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Molecular Characterization and Pathogenicity of *Colletotrichum* on Banana Fruits: Wound Effects on Virulence and Cross-Infection

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Abstract: For this article, we evaluated whether wounds would affect the pathogenicity and virulence of *Colletotrichum* sp. isolates on *Musa* spp. banana cultivars. We further assessed the potential of cross-colonization with other fruit species and investigated the molecular and phylogenetic characterization of the most virulent isolates. Firstly, we collected dwarf bananas showing anthracnose symptoms from commercial markets in the city of Gurupi, Tocantins State, Brazil, and isolated *Colletotrichum* sp. under controlled conditions prior to identification. The virulence was assessed on wounded and unwounded banana fruits, identifying the most virulent isolate by exposure tests on fruits of the "prata", "maçã", "marmelo", and "terra" banana cultivars. We also subjected specimens of mango (*Mangifera indica*), papaya (*Carica papaya*), and apple (*Malus domestica*) fruits to the exposure tests. Our results indicated that pathogenicity varies with the isolate (with C2, C8, and C10 as the most virulent), fruit condition (wounded fruits are the most susceptible), and cultivars (terra, marmela, and maça are the most susceptible). All isolates were more virulent on wounded bananas, while those on unwounded ones showed lower virulence. Among the banana cultivars, "prata" fruits were the most susceptible, regardless of wounding. Additionally, *Colletotrichum* isolates from dwarf bananas were pathogenic to mango, papaya, and apple fruits. Furthermore, our results demonstrated that the most virulent isolates belong to the species *C. musae*. Collectively, our findings reinforce the relevance of minimizing post-harvest wounds on banana fruits and highlight the risks of cross-infection when storing bananas alongside other fruit species.

Keywords: anthracnose; tropical fruits; post-harvest diseases; cross-infection

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1. Introduction

The genus *Colletotrichum* is one of the most significant fungal pathogens in agricultural production because it is the causal agent of anthracnose in several crops [1–3]. This

pathogen is especially prevalent in tropical and subtropical regions, where climatic conditions and a wide variety of susceptible plant species facilitate its spread. It can impact crops at various stages, including post-harvest; however, infection during the post-harvest or ripening stages is responsible for major economic losses [4]. Due to the formation of black to dark brown sunken lesions on the fruit surface, anthracnose reduces fruit quality and marketability [5–7].

The banana (*Musa* spp.) is one of the most widely consumed tropical fruits in the world and has gained significant importance in the global market over the past 60 years. This popularity is due to its high nutritional value, digestibility, appealing texture, pleasant aroma, and convenience as both a dry and fresh snack, making it a favorite among people of all ages [8,9]. However, like other tropical fruits, bananas are delicate and are vulnerable to external factors during their growth, transportation, and storage, which can severely impact their quality and lead to significant post-harvest losses. *Colletotrichum musae* is the pathogen associated with anthracnose in banana crops, which is the most significant post-harvest disease, potentially causing 30–40% losses of marketable fruit [5,10]. This pathogen can infect bananas in the field, remaining dormant until conditions become favorable for its development [11]. In climacteric fruits like bananas, anthracnose symptoms typically appear during the ripening phase, manifesting as peel blemishes, brown to black sunken lesions, and orange to salmon-colored acervuli [12–14]. The severity of the disease depends on factors such as the banana cultivar, storage conditions, and the specific pathogen isolate.

In the literature, several studies have reported the dependence of cultivar species on the colonization of *Colletotrichum* spp. [3,15], including banana cultivars and *C. musae* [16,17]. Moreover, multiple *Colletotrichum* species have been found to infect or colonize the same host plant [18,19]. Additionally, the pathogenicity and virulence of *Colletotrichum* isolates vary significantly [2,20,21]. This variability can be attributed to the genetic differences among isolates and the unique biochemical mechanisms that each cultivar employs to recognize and defend against pathogens. Furthermore, in both cases, the presence or absence of injuries in the host also influences the aggressivity and severity of the pathogen [22,23].

Chemical applications using cupric products, strobirins, dithiocarbamates, benzimidazole, triazole compounds, prochloraz, imazalil, and chorothalonilis are commonly employed to control anthracnose in fruits [6,24]. For bananas, soaking the fruits in tedocor, carbendazim, prochloraz, prochlorin, or thiabendazole is a typical method to prevent and control post-harvest disease [25]. However, due to the harmful effects of these chemicals, identifying the aggressive isolates of *Colletotrichum* and resistant cultivars may offer a more effective approach to disease control [3,4], serving as a foundation for future management-integrated programs. Research is needed to explore the pathogenic variability of *Colletotrichum* species across various banana cultivars, thereby enhancing our understanding of their host range. As bananas are sensitive to the injuries that can occur during harvesting, packaging, and transportation, various microorganisms can penetrate the skin integuments through wounds. Few studies have evaluated the influence of lesions on fruit peels and the development of anthracnose in different banana cultivars and other fruits. Additionally, the potential for cross-infection among intraspecific isolates from banana cultivars and the fruits of other plant species should be investigated. Therefore, this study aims to evaluate the pathogenicity and virulence of *Colletotrichum* sp. isolates on different banana cultivars, as well as the molecular characterization and potential cross-infection of *Colletotrichum* sp. obtained from dwarf banana (*Musa cavendishii* L.) fruits to mango, papaya, and apple fruits.

