EFFECT OF TEMPERATURE AND LEAF WETNESS ON *Phoma tarda* AND PHOMA LEAF SPOT IN COFFEE SEEDLINGS

Emi Rainildes Lorenzetti[1], Edson Ampélio Pozza[2], Paulo Estevão de Souza[3],
Leandro Alvarenga Santos[4], Eduardo Alves[5], André Costa da Silva[6], Fernanda Gonçalves Martins Maia[7],
Rejane Rodrigues da Costa Carvalho[8]

ABSTRACT: Phoma leaf spot is a major disease of coffee plants in regions with specific conditions of temperature and humidity. Despite the importance of environmental variables for disease progression, so far they have been poorly understood. Thus, we assessed the effect of different temperatures (15, 20, 25 and 30 oC) on mycelial growth, production and germination of conidia of *Phoma tarda*; and the same effect of temperature (15, 20, 25 and 30 oC) and leaf wetness duration (6, 12, 24, 36 and 48 hours) on fungal infection and severity of disease over time in coffee plants. Disease assessment was integrated in the area using the disease progress curve (AUDPC) and the infection processes were observed by scanning electron microscopy (SEM). The optimal temperatures for fungus growth, conidial production and germination in vitro were 22.9, 29.8, and 25.1 °C, respectively. Temperatures ranging from 15-20 °C significantly increased germ tube length and provided favorable conditions for pathogen infection through observation of early events such as cuticle separation from leaf tissue. The same temperature range combined with increasing leaf wetness periods, reached the highest value of the AUDPC of phoma leaf spot. Thus, it was found that both temperature and leaf wetness duration in the variables assessed and the difference in relation to temperature for in vitro growth and sporulation for in vivo tests were important.

Index terms: Epidemiology, *Coffea arabica*, scanning electron microscopy, growth, sporulation.

EFEITO DE TEMPERATURA E MOLHAMENTO FOLIAR EM *Phoma tarda* E MANCHA DE PHOMA EM MUDAS DE CAFEEIRO

RESUMO: Mancha de Phoma é uma das principais doenças do cafeeiro em zonas com condições específicas de temperatura e umidade. Apesar da importância das variáveis ambientais para o progresso da doença, até agora essas têm sido mal compreendidas. Avaliou-neste estudo, o efeito de diferentes temperaturas (15, 20, 25 e 30 ºC) sobre o crescimento micelial, produção e germinação de conídios de Phoma tarda, e o efeito da temperatura (15, 20, 25 e 30 ºC) e da duração do período de molhamento (6, 12, 24, 36 e 48 horas) sobre a infecção fúngica e severidade da doença, ao longo do tempo em plantas de café. A avaliação da doença realizada através da área abaixo da curva de progresso da doença (AACPD) e os processos de infecção foram observados por microscopia eletrônica de varredura (MEV). As temperaturas ótimas para o crescimento de fungos, produção de conídios e germinação foram de 22,9, 29,8, 25,1 °C, respectivamente. Temperaturas variando entre 15-20 °C aumentaram, significativamente, o comprimento do tubo germinativo e proporcionaram condições favoráveis para a infecção pelo patógeno através da observação dos primeiros eventos, tais como a separação da cutícula do tecido foliar. A mesma faixa de temperatura combinada com períodos crescentes de molhamento foliar, no máximo, 48 horas, atingiu os maiores valores da AACPD da Mancha Phoma. Assim, verificou-se que, tanto a temperatura quanto a duração do molhamento para testes in vivo e a influência da temperatura para o crescimento e esporulação in vitro foram importantes.

Termos para indexação: Epidemiologia, Coffea arabica, microscopia eletrônica de varredura, doenças do cafeeiro.

1 INTRODUCTION

Coffee stands out as a socially and economically profitable crop in Brazil. The state of Minas Gerais alone accounts for approximately 50% of the national coffee production, mostly concentrated in the south region (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2010). Among many crop limiting factors, diseases such as coffee rust (*Hemileia vastatrix* Berk. & Broome), brown eye spot (*Cercospora coffeicola* Berk. & Cooke) (POZZA; CARVALHO; CHAUFOUN, 2010), and phoma leaf spot (*Phoma tarda* (Stewart) Boerema & Bollen) (*P. tarda*) (SALGADO et al., 2009) stand out for causing significant yield and quality losses. Although such diseases are limited to certain regions with specific climatic conditions, the incidence of phoma leaf spot has significantly increased in coffee crops in recent years in areas where it has never occurred before.

[1]Instituto Federal de Educação, Ciência e Tecnologia do Sudeste de Minas Gerais - Câmpus Rio Pomba - 36180-000 - Rio Pomba - MG - emi.lorenzetti@ifsudestemg.edu.br
[2,3,4,5]Universidade Federal de Lavras /UFLA - Departamento de Fitopatologia/DFP - Cx. P. 3037 - 37.200-000 - Lavras -MG eapozza@dfp.ufla.br, pauleste@dfp.ufla.br, leandro.alvarenga.s@hotmail.com, ealves@dfp.ufla.br
[6]Universidade Federal de Viçosa - Departamento de Fitopatologia/DFP - 36.570-000 - Viçosa-MG - andrec_agro@yahoo.com.br
[7]Universidade Federal de Uberlândia - Cx. P. 593- 38400-902 - Uberlândia - MG - feagrosal@yahoo.com.br
[8]Universidade Federal Rural de Pernambuco - Departamento de Agronomia - 52171- 900 - Recife - rejanercosta@yahoo.com.br

The disease can cause tip and branch dieback with necrosis of rosettes and flowers, which significantly reduce crop yield. As growing area has been expanded to regions with mean annual temperature above 23 °C, such as northern of Minas Gerais state and northeastern Brazilian states, researchers had thought that phoma spot was unlikely to occur (POZZA; CARVALHO; CHAUFOUN, 2010); however, many reports of disease occurrence have been cited.

