



Plant Protection

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Occurrence of rust in *Myrcianthes pungens* (O. Berg) D. Legrand caused by *Austropuccinia psidii* in the state of Rio Grande do Sul

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Abstract - Guabiju tree (*Myrcianthes pungens*) belongs to the Myrtaceae family, with wide occurrence in Rio Grande do Sul (RS), southern Brazil, demonstrates great commercial potential regarding the consumption of its fresh fruit, which has a sweet taste and can be used in drinks, jellies and ice creams, in addition to its nutraceutical properties. As their main characteristic, rusts present the formation of orange pustules containing urediniospores of the pathogen on affected organs. The action of the pathogen causes deformation of stems, leaves, flowers and fruits, thus interfering with the physiological processes of the plant. Thus, the present work aimed at presenting information on the occurrence and confirmation of the causal agent of guabiju rust, in addition to reporting the accessions most susceptible and resistant to *Austropuccinia* in the guabiju working collection of Eldorado do Sul, RS, Brazil. The diagnosis of the disease was based on symptoms, observation of fungal structures by light microscopy and molecular analyses. From microscopy, ellipsoid to ovoid and slightly equinulate urediniospores were observed, characteristic of *Austropuccinia* sp. The sequence of the internal transcribed spacer (ITS) region of the isolate showed 99.06% similarity with sequences from the same region of *A. psidii* deposited on the nucleotide database - GenBank (NCBI). This is the first report of rust associated with guabiju in the state of Rio Grande do Sul, Brazil. From the diagrammatic scale developed, it was possible to identify different levels of susceptibility to *A. psidii* in guabiju accessions under study.

Index Terms - Myrtaceae, Genetic Resources, Native Fruits of Brazil, Guabiju, Phytopathogen.

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Ocorrência de ferrugem em *Myrcianthes pungens* (O. Berg) D. Legrand causada por *Austropuccinia psidii* no estado do Rio Grande do Sul

Resumo - Guabiju (*Myrcianthes pungens*), pertencente à família Myrtaceae, com ampla ocorrência no Rio Grande do Sul (RS), apresenta grande potencial para exploração de seus frutos no consumo *in natura*, com sabor adocicado, podendo compor bebidas, geleias e sorvetes, além de possuir propriedades nutracêuticas. As ferrugens, como característica principal, apresentam a formação de pústulas alaranjadas contendo urediniósporos do patógeno sobre os órgãos afetados. A ação do patógeno causa deformação dos órgãos: caules, folhas, flores e frutos, interferindo, assim, nos processos fisiológicos da planta. Assim, o presente trabalho teve como objetivo apresentar informações sobre a ocorrência, confirmação do agente causal da ferrugem do guabijuzeiro, além de relatar os acessos mais suscetíveis e resistentes a *Austropuccinia* na coleção de trabalho de guabijuzeiros em Eldorado do Sul-RS. A diagnose da doença foi realizada com base nos sintomas, na observação das estruturas fúngicas por microscopia óptica e por análises moleculares. A partir da microscopia, observaram-se urediniósporos elipsoides a ovoides e levemente equinulados, característicos de *Austropuccinia* sp. A sequência da região *internal transcribed spacer* (ITS) do isolado apresentou 99,06% de similaridade com sequências da mesma região da espécie *A. psidii* depositadas no banco de dados nucleotídeos - GenBank (NCBI). Este é o primeiro relato de ferrugem associada a guabijuzeiros no Estado do Rio Grande do Sul, Brasil. A partir da escala diagramática desenvolvida, foi possível identificar diferentes níveis de suscetibilidade a *A. psidii* nos acessos de guabijuzeiros avaliados.

Termos para Indexação - Myrtaceae, Recursos Genéticos, Frutas Nativas do Brasil, Guabiju, Fitopatógeno.

Introduction

Guabiju tree (*Myrcianthes pungens*) belongs to the Myrtaceae family, with wide occurrence in Rio Grande do Sul (RS), southern Brazil, demonstrates great commercial potential regarding the consumption of its fresh fruit, which has a sweet taste and can be used in drinks, jellies and ice creams, in addition to its nutraceutical properties. Guabiju trees can also be used as ornamental plants. The species is suitable for urban tree planting, landscaping, domestic orchards, and reforestation – as it may be a fruit source for large-sized birds and serve as stabilization for riverbanks. It is also a honey plant (LORENZI et al., 2006; WOLFF et al., 2009).

To date, there are few reports of pests and diseases affecting guabiju in Brazil. However, there are several studies confirming that fungi belonging to the Puccinioniaceae family cause diseases of economic importance in species of the Myrtaceae family (BERGAMIN FILHO; AMORIM, 1996; FIGUEIREDO; PASSADOR, 2008). Regarding the Puccinioniaceae family, *Austropuccinia psidii* (G. Winter) Beenken (syn. *Puccinia psidii* Winter) stands out, which has a wide range of hosts, being reported in more than 73 genera and 445 species of the Myrtaceae family (CARNEGIE; GIBLIN, 2020). Among the hosts already reported are guava (*Psidium guajava* L.) (APARECIDO, 2001), eucalyptus (*Eucalyptus* spp.) (HAWKSWORTH et

al., 1995), jambo (*Syzygium jambos* (L.) Alston (MOHALI AND AIME, 2016), as well as fruit trees native to southern Brazil, such as pitangueira-preta or guamirim (*Eugenia florida* DC.), guabiroba (*Campomanesia xanthocarpa* O. Berg.), cerejeira-do-rio-grande (*Eugenia involucreta* DC.) (RUIZ et al., 2017), jabuticaba (*Myrciaria cauliflora* (Mart.) O. Berg.) (NASCIMENTO; MELO, 2013) and uvaia (*Eugenia pyriformis* Cambess) (PIERI, 2012). In Brazil, rusts cause large losses due to environmental conditions favorable for the development of the disease (FIGUEIREDO; PASSADOR, 2008; APARECIDO; VALE, 2012).

As their main characteristic, rusts present the formation of orange pustules on affected organs. The action of the pathogen causes deformation of stems, leaves, flowers and fruits, thus interfering with the physiological processes of the plant (FERREIRA, 1989). According to Vasconcelos et al. (1998), by affecting new branches, the fungus reduces plant vigor, affecting subsequent fruit production in guava trees. The *Puccinia psidii* (syn. *Austropuccinia psidii*) species was first described by Winter (1884) on guava trees in São Francisco do Sul, state of Santa Catarina, Brazil.

