UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

FACULDADE DE CIÊNCIAS AGRONÔMICAS
UNESP | BOTUCATU

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CROSTA-NEGRA DA SERINGUEIRA: resistência clonal e identificação do agente causal

Botucatu

2024

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Tese apresentada à Faculdade de Ciências Agronômicas da Unesp Câmpus de Botucatu, para obtenção do título de Doutor em Agronomia (Proteção de Plantas).

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Coorientador(a): Prof. Drª Ana Carolina Firmino

Botucatu

2024

A635c

Antonio, Gabriel Leonardi
CROSTA-NEGRA DA SERINGUEIRA : resistência clonal e identificação do agente causal / Gabriel Leonardi Antonio. -- Botucatu, 2024
68 p. : il., tabs., fotos

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agronômicas, Botucatu
Orientador: Edson Luiz Furtado
Coorientadora: Ana Carolina Firmino

1. Fitopatologia. 2. Patologia Florestal. 3. Doenças da Seringueira. 4. Crosta-negra. 5. Reisistência Clonal. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agronômicas, Botucatu. Dados fornecidos pelo autor(a).

Impacto potencial desta pesquisa

A crosta-negra, doença foliar que acomete a seringueira (*Hevea brasiliensis* Muell.), foi descrita inicialmente em 1899, na região amazònica, tendo como agente causal o fungo *Phyllacora huberi.* Em 1990 o fungo *Rosenscheldiella heveae* também foi associado a doença. A doença se manifesta por meio de áreas cloróticas acompanhadas de pontos necróticas na face superior dos folíolos e por incrustrações estromáticas dos patógenos na face inferior, que formam placas negras. Com o passar do tempo a doença provoca queda precoce de folhas e redução da produtividade dos seringais. No Estado da Bahia foram relatadas perdas de produtividade de até 38% em decorrência da doença, além disso a crosta-negra tem sido observada com frequência em seringais do Estado São Paulo, nas principais regiões produtoras. A identificação dos agentes causais foi realizada há mais de três décadas, porém sem a utilização de ferramentas de biologia molecular. Além disso, existem poucas informações na literatura sobre a reação dos clones mais utilizados no Brasil a esses patógenos e acerca das condições climáticas ideais para o desenvolvimento da doença. O presente trabalho demonstrou que a doença pode estar relacionada a gêneros de fungos ainda não relatados como agentes causais da crosta-negra, identificou clones com alto potencial produtivo que apresentam diferentes graus de resistência a doença e constatou que períodos de temperaturas amenas antecedidos por precipitações pluviométricas favorecem a doença. Todas essas informações podem auxiliar os produtores de seringueira no manejo da doença e contribuir para melhorar o entendimento sobre a resistência genética e epidemiologia da crosta-negra.

Potential Impact of this research

Black crust, a foliar disease that impacts the rubber tree (*Hevea brasiliensis* Muell.), was first documented in 1899 in the Amazon region, with the fungus *Phyllacora huberi* identified as the causative agent. In 1990, another fungus, *Rosenscheldiella heveae*, was also associated with this disease. This disease presents itself with chlorotic areas and necrotic spots on the upper surface of the leaflets. On the lower surface, there are stromal incrustations of the pathogens that develop into black plaques. Over time, this disease results in early leaf drop and decreases the productivity of rubber plantations. In Bahia, the disease resulted in reported productivity losses of up to 38%. Additionally, black scab has been frequently observed in rubber plantations in São Paulo, a major producing region..The identification of the causal agents occurred more than two decades ago, but it was conducted without the use of molecular biology tools. Unfortunately, there is limited information available in the literature regarding the response of the primary commercial clones used in Brazil to these pathogens and the optimal climatic conditions for disease development. The current study has shown that the disease could be linked to fungal genera previously unreported as causal agents of black crust. It has also identified high-productivity clones that exhibit varying degrees of resistance to the disease. Additionally, the study found that periods of mild temperatures preceded by rainfall are conducive to disease development. This information can be invaluable for rubber producers in effectively managing the disease and can also contribute significantly to enhancing our understanding of genetic resistance and the epidemiology of black crust.

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: CROSTA-NEGRA DA SERINGUEIRA: resistência clonal e identificação do agente causal

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Botucatu, 01 de fevereiro de 2024

Aos meus pais, Carmen e Reinaldo, dedico

AGRADECIMENTOS

A minha irmã Luisa, por toda o carinho.

A minha namorada, Juliana, pelo carinho, amor e paciência durante esse período.

A toda minha família, tios, primos, padrinhos, sogros, cunhados e amigos.

Ao Prof. Dr. Edson Luiz Furtado, por sempre indicar o melhor caminho a se seguir.

A Prof. Drª. Ana Carolina Firmino, por todos os anos de parceria, ensinamentos e conquista, desde a graduação.

A todos os professores e funcionários do Departamento de Proteção de Plantas da FCA.

Aos motoristas da FCAT, Alan e Roberto, pelas viagens para a realização das coletas.

Aos alunos do laboratório de Fitopatologia da FCAT, Túlio, Marcela, Thaís, Louyne e Dhonatã pelo auxílio e parceria.

Aos doutorandos Ierli, Lisandro e Lucas pela ajuda durante o decorrer do curso.

Ao APTA, em nome dos pesquisadores Ivan e Scaloppi, e dos estagiários do Centro de Seringueira em Votuporanga, pela contribuição para o andamento da pesquisa.

As Repúblicas Buscapé e EterNaMent pelo período que passei durante o doutorado.

À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), pelo apoio financeiro no projeto (Processo n° (2020/11518-4)).

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil – CAPES – Código de financiamento 001.

“No começo pensei que estivesse lutando para salvar seringueiras, depois pensei que estava lutando para salvar a Floresta Amazônica. Agora, percebo que estou lutando pela humanidade”

GOOGLE homenageia seringueiro e ativista Chico Mendes. **O Povo**. Redação online. 15. jan. 2015. Disponível em: https://www.opovo.com.br/noticias/tecnologia/2015/12/google-homenageia-seringueiro-e-ativista-chico-mendes.html. Acesso em: 20 fev. 2024.

RESUMO

Crosta-negra é uma doença foliar da seringueira (*Hevea brasiliensis* Muell.), cujo os sintomas são a formação de placas negras com incrustações estromáticas dos agentes causais na parte abaxial dos folíolos que são refletidas através de áreas cloróticas e pontos necróticos na face adaxial dos tecidos afetados. O primeiro fungo relacionado a crosta-negra foi *Phyllacora huberi* P. Henn., em 1899, na região amazônica, posteriormente, em 1990, *Rosenscheldiella heveae* também foi relatado como agente causal. Por ser considerada uma doença secundária durante as últimas décadas, existem poucos estudos acerca da taxonomia dos agentes causais da doença, epidemiologia e métodos de controle da crosta-negra. Atualmente não há produtos registrados para o controle dessa enfermidade ou estudos acerca da resistência dos clones comerciais mais utilizados no Brasil. O objetivo desse estudo foi identificar fontes de resistência a crosta-negra, as condições climáticas que favorecem o desenvolvimento da doença e avançar nos estudos relativos a identificação dos agentes causais. Para isso, 21 clones de seringueira, implantados em jardim clonal, foram avaliados durante dois anos, em oito avaliações. Em cada avaliação foram coletados 45 folíolos de três plantas diferentes do mesmo clone, sendo que em cada planta eram retirados 15 folíolos do baixeiro, 15 do terço médio e 15 da copa da planta, totalizando 135 folíolos por clone em cada coleta. Os folíolos foram então levados ao laboratório, escaneados, e a porcentagem da área foliar com sintomas de crosta-negra avaliada através do software Leaf Doctor. Os resultados foram analisados por meio do teste Scott Knott a nível de probabilidade de 5%. Visando avaliar a influência climática no desenvolvimento da doença durante o período de avaliações, as informações meteorológicas da área do experimento, Votuporanga-SP, foram coletadas de uma estação meteorológica presente no local.. A identificação do agente causal ocorreu a partir de isolamentos regiões de tecidos lesionadas de folíolos do clone RRIM 600. Após os isolamentos, foram realizadas análises morfológicas por meio de microscopia ótica e moleculares através do sequenciamento parcial da região ITS-5.8S rDNA para determinar o gênero do fungo. Além disso foram conduzidos testes de patogenicidade em casa de vegetação com as fases sexuadas e assexuadas dos fungos isolados. Os clones IAC300, IAC 418, IAC503, PB311 e RRIM600 apresentaram as maiores porcentagens de área foliar com sintomas da doença, enquanto IAC511 não apresentou lesões e IAC301, IAC 411, IAC501, IAC507 e IRCA111 tiveram uma baixa porcentagem de área foliar lesionada. Também foi constatado que a doença se manifesta com maior severidade em períodos com precipitações pluviométricas seguidas de temperaturas amenas. Em relação ao agente causal, o fungo teleomórfico *Davidiella* sp. foi identificado como agente causal da crosta-negra. A fase anamórfica do patógeno corresponde a *Cladosporium* sp.

Palavras-chave: *Davidiella*; *Cladosporium*; resistência clonal; epidemiologia.

