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Incidence of fusariosis in Montes Claros and effects of temperature and photoperiod on the development of *Fusarium guttiforme*

Camilo Hermes Braga

Departamento de Fitotecnia, Federal University of Ceará, Brazil.
E-mail: camilo.braga@ufc.br

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We examined the incidence of fusariosis in Montes Claros–MG and determined the effect of temperature and photoperiod on the development of *Fusarium guttiforme*. Four family production units were investigated, from which four isolates of *F. guttiforme* were isolated. For each isolate, two experiments were set up to assess mycelial growth for 144 h in environments with different temperatures and either a 12 h photoperiod or continuous darkness. To evaluate the development of isolates of *F. guttiforme*, we used a portion sub-divided randomized block design. The number of conidia produced by each isolate was counted according to the above-mentioned conditions. All production units had occurrence rates ranging from 12.2 to 64.4%. The fungal isolates showed a higher mycelial growth rate when incubated at 25°C under a 12 h photoperiod. However, the production of conidia varied depending on the temperature and on the isolate. With these overall results better understand how the spread of this fungus occurred so quickly. We may use this information to suggest management practices that reduce the incidence of pineapple fusariosis.

Key words: Light, mycelial growth rate, *Fusarium guttiforme*, temperature.

INTRODUCTION

Brazil has an average pineapple productivity of 40 t ha⁻¹, below the average of 50 t ha⁻¹ achieved by other producer countries. However, the country is the largest producer of pineapples in the world, with a production of 2.49 million t yr⁻¹. Paraíba is the main pineapple-producing state, accounting for 17.9% of the national production, followed by Minas Gerais, which accounts for 17.5% of the national production (Cristani et al., 2010; Aquije

et al., 2011).

According to Ploetz (2005), fusariosis was first described in Argentina and Brazil, and more recently in Uruguay, Paraguay, and Bolivia, and subsequently has caused large losses in pineapple production in these countries. One of the factors hampering pineapple productivity in the municipality of Montes Claros, Northern Minas Gerais, is plant health. Farmers in the region report

that their main activity was the production of pineapple. However, in 1980, several farmers abandoned pineapple propagation because of the damage caused by fusariosis. The continuous purchase of pineapple seedlings from neighbouring farmers, including those that have ceased growing this particular crop due to the occurrence of this disease, may have caused the rapid spread of the disease in the region. Currently, a few farmers continue to maintain their pineapple plantations, although close to 100% of the crops are infected by the fungus.

Fusariosis is caused by the fungus *Fusarium guttiforme* Nirenberg and O'Donnell (Syn.: *F. subglutinans*) (Wollen and Reinking) Nelson, Tousson and Marasas f. sp. *ananas* Ventura, Zambolim and Gilbertson) (Aquiye et al., 2011). This fungus does not produce chlamydo-spores, but can remain in pineapple plants in the epiphytic form (Ventura and Zambolim, 2002). According to Alves and Nunes (2008), the survival of *F. guttiforme* in the soil may vary according to the percentage of organic matter in the substrate. However, this fungus does not remain for more than 4 months in the soil without a host.

On the basis of the accounts of farmers and the incidence of the disease in pineapples, there is a need to understand how abiotic and biotic factors contribute to the survival and spread of this fungus across the region to establish disease management practices that are appropriate for use by farmers. Doohan et al. (2003) reported that temperature, water availability, aeration, and light are the environmental factors that strongly influence the production of *Fusarium* sp. inoculum. The species *F. graminearum* and *F. culmorum* have been recognized for their optimal growth at a specific temperature and in a potato dextrose agar (PDA) culture medium (Brennan et al., 2003).

In addition to temperature, light can also affect fungal development. According to Leach (1967), light stimulates asexual and sexual reproduction in most fungi and this effect is correlated to nutrition and temperature. The quality and intensity of light can also affect conidial germination, vegetative growth rate, formation of reproductive structures, spore pigmentation, and shape and size of most fungal species (Minussi et al., 1977). According to Coutinho (2010), isolates of *F. guttiforme* from three Brazilian states showed variations when exposed to ambient light, substrate, and different temperatures. The combination of alternating or continuous lighting and PDA culture medium promotes a greater discriminatory power among fungal isolates, indicating that even colonies of the same sub-species may exhibit variation in growth and reproduction when exposed to the same environment.