In eucalyptus, the action of the pathogen occurs on leaves and shoots (KRUGNER; AUER, 1997), with lesions that begin with chlorotic punctuations that turn into pustules, exposing yellow urediniospores (KRUGNER; AUER, 1997). For the other myrtaceae reported, in addition to the pathogen attacking leaves and shoots, symptoms also occur in buds, flower buds, branches and developing fruits (NASCIMENTO; MELO, 2013).

The causal agents of rust are obligate parasites (biotrophs) (APARECIDO, 2001;

FIGUEIREDO; PASSADOR, 2008), which remove the nutrients they need directly from the host's living cells through haustoria (FERREIRA, 1989). The spread of the disease occurs through the dispersal of urediniospores by wind, rain, irrigation or splashing water, and by insects and birds.

Thus, the present work aimed at presenting information on the occurrence and confirmation of the causal agent of guabiju rust, in addition to reporting the accessions most susceptible and resistant to *Austropuccinia* in the guabiju working collection of the Federal University of Rio Grande do Sul (UFRGS) in Eldorado do Sul, RS, Brazil.

Material and methods

Climate and Soil

The work collection located at the Agronomic Experimental Station (EEA) of UFRGS, in Eldorado do Sul, RS (30° 06' S and 51° 40' W), has average altitude of 60 meters above sea level. The soil of the site is a Typical Dystrophic Red Argisol (SANTOS et al., 2018). The climate is characterized as humid subtropical, classified as Cfa by Köppen; the average annual temperature is 18.8 °C, the average annual precipitation is 1,455 mm and the average annual relative humidity is 77% (BERGAMASCHI et al., 2013). Shows the meteorological data collected on site (Figure 7).

Study Plants

The working collection consists of 16 accessions (10 plants per accession) from seeds previously collected in different locations in RS: Maquiné, Guabiju, Cachoeira do Sul, Bento Gonçalves, Porto Alegre and Santa Maria, from which seedlings were obtained and allocated in the EEA of UFRGS. Planting

spacing is 7.0 x 6.0 m. The collection was installed in 2013, where in the planting row, between every two guabiju plants, a bracatinga (*Mimosa scabrella*) seedling was planted, contributing to nitrogen fixation in the soil. This culture intercropping was chosen because, for the establishment of guabiju seedlings, it was necessary to have shading in the area, since in the successional dynamics of forests, guabiju is considered a late-secondary species. In May 2018, bracatinga trees were eliminated from the area (with plants cut close to the ground and removal of branches from the area). The age of guabiju trees was 6 years at the beginning of the study and the average plant height was 3.4 meters. This project was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) with code AE7A83F and under the title: *Characterization study of the guabiju tree*.

Sampling

Samples of different accessions from the working collection were used. Samples were composed of young leaves and fruits with the presence of urediniospores, which were collected and photographed. Subsequently, the material was sent to the Laboratory of Plant Virology of the Department of Plant Health, Faculty of Agronomy, UFRGS.

Microscopic Analysis

The disease was diagnosed based on symptoms and observation of urediniospores by microscopy. For morphological characterization, with the aid of a sterilized histological needle, urediniospores were transferred to a microscope slide containing a drop of sterilized deionized water. Ten urediniospores

were randomly observed under microscope at 40x magnification. Each urediniospore was photographed under light microscope and measured for length and diameter evaluation using the Leica Application Suite software version 4.12.0.

DNA extraction

Urediniospores present in the plant material were collected and DNA extraction was performed according to Pocovi et al. (2010) with modifications, which consisted of reducing the volume of reagents by half and changing the reagents of the extraction buffer, where 140 mM D-sorbitol and 30 mM N-lauroylsarcosine were not added.

After extraction, the DNA was quantified in spectrophotometer (Nanodrop Thermo Scientific model 2000) with absorbance being determined at A260 nm and A280 nm. The DNA concentration was adjusted to 50 ng/μL using ultrapure water.

PCR, Sequencing and Phylogenetic Analysis

The molecular identity of the isolate was determined by PCR amplification and sequencing of the internal transcribed spacer regions (ITS), 5.8S rDNA and parts of 18S and 28S rDNA using the following primers: ITS1 (5'-TCCGTTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (WHITE et al., 1990). The PCR mix (15 μL) contained 2.0 μL of genomic DNA, 0.3 μL of each primer (25 μmol/L), 10.03 μL of sterile ultrapure water, 0.12 μL of Taq Platinum PCR, 1.50 μL of 10× PCR buffer, 0.45 μL of MgCl₂ (1.5 mmol/L) and 0.3 μL of dNTPs (0.2 mmol/L). The PCR amplification program consisted of 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 1 min, with final extension of 72 °C for 10 min.

The PCR product was submitted to 1.5% agarose gel (weight/volume) in 0.5X Tris/Borate/EDTA (TBE) buffer at 90V for 80 minutes using loading buffer containing Blue Green dye (LGC) and visualized under UV light using a gel imaging system (L-PIX TOUCH, Loccus). Fragment size was estimated in relation to a standard 1kb molecular weight marker (Qiagen, USA).

The PCR product was purified using the ReliaPrep™ DNA Clean-Up and Concentration System (Promega, Madison, USA) and sequenced in both directions using the same PCR primers produced by company ATCGene Molecular Analysis, Porto Alegre, RS, using AB-3500 automatic sequencer (Applied Biosystems, USA). The sequences obtained were edited in the BioEdit 7.0.5.3 software and consensus sequences were analyzed using the Molecular Evolutionary Genetics Analysis software (MEGAX) (KUMAR et al., 2018), built with the ClustalW algorithm and compared in the NCBI GenBank database. The nucleotide sequence similarity of the isolate was calculated using the BLAST software (Basic Local Alignment Search Tool). Phylogenetic analyses were performed using the Maximum Likelihood method and the MEGAX software. Reference sequences corresponding to the ITS gene from *Austropuccinia psidii* species previously deposited in the Genbank were also added to the analyses. *Puccinia graminis* f. sp. *tritici* sequence was used to form the external group.

Diagrammatic Scale

A diagrammatic scale was constructed (Figure 1) using the model proposed by Godoy et al. (2006) of *Glycine max* adapting to *Myrcianthes pungens* and changing the maximum

disease threshold observed in the field. The representation of symptoms includes tissues that have become necrotic caused by pustules and coalescing lesions.