ABSTRACT

Black crust is a foliar disease that affects the rubber tree (*Hevea brasiliensis* Muell.). Its symptoms include the development of black plaques with stromal incrustations of the causal agents on the abaxial side of the leaflets. These symptoms are reflected through chlorotic areas and necrotic spots on the adaxial side of the affected leaflets. The first fungus related to the disease was *Phyllacora huberi* P. Henn., in 1899, in the Amazon region, later, in 1990, *Rosenscheldiella heveae* was also reported as the causal agent of black crust. Over the past few decades, black crust has been considered a secondary disease, and as a result, there has been limited research on the taxonomy of the disease's causal agents, its epidemiology, and methods for its control. Currently, there are no registered products for controlling this disease, and there are also no studies on the resistance of the most commonly used commercial clones in Brazil. The aim of this study was to identify sources of resistance to black crust, determine the climatic conditions that promote disease development, and make progress in research related to the identification of causal agents. To achieve this goal, 21 rubber tree clones planted in a clonal garden were assessed over a period of two years, with a total of eight evaluations conducted. During each evaluation, 45 leaflets were gathered from three distinct plants of the same clone. These leaflets included 15 from the bottom of the plant, 15 from the middle third, and 15 from the crown, resulting in a total of 135 leaflets collected per clone in each sampling. Subsequently, the collected leaflets were transported to the laboratory, where they were scanned, and the percentage of leaf area exhibiting black crust symptoms was assessed using the Leaf Doctor software. The obtained results were subjected to analysis using the Scott Knott test at a significance level of 5%. To assess the climatic impact on disease development during the evaluation period, meteorological data from the experiment area in Votuporanga-SP were collected from an on-site meteorological station. The causal agent was identified through the isolation of tissue regions from leaflets of the RRIM 600 clone. After the samples were isolated, morphological analyses were performed using optical microscopy, and molecular analyses were conducted by partially sequencing the ITS-5.8S rDNA region to determine the fungus's genus. Furthermore, pathogenicity tests were carried out in a greenhouse using the sexual and asexual phases of the isolated fungi. Clones IAC300, IAC 418, IAC503, PB311, and RRIM600 exhibited the highest percentages of leaf area with disease symptoms, while IAC511 displayed no lesions. Additionally, IAC301, IAC411, IAC501, IAC507, and IRCA111 had a low percentage of lesioned leaf area.. It was also found that the disease manifests itself more severely in periods with rainfall followed by mild temperatures. Regarding the causal agent, the teleomorphic fungus *Davidiella* sp. was identified as the causal agent of black crust. The anamorphic phase of the pathogen corresponds to *Cladosporium* sp.

Keywords: *Davidiella*; *Cladosporium*; clonal resistance; epidemiology.

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LISTA DE ABREVIATURAS E SIGLAS

IAC	Instituto Agronômico de Campinas
IRCA	Institute de Recherches sur le Caoutchouc
PB	Prang Besar
PC	Promotion Clone
PR	Proesfstation voor Rubber
RRIM	Rubber Research Institute of Malaysia

SUMÁRIO

INTRODUÇÃO GERAL

A seringueira (*Hevea brasiliensis* Müell. Arg.), é uma espécie arbórea pertencente a família Euphorbiaceae que tem como centro de origem a Amazônia (Alvarenga, 2021). Esta espécie possui grande importância econômica devido à produção de látex, fonte de borracha natural que é matéria prima para produtos como pneus e luvas cirúrgicas (Alvarenga, 2021).

O Sudeste Asiático concentra cerca de 90% da produção de borracha natural mundial, sendo Indonésia, Vietnã e Tailândia os principais países produtores (FAO, 2022). Segundo o IBGE a área de seringais no Brasil em 2021 era de 177,8 mil hectares com uma produção de borracha natural de 399,8 mil toneladas. Em 2021 a produção brasileira foi suficiente para atender 49% da necessidade do mercado nacional, sendo o restante importado (Brisolara; Barros; Amaral, 2023).

No Brasil, a área de seringais aumentou 84% entre 2003 e 2021 e atualmente o Estado de São Paulo concentra cerca de 70% da produção nacional de borracha natural (Brisolara; Barros; Amaral, 2023). Dentro do território brasileiro, a seringueira é cultivada em 17 estados, desde a região Norte até o norte do Estado do Paraná (Gasparotto *et al.*, 2012). A heveicultura é responsável por cerca de 80 mil empregos no Brasil, sendo que a cultura gera um posto de trabalho para cada 3,5 hectares cultivados, além disso os seringais possuem importância ambiental e proporcionam qualidade de vida aos produtores por meio da geração de renda (Scaloppi Junior; Freitas; Gonçalves, 2015; Braga, 2016).

Apesar de ser uma cultura em franca expansão, o preço da borracha natural é muito variável e em alguns anos produtores podem ter sua margem de lucro reduzida (Brisolara; Barros; Amaral, 2023). Além da variação do preço, outros fatores podem reduzir a produtividade e consequentemente a rentabilidade dos produtores, entre eles estão os problemas fitossanitários.

Doenças foliares como o mal-das-folhas, a antracnose, o oídio e a mancha aureolada, que tem como agentes causais *Pseudocercospora ulei*, *Colletotrichum* ssp., *Oidium heveae* e *Rhizoctonia solani*, respectivamente, estão entre os fatores que reduzem a produtividade dos seringais. Nos últimos anos, uma outra doença foliar, a crosta-negra, passou a acometer os seringais de forma expressiva.

Na literatura, os fungos *Phyllachora huberi* e *Rosenscheldiella heveae* são associados a doença. *P. huberi* foi descrito como patógeno de seringueira por P. Henn em ,1899, na região amazonica, e *R. heveae* por Junqueira e Bezerra (1990). (Stradioto Papa *et al.*, 2013; Gonçalves; Goes, 2017). Porém, nesses trabalhos são utilizadas apenas observações morfológicas.

A manifestação mais significativa da crosta-negra passou a ocorrer devido às mudanças climáticas e fatores como expansão dos seringais para diferentes localidades com diferentes condições ambientais, com isso essa enfermidade passou a causar prejuízos devido à queda precoce das folhas, fato que pode reduzir em até 38% a produção de borracha (Stradioto Papa *et al.*, 2013; Gonçalves; Goes, 2017; Virgens Filho *et al.*, 2022).

Os sintomas da doença são expressos através de áreas cloróticas e pontuações necróticas da face adaxial dos folíolos, que são acompanhadas pela presença de placas negras circulares na face abaxial dos folíolos. Nessas placas estão presentes incrustações estromáticas que contém estruturas reprodutivas dos agentes causais (Gasparotto *et al.*, 2012; Gomes *et al.*, 2023). A ocorrência da crosta-negra facilita a entrada de outros patógenos, como *Colletotrichum*, devido as fissuras provocadas pelas placas negras secas no tecido foliar (Bezerra, 2020; Gasparotto *et al.*, 2012).

Diversos países da América do Sul como Venezuela, Trinidad, Colômbia, Bolívia, Peru e Suriname possuem relatos da doença (Sterling; Rodriguez, 2018).

No Brasil, ela era frequentemente encontrada no Acre, Amapá, Amazonas, Pará, Rondônia e Mato Grosso, porém em 2011 a crosta-negra foi registrada em São Paulo, na Região de Tupã. Em seguida, no ano de 2012, ela foi observada, em muitas áreas, nas regiões de Votuporanga, Barretos e São José Do Rio Preto (Gonçalves; Goes, 2017). Na Bahia a crosta-negra é apontada como uma das causas da queda precoce de folhas da seringueira, levando a perdas de produtividade de até 38% (Virgens Filho *et al.*, 2022).

Os novos relatos da doença no campo trazem a necessidade de novos estudos sobre a identificação do(s) agente(s) causal(is) e da busca de alternativas para o manejo da crosta-negra. Atualmente, não há fungicidas registrados para o controle dessa doença no Brasil (Agrofit, 2023) e nem estudos de resistência com os clones mais cultivados no país.

Devido a esses fatores, essa pesquisa teve como objetivo avançar na caracterização de fungos associados a crosta-negra da seringueira, buscar fontes de resistência em clones comercias e analisar a influência das condições climáticas no desenvolvimento da doença.

CAPÍTULO 1

RUBBER TREE RESISTANCE TO BLACK CRUST: EVALUATION IN CLONAL GARDEN[1]

ABSTRACT

The occurrence of black crust, a disease previously considered secondary, has become a cause for concern due to early leaf fall in rubber trees (*Hevea brasiliensis*). This study aimed to identify sources of resistance to black crust in 21 clones of rubber trees in a clonal garden. To achieve this, the disease severity was evaluated through eight assessments conducted over a two-year period. In each evaluation, fifteen leaflets from three different parts of the plant (bottom, middle third, and crown) were randomly collected, resulting in a total of 45 leaflets per plant. These leaflets were then taken to the laboratory, and the software Leaf Doctor was used to measure the percentage of leaf area exhibiting symptoms of black crust. The results were analyzed using the Scott Knott Test at a 5% probability level. The clones with the highest infected leaf area observed during the evaluations were IAC300, IAC418, IAC503, PB311, and RRIM600. Clone IAC511 did not show any symptoms of the disease in any of the evaluations. Clones IAC301, IAC411, IAC501, IAC507, and IRCA111 also demonstrated some resistance to the disease, as they exhibited low percentages of affected leaf areas. Additionally, this study revealed the progression of the disease over the evaluation period. The infection initially starts on the youngest leaves (crown) in March and subsequently intensifies in the middle third of the plant, corresponding to the youngest leaf previously infected in the crown. Furthermore, the correlation of climatic data collected in the experimental area over the two-year evaluation period indicated that the disease manifests with greater severity when the average temperatures fall below 25 ºC after periods of heavy rainfall, followed by dry periods with low relative humidity. The data presented in this study significantly contribute to the management of this disease in the field by identifying resistant materials and enhancing our understanding of the pathogen's life cycle.

Keywords: *Clonal reaction, Cladosporium, Hevea brasiliensis.*

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1 Capítulo redigido de acordo com as normas do periódico **Forest Pathology**

1.1 INTRODUCTION

The rubber tree (*Hevea brasiliensis* Muell.) is a thriving crop in Brazil, experiencing an 84% growth in planted area between 2003 and 2021 (Brisolara; Barros; Amaral, 2023). Rubber farming contributes to approximately 80,000 direct jobs. Beyond its economic significance, this crop holds great environmental importance in Brazil, being the species' origin center (Scaloppi Junior; Freitas; Gonçalves, 2015). Latex extracted from the rubber trees serves as a raw material for various products, including tires and surgical gloves.

In Brazil, the rubber tree contends with several foliar diseases, including South American leaf blight (caused by *Pseudocercospora ulei*) and anthracnose (caused by *Colletotrichum* spp.) (Furtado; Gasparotto; Pereira, 2016). São Paulo boasts the largest latex production in Brazil, and recently, key producing regions in the state, including municipalities like Votuporanga, São José do Rio Preto, and Barretos, have experienced the emergence of a disease known as black scab, caused by *Cladosporium* sp. (Somain; Droulers, 2016; Gonçalves; Goes, 2017; Gomes *et al.*, 2023) This disease has been associated with productivity losses of up to 38% in the state of Bahia (Viirgens Filho *et al.*, 2022).