2. Materials and Methods

2.1. Collection and Identification of Isolates

Initially, banana fruits from the dwarf banana cultivar (*M. cavendishii*) were collected from 10 commercial locations in Gurupi (11°43′4″ S, 49°04′07″ W), State Tocantins, Brazil. Healthy, uniformly sized fruits with a slightly yellowish color and at an early stage of maturation, suitable for fresh consumption, were purchased from local stores. The fruits were washed with neutral soap and rinsed three times in sterile water. After sanitization, the fruits were placed in plastic trays and incubated for 7 days at 27 °C. Following this period, fruits showing anthracnose symptoms, characterized by depressed brown lesions, were selected. Using a scalpel, symptomatic lesion pieces were excised and cleaned. Disinfestation involved immersing the tissues in 50% alcohol for 30 s, followed by 1% sodium hypochlorite for 40 s and then rinsing three times with sterile water. The cleaned lesion pieces were then transferred to 9 cm Petri dishes containing potato, dextrose, and agar (PDA) and incubated at 27 °C with a 12-hour photoperiod. Once the fungi grew from the lesions, the colonies were subcultured onto new PDA Petri dishes to obtain pure cultures. To identify the isolates at the genus level, slides of the fungus's assimilative and reproductive structures were examined under an optical microscope. Identification was based on the classic literature [26,27]. The Petri dishes containing the isolates were then stored and maintained in the Mycological Collection of the Phytopathology Laboratory at the Gurupi Campus of the Federal University of Tocantins.

2.2. Area Under the Disease Progress Curve (AUDPC) Tests of Colletotrichum sp. Isolates

For evaluating the area under the disease progress curve (AUDPC) for the 27 *Colletotrichum* sp. isolates, healthy dwarf banana fruits were used. After aseptic treatment with 50% alcohol and 1% sodium hypochlorite, followed by rinsing in sterile water, the fruit surface was inoculated with the isolates, using both "wounded" and "unwounded" fruits. Injuries were made using a sterilized scalpel, then a 6 mm disc mycelium disc was placed at the site of each wound. The inoculated fruits were placed on separate trays and stored in a humid chamber with 85 ± 3% relative humidity and a temperature of 27 ± 3 °C. Every 48 h, the lesion area and diameter were measured with a digital caliper until the tenth day [1]. To confirm the causal agent of the infection, reisolation was performed on PDA growth medium, and the fungus was identified under an optical microscope, ensuring that all stages of Koch's postulates were fulfilled. The AUDPC was estimated from the diameter of the lesions (mm), using the equation: $AACPD = \Sigma (y_i + y_{i+1})/(t_{i+1} - t_i)$, where y_i and y_{i+1} are the severity values observed in two consecutive evaluations and t_i is the interval between evaluations [28,29].

2.3. Virulence of Colletotrichum sp. Isolates in Dwarf Banana Fruits, Both Wounded and Unwounded

For the virulence bioassay, we selected three isolates that caused the largest lesions on dwarf banana fruits unwound during the pathogenicity test (Section 2.2. AUDPC). Initially, the isolates "C2", "C8", and "C10" were inoculated into the dwarf banana fruits, following the same procedure as described in Section 2.1. Subsequently, lesion assessments (virulence) were recorded every 24 h for 10 days using a digital caliper. Following the same procedures, the virulence of these same isolates was tested in other banana cultivars: "prata", "maçã", "marmelo", and "terra".

2.4. Evaluation of Cross-Infection of Colletotrichum sp. Obtained from Banana

The isolates "C2", "C8", and "C10" were used to investigate the potential for cross-infection. Healthy, uniform papaya, mango, and apple fruits, purchased from local stores

at a suitable stage of ripeness for consumption, were selected. Initially, the fruits were washed with neutral soap and rinsed three times in sterile water. A 6 mm diameter disc of mycelium was then placed on the surface of the fruit peel, on fruits both with and without wounds. The inoculated fruits were placed on separate trays and stored in a humid chamber with a relative humidity of 85 ± 3% and a temperature of 27 ± 3 °C. Every 48 h, lesion measurements were taken using a digital caliper until the tenth day.

2.5. DNA Extraction, PCR, and Sequencing

Considering the epidemiological importance of the disease, the three isolates (C2, C8, and C10) that caused the largest lesions on unwounded dwarf banana fruits during the pathogenicity test were selected for molecular analysis. *Colletotrichum* isolates were grown on PDA media at 25 ± 2 °C for 7 days under 12 h of light. Genomic DNA was extracted using the CTAB (cetyl trimethyl ammonium bromide) protocol, as described previously [2,30]. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) partial region was amplified for all isolates to identify haplotype diversity through DnaSP v.6 [31]. The sequences thus obtained were compared with those from GenBank using the BLAST tool. The following loci were amplified for multilocus analyses: the intergenic spacer between DNA lyase and the mating-type locus MAT1-2-1 (APN2/MAT-IGS), and the intergenic spacer between GAPDH glutamine synthetase (GS) and β-tubulin (TUB2). These genomic regions are reported to be the most informative for identifying species from several *Colletotrichum* species complexes [32]. PCR amplifications were performed in a 12.5 µL volume reaction containing 4 µL PCR-grade water, 1 µL DNA template, 0.625 µL of each primer (10 µM), and 6.25 µL 2× PCR master mix (Promega GoTaq Master Mix; Madison, Wisconsin, USA). The PCR products were visualized in a 1.5% agarose/TAE gel by electrophoresis. The primers and cycles used in this study [33–35] are listed in Supplementary Table S1. PCR products were purified by ethanol and ammonium acetate precipitation and then sequenced on an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA) on a DNA sequencing platform located at the Laboratório de Bioinformática e Biologia Evolutiva–LABBE of the Universidade Federal de Pernambuco (Pernambuco, Recife, Brazil). Sequence reads were assembled into contigs and edited using the Staden package v.2.0.0 (1998) [36].

