Thermotherapy in 'dwarf silver' banana produced in the semi-arid region of Minas Gerais

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ABSTRACT

Anthracnose stands out among the rots that can occur in the postharvest phase of bananas. The objective of this study was to evaluate thermotherapy in the control of anthracnose in 'Prata Anã' banana produced in the North of Minas Gerais. Bananas of the Prata Anã variety were divided into bouquets of three fruits and inoculated with Colletotrichum musae. The bouquets were submitted to thermotherapy at five temperatures (40, 44, 48, 52 and 56 °C) for four immersion times (4, 8, 12 and 16 minutes) and control. The treatments were repeated five times in a completely randomized design. The area under the incidence progress curve (AACPI) and the area under the severity progress curve (AACPS) were calculated. To evaluate the efficiency of thermotherapy compared to chemical control, the fruits were submitted to the following treatments: thermotherapy with and without fungicide application and fruits treated only with fungicide, with the fruits without thermotherapy and without fungicide application being the control. After fifteen days of storage, the fruits were evaluated for anthracnose severity. The results of the experiments were submitted to analysis of variance and regression and the means were compared using the Scott-Knott test ($p<0.05$). The control was compared to the treatments using Dunnett's test ($p<0.05$). The lowest AACPI values for anthracnose were observed at a temperature of 48 °C for 8 and 16 minutes. Thermotherapy at 52 °C after 8 minutes of immersion, in addition to reducing the AACPS of anthracnose in the fruits by up to 81.6%, delayed their maturation. The fruits submitted to thermotherapy at 56 °C showed greater disease severity and resulted in the advancement of their maturation. Thermotherapy reduces the severity of anthracnose in fruits in a similar way to the use of fungicide, but the association of both results in better control of the disease in 'Prata Anã' banana. Thermotherapy is an efficient technique to reduce the severity of the disease in 'Prata Anã' bananas produced in the north of Minas Gerais.

Keywords: *Colletotrichum musae*, Thermotherapy, Incidence, Severity.

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INTRODUCTION

Several rots can occur in the postharvest phase of bananas, with greater emphasis on anthracnose caused by the fungus *Colletotrichum musae* (Berk & Curt.), which is one of the main postharvest diseases and leads to significant losses of up to 40% of production (COELHO *et al*., 2010).

The control of banana anthracnose is mainly carried out by chemical and physical methods. Among the physical methods, hydrothermal treatment has been tested by several researchers for the postharvest management of the disease (SPONHOLZ *et al*., 2004; MORAES *et al*., 2005; MORAES *et al*., 2006; NOLASCO *et al*., 2008; SILVA *et al*., 2008; RAMIREZ *et al*., 2011).

Heat can directly control pathogens by denaturing proteins, releasing lipids, destroying hormones, reducing metabolic reserves or injuries, inhibiting or delaying germ tube elongation, or inactivating spores. The use of high temperatures also aims to control fungal diseases by the expression of plant defense genes (BARKAI-GOLAN and PHILLIPS, 1991; FERGUSON, *et al*., 2000). However, the ideal temperature for the control of anthracnose and the exposure time of the fruits varies among the different studies carried out by the researchers.

The climatic conditions of the region where the fruits were produced can influence the response to hydrothermal treatment. Field studies have shown that environmental factors directly influence banana physiology (CABRERA and GÁLAN SAÚCO 2005; TAULYA *et al*., 2014; LIMA *et al*., 2015).

According to Golan and Phillips (1991), the efficiency of hydrothermal treatment in fruits is related to the climatic conditions close to the harvest of the bunches. Differences in the efficiency of thermotherapy were also observed by Shiffmann-Nadel and Cohen (1966) in fruits produced at different growth temperatures and cultural practices of the cultivars and harvested at various stages of maturity.

Environmental factors are directly involved in the incidence and severity of the disease, influence the various phases of the pathogen's life cycle and also the development of the host, being important for the infection of pathogens (ADASKAVEG et al. 2002, MAFIA et al. 2011, OLIVEIRA et al. 2011).

Thus, the objective of this study was to evaluate thermotherapy in the management of anthracnose in 'Prata Anã' banana produced in the North of Minas Gerais.

MATERIAL AND METHODS

The bunches of 'Prata Anã' bananas were obtained from an area with commercial plantation in the municipality of Nova Porteirinha – MG, located at 15° 48' 09" S and 43° 18' 32" W, with an altitude of 533 m, an average annual temperature of 27.5 °C and a climate, according to Köppen's classification of type Aw, characterized by rainfall concentrated in the summer and dry in the winter.

