RUFINO, C. P. B.; NOGUEIRA, S. R.; ARAÚJO, C. S.; ROSSI, A. J. D.; ANDRADE NETO, R. C.; LUNZ, A. M. P.; MACEDO, P. E. F. Chemical control of *Colletotrichum gloeosporioides* on seedlings of single assai palm. **Revista de Agricultura Neotropical**, Cassilândia-MS, v. 10, n. 2, e7264, Apr./June, 2023. ISSN 2358-6303. DOI: https://doi.org/10.32404/rean.v10i2.7264.

Chemical control of *Colletotrichum gloeosporioides* on seedlings of single assai palm

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Received: 04/11/2022; Accepted: 07/03/2023.

ABSTRACT

Anthracnose (*Colletotrichum gloeosporioides*) is the main disease affecting single assai palm seedlings and, therefore, a limiting factor to the production of quality seedlings. So far, there are no fungicide recommendations for the control of *C. gloeosporioides* in *Euterpe precatoria*. Thus, this study aimed to investigate the action of fungicides, such as pyraclostrobin + fluxapyroxad, as a possible alternative for controlling anthracnose in single assai palm seedlings. The experiment was installed in the nursery at Embrapa Acre, in a randomized block design, with seven treatments (0 – 0.155 – 0.187 – 0.218 – 0.250 – 0.281 – 0.312 µl of fungicide), with four replications of five plants. Evaluations were conducted for ten months regarding seedling height, stem diameter, number of leaves, shoot dry mass, root dry mass, total dry mass, and Dickson Quality Index. A visual scale ranging from 1 to 9 was used to assess the severity of the disease. The data were submitted to analysis of variance and regression analysis. Under the study conditions, the application of the active ingredient pyraclostrobin + fluxapyroxad reduced anthracnose severity by an average of 36.94% in single assai palm, providing better quality seedlings in the nursery.

Keywords: Assai palm, Pyraclostrobin + fluxapyroxad, Severity.

Controle químico de *Colletotrichum gloeosporioides* em mudas de açaizeiro solteiro

RESUMO

A antracnose (*Colletotrichum gloeosporioides*) é a principal doença que acomete mudas do açaizeiro solteiro e, portanto, um fator limitante à produção de mudas de qualidade. Até no momento não existir recomendações de fungicidas para o controle de *C. gloeosporioides* em *Euterpe precatoria*. Assim, o objetivo deste estudo foi investigar a ação de fungicidas, como piraclostrobina + fluxapiroxade, para uma possível alternativa de controle da antracnose em mudas de açaizeiro solteiro. O experimento foi instalado no viveiro da Embrapa Acre, em delineamento em blocos casualizados, com sete tratamentos (0 – 0,155 – 0,187 – 0,218 – 0,250 – 0,281 – 0,312 µl do fungicida), com quatro repetições utilizando cinco plantas. As avaliações foram realizadas por um período de 10 meses quanto à altura da muda, diâmetro do colo, número de folhas, massa seca da parte aérea, massa seca da raiz, massa seca total e o Índice de Qualidade de Dickson. Para avaliar a severidade da doença utilizou-se uma escala visual de notas variando de 1 a 9. Os dados foram submetidos à análise de variância e à análise de regressão. Nas condições do estudo, a aplicação do ingrediente ativo piraclostrobina + fluxapiroxade reduziu em média de 36,94% da severidade da antracnose em mudas de açaizeiro solteiro proporcionando mudas de melhor qualidade, em viveiro.

Palavras-chave: Açaizeiro; piraclostrobina + fluxapiroxade; severidade.

1. Introduction

The *Euterpe precatoria* Martius, also known as single assai palm, is a species with solitary habit naturally found on dry land, as well as in flooded areas, in the Brazilian states of Acre, Roraima, Amazonas, Rondônia, and Pará (Henderson, 1995). It is considered one of the most important species of the genus *Euterpe* due to its agronomic, technological, nutritional, and economic potential (Yuyama et al., 2011).