The *in vitro* characterisation of the growth and reproduction of fungi provides important information on the cultivation and production of viable inoculum. In addition, it leads to a better understanding of the development of microbial colonies in each region, which allows the selection of inoculants with increased virulence

that can be used in plant breeding programs. We examined the incidence of fusariosis in four family production units in the municipality of Montes Claros–Minas Gerais and determined the influence of temperature and photoperiod on the development of *F. guttiforme* thriving in these areas.

MATERIALS AND METHODS

Four family production units in the rural community of Chapadinha, in Montes Claros–MG (19°54'38"S, 43°56'48"W) were selected for this study. This community is located 27 km from the urban area, where pineapple has been farmed for more than 20 years and has been naturally infested by fusariosis.

All areas allocated to the agroecological production of pineapple, which were identified using a survey conducted by the residential community association, were evaluated. During the survey period, the farms showed plants in their propagative and reproductive phases of fusariosis, based on disease symptoms that were observable throughout the plant cycle (Ventura and Zambolim, 2002). The average temperature of the study area ranged from 25 to 30°C and the relative humidity remained above 60%. The croplands were conventionally called Family Production Unit 1 (UPF 1), UPF 2, UPF 3, and UPF 4, and all plantations were dry land.

In all the UPFs, weed management was accomplished through manual weeding. The farmers themselves planted UPFs 1 and 3 with seedlings, whereas UPFs 2 and 4 were inoculated with seedlings acquired from other farmers. Liming and fertilisation with phosphate and ammonium sulphate (without soil chemical analysis) were conducted in UPF 2 only. Except for UPF 4, all UPFs underwent crop rotation using pineapple and cassava.

The incidence of fusariosis in each family production unit was determined by counting the number of diseased plants according to the methodology of Embrapa (2005). We evaluated 500 plants ha⁻¹. Sampling was carried out in a zigzag pathway, sampling 10 points. Plants were considered as diseased when they presented at least one of the following symptoms: gum exudation, curvature of the apex, shortening of the stem, abnormal shape of a cup, change in the leaf rosette configuration, death of the apex of the stem, and stunting. The incidence data were expressed as a percentage of plants with symptoms.

In vitro development of *F. guttiforme*

After surveying the incidence of pineapple fusariosis in each property, plants with disease symptoms were collected and transported to the laboratory. The plants were washed in distilled water and liquid detergent and the leaves and fruits were isolated. At the edges of the damaged tissues, approximately 0.5 cm² fragments were removed, and then immersed in 70% ethanol for 30 s, followed by immersion in 2% sodium hypochlorite for 1 min. Subsequently, the fragments were washed three

times in sterile distilled water. After eliminating the excess moisture, the fragments were placed in 9 cm Petri dishes containing PDA culture medium and incubated at $25 \pm 2^\circ\text{C}$ under a 12 h photoperiod. All procedures were performed in a laminar flow hood under aseptic conditions. Upon growth of *Fusarium* colonies, these were transferred to Petri dishes containing PDA medium and cultured under the conditions previously described. *F. guttiforme* was then isolated and identified (Nelson et al., 1994) using the following designation: isolates from leaves (PLA 1 and PLA 2) and isolates from fruits (FRU 1 and FRU 2). We use two isolates of each plant for greater genetic variation.

After isolation and identification of the isolates, a monospore culture was prepared. Briefly, a sample of sporulated culture was placed in a test tube containing 10 ml of sterile distilled water, stirred, and subjected to serial dilution (four times). An aliquot containing 1 ml of the diluted suspension was placed on the surface of Petri dishes containing agar (20%) culture medium and incubated at a constant temperature of 25°C under light, in incubators with four fluorescent lamps (2500 Lux). After a 9 h incubation and growth of the conidial germination tubes, one conidium was isolated under an optical microscope objective. This fragment containing the conidia was transferred to a test tube containing the PDA culture medium and incubated at a constant temperature of 25°C under a 12 h photoperiod. After 15 days, the cultures were inoculated into 9 cm Petri dishes containing the PDA medium. Single-spore cultures of each isolate were assessed for pathogenicity according to the methodology described by Oliveira et al. (2011).

