

NOTA PRÉVIA

INFECTION PROCESS OF *Cercospora coffeicola* IN IMMATURE COFFEE FRUITS

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(Received: September 20, 2018; accepted: December 06, 2018)

ABSTRACT: Cercosporiosis is caused by *Cercospora coffeicola* and represents a very important coffee plants phytosanitary problem. Catuaí IAC 144 and Topázio cultivars at the F2 stage were inoculated with a conidial suspension. Samples were collected at 4, 8, 12, 24, 36, 48, 72, 96, and 168 hours after inoculation (hai) for scanning electron microscope studies. Fungal germination in epidermal surface occurred four hai; penetration only through epidermal wounds, without appressoria, started at 12 hai while the mycelial colonization occurred at 72 hai. The infection in immature coffee fruits implies brown eyespot control management anticipation need, even before the first symptoms.

Index terms: Brown eyespot, *Coffea arabica*, scanning electron microscopy.

PROCESSO INFECCIOSO DE *Cercospora coffeicola* EM FRUTOS DE CAFÉ IMATUROS

RESUMO: Cercosporiose é causada por *Cercospora coffeicola* e representa grave problema fitossanitário do cafeeiro. Frutos de cv. Catuaí IAC 144 e Topázio no estágio F2 foram inoculados com suspensão conidial. Amostras foram coletadas 4, 8, 12, 24, 36, 48, 72, 96, e 168 horas após a inoculação (hai) para estudos de microscopia eletrônica de varredura. A germinação conidial na superfície epidérmica ocorreu quatro hai; a penetração na epiderme ocorreu através de ferimentos, sem formação de apressórios, 12 hai, já a colonização micelial interna ocorreu 72 hai. A infecção de frutos imaturos implica na necessidade de antecipação no manejo da mancha de olho pardo, mesmo antes dos primeiros sintomas.

Termos para indexação: Mancha de olho pardo, *Coffea arabica*, microscopia eletrônica de varredura.

One of the main phytosanitary problems of coffee is cercosporiosis, also known as brown eye spot disease, whose etiological agent is the fungus *Cercospora coffeicola* Berkeley & Cooke. The pathogen can infect leaves and fruits of coffee tree, causing losses of 15% to 30% (GUIMARÃES; MENDES; BALIZA, 2010), and can affect the final product quality: consumers' cup of coffee (POZZA *et al.*, 2010). This adverse effect on quality is due to change in staining and, as proportion of diseased grains increases, a reduction in grain size, total sugars, total solids, and pH (LIMA; POZZA; SANTOS, 2012).

In order to reduce this infection and to guarantee quality to final product, adequate management strategies for cercosporiosis control must be employed. Therefore, it is necessary to know how the pathogen germinates and penetrates the fruit, whether by stomata or wounds, or by appressoria formation. The infection process of *C. coffeicola* has been frequently studied and characterized in foliar tissues of coffee (SOUZA *et al.*, 2011) but little is known about how this

process occurs in fruits. Infections occurrence during the early flowering stages has been frequently reported in the field; however there is not scientific confirmation. Our results explain how this pathogen penetrates the coffee fruit and helps to determine when coffee grain disease monitoring and biological control agents and fungicides application should start. The objective in this study was to describe the infection process of *Cercospora coffeicola* in coffee fruit.

The isolate of *C. coffeicola* used was CML 2489 and was deposited in mycological collection of Federal University of Lavras (UFLA) in Lavras, Minas Gerais, Brazil. Its sporulation was induced as described by Souza *et al.* (2011), using a conidia suspension of 6×10^4 conidia.mL⁻¹ to inoculate the pathogen. The trials were carried out with fruit obtained from detached branches of the middle third of cv. Catuaí IAC 144 and cv. Topázio sampled from the UFLA Coffee Growing Field in November 2013. It was used two branches of each cultivar with 10 fruit rosettes in F2 stage ('pin head' stage, with green color and 3.1 to 4 mm). It was

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performed the fruits disinfestation by immersion in 1 mg L⁻¹ sodium hypochlorite (twice), followed by rinsing with distilled water and drying on filter paper in a laminar flow chamber for 2 hours; afterwards, the samples were placed in a plastic tray.