Although some technologies and innovations have been developed to optimize the seedling production of the single assai palm (Araújo et al., 2020, Almeida et al., 2018), it is important to establish ways to control anthracnose caused by the fungus *Colletotrichum gloeosporoides* in this palm, as it is the main disease that affects this fruit species in the nursery stage. Due to its high incidence and severity, the previously mentioned disease can lead to up to 70% of seedling death if effective management methods are not adopted, making the implantation and expansion of orchards unfeasible (Nogueira et al., 2016, Nogueira et al., 2017).

The pathogen attacks the younger leaves, initially causing dark and irregular spots with a light brown center and dark border. Later, it leads to scorching and the consequent death of plants (Nogueira et al., 2017), which reassures the need to establish effective control of the disease. Even though there are many mechanisms to control plant diseases, the main and most important strategy to treat those caused by fungi remains the use of chemical control (Silva et al., 2020, Finotto et al., 2011) because it is, in most cases, efficient and economically viable to ensure satisfactory production (Amorim et al., 2011; Finotto et al., 2011).

Currently, no active ingredient is registered in the Ministry of Agriculture, Livestock and Food Supply (MAPA) and/or in the Fungicide Resistance Action Committee (FRAC-BR) to control anthracnose in seedlings of assai palm, a fact that motivated the development of the referred study. Thus, this study aimed to evaluate the efficiency of foliar application of pyraclostrobin + fluxapyroxad fungicide (Orkestra®) on the control of anthracnose in *E. precatoria* seedlings, under nursery conditions, in Acre State, Brazil.

2. Material and Methods

The experiment was conducted from March 2018 to January 2019 at the Embrapa Acre experimental field nursery in Rio Branco, Acre, Brazil (10º1'30"S, 67º42'18"W, and 160 m above sea level). The climate of the region is Am, according to the Köppen classification, with average temperatures from 21 °C to 31 ºC, rainfall of 1,648.94 mm year^{-1}, and relative humidity of 83% (Agritempo, 2021). The temperatures and relative humidity inside the study environment were recorded using a datalogger model AK 174, from which a maximum temperature of 30.4ºC and minimum of 23.2ºC, with an average of 26.8ºC, and relative humidity of 88.9% were recorded. The experimental design was randomized blocks, with seven treatments, four replications, and five plants per plot.

The treatments consisted of seven doses of the commercial fungicide pyraclostrobin 333 gL^{-1} + fluxapyroxad 167 gL^{-1}: 0 – 0.155 – 0.187 – 0.218 – 0.250 – 0.281 – 0.312 µl, diluted in 250 ml of distilled water. In the nursery, the assai seeds were sown in the sand and emerged around 45 days after. Afterward, the selection of standardized seedlings, pre-emerged presenting 4.0 cm long, was conducted, and transferred to black polyethylene plastic bags with a volumetric capacity for 1 dm³ filled with a substrate in the proportion of 2.5 soil + 1.5 sand. During the permanence of the seedlings in the nursery with 50% shading net, daily irrigations were performed with a 2-minute irrigation shift every four hours.

The treatment applications began when the seedlings presented around three fully expanded leaves. They were made by biweekly spraying, totaling 20 applications over ten months, using a Guarany compression spray with a maximum pressure of 3.0 bar (45 psi) and an adjustable spray tip. Subsequently, evaluations of the disease severity were performed. For that, were created a diagrammatic scale with scores ranging from 1 to 9 (Figure 1), where 1 represented the absence of symptoms, and 9 was attributed to a dead leaf due to the incidence of the disease. The scores were attributed to each plant individually, every fifteen days for 10 months, totaling 20 severity evaluations.

From the data of the severity assessments, the area under the disease progress curve (AUDPC) was calculated using the formula proposed by Campbell e Madden (1990): $AUDPC = \sum [(X_i + X_{i+1})/2](t_{i+1} - t_i)$. At ten months after the subculture, the following variables were measured: plant height in cm (PH), with the aid of a graduated ruler, from the soil surface to the emission of the highest leaf's leaflet; stem diameter in mm (SD), with the aid of a digital caliper, measured at 1 cm above the soil; the number of leaves in leaves plant^{-1} (NL). In addition, shoot dry mass (SDM), root dry mass (RDM), and total dry mass (TDM) were determined in grams and obtained after being packed in Kraft paper bags and oven-dried at 55 ºC until constant mass and, later, weighed on a digital scale.