The symptoms of black crust are chlorotic areas and necrotic spots on the upper surface (adaxial part) of the leaflets, which correspond to the black circular plaques found on the lower surface (abaxial part). These plaques coincide with the fungal stromal incrustations (Gasparotto *et al.*, 2012). The occurrence of black crust facilitates the entry of other pathogens, such as *Colletotrichum*, as the drying black plaques cause leaf tissue rupture, providing a gateway for other pathogens (Gasparotto *et al.*, 2012).

Currently, no chemicals are registered for controlling this disease on rubber trees in Brazil (Agrofit, 2023). Nonetheless, some studies suggest the effectiveness of applying certain fungicides, such as benomyl (0.075%), triadimefon (0.025%), and tebucanozol (a mixture of strobirulin, azoxystrobirulin, and a triazole), in controlling this disease in clonal gardens (Trindade *et al.*, 1999 cited in Vigens Filho *et al.*, 2022; Cabral Júnior, 2020). However, chemical control of rubber tree diseases in the field has certain restrictions. Effective fungicide action requires homogeneity in leaf area or crown development. Treatment should be initiated before the epidemic advances too far, and application needs to be fast and accurate (Guyot *et al*., 2001).

Utilizing resistant clones emerges as the optimal choice for managing black crust. Researchers conducted studies in the southern region of Bahia and in French Guiana, identifying several clones that showed resistance to black crust (Virgens Filho, 2022; Le Guen; Seguin; Mattos, 2000). These findings demonstrate that genetic control represents a potential alternative for managing the disease.

In response to the need for further studies on the genetic control of black crust, this study aimed to evaluate the reaction of different clones in a clonal garden regarding their resistance to black crust. The study was based on the main clones studied by Antonio *et al.* (2021), who identified anthracnose-resistant clones. The study aimed to identify clones with multiple resistance to reduce control costs for rubber tree producers.

1.2 MATERIAL AND METHODS

The experiment was installed at the Center of Rubber Tree and Agroforestry Systems, Agronomic Institute - IAC in the city of Votuporanga (20°25’13’’S, 49°58’42’’W), São Paulo State. During the experiment, data on average, minimum and maximum temperatures, humidity and precipitation were collected, always considering the 30 days prior to the collection of leaflets, at a meteorological station located close to the leaflet collection area. The data were used to assess the influence of climatic factors on the development of the disease.

The evaluated plants are established in the clonal garden and have an average age of 8 years. They are planted with a spacing of 1.5 m between rows and 0.7 m between individual plants. The arrangement ensures that each planting line features only one clone. No pruning occurred in the clonal garden during the evaluation period.

The study area has a documented history of black crust occurrence. Consequently, the evaluations were conducted in the natural field conditions, without the requirement of artificial inoculations.

A total of twenty-one rubber tree clones were included in the evaluation. Among them, one clone, RRIM 600, is commonly used by producers and is known to be susceptible to the disease. The remaining twenty clones, namely IAC 300, IAC 301, IAC 406, IAC 410, IAC 411, IAC 412, IAC 418, IAC 500, IAC 501, IAC 502, IAC 503, IAC 505, IAC 506, IAC 507, IAC 511, IAC 512, IRCA 111, PB 311, PB 350, and PC 119, show promise for field cultivation.

The severity of black crust was determined by assessing the percentage of leaf area displaying disease symptoms. Leaflets were collected over a period of two years, with a total of eight collections conducted in the months of March 2021, May 2021, August 2021, November 2021, February 2022, May 2022, August 2022, and November 2022. For each collection, fifteen leaflets were randomly collected from three different locations on the plant: the bottom, middle third, crown. This resulted in a total f forty-five leaflets per plant and one hundred and fifteen leaflets per clone in each collection.

The leaflets were collected from three different plants of each clone. To ensure consistency, the plants used for data collection were marked for subsequent collections. The three selected plants were placed at varying positions within the planting line: one near an end, another in the center, and the last closer to the opposite end. The first 4 plants at each end were not considered for potential marking to eliminate the border effect.

The collected leaflets from the clonal garden were transported to the laboratory for further analysis. In the laboratory, the leaflets were scanned, and the percentage of area exhibiting disease symptoms was measured using the Leaf Doctor program (Pethybridge; Nelson, 2015). The data on the percentage of injured leaf area were subjected to the Scott-Knott test at a 5% probability level using the SISVAR program (Ferreira, 1998). Comparisons were conducted for clones, plant parts, and the interaction of these factors in each collection and overall.

1.3 RESULTS AND DISCUSSION

The evaluation results revealed that several clones exhibited different levels of susceptibility to black crust disease. Among the evaluated clones, IAC300, IAC418, IAC503, PB311, and RRIM600 exhibited higher percentages of disease-affected leaf areas (Table 1, Figure 1). On the other hand, in the clone IAC 511 no disease symptoms were observed in any of the collected samples across all periods. Notably, clones IAC301, IAC411, IAC501, IAC507, and IRCA111 exhibited low percentages of disease-affected leaf areas (Table 1, Figure 1).

Furthermore, it is worth mentioning that the clones IAC511 and IAC507 possess the additional advantage of higher productivity compared to the commonly planted clone in São Paulo, RRIM600 (Scaloppi Junior; Freitas; Gonçalves, 2015).

The clones belonging to the IAC500 series evaluated in this experiment have favorable productive characteristics such as latex production precocity, occurring at five and a half years, and adequate stem perimeter for tapping. Clones IAC507 and IAC511 exhibit a greater increase in stem perimeter even after the start of exploitation, indicating sustained growth during the exploitation period (Scaloppi Junior; Freitas; Gonçalves, 2015).

These clones also exhibited lower severity rates in an experiment evaluating leaf anthracnose (Antonio *et al.*, 2021). This characteristic is advantageous for the management of foliar diseases in rubber trees plantations.

Conversely, the results suggest that clones with high productive potential, like IAC 418 and IRCA 111, which boast average yields 46% and 32% higher than the RRIM 600 clone (Alem *et al.*, 2015; Gonçalves *et al*., 2007), respectively, exhibited a high percentage of injured leaf area.

A potential strategy for utilizing these clones in areas affected by black scab could involve polyclonal planting. In this approach, approximately four to five clones are planted in alternating rows (Gasparotto *et al.*, 2012). By intermixing susceptible and resistant clones, this method aims to decrease pathogen inoculum pressure compared to planting a single susceptible clone. The resistant plants act as a barrier to spore dispersal and reduce the number of reproductive structures of the pathogen (Rios *et al.*, 2021). Polyclonal planting serves as a cultural control method for South American leaf light (Gasparotto *et al.*, 2012).

According to Virgens Filho *et al.* (2022), in the black crust pathosystem, clones such as CMB 75, IAN 6590, SIAL 1005, and Fx 3864 exhibit resistance in small-scale clone competition experiments. However, these clones possess certain characteristics that hinder their commercial cultivation, including susceptibility to *P. ulei* and mites, vulnerability to wind damage, and low yield potential. As a result, these authors suggest utilizing these clones as a source of resistance in genetic improvement programs with the goal of disease control.

In the study conducted by Le Guen, Seguin and Mattos (2000), 200 clones were evaluated for resistance to black crust in field conditions. Out of these, 42 clones derived from *H. brasiliensis* and *H. benthamiana* were identified as resistant. However, the authors noted that some of the initially resistant clones became susceptible to the disease during the evaluation period. This variation in disease reaction, according to the authors, can be attributed to the interaction between genotype and the environment. Additional monitoring of black crust symptom development in 16 clones over a period of at least three years in French Guiana (Rivano, unpublished data, cited in Le Guen; Seguin; Mattos, 2000) revealed that clone resistance can vary depending on the planting location. For instance, clones IRCA 519 and RO 38, which exhibited resistance to black crust in Mato Grosso, were found to be susceptible in French Guiana.

Gomes (2023), conducted observations that revealed the presence of various *Cladosporium* species as the causal agents of black crust in different regions. These findings highlight the existence of diverse interactions between the pathogen and the host, which aligns with the observations made by Le Guen, Seguin and Mattos (2000). Presence of different *Cladosporium* species as causative agents of black crust in various regions implies the existence of regional variations in the disease dynamics and emphasizes the importance of considering local factors when studying and managing black crust.

Observing the disease progression (Figure 3), considering all clones, it becomes apparent that the peak severity occurred in May 2022.. In an experiment involving *Cladosporium* inoculation in rubber tree seedlings, Gomes *et al.* (2023) reported that stromal cavities started to appear after 120 days, coinciding with the peak of leaflet damage. Around 120 days before the May 2022 collection, there was a noticeable accumulation of precipitation in the period leading up to the February 2022 collection (Figure 3). Precipitation contributes to leaf wetness, which is highly correlated with the infection rate of the causal agent of black scab (Anjos, 2023), in addition, temperatures close to 20° and 25°C favor the development of the pathogen (Anjos, 2023) and they were observed in May 2022. Therefore, the infection may have started during the period with the greatest leaf wetness and developed until reaching its peak after 120 days.

This behavior was also noted in individual clones. RRIM 600 exhibited higher percentages of injured leaf area in the second year of evaluation (Tables 2 to 9), which may be attributed to elevated relative humidity and precipitation, along with temperatures closer to those conducive to pathogen development. It is recognized that under non-ideal environmental conditions, disease severity can decrease (Amorim; Rezende; Bergamin Filho, 2011).

In rubber trees, similar interactions have been previously reported in an experiment that examined the defoliation caused by *Colletotrichum* in different rubber-producing regions. The study found that clones GT1 and IAN 873 exhibited lower defoliation rates in the Matão region compared to Piracicaba, where they were highly susceptible to the pathogen. The authors attributed this variation in clone behavior to regional climatic conditions that differed between the two locations. These climatic conditions favored disease expression in the field, along with the presence of genetic variability within the fungus (Furtado *et al.*, 1994).

Table 1: Black crust severity (% diseased leaf area) in different rubber tree clones evaluated at different times.

