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ORIGINAL ARTICLE

Cladosporium spp. associated with black crust on rubber trees in Brazil

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ABSTRACT

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In recent years, a disease called black crust has caused damage to rubber trees in Brazil, since it leads to rapid leaf fall and consequently low latex production. Until the 1990s, black crust was associated with the fungi *Phyllachora huberi* and *Rosenscheldiella heveae*, but in 2022, after analysis of samples collected in the states of Minas Gerais and São Paulo, different species of the fungus *Cladosporium* ssp. were isolated from rubber tree leaves showing black crust in their leaflets. After morphological and phylogenetic analysis, using partial sequences of ITS-5.8S rDNA, α-elongase and actin regions, the isolates were identified as *Cladosporium pseudocladosporioides, C. tenuissimum, C. cladosporioides, C. perangustum* and *C. xanthochromaticum.* Pathogenicity tests with these isolates, in a greenhouse, demonstrated rapid appearance of symptoms such as apical death, damage to stems, intense defoliation and presence of black crust on stems and leaves. All isolates were pathogenic but had variable aggressiveness. This is the first report of *Cladosporium* causing black crust on rubber trees in Brazil.

Keywords: *Hevea brasiliensis* Müell, fungus, defoliation.

RESUMO

Gomes, M.E.; Antonio, G.L.; Oliveira, T.L.; Perfeito, D.M.; Anjos, L.V.S.; Gonçalves, E.C.P.; Fischer, I.H.; Scaloppi Junior, E.J.; Furtado, E.L.; Firmino, A.C. ***Cladosporium* spp. associado a crosta negra em seringueira no Brasil**. ***Summa Phytopathologica,*** v.49, p.1-3, 2023.

Nos últimos anos, uma doença chamada crosta negra tem causado danos à seringueira no Brasil, pois esta doença causa a rápida queda das folhas e como consequência a baixa produção de látex. Até a década de 1990, a crosta negra era associada aos fungos *Phyllachora huberi* e *Rosenscheldiella heveae*, mas em 2022, após análise de amostras coletadas nos estados de Minas Gerais e São Paulo, diferentes espécies do fungo *Cladosporium* ssp. foram isoladas de folhas com crosta negra em folíolos de seringueira. Após análise morfológica e filogenética, com sequências parciais das regiões ITS-5.8S rDNA, α-elongase e actina, os isolados foram identificados como *Cladosporium pseudocladosporioides*, *C. tenuissimum*, *C. cladosporioides*, *C. perangustum* e *C. xanthochromaticum*. Testes de patogenicidade com estes isolados, em casa de vegetação, demonstraram o rápido aparecimento de sintomas como morte apical, danos aos caules, intensa desfolha e presença de crosta negra nos caules e folhas. Todos os isolados foram patogênicos, porém com agressividade variada. Este é o primeiro relato de *Cladosporium* ssp. causando crosta negra em seringueiras no Brasil.

Palavras-chave: *Hevea brasiliensis* Müell, fungo, desfolha.

Black crust has been a cause of concern for the rubber industry due to formation of leaf spots and induction of early leaf fall. The main symptoms of this disease are circular black plaques on the abaxial surface and chlorotic areas on the adaxial surface of leaflets. There was an approximately 38% drop in latex production in Bahia due to this disease affecting rubber trees (8).

Phyllachora huberi was the first fungus associated with black crust attacking rubber tree crops, in 1899, in the Amazon region. In 1990, *Rosenscheldiella heveae* was also associated with this same disease. Simultaneously, isolates of *Cladosporium* were obtained from black crust lesions, but no in-depth studies were conducted for this case. Since then, there have been few updates on the disease because it was considered secondary for some years (3, 4, 5).

Studying the pathogens associated with black crust is essential to understand the epidemiology and to develop management tools for this disease. Therefore, the current study aims to identify *Cladosporium* isolated from black crust affecting rubber trees in two important cultivation regions. In 2022, attacked leaves of clone RRIM600 were collected from rubber tree crops severely affected by black crust in the cities of Campina Verde (Minas Gerais State) and Barretos (São Paulo State). Those leaves were taken to the laboratory for fungal isolation according to the method described by Junqueira & Bezerra (5).