Although most often endemic, phoma spot can sometimes occur in explosive epidemics due to cold climate, heavy rain, strong winds and hail, which cause leaf damage and allow to the entrance of the pathogen. These conditions, associated with leaf wetness caused by localized rainfall, dew, and fog eventually favor disease (POZZA; CARVALHO; CHAUFOUN, 2010). In addition, unusual climatic instability in recent years has been hampering the disease management (POZZA; ALVES, 2008).

Temperature of 23 °C has been cited as optimal for mycelial growth of *Phoma tarda* and increasing infection of wounded leaves (FIRMAN, 1965). In the field, intermittent periods of cold weather combined with temperatures from 18 to 19 °C, cold wind and altitude above 900 m are highly favorable microclimatic conditions, especially in barren areas temperatures from 16 to 20 °C and rainfall above 4 mm/day as highly favorable for this fungal infection (POZZA; ALVES, 2008).

Despite previous research, the specific environmental conditions that favor infection by isolates of *P. tarda* and progress of phoma leaf spot, especially temperature and duration of leaf wetness, are as yet very poorly explored. However, such conditions have been extensively cited as having a direct influence on the increase of disease levels in susceptible crops (DUTHIE, 1997). Thus, elucidating the pathogen behavior in these conditions can be useful to explain why the disease is occurring in regions apparently unfavorable to the pathogen and in periods historically showing low intensity. In addition, the study can contribute to disease management.

Therefore, our objective was to evaluate the effect of temperature on mycelial growth, production and germination of conidia of *P. tarda* in vitro, and the effect of temperature and leaf wetness duration on infection and severity of phoma leaf spot in coffee seedlings.

2 MATERIALS AND METHODS

Obtaining the isolate

We used the CMI-720 isolate of *P. tarda* from coffee leaves (*Coffea arabica* L.) of plants from Santo Antonio do Amparo, Minas Gerais state, Brazil, provided by the Coleção Micológica de Lavras - CML, Department of Plant Pathology, UFLA. The fungus was grown in liquid malt extract and incubated under a photoperiod of 12 hours at 20 °C in germination chamber BOD type.

Fungus growth, conidia production and germination

To assess mycelial growth, we used 9 cm diameter Petri dishes and 15 mL of liquid malt extract. A 9 mm diameter plug of fungus mycelium grown for 10 days was placed in the center of each plate. The dishes were conditioned at different increasing temperatures (15, 20, 25, and 30 ºC) with a photoperiod of 12 hours in germination chambers. The trial was a completely randomized design with three replications, each consisting of five Petri dishes. Colony diameter was measured every 48 hours in orthogonal position, until one of the treatments reached the full diameter of the Petri dish. We used the values to calculate mycelial growth (MG), based on the formula:

$$MG = \sum (D - Db)/N$$

(D = colony diameter, Db = mean colony diameter on the day before, N = number of days after inoculation).

Conidia production was evaluated at the twenty-fourth day of incubation after all treatments reached maximum growth. Then, each plate received 15 mL of water and Tween 20 (Sorbitan monolaurate ethoxylate) 1%. The colony surface was scraped with a Drigalsky spatula, and spores of each dish were filtered through cheeseclothe and suspended. For each spore suspension, three 0.1 mL aliquots were separately transferred to a hemacytometer and spores were counted under light microscope. The experiment was a completely randomized design with three replicates, two counts per replication.

To evaluate spore germination, we used 9 cm diameter Petri dishes with 10 mL of water agar. After solidification, we added 200 µL of spore suspension from a culture grown for 24 days at 25 °C in concentration of 2×10^5 spores/mL per dish. The plates were incubated in a growth

chamber under fluorescent light for 12 hours at 15, 20, 25, and 30 oC in a completely randomized design with three replicates, three counts per dish. Immediately after incubation period, conidia germination was paralyzed with lactophenol. The percentage of spore germination was assessed by counting a sample of 200 spores per quadrant, using light microscopy. Spores were considered germinated when the germ tube was equal to or greater than its diameter. The percentage was determined in three replicates over time.

For statistical analysis we used SAS software (STATISTICAL ANALYSIS SYSTEM INSTITUTE - SAS INSTITUTE, 2010). Each trial was replicated three times, and all data were subjected to pooled analysis over time. Then, analysis of variance was performed. The significant variables in F-test were subjected to adjustment of regression models, and the first order derivatives of the regression equations were calculated to determine the optimum temperature and the point of maximum growth, production and germination of conidia.

Effect of wetness and temperature on the severity of phoma leaf spot

The experiment was carried out in growth chambers under different incubation temperatures and periods of leaf wetness with coffee seedlings of Topazio MG1190 cultivar with approximately 8 pairs of leaves. The cultivar was chosen due to its higher susceptibility to phoma leaf spot (BARGUIL, 2004).