To evaluate the incidence (number of plants in the accession that presented some symptoms of the disease at the time of evaluation) and severity (percentage of infected leaf area) of the disease among the 16 accessions in the collection, two evaluations were carried out in January 2019, assigning grades from 0 to 5, representing the percentage amplitude of the infected leaf area (grade 0: absence of symptoms (0%); grade 1: 1 to 5%; grade 2: 6 to 21%; grade 3: 22 to 37%; grade 4: 38 to 50% and grade 5: 51 to 75%) (Figure 1).

With the aid of the proposed scale, the severity of the disease was estimated in the different accessions. Evaluations were visual in the four quadrants of the entire plant.

Statistical analysis

Data obtained were submitted to analysis of variance (ANOVA), and means were compared by the Scott-Knott test at 5% probability level. Analyses were performed using the R Studio statistical software version 2022.07.0.548 (RStudio, 2022).

Results and discussion

Morphology and Taxonomy

Through evaluations carried out by optical microscopy, the presence of urediniospores, typical structures of the genus *Austropuccinia* spp., was observed (Figure 2). Ellipsoidal to ovoid and slightly equinulate urediniospores with length and diameter dimensions ranging from 18.8 to 22.5 and 13.4 to 18.8 μm were observed (Figure 2).

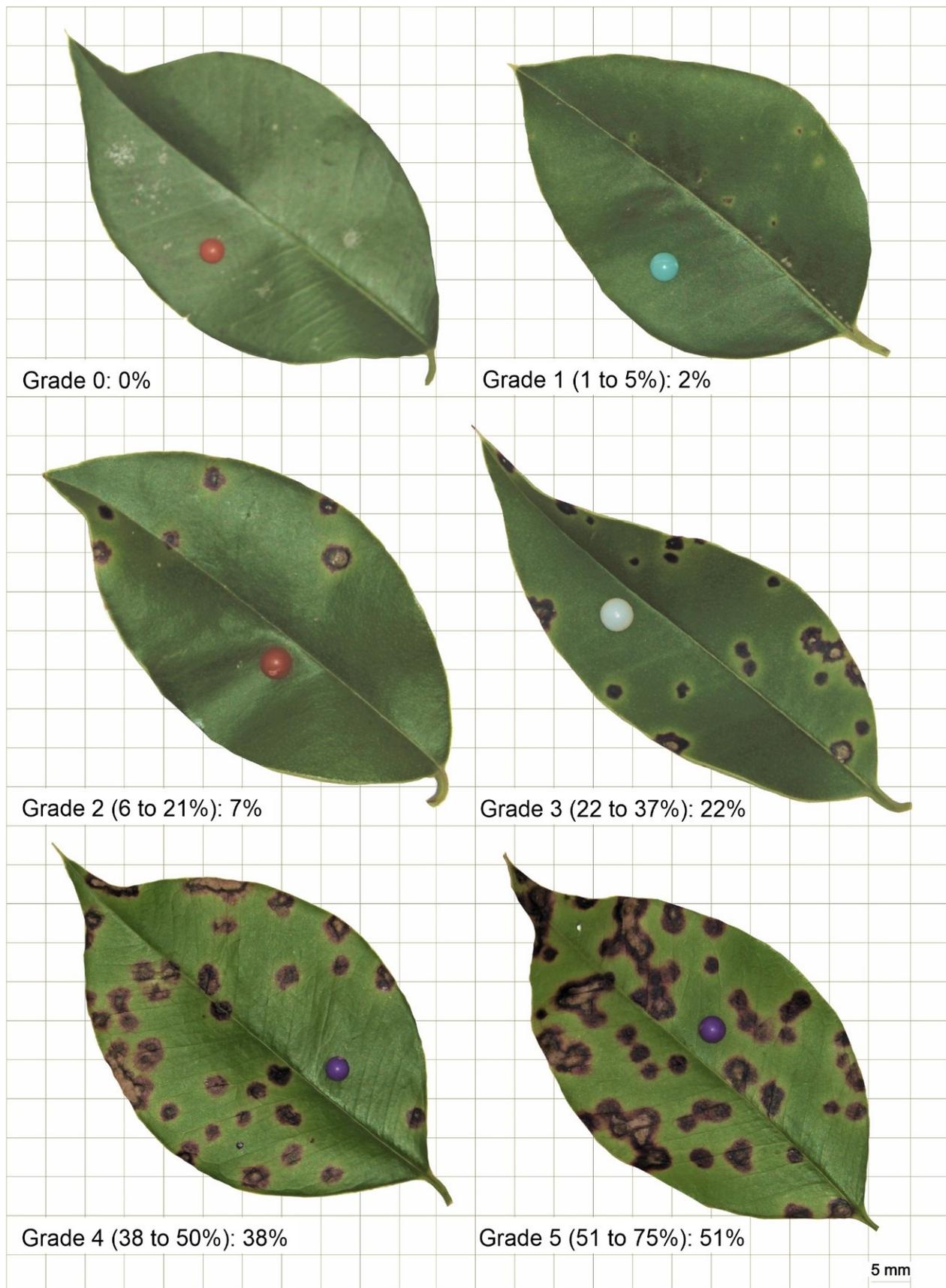


Figure 1. Diagrammatic scale adapted to evaluate rust in *Myrcianthes pungens*. Grade and percentage amplitude of infected leaf area (%), grade 0: absence of symptoms (0 %); grade 1: 1 to 5%; grade 2: 6 to 21%; grade 3: 22 to 37%; grade 4: 38 to 50% and grade 5: 51 to 75%. Eldorado do Sul-RS, 2019.