Ten isolates of the genus *Cladosporium* were obtained: one from Minas Gerais (CN4) and nine from São Paulo (CN6, CN8, CN10, CN11, CN12, CN17, CN22, CN24 and CN26). These isolates were used for pathogenic and molecular characterization. Pathogen inoculation in the seedlings was carried out by spraying 50mL of a suspension of 10^6 conidia.mL^{-1} *Cladosporium*, obtained from Petri dishes, in which the isolates were grown in PDA medium. Following inoculation, the seedlings were incubated at 25 ± 2ºC for 24 hours under high humidity conditions (above 80% RH) in the laboratory. Subsequently, the seedlings were taken to the greenhouse and irrigated daily. Each isolate was inoculated into three plants. The experiment was repeated twice and conducted in a completely randomized design. Symptom assessment was performed weekly for six months. Fragments of tissues with symptoms were collected for re-isolation of the fungus.

After confirming the pathogenicity of isolates, DNA was extracted from the structures formed by the fungus in a Petri dish, according to the method developed by Murray & Thompson (6) with modifications. The obtained DNA was sequenced for the identification of isolates. Such identification was based on the regions ITS-5.8S rDNA, part of the actin genes (act) and translation elongation factor 1-alpha (tef1), according to the methodology described by Bensch et al. (2). The obtained sequences were edited with the software Mega 11.0 and compared with those deposited in GenBank (7).