Three pairs of leaves in the plant upper third were marked. Then, seedlings were inoculated with a suspension of 2 x 105 spores / mL containing 1% Tween 20 (LIMA et al., 2010). After inoculation, the plants were enclosed in clear plastic bags and remained at saturated humidity for 6, 12, 24, 36, and 48 h, thus simulating periods of leaf wetness. Incubation temperatures in growth chambers were 15, 20, 25, and 30 °C (± 3 ºC) under a 12 hours photoperiod. After the plastic bags were removed, plant humidity was maintained around 80% by a humidifier. We used a completely randomized design with four replications, two plants per replicate, in a 4x5 factorial arrangement (four temperature levels and five periods of leaf wetness) totaling 40 seedlings. The trial was replicated twice.

We evaluated the severity of disease using the diagram proposed by Salgado et al. (2009) in 10 assessments every seven days, starting after the onset of symptoms. Data was converted to the area under the disease progress curve (AUDPC) over time.

Both trials were subjected to pooled analysis. Then, we proceeded with the analysis of variance of the 4x5 factorial. As soon as significant interactions in the analysis of variance F-test occurred, we fitted the regression models. Statistical analysis was performed on SAS (SAS INSTITUTE, 2010) and Sigma Plot (Sigma Plot ® - Version 10.0 - Systat Software Inc.).

Scanning electron microscopy (SEM) studies

Seedlings of coffee cultivar Topazio MG 1190 with eight pairs of leaves were infected with a spore solution of 2 x 105 spores/mL of distilled water containing a commercial spreader-sticker (alkylphenol polyglycol ether) 0.1 m/L in areas delimited by plastic tape. Next, waxed paper moistened with distilled water was placed at the inoculation site to fix the spores on the leaves. After inoculation process, the plants were enclosed in clear plastic bags, moistened and kept at saturation humidity for 6, 12, 24, 36, and 48 hours, thus simulating the periods of leaf wetness. Incubation temperatures in growth chambers were 15, 20, 25, and 30 °C (± 3 ºC) under a 12 hours photoperiod.

The trial was a completely randomized design with two plants per treatment. After each period of leaf wetness, samples (0.5 x 0.5 cm) were fixed in modified Karnovsky, for a 24-hour period. After fixation the material was immersed three times in cacodylate buffer, 10 minutes in each immersion. Then, it post-fixed in 1% osmium tetroxide aqueous solution for 1-2 hour and then dehydrated in a crescent series of acetone solution (25, 50, 70, and 90 for 10 minutes and three times for 10 minutes at 100%). Afterwards, the samples are transferred to a critical point dryer Balzers CPD 030 (Balzers, Liechtenstein, Germany) to complete the drying process with carbon dioxide as a transition fluid. The specimens obtained are mounted on aluminum stubs, with a double-stick carbon tape put on a film of aluminum foil, coated with gold using a sputter Balzers SCD 050 (Balzers, Liechtenstein, Germany), and kept in desiccator with silica gel until observing. SEM analyses were conducted using a Leo EVO 40 electron microscope (Leo Electron Microscopy, Cambridge, UK), and the images generated at various magnifications were digitally recorded.

The germ tubes of a spore sample were measured at different temperatures in the 36-hour wetness. The trial was a completely randomized design, with ten conidia measured. Measurement of spores was performed with the software SmartSEM (V.05.04.03.00, CARL ZEISS, 2010).

3 RESULTS AND DISCUSSION

Pooled analysis showed no significant difference between replications over time, thus data referred to the average of trials. All variables analyzed in vitro, i.e., micelial growth, production and germination of conidia were significant at F test.

Mycelial growth curve showed a pattern identical to the curve of pathogen grown in culture medium, with the point of maximum rate, minimum and maximum temperatures reaching threshold values. The lowest growth temperatures were 15 °C with minimum at 30 °C (Fig. 1a). The optimum temperature for mycelial growth (IVCM) for the isolate IMC 720 of *P. tarda* was 22.9 °C. After 12 days, the fungus colony filled the entire Petri dish.

Conidial production increased with increasing temperature (Fig. 1b), and at 30 ºC there was a slight drop in the number of conidia. The first order derivatives showed that optimum temperatures for conidial production and conidial germination were 29.8 and 25.1 °C, respectively (Fig. 1c).

Conidial production increased with increasing temperature (Fig. 1b), and at 30 ºC there was a slight drop in the number of conidia. The first order derivatives showed that optimum temperatures for conidial production and conidial germination were 29.8 and 25.1 °C, respectively (Fig. 1b; 1c). Germination showed differences between in vitro and in vivo responses. On water agar plates, the most favorable temperature was 25.1 ºC, showing 100% germination. The experiment with in SEM (Scanning Electron Microscope) revealed the first spores germinated in the 12-hour leaf wetness at 20 °C in coffee leaves (Fig. 2). The highest growth of germ tube was also observed at 20 ºC (Fig. 3 and Fig. 4).

The interaction was significant for the variables temperature and leaf wetness (p <0.05) (Fig. 5) for AUDPC. The highest AUDPC values occurred between temperatures of 15 and 20 °C (± 3 °C) combined with increasing leaf wetness, reaching the maximum rate in 48 hours. However, the largest in vitro mycelial growth occurred at a higher temperature, 23 °C.