Bunches were harvested in the months of November and December at pre-climacteric stage or maturation stage 2 (green fruits with yellow traces), according to the Von Loesecke scale (PBMH and PIF 2006), plummeted, and the central bunches were selected for uniformity of the fruits during maturation. The bunches were transported to the Post-harvest Pathology Laboratory in plastic boxes protected with paper to avoid injuries.

In the laboratory, the bunches were subdivided into bouquets of three fruits. The bouquets were washed with neutral detergent and dried in the environment. They were then atomized with a 2.5x106 $^{esporos.mL-1}$ suspension of *C. musae*, obtained from colonies cultivated in PDA, for seven days, in order to standardize the infection. After inoculation, the bouquets were placed in plastic trays for 24 hours. After this period, the bouquets were immersed in a thermostatic bath with water heated at different temperatures: 40, 44, 48, 52 and 56 °C, for different immersion times: 4, 8, 12 and 16 minutes. After treatment, all bouquets were cooled in water at room temperature. The additional treatment (control) consisted of fruits without thermotherapy.

The experimental design was completely randomized, in a 5 x 4 factorial scheme (5 temperatures x 4 immersion times) and the control. Each treatment consisted of five replicates containing a bouquet with three fruits each.

The bouquets were stored in expanded polystyrene trays and kept in a refrigeration chamber (25±1 °C and 80±5% RH) for 3, 6, 9, 12 and 15 days, when the disease intensity evaluations were performed.

The intensity of anthracnose in fruits was evaluated by incidence and severity. The incidence was obtained by the number of fruits affected per repetition, and these values were expressed as percentages per treatment. For the severity variable, the diagrammatic scale developed by Moraes *et al*. was adopted. (2008) with disease severity ranging from 0.5 to 64%. With the results of incidence and severity, the Area Under the Incidence Progress Curve (AACPI) and the Area Under the Severity Progress Curve (AACPS) were calculated, and disease progress curves were constructed according to the formula of Shaney and Finney (1977).

To evaluate the efficiency of thermotherapy compared to chemical control in the control of anthracnose, banana bouquets containing three fruits were atomized with a suspension of 2.5x106 spores mL-1 of *C. musae*, obtained from colonies cultivated in PDA, for seven days. After inoculation, the bouquets were incubated in a humid chamber at 25 °C for 24 hours. After this period, the bouquets were submitted to the following treatments: hydrothermal treatment (52 °C for eight minutes) with subsequent application of fungicide (Imazalil at a concentration of 2 mL.L-1), fruits submitted to hydrothermal treatment without fungicide application and fruits treated only with fungicide. The control consisted of fruits without hydrothermal treatment and without fungicide application.

The fruits were stored in expanded polystyrene trays and kept in a refrigerated chamber (25±1 °C and 80±5% RH). The statistical design was completely randomized.

The intensity of anthracnose in the fruits was evaluated by evaluating the severity after 15 days of fruit storage, using the scale of **Moraes et al. (2008).**

The data obtained in the experiments were submitted to analysis of variance and regression of the means compared by the Scott-Knott test at 5% probability, using the "Sisvar" software (FERREIRA, 2011). **The control was compared with the other treatments by Dunnett's test at 5% probability** using the *SAS* **software.**

RESULTS AND DISCUSSION

As there was no adjustment of regression models, the means of the AACPI of anthracnose in the banana 'Prata Anã' were compared by the Scott-Knott test ($p<0.05$).

Evaluating the binomial, water temperature x fruit immersion time, the lowest values of the AACPI of anthracnose were observed when using the hydrothermal treatment at 48 °C for 8 and 16 minutes, not differing from each other. The reduction of AACPI in the treated fruits in relation to the control was observed in the treatments at 40 °C for 8 and 16 minutes, 44 °C for 4, 8, 12 and 16 minutes, 52 °C for 8, 12 and 16 minutes and 56 °C for 8 minutes, all with a reduction of the disease by 11.11%, with the exception of hydrothermal treatments at 48 °C for 8 and 12 minutes. both reduced the AACPI of anthracnose by 40.74% (Table 1).

