EFFECT OF LIGHT AND TEMPERATURE ON *Cercospora coffeicola* AND *Coffea arabica* PATHOSYSTEM

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ABSTRACT: The mycelial growth rate (MGR), *in vitro* production of cercosporin, and intensity (incidence and severity) of Cercospora leaf spot on coffee seedlings 'Catuaí Vermelho IAC 144' were evaluated under different light intensities (80, 160, 240, and 320 µmol m^{-2} s^{-1}) and temperatures (17, 21, 25, and 29°C). Dark condition (0 µmol m^{-2} s^{-1}) was also included in *in vitro* experiments. *In vivo*, were evaluated incidence, severity, rates of chlorophyll a, b, and total in healthy (without symptoms) and sick tissues (with symptoms), and the photosynthetic rate and variables affecting it, were also evaluated. All the experiments were done at least two times. In *in vitro* experiments, the highest mycelial growth rate (MGR) was observed at 24.1°C in the dark condition (0 µmol m^{-2} s^{-1}), while the highest amount of toxin occurred at 24.9°C and light intensity 320 µmol m^{-2} s^{-1}. When dishes were incubated in the dark, the lowest levels of cercosporin were produced by the pathogen, regardless of temperature, thus confirming the importance of light in the activation of toxin production. In *in vivo* experiments, the highest incidence and severity progress of the disease were observed at 21°C. With respect to the amounts of chlorophyll a, b, and total, regardless of treatment, the lowest levels were found in the area of the leaf with symptons compared to the area without symptoms. Photosynthesis correlated positively with temperature and negatively with internal carbon. These results elucidate the effect of the interaction between temperature and light intensity, contributing to future studies with this pathosystem.

Index terms: Epidemiology, cercosporin, Cercospora leaf spot.

EFEITO DA LUMINOSIDADE E DA TEMPERATURA NO PATOSSISTEMA
Cercospora coffeicola E CAFEEIRO

RESUMO: O índice de velocidade de crescimento micelial (IVCM), a produção de cercosporina e a intensidade (incidência e severidade) da cercosporiose do cafeeiro em mudas de cafeeiro cv. Catuaí Vermelho IAC 144, foram avaliadas em diferentes intensidades luminosas (80, 160, 240 e 320 µmol m^{-2} s^{-1}) e temperaturas (17, 21, 25 e 29°C). Para os experimentos in vitro, adicionou-se o tratamento escuro (0 µmol m^{-2} s^{-1}). In vivo, avaliou-se a incidência e a severidade da doença, as taxas das clorofilas a, b e total em tecidos doentes (com sintomas) e sadios (ausência de sintomas), a taxa fotossintética e variáveis que a afetam. Todos os experimentos foram realizados no mínimo duas vezes. Nos experimentos in vitro, o maior IVCM foi observado na temperatura de 24,1°C, no tratamento escuro (0 µmol m^{-2} s^{-1}). Já a maior quantidade da toxina foi observada na temperatura de 24,9°C e na intensidade luminosa de 320 µmol m^{-2} s^{-1}. Quando as placas foram incubadas no escuro, as menores taxas de cercosporina foram produzidas pelo patógeno, independente da temperatura, confirmando a importância da luminosidade na ativação e produção da toxina. Para o experimento in vivo, observou-se o maior progresso da incidência e da severidade da doença à 21°C. Com relação às taxas de clorofilas a, b e total independente do tratamento, as menores taxas foram observadas na área da folha com sintomas em relação às áreas sem sintomas. A fotossíntese correlacionou-se positivamente com a temperatura e negativamente com o carbono interno. Os resultados deste trabalho elucidam o efeito da interação entre temperatura e intensidade luminosa, colaborando para estudos futuros com este patossistema.

Termos de indexação: Epidemiologia, cercosporina, cercosporiose do cafeeiro.

1 INTRODUCTION

Cercospora leaf spot caused by the fungus *Cercospora coffeicola* Berk. & Cooke is one of the oldest known and most important coffee diseases. The pathogen infects seedlings from nursery to trees in field production. In the nursery, the disease causes severe defoliation thus reducing growth of seedlings, which become inappropriate for planting and marketing (POZZA; CARVALHO; CHALFOUN, 2010). In mature fields, in addition to leaf drop the disease causes premature fall, breaking during pith processing of grains, which reduces production and can also reduce quality of beverage, thus causing depreciation (LIMA; POZZA; SANTOS, 2012).

Intensity of symptoms, pathogen infection ability, and plant physiological responses to penetration and colonization are mainly influenced by environmental variables such as temperature, leaf wetness, light, water availability, and soil fertility. Light and temperature are particularly important for affecting chlorophyll thus directly influencing photosynthesis (WAGGONER, 1960).

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To the host, light is essential for the process of producing the biochemical energy required for growth and production of plant species (TAIZ; ZIEGER, 2013). However, high light intensities saturate the photosynthetic apparatus causing severe photo inhibition which can be further aggravated by water deficit and temperature extremes (DAMATTA et al., 2007). In turn, temperature has an important role in controlling the speed of chemical reactions which drive plant growth and development (LEHNINGER; NELSON; COX, 2011).

Light and temperature are capable of inducing or inhibiting vegetative growth and reproductive process of most fungi. In plant pathogens, temperature modulates the production of proteins and enzymes are responsible for maintaining fungal cells, and can influence colony growth, sporulation and germination of conidia, as well as the incubation period and progress of disease (SCHUMANN; D'ARCY, 2012). In studies of *Cercospora* species, temperatures from 25 to 27°C were suitable for production of conidia (BRUNELLI et al., 2006). Light exerts a direct effect, inducing or inhibiting the formation of reproductive structures, although some species are indifferent to quantity or quality of light. Echandi (1959) reported the relationship between *C. coffeicola* and light intensity, as the genus *Cercospora* produce cercosporin toxin which is activated in the presence of light, resulting in necrosis of plant cells, however, the effect was not quantified (DAUB; HERRERO; CHUNG, 2005). According to Echandi (1959), light was important in the occurrence and progression of disease in coffee plants, even before the toxin was discovered, but few quantitative studies have been conducted to date. Like *C. beticola* in sugar beets and *C. coffeicola* in coffee, the toxin does not affect penetration and infection, but influences pathogen colonization after infection (DAUB; CHUNG, 2007). This fact justifies more severe cercospora infections in full-sun plantations when compared to shaded plantations.