2.6. Phylogenetic Analyses

Sequences from the *Colletotrichum* ex-type and reference isolates from previous studies were retrieved from GenBank and included in the phylogenetic analyses. Multiple sequence alignments (MSA) were generated with the online version of MAFFT 7 using the Q-INS-i iterative refinement method [37,38]. For the multilocus analysis, the loci were concatenated using Sequence Matrix v. 1.8 [39].

A phylogeny for each locus and concatenated alignments were inferred using maximum likelihood (ML). ML analyses were performed using IQ-TREE v. 2.1.2 [40], keeping identical sequences in the alignment. The ML tree search was estimated using a specific substitution model for each locus. Model parameters were separately estimated for each partition using ModelFinder [41,42], allowing each partition to have its evolution rate (-m MFP-p). The best ML tree was found after 1000 iterations with a perturbation strength of 0.2. ML analyses were carried out with 1000 bootstrap pseudoreplicates under the GTR-GAMMA model (-m GTRGAMMA-p 12345-k-f a–N 1000-x 12345). The species were recognized by utilizing the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) criteria, as described previously [43,44].

2.7. Morphological Characterizations

For phenotypic characterization of the new species, mycelial plugs 5 mm in diameter were taken from the margin of 7-day-old colonies and transferred to the center of Petri

dishes containing PDA. The culture was maintained in the same incubation conditions and the colony features were recorded from 7-day-old colonies. The conidia from PDA were prepared for examination under a microscope by mounting them in a solution of 10% lactic acid. A modified version of the slide culture technique developed by Johnston and Jones [45] was used to induce the formation of appressoria. In this method, a small block of agar-agar medium (4%) was placed on a sterile microscope slide, and conidial masses were spread on the top edges of the block. The block was then covered with a sterile coverslip and left to incubate in the dark at a temperature of 25 °C for 24 h. Microscopic images of the samples were captured using a Nikon Eclipse Ni-U transmitted light microscope equipped with a DS151 L3 digital camera.

2.8. Statistical Analysis

The difference in the area under the disease progress curve (AUDPC) of the same isolate between fruits with and without wounds was estimated by a *t*-test ($p < 0.05$). Virulence results were examined through linear and non-linear regression, a one-way ANOVA, and test-*t* $p< 0.05$ (after 10 days), with the normality and variance assumptions checked. All statistical analyses were performed using SigmaPlot software (Systat Software, San Jose, CA, USA), version 12.0.

3. Results

3.1. Morphological Characterization

The basic growth pattern, colony characteristics on PDA growth medium, and spore morphology were observed to identify the morphological groups associated with the genus *Colletotrichum* sp. (Figure 1A). The colonies exhibited colors ranging from white to light gray, with a similar reverse side. They varied in aerial mycelium formation, ranging from flaky with no visible conidia to sparse, submerged, and well-sporulated mycelia (Figure 1B,C). Most isolates displayed masses of orange-colored conidia on the colony surface. The conidia were hyaline, straight, and featured rounded appendages. At the end of the evaluations, the three isolates that showed the best responses to the analyzed variables were selected, and the length and width of 30 conidia were measured, yielding an average size of 23.5 µm by 9.7 µm.