Most phytopathogenic microorganisms have a lethal thermal point at temperatures in the range of 45 to 60 °C (COCHRANE, 1958; DEVERALL; 1965; LIU *et al*., 1997), as noted by Tanaka *et al*. (2003) in the control of the incidence of species of the genus *Colletotrichum* spp. in the solar heat treatment of water.

Several studies carried out in other pathosystems have demonstrated, in a similar way to this work, that thermotherapy was efficient in reducing, but not in suppressing, the incidence of diseases. Sponholz *et al.* (2004) working with 'Prata' bananas treated at 45 °C, found that thermotherapy did not prevent the incidence of anthracnose and the percentage of the injured area reached the maximum 12 days after treatment. In Cajá, Brito *et al*. (2008) also observed that hydrothermal treatment at 50 °C did not control the appearance of rot in the fruits.

Regarding the AACPS of anthracnose, a significant interaction ($p<0.05$) was observed between the following factors: water temperature and immersion time.

A quadratic behavior was observed in the regression plots of the disease of the disease in the fruits due to the increase in water temperature (Figure 1).

Thermotherapy with immersion of the fruits for 4, 8 and 12 minutes showed AACPS values that decreased and obtained a subsequent increase from the temperatures of 47.7 °C, 45.8 °C and 48.16 °C, respectively. The increased severity of anthracnose can be attributed to the fact that the higher exposure temperatures resulted in physical damage to the fruits, facilitating infection of *C. musae*. The potentiation of the fungal action with the increase in the exposure period was also observed by Moraes *et al*. (2005), verifying an increase in the incidence of *C. musae* when the period of exposure of the fruits to heat treatment was prolonged.

In the immersion time of 16 minutes, an inverse behavior was observed, as there was progress of the AACPS of the disease until it reached a temperature of 43.6 °C, followed by a reduction with the increase in temperature. The reduction of AACPS at the 16-minute immersion time was due to the greater exposure of the fruits at high temperatures.

One of the mechanisms responsible for the death of phytopathogens is the denaturation of proteins and enzymes, which are important for cellular metabolism. Thus, immersion of fruits in water heated from 50 °C to 55 °C for 10 minutes has been considered a standard method for the control of several fungal diseases in postharvest (LIU *et al*. 1997).

The lowest values of the AACPS of anthracnose by the regression plot were observed in the treatments at 48 °C when immersed at 4, 8 and 12 minutes, verifying that there is no need for such high temperatures to obtain satisfactory results in bananas of the Prata Anã variety.

The control of pathogens by hydrothermal treatment occurs when spores in quiescent infections are present on the surface or in the first cell layers of the fruit (Silveira et al. 2005), a fact that occurs with the fungus *C. musae* that causes anthracnose in bananas. Some authors report that the effect of thermotherapy on postharvest disease control is due to the reduction in fungal spore viability and induction of resistance (CABRERA and DOMÍNGUEZ, 1998; PESSOA *et al*., 2007).

Analyzing the interaction between the factors water temperature and fruit immersion time, it was found that in the 4-minute time, the temperatures of 44, 48 and 52 °C presented the lowest values of the AACPS of anthracnose, not differing from each other (Table 2).

In the 8-minute immersion time, it was observed that thermotherapy at 56 °C presented the highest AACPS value, differing from the other temperatures used. When the fruits were immersed for 12 minutes, it was found that the lowest AACPS values were found at temperatures of 48 and 52 °C, with a reduction of the disease in 81.2 and 88.6%, respectively, with no difference between them. After 16 minutes of immersion, there was no difference between the temperatures in the treated fruits.

Sponholz *et al*. (2004) reported that when 'Prata' banana fruits were exposed to 50 °C for 15 minutes of immersion 12 days after treatment, the percentage of injured area was 25%, a value higher than that found in this study. Nolasco *et al*. (2008) were able to control rots in 'Prata' banana until the 12th day of evaluation with the binomials time/temperature of immersion in water at 50 °C for 6 and 12 minutes.

Setting the water temperature at 40 °C showed the lowest AACPS values after 8 minutes of immersion and a reduction of up to 63.8% compared to the control. At 44 °C, the immersion time of 8 minutes resulted in a reduction of 62.6% in relation to the control, but there was no difference between the immersion times used. At 48 °C, all immersion times reduced the AACPS, differing from the control by up to 81.6%.

There was no difference between the immersion times at the temperature of 52 °C. There was a difference in immersion times in relation to the control after 8 minutes, with a reduction of up to 88.6%. This temperature presented the lowest AACPS values of anthracnose compared to the control, proving to be the most efficient treatment. In addition, the fruits treated at this temperature remained with green traces, showing a late maturation.