Besides affecting disease and photosynthesis, light can directly affect cultural characteristics of *C. coffeicola,* since colony color in culture varies depending on lighting conditions. In the dark, colonies vary from greenish-black during early growth and milky-white to brown at the end (MARTINS; MAFFIA; MIZUBUTI, 2008). In the presence of light toxin production may occur as a red pigment, and hence mycelium color ranges from light to intense red, depending on the ability of the isolate to produce pigment.

Even brief exposure to light can stimulate pigment production (DAUB; HERRERO; CHUNG, 2005).

Thus, the influence of different light intensities and temperatures on mycelium growth, production of cercosporin, and *in vivo* on the incidence and severity of disease, chlorophyll content, and physiological variables were evaluated in coffee seedlings.

2 MATERIAL AND METHODS

Obtaining isolates

Coffee leaves infected with the pathogen, collected in Lavras, Minas Gerais state, were used for obtaining ten isolates of *C. coffeicola*, which were incubated at 25°C/12 hours photoperiod in malt medium (20g malt extract, 20g agar and 1000 mL distilled water). Each experiment that is described below was performed twice in the time (two trials).

Pathogen sporulation and pathogenicity test to choose the isolate

For obtaining conidia of *C. coffeicola,* 12 plugs of mycelium 0.5 cm diameter were removed from the edges of colonies in Petri dishes and transferred to 30 mL tomato juice medium (STT) in 125 mL Erlenmeyer flasks. Medium was composed of 200 mL Super Bom® tomato juice, 3.2 g CaCO$_3$ and 800 mL distilled water. The flasks with medium and fungus were kept under continuous stirring at 120 rpm at 25°C approximately. After four days, the content of each flask was poured in solid tomato juice, in the previous medium (STT) plus 16 g agar per liter of medium, in two Petri dishes, which were kept open in BOD chamber at 25°C under continuous light. When the medium dehydrated after two days, 5 mL distilled water was added to each plate, which was then lightly scraped with a glass rod. Next, the suspension was filtered through a layer of sterile gauze. The concentration of conidia was estimated using a hemacytometer in each filtrate isolate, based on the average of readings in both slide fields. This procedure was repeated four times, totaling eight readings. Based on these readings, the inoculum suspension was calibrated to 2×10^4 conidia mL^{-1} after dilution in water.

Using this suspension, a pathogenicity test of ten isolates was performed by spraying to the point of flowing on all leaves in seedlings of 'Catuaí Vermelho IAC 144' with four to five pairs of leaves, grown in standard substrate (700 liters

of soil and 300 liters of manure per cubic meter of mixture) plus 1kg P_2O_5 (5 kg superphosphate) and 0.3 kg K_2O (0.5 kg potassium chloride). Then, all leaves were inoculated with the suspension and the inoculated plants were kept in relative humidity above 90% for 72h in growth chamber at 21°C. A randomized block design was used with three replicates, two plants each. The incidence and severity of disease were evaluated in all leaves, 20 days after inoculation. Incidence was determined by counting number of leaves with symptoms in relation to total inoculated leaves. Severity (percentage of leaf area with symptoms) was measured in all leaves with incidence, according to the scale by Custódio et al. (2011).

Cercosporin production test to choose the isolate

Cercosporin production was assessed by incubating mycelium plugs of each isolate on malt medium, in BOD chamber at 25°C/12 hours photoperiod. After 14 days of incubation, three plugs 0.5 cm diameter with mycelium growth were removed from the edge of dishes of each isolate, which were immersed in 8 mL KOH 5N for four hours at 25°C in the dark for extracting cercosporin. Then, absorbance was measured using a spectrophotometer Beckman DU 640B. Toxin concentration in the extract was estimated by using the molar extinction coefficient of 23300 to A_{480} nm readings (JENNS; DAUB; UPCHURCH, 1989) in each treatment. This assay was performed in triplicate.

Significant variables were assessed by Tukey's test at 5% probability. The isolate capable of causing the highest severity of disease and greatest ability to produce toxin cercosporin in the culture medium was then chosen to do all the experiment that is described below.

Effects of light intensity and temperature on mycelium growth of *C. coffeicola*

The mycelium growth of the isolate chosen was evaluated in different conditions of light and temperature. Plugs with malt medium and mycelium 0.5 cm diameter were removed from the edge of colonies. Then one plug was placed at the center of Petri dishes (9 cm) with malt, which were incubated in their respective treatments. A randomized block design was used with six replicates, each consisting of a Petri dish, in factorial analysis of variance (5x4). Factor 1 was light intensities 0, 80, 160, 240, and 320 μmol m^{-2} s^{-1}, and

factor 2 the temperatures 17, 21, 25, and 29°C, totaling 20 treatments. A stepped structure with 400 Watt metal lamps was used for light intensity, which varied with distance of seedlings from lamps. Evaluation was made at two-day intervals by measuring colony diameter in both orthogonal growth directions, then the average was calculated, totaling seven evaluations.

Then, the mycelial growth rate (MGR) was calculated using the Maguire (1962) formula.

***In vitro* production of cercosporin**

After assessing mycelial growth, toxin cercosporin was extracted from each experiment in each treatment as previously described (JENNS; DAUB; UPCHURCH, 1989).

Seedling inoculation and assessment of intensity disease

Seedlings of 'Catuaí Vermelho IAC 144' grown in standard substrate (700 liters of soil and 300 liters of manure per cubic meter of mixture) plus 1kg P_2O_5 (5 kg superphosphate) and 0.3 kg K_2O (0.5 kg potassium chloride) with four to five pairs of leaves were inoculated as described in the pathogenicity test. The experiment was conducted in growth chambers in randomized block design with three replicates, each consisting of two plants, in factorial analysis of variance (4x4). Factor 1 was light intensities 80, 160, 240, and 320 μmol m^{-2} s^{-1}, and factor 2 the temperatures 17, 21, 25, and 29°C, totaling 16 treatments. After inoculation, seedlings were subjected to the conditions mentioned and incubated in humid chamber above 90% relative humidity for 72 hours in a controlled environment. Different light intensities were obtained as in the previous experiment. After incubation period, the incidence and severity of disease were assessed at five-day intervals as previously described, totaling five evaluations in each trial.

