

# Control of leaf anthracnose of peach palm with fungicides: a valid strategy for seedling nurseries, but not for young plantations<sup>1</sup>

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#### ABSTRACT

This work aimed to evaluate the efficacy of fungicides for controlling anthracnose (*Colletotrichum gloeosporioides*) on peach palm (*Bactris gasipaes* var. *gasipaes*) seedlings in nursery beds and in newly planted peach palms in the field. The nursery experiment included two fungicide combinations: [thiophanate-methyl + chlorothalonil (TM + C) and pyraclostrobin + epoxiconazole (P + E)], three application intervals (7, 14, and 21 days), and an additional nontreated control. The two field experiments with newly planted peach palms tested the fungicide combinations TM + C at 15-day intervals for 12 months after transplanting, comparing to the control without fungicide application. The severity of anthracnose was assessed, and it was used for calculating the area under the disease progress curve (AUDPC). The fungicides TM + C and E + P applied at 7-days intervals were effective in the control of anthracnose in peach palm seedlings, resulting in the lowest severities and AUDPC. The fungicide TM + C reduced the maximum disease severity and AUDPC under field conditions. However, differences in the final severity of anthracnose in the field is not necessary.

Keywords: Bactris gasipaes; Colletotrichum gloeosporioides; fungicides; fungus; palm's heart.

#### **INTRODUCTION**

Palm heart is an important forest product for food in Brazil and several other countries. Also, it has been an important source of income, especially for small farmers in some rural areas of South and Central America (Santos *et al.*, 2011; Penteado *et al.*, 2014). A major source of palm heart in Brazil until a few decades ago was jussara-palm (*Euterpe edulis* Martius) obtained by extractivism in the Atlantic Forest along the Brazilian coast (Santos *et al.*, 2011). However, indiscriminate exploitation has led this palm to be included in the list of plants at risk of extinction in the country (CNCFlora, 2020). One of the alternatives found to overcome this problem was to promote the cultivation of new species of palm for palm heart, among them the peach palm (*Bactris gasipaes* Kunth var. *gasipaes* Henderson) (Figure 1 A), a palm tree native to the Amazon region (Santos *et al.*, 2011). Currently, the cultivation of peach palm plays an important role in the conservation of jussara-palm, especially in the most populous states in the northeast, southeast, and south regions of Brazil. This palm differs from the jussara-palm because it can form tillers. The resulting clumps allow more than one harvest per year

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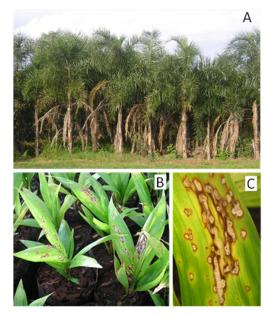
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and extend production for several years. In addition, peach palm harvest starts at 18 months of age. In addition, peach palm is the only commercial source of palm heart that does not darken due to oxidation, allowing its commercialization for fresh consumption in different types of cuts (Santos *et al.*, 2011; Penteado *et al.*, 2014).



**Figure 1:** Three-year-old peach palm plantation (A); Peach palm seedlings in a nursery with leaves damaged by anthracnose, caused by the fungus *Colletotrichum gloeosporioides* (B); and Coalescing necrotic lesions on leaf of peach palm seedling with acervuli (dark spots) of *C. gloeosporioides* in the center of lesion (C).

The increase in the planted area of peach palm has been accompanied by an increase in damage caused by diseases, not only in field but also in seedling nurseries; the diseases included: anthracnose (Santos *et al.*, 2011; Mafacioli *et al.*, 2009; Santos *et al.*, 2001), fusariosis (Jarek *et al.*, 2018) and stipe basal rot (Lopes *et al.*, 2019). In fields and in seedling nurseries, anthracnose caused by the fungus *Colletotrichum gloeosporioides* (Penz.) Penz. & Sac., is the main foliar disease and affects the development of seedlings in nurseries and young plants in new plantations (Mafacioli *et al.*, 2006a; Mafacioli *et al.*, 2008).