This confirms, as reported by Lobo *et al*. (2000) working with Cavendish bananas treated at 50 °C for 15 min, that heat treatments of immersion at elevated temperatures produce alteration of ripeness. Similar results were found by Wall (2004) when applying thermotherapy (48, 49 and 50 °C) for 15 and 20 minutes in bananas, where an alteration in ethylene synthesis was presented. In a study carried out, Chillet *et al*. (2006) observed that fruits at an advanced stage of maturation are more susceptible to infections by *C. musae*, while unripe fruits or those at the beginning of maturation are more resistant to infection. Ramirez *et al*. (2011) also reported that treatment at 55 °C and a soaking time of 5 minutes slows down the ripening of Cavendish bananas.

Generally, unripe fruits are more resistant to pathogens, due to the presence of phytoalexins and other compounds (Pessoa & Oliveira 2006). Tannins, found in these fruits, are examples of high molecular weight phenolic compounds that precipitate proteins, assuming a role in plant protection against pathogens, since they have the ability to form complexes with proteins and polysaccharides, inactivating enzymatic reactions. These biochemical reactions mischaracterize the enzymes, preventing them from being used in the normal growth processes of the fungus (Haslam 1996, Efraim et al. 2006).

Using the water temperature at 56 °C, it was observed that all times of exposure were harmful to the fruits. Immersion for 4 and 8 minutes caused rapid maturation of the fruits that favored the development of the disease, increasing the AACPS values by 78.3%. After 12 minutes of immersion, there was scalding in the treated fruits, which resulted in a reduction in the AACPS, but making the fruits unfeasible for commercialization.

According to Moraes et al. (2005), the injuries caused by hydrothermal treatment can cause an increase in weight loss, peel discoloration, increased susceptibility to fungi and a reduction in postharvest life. Sponholz et al. (2004) and Moraes et al. (2005), working with *C. musae* in bananas, observed that the increase in temperature reduced the injured area. However, very high temperatures around 56 °C, associated with longer exposure intervals, 9 and 12 minutes, led to injuries to the epidermis of the fruits that are more harmful to the commercialization than the action of the pathogen itself, due to the poor visual aspect on the surface of the same. Ramirez et al. (2011) evaluated the effect of thermotherapy on the latex secretion of freshly cut bananas, and found that bananas exposed to 55 °C became darker and showed symptoms of burning due to high temperature.

Temperatures of 50, 55 and 56 °C are harmful to the fruits, causing scalding and hardening of the pulp, impairing marketing due to the poor visual appearance of the fruits (ARMSTRONG, 1982; RAHMAN *et al*., 1994; REYES *et al*., 1998; PESSOA *et al*., 2009). Heat treatment can promote external and/or internal damage to fruit tissues. Temperature control and exposure time are extremely important for its use alone or in combination with other control methods (CHITARRA and CHITARRA, 2005).

Figure 2 shows that there was a difference in the severity of anthracnose between the fruits submitted to hydrothermal treatment with the fungicide Imazalil and the fruits submitted only to hydrothermal treatment and only with the fungicide. Thermotherapy reduced the severity of anthracnose in the fruits in a similar way to the use of fungicide, not differing statistically from each other, but more efficient results were those in which bananas were treated with thermotherapy in conjunction with the fungicide Imazalil, which practically controlled the disease, presenting only 0.5% of anthracnose in the fruits. Thus, it was verified that the association of hydrothermal treatment/chemical treatment was efficient in the management of anthracnose in 'Prata Anã' banana.

By Dunnett's test at 5% probability, it was observed that there was a difference in the severity of the disease between the control and the fruits treated by the hydrothermal treatment with fungicide and by the hydrothermal treatment without fungicide, which can be expressed in a reduction of the disease in 20.99% and 19.24%, respectively. Even with the onset of the disease, the lesion caused in the fruits treated only with fungicide was significantly lower than in the control, with a reduction of 19.58% of the disease.

These results corroborate those found by Coelho *et al.* (2010) studying the postharvest control of anthracnose of 'Prata Anã' banana treated with the fungicide Imazalil. The authors found a smaller effect on the control of *C. musae*, not completely controlling the disease.