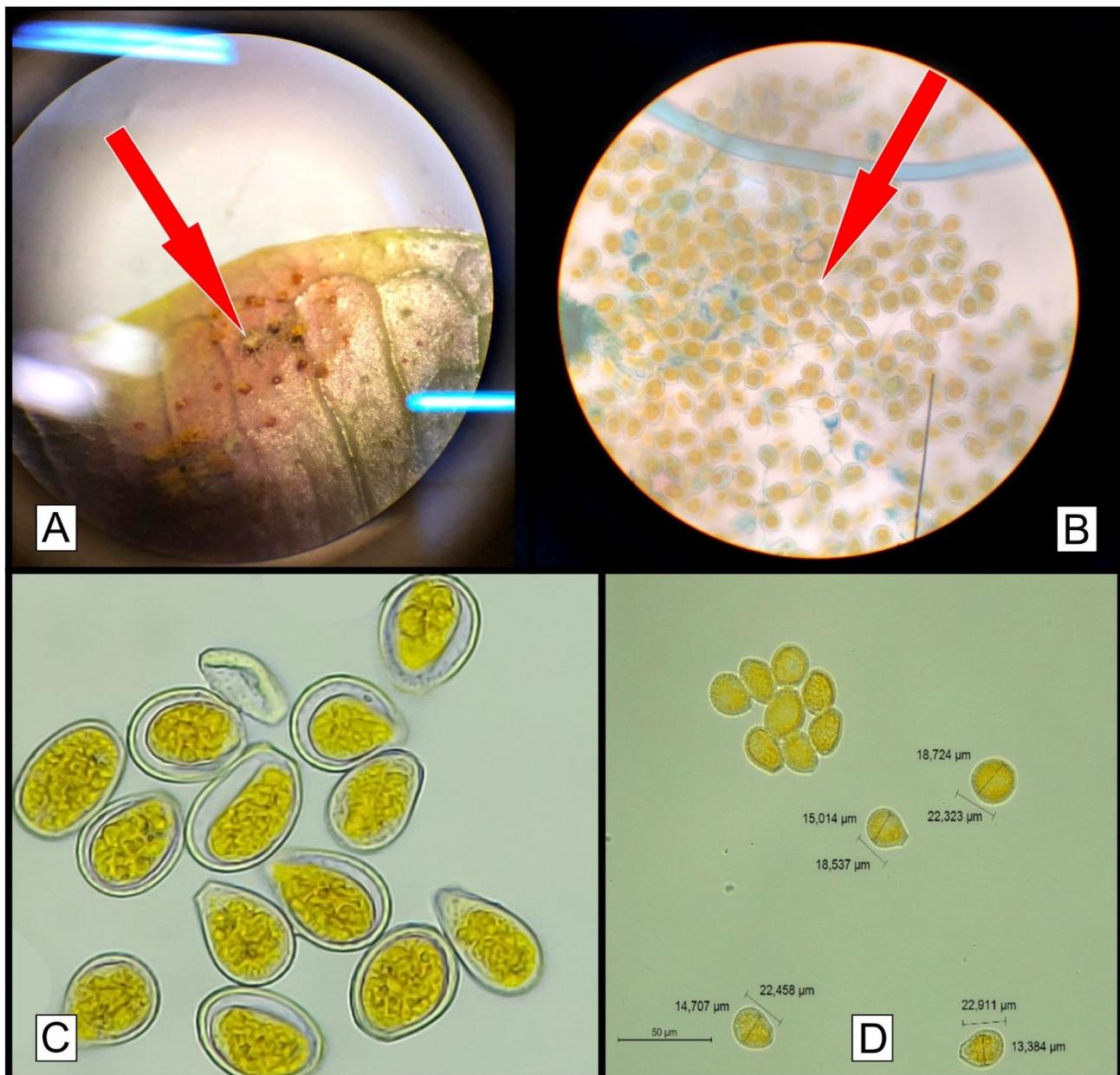


Figure 2. *Austropuccinia psidii*, optical microscope image. Pustules in *Myrcianthes pungens* leaves with rust symptoms (A). Urediniospores (B). Ellipsoidal to ovoid and completely equinulate urediniospores (C). Measurements of urediniospores (length and diameter) of *Austropuccinia psidii* with dimensions ranging from 18.8 to 22.5 and 13.4 to 18.8 μm , collected from the surface of guabiju fruits and observed under light microscope (D). Porto Alegre-RS, 2021.

The ITS gene sequence of the isolate showed 99.06% similarity with sequences from the same region of the *Austropuccinia psidii* species deposited on the nucleotide database - GenBank (NCBI). In the phylogenetic analysis, using the Maximum Likelihood method, the isolate belongs to the *Austropuccinia psidii* (sin. *Puccinia psidii*) species (Figure 3). The results of the present work are corroborated by Pérez et al. (2010), who analyzed infection by

Austropuccinia psidii in eucalyptus and native myrtaceae in Uruguay and reported infection of the pathogen in a guabiju plant in the municipality of Tacuarembó.

Incidence and Severity of the Infection in Accessions

The fungus attacks new leaves and herbaceous branches of shoots, flower buds and developing fruits. In the initial infection phase,

leaves, flowers and fruits show bright yellow pustules (Figure 4). Initially, small yellow and necrotic punctuations appear on leaves, and as they evolve, they become circular spots

covered by a yellowish powdery mass composed of urediniospores and teliospores. Over time, the powdery mass disappears, leaving the necrotic area dry.