Anthracnose occurs in all Brazilian regions that plant peach palm (Alves & Batista, 1983; Pizzinato *et al.*, 1996; Poltronieri *et al.*, 1999; Santos *et al.*, 2011; Mafacioli *et al.*, 2009). Anthracnose affects the leaves of the peach palm, characterized by rounded and depressed lesions that can affect all the leaves of the crown (Figure 1 B-C), reducing the photosynthetically active leaf area, impairs growth and the formation of the heart of palm in the apical region of the plants. It is known from field observations, that anthracnose causes high losses in the nursery and that the disease continues to occur after out planting in the field (Santos et al., 2011; Mafacioli et al., 2009; Santos et al., 2001), including fruit production (Vida et al., 2006). Because peach palm has only more recently been cultivated, there is a lack of information for efficient management of anthracnose. Although genetic variation exists in peach palm and eventual development of resistant cultivars or clones will occur, currently planted genotypes are susceptible (Khalil Filho et al., 2011; Santos et al., 2011). Because attempts to manage the disease by application of fungicides in nurseries and plantations is usual (Santos et al., 2011), better evaluation of treatment efficacy is needed. Therefore, the objective of this work was to evaluate the use of fungicides to control anthracnose in seedlings in nurseries and in the first year of planting of the peach palm.

### **MATERIAL AND METHODS**

# Fungicides to control anthracnose in seedlings in peach palm nursery

The experiment was conducted in a peach palm nursery in Garuva, SC, Brazil, at 26°01' 37,25" S, 48°50' 12,63" W and 19 m above sea level. The experiment followed the double factorial scheme (3x3) in a completely randomized design with four replications. The factors evaluated were fungicides: a) without fungicide, b) thiophanate-methyl + chlorothalonil (Cerconil PM 50®) and c) pyraclostrobin + epoxiconazole (Opera®). Applications were made at 7, 14 and 21 days, totaling 30, 15 and 10 applications, respectively. The concentrations of fungicides were 0.5 and 1.25 g/L of the active ingredients thiophanate-methyl + chlorothalonil, respectively, and 0.33 and 0.12 mL/L of the active ingredients pyraclostrobin + epoxiconazole, respectively. The sprays were applied with a backpack sprayer using a conical nozzle. The spray volume was approximately 10 mL/plant. Each experimental unit was a plot comprised of 100 peach palm seedlings. Seedling infections resulted from naturally occurring inoculum.

Seedlings of 3 to 4 cm in height, 0.3 to 0.4 cm neck diameter and with one to two open leaves were transplanted into polyethylene plastic bags (6 cm in diameter and 15 cm in height) with commercial substrate and placed in nursery bags. Plots were separated with side walls made of plastic film during the entire evaluation period to limit spray to the treated plot and prevent contact of plants in adjacent plots. The plants were exposed to ambient conditions and seedling irrigation was carried out whenever there was a drought longer than 7 days. During the experiment, weeds were manually removed.

Ten seedlings per plot were randomly marked to be used in all evaluations. The severity (percentage of diseased leaf area) of anthracnose was evaluated with an interval of 30 days from the transplant of the seedlings, for a period of 7 months, evaluating all the leaves of each marked plant. The leaves were photographed with a digital camera and the images were analyzed with the ASSESS © 2.0 software (Image Analysis Software for Plant Disease Quantification. American Phytopathological Society, St. Paul.). From severity data, the disease progress curve over time and the area under the disease progress curve (AUDPC) were obtained (Shaner & Finney, 1977). The height of the plants and the diameter of the stem were determined 210 days after transplanting (DAT). The height was measured from the neck of the plant until the insertion of the spear leaf (leaf that has not yet opened). The stem diameter was measured approximately 2 cm above the ground.

The control efficiency (C) of fungicides and the application interval was calculated using the formula %C =  $[(D-T)/D] \times 100$ , where D is the AUDPC in the control and T is the AUDPC in the treatments. The Shapiro-Wilk test was applied to verify the normality of the data, and the Cochran's test, was used to assess homoscedasticity. Further, data were subjected to analysis of variance (ANO-VA). Whenever the interaction was not significant, the model was reduced to test only the main effects. Whenever a main factor was significant ( $P \le 0.05$ ), treatment means were compared based on the Scott-Knott test ( $P \le 0.05$ ). All analyses were conducted using the SISVAR version 4.6 (Ferreira, 2011).