Rubber tree clones	2021				2022			
	March*	May	August	November	February	May	August	November
IAC 300	0.05	0.35C	0.65C	3.24D	1.47 C	3.22 F	0.00 A	0.10 A
IAC 301	0.00	0.02 A	0.00 A	0.48B	0.20 A	0.36 A	0.45 A	0.00 A
IAC 406	0.00	0.14B	0.01 A	1.25C	0.50 A	1.30 C	1.31 C	0.94 C
IAC 410	0.00	0.16B	0.23B	0.76B	0.031A	0.24 A	0.33 A	0.68 B
IAC411	0.00	0.00 A	0.01 A	0.10 A	0.27A	0.55 A	0.06 A	0.17 A
IAC412	0.00	0.04 A	0.30B	0.00 A	0.18 A	0.28 A	2.93 E	1.06 C
IAC418	0.01	0.10B	0.03 A	0.21B	0.94 B	2.73 E	1.88 D	0.55 B
IAC500	0.00	0.00 A	0.05 A	0.15B	0.90 B	2.15 D	0.33 A	0.10 A
IAC501	0.00	0.00 A	0.00 A	0.37B	0.10 A	0.02 A	0.01 A	0.00 A
IAC502	0.00	0.02 A	0.03 A	0.15B	0.13 A	2.21D	0.30 A	0.14 A
IAC503	0.00	0.05 A	0.02 A	1.19C	1.13 B	2.63 E	0.93 B	0.00 A
IAC505	0.00	0.00 A	0.33B	0.17B	0.03 A	0.29 A	1.11 C	1.05 C
IAC506	0.00	0.00 A	0.00 A	0.03 A	0.01 A	0.86 B	1.17 C	0.00 A
IAC507	0.00	0.01 A	0.00 A	0.00 A	0.00 A	0.24 A	0.16 A	1.12 C
IAC511	0.00	0.00 A	0.00 A	0.00 A	0.00 A	0.00 A	0.00 A	0.00 A
IAC512	0.00	0.04 A	0.26 B	0.00 A	0.10 A	0.93 B	0.84 B	1.07 C
IRCA111	0.00	0.00 A	0.13 A	0.00 A	0.00 A	1.15 C	0.25 A	0.03 A
PB311	0.00	0.01 A	0.37 B	1.32C	0.40 A	2.56 E	1.54 D	0.21 A
PB350	0.00	0.02 A	0.06 A	0.28B	0.01 A	2.41 D	2.73 E	0.08 A
PC119	0.00	0.00 A	0.00 A	0.00 A	0.00 A	1.50 C	1.40 C	0.16 A
RRIM600	0.00	0.05 A	0.13B	0.05 A	0.83 B	4.35 G	1.29 C	0.67 B
CV	-	20.11	32.05	60.26	37.15	43.05	40.32	37.12

CV: coefficient of variation of data transformed into square root - SQRT (Y). The means followed by the same capital letters in the columns do not differ from each other in the Scott-Knott test at 5% probability. * there was no significant difference in the column by the 5% F test

INSERT FIGURE 1

Based on the analysis of the overall averages of the percentage of diseased leaf area in different parts of the plant and considering the data of the most susceptible clone (IAC300), the progression of the disease was observed over the two-year evaluation period (Figure 2; Tables 2 to 9) in various plant sections.

The youngest leaves (crown) show similar disease severity to the middle third in March/2021 to February/2022, however in May/2022 the severity is higher, this increase in severity is associated with the emergence of new shoots prompted by summer rains and the subsequent refoliation following natural senescence during the winter, respectively. This observation supports the pathogen cycle described by Gasparotto *et al.* (2012). According to their findings, newly formed leaves play a crucial role as an entry point for the fungus. The pathogen survives in the leaf debris on the ground, and under favorable environmental conditions, such as rainy periods, it releases its spores. As time progresses, the infected younger leaves in the crown become the middle third of the plant, which could explain the higher disease severity observed in this plant section.

The black crust occurrence, in general, was more pronounced in the middle third of the plant, particularly when compared to the lower portion, where there is minimal or no disease presence. Various external factors, including microclimate influences, lower light intensity and higher concentration of old leaves in the bottom of plants, may have contributed to this disparity. Leaves in a more advanced phenological stage tend to exhibit resistance or reduced susceptibility to pathogens. In this case, when the leaf reaches maturity, several genes potentially associated with defense mechanisms are expressed (Kus *et al.*, 2002). These factors could explain the lower infection rate observed in the lower part of the rubber tree, as these leaves are the oldest.

INSERT FIGURE 2

On general, clones with varying percentages of leaf area affected by black scab were identified. IAC 511 showed no lesions, while IAC301, 411, 501, 507, and IRCA111 displayed a low percentage of injured leaf area, making them potential alternatives for disease management. Furthermore, it provided insights into the behavior of the fungus in the field, highlighting its greater severity in younger leaves, particularly in the middle third of the plants, during periods with mild temperatures. These observations lay the foundation for further research involving clones that displayed a low disease percentage and contribute to the enhancement of evaluation methods based on the plant's growth stage.

Table 2: Black crust severity (% diseased leaf area) in different parts of rubber tree clones in March 2021.

Rubber tree clones	Part of the plant		
	Crown**	Middle third	Bottom**
IAC 300	0.0 a	0.16 Bb	0.0 a
IAC 301*	0.0	0.0 A	0.0
IAC 406*	0.0	0.0 A	0.0
IAC 410*	0.0	0.0 A	0.0
IAC411*	0.0	0.0 A	0.0
IAC412*	0.0	0.0 A	0.0
IAC418	0.0 a	0.0 Aa	0.24 b
IAC500*	0.0	0.0 A	0.0
IAC501*	0.0	0.0 A	0.0
IAC502*	0.0	0.0 A	0.0
IAC503*	0.0	0.0 A	0.0
IAC505*	0.0	0.0 A	0.0
IAC506*	0.0	0.0 A	0.0
IAC507*	0.0	0.0 A	0.0
IAC511*	0.0	0.0 A	0.0
IAC512*	0.0	0.0 A	0.0
IRCA111*	0.0	0.0 A	0.0
PB311*	0.0	0.0 A	0.0
PB350*	0.0	0.0 A	0.0
PC119*	0.0	0.0 A	0.0
RRIM600*	0.0	0.0 A	0.0
CV	4.28		

CV: coefficient of variation of data transformed into square root - SQRT (Y). Means followed by the same uppercase letters in the columns and the same lowercase letters in the rows do not differ in the Scott-Knott test at 5% probability. * there was no significant difference in the row by the 5% F-test in the row.** there was no significant difference in the column by the 5% F-test.

Table 3: Black crust severity (% diseased leaf area) in different parts of rubber tree clones in May 2021.

Rubber tree clones	Part of the plant		
	Crown	**Middle third****	**Bottom**
IAC 300	0.52 Cb	0.2A a	0.31 Ba
IAC 301*	0.02 A	0.03	0.0 A
IAC 406*	0.38 C	0.05	0.0 A
IAC 410	0.4 Cb	0.03 a	0.07 aA
IAC411*	0.0 A	0.0	0.0 A
IAC412*	0.1 A	0.03	0.0 A
IAC418*	0.22 Ab	0.0 a	0.07 Aa
IAC500*	0.0 A	0.0	0.0 A
IAC501*	0.0 A	0.0	0.0 A
IAC502*	0.0 A	0.07	0.0 A
IAC503*	0.0 A	0.14	0.0 A
IAC505*	0.03 A	0.08	0.0 A
IAC506*	0.0 A	0.0	0.0 A
IAC507*	0.02 A	0.02	0.0 A
IAC511*	0.02 A	0.0	0.0 A
IAC512*	0.07 A	0.05	0.0 A
IRCA111*	0.0 A	0.0	0.0 A
PB311*	0.04 A	0.0	0.0 A
PB350*	0.05 A	0.03	0.0 A
PC119*	0.0 A	0.0	0.0 A
RRIM600*	0.15 Ba	0.0 b	0.0 Ab
CV	20.40		

CV: coefficient of variation of data transformed into square root - SQRT (Y). Means followed by the same uppercase letters in the columns and the same lowercase letters in the rows do not differ in the Scott-Knott test at 5% probability. * there was no significant difference in the row by the 5% F-test in the row.** there was no significant difference in the column by the 5% F-test.

Table 4: Black crust severity (% diseased leaf area) in different parts of rubber tree clones in August 2021.

Clones	**Part of the plant**		
	Crown	**Middle third**	**Bottom****
IAC 300	1.04Db	0.76Cb	0.18a
IAC 301*	0.0A	0.0 A	0.0
IAC 406*	0.0A	0.0 A	0.05
IAC 410*	0.20B	0.25B	0.20
IAC411*	0.02 A	0.0 A	0.0
IAC412	0.20 Ba	0.68Cb	0.0a
IAC418*	0.01 A	0.0 A	0.01
IAC500*	0.0A	0.13B	0.0
IAC501*	0.0A	0.0 A	0.01
IAC502*	0.01 A	0.0 A	0.0
IAC503*	0.04 A	0.02 A	0.0
IAC505	0.5 Cb	0.5Cb	0.0a
IAC506*	0.0 A	0.0 A	0.01
IAC507*	0.0 A	0.0 A	0.0
IAC511*	0.0 A	0.0 A	0.0
IAC512	0.30 Cb	0.48Cb	0.0a
IRCA111	0.32 Cb	0.0 Aa	0.06a
PB311	0.14 Ba	0.62Cb	0.35b
PB350*	0.15 B	0.04 A	0.0
PC119*	0.0A	0.0 A	0.0
RRIM600	0.12Bb	0.28Bb	0.0a
CV	32.62		

CV: coefficient of variation of data transformed into square root - SQRT (Y). Means followed by the same uppercase letters in the columns and the same lowercase letters in the rows do not differ in the Scott-Knott test at 5% probability. * there was no significant difference in the row by the 5% F-test in the row.** there was no significant difference in the column by the 5% F-test.