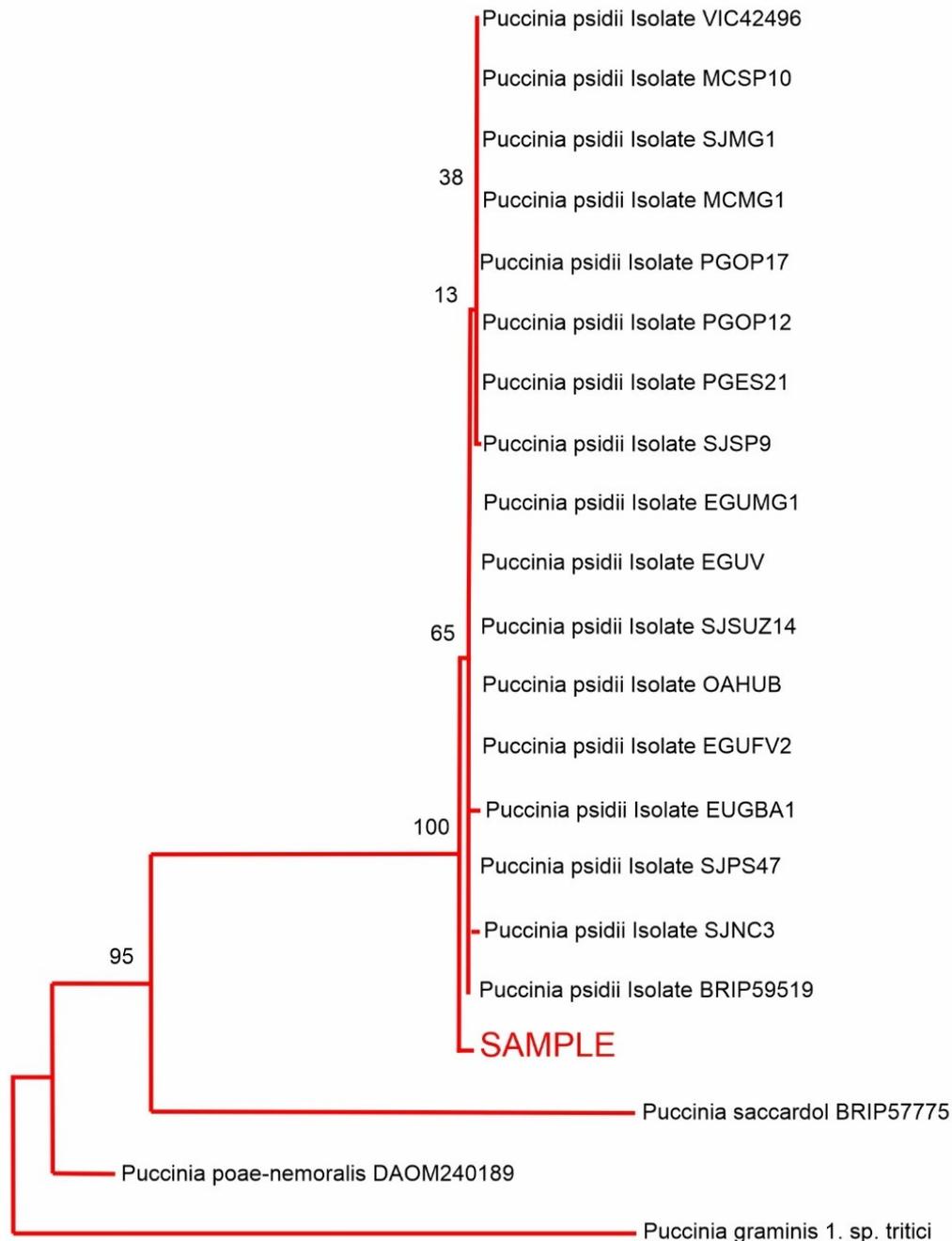


Figure 3. Phylogenetic tree demonstrating the evolutionary relationships between the ITS gene sequences of the isolated obtained and sequences of related isolates present in the nucleotide database - GenBank.

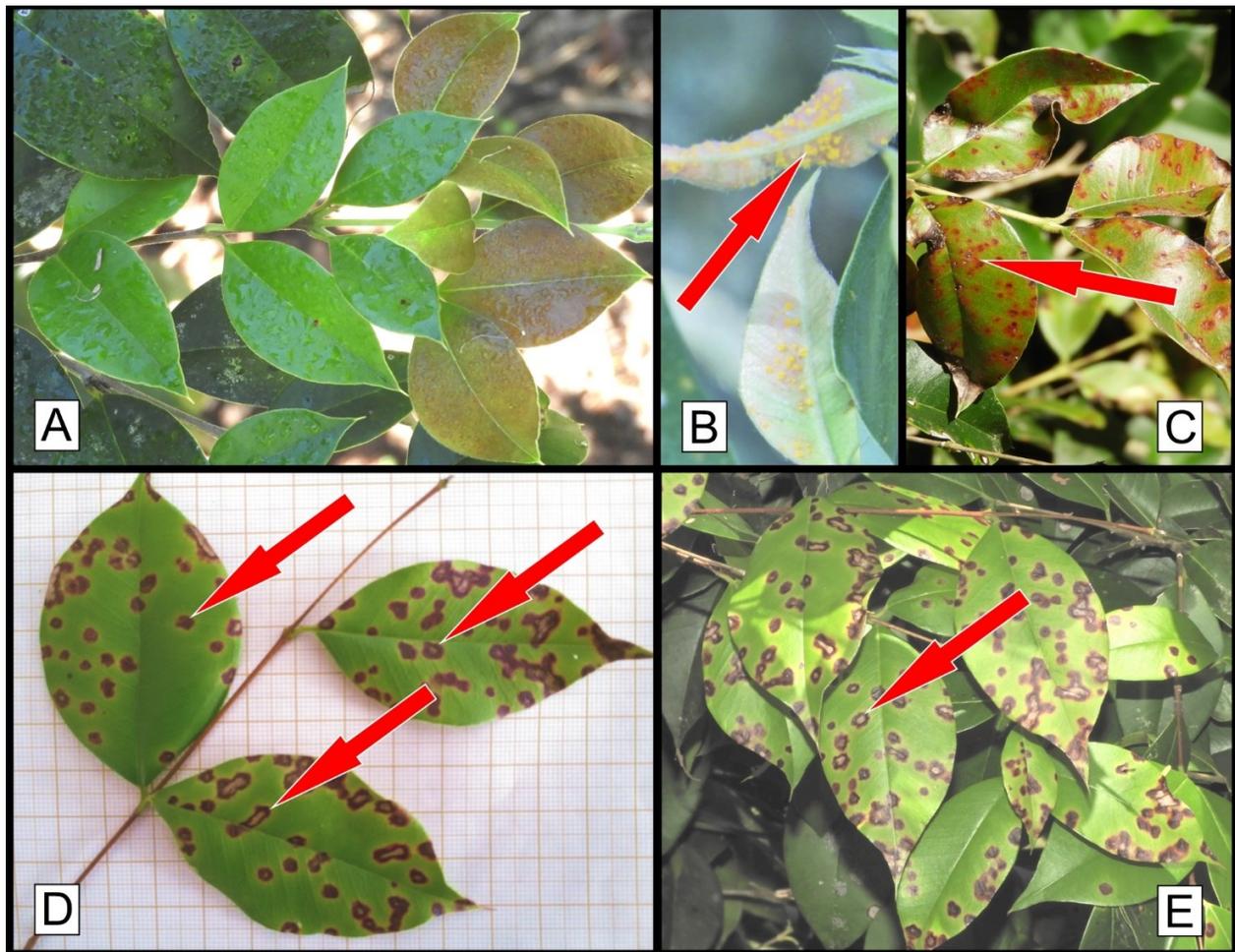


Figure 4. Guabiju (*Myrcianthes pungens*) leaves without lesions (A); bright yellow pustules, pathogen urediniospores (B); leaves with initial lesions (C); fully expanded leaves, brown lesions with dark edges (D) and circular lesions with yellow halos (E) caused by the fungus *Austropuccinia psidii*. Eldorado do Sul-RS, 2019.