# Fungicides to control anthracnose in newly planted peach palms

Two experiments were conducted in two areas in the municipality of Paranaguá, PR, Brazil, in an area with flat relief (experiment 1) at 25°35'58,77" S, 48°37' 50,77" W and 28 m above sea level level and another area with wavy relief (experiment 2) at 25°36' 03,01" S, 48°37' 53,26" W and 35 m above sea level. In both areas the soil was of the Inceptisol order and medium texture. These two experiments were carried out at the same time, concomitantly with the experiment with seedlings from the nursery.

The experiments were carried out in a randomized block design with two treatments and 11 repetitions. Each repetition consisted of two plants. The treatments consisted of spraying 0.5 and 1.25 g/L of water of the active ingredients thiophanate-methyl + chlorothalonil, respectively, at 15-day intervals, totaling 24 applications during 1 year; and the control, where the plants were not sprayed with fungicide. The sprays were applied with a backpack sprayer using a conical nozzle. The spray volume used ranged from 20 to 40 mL/ plant. Each experimental unit consisted of a plant. Seedling infections by the pathogen occurred naturally.

The seedlings were planted in May and were acquired in a commercial nursery that had approximately 2% severity of anthracnose, had four to six open leaves and an approximately 7 months. The soil was prepared by mowing, digging the holes, and planting the seedlings. The seedlings were planted at a spacing of 2 m between rows and 1 m between plants. Weed control was carried out by mowing throughout the period. Fertilization was not applied at planting time. After 30 days, the first chemical topdressing was applied with 50 g of fertilizer per seedling of the formula 10-10-10 (N-P-K) and repeated every 30 days.

The severity (percentage of leaf area with anthracnose) was assessed at 30-day intervals, for 12 months after planting the seedlings in the field. All leaves of each plant were evaluated, and the average severity of each plant was obtained. To assess the severity, the leaves were photographed with a digital camera and the images were analyzed with the ASSESS © 2.0 software. From severity data, the disease progress curve over time and the area under the disease progress curve (AUDPC) were obtained (Shaner & Finney, 1977). Plant height and stem diameter were determined at 360 days after planting (DAP). Height was measured from the soil surface until the insertion of the spear leaf (leaf that has not yet opened). Diameter was measured approximately 4 cm above the ground.

The climate variables measured were: precipitation; average relative humidity (RH); and maximum, average, and minimum temperature. These data were obtained at meteorological station located at 15 km of the experiments (Estação Experimental do IAPAR, at 25°30' 32,00" S, 48°48' 31,20" W W and 50 m above sea level).

The Shapiro-Wilk test was applied to verify the normality of the data, and the Cochran's test, was used to assess homoscedasticity. Further, data were subjected to analysis of variance (ANOVA). The treatments means were compared based on the Student t-test ( $P \le 0.05$ ). All analyses were conducted using the SISVAR version 4.6 (Ferreira, 2011).

# RESULTS

#### Anthracnose control in nursery with fungicides

The application of fungicides in the seedling production stage reduced the severity of anthracnose over time. The highest observed severity was 10.5% in the control and the lowest observed severities were 0.5 and 0.4% when the fungicides thiophanate-methyl + chlorothalonil and pyraclostrobin + epoxiconazole, respectively, were applied at intervals of 7 days (Figure 2).

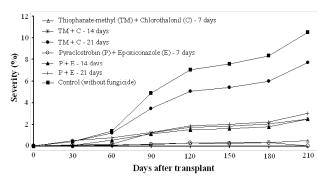


Figure 2: Progress curves of anthracnose severity in peach palm seedlings in nursery under different treatments with fungicides and spray intervals.

The interaction of factors fungicide and application intervals was significant ( $P \le 0.05$ ) for maximum severity

and AUDPC variables. Significant differences were observed when compared to the control when the fungicides thiophanate-methyl + chlorothalonil and pyraclostrobin + epoxiconazole, these both combinations of fungicides did not differ when applied at intervals of 7 and 14 days. With 21-day application intervals, the fungicide thiophanate-methyl + chlorothalonil did not differ from the control. The 7-day application interval was the most efficient in controlling anthracnose for both fungicides combinations (Table 1). Based on AUDPC, the fungicide application provided control efficiency of 96-97% and 76-78% for applications with intervals of 7 and 14 days, respectively, for both combinations of fungicides. With the 21-day interval between applications, there was a difference between the pyraclostrobin + epoxiconazole and thiophanate-methyl + chlorothalonil combinations, which provided 71 and 27% of control, respectively.