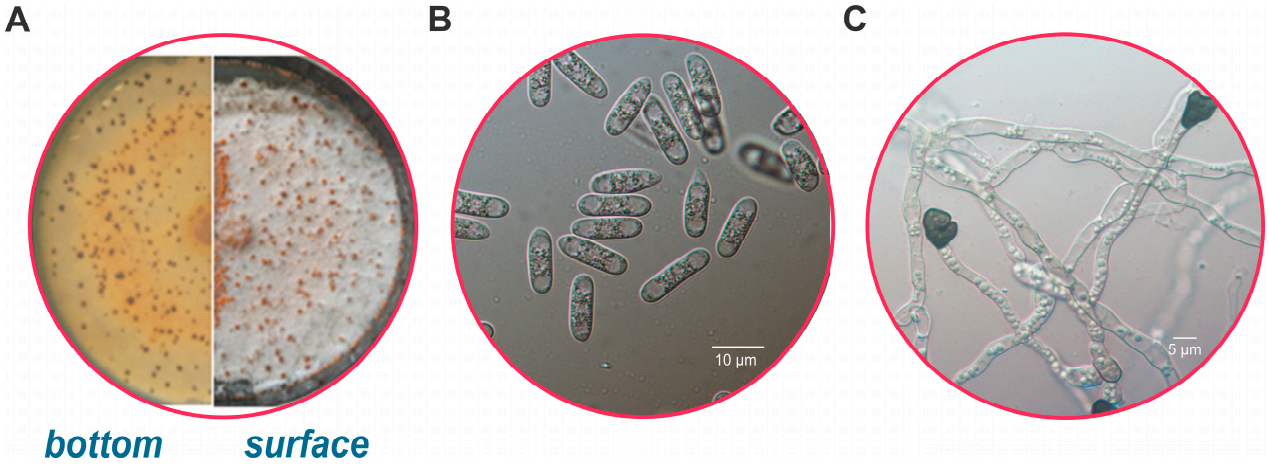

Figure 1. Morphological aspects of the colonies in PDA Petri dishes: the bottom and surface of *Colletotrichum* sp. isolated from dwarf bananas (**A**), conidia formed in PDA medium (**B**), and appressorium formation (**C**).

3.2. Pathogenicity and AUDPC of Isolates in Dwarf Banana

The pathogenicity results revealed the effects of 27 *Colletotrichum* sp. isolates on banana fruits under two exposure conditions. When the fruits were wounded, all isolates at the inoculation sites developed dark brown necrotic lesions that became depressed as the pathogen colonized the tissues, confirming their pathogenicity. In the absence of a wound, 19 of the isolates exhibited similar symptoms (Figure 2). Five days after inoculation, pinkish/orange conidial cirri (masses of spores) were observed under both conditions. To confirm the causal agent of the infection, the pathogenic isolates were reisolated on PDA growth medium, and the fungi were identified under an optical microscope, fulfilling all stages of Koch's postulates. The assessment of the area under the disease progress curve (AUDPC) showed that all isolates affected the bananas when wounds were present. Although the AUDPCs for isolates 8, 22, and 25 were similar under both conditions, a greater area of infection was noted for isolates 2, 8, and 10 in the absence of wounds on the dwarf banana (Figure 2). This indicates the potential of these three isolates (C2, C8, and C10) to penetrate tissues through physical and/or biochemical mechanisms.

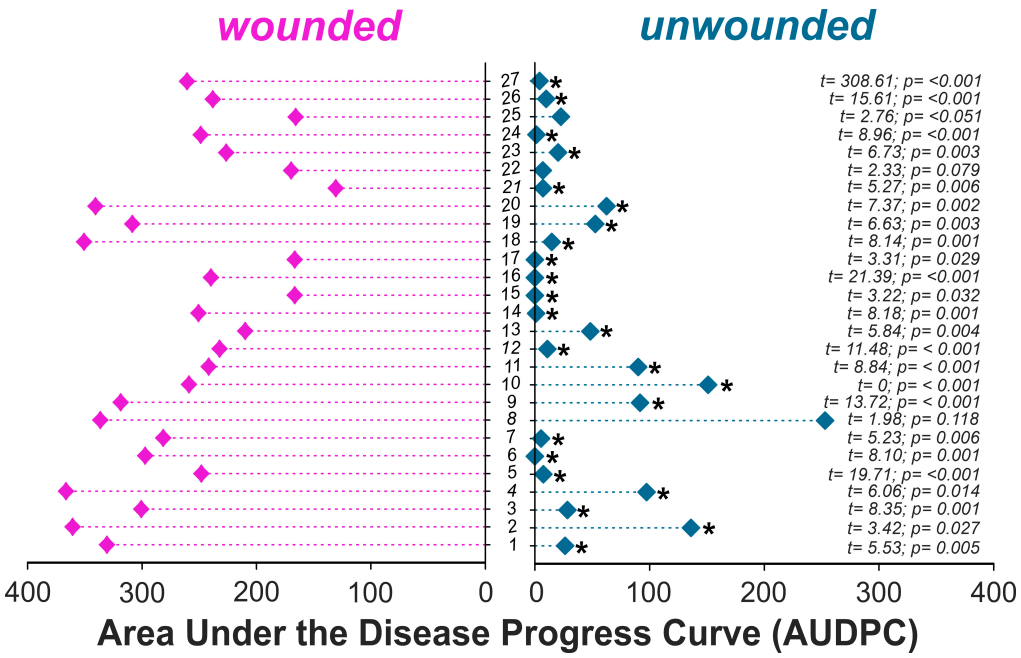

Figure 2. The area under the disease progress curve (AUDPC) after 10 days of inoculation of 27 *Colletotrichum* sp. isolates in dwarf banana fruits, both with and without wounds, as shown in the center. Symbols represent the mean values of three replicates, while asterisks indicate significant differences for the same isolate between wounded and unwounded fruits (*t*-test, $p < 0.05$).

3.3. Virulence of Colletotrichum sp. Isolates in Dwarf Banana

Virulence increased over time in both fruits with lesions on the skin (Figure 3A) and fruits without lesions (Figure 3B); no differences were seen between isolates in injured fruits ($H = 1.86$; $p = 0.439$) and non-injured fruits ($F_{2,6} = 0.88$; $p = 0.459$) after 10 days. However, the infections caused by the isolate C2 ($t = 2.88$; $df = 4$; $p = 0.044$) and the isolate C10 ($t = 5.99$; $df = 4$; $p = 0.0039$) were greater when wounds were made in the skin of the fruit than in fruit without wounds, and were without variation for the isolate C8 ($t = 1.83$; $df = 4$; $p = 0.140$) (Figure 3C).