Table 5: Black crust severity (% diseased leaf area) in different parts of rubber tree clones in November 2021

Clones	Part of the plant		
	Crown	Middle	Baixeiro**
IAC 300	0.52Ca	9.09Eb	0.12a
IAC 301	0.22Bb	1.21Cc	0.0a
IAC 406	2.32Db	1.45Cb	0.0a
IAC 410	2.31Db	0.0Aa	0.0a
IAC411*	0.3B	0.0A	0.0
IAC412*	0.0 A	0.0A	0.0
IAC418*	0.3B	0.12 A	0.20
IAC500*	0.47C	0.0A	0.0
IAC501	0.22Ba	0.9Bb	0.0a
IAC502*	0.0 A	0.5B	0.0
IAC503	1.34Db	2.22Dc	0.0a
IAC505*	0.41C	0.16 A	0.0
IAC506*	0.0 A	0.0A	0.0
IAC507*	0.0 A	0.08 A	0.0
IAC511*	0.0 A	0.0A	0.0
IAC512*	0.0 A	0.0A	0.0
IRCA111*	0.0 A	0.0A	0.0
PB311	2.53Db	0.67Ba	0.76a
PB350	0.75Cb	0.07 Aa	0.0a
PC119*	0.0 A	0.0A	0.0
RRIM600*	0.15B	0.0A	0.0
CV	60.05		

CV: coefficient of variation of data transformed into square root - SQRT (Y). Means followed by the same uppercase letters in the columns and the same lowercase letters in the rows do not differ in the Scott-Knott test at 5% probability. * there was no significant difference in the row by the 5% F-test in the row.** there was no significant difference in the column by the 5% F-test.

Table 6: Black crust severity (% diseased leaf area) in different parts of rubber tree clones in February 2022.

Clones	Part of the plant		
	Copa	Terço médio	Baixeiro**
IAC300	1.44 Bc	2.57 Cb	0.40a
IAC301*	0.52 A	0.07 A	0.00
IAC406	0.69 Ab	0.83Ab	0.00a
IAC410*	0.10 A	0.00 A	0.00
IAC411	0.00Aa	0.82Ab	0.00a
IAC412*	0.00A	0.04 A	0.53
IAC418	1.35Bb	1.48Bb	0.00a
IAC500	0.83Ab	1.88Bb	0.00a
IAC501*	0.00A	0.32 A	0.00
IAC502*	0.13A	0.22 A	0.07
IAC503	0.23Aa	3.14Cb	0.03a
IAC505*	0.00 A	0.09 A	0.00
IAC506*	0.01 A	0.01 A	0.00
IAC507*	0.00 A	0.00 A	0.00
IAC511*	0.00 A	0.00 A	0.00
IAC512*	0.27 A	0.06 A	0.00
IRCA 111*	0.00 A	0.00 A	0.01
PB 311*	0.69 A	0.34 A	0.16
PB 350*	0.00 A	0.00 A	0.00
PC 119*	0.00 A	0.00 A	0.00
RRIM 600	2.04 Bb	0.45 Aa	0.00a
CV	37.08		

CV: coefficient of variation of data transformed into square root - SQRT (Y). Means followed by the same uppercase letters in the columns and the same lowercase letters in the rows do not differ in the Scott-Knott test at 5% probability. * there was no significant difference in the row by the 5% F-test in the row.** there was no significant difference in the column by the 5% F-test.

Table 7: Black crust severity (% diseased leaf area) in different parts of rubber tree clones in May 2022.

Clones	**Part of the plant**		
	Copa	**Terço médio**	**Baixeiro**
IAC 300	2.06Ba	6.32 Eb	1.30Ba
IAC301*	0.85B	0.25A	0.00A
IAC406	1.02Bb	2.70Cb	0.15Aa
IAC410*	0.31A	0.20A	0.22A
IAC411	0.10Aa	1.35Bb	0.21Aa
IAC412*	0.52A	0.24A	0.10A
IAC418	0.83Ba	6.04Eb	1.34Ba
IAC500	0.68Aa	2.94Cb	2.84Cb
IAC501*	0.05A	0.02A	0.02A
IAC502	1.14Ba	1.86Ba	3.64Db
IAC503	1.13Ba	2.75Ca	4.02Db
IAC505*	0.24A	0.27A	0.36A
IAC506	0.94Bb	1.09Bb	0.56Aa
IAC507*	0.20A	0.52A	0.00A
IAC511*	0.00A	0.00A	0.01A
IAC512	1.71Bb	0.72Aa	0.38Aa
IRCA 111*	0.87B	1.19B	1.40B
PB 311	5.01Dc	2.07Bb	0.61Aa
PB 350	3.94Cb	3.11Cb	0.18Aa
PC 119*	1.40B	1.20B	1.92C
RRIM 600	5.49Db	4.53Db	3.03Ca
CV	43.36		

CV: coefficient of variation of data transformed into square root - SQRT (Y). Means followed by the same uppercase letters in the columns and the same lowercase letters in the rows do not differ in the Scott-Knott test at 5% probability. * there was no significant difference in the row by the 5% F-test in the row.** there was no significant difference in the column by the 5% F-test.

Table 8: Black crust severity (% diseased leaf area) in different parts of rubber tree clones in August 2022.

Clones	Part of the plant		
	Copa	Terço médio	Baixeiro
IAC 300*	0.00A	0.00A	0.00A
IAC301*	0.00Aa	0.70A	0.67A
IAC406	2.20Cb	0.78Aa	0.96Aa
IAC410*	0.00A	0.55A	0.46A
IAC411*	0.07A	0.12A	0.01A
IAC412	3.64Db	3.60Cb	1.57Ba
IAC418	3.22Dc	1.71Bb	0.72Aa
IAC500*	0.00A	0.69A	0.31A
IAC501*	0.02A	0.00A	0.00A
IAC502*	0.10A	0.32A	0.50A
IAC503*	1.21B	0.65A	0.94A
IAC505	0.71Ba	0.27Aa	2.34Bb
IAC506	1.92Cb	1.16Bb	0.44Aa
IAC507*	0.11A	0.35A	0.03A
IAC511*	0.00A	0.00A	0.00A
IAC512	2.17Cb	0.16Aa	0.18Aa
IRCA 111*	0.06A	0.60A	0.10A
PB 311	3.74Db	0.90Ba	0.00Aa
PB 350	2.71Cb	3.77Cb	1.72Ba
PC 119	0.95Bb	0.65Aa	2.62Bc
RRIM 600	0.46Aa	1.36Bb	2.07Bc
CV	40.08		

CV: coefficient of variation of data transformed into square root - SQRT (Y). Means followed by the same uppercase letters in the columns and the same lowercase letters in the rows do not differ in the Scott-Knott test at 5% probability. * there was no significant difference in the row by the 5% F-test in the row.** there was no significant difference in the column by the 5% F-test.

Table 9: Black crust severity (% diseased leaf area) in different parts of rubber tree clones in November 2022.

Clones	Part of the plant		
	Copa	Terço médio	Baixeiro
IAC 300*	0.00A	0.02A	0.02A
IAC 301*	0.00A	0.01A	0.00A
IAC 406	0.00Aa	2.82Cb	0.00Aa
IAC 410	0.16Aa	0.00Aa	1.90Cb
IAC 411*	0.00A	0.00A	0.52A
IAC 412	0.62Aa	0.20Aa	2.36Cb
IAC 418	0.00Aa	0.92Ab	0.73Ab
IAC 500*	0.00A	0.31A	0.00A
IAC 501*	0.00A	0.00A	0.00A
IAC 502*	0.00A	0.44A	0.00A
IAC 503*	0.00A	0.00A	0.00A
IAC 505	2.18Bb	0.00Aa	0.97Ba
IAC 506*	0.00A	0.00A	0.00A
IAC 507	0.60Aa	2.76Cb	0.00Aa
IAC 511*	0.00A	0.00A	0.00A
IAC 512	1.97Bb	0.18Aa	1.06Bb
IRCA 111*	0.03A	0.08A	0.00A
PB 311*	0.10A	0.22A	0.34A
PB 350*	0.07A	0.08A	0.10A
PC 119*	0.26A	0.00A	0.24A
RRIM 600	0.34Aa	1.61Bb	0.08Aa
CV	36.99		

CV: coefficient of variation of data transformed into square root - SQRT (Y). Means followed by the same uppercase letters in the columns and the same lowercase letters in the rows do not differ in the Scott-Knott test at 5% probability. * there was no significant difference in the row by the 5% F-test in the row.** there was no significant difference in the column by the 5% F-test.

INSERT FIGURE 3

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP (2020/11518-4), and Agência Paulista de Tecnologia dos Agronegócios (APTA).