The interaction of factors fungicide and applications intervals was significant ( $P \le 0.05$ ) for plant height. The greatest plant heights were observed when the fungicides thiophanate-methyl + chlorothalonil and pyraclostrobin + epoxiconazole were sprayed at 7-day intervals (Table 1). There was no effect of fungicides and application intervals for stem diameter ( $P \ge 0.05$ ).

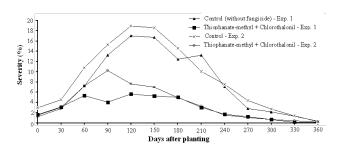
Maximum severity (%) Spray intervals (days) 7 14 21 Fungicides 10.5\* Control 10.5 Aa Aa 10.5 Aa Thiophanate-methyl + chlorothalonil chlorothalonil 0.5 Cb 2.5 Bb 7.7 Aa Pyraclostrobin + epoxiconazole 0.4 Bb 2.5 Ab 3.0 Ab AUDPC Spray intervals (days) 7 14 21 Fungicides 1043.77 Aa Control 1043.7 1043.7 Aa Aa Thiophanate-methyl + chlorothalonil 39.7 Cb 248.7 Bb 756.5 Aa Pyraclostrobin + epoxiconazole 34.7 Bb 231.0 Ab 300.0 Ab Plant height (cm) Spray intervals (days) 7 14 21 Fungicides 11.0 Ab Control 11.0 Ab 11.0 Ab Thiophanate-methyl + chlorothalonil 11.5 Cb 13.7 Aa 12.5 Ва Pyraclostrobin + epoxiconazole 13.5 12.0 Ba 12.5 Ba Aa

 Table 1: Maximum severity (%), area under the disease progress curve (AUDPC) of anthracnose of peach palm and plant height (cm) under different treatments with fungicides and spray intervals.

\* Means followed by the same uppercase letters in the row and lowercase letters in the column do not differ significantly by the Scott-Knott test at 5% probability.

#### Anthracnose control in field

Anthracnose in peach palm plants, in both experimental areas, both in the treatment with thiophanate-methyl + chlorothalonil and in the treatment without fungicide, showed the same pattern of disease progress curve (Figure 3). The severity at 30 DAP was approximately 2% for both treatments and experiments. The maximum severity values were observed at 120 DAP in the control with a severity of 16.9 and 18.9% for experiments 1 and 2, respectively. From that point on, the severity of the disease decreased and in the last evaluation (360 DAP) the final severity observed was between 0.0 and 0.3% (Figure 3, Table 2).



**Figure 3:** Progress curves of anthracnose severity in peach palm plants in the first year after planting in two areas (Experiments 1 and 2), with and without treatment with thiophanate-methyl + chlorothalonil.

**Table 2**: Maximum severity (Sev<sub>max</sub>) (%), final severity (Sev<sub>fin</sub>) (%) and area under the disease progress curve (AUDPC) of anthracnose of peach palm sprayed or not with thiophanate-methyl + chlorothalonil fungicides at 15-day intervals in the first year after planting in the field in two experimental areas.

Treatments	Experiment 1			Experiment 2		
	Sev <sub>max</sub> (%)	Sev <sub>fin</sub> (%)	AUDPC	Sev <sub>max</sub> (%)	Sev <sub>fin</sub> (%)	AUDPC
Control	16.9* a	0.3 a	2905.9 a	18.9 a	0.2 a	3306.6 a
Fungicide	5.6 b	0.0 a	1068.5 b	10.1 b	0.1 a	1411.9 b

\* Means followed by the same letters in the column do not differ significantly by the Student t-test at 5% probability.

In the two experimental areas (experiments 1 and 2) there were significant differences between the fungicide thiophanate-methyl + chlorothalonil and the control for maximum severity and AUDPC. However, there was no significant difference in the final severity of anthracnose

(Table 2). No differences in plant height were observed for experiment 1, but for experiment 2, a 5.6 cm gain in height was observed when the fungicide was used. There was no difference in stem diameter between the control and when the fungicide was used in both experiments (Table 3).

Table 3: Plant height (cm) and stem diameter (cm) at 360 days after planting peach palm, sprayed or not with thiophanate-methyl +
chlorothalonil fungicide at 15-day intervals in the first year after planting in the field in two experimental areas.