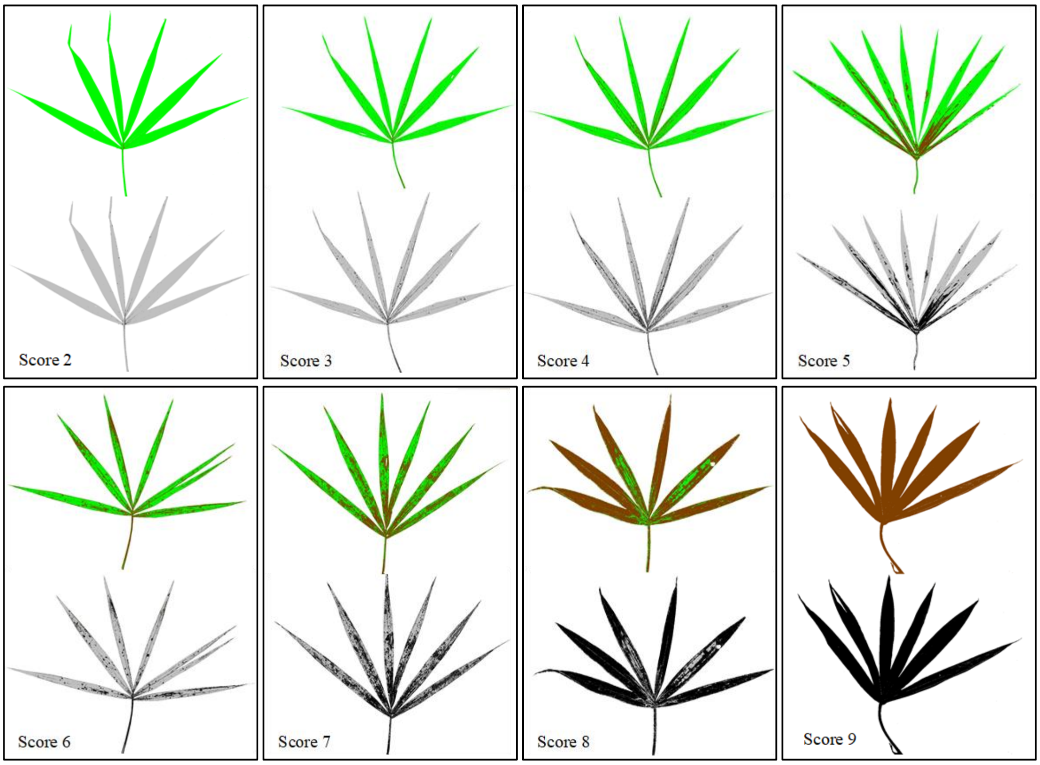

Figure 1. Diagrammatic scale to evaluate the severity of anthracnose in leaves of single assai palm based on scores ranging from 1 to 9. Where: 1 - 0% of the leaf area with lesions; 2 - 0.78 to 4% of the leaf area with lesions; 3 - 5 to 11% of the leaf area with lesions; 4 - 12 to 21% of the leaf area with lesions; 5 - 22 to 33% of the leaf area with lesions; 6 - 34 to 47% of the leaf area with lesions; 7 - 48 to 84% of the leaf area with lesions; 8 - 85 to 99% of the leaf area with lesions; 9 - 100% of the leaf necrosis due to the disease. Photo: Sonia Nogueira.

Finally, the seedling quality was determined using the Dickson Quality Index (DQI) (Dickson et al., 1960), obtained by the equation:

$$DQI=\frac{TDM\ (g)}{\left(\frac{PH\ (cm)}{SD\ (mm)}\right)+\left(\frac{SDM\ (g)}{RDM\ (g)}\right)}$$

The data were submitted to the Shapiro-Wilk and Bartlett tests to verify the normality of errors and homogeneity of variances, respectively. The analysis of variance was performed by the F test at the significance level of 5%. The data were submitted to regression analysis, being adjusted through polynomial equations using the statistical software Sisvar (Ferreira, 2011).