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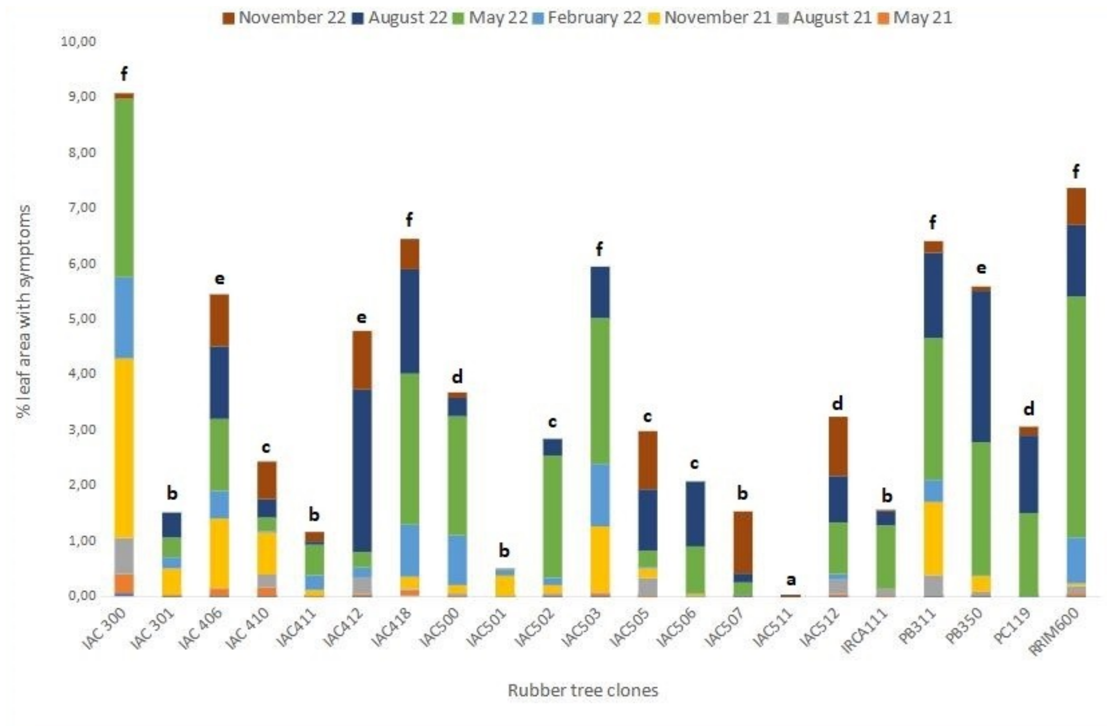
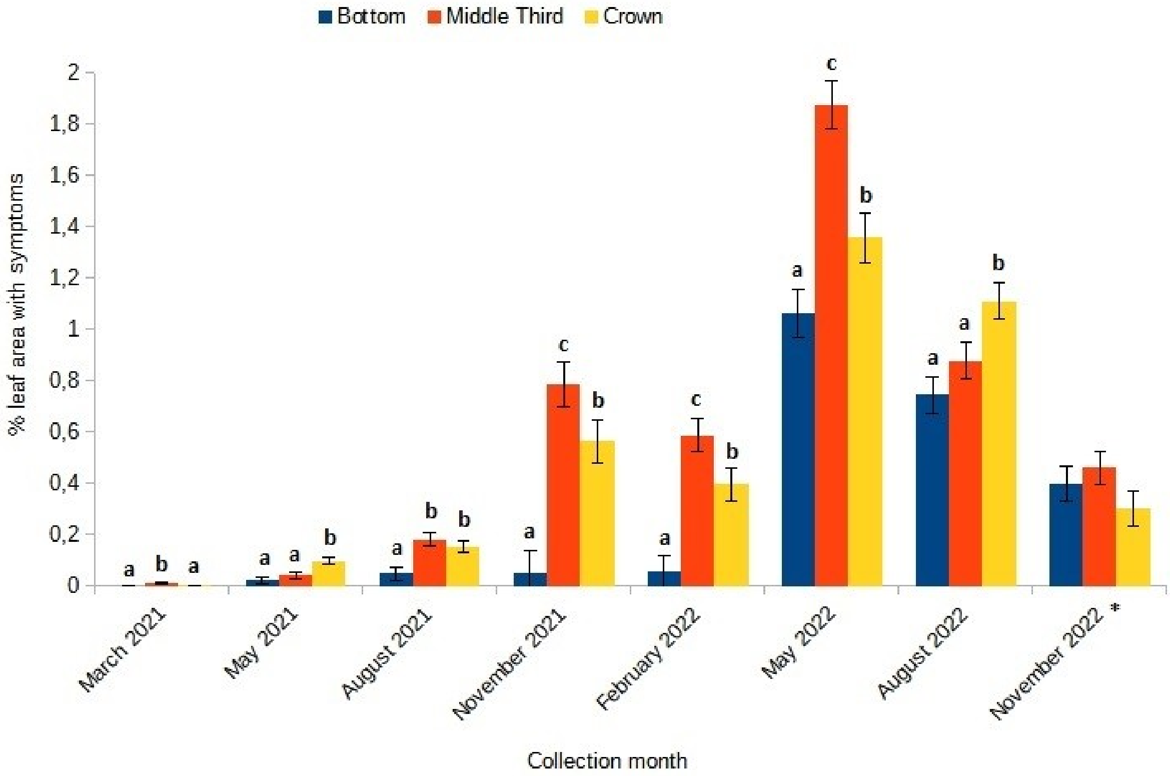
Figure Legends

Figure 1: Cumulative severity (% leaf area with symptoms) of black crust in various rubber tree clones, over the eight collections.



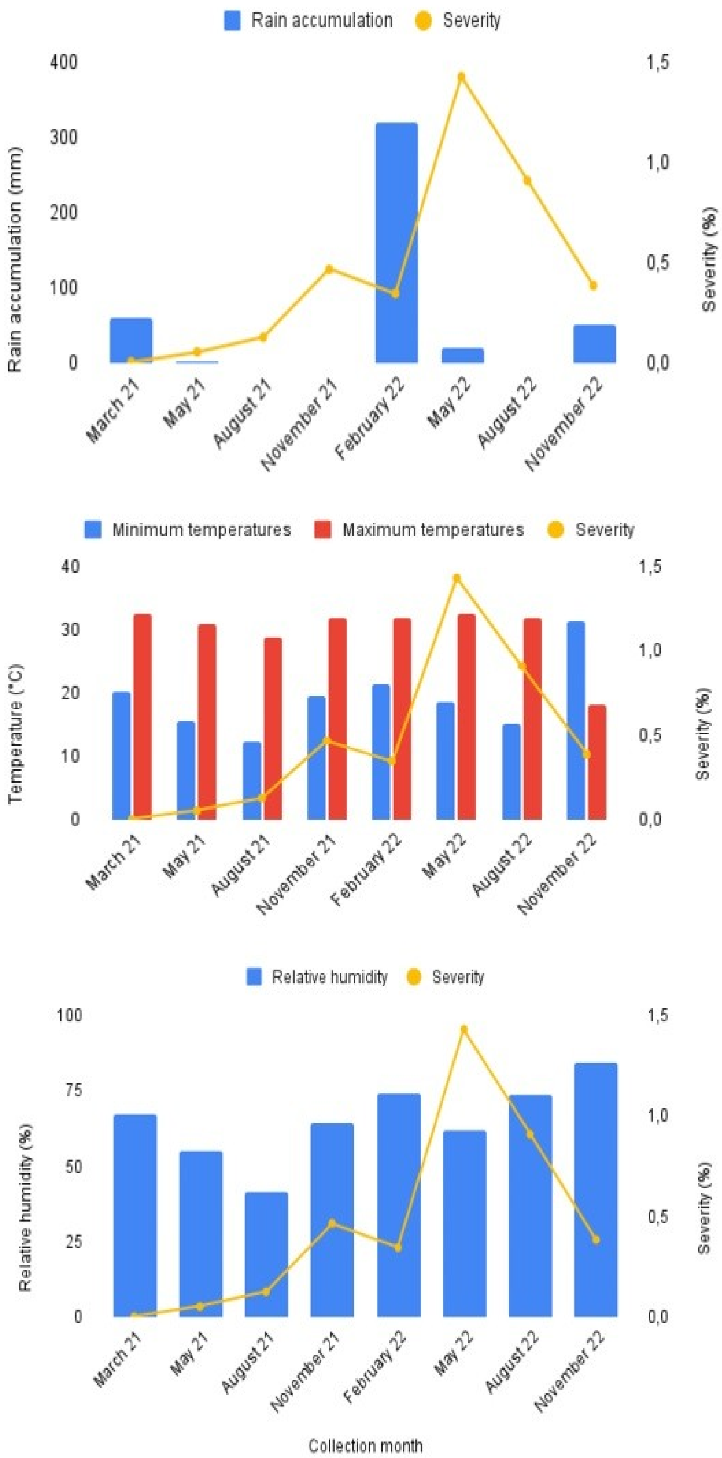
Means followed by the same letters do not differ from each other in the Scott-Knott test at 5% probability.

Figure 2: Overall mean black crust severity (% leaf area with symptoms) on different parts of the plant at different collections.



Means followed by the same letters do not differ from each other in the same collection by the Scott-Knott test at 5% probability * there was no significant difference in the column by the 5% F test.

Figure 3: Black crust severity (% leaf area with symptoms) and different climatic data (temperatures, relative humidity and rainfall) measured in the 30 days immediately prior to each collection during collection periods.



CAPÍTULO 2

TELEOMORPHIC PHASE OF *Davidiella* sp. CAUSING BLACK CRUST ON RUBBER TREES[2]

ABSTRACT

Black crust has caused damage to the rubber tree (*Hevea brasiliensis* Muell.) in Brazil due to the early leaf fall it causes. There is little information about this disease, and its occurrence has always been associated with the fungi *Phyllachora huberi* and *Rosenscheldiella heveae*, which could occur together or separately. In 2023, *Cladosporium* sp. has been linked to the disease. The goal of this work was to progress the identification of the causal agent of black crust. Rubber tree leaflets from the RRIM 600 clone, collected in Barretos, São Paulo, were transported to the laboratory. Following morphological and genetic analyses, including sequencing of the ITS-5.8S rDNA region, the teleomorphic phase of *Davidiella* sp. was identified. Part of these ascospores produced in the sequenced stroma were used to carry out pathogenicity tests in a greenhouse. The spots on the inoculated leaves show stroma formation after 3 months. Both the ascospores from the stroma collected in the field and those formed in the greenhouse presented bitunicate asci measuring approximately 32µm in length and 13µm in width, containing fusiform ascospores with average dimensions of 11µm x 3.2µm in length and width. Based on these analyses, it can be affirmed that this constitutes the first report of the teleomorphic phase of *Davidiella* sp. associated with black crust. The anamorphic phase, *Cladosporium* sp., had already been reported. This information aids in comprehending the disease cycle in the field.

Keywords: Sexual phase, leaf disease, Capnodiales.

2 Capítulo redigido de acordo com as normas do periódico **Forest Pathology**

2.1 INTRODUCTION

Although Brazil has a significant area of rubber plantation (Brisolara; Barros; Amaral, 2023), the increase in latex productivity in the country is limited by factors such as phytosanitary problems.

Among the most important foliar diseases of the rubber tree are leaf blight, anthracnose, powdery mildew and areolate spot, caused by *Microcyclus ulei*, *Colletotrichum* ssp., *Oidium heveae* and *Rhizoctonia solani*. Black crust is also a disease that affects rubber plantations, but it has always been considered of little importance (Gasparotto *et al.*, 2012; Furtado; Gasparotto; Pereira, 2016) has increased, being present in the states of Bahia, Espírito Santo, Mato Grosso do Sul and São Paulo (Gonçalves e Goes, 2017; Virgens Filho *et al*., 2022). There is a report of a 38% reduction in rubber production between 2017 and 2020 in areas attacked by this disease in Bahia (Virgens Filho *et al.*, 2022).