	Plant he	ight (cm)	Stem diameter (cm)		
Treatments	<b>Experiment 1</b>	Experiment 2	Experiment 1	Experiment 2	
Control	42.0 a	38.0 b	3.7 a	3.6 a	
Fungicide	42.8 a	43.6 a	3.9 a	3.8 a	

\* Means followed by the same letters in the column do not differ significantly by the Student t-test at 5% probability.

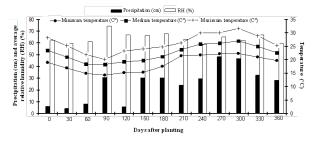
The temperature during the experiments ranged from 14.3 to 31.7 °C with an average temperature of 22.2 °C. The average relative humidity was of 64.1%. The total precipitation was 3243.1 mm (Figure 4).

# DISCUSSION

There are few studies in the literature on anthracnose of peach palm (Mafacioli *et al.*, 2006b; Tessmann *et al.*, 2005; Santos *et al.*, 2011). This study contributes to a better understanding of this pathosystem. We observed that anthracnose is an important factor for the production of peach palm seedlings and that chemical control is a useful tool to be used to obtain high sanitary quality seedlings. In the first year of planting, there is no need to control the disease with fungicides under the field conditions evaluated.

In studies carried out in Paraná and Santa Catarina states, anthracnose was found in 100% of the peach palm nurseries and plantations (Santos *et al.*, 2001; Bellettini *et al.*, 2012). These authors reported that the disease causes a reduction in the quantity and quality of seedlings in the

nurseries, depreciating their commercial value. At this stage, abiotic factors, such as high plant density, moisture accumulation and lack of adequate control, predispose the seedlings to the disease (Santos *et al.*, 2001). The use of seedlings of peach palm with high sanitary quality is extremely important, mainly for avoiding or reducing the amount of inoculum of the pathogen taken to the field (Santos *et al.*, 2001; Santos *et al.*, 2011). In this study, the fungicides thiophanate-methyl + chlorothalonil and pyraclostrobin + epoxiconazole, applied at intervals of 7 days, were effective in controlling anthracnose in peach palm seedlings, with final anthracnose severity below 0.5%. To our knowledge, this is the first study on chemical control of anthracnose in peach palm leaves.



**Figure 4:** Values of meteorological variables recorded during the epidemics in the experiments in peach palm plants in the first year of planting.

The progress of anthracnose in the nursery increased after transplanting, reaching the highest severity values at the end of seedling production (210 DAT). In the seedling production stage, the increase in the anthracnose progress curve may have been to the favorable microclimate formed around the plants, resulting from the high density of plants, high ambient humidity and favorable temperature, concomitantly with the increase in the availability of susceptible host tissue due to the growth and production of new leaves. Colletotrichum species in general causes disease in conditions of high humidity because the release and dispersion of spores are influenced by the amount of free water in the environment, requiring at least 1 to 4 hours of high humidity at optimal temperatures (20 to 30 °C) for the germination of spores and tissue invasion (Lopez, 2001). Anthracnose mainly affects young leaves (Santos et al., 2001; Mafacioli et al., 2009) and lesions can occur throughout the leaf blade leading to the coalescence of lesions and leaf drying (Santos et al., 2001; Mafacioli et al., 2009). The increase in severity over time in the seedling occurs due to the lower rate of new leaf emission and lower rate of senescent leaves, making it possible to evaluate the same bifid leaf for

a longer time. Seedlings were transplanted with one to two leaves, and at the end of seedling production, plants with a maximum of six leaves were observed.