For myrtaceae in general, the lesions appear separately at first. However, when the host has high susceptibility and favorable environmental conditions, lesions coalesce and can affect the entire leaf (Figure 4), resulting in deformation and death of the leaf blade (Figure 5), causing tissue loss, dryness, defoliation and death of branches (Figure 5) (BURNETT; SCHUBERT, 1985; SUSSEL, 2010). Values above 75% of infected leaf area on the adaxial surface are rarely found in the field, as rust causes rapid leaf senescence and plant defoliation.

Studies carried out by Mangone et al. (2017) in guabiju leaves reported that telia and urediniospores develop more frequently on the abaxial surface of leaves due to the higher

concentration of stomata. In the early stages of telia development, epidermal cells elongate and the palisade and spongy parenchyma begin to collapse and disorganize, respectively. At advanced stage, telia detach from the epidermis and cuticle and the parenchymal mesophyll becomes disorganized. Urediniospores occupy the mesophyll and elevate the abaxial epidermis. The total leaf blade thickness is reduced to 46% in spots with telia and 45% in spots with urediniospores. Based on information from the histological study by Mangone et al. (2017), the most suitable way to control rust through applications with fungicides should be mainly directed on the abaxial surface of leaves, since this is where infection begins.

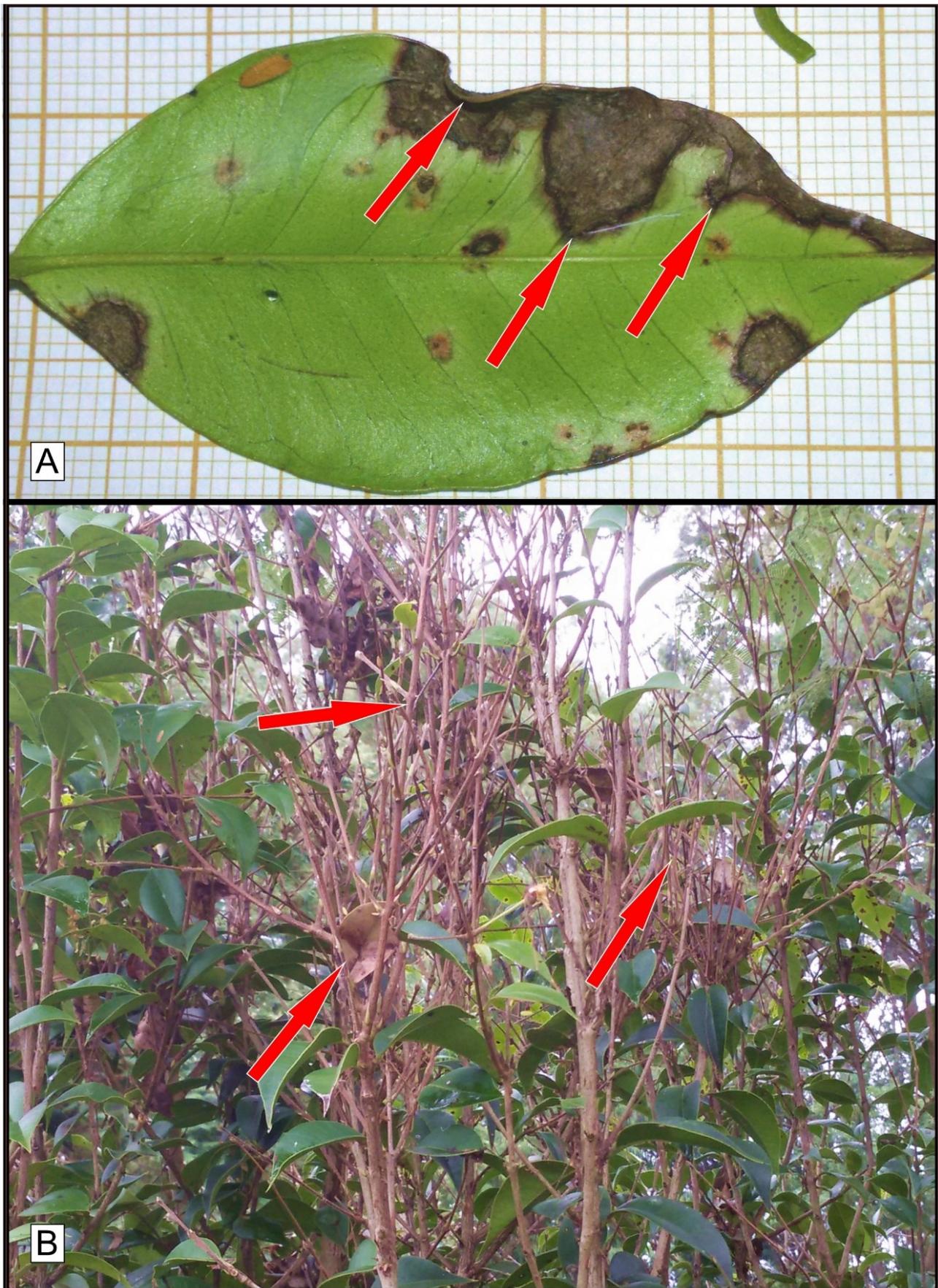


Figure 5. Guabiju (*Myrcianthes pungens*) leaves with deformation and death of the leaf blade, a symptom of the action of *Austropuccinia psidii* (A). Leaves with advanced attack by *A. psidii* resulting in the drying of leaves and consequently falling of the organ (B). Eldorado do Sul, RS, 2019.

In myrtaceae in general, when infection attacks developing fruits, it causes fruit drop, mummifications or necrotic lesions (SILVEIRA, 1951). In guava, the pathogen also affects flower buds, causing abortion and drop. According to Junqueira et al. (2001) in the initial development phase, flower buds present circular lesions with variable diameter and covered by a

yellowish powdery mass, causing partial or total loss of production in guava trees. When fruits remain on the plant, they become deformed, compromising them for fresh consumption (JUNQUEIRA et al., 2001; SUSSEL, 2010). In the present study, the presence of pustules on flower buds was observed (Figure 6).