The fruit branch cut site was introduced in moist phenolic foam and wrapped in plastic film, with nutrient solution from Hoagland and Arnon (1950), to ensure nutrition for the fruit during the experiment period. The inoculation was performed using 100 µL of conidial suspension, applied to F2 stage fruits surface, just after petals fell. The samples were incubated at 25 ± 2 °C and 90 ± 5% relative humidity with continuous white fluorescent light. The samples were processed to electron microscope analysis as done by Silva et al. (2017). Ten fruit units from each cultivar were collected at 4, 8, 12, 24, 36, 48, 72, 96, and 168 hours after inoculation (hai); fragments of 25 mm² were fixed with Karnovsky solution (pH 7.2) and post-fixed with 1% osmium tetroxide solution and dehydrated with acetone series. The specimens were dried in a Critical Point Apparatus (Baltec CPD 030 Balzers) followed by gold coating on a Sputter Coater Evaporator (Baltec SCD 050 Balzers). The acquisition of the images was performed with at the Electron Microscopy Lab in Federal University of Lavras using the Zeiss LEO EVO 40 XVP MEV and Smart Sem software, at 20 Kv and 9 mm working distance. The images obtained were edited using Corel Draw software.

Our analysis showed the germination of some *C. coffeicola* conidia on the coffee fruit surface at four hai (Figure 1A) and this was verified for both cultivars; germination occasionally showed two germ tubes (Figure 1A, arrows) and most of the conidia germinated at eight hai. The germ tubes grew towards the stomata in an attempt to penetrate them, but as they not clearly penetrate directed the sub-stomatal cavity, demonstrating a possibly absence of chemotropism to the stomata (Figures 1 B and C). However it was observed that the germ tubes clearly grew towards the wounds (Figures 1 D to G), thus showing a probable wounds chemotropism. In fact, the penetration of the pathogen occurred only through lesions on the fruits epidermis (Figures 1 D to G), for both coffee cultivars. Germ tubes developed and moved on open stomata but did not penetrate these natural openings (Figures 1 B and C). The penetration of *C. coffeicola* into the fruit occurred at 12 hai (Figure 1 D) and in subsequent evaluations: 24 hai

(Figures 1 E and F), 36 hai (Figure 1 G), and 168 hai (Figure 1 H). It was not observed appressoria formation, reinforcing the passive penetration hypothesis, by wounding. Samples evaluated at 168 hai had internal mycelial tissue colonization (Figure 1 H). No symptoms were observed during the evaluation.

In our work, the presence of germinative tubes in fruits at four hai was verified on the fruit epidermal surface. Similarly, Souza et al. (2011) observed *C. coffeicola* conidial germination both on adaxial and abaxial in coffee leaves surfaces at four hai. These authors also observed the growth of the germinative tubes towards stomatal openings, as occurred in the fruit; however, they observed penetration by stomata. They also verified that penetration into coffee leaves occurred at 36 hai and was mainly through the stomata on the epidermal surface or, occasionally, due to wounds in the epidermis, also without the appressoria formation. Some *Cercospora* species penetrate leaf tissues in this way, such as *C. arachidicola* (SMITH; PAUER; SHOKES, 1992), and *C. moricola* (GUPTA et al., 1995); however, *C. henningsii* penetrates directly through the epidermis of leaf tissues, and also without appressoria formation (BABU et al., 2009; PAULA et al., 2015). In this way, the infection process of *Cercospora* spp. may differ depending on the organ of the plant or the studied pathosystem.

The importance of infection process of *C. coffeicola* studies in immature fruit at young stages, shortly after the dry petals fall, is evidenced with the present results because certain species of cercosporioides fungi can go through long periods of latency; as example, *Pseudocercospora musae* has a latency periods around 40 days (FREITAS et al., 2017). That is, penetration occurs and symptoms are observed days or months after this process. Therefore, it is recommended starting control measures in the early fruiting stages from F2 to F5. The germination of *C. coffeicola* on the surface of the fruit from coffee cv. Catuaí IAC 144 and Topázio in the F2 stage is observed at four hai and its penetration only occurs by wounds in the epidermis and after 12 hai. Additionally, its colonization in the fruit's internal tissues occurs after 72 hai.

In order to evaluate brown eye spot in yellow and red coffee fruits, diagrammatic scales were validated by Paula et al. (2016). These scales are important to assessing the severity progress in already infected fields and compare control treatments.