A graph for disease progress over time was plotted for both incidence and severity. To compare the treatments, the area under the disease progress curve for incidence (AUDPCI) and severity (AUDPCS) was calculated according to Shanner and Finey (1977). Analysis of variance was performed on the results.

Evaluation of chlorophyll

Fresh leaf tissue of coffee seedlings in different shades of green chlorophyll, were measured for chlorophyll content for calibration

purposes by a portable SPAD-502®. After reading, 0.2 g of plant tissue was weighed, macerated in liquid nitrogen, and transferred to tubes containing 10 mL 80% acetone (v/v), remaining for 24 hours in a cold room, protected from light. After 24 hours, the extracts were filtered and read in spectrophotometer at wavelengths 663 and 645 nm for chlorophyll a and b, respectively. Measurements of chlorophyll (mg gMF^{-1}) were made according to Whitham, Blaydes and Devlin (1971).

After the calibration curve was made, symptomatic leaves were marked in all treatments to monitor chlorophyll readings with the progress of disease. A factorial analysis of variance (4x4x2) was used, where factor 1 was light intensities 80, 160, 240, and 320 µmol m^{-2} s^{-1}; factor 2 was temperatures 17, 21, 25, and 29°C; and factor 3 was leaf section (without/with symptoms), totaling 32 treatments. Readings were made with portable SPAD-502® on the same day of evaluation of the incidence and severity of disease. Ten readings were performed per leaf, five in healthy areas (without symptoms) and five in sick areas (with symptoms). Based on the results, the levels of chlorophyll a, b, and total were determined in each experiment and each treatment over time.

Gas exchange

At the end of the experiment, net photosynthetic rate (A), intercellular CO_2 concentration (Ci), stomatal conductance (Cond), transpiration (Tr), and leaf temperature (Tleaf) were evaluated in symptoms and without symptoms leaves, using a portable infrared gas analyzer (IRGA-ADC-LCA4®). Carboxylation efficiency was obtained by A/Ci relationship. This variables were measured in both trials. Then these data were correlated (Pearson's correlation) with temperature, light, and incidence of disease.

Statistical analysis of the experiments

Data were subjected to Levene's test (1960) to assess homogeneity and variance of residuals. As data met the assumptions of analysis of variance, F test was performed for variables at 5% probability in SAS® software. In the case of significant variables, linear regression models were fit to the quantitative variables. For estimating chlorophyll in without and with symptoms leaves, the F test of analysis of variance was used to discriminate treatments. Adjusted models were plotted in Sigma Plot®, and response surface graphs in R programam.

3 RESULTS AND DISCUSSION

The combined analysis of both trials in both experiments (*in vitro* and *in vivo*) were not significant, so far as the results refer to their respective averages.

Pathogenicity test and choice of isolate

According to Tukey's test, isolate 8 had higher incidence and severity of disease and higher *in vitro* production of cercosporin, which justified the choice of this isolate to perform the experiments (Fig. 1), especially because there is a relationship between production of cercosporin and aggressiveness disease (DAUB; EHRENSHAFT, 2000).

Mycelial growth and cercosporin production

A significant interaction was found between light intensity and temperature for mycelial growth rate (MGR) and cercosporin production. At the lowest temperature, 17°C, MGR ranged from 1.19 to 1.54, reducing from dark to the highest light intensity. This pattern in relation to light kept the same at all temperatures, and MGR kept reducing up to 21°C. From this temperature, MGR increased up to 24.1°C at all light intensities and then kept drastically reducing up to 29°C, where the lowest MGR values were found (Fig. 2). The highest MGR, 1.67, occurred at 24.1°C and in the dark.

