2008).

The highest average yield was 3178 kg ha<sup>-1</sup> or 53 sc ha<sup>-1</sup> for treatment T8 (trifloxystrobin + prothioconazole - Fox<sup>®</sup> and fluazinam - Frowncide<sup>®</sup>) (Table 1). The treatment with the combined chemical-biological application yielded 1085 kg ha<sup>-1</sup> (18 sc ha<sup>-1</sup>) more than the untreated control. Thus, 100 ha, under the same conditions, could yield an additional 108,500 kg (1800 sc 100 ha<sup>-1</sup>) / 100 ha of beans, which, at current prices (R\$ 205.00 sc) would provide an additional R\$ 369,000.00. Given spraying costs per hectare of R\$ 11.10 (Richetti and Roese, 2008), the spraying costs on 100 hectares would be R \$ 1110.00 per application.

In the T8 treatment (Table 1), the fungicide trifloxystrobin + prothioconazole - Fox<sup>®</sup> for 1 h costs R\$ 65.00 per hectare (R\$ 130.00 per liter; dosage 0.5 L / ha<sup>-1</sup>) or R\$ 6,500.00 per application on 100 ha. Similarly, the fungicide fluazinam - Frowncide<sup>®</sup> also costs R\$ 65.00 per ha (R\$ 130.00 per liter; dosage of 0.5 L / ha), which would cost an additional R\$ 6500.00 per application on 100 ha. Thus, a single application on 100 ha of the fungicides in the T8 treatment would cost R\$ 13,000.00



**Figure 3.** Average yields (kg / ha) transformed by  $\sqrt{x + 10}$  using different types of biological and chemical combinations applied on beans cv. Pérola during the winter crop under central pivot irrigation (2015) [Means followed by the same letter do not differ by Tukey test (P ~ 0.05)].



**Figure 4.** Mean area under the white mold progress curve (AUWMPC) versus yield (kg / ha) of different combinations of biological and chemical treatments to bean cv. Pérola in the winter crop under central pivot system.

(Carneiro et al., 2015).

Finally, the total cost of three applications of T8 on 100 ha would be R\$ 58,500.00 (3 x (spraying cost of R\$ 1110 + fungicide cost of R\$ 13,000)). Therefore, the net revenue on 100 ha gained by using the T8 treatment, rather than the untreated control no treatment, would be

R\$ 307,170.00 per 100 ha (Carneiro et al., 2015).

Yield increases in the experiment were explained by AUDPC (84.3%) (Figure 4), including highly correlated variables (growth rate of  $-0.6143\% \text{ day}^{-1}$ ) fit to a linear model. The control treatment (without any applications) showed that higher AUDPC was related to lower yields.



Contrary to our study, isolated fungicide active ingredients (not mixed with other active ingredients) and isolated treatments of *T. harzianum* in two consecutive harvests were shown to be more efficient at controlling the severity and incidence of WM and improving yield than treatments containing pure fungicides (fluazinam) in the 2009-2010 crop (Sumida et al., 2015).

T7 and T9 were strongly correlated with higher yield and lower AUDPC (Figure 4). Decreased WM, which was influenced by physiological resistance and plant architecture, had little influence on yield but reduced AUDPC (Görge et al., 2003).

## Conclusion

Single applications of biological control agents (T2, T3 and T4), applied at different rates and times, had statistically similar effects on WM incidence in common beans throughout the evaluation period.

A combination of a biological control agent (*B. subtilis*) and two active chemical control ingredients (trifloxystrobin + prothioconazole and fluazinam) produced the greatest reduction in AUDPC. The treatments using only biological control agents produced greater reductions in AUDPC than did the treatment without a combination of controls strategies.

T8 (three applications), with two chemical treatments and 3 applications over the bean growth cycle, produced numerically higher yields that were statistically equal to the yields of the treatments with combinations of biological and chemical agents (T5, T6, T7 and T9, four applications).

The highest control efficiency related AUDPC (77.9%) and yield efficiency (51.8%) were in T7 and T8 respectively.

The yield increases from the combined chemical and biological treatments reduced AUDPC by 84.3%. T8 increased yields, lowered final production costs and reduced the incidence of white mold, which the crop converted into yield gains.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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