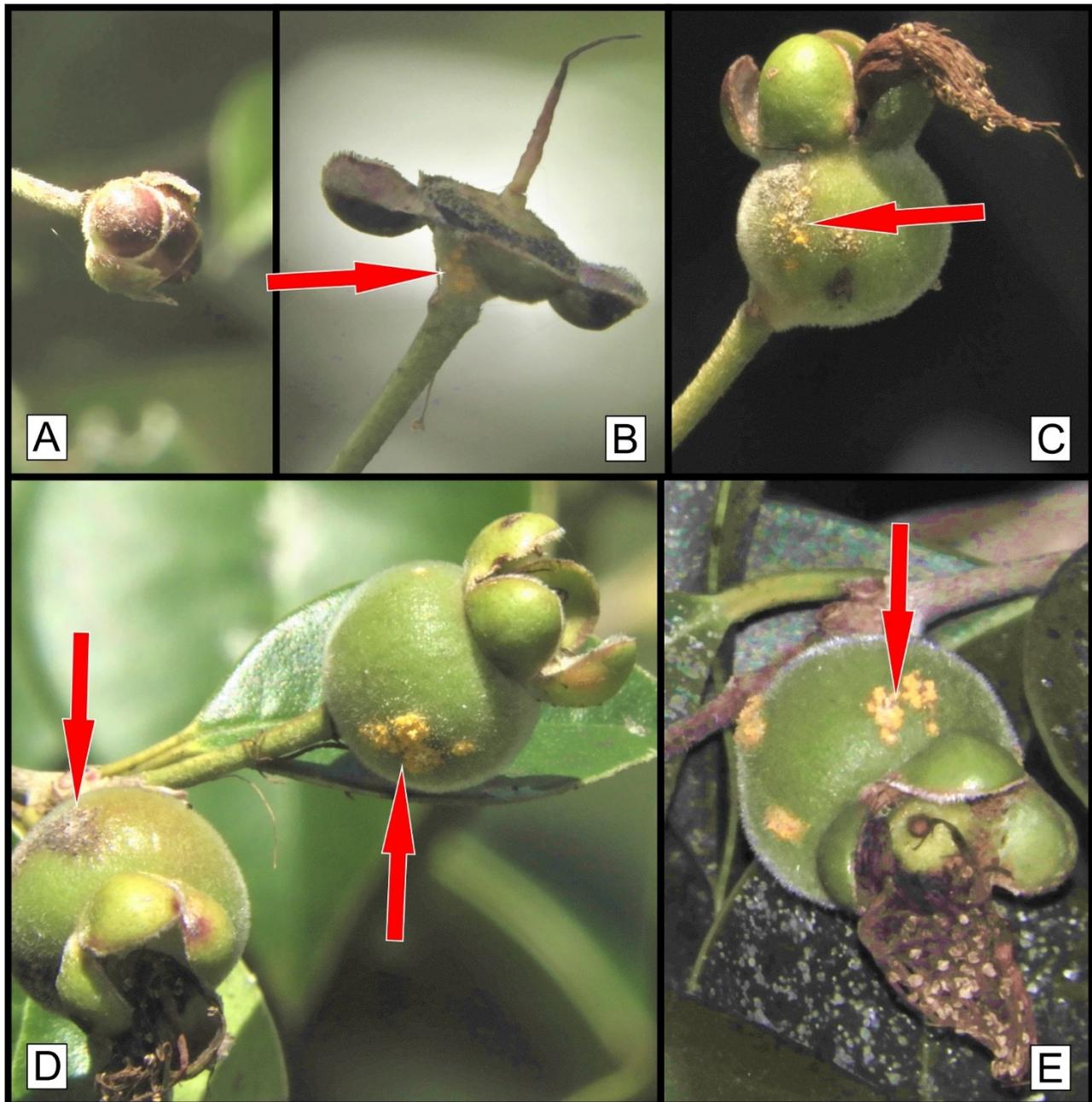


Figure 6. *Myrcianthes pungens* floral buds with symptoms caused by *Austropuccinia psidii* (A) flower after the drop of petals, covered by urediniospores (B). *A. psidii* infection in developing *M. pungens* fruits with lesions covered by urediniospores. More advanced infection, formation of spots (C) and pustule in the initial infection phase (D-E). Eldorado do Sul-RS, 2019.

Affected flowers had 100% abortion and drop and when infection occurred in fruits, they also dropped from the plant (Figure 6).

In this work, the presence of the disease in guabiju seedlings was not evaluated; however, according to literature, the disease can also occur at this stage. Ruiz et al. (2017) evaluated the susceptibility of myrtaceae seedlings to *Puccinia* sp. in the state of São Paulo and observed that guabiju showed symptoms of the disease in eight of ten seedlings evaluated. The authors also found that the incubation period of *Puccinia* sp. was five days and the latency period was 15 days in guabiju.

In search for accessions more resistant to rust, the susceptibility of the guabiju working collection to *Austropuccinia psidii* was evaluated, where 90% of plants showed some symptoms of the disease, with significant differences among accessions in the incidence and severity of the disease (Table 1). Assessing the incidence of affected plants, significant difference among accessions was observed (Table 1). Accession G05 differed from the others, presenting incidence in 30% of plants. Also with significant difference, accession G08 appeared with incidence of 50%. The other accessions: G01, G02, G03, G04, G06, G07, G09, G10, G11, G12, G13, G14, G15 and G16 presented at least 80% of affected plants.

Assessing the severity of the disease, accession G03 proved to be more susceptible. Accession G05, on the other hand, showed the highest level of resistance. The other accessions showed intermediate behavior against the disease.

In order to insert guabiju in the commercial agricultural matrix, the search for more resistant accessions is extremely important. As

the accession or cultivar shows less resistance, being more susceptible to the pathogen, the more the producer will have to spend resources to control the disease.

Table 1. Average grades (0 to 5) assigned according to the adapted diagrammatic scale, incidence (number of affected plants) and severity in the different accessions of the guabiju working collection (*Myrcianthes pungens*) at UFRGS. Eldorado do Sul, RS, 2020.