In this study the highest mycelial growth rate (MGR) was found at 24.1°C in the dark. Although fungi had grown in the dark, there was low cercosporin production under these conditions probably because the fungal genes responsible for toxin production were not activated in the dark. Thus, as fungi used no energy for toxin production, this energy may have been used in their own growth. Echandi (1959) also found increased mycelial growth of three isolates of *C. coffeicola* at 24°C and a reduced fungal growth at 30°C, results close to those reported in the present study, however, the author did not evaluate the relationship between temperature and light intensity. Souza, Maffia and Mizubuti (2012) studied different isolates of *C. coffeicola* and found higher growth at 26°C than at 18 or 22°C, but the study neither evaluated temperature 24°C nor adjusted regression equations. Other species of *Cercospora* had the following optimum growth temperatures: 27°C for *C. beticola* and *C. apii*; 25°C for *C. sojina*; 20 to 25°C for *C. carotae* and 25 to 28°C for *C. piaropi*. (GROENEWALD; GROENEWALD; CROUS, 2005).
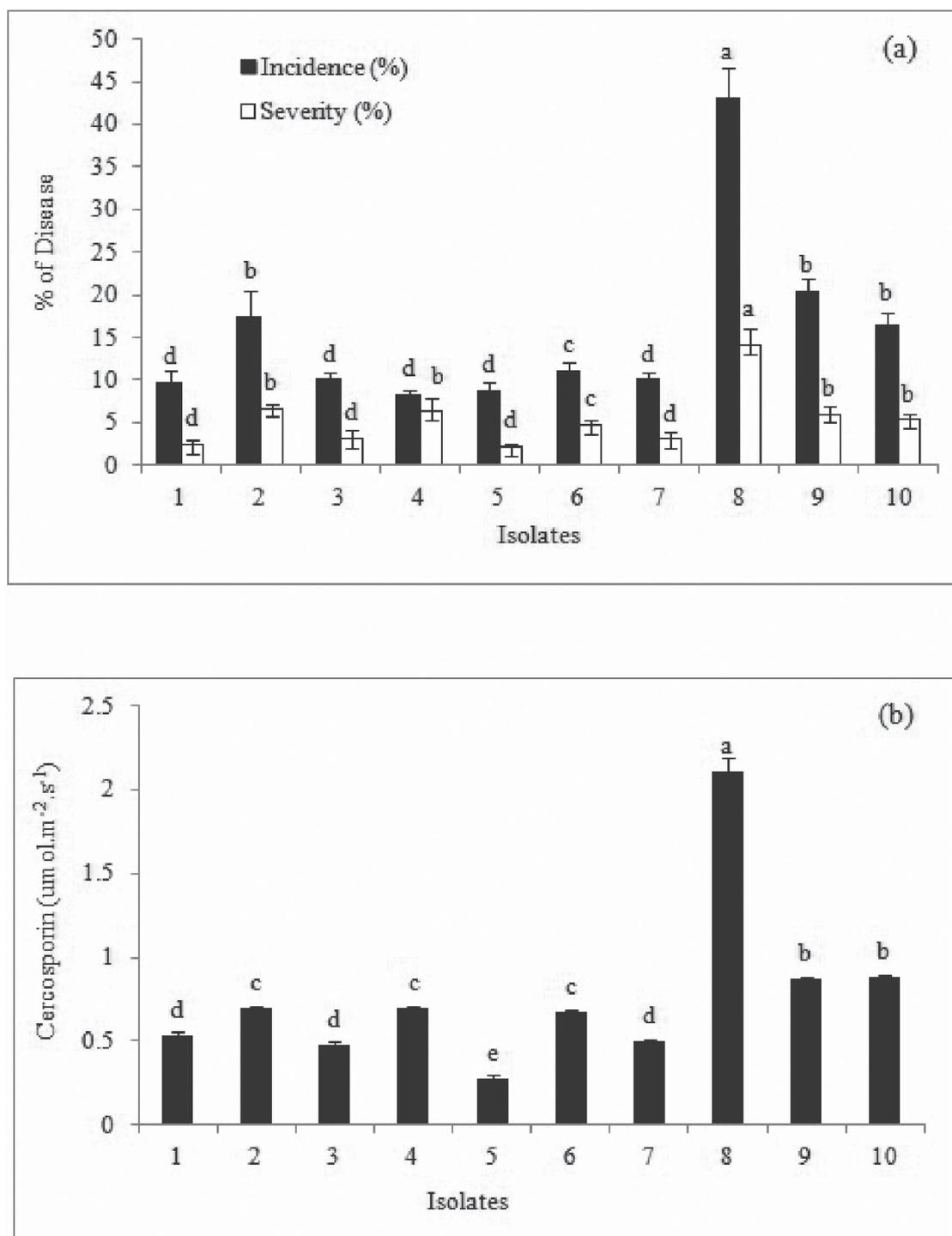
FIGURE 1 - Incidence and severity of disease (a) and *in vitro* production of cercosporin (b) to different isolates of *Cercospora coffeicola*.

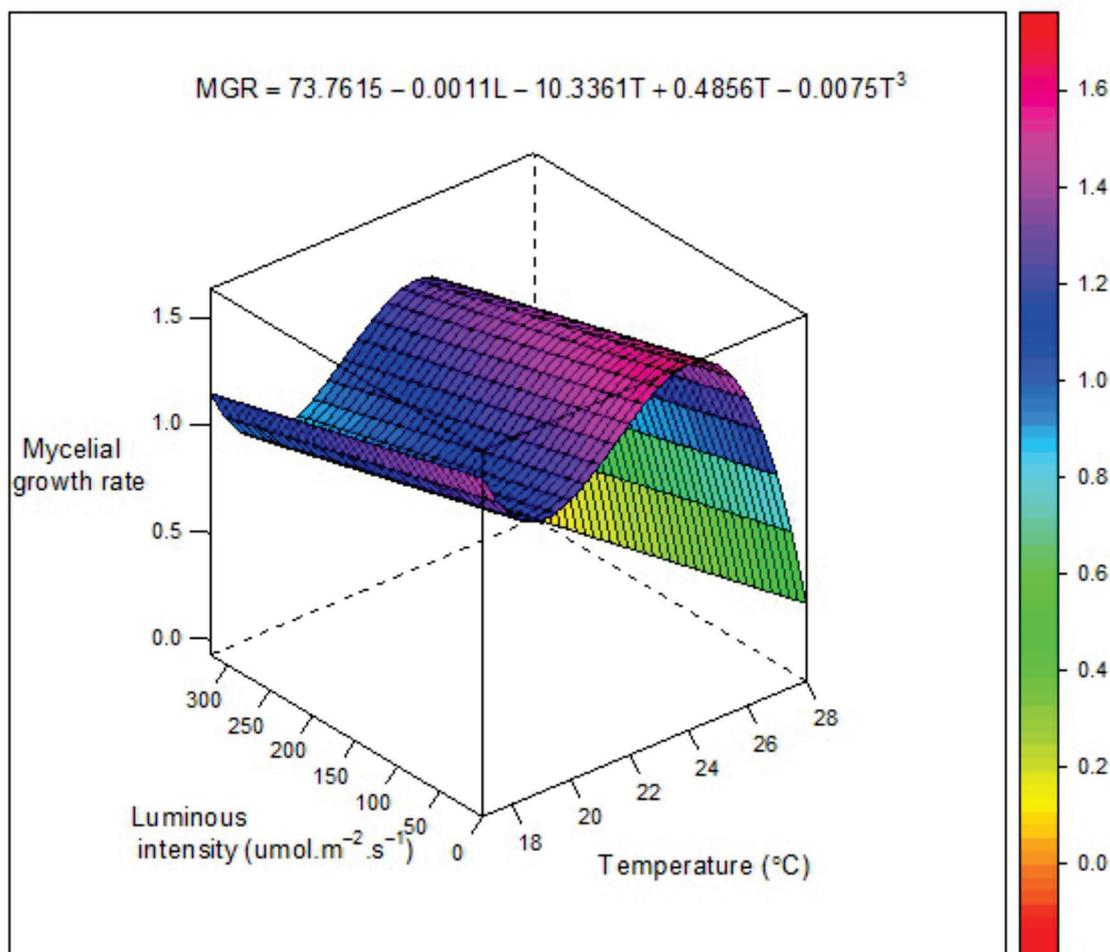$$MGR = 73.7615 - 0.0011L - 10.3361T + 0.4856T - 0.0075T^3$$

FIGURE 2 - Mycelial growth rate (MGR) versus temperature (T) and light intensity (IL).