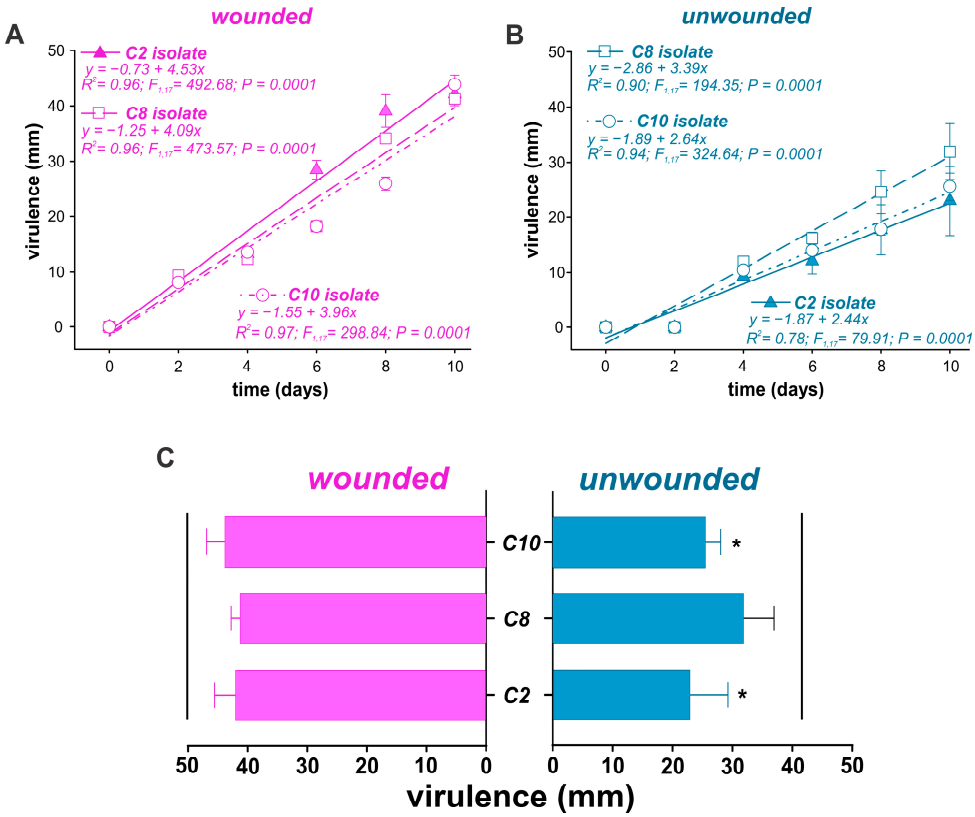

Figure 3. Increase of the virulence of *Colletotrichum* sp. isolates in dwarf banana fruits with wounds (**A**) and without wounds (**B**) over time, and final virulence after 10 days (**C**). (**A**,**B**) Symbols are the mean of three replicates and vertical lines represent the standard error (SE). The horizontal bars (**C**) showed the mean (±SE) of three replicates and asterisks (*) on the bars indicate the statistical difference between treatments in the same isolate according to the *t*-test ($p < 0.05$). Horizontal bars grouped by vertical lines indicate that no statistical difference ($p > 0.05$) among the isolates was seen for wounded and unwounded fruits. We used a Tukey test (for parametric data) or the Kruskal–Wallis test (for nonparametric data).

3.4. Virulence of Colletotrichum sp. Isolate "C8" in Four Banana Cultivars

Since isolate "C8" caused the most significant and most severe infection in inoculated dwarf banana fruits without prior wounds (as shown in Figure 2), it was selected for the evaluation of virulence in four banana cultivars. Our results indicated that *Colletotrichum* colonization on bananas varied with the cultivar (Figure 4), with a constant increase in virulence in the four banana varieties regardless of whether the fruit was wounded (Figure 5A) and in the absence of wounds (Figure 5B). After 10 days, the virulence was similar in all varieties of banana with injuries ($F_{3,8} = 3.33$; $p = 0.077$); in non-injured fruits, the banana variety "prata" was most susceptible. When the susceptibility of the same banana variety was evaluated after 10 days, the "terra" banana ($t = 14.55$; $df = 4$; $p = 0.0001$), "marmela" banana ($t = 6.02$; $df = 4$; $p = 0.0038$), and "maçã" banana ($t = 7.09$; $df = 4$; $p = 0.0021$) were more susceptible when the fruit had wounds on the skin, while the "prata" banana showed no difference in both conditions ($t = 1.66$; $df = 4$; $p = 0.170$) (Figure 5C).