Accession	Incidence (%)	Severity
G01	100 a	3.20 d ¹
G02	100 a	3.55 c
G03	100 a	4.33 a
G04	90 a	2.30 e
G05	30 c	0.30 i
G06	100 a	2.80 d
G07	100 a	1.65 f
G08	50 b	0.85 h
G09	100 a	3.95 b
G10	100 a	3.60 c
G11	100 a	3.00 d
G12	100 a	2.45 e
G13	100 a	3.80 b
G14	100 a	1.20 g
G15	80 a	2.55 e
G16	90 a	2.66 e
CV (%)	5.87	6.75

¹ Means followed by the same letters in the column do not differ statistically by the Scott-Knott test ($P < 0.05$)

In the month of January 2019, month of assessments of incidence and severity of the disease in accessions, the accumulated precipitation was 79.65 mm, and 16 days of the month were rainy (Figure 7). In addition, in the 16 rainy days, nine of them were in sequence (from 12/Jan to 20/Jan/2019), reinforcing the long period of high humidity to which plants were submitted. As reported by Ruiz et al. (1989) in eucalyptus, for infection to occur, conditions of high relative humidity, close to or equal to 100%, and presence of free water between 6 and 24 hours of leaf wetness are necessary. With the sequence of nine rainy days,

the environmental condition for *Austropuccinia psidii* infection was extremely favorable to the development of the disease,

with a long period of high relative humidity of approximately 216 continuous hours with leaf wetness.

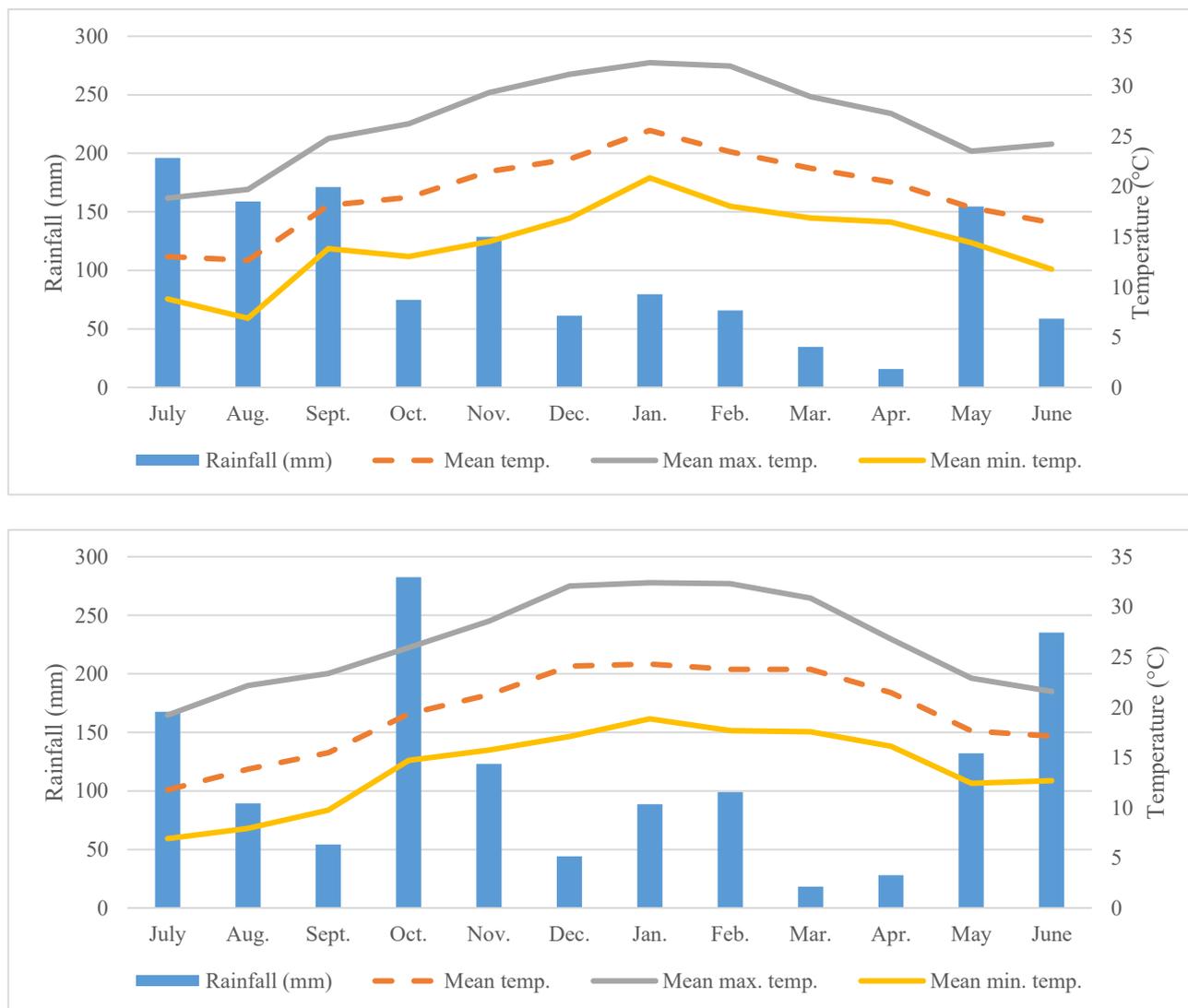


Figure 7. Meteorological data from July/2018 to June/2019 (A) and from July/2019 to June/2020 (B), monthly average precipitation, monthly maximum, average and minimum temperature of EEA-UFRGS, Eldorado do Sul- RS, Brazil.

Another important factor is temperature, and according to the same authors (RUIZ et al., 1989; APARECIDO;VALE, 2012), for eucalyptus, the temperature must be between 10 and 30 °C, with optimal temperature being 23 °C. In January 2019, the average temperature was the highest for the period (July/2018/ to June/2019) with 25.61 °C, with maximum temperature reaching 32.36 °C and minimum reaching 20.89 °C. Therefore,

the environmental conditions were close to ideal for the development and multiplication of the pathogen, combined with the host condition, which at the time was emitting new vegetative shoots.

According to the results obtained, these represent an indication of the behavior of accessions evaluated against *Austropuccinia psidii*; however, further studies are needed to evaluate the incubation period and latency,

in addition to incidence and severity at different seasons to better determine the variability presented among accessions.

Conclusion

The results obtained in this study allowed concluding that guabiju rust is caused by *Austropuccinia psidii*. This is the first report of rust in a guabiju population in the state of Rio Grande do Sul, Brazil. With the diagrammatic scale, it was possible to identify differ-

ent levels of susceptibility to rust in accessions present in the collection.

Acknowledgments

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