3. Results and Discussion

There were statistical differences ($p<0.05$) for all variables analyzed. A quadratic regression adjustment was observed for the variables PH (Figure 2 A), SD (Figure 2 B), and NL (Figure 2 C), whose appropriate doses were 0.223 µl, 0.212 µl, and 0.167 µl, respectively, with maximum estimated values corresponding to 31.50 cm, 16.45 mm, and 4.70 leaves plant^{-1}. The seedling height and the stem diameter are the most used morphological variables to assess seedling quality (Melo et al., 2018) since the first plays an important role in the initial growth after the transplanting to the field (Rossa et al., 2010; Melo et al., 2018), and the second is considered the main indicator of seedling survival in the field (Dutra et al., 2015) because it influences the nutrients and water translocation to the entire plant, as well as in metabolic and photosynthetic processes, resulting in mass gain (Oliveira et al., 2013).

The fungicide doses significantly affected the SDM (Figure 3A), RDM (Figure 3B), and TDM (Figure 3C) and were adjusted to second-degree equations. The maximum points were obtained at the doses of 0.253 µl for the SDM, 0.241 µl for RDM, and 0.247 µl for the TDM, with estimated values of 7.14 g, 4.91 g, and 12.05 g, respectively. It is important to highlight that biometric variables and dry mass accumulation should not be individually analyzed when evaluating seedling quality (Araújo et al., 2020).

Thus, the Dickson Quality Index (DQI) is an excellent tool to assist in the process of choosing the best quality plants in a nursery, as it is one of the most complete indexes to assess the seedling quality since in its calculation, both robustness and biomass distribution are considered (Eloy et al., 2013). After being determined, the DQI was adjusted to the polynomial regression model, whose maximum point was obtained at 0.241 µl of pyraclostrobin + fluxapyroxad, with an estimated value of 3.57 (Figure 4).