Although the existence of *C. musae that* are resistant to fungicides has been verified, Chillet *et al*. (2006) suggest that the onset of the disease should not be explained only by this reason, but also by the quality of the banana, which is greatly influenced by edaphoclimatic factors. According to research conducted by the authors, there may be a relationship between the mineral status of the plant and the susceptibility of the fruit to the disease.

TABLE 1. Area Under the Incidence Progress Curve (AACPI) for anthracnose in 'Prata Anã' banana, submitted to thermotherapy at different temperatures and immersion times.

Immersion Time (minutes)	Water Temperature (°C)[1]				
	40	44	48	52	56
4	468,75 aA	450,00 aA*	468.75 aB	468,75 aA	468,75 aA
8	450,00 bA*	450,00 bA*	300,00 aA*	450,00 bA*	450,00 bA*
12	468,75 bA	450,00 bA*	300,00 aA*	450,00 bA*	468,75 bA
16	450,00 aA*	450,00 aA*	468.75 aB	450,00 aA*	468,75 aA
CV (%)	5,36				
Witness	506,25				

[1]Means followed by the same letter, lowercase in the row and uppercase in the column, do not differ from each other according to the Scott-Knott test ($p<0.05$). Means with an asterisk (*) differ from the control by Dunnett's test ($p<0.05$).

TABLE 2. Area Under the Severity Progress Curve (AACPS) for anthracnose in 'Prata Anã' banana, submitted to hydrothermal treatment at different temperatures and immersion times.

Immersion Time (minutes)	Water Temperature (°C)[1]				
	40	44	48	52	56
4	91,25 bB	56,50 aA	38,75 aA*	47,75 aA	107,75 bC
8	38,50 aA*	39,25 aA*	19,25 aA*	17,75 aA*	187,00 bC*
12	61,25 bA	51,00 bA	19,75 aA*	12,00 aA*	75,75 bB
16	38,00 aA*	51,50 aA	39,00 aA*	19,00 aA*	23,25 aA*
CV (%)	23,84				
Witness	104,89				

[1]Means followed by the same letter, lowercase in the row and uppercase in the column, do not differ from each other according to the Scott-Knott test ($p<0.05$). Means with an asterisk (*) differ from the control by Dunnett's test ($p<0.05$).

FIGURE 1. Area Under the Severity Progress Curve (AACPS) for anthracnose in Prata Anã' banana, over 15 days, submitted to thermotherapy for 4 minutes of immersion (A), 8 minutes of immersion (B), 12 minutes of immersion (C) and 16 minutes of immersion (D).