Thus, the optimal temperature for mycelial growth differs between species.

The highest cercosporin production, 1.43 μmol.L^{-1} was found at 24.9°C (Fig. 3) with increased toxin from lowest to highest light intensity. At temperatures below and above this value cercosporin production decreased at all light intensities with a slight increase at 17°C, but always below 24.9°C. At all temperatures toxin production increased with the highest light intensity while the lowest was found in the dark, contrary to MGR behavior.

The highest amount of cercosporin was found at 24.9°C and light intensity 320 μmol m^{-2} s^{-1}, followed by 240, 160, 80, and 0 μmol m^{-2} s^{-1},

contrary to the results for mycelial growth rate (MGR). This confirms the crucial role of both light and interaction between temperature and light in toxin production and activity, which increase pathogen aggressiveness. In *C. asparagine*, *C. beticola* and *C. kikuchii* the highest toxin production occurred at 20 to 30°C and in vitro production of cercosporin varies among species and isolates (JENNS; DAUB; UPCHURCH, 1989). In the presence of light, cercosporin is toxic to organisms such as plants, bacteria, fungi, rats and only species of *Cercospora* and other fungi producing toxin with activity similar to cercosporin resist to its oxidizing action (DAUB; CHUNG, 2007).
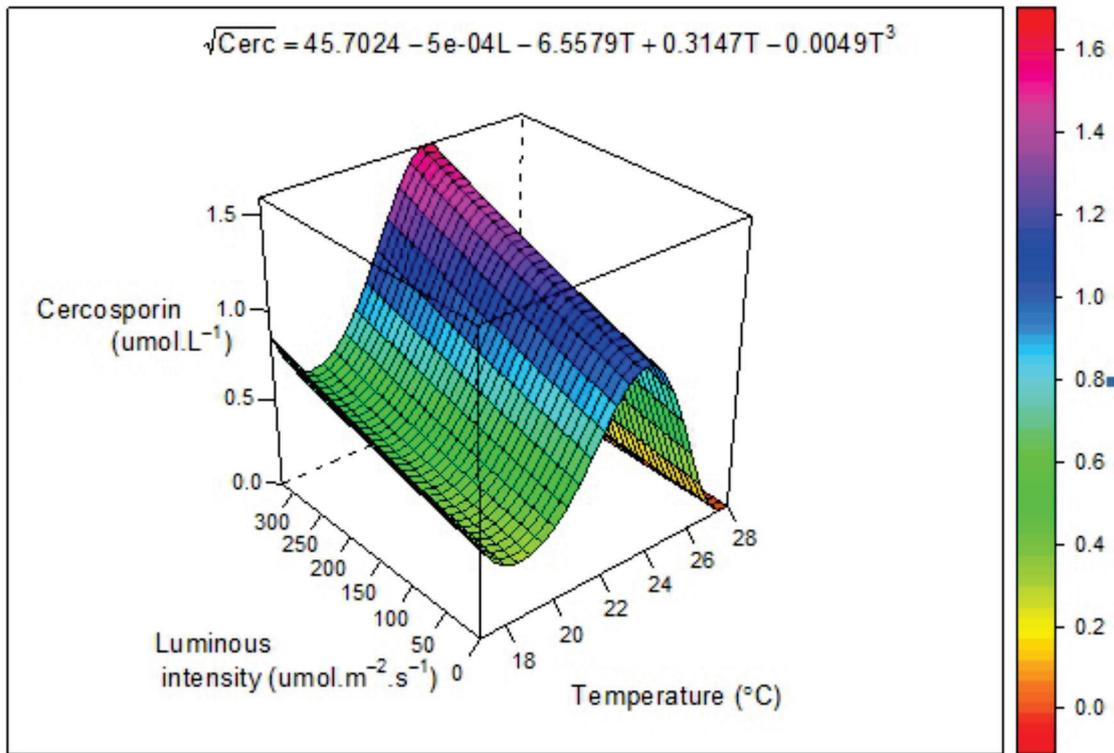
$$\sqrt{Cerc} = 45.7024 - 5e\text{-}04L - 6.5579T + 0.3147T - 0.0049T^3$$

FIGURE 3 - Cercosporin production (μmol L^{-1}) versus temperature (T) and light intensity (IL).

Progress curves of Cercospora leaf spot in coffee seedlings

The first symptoms of disease appeared 22 days after inoculation, and from this date the rate of progress leading to the exponential phase of disease occurred. The progress curve of incidence varied according to light intensity versus temperature. The highest progress of disease was found at 21°C, reaching 31.2% incidence at light intensity 80 μmol m^{-2} s^{-1} and 12.7% incidence at light intensity 320 μmol m^{-2} s^{-1}. At temperatures 17, 25, and 29°C the highest progress occurred at intensity 320 μmol m^{-2} s^{-1} followed by 80 μmol m^{-2} s^{-1} (Fig. 4).

With respect to the severity of disease, the behavior was similar to that of incidence. The highest progress rates occurred at 21°C, at intensity 80 μmol m^{-2} s^{-1} (9.9%) followed by 320 μmol m^{-2} s^{-1} (9.7%). At temperatures 17, 25, and 29°C the highest rate occurred at intensity 320 μmol m^{-2} s^{-1} followed by 80 μmol m^{-2} s^{-1} (Fig. 5).

The incidence and severity of disease in *in vivo* experiments were higher at 21°C at the extremes of light intensity, 80 and 320 μmol m^{-2} s^{-1}. In a study to assess the effect of different temperatures on the severity of disease in coffee seedlings, Souza, Maffia and Mizubuti (2012) found that severity was higher at 22°C, close to the temperature reported herein; however, the study did not describe temperature interaction with light. The light extreme 320 μmol m^{-2} s^{-1} was optimal for *in vitro* production of cercosporin, thus it could be related to pathogen aggressiveness at 22°C. This light intensity fits the range required for proper growth and development of coffee plant C3 in shaded area. This plant is morphologically and physiologically adapted to this environment, with low saturation irradiance ranging 300-600 μmol m^{-2} s^{-1} (KUMAR; TIESZEN, 1980).