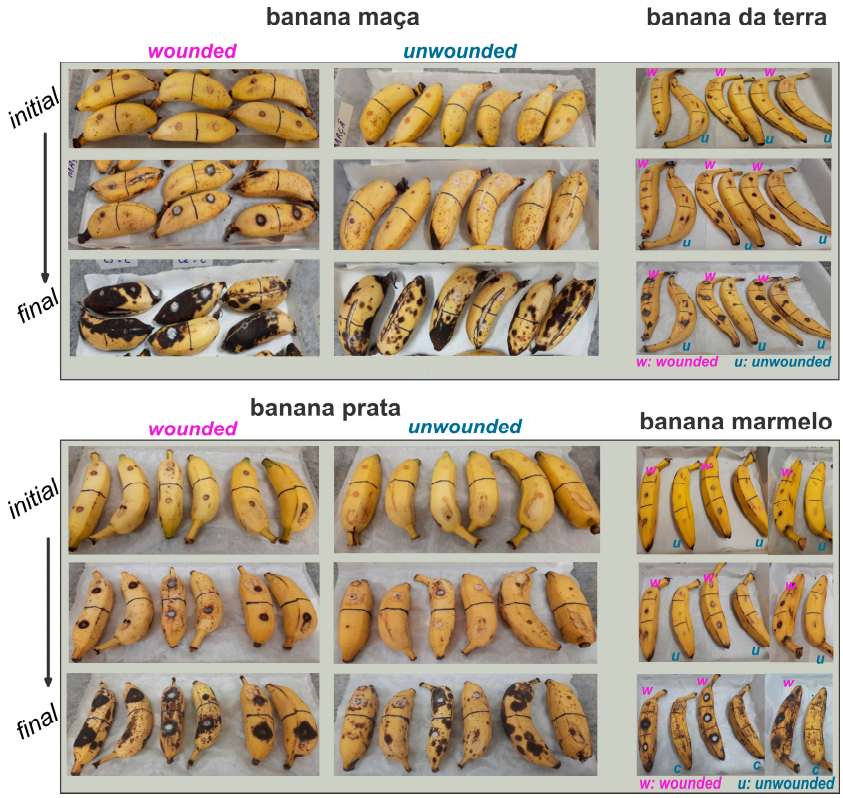

Figure 4. Virulence of isolate "C8" of *Colletotrichum* sp. in four banana cultivars in the presence and absence of a wound, in the initial, intermediary, and final phases (10 days).

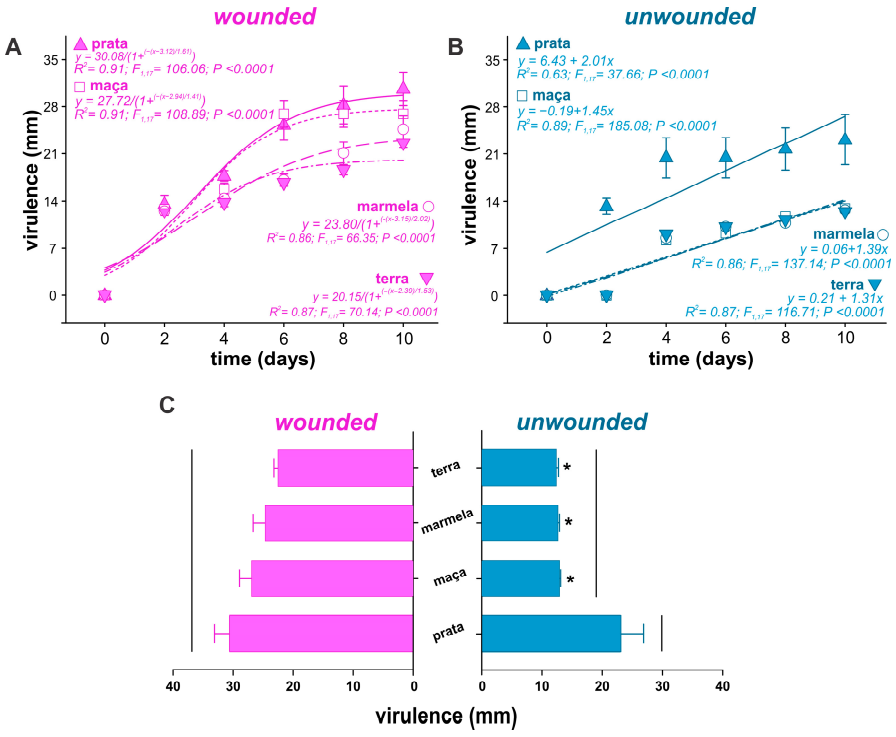

Figure 5. Virulence of the isolate "C8" of *Colletotrichum* sp. for banana cultivars in the presence (**A**) and absence (**B**) of a wound, and final virulence after ten days (**C**); symbols are the mean of three replicates and vertical lines show the standard error (SE). The bars (**C**) show the mean (± SE) of three replicates, and asterisks (*) on the bars indicate the statistical difference between the same variety of bananas at the test-*t* ($p < 0.05$); vertical lines above the bars at the same level indicate that no statistical difference was found by the Tukey test, $p < 0.05$.

3.5. Cross-Infection of Colletotrichum sp. Obtained from Bananas to Other Fruit Species

Our results demonstrated that cross-inoculation occurred in the three isolates tested on papaya and apple fruits, regardless of whether the fruits were injured. However, isolate "C10" was not pathogenic to apples without wounds. In mango, only the isolates "C2" and "C8" affected the fruit when it was injured (Figure 6). Additionally, when the isolate was pathogenic, initial symptoms appeared earlier in the injured fruits (Figure 6).