In the ultrastructural analysis of samples under temperatures from 15 to 20 ºC and 24-hours leaf wetness, the cuticle began to separate from leaf tissue due to physiological processes that allow the pathogen to breach the host cuticle (Fig. 4 b). The analysis by scanning electron microscopy showed 60% of reduction in germ tube growth at 30 °C when compared with temperatures of 15 and 20 °C, thus confirming that the highest severity rates occur at these levels.

After 36 hours of leaf wetness (Fig. 4) the cuticles were clearly separated from leaves in all treatments, regardless of germ tube growth. Germ tube length increased from 15-20 °C, showing maximum rate at 18.11 °C, thus explaining the highest severity rates found at temperatures from 15-20 °C combined with increasing leaf wetness duration (Fig. 6).

Phoma leaf spot is a serious phytosanitary issue and a limiting condition for coffee crops in specific areas. Little data about the factors involved in infection of *P. tarda* and its interaction with coffee plant is currently available. In one of the few reports, Firman (1965) obtained temperatures levels similar to those in this study, although only for mycelial growth of fungi.
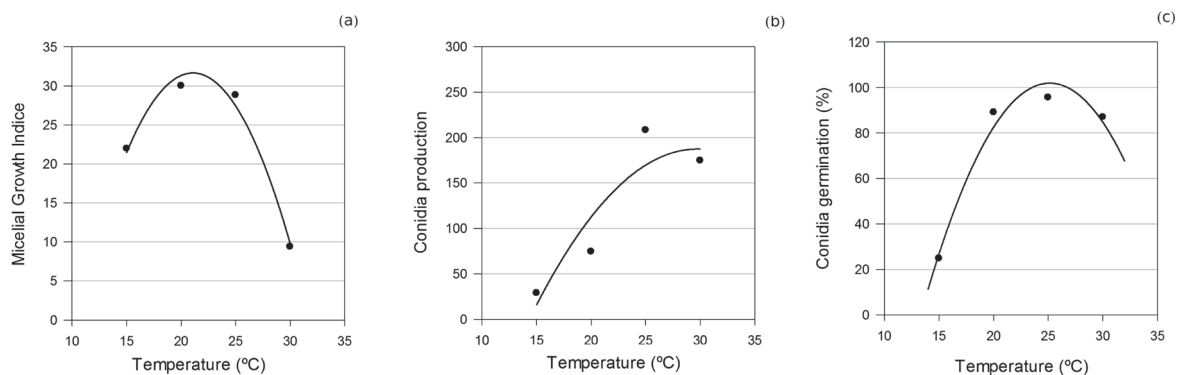

FIGURE 1- In vitro tests with *Phoma tarda* from coffee. (a) Mycelial growth. (b) Conidia production. (c) Conidia germination (%).
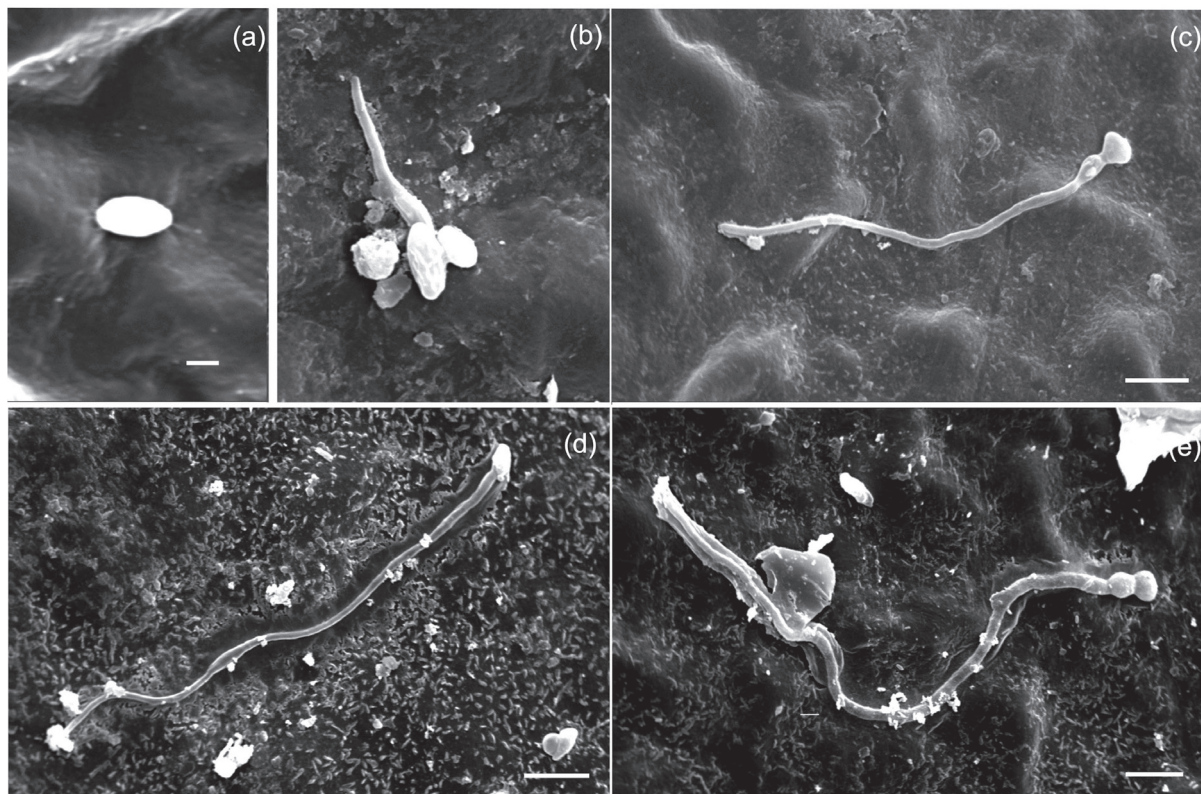
FIGURE 2- Scanning electron micrographs of *Phoma tarda* conidia germination at 20ºC, The figures present a *P. tarda* conidia in the germination process along the time (crescent order)– (a) wetness of 6h. (Bar = 2µm) (b) wetness of 12h. (Bar = 2µm) (c) wetness of 24h.(Bar = 10µm) (d) wetness of 36h.(Bar = 10µm) (e) wetness of 48h.(Bar = 10µm).