Area under the disease progress curve (AUDPC)

Results of area under the disease progress curve for incidence (AUDPCI) and severity (AUDPCS) were not significant according to F test.
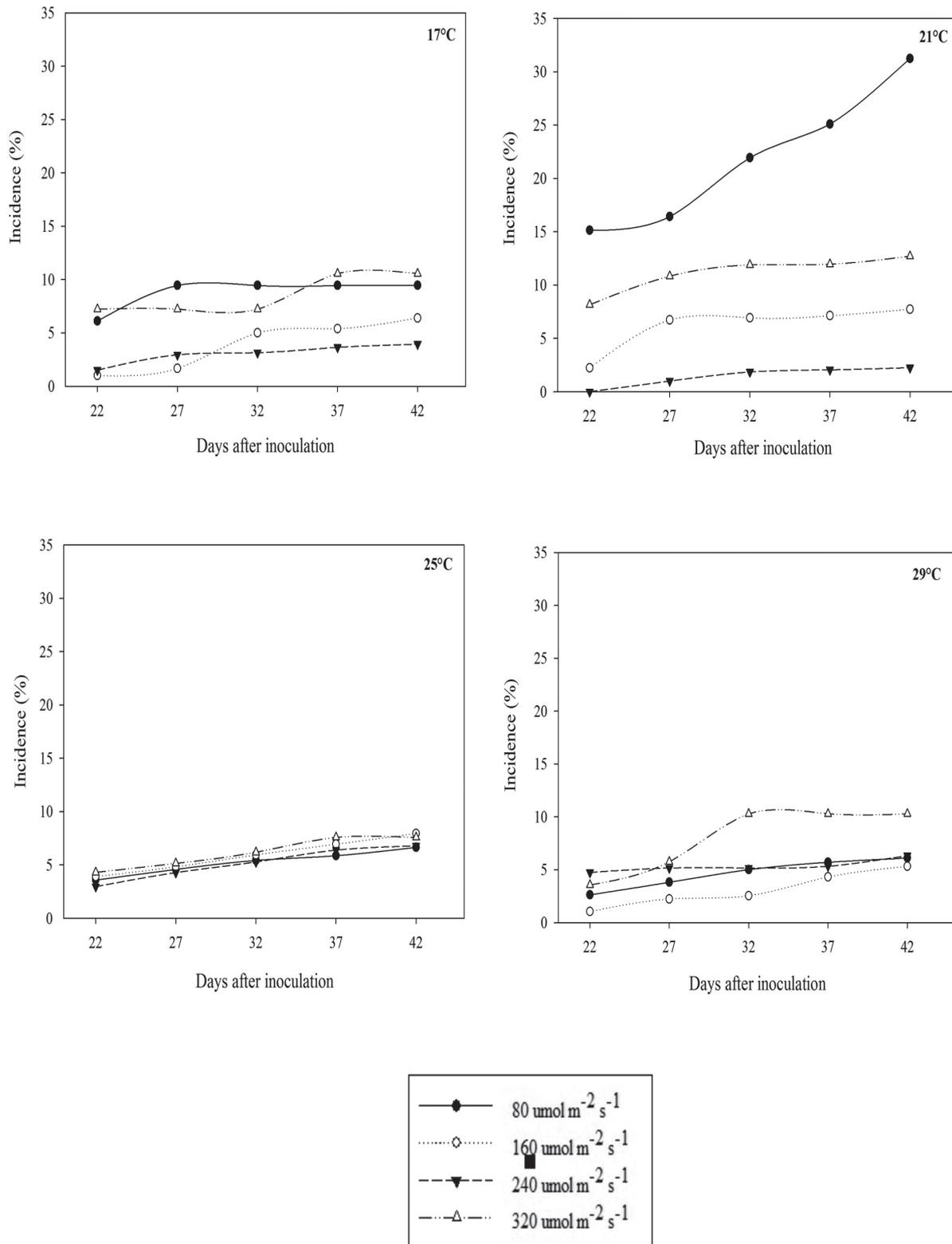
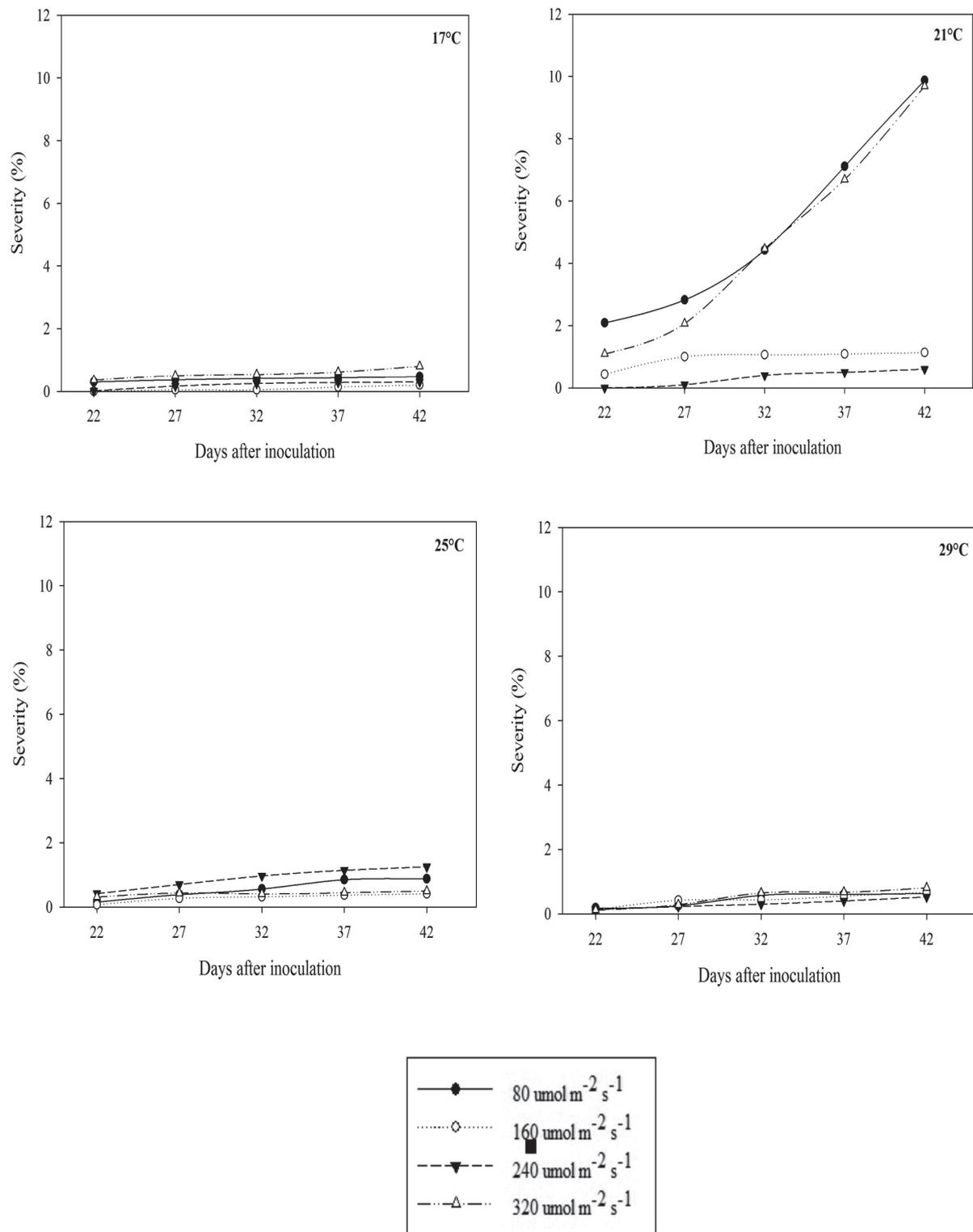FIGURE 4 - Progress curves of Cercospora leaf spot incidence at different temperatures and light intensity.