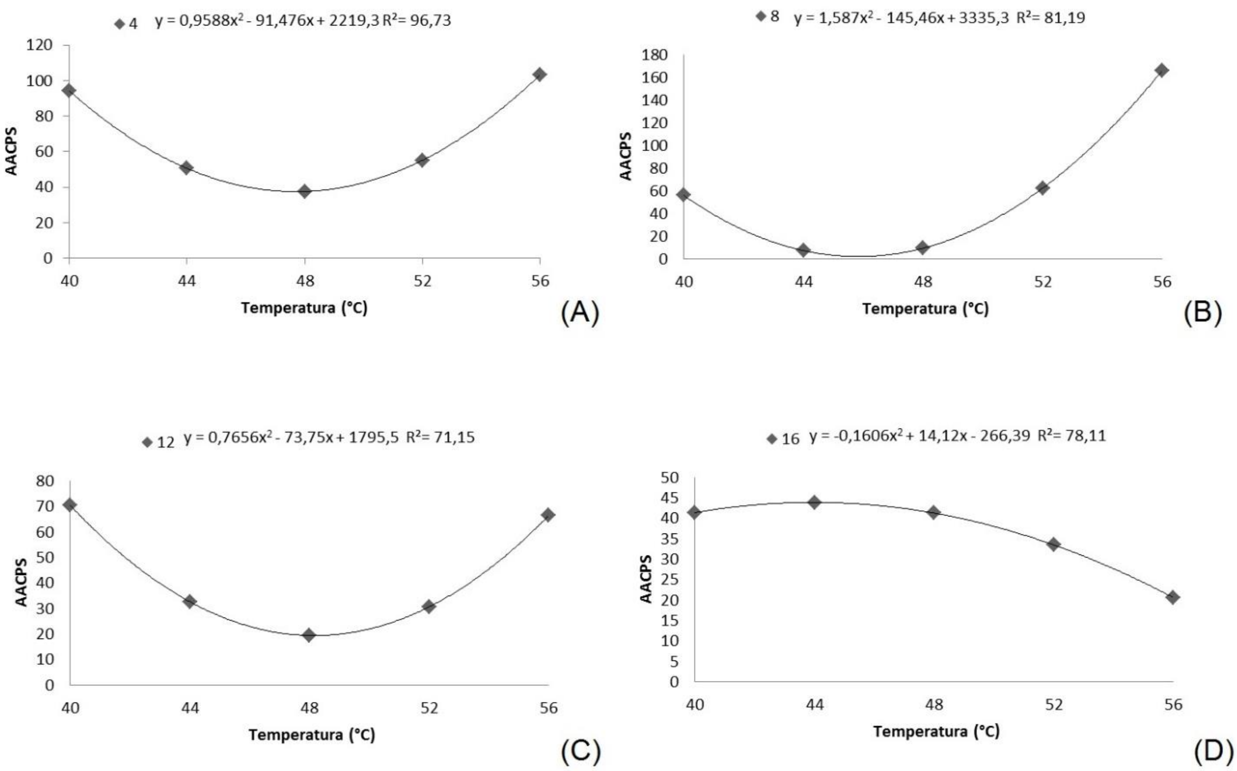
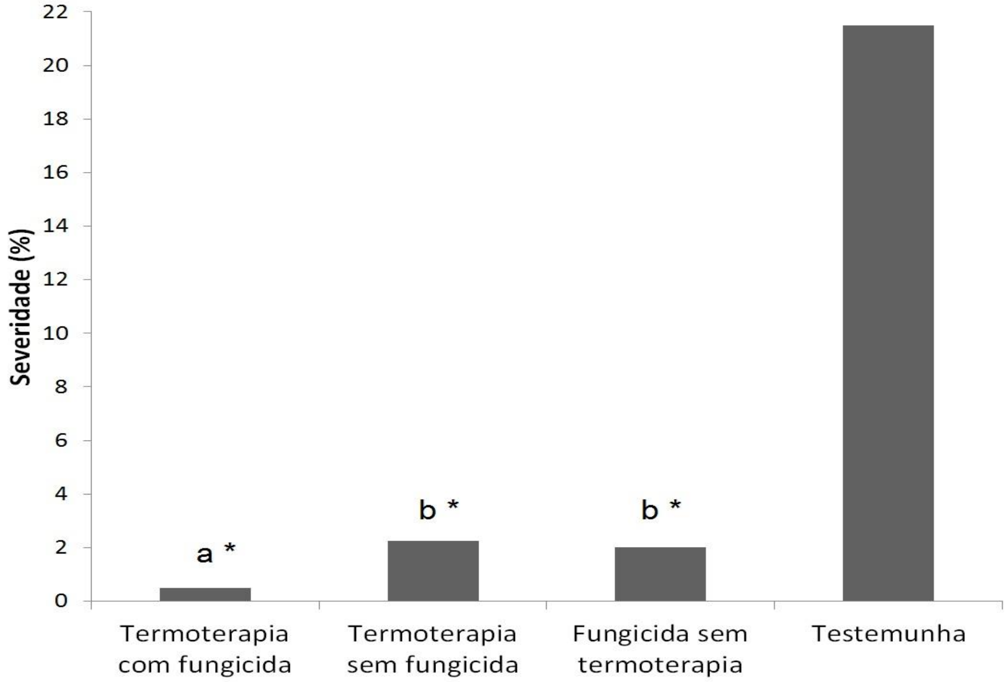


FIGURE 2. Severity of anthracnose in 'Prata Anã' banana submitted to hydrothermal (52°C/8'), chemical (Imazalil 2 mL.L-1) and combination of both, after fifteen days of storage, expressed as percentage of lesion area/fruit. Means followed by the same letter did not differ from each other according to the Scott-Knott test (p<0.05). Means with an asterisk (*) differ from the control by Dunnett's test (p<0.05).



CONCLUSIONS

Thermotherapy at 52 °C after 8 minutes of immersion reduces the AACPS of anthracnose in dwarf bananas in up to 81.6% of the injured area and delays their maturation.

The association of hydrothermal treatment with the fungicide Imazalil results in better control of anthracnose in 'Prata Anã' banana.

Thermotherapy is an efficient technique to reduce the severity of the disease in 'Prata Anã' bananas produced in the north of Minas Gerais.

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