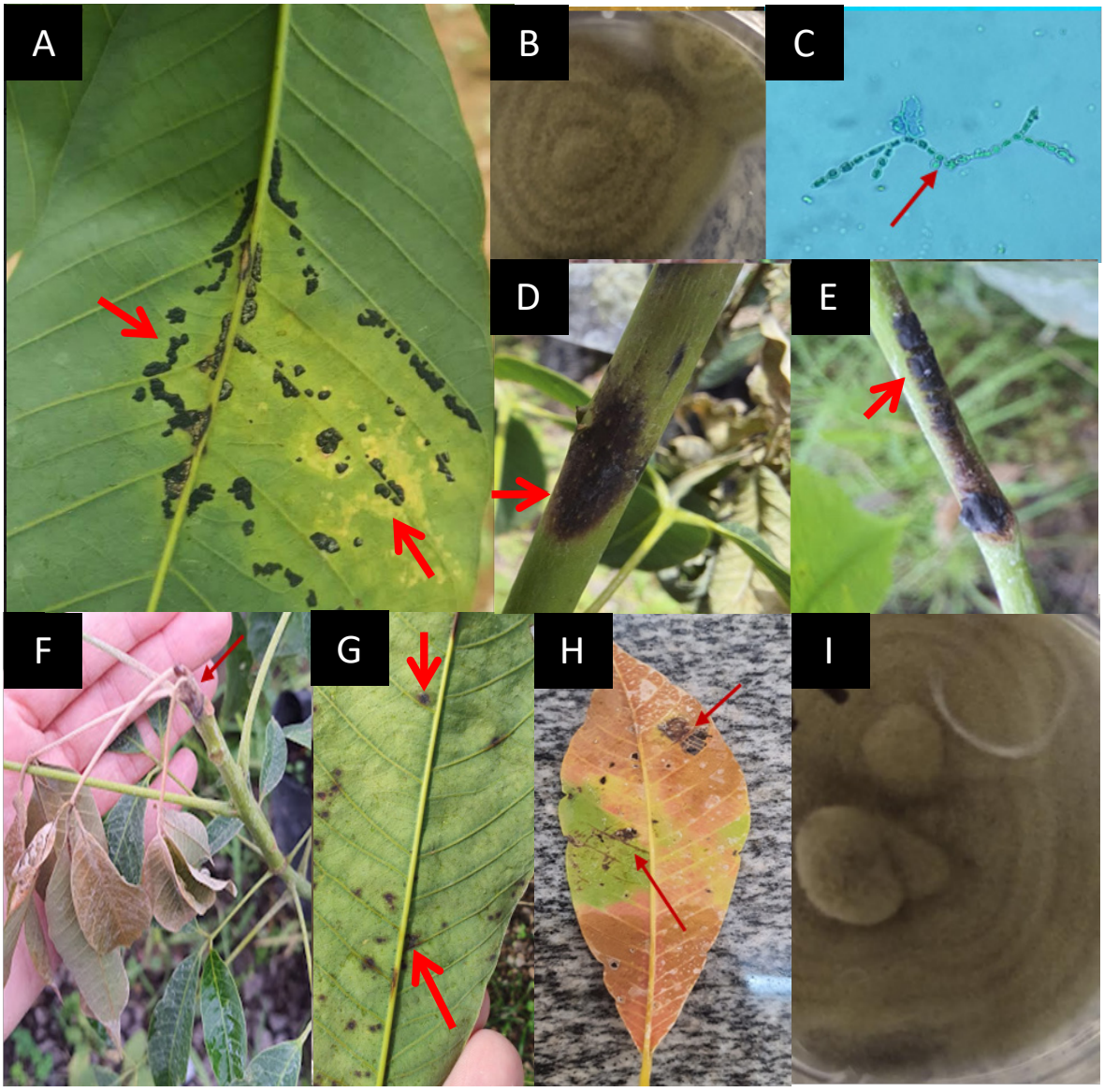

Figure 1: **A:** Symptoms in the field. **B and C**: *Cladosporium* colony (isolate CN4) and spores of the fungus used in pathogenicity tests; **D and E**: Symptoms of black crust on stalks after 30 days of inoculation. **F**: Symptoms of stem lesions and apical death of seedlings after 15 days of inoculation; **G and H:** Symptoms of black crust and "green islands" on the leaves after 120 days of inoculation, **I:** Colony of *Cladosporium* re-isolated from leaves with black crust.

All isolates were pathogenic but had variable aggressiveness. The isolates CN4, CN10, CN12, CN17, CN22 and CN26 caused apical death, defoliation and injury to the stalk, stem and leaf of plants (Figure 1) after 15 days of inoculation on average, while the other isolates only caused leaf damage after 30 days of inoculation. After 120 days of inoculation, seedlings started to show small black crusts, characteristic of this disease, and "green islands'' around the black crusts. Fungi of the genus *Cladosporium* were re-isolated from tissue fragments that presented lesions (Figure 1).

The variation in symptoms between isolates can be explained based on two factors. The first factor is associated with the temperature in the greenhouse during the studies, which was around 38°C, as measured by internal thermometers. The high temperatures may have influenced the appearance of typical symptoms of the disease, since black crust only started to appear in early June, when temperatures are lower in Dracena region. This hypothesis corroborates the data obtained by Anjos (1), who found that this fungus prefers milder temperatures for its development.

The second factor is the genetic variation of isolates. According to genetic analysis, five species of *Cladosporium* (*C. pseudocladosporioides, C. tenuissimum, C. cladosporioides, C. perangustum* and *C. xanthochromaticum*) were found, corroborating the morphological analyzes performed by Bensch et al. (2). Four of five isolates (CN6, CN8, CN11 and CN24) that belong to the species *C. xanthochromaticum* showed late and less aggressive symptoms. It is important to emphasize that five different species were found in a set of ten isolates. On a farm in the city of Barretos, all five species were identified, indicating the high genetic diversity of this fungus within a small geographic space. This shows the importance of greater geographic coverage for the screening of black crust, since this fungus has rapidly spread and adapted to different regions of Brazil (8).

This is the first report of *C. pseudocladosporioides, C. tenuissimum, C. cladosporioides, C. perangustum* and *C. xanthochromaticum* associated with black crust in rubber trees.

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