In other pathosystems, mycelial growth always shows a similar trend in optimum temperature range, which is favorable for pathogen development. The behavior was similar in other coffee plant diseases. The optimum temperature for growth of different isolates of *Colletotrichum spp.*, which causes coffee blister spot, ranged from 22 to 28 °C, and temperatures of 15 and 35 °C caused a drastic reduction in mycelial growth (DIAS et al., 2005). As for *Hemileia vastatrix*, the coffee rust, the urediniospore maximum germination occurred at 24 °C, and infectivity reduced with increasing temperature (MONTOYA; CHAVES, 1974).

Temperature and light are important factors for mycelial growth and production of fungal spores in vitro. However, conditions favoring fungal growth do not necessarily favor sporulation (NOZAKI; CAMARGO; BARRETO, 2004), which was observed for *P. tarda*. The temperature in vitro showing the highest production of conidia was close to 30 °C, whereas lower temperatures around 23 °C favored mycelial growth. Different optimum temperatures for mycelial growth and sporulation were also observed in the pathosystem *Stenocarpella x maize* (CASA et al., 2007). It is possible that reduced growth resulted in more effort to increase sporulation in an attempt to ensure the survival of fungi in the environment.

Salgado et al. (2003) assessed the MS-13 isolate of *P. tarda* collected in southern Minas Gerais state and grown in culture medium V8 agar. The authors found that the optimum temperature and number of days that best favored conidial production were 15 °C in a period from 20 to 24 days. However, our study showed different outcomes (29.8 °C), since rise in incubation temperature definitely increased conidial production. Among other factors, such dissimilar results are due to the different culture media employed. Some media are more favorable for sporulation of fungi than others, as they have complex carbohydrates. These compounds are less capable of producing vegetative hyphae, although more capable of producing spores (NOZAKI; CAMARGO; BARRETO, 2004).
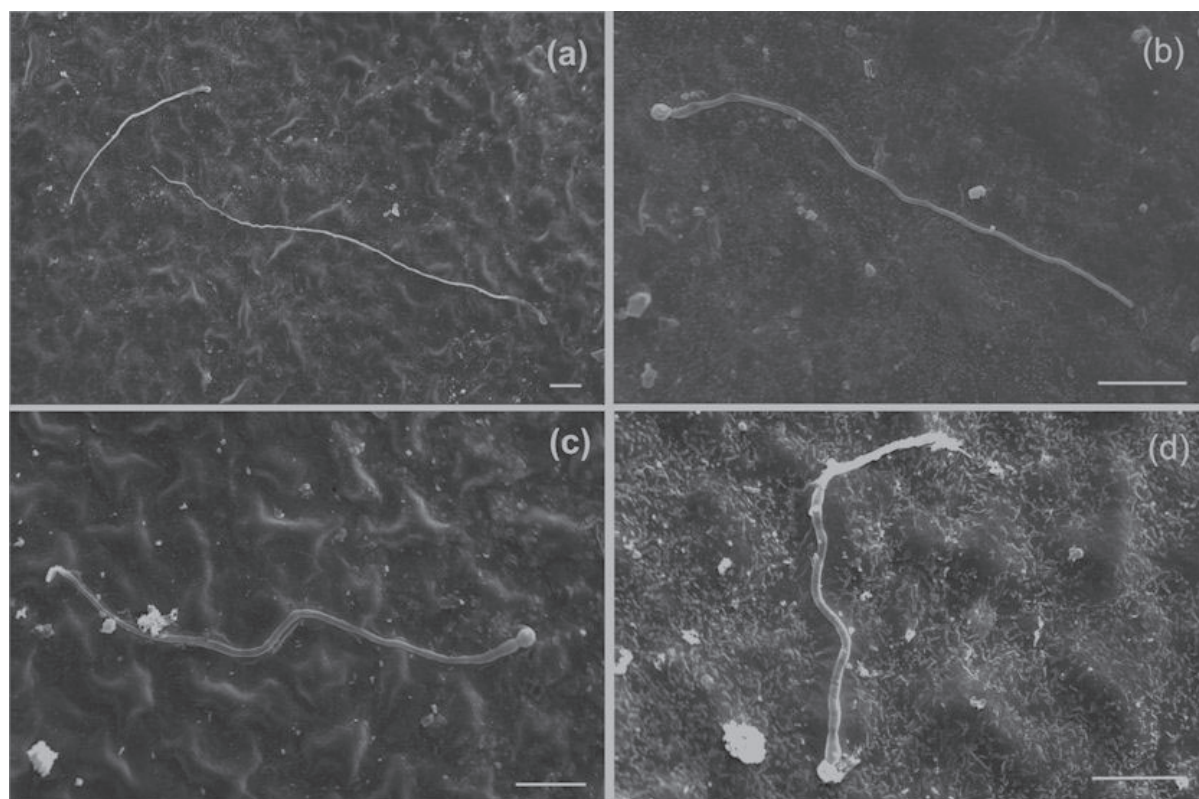
FIGURE 3 - Scanning electron micrographs of *Phoma tarda* conidia germination in wetness of 36 h. The figures present a *P. tarda* conidia in the germination process in the same time, in different temperatures (a) 15ºC, (b) 20ºC,(c) 25ºC, (d) 30ºC. (Bar = 10µm).