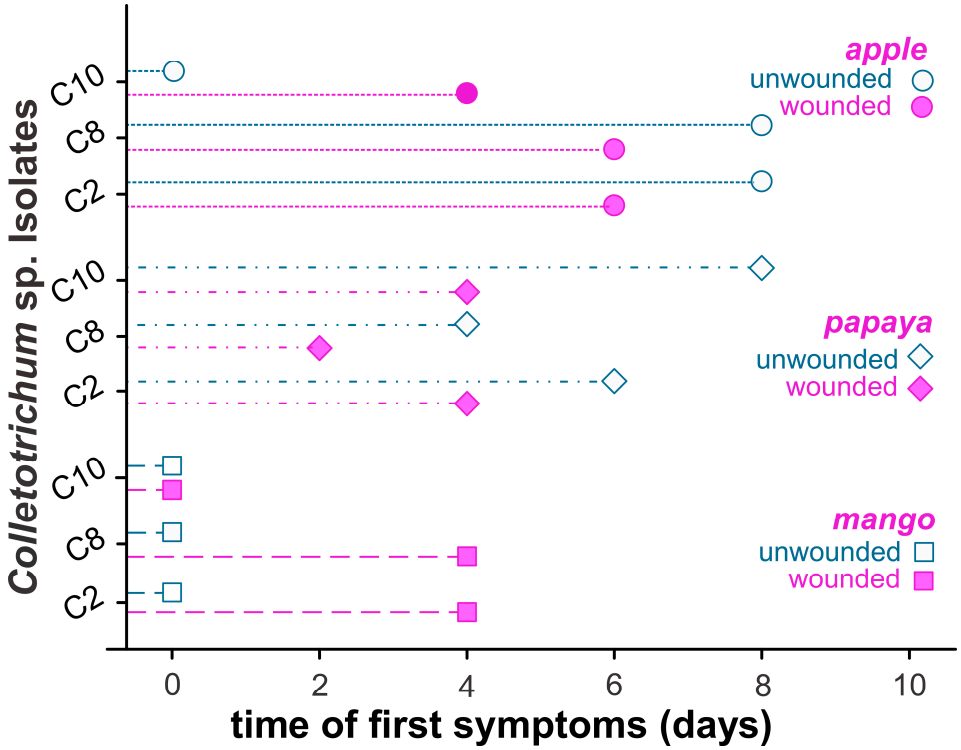

Figure 6. Cross-infection of *Colletotrichum* sp. obtained from dwarf banana to mango, papaya, and apple fruits; the horizontal axis (*x*) shows the time of onset of the initial symptoms of *Colletotrichum* sp. in each fruit species (three replicates); "0" indicates that no pathogenic symptoms were observed during 10 days of evaluation.

3.6. Sequencing and Phylogenetic Analyses

A preliminary analysis among the three isolates (C2, C8, and C10) using partial GAPDH sequences identified one haplotype among the *Colletotrichum* banana isolates. The BLAST analysis indicated that isolates presented a high similarity with species from one of the *Colletotrichum* species complex: one haplotype in *C. gloeosporioides sensu lato*. Three isolates were aligned within the *C. musae* clade in the multilocus analysis, with strong support (100%) in the ML multilocus analyses (Figure 7, Table S2).