To determine the mycelial growth rate and conidial production of the four isolates, two treatments were conducted, in which each isolate was subjected to either a photoperiod of 12 h or continuous darkness. For each treatment, we measured the effect of temperature and time (incubation period) on mycelial growth rate. In each treatment, the experimental design was completely randomised in a 3 6 4 the sub-divided plot scheme, using three temperatures (20, 25, and 30°C), six incubation periods (24, 48, 72, 96, 120 and 144 h) and four isolates of *F. guttiforme* (PLA 1, PLA 2, FRU 1 and FRU 2) with three replicates. Each plot consisted of a Petri dish containing the PDA culture medium and a 0.7 cm diameter colony disc of the isolate. During the 6-day incubation period, the diameters of colonies in two orthogonal axes were measured every 24 h. The mycelial growth rate was calculated as the difference between the measured diameter and the previous diameter.

To assess the amount of conidia produced by each isolate, two 0.7 cm diameter discs with mycelium were withdrawn from the edges of the colony from each replicate at the end of each experiment. The discs were then transferred to test tubes containing 20 ml of sterile distilled water and stirred to promote the shedding of conidia. The number of macroconidia and microconidia in suspension were counted using a Neubauer chamber type and an optical microscope. Data were subjected to

variance analysis and regression analysis. When necessary, we compared the means using the Tukey test ($P < 0.05$).

RESULTS AND DISCUSSION

The incidence of fusariosis in the family production units (12.2 to 64.4%) was higher than the recommended 1% control level of the disease. Some of the factors that may have contributed to the high number of diseased plants in the units were the relative humidity, which remained above 60% during the evaluation period; the average temperature, which ranged from 20 to 30°C ; and the phenological stage of the crop (breeding/propagation). Such factors contributed to the high levels of fusariosis in some pineapple crops in the municipality of Santa Rita-PB (52.2 to 60.0%) (Oliveira et al., 2011). According to these authors, the flowering of plants at specific temperature and relative humidity conditions were favourable to the production of seedlings and the occurrence of fungal infections, thus increasing the number of infected plants.

UPF 1 showed a lower incidence of diseased plants (12.2%) than that observed in the other units. The pineapple field in this production unit was only 2 years old, and the planting area was located between sparse native Cerrado vegetation, which allows a spatial isolation in relation to other pineapple plantations in the region. The seedlings used in this pineapple field were selected from other plantations of the same farmer. Such seedlings may have served as an inoculation mechanism for this pathogen in the new planting area because *F. guttiforme* can infect the whole plant and some seedlings lightly infected by the fungus may not have been identified by the farmers and acted as a source of the initial inoculum in the new cultivation areas (Verzignassi et al. 2009; Oliveira et al., 2011).

UPFs 2 and 3 showed a two-fold incidence of fusariosis at 24.22 and 26.2%, respectively, relative to that observed in UPF 1. However, these plantations are near other older pineapple fields (soca type), which were abandoned by farmers due to the high incidence of *Fusarium*. Fertilisation was not performed in these UPFs, although crop rotation using pineapple and cassava was conducted, which may contribute to the lower fruit damage caused by the disease.

In contrast, UPF 4 presented an extremely high incidence of fusariosis (64.4%). Its fruit production was extensively impaired, with a low inflorescence. This property also underwent liming and fertilisation with duck waste phosphate and ammonium sulphate. However, the correction and fertilisation of the soil were not based on its chemical and physical analysis. It may be possible that the soil pH correction with lime contributed to the high incidence of fusariosis in this unit. This has been described by Rodrigues et al. (2002), in which the use of limestone favoured the development of *Rhizoctonia solani* in beans. However, the level of nitrogen may have

Table 1. Mycelial growth (cm) of four isolates of *F. guttiforme* temperature and six of incubation time and 12 h photoperiod.