This disease's main symptom is the appearance of circular black plaques on the abaxial surface of the leaflets and chlorotic areas, a reflection of the black plaques, on the adaxial surface. The black plaques are associated with the stromal cavities formed by the fungus (Gasparotto *et al.*, 2012). The fungus *P. huberi* was the first associated with the disease in Brazil, in 1899, in the Amazon region, followed by the fungus *R. heveae*, in the 1990s (Junqueira; Bezerra, 1990). Since then, there are few updates on the disease because it has always been considered secondary in rubber trees (Gasparotto *et al*., 2012). Recent studies with black crust indicate that another pathogen could be involved in this disease, since in samples collected by

Gomes (2023), no evidence of the presence of *P. huberi* and *R. heveae* was found in the stroma of the leaves. This study aimed to progress the identification of the causal agents of black crust.

2.2 MATERIAL AND METHODS

In 2023, attacked leaves of the RRIM600 clone were collected, coming from rubber plantations with high levels of black crust, in the city of Barretos (São Paulo State-Brazil) (20°33’33’’S, 48°34’8’’W) (Figure 1). These leaves were taken to the laboratory and the parts containing the stroma were cut to better match. They were subsequently subjected to superficial desinfestation with alcohol (70%), sodium hopochloride (2%) and sterilized water, for one minute at each stage. Then stroma were placed in a humid chamber for 48 hours at 12°C to produce ascospores following the method described by Junqueira and Bezerra (1990) and Gasparotto e*t al.* (2012). Each stroma with the ascospores on its surface (Figure 1) was divided into two pieces, which were destined for DNA extraction and the other for pathogenicity testing at greenhouse on seedlings of the RRIM600 clone. Notably, 25 stromas were found that produced ascospores on their surfaces, with an average of three stromas from each leaf collected.

Molecular identification of samples

To remove DNA from estromes, the methodology developed by Murray and Thompson (1980) was followed, with modifications. The DNA obtained was diluted to working concentrations of 20 $ng.mL^{-1}$ and identification was based on sequencing of the ITS-5.8S rDNA regions of the samples. The sequences obtained were edited using the Mega 11.0 program (Tamura *et al*., 2021) and compared with those deposited in GenBank.

Pathogenicity tests

This step was carried out in a greenhouse with 6-month-old rubber tree budded plants from the RRIM600 clone. For pathogenic characterization, 25 fragments of each stroma were inoculated into the abaxial part of a rubber tree leaf, located in the upper part of the plant. The inoculated area was located using circular adhesive tape, which also had the function of making the stroma fragment (Figure 1). Symptom assessment was carried out weekly for six months. Fragments of tissue with symptoms were collected for production and identification of ascospores.

Morphological analysis

Part of the ascospores produced from each stroma were collected with a needle and placed on slides containing lactophenol. Then, these slides were sealed with nail polish and taken for analysis using a video camera system installed in an Opton® optical microscope, model TA-0124XS. The image was transmitted to a computer and analyzed using the EDN-2 program. Measurements were taken for 50 ascospores from each stroma.To calibrate the equipment, a micrographed slide (Carl Zeiss®) was used.

2.3 RESULTS AND DISCUSSION

Of the 25 stroma sequenced, seven were identified as *Davidiella* sp. The dimensions of the asci and ascospores in the samples obtained, as well as the morphology of these structures, were similar to those described by Bensch *et al*., (2018). Both the ascospores from the stroma collected in the field and those formed in the greenhouse presented bitunicate asci measuring approximately (24-41) 32µm x 13µm (7-15), in length and width respectively, containing fusiform ascospores with average dimensions of 11µm (7-14) in length and 3.2µm (3-3.5) in width.

In relation to the other sequences obtained, they were of low quality, or had a high analogy with fungi that normally live in the phylloplane of the leaf, such as *Fusarium* sp. and *Trichoderma*. This indicates that the extraction method must be optimized and more sensitive to work only with the low quantity of ascospores collected.

Despite the problems encountered in sequencing, all inoculated stroma were pathogenic, with black crust symptoms starting around 2 months after inoculation and after 3 months it was already possible to verify the presence of stroma in the leaves (Figure 1). The delay in the onset of symptoms may be related to factors such as the temperature of the greenhouse during the beginning of the studies, which was around 38°C, measured by internal thermometers. Anjos (2023), had already confirmed that this disease develops better in mild temperatures (between 20 and 25°C), and that high temperatures inhibit its growth, this would explain why the stroma appeared on the leaf only in the month of June, when the temperatures were lower. The results obtained also corroborate the studies developed by Gomes *et al.*

(2023), which found different species of *Cladosporoium* associated with black crust on rubber trees, as according to Braun *et al.* (2003) and Bensch *et al.*, (2018) *Davidiella* sp., is the teleomorphic phase of *Cladosporium*.

INSERT FIGURE 1

Given this information, the pathogen cycle becomes clearer in the field, as it is understood that the fungus can survive in asexual form in the stroma and stroma in the leaves that fall to the ground in winter, when the natural defoliation of the rubber tree occurs, making the ascospores of *Davidiella* formed in these stroma the main source of primary inoculum of the pathogen to initiate an infection cycle in newly formed leaves at the time of emission of new leaves by the plant (Gasparotto *et al*., 2012), and the spores of the asexual phase, corresponding to the *Cladosporium*, as responsible for the secondary cycle of the disease in the plant. This fact suggests that black crust has a life cycle similar to that of South American leaf spot, caused by the fungus *Pseudocercospora ulei* (*Microcyclus ulei*).

2.4 CONCLUSIONS

This is the first report of teleomorph phase of *Davidiella*, associated to black crust in rubber tree.

ACKNOWLEDGMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, Fundação de Amparo à

Pesquisa do Estado de São Paulo, FAPESP (2020/11518-4 e 2020/14082-2), and Agência Paulista de Tecnologia dos Agronegócios (APTA).

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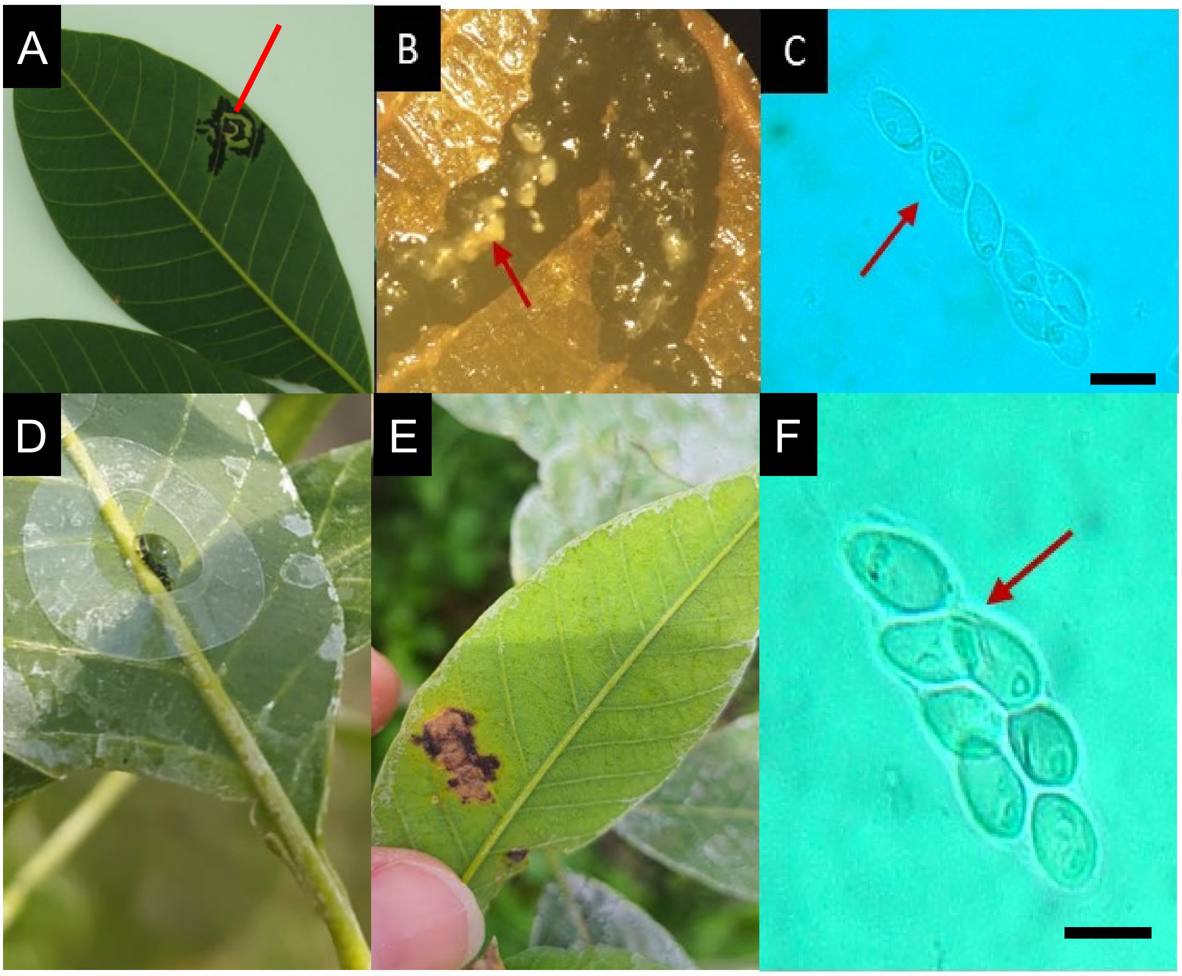
Figure 1: Morphlogical characterization of the black crust and the causal agent *Davidiella* sp.



A. Symptoms of the Black crust witch Stromas in the field. **B and C**; Stromas with ascospores; **D.** Inoculation of the leaf witch stromas parts. **E.** Symptoms of the black crust 3 months after inoculation; **F.** Ascos and ascospores from reisolation os the stromas from greem house plants.

CONSIDERAÇÕES FINAIS

As informações obtidas nos dois capítulos demonstram que:

A utilização de clones resistentes é uma alternativa para o controle da crosta negra;

O fungo *Davidiella* sp., em sua fase teleomórfica, foi associado a crosta negra pela primeira vez;

Ambos os pontos são importantes para o desenvolvimento de estudos voltados para etiologia e controle da crosta negra.