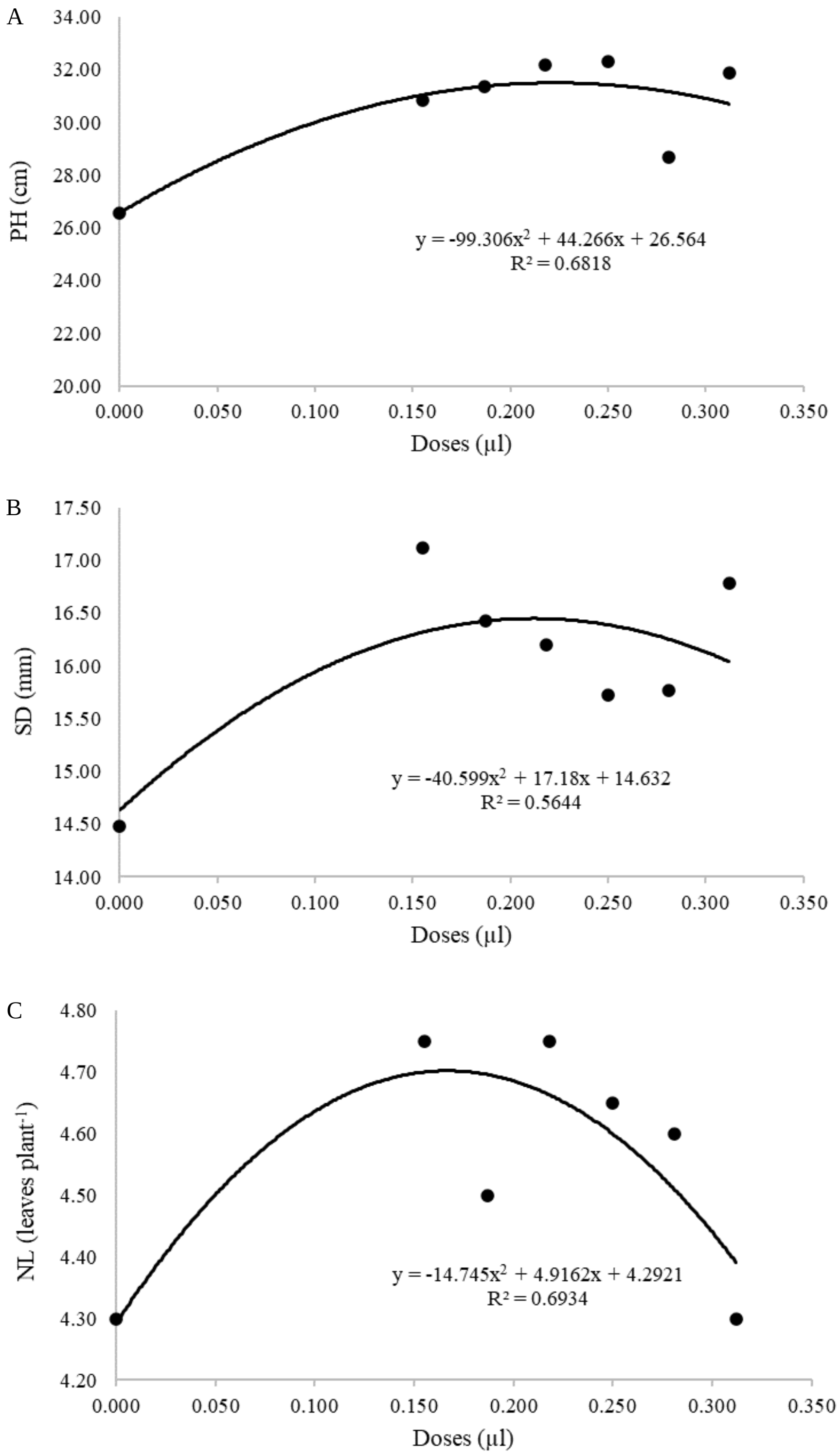

Figure 2. Plant height (PH - A), stem diameter (SD - B), and number of leaves (NL - C) of single assai palm seedlings, at ten months after subculture, submitted to different doses of pyraclostrobin + fluxapyroxad (µl) for the control of anthracnose.

The DQI obtained in the present study was higher than those reported by Araújo et al., (2020) and Almeida et al., (2018) but lower than the stipulated by Welter et al., (2014) for the *E. oleracea* species, indicating that this index may vary according to species, management conditions, and interaction between them. Currently, there is no commercial fungicide registered for use in nurseries or even in the field to control anthracnose (*Colletotrichum gloeosporoides*) in single assai palm, being fundamental studies on active principles. From the scores in the diagrammatic scale, it was possible to verify that the area under the disease progress curve (AUDPC) was adjusted to a decreasing linear severity model in contrast to the increase of the fungicide doses. A reduction of 36.94% in severity was observed at 0.281 µl (Figure 5), evidencing the efficiency of the active ingredient in controlling anthracnose in this palm species.