Another explanation for the difference is the variability of the isolates. Other studies confirm that different isolates varied their conidial production (AIUCHI et al., 2008; DIAS et al., 2005). *Cercospora coffeicola* isolates from different locations also showed variation in mycelial growth (SOUZA, 2007). This difference may be due to genetic variability of species, with great variation in morphological and biochemical aspects among isolates, which is related to their high climatic adaptability. Variations also occur among isolates of *Phoma*, as infectivity and severity are limited by environmental conditions such as temperature, humidity, light intensity and altitude (GOMEZ; BUSTAMANTE, 1977). Isolates of *Phoma* from coffee growing areas with higher altitude and humidity were most pathogenic in Costa Rica (FERNANDEZ, 1986). When comparing conidial germination in culture medium in vitro in the electron microscopy trial, we found that temperature around 25 °C provided the highest percentage of germination, whereas the ideal temperature for coffee leaves was around 20 oC. In natural substrates such as leaves, conidia are longer in *Bipolaris sorokiniana* (Sacc.) Schoemaker (BARBA; REIS; FORCELINI, 2004) like observed in coffee leaves.

Variations in germination in different substrates were also observed in coffee leaves and rust spores. On agar-water plates, the germination percentage of urediniospores was higher than in coffee leaves, which is probably due to uniformity and constancy of the temperature and humidity achieved in the culture medium (MONTOYA; CHAVES, 1974). As in *Potebniamyces pyri* (Berk. & Broome), conidial germination did not occur at 35 °C, and at threshold temperatures 0 and 30 °C the germ tube elongation decreased (LIU; XIAO, 2005).

Low temperatures ranging from 15 to 20 °C and leaf wetness periods over 36 hours are optimal conditions for fungal infection in coffee seedlings, thus confirming the temperature levels from 16 to 20 °C observed by Pozza and Alves (2008).
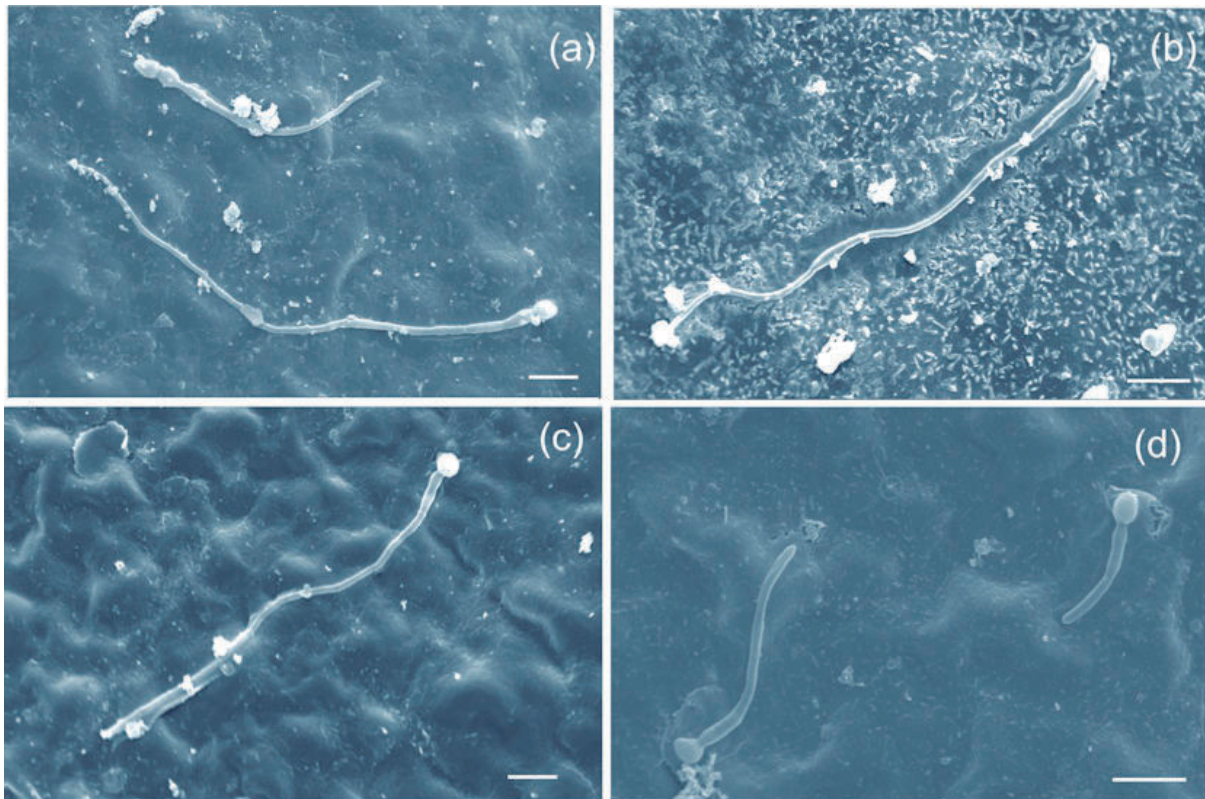
FIGURE 4 - Scanning electron micrographs of *Phoma tarda* conidia germination in wetness of 48 h. The figures present a *P. tarda* conidia in the germination process in the same time, in different temperatures (a) 15ºC, (b) 20ºC,(c) 25ºC, (d) 30ºC. (Bar = 20μm).
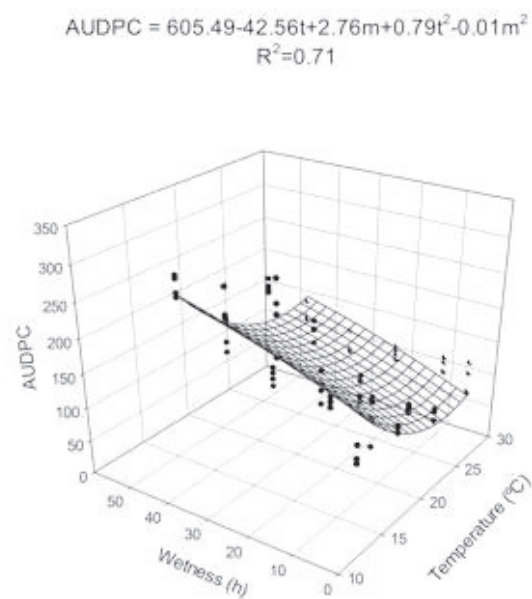
$$AUDPC = 605.49 - 42.56t + 2.76m + 0.79t^2 - 0.01m^2$$
$$R^2 = 0.71$$