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APÊNDICE A – Arquivo suplementar 1

Parentais dos clones utilizados nas avaliações de resistência de seringueira a crosta-negra

Clones	Parentais	Origem
IAC 300	RRIM 605 (Tjir 1 x PB 49) x AVROS 363RRIM 605 (Tjir 1 × PB 49) × AVROS 353 (AVROS 164 × AVROS 160)	Brasil
IAC 301	RRIM 501 (Pil A 44 × Lun N) × AVROS 1518 (AVROS 214 × AVROS 317)	Brasil
IAC 406	IAN 873 (PB 86 × FA 1717) × RRIM 600 (Tjir 1 × PB 86)	Brasil
IAC 410	PB 86 × PB 235 [PB 5/51 (PB 56 × PB 24) × PB S/78 (PB 49 × PB 25)]	Brasil
IAC411	GT 711 ill.	Brasil
IAC412	IAN 873 (PB 86 × FA 1717) × GT 711	Brasil
IAC418	RRIM 600 (Tjir 1 × PB 86) ill.(1)	Brasil
IAC500	RRIM 600 (Tjir 1 × PB 86) ill.	Brasil
IAC501	RRIM 526 (Pil B 58 × Pil D 65) ill.	Brasil
IAC502	IAC 41 [RRIM608 (Tjir 33 × Tjir 1) × AVROS 1279 (AVROS 156 × AVROS 374)] ill.	Brasil
IAC503	Fx 3899 (F 4542 (1) × AVROS 363) ill. IAN 873 (PB 86 × FA 1717) ill.	Brasil
IAC505	IAN 873 (PB 86 x FA 1717) ill. IAN 873 (PB 86 × FA 1717) ill.	Brasil
IAC506	AVROS 1513 ill	Brasil
IAC507	IAC 90 [RRIM 507 (Pil B 84 × Pil A 44) × Fx 25(F 351 × AVROS 49)]	Brasil
IAC511	IAC 15 [RRIM 504 (Pil A 44 × Lun N) × RRIM 600(Tjir 1 × PB 86)] ill.	Brasil
IAC512	Fx 25 (F 351 × AVROS 49) ill.	Brasil
IRCA111	RRIM 507 (Pil B 50 x Pil B 84) x Fx 25 (F 361 x AVROS 49)	Costa do Marfim
PB311	RRIM 600 (Tjir 1 × PB 86) × PB 235 [PB 5/51 (PB 56 × PB 24) × PB S/78 (PB 49 × PB 25)]	Malásia
PB350	RRIM 600 (Tjir 1 x PB 86) x PB 235 [(PB 5/51(PB 56 x PB 24)]	Malásia
PC119	GT 1 x RRIM 703 [RRIM 600(Tjir 1 x PB 86) x RRIM 500 (Pil B 84 x Pil A 44)]	Malásia
RRIM600	Tjir 1 x PB 86	Malásia

(1) ill. Ilegítimo (genótipo obtido a parir de polinização aberta de planta matriz)

IAC: Instituto Agronômico de Campinas; IRCA: Institute de Recherches sur le Caoutchouc; PB: Prang Besar; PC: Promotion Clone; PR: Proesfstation voor Rubber RRIM: Rubber Research Institute of Malaysia; Tjir: Tjirandji; F: Ford; Fx: Ford cross; IAN: Instituto Agronômico do Norte; AVROS: Algemene Veriniging Rubberplanters Oostkust Sumatra

APÊNDICE B – Arquivo suplementar 2
Demonstração do funcionamento do software Leaf Doctor, utilizado para aferir a severidade da crosta-negra nos folíolos de seringueira

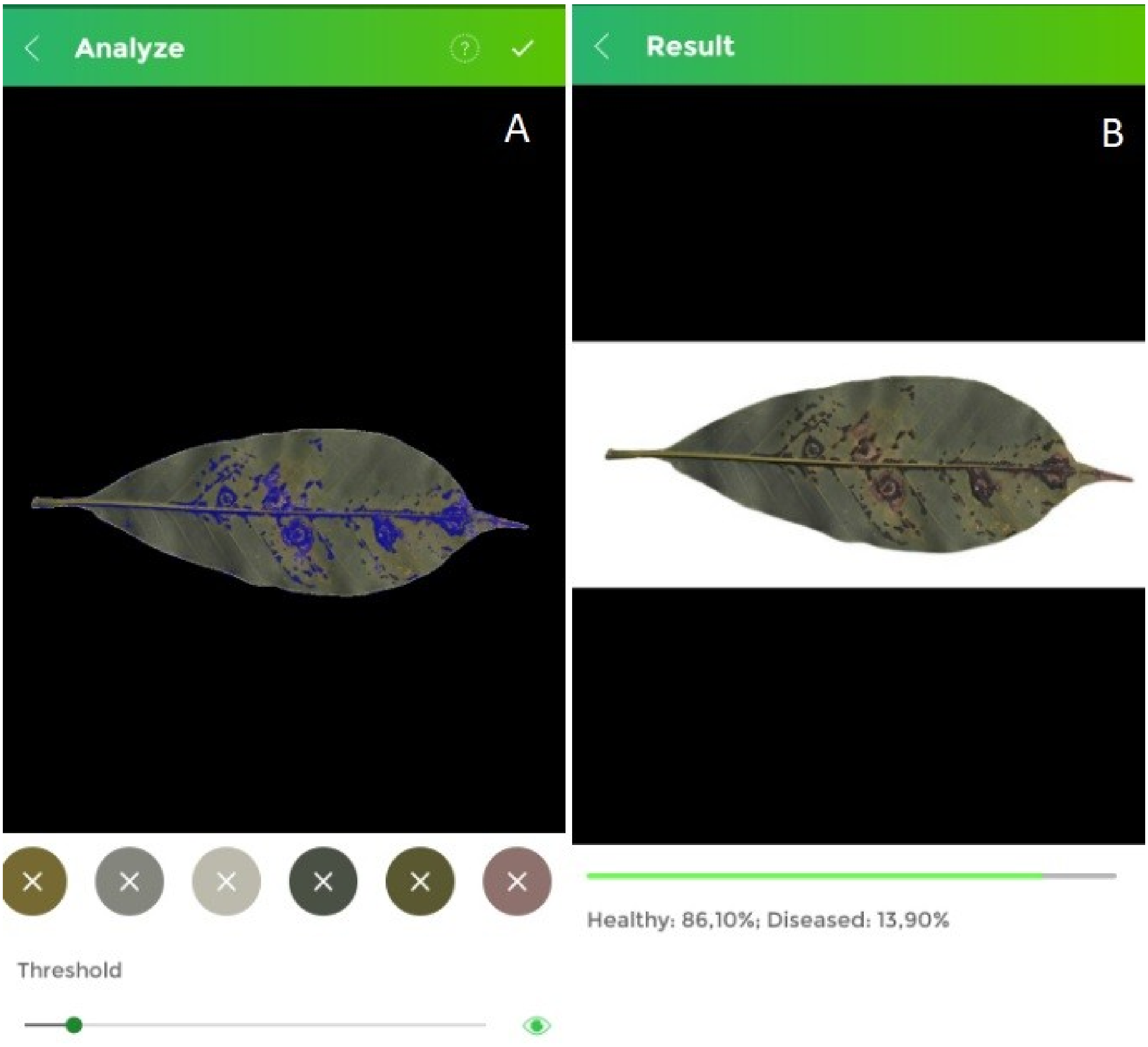
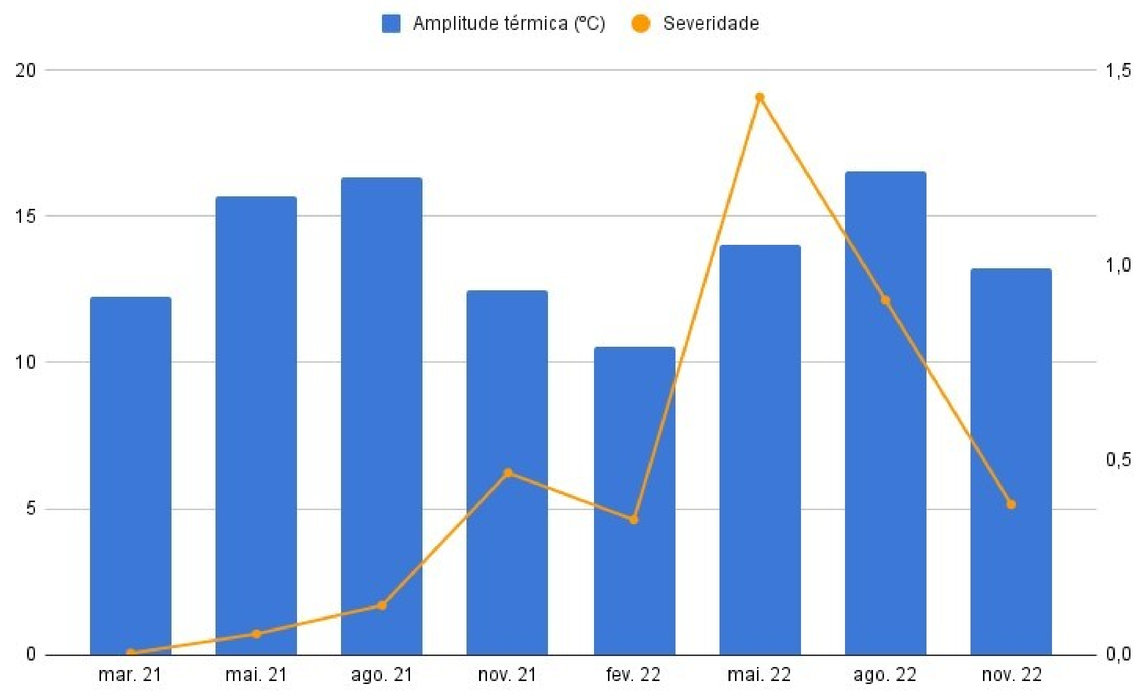


Figura A: a área em azul demonstra a superfície do folíolo que o software Leaf Doctor identificou como lesionada; Figura B: indicação da porcentagem da área identificada como lesionada

APÊNDICE C – Arquivo suplementar 3
Correlação entre amplitude térmica e severidade da crosta-negra durante dois anos de avaliação, em diferentes clones de seringueira, em jardim clonal



APÊNDICE D – Arquivo suplementar 4

Ilustração do ciclo da crosta-negra da seringueira