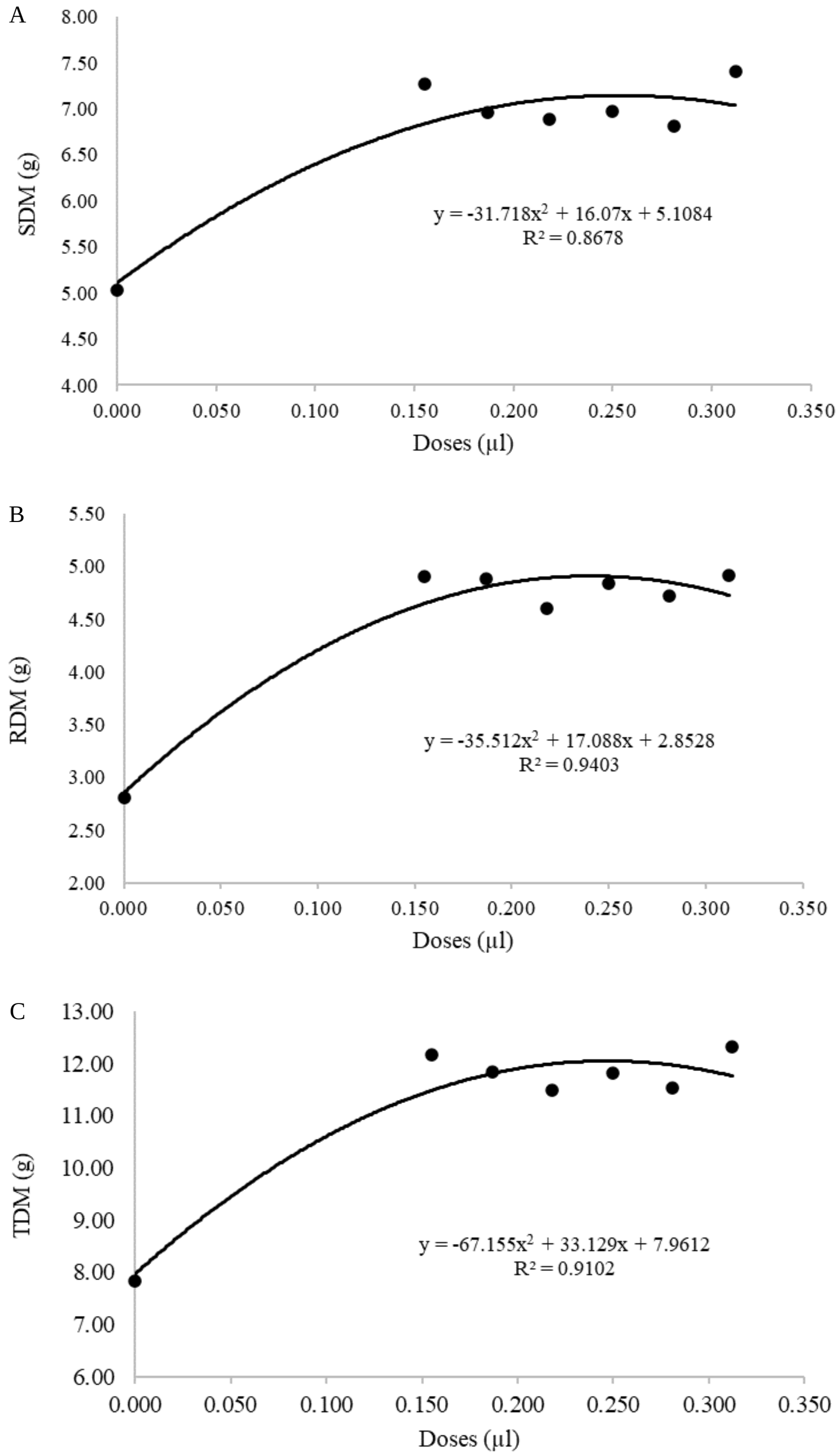

Figure 3. Shoot dry mass (SDM - A), root dry mass (RDM - B), and total dry mass (TDM - C) of single assai palm seedlings at 10 months after subculture, submitted to different doses of pyraclostrobin + fluxapyroxad (µl) for the control of anthracnose.

The results observed in this study are in line with those pointed out by Oliveira et al., (2021), who found that pyraclostrobin + fluxapyroxad reduced up to 55% the AUDPC of anthracnose (*Colletotrichum truncatum*) in soybean plants. Xavier and Vallad (2020) observed lower disease severity and greater control after evaluating this active ingredient for controlling the leaf spot on pomegranates. Ribeiro et al., (2019) studied pyraclostrobin + fluxapyroxad, associated with the protective fungicide Mancozeb, and observed less progress of the target spot (*Corynespora cassiicola*) in soybean. Godoy et al., (2017) also attested to the efficiency of controlling target spot severity in soybeans using this active ingredient. Above all, the prolongated use of fungicides can select resistant fungal populations. Therefore, it is of paramount importance fungicides are described for the control of diseases that are applied at appropriate times and intervals because their effect reduces when applied incorrectly, and consequently, mutations of new species of more virulent fungi can arise (Materatski et al., 2019).