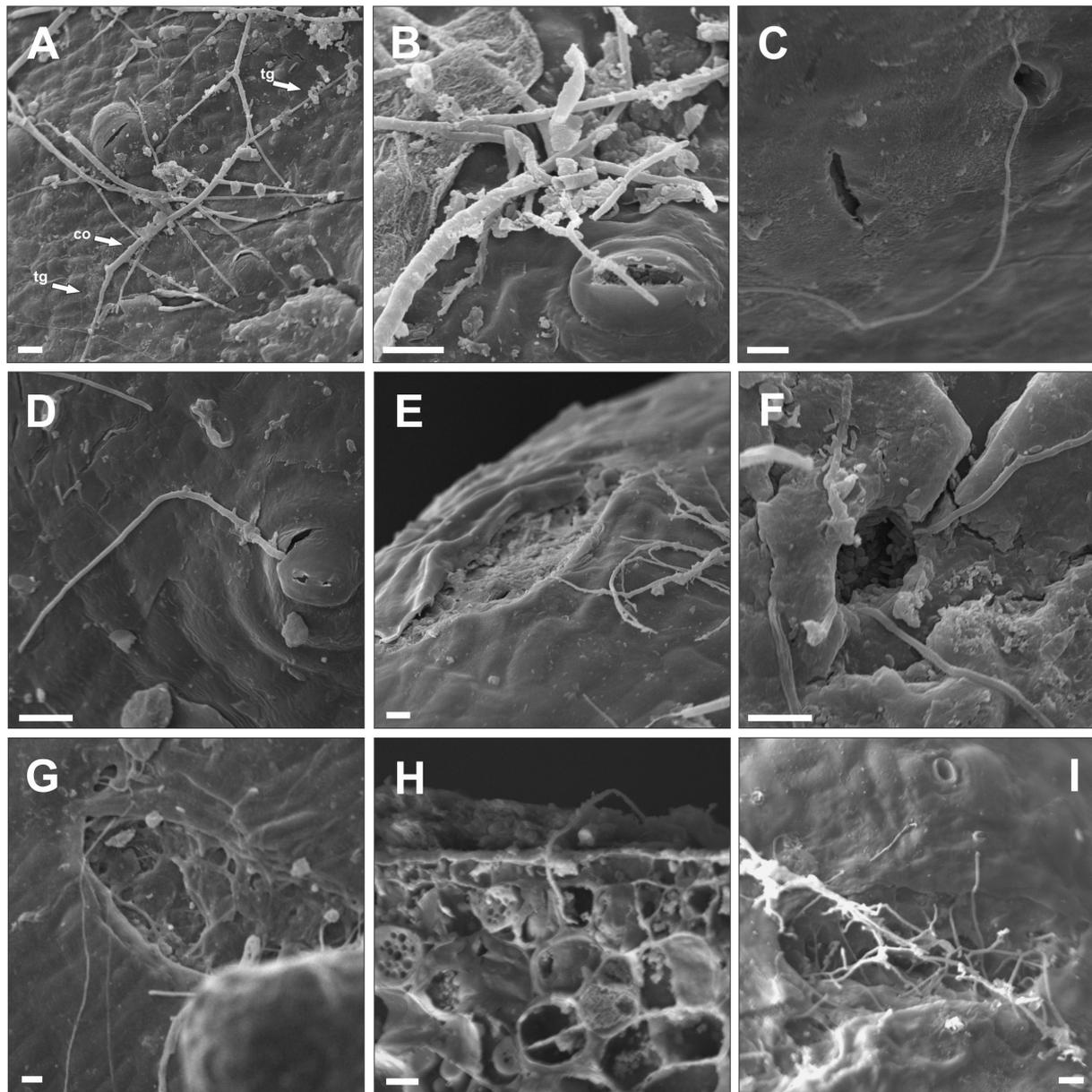


FIGURE 1 - Scanning Electron Micrographs. **A.** Germination of *Cercospora coffeicola* conidia four hours after inoculation (hai) in fruits of coffee at F2 stage. **B** and **C.** Growth of germinative tubes on stomata at eight hai. Penetration by wound at 12 (**D**), 24 (**E** and **F**) and 36 (**G**) hai. **H.** Cross-section of a penetration through injury at 168 hai. **I.** Internal mycelial colonization of tissues by *C. coffeicola* at 72 hai. Co: conidium, tg: germ tube. Scale bars = 10 μ m.

However, once the latent infection is possible in immature fruits, the preventive control is required, especially under optimal disease conditions, such as mild temperatures, high relative humidity, soaked soil, cold winds, nutritional deficiencies (GODOY; BERGAMIN FILHO; SALGADO, 2016) and intense sunlight, which may increase cercosporin production (SILVA et al., 2016). The control methods to reduce the initial

C. coffeicola inoculum include avoiding favorable conditions, cultural management (VASCO et al., 2015), chemical and or biological control (GODOY; BERGAMIN FILHO; SALGADO, 2016). In addition, it is possible that the use of resistant varieties contributes to the reduction of disease severity, and in consequence, fruit infection by the pathogen. (BOTELHO et al., 2017).

The *Cercospora coffeicola* infection in immature coffee fruits is very important, and implies in brown eyespot control management anticipation need, even before the first symptoms. Thus, the control actions should be preventive, since it is only possible to observe this infection through microscope.

ACKNOWLEDGMENTS

The authors thanks the FAPEMIG (Minas Gerais Research Foundation), the CAPES (Brazilian Federal Agency for the Support and Evaluation of Graduate Education), and the CNPq (National Council of Technological and Scientific Development) for financial support.

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