FIGURE 5 - Effect of temperature and leaf wetness on AUDPC of *Phoma tarda* in coffee seedlings.
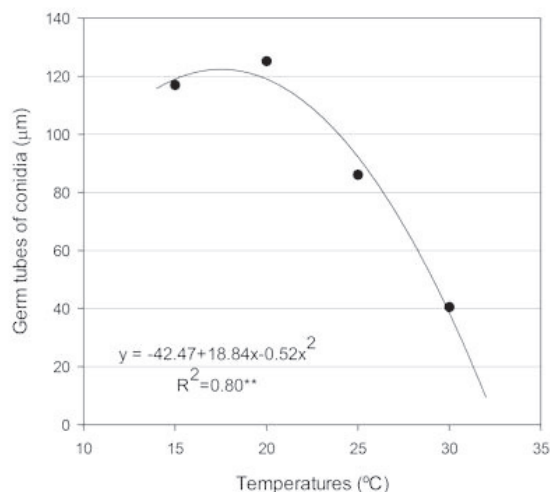
FIGURE 6 - Measurement of germ tubes of *Phoma tarda* a function of temperature.

Differences between in vitro and in vivo conditions can be explained by the interaction with host in trials in vivo. Such interaction comprises the inherent ability of the fungus to attack and the ability of the plant to defend itself (POZZA; ALVES, 2008). Besides environmental factors such as temperature and leaf wetness, variability of isolate and host can also change the infection process (TRAPERO-CASAS; KAISER, 1992). Thus, other coffee cultivars and *Phoma* isolates may show different responses. In Colombia, isolates of *Phoma* sp. from crops planted at different altitudes showed differences in pathogenicity, and those from higher altitudes were the most pathogenic (GOMEZ; BUSTAMANTE, 1977).

As low temperatures are conditions favorable for phoma leaf spot, climate change and increases in global temperatures will certainly reduce areas favoring the disease in future scenarios (MORAES et al., 2010). However, such environmental changes can lead to plant and pathogen adaptations (EASTBURN; MCELRONE; BILGIN, 2011).

In the SEM trial, cuticle separation from leaves was shown as part of the process of infection by *Phoma tarda*. Fungal spores have a small amount of cutinase that releases cutin monomers when in contact with plant cell walls. Monomers trigger cutinase gene expression in the fungus, thus degrading the cuticle layer. The fungus penetrates the cuticular barrier by combining the physical and enzyme processes promoted by fungal cutinase (KOLATTUKUDY et al., 1995).

Considering the importance of the disease and the lack of data regarding its epidemiology, this study provides relevant information to further studies on the pathosystem, which can help establish strategies for disease management in field conditions.

4 CONCLUSION

This study showed the effect of low temperatures, around 20 ºC, combined with conditions of prolonged leaf wetness, maximum of 48 hours, on the highest value of area under the progress curve of phoma leaf spot in coffee plants.

5 ACKNOWLEDGEMENTS

6 REFERENCES

AIUCHI, D. et al. Variation in growth at different temperatures and production and size of conidia in hybrid strains of *Verticillium lecanii* (Lecanicillium spp.) **Applied Entomology Zoology,** Tokyo, v. 43, p. 427-436, 2008.

BARBA, J. T.; REIS, E. M.; FORCELINI, C. A. Efeito do substrato na morfologia de conídios de *Bipolaris sorokiniana* e da densidade de inóculo na intensidade da mancha marrom em cevada. **Fitopatologia Brasileira,** Brasília, v. 29, p. 1-6, 2004.