Time (h)	Temperature (°C)	Isolates				C.V. (%)
		FRU 1	FRU 2	PLA 1	PLA 2	
24	20	0.36 ^{AB}	0.44 ^{Aa}	0.11 ^{AB}	0.13 ^{Aa}	18.24
	25	0.68 ^{Aab}	0.74 ^{Aa}	0.38 ^{AB}	0.77 ^{Aa}	
	30	1.05 ^{Aa}	0.82 ^{Aa}	1.13 ^{Aa}	0.55 ^{Aa}	
48	20	1.22 ^{Ab}	1.54 ^{Ab}	1.07 ^{Ab}	1.38 ^{Aab}	
	25	2.17 ^{Aa}	2.32 ^{Aa}	2.30 ^{Aa}	1.89 ^{Aa}	
	30	1.58 ^{Ab}	1.46 ^{Ab}	1.40 ^{Ab}	1.20 ^{Ab}	
72	20	1.73 ^{Aab}	1.53 ^{Ab}	1.50 ^{Aab}	1.64 ^{Aab}	
	25	2.11 ^{Aa}	2.18 ^{Aa}	1.89 ^{Aa}	1.73 ^{Aa}	
	30	1.53 ^{Ab}	1.67 ^{Aab}	1.33 ^{Ab}	1.17 ^{Ab}	
96	20	1.60 ^{Aab}	1.46 ^{Aa}	1.38 ^{Aa}	1.44 ^{Aab}	
	25	1.95 ^{Aa}	1.87 ^{Aa}	1.61 ^{Aa}	1.73 ^{Aa}	
	30	1.39 ^{Ab}	1.44 ^{Aa}	1.10 ^{Aa}	1.04 ^{Ab}	
120	20	1.26 ^{Aa}	1.39 ^{Aa}	1.15 ^{Aa}	1.09 ^{Aab}	
	25	1.40 ^{Aa}	1.19 ^{Aa}	1.49 ^{Aa}	1.53 ^{Aa}	
	30	1.28 ^{Aa}	1.30 ^{Aa}	1.07 ^{Aa}	0.98 ^{Ab}	
144	20	1.41 ^{Aa}	1.41 ^{Aa}	1.29 ^{Aa}	1.29 ^{Aa}	
	25	0.00 ^{Bb}	0.00 ^{Bb}	0.62 ^{Ab}	0.65 ^{Ab}	
	30	0.92 ^{Aa}	1.14 ^{Aa}	0.86 ^{Aab}	0.77 ^{Aab}	

Means followed by the same uppercase letter, in the column, and same lower case, in the line, are not statistically different (Tukey test) ($p < 0.05$).

been excessive for the pineapple plants, thus increasing their susceptibility to *F. guttiforme*. According to Santos et al. (2010), the application of nitrogen in the form of ammonia to soils at low pH, such as that of the Cerrado, may favour increased disease severity.

Another factor that contributes to the longevity and spread of inoculum is the age of the plantations. The pineapple is essentially a perennial crop and can have multiple cycles for many years in the same location, giving rise to what is commonly called 'soca' (Lima et al., 2002). This type of farming was observed in the family production units surveyed. According to Ventura and Zambolim (2002), the fungus can remain in the saprophytic form on the pineapple leaves, but also in other plants present in cultivated areas. Thus, when the 'soca' areas are abandoned, they serve as a source of inoculum for neighbouring crops and also permit the maintenance of *F. guttiforme* in the location, even after the death of the pineapple plant.

In vitro* development of *F. guttiforme

When incubated under a 12 h photoperiod, the mycelia growth rate of all isolates was influenced by the interaction of temperature with the incubation period and

isolates. With a photoperiod of 12 h, all *F. guttiforme* isolates exhibited the highest growth rates when incubated at 25°C (Tables 1 and 2). The isolated PLA 1 and PLA 2 grew better at 25°C after 144 h of incubation (Table 1). This is because the mycelium of FRU 1 and FRU 2 isolates had occupied the entire petri dish, and therefore, the growth rate was equal to zero in this time (Table 1). This indicates that the isolated FRU 1 and FRU 2 can be more vigorous than the single PLA 1 and PLA 2. For the PLA 1 isolate, a temperature of 30°C during the first 24 h resulted in the highest growth rate, when incubated under a 12-h photoperiod (Table 1). For the PLA 1 and PLA 2 isolates, which were derived from leaves, we found that, with 81.73 to 80.82 h of incubation at 25°C under a photoperiod of 12 h, there was a higher rate of mycelial growth (2.05 and 1.91 cm, respectively) (Table 2). The FRU 1 and FRU 2 isolates, which were derived from fruits, showed the highest growth rate (2.26 cm) after 75 to 77 h of incubation at 25°C under a 12 h photoperiod (Table 2).

The four *F. guttiforme* isolates produced the same number of conidia when incubated under a 12 h photoperiod. The fungus, regardless of the isolate used, showed a positive linear conidia production ($\hat{y} = -2.932539 + 0.219633x$, $R^2 = 0.83$) between 20 and 30°C

Table 2. Equations of four isolates of *F. guttiforme* incubated at different temperatures for 144 h of in a 12 h photoperiod.