FIGURE 5 - Progress curves of severity of disease at different temperatures and light intensity.

Analysis of chlorophyll a, b, and total

According to F test, the variables of light, temperature and leaf section (with/without symptoms) were individually significant for chlorophyll a, b, and total, but there was no interaction between them. The amount of chlorophyll in tissues with symptoms was always lower in relation to the without symptoms side. Regarding temperature, the smaller quantities of chlorophyll a, b, and total were found at 24, 22.6, and 20.3°C, respectively, with the highest production always occurring at 17°C (Fig. 6).

With respect to light intensity, the highest amounts of chlorophyll a, b, and total were found at 217, 133, and 290 μmol m^{-2} s^{-1}, respectively, while the lowest occurred at 320 μmol m^{-2} s^{-1} (Fig. 7).

Cercospora leaf spot causes physiological disorder in plants, such as leaf yellowing, reduction in photosynthetic area, and leaf drop. One cause of leaf yellowing is the reduction in leaf chlorophyll content. According to the results, it was found that independently of temperature and light intensity the levels of chlorophylls a, b, and total were consistently lower in the area infected than in the healthy leaf side. This was due to destruction of chlorophyll or chloroplasts in the yellowish halo in Cercospora leaf spot lesions (REZENDE et al., 2011). It was also found that, regardless of temperature, from the intensity 160 μmol m^{-2} s^{-1} the decrease in amounts of chlorophyll was accentuated. This was probably because from that light intensity the infectious process is favored, which affect chlorophyll content and consequently the photosynthetic area.
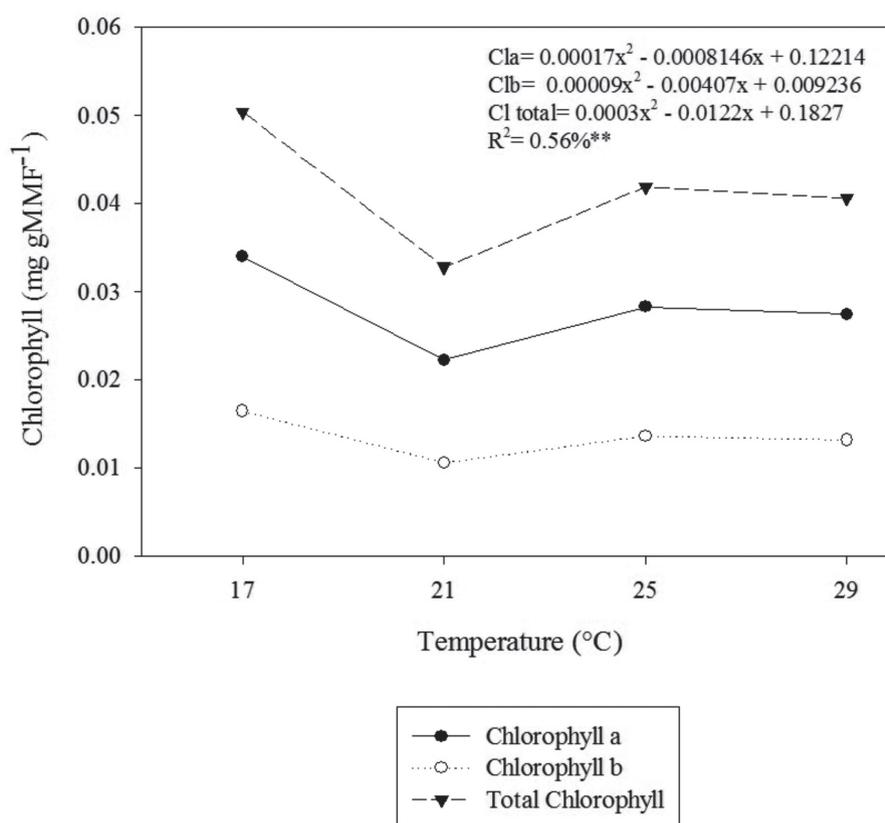

The equations shown in the figure:

$$Cla = 0.00017x^2 - 0.0008146x + 0.12214$$
$$Clb = 0.00009x^2 - 0.00407x + 0.009236$$
$$Cl\ total = 0.0003x^2 - 0.0122x + 0.1827$$
$$R^2 = 0.56\%**$$