BARGUIL, B. M. I**ndução de resistência e reação de cultivares de** *Coffea arabica* **L. a** *Phoma costarriciensis Echandi*. 2004. 64 p. Dissertação (Mestrado em Fitopatologia) - Universidade Federal de Lavras, Lavras, 2004.

CASA, R. T. et al. Efeito da temperatura e de regimes de luz no crescimento do micélio, germinação de conídios e esporulação de *Stenocarpella macrospora* e *Stenocarpella maydis*. **Fitopatologia Brasileira**, Brasília, v. 32, p. 137-142, 2007.

COMPANHIA NACIONAL DE ABASTECIMENTO. **Acompanhamento da safra brasileira café safra 2010:** segunda estimativa, maio/2010. Brasília, 2010.

DIAS, M. D. et al. Efeito da temperatura no crescimento micelial, produção e germinação de conídios de *Colletotrichum* spp. isolados de *Coffea arabica* L. **Ciência & Agrotecnologia,** Lavras, v. 29, p. 545-552, 2005.

DUTHIE, J. A. Models of the response of foliar parasites to the combined effects of temperature and duration of wetness. **Phytopathology,** Saint Paul, v. 87, p. 1088-1095, 1997.

EASTBURN, D. M.; MCELRONE, A. J.; BILGIN, D. D. Influence of atmospheric and climatic change on plant-pathogen interactions. **Plant Pathology**, Honolulu, v. 60, p. 54-69, 2011.

FERNANDEZ, J. M. **El derrite del cafeto:** su control y algunos aspectos de su biología. San José: Ministerio de Agricultura y Ganadería, 1986.

FIRMAN, I. D. Some investigations on a disease of *Coffea arabica* caused by *Ascochyta tarda*. **Transactions of the British Mycological Society,** Cambridge, v. 48, p. 161-166, 1965.

GOMEZ, R.; BUSTAMANTE, E. Influencia de la luz y la temperatura en el desarrollo de la muerte descedente del cafeto causado por *Phoma* sp. **Fitopatologia Colombiana**, Palmira, v. 6, p. 73-80, 1977.

KOLATTUKUDY, P. E. et al. Surface signaling in pathogenesis. **Proceedings of the National Academy of Sciences of The United States of America**, Washington, v. 92, p. 4080-4087, 1995.

LIMA, L. M. et al. Relação nitrogênio/potássio com mancha de Phoma e nutrição de mudas de cafeeiro em solução nutritiva. **Tropical Plant Pathology,** Brasília, v. 35, p. 223-228, 2010.

LIU, Q.; XIAO, C. L. Influence of nutrient and environmental factors on conidial germination of *Potebniamyces pyri*. **Phytopathology**, Saint Paul, v. 95, p. 572-580, 2005.

MONTOYA, R. H.; CHAVES, G. M. Influência da temperatura e da luz na germinação, infectividade e período de geração de *Hemileia vastatrix* Berk. & Br. **Experientiae**, Viçosa, v. 18, p. 239-266, 1974.

MORAES, W. B. et al. Impacto das mudanças climáticas globais na distribuição espaço-temporal da mancha de phoma do cafeeiro no Brasil. In: CONGRESSO BRASILEIRO DE METEREOLOGIA, 16., 2010, Belém. **Anais**... Belém: CBMET, 2010. Available from: <http://www.cbmet2010.com/anais/4.html>. Access in: 10 Mar. 2011.

NOZAKI, M. H.; CAMARGO, M.; BARRETO, M. Caracterização de *Diaporthecitri* em meios de cultura e diferentes condições de temperatura e luminosidade. **Fitopatologia Brasileira**, Brasília, v. 29, p. 429-432, 2004.

POZZA, E. A.; ALVES, M. C. Impacto potencial de mudanças climáticas sobre as doenças fúngicas do cafeeiro no Brasil. In: GHINI, R.; RAMADA, E. (Ed.). **Mudanças climáticas**: impactos sobre doenças de plantas no Brasil. Brasília: EMBRAPA, 2008. p. 220-238.

POZZA, E. A.; CARVALHO, V. L.; CHALFOUN, S. M. Sintomas de injúrias causadas por doenças do cafeeiro. In: GUIMARÃES, R. J.; MENDES, A. N. G.; BALIZA, D. P. (Ed.). **Semiologia do cafeeiro.** Lavras: UFLA, 2010. p. 67-106.

SALGADO, M. et al. Escala diagramática para avaliação da severidade da mancha de Phoma do cafeeiro. **Tropical Plant Pathology**, Brasília, v. 34, p. 422-427, 2009.

SALGADO, M. et al. Influência da temperatura e do tempo de incubação no crescimento micelial e produção de conídios in vitro de espécies de Phoma do cafeeiro. In: SIMPÓSIO DE PESQUISA DOS CAFÉS DO BRASIL; WORKSHOP INTERNACIONAL DE CAFÉ & SAÚDE, 3., 2003, Porto Seguro. **Anais.**.. Porto Seguro, 2003. p. 202-203.

SOUZA, A. G. C. **Variabilidade fisiológica de isolados de** *Cercospora coffeicola*. 2007. 31 p. Dissertação (Mestrado em Fitopatologia) - Universidade Federal de Viçosa, Viçosa, 2007.

TRAPERO-CASAS, A.; KAISER, W. J. Development of *Didymella rabiei*, the teleomorph of *Ascochyta rabiei*, on chickpea straw. **Phytopathology**, Saint Paul, v. 82, p. 1261-1266, 1992.