Isolates	Temperatures (°C)	Equations	R ²
FRU 1	20	$\hat{y} = -0.415894 + 0.0423970*x - 0.000215364*x^2$	0.82
	25	$\hat{y} = -0.753487 + 0.0781892**x - 0.000507220**x^2$	0.95
	30	$\hat{y} = 0.672379 + 0.0225090*x - 0.000145829*x^2$	0.88
FRU 2	20	$\hat{y} = -0.0772617 + 0.0345622*x - 0.000175280*x^2$	0.73
	25	$\hat{y} = -0.546431 + 0.0746039**x - 0.000496448**x^2$	0.93
	30	$\hat{y} = 0.324954 + 0.0289570*x - 0.000166189*x^2$	0.81
PLA 1	20	$\hat{y} = -0.644221 + 0.0423274*x - 0.000209196*x^2$	0.84
	25	$\hat{y} = -0.590900 + 0.0645372*x - 0.000394826*x^2$	0.71
	30	$\hat{y} = 1.03019 + 0.00868136*x - 0.0000699241*x^2$	0.82
PLA 2	20	$\hat{y} = -0.587712 + 0.0455603*x - 0.000237633*x^2$	0.71
	25	$\hat{y} = -0.150864 + 0.0512199*x - 0.000316881**x^2$	0.88
	30	$\hat{y} = 0.195624 + 0.0230223*x - 0.000135064*x^2$	0.74

* Significant t test ($p < 0.05$). ** Significant t test ($p < 0.01$).

Table 3. Mycelial growth (cm) of *F. guttiforme* in three temperatures and six of incubation time and continuous darkness.

Temperature(°C)	Incubation time (h)					
	24	48	72	96	120	144
20	0.09 ^b	1.00 ^d	1.21 ^{ab}	1.20 ^{ab}	1.23 ^a	1.12 ^d
25	0.42 ^a	1.36 ^a	1.39 ^a	1.39 ^a	1.36 ^a	1.50 ^a
30	0.49 ^a	1.04 ^b	1.01 ^b	1.01 ^b	0.92 ^b	0.91 ^c
C.V.(%)	20.42					

Means followed by the same letter in the column are not statistically different (Tukey test) ($p < 0.05$).

Table 4. Mycelial growth (cm) of four isolates of *F. guttiforme* and six of incubation times and continuous darkness.

Isolates	Incubation times (h)					
	24	48	72	96	120	144
FRU 1	0.43 ^a	1.11 ^a	1.19 ^a	1.19 ^a	1.10 ^a	1.49 ^a
FRU 2	0.33 ^a	1.21 ^a	1.28 ^a	1.27 ^a	1.27 ^a	1.22 ^{ab}
PLA 1	0.27 ^a	1.12 ^a	1.18 ^a	1.17 ^a	1.17 ^a	1.11 ^d
PLA 2	0.29 ^a	1.09 ^a	1.17 ^a	1.16 ^a	1.15 ^a	0.88 ^c
C.V.(%)	20.42					

Means followed by the same letter in the column are not statistically different (Tukey test) ($p < 0.05$).

30°C, where conidia production was expressed N° conidia x 10⁵ ml⁻¹.

We note that in continuous darkness was not influenced by the triple interaction between the factors. However, there was a significant interaction between incubation time and temperatures and there was also the

effect of interaction between isolate and incubation time (Tables 3 and 4, Figures 1 and 2).

The isolates presented the highest average rate of mycelial growth when cultivated in continuous darkness at a temperature of 25°C, in all of the incubation time (Table 3 and Figure 1). We also observed a pattern of