FIGURE 6 - Behavior of chlorophyll a, b, and total according to temperature.

$$Cla= -0.000001x^2 + 0.000127x + 0.019573$$
$$Clb= -0.000001x^2 + 0.000063x + 0.009236$$
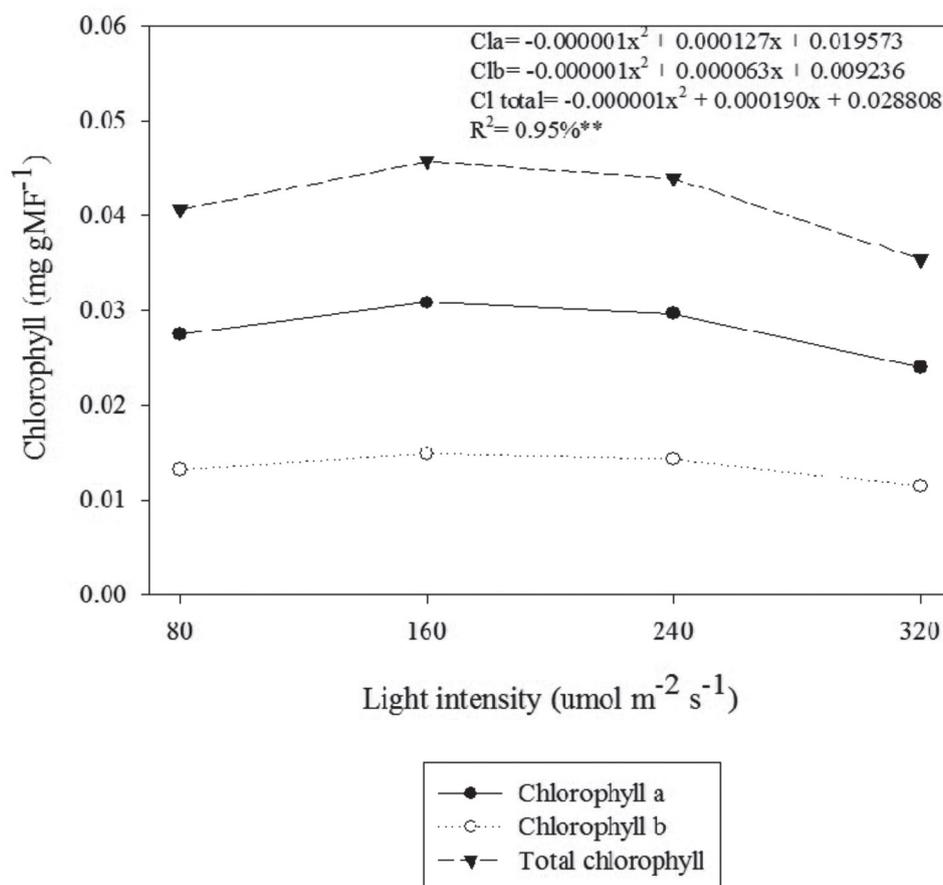$$Cl\ total= -0.000001x^2 + 0.000190x + 0.028808$$
$$R^2= 0.95\%**$$

FIGURE 7 - Behavior of chlorophyll a, b and total according to light intensity.

Correlation between physiological variables and temperature and light intensity

Significant negative correlations were found between temperature (°C) and internal carbon (Ci), and positive relationship between temperature and leaf temperature (Tleaf). Photosynthesis was negatively correlated with Ci and positively with Tleaf. There was positive correlation between stomatal conductance (Cond) and transpiration (Tr). These results were found in both with and without symptoms leaves (Table 1).

According to the results, positive correlation between temperature and photosynthesis and negative correlation between internal carbon and photosynthesis imply that Rubisco was probably functional, as internal carbon was being consumed to perform photosynthesis which had been facilitated by increased temperature. Positive correlation between stomatal conductance and transpiration is justified, as transpiration also occurs through open stomata.

In this study, both light intensity and temperature influenced plant physiology, directly affecting photosynthesis (TAIZ; ZEIGER, 2013). Light intensity and temperature levels, as well as water and nutrients are important factors in photosynthetic activity in coffee plants. Furthermore, quality and intensity of light can affect physiological responses of pathogens, such as conidia production, pigmentation, sexual development (PURSCHWITZ et al., 2006), germination, formation of resistance structures, and size and shape of conidia. Temperature influences the occurrence and progress of diseases and also affects pathogen sporulation, lesion expansion, incidence and severity of fungal diseases (PAUL; MUNKVOLD, 2005).

TABLE 1 - Correlation between physiological variables and temperature and light intensity for leaves without and with symptoms.

Variables - Leaves without symptoms

	Ci	Tr	Tleaf
Temp (°C)	-0.63*	0.27 ns	0.90*
Light intensity	-0.16 ns	-0.39 ns	-0.14 ns
Photosynthesis	-0.87*	0.35 ns	0.77*
Cond	0.03 ns	0.95*	-0.06 ns

Variables - Leaves with symptoms

	Ci	Tr	Tleaf
Temp (°C)	-0.66*	0.27 ns	0.56*
Light intensity	-0.11ns	-0.37 ns	-0.35 ns
Photosynthesis	-0.76*	0.21 ns	0.52*
Cond	0.10 ns	0.87*	-0.31 ns

Ci: internal carbon   Tleaf : leaf temperature   Cond: stomatal conductance   Tr : transpiration
Ns= no significant   * significant 0.05

4 CONCLUSION

This study showed interaction between temperature and light intensity affecting the mycelial growth rate and cercosporin production. The highest MGR, 1.67, occurred at 24.1°C and in the dark (0 µmol m^{-2} s^{-1}) and the highest amount of cercosporin production was found at 24.9°C and light intensity 320 µmol m^{-2} s^{-1}.

The progress of brown eye spot was also affected for the temperature and light intensity. The highest progress of disease was found at 21°C, reaching 31.2% of incidence and 9.9% of severity at light intensity 80 µmol m^{-2} s^{-1}.

For chlorophylls, temperature and light intensity were individually significant for chlorophylls a, b, and total, but there was no interaction between them.

5 ACKNOWLEDGEMENTS

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