In the case of field planting, the progress curve had a different behavior, with the severity increasing right after planting until reaching the maximum severity of the disease, subsequently showing a marked reduction in the severity. The same behavior of the progress curve observed in the nursery was also observed in the field until 120 days after planting (DAP). This can be explained by the fact that in the first year of field planting, despite the increase in the number of leaves and leaf expansion, the growth of plants in relation to height, stem diameter and number of leaves was slower between 120 and 150 DAT (data not shown) with a lower rate of new leaf production and a lower rate of senescent leaves. At this point, the plants had an average of 3.5 leaves per plant (data not shown). It is important to note that during this period, the plants are stressed by the transplanting to the field, and their leaves might yellow (Mora Urpi, 1984). According to Morsbach et al. (1998), in this stage the plants form the root system, and their growth is slow. Considering the slower plant growth, favorable environment and present pathogen, an increase in the disease progress curve was observed with greater severity at 120 DAT (severity close to 20%). However, from 120 DAP a reduction in severity was observed to values close to 0% at the end of 1 year of planting. This can be explained by the fact that a greater growth of plants was observed from 150 DAP (data not shown), with a higher rate of new leaf production and a higher rate of senescent leaves. At 360 DAP the plants had an average of 9.5 leaves per plant (data not shown). Over time, the old leaves that were formed still in the nursery and that had the disease became senescent and detached from the plant. The production of new leaves causes a dilution effect of severity because the healthy area increases in greater proportion than the diseased area, in addition to changes in leaf phenology. Peach palm has bifid leaves in its young stage and, in contrast, pinnate leaves when the plant reaches an adult stage (Morsbach et al., 1998; Khalil Filho et al., 2011). In this work, close to 240 DAP, the plants stopped forming bifid leaves and increased the formation of pinnate leaves, modifying the architecture of the canopy, which may have contributed to create unfavorable conditions to the disease due to the reduction of the wetness period because of the greater ventilation and brightness. In addition, unlike the nursery where there is a high density of plants per area, in definitive planting there is a low density of plants per area with a spacing of 2 m between rows and 1 m between plants (Neves *et al.*, 2011; Santos *et al.*, 2011). The climatic conditions did not have great variations that could explain the fact that the progress curve has grown and then presented a great fall. From 150 DAP, greater precipitation was observed, temperatures were in an optimal range of development of the pathogen and a drastic reduction in the severity of the disease was observed (Figure 4).

In field conditions, although the fungicide thiophanate-methyl + chlorothalonil applied at 15 day intervals reduced the disease over time compared to not applying the fungicide, it was observed that the final severity between treatments and experiments ranged from 0 to 0.3%. In addition to the final severity being very low, the disease did not affect stem diameter and plant height in experiment 1. Despite the 5.6 cm height gain observed with the application of the fungicide in experiment 2, this gain is not advantageous when having to perform 24 fungicide applications during the year. Therefore, there is no need for chemical control of anthracnose during the first year of cultivation under the conditions of the experiments. The maximum disease severity observed in the control treatment of approximately 20% is considered high for this pathosystem. As it is an emerging disease, there is no published information about maximum severities that are found in the fields under different conditions. According to our experience visiting different locations of production of the crop, we did not observe much greater severities than those observed in the places of these experiments. This ensures that the conditions under which the experiments were conducted were favorable for the occurrence of the disease. The seedlings used for planting were around 2% in severity, but a 10.5% severity was observed in the control treatment in seedlings produced in nurseries. Therefore, it is not possible to infer whether anthracnose can cause damage if seedlings with greater severity are planted. However, it is assumed that due to the small number of seedling leaves, 4 to 6 leaves per plant (Neves et al., 2011), a greater reduction in leaf area due to the disease should compromise the initial establishment of plants in the field. Thus, the planting of seedlings with high sanitary quality can be an important point for the success of planting and this can be guaranteed with the use of fungicides in the nursery, as shown in this study.

To the best of our knowledge, this is the first study that demonstrates the effectiveness of the use of fungicides in controlling anthracnose of peach palm in the production of seedlings in nurseries and first year of plantation establishment, as well as the epidemiological behavior regarding the disease progress curve. This study will serve as a basis for future studies, such as evaluation of new fungicide molecules and the influence of chemical, physical and microbiological conditions of substrates in the production of seedlings, as well as different environmental conditions and genetic materials, and other control measures that may help in the integrated management of the disease.

# CONCLUSIONS

Fungicides are useful tools for the management of anthracnose in peach palm seedlings in nurseries. The fungicides thiophanate-methyl + chlorothalonil and pyraclostrobin + epoxiconazole applied at 7-day-intervals showed the greatest efficacy in the control of anthracnose in peach palm seedlings.

The use of fungicide has been shown not to be a valid field strategy for the management of leaf anthracnose in young plantations under the field conditions evaluated, since healthy seedlings are used.

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We have no conflict of interest to declare.

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