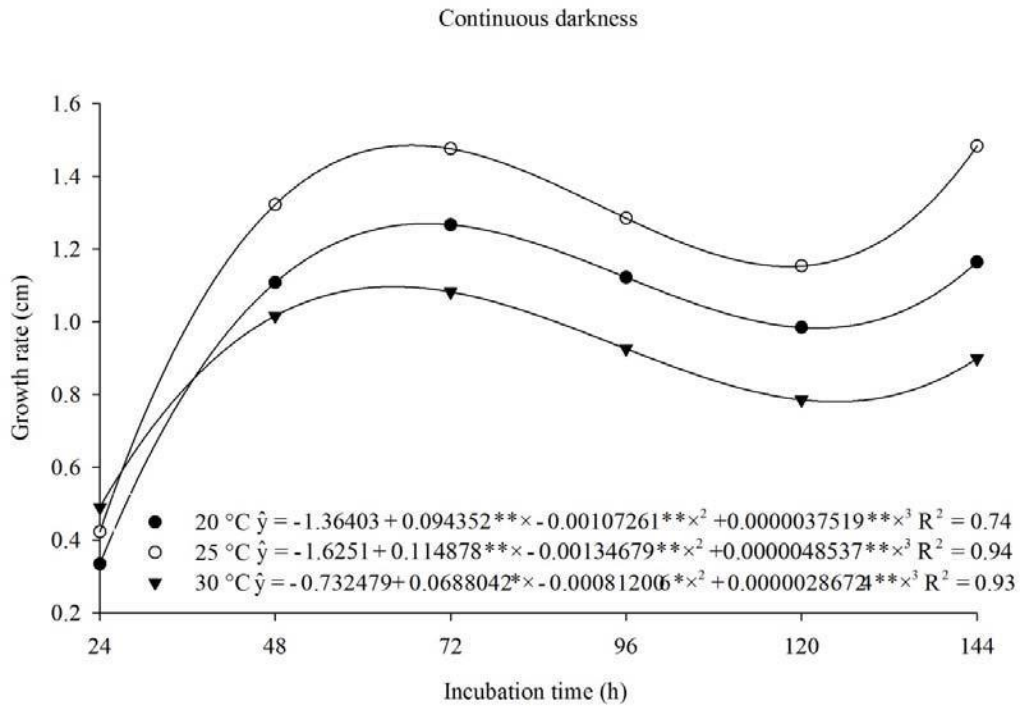


Figure 1. Growth rate of the *F. guttiforme* as a function of incubation time and different temperatures. * Significant t test ($p < 0.05$). ** Significant t test ($p < 0.01$).