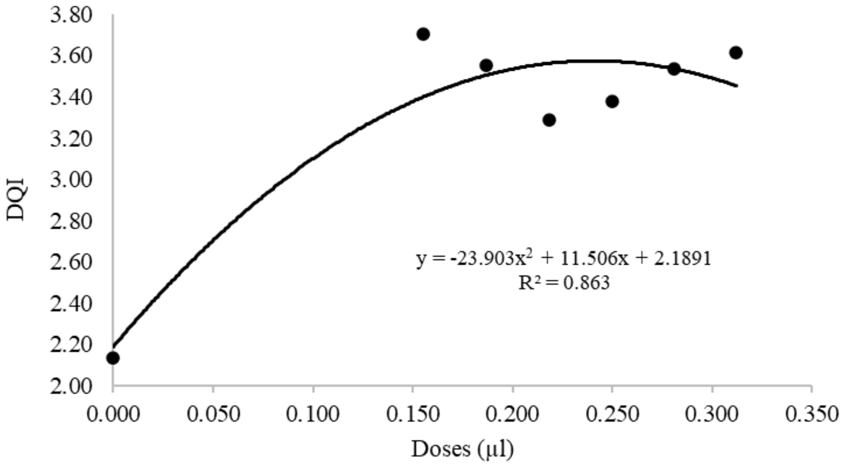

Figure 4. Dickson Quality Index (DQI) of single assai palm seedlings at 10 months after subculture submitted to different doses of pyraclostrobin + fluxapyroxad (µl) for the control of anthracnose.

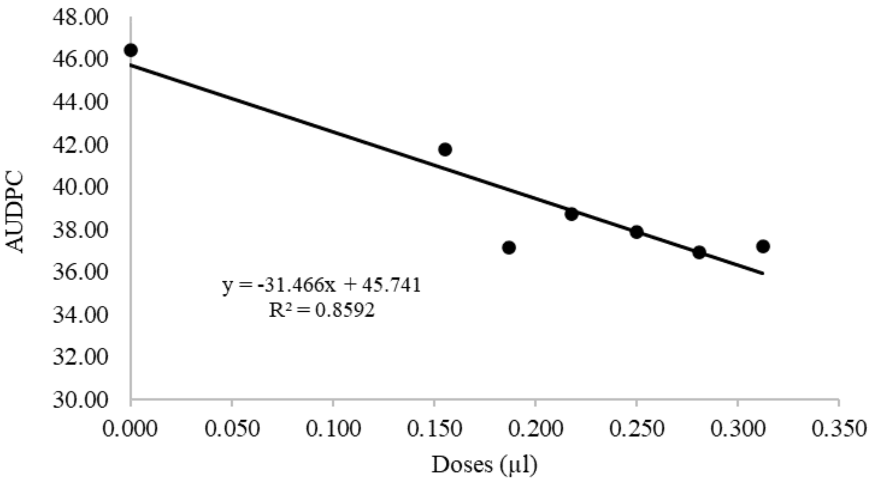

Figure 5. Area Under the Disease Progress Curve (AUDPC) of single assai palm seedlings at 10 months after subculture, submitted to different doses of pyraclostrobin + fluxapyroxad (µl) for the control of anthracnose.

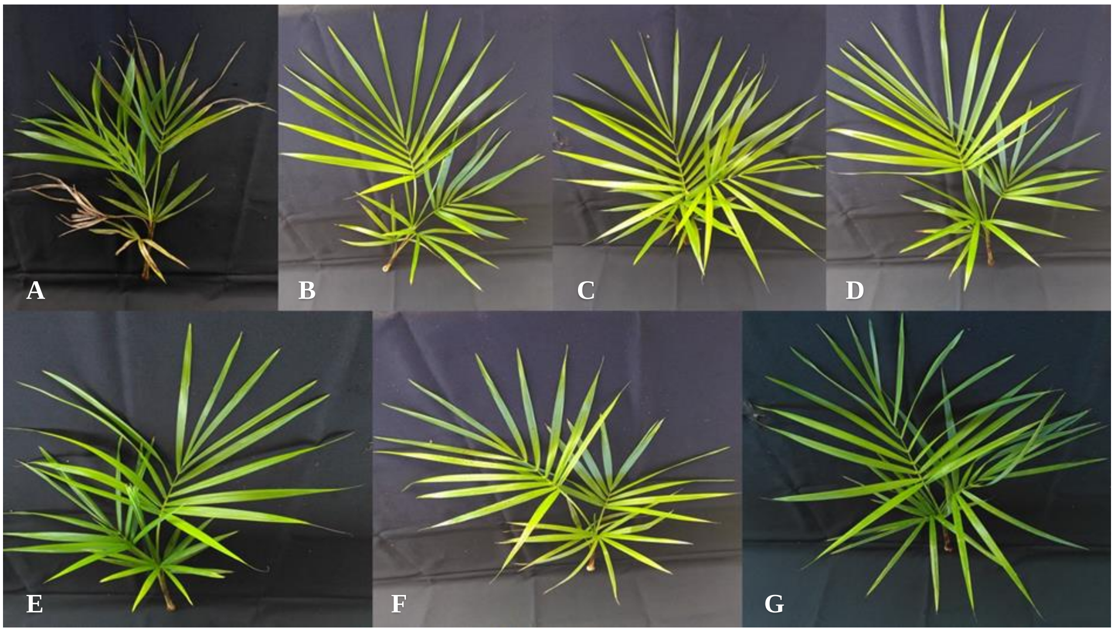

Figure 6. Single assai palm seedlings at 10 months after subculture submitted to the application of doses of pyraclostrobin + fluxapyroxad (µl) for the control of anthracnose. (A) Control - (B) 0.155 µl - (C) 0.187 µl - (D) 0.218 µl - (E) 0.250 µl - (F) 0.281 µl, and (G) 0.312 µl.

Certain fungicides provide modes the action described as gene-specific in terms of resistance induction. Specifically for *Colletotrichum* spp., fungicides based on strobilurin, for example, intervene in the mitochondrial respiratory pathway by binding to the center of cytochrome b of the pathogenic fungi (Avila-Adame and Köller, 2003), while the ones based on benzimidazole bind to β-tubulin and thus prevent the formation of the microtubules (Vela-Corcía et al., 2018).

Although the main objective of this study was to investigate the efficiency of pyraclostrobin and fluxapyroxad on the control of anthracnose, since mutations can occur, it is important to associate with other fungicides whose efficiency is proven. Nogueira et al., (2017) described that fungicides pyraclostrobin + epoxiconazole and trifloxystrobin + tebuconazole are promising for controlling anthracnose in *E. precatoria* under nursery conditions. According to Carraro et al., (2019), the control of anthracnose is extremely important for fruit yield. In commercial orchards that do not control this disease, its incidence can reach up to 80%, resulting in reduced yield.

According to Amorim et al., (2011), one of the options for managing plant diseases is using chemical control, which, in most cases, is effective in ensuring good production parameters. In general, the results of this study demonstrated that for the area under the disease progress curve (AUDPC) of single assai palm, all doses of fungicide contributed significantly to disease reduction when compared to the control treatment (Figure 6).

4. Conclusions

The spray application of the active ingredient pyraclostrobin + fluxapyroxad reduces the severity of anthracnose in single assai palm and provides seedlings with better quality.

Authors' Contribution

All authors contributed to the interpretation and research design. Conceição Paula Bandeira Rufino and Anderson José Danielsson Rossi were responsible for installing, conducting, and evaluating the experiment. Conceição was also responsible for writing the first version of the manuscript, which was passed on to the other authors for reading, corrections, and contributions. Cleyton Silva de Araújo was responsible for the statistical analyses and manuscript translation. Also, Sônia Regina Nogueira, Aureny Maria Pereira Lunz, and Romeu de Carvalho Andrade Neto were responsible for the *Euterpe precatoria* project at the Brazilian Agricultural Research Corporation (Embrapa) and contributed to the result interpretation and critically reviewed the manuscript, along with analyst Paulo Eduardo França de Macedo.

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