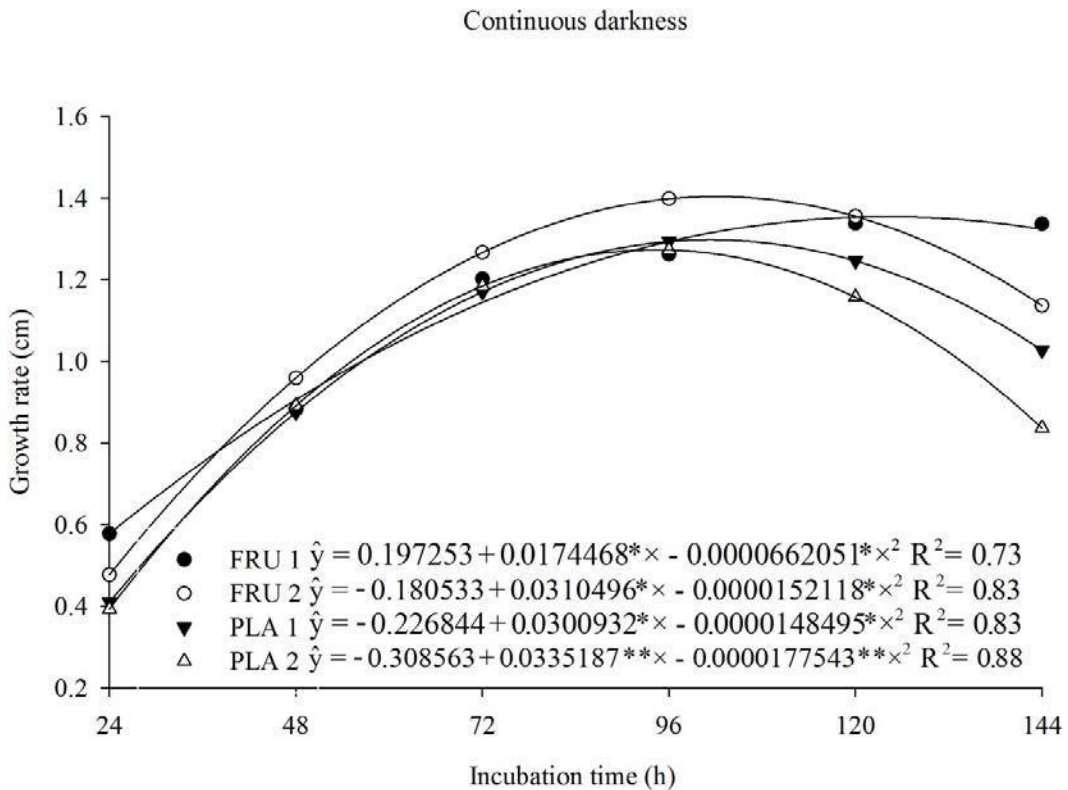


Figure 2. Mycelial growth as a function of incubation time and of the four *F. guttiforme* isolates.* Significant t test ($p < 0.05$). ** Significant t test ($p < 0.01$).

Table 5. Number of conidia produced by four *F. guttiforme* isolates subjected to different incubation temperatures in continuous darkness.

Isolates	N° of conidia ml ⁻¹
PLA 1	2.42 10 ⁵ b
PLA 2	2.95 10 ⁵ b
FRU 1	4.17 10 ⁵ a
FRU 2	2.35 10 ⁵ b
C.V. (%)	48.03

Means followed by the same letter in the column are not statistically different (Tukey test) ($p < 0.05$).

growth of the fungus incubated in continuous darkness, common will all temperatures studied, where the first 24 h there was a significant increase in the growth rate, and then a range (24-120 h) where the growth rate remained constant (Figure 1). The isolates achieved the highest growth rate after 102 h of incubation in continuous darkness, except for the isolated FRU 1, which had the highest growth rate after 131 h of incubation also in continuous darkness (Figure 2). Demonstrating that isolates can propagate quickly, even in the absence of light, for example, when infecting pineapple fruits.

When the four isolates were incubated in continuous darkness, there was no interaction effect between treatments (fungal isolates and incubation temperature) and the number of conidia produced. The FRU 1 isolate produced the greatest number of conidia, more than twice that of the PLA 1, PLA 2, and FRU 2 isolates, regardless of the incubation temperature (Table 5).

In general, the isolates showed a higher rate of mycelial growth when incubated under a 12 h photoperiod. The same growth pattern was observed for *Stenocarpella macrospora* and *Stenocarpella maydis* (Casa et al., 2007). However, the production of conidia varied among isolates. The FRU 1 isolate showed a higher rate of sporulation when maintained in continuous darkness. As for the other isolates, alternation or absence of light did not affect sporulation. Devi and Singh (1994) previously demonstrated that the growth rate of *F. moniliforme* was higher when incubated with continuous light and sporulated best in continuous darkness.

Doohan et al. (2003) reported that temperature and light influence the reproduction of *Fusarium* sp. We have verified their results in our experiments, in which each isolate showed a distinct growth pattern when incubated in continuous darkness or under a 12 h photoperiod. The alternation of light provided the highest growth rate of the isolates. However, when these isolates were grown in continuous darkness, the production of conidia of the FRU 1 isolate differed from that of the others. According to Leach (1967), light stimulates the sexual and asexual reproduction of fungi and this stimulus is correlated to temperature. This was also observed in the present study, because when the *F. guttiforme* isolates were exposed to a photoperiod of 12 h, the conidia production increased at higher temperatures. However, when they were subjected to continuous darkness, conidial

production of three of the four isolates was not affected by the increase in temperature.

In this study, we used PDA culture medium and the optimum growth temperature of *F. guttiforme* isolates was 25°C. This corroborates the data presented by Bueno et al. (2007) and Basseto et al. (2011), who reported that the optimal temperature range for the growth of the three *F. oxysporum* strains was from 25 to 26°C.

In summary, the incidence of fusariosis in family production units studied in the rural community of Chapadinha, Montes Claros, Minas Gerais was high, ranging from 12.2 to 64.4%. The *F. guttiforme* isolates showed a higher rate of mycelial growth when incubated under a 12 h photoperiod and in continuous darkness and at 25°C. A 12 h photoperiod and a temperature of 30°C leads to an increase in conidia production in *F. guttiforme*.

The four isolates showed a lot adapted to temperature conditions of the region, due to their high growth rate and higher production of conidia in temperature above 25°C, conditions common to the Brazilian Cerrado region where the study was conducted. This makes it easy to understand why the spread and establishment of the fungus by pineapple plantation the region occurred so quickly.

Conflict of Interest

The authors have not declared any conflict of interest.

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