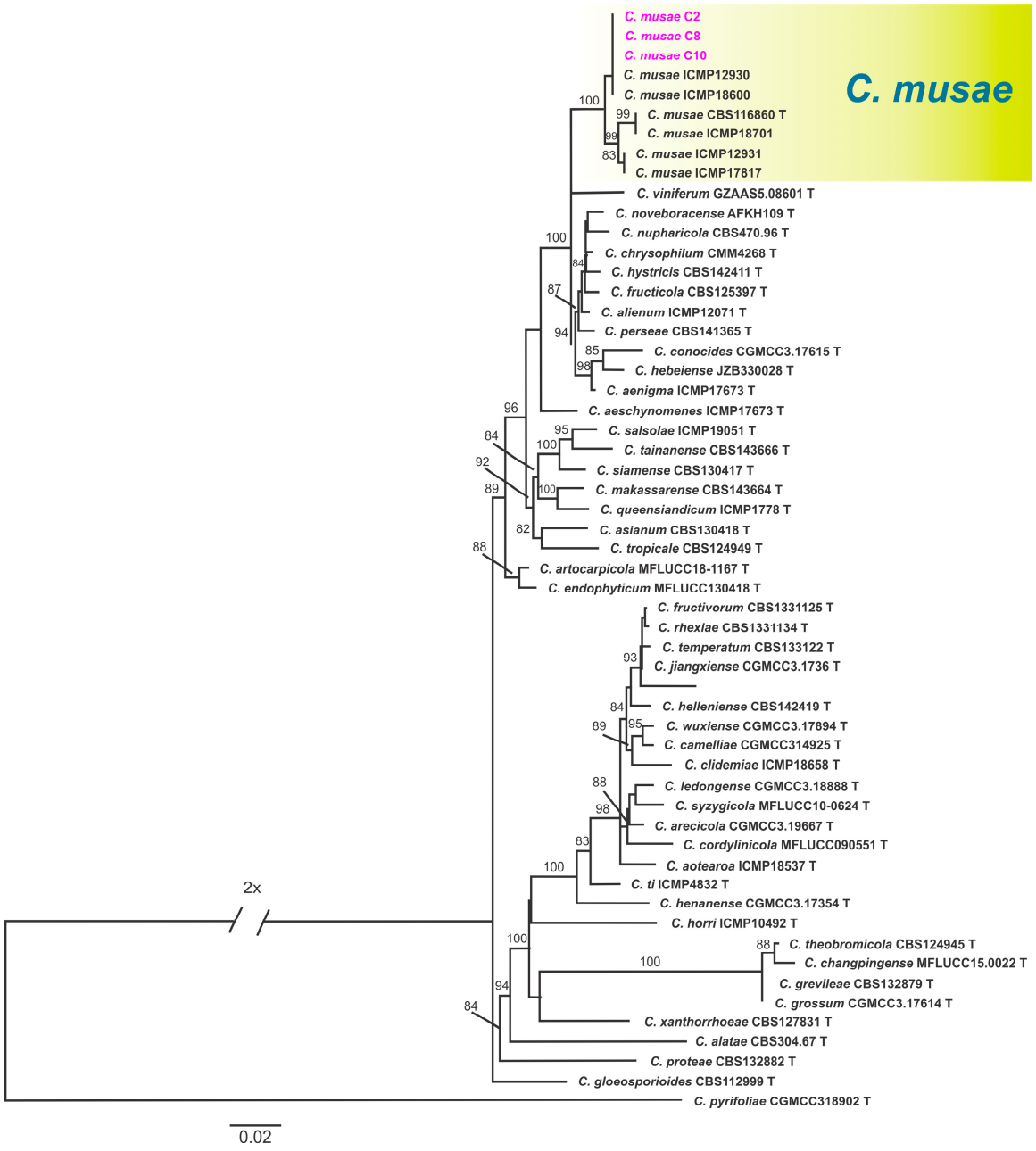

Figure 7. Maximum likelihood tree of the *C. gloeosporioides* species complex inferred on IQ-TREE from a concatenated alignment of APN2/MAT-IGS, GAPDH, GS, and TUB2. Significant supports for ML (SH-alrt bootstrap $\geq$ 80) are shown above the nodes. The tree was rooted with *Colletotrichum pyrifoliae*. Ex-type isolates are indicated with "T" at the taxa labels' ends. Isolates from *Musae* spp. are highlighted in bold. The scale bar indicates the average number of substitutions per site.

4. Discussion

Our results indicated that all tested isolates were pathogenic when the protective skins of the fruits were ruptured by wounds. Notably, three isolates were virulent, even in fruits without injuries. While pathogenicity in these uninjured fruits was delayed and resulted in larger necrotic areas, virulence was lower over time. Similarly, the virulence of these isolates varied among different banana species, paralleling the findings on cross-inoculation.

Fruits of dwarf bananas with injuries exhibited the largest necrotic areas (AUDPC) ten days post-inoculation. In contrast, not all isolates caused disease in "unwounded" conditions. In both cases, we confirmed the causal agent of fruit damage through Koch's postulates, concluding that all isolates obtained from reisolation had characteristics similar to

those used in the fruit inoculation process. Our results align with those of Santos et al. [46], who evaluated the genotypic and pathogenic diversity of *C. musae* in bananas, noting that all isolates were pathogenic in fruits with injuries. Similarly, Pessoa et al. [47] found that *C. musae* specimens that were inoculated and without injury exhibited a lower rate of lesions compared to inoculations with injuries. The "unwounded" condition also yielded lower averages in AUDPC evaluations. Although it has been noted that *Colletotrichum* spp. possess specific penetration mechanisms that can be effective regardless of the presence of wounds or natural openings [48,49], other studies report that injuries in the host can facilitate the entry of phytopathogens and promote colonization [22,23,50,51]. This can accelerate and enhance the infection and colonization processes [52]. Additionally, banana peel contains various compounds, such as hydroxycinnamic acids, flavonoids, tannins, alkaloids, phlorotannins, glycosides, catecholamines, and anthocyanins, which exhibit antifungal and antibacterial activity [53–55]. Consequently, in damaged fruit, these defenses may be weakened, facilitating the colonization of microorganisms.

Our results also indicate that pathogenicity varied among the collected isolates. In this context, differences were found among *Colletotrichum* sp. isolates, likely due to genetic variability within the same species, which affected their pathogenic capacity [20]. According to Barguil et al. [56], the non-pathogenicity of certain isolates in their original hosts may relate to the pathogen's endophytic habits or a loss of pathogenicity in those isolates. Additionally, Farias Couto et al. [57] demonstrated that *C. musae* isolates can differ significantly, whether inoculated in their original cultivar or isolated from one cultivar and inoculated in another, and some may even be non-pathogenic.

This study also demonstrates the ability of the fungus to cross-infect popular banana cultivars, with variability in virulence among *Colletotrichum* sp. isolates and banana species. With injuries, all four banana species were similarly colonized, but in the absence of wounds, the "terra", "marmelo", and "maçã" cultivars were less affected, while the "prata" cultivar was the most affected after 10 days, regardless of the wound. Similar findings were reported by Ootani et al. [58], who noted that the "prata" and "maçã" banana cultivars were more susceptible to *C. musae*. Differences in susceptibility among banana cultivars have been attributed to complex biochemical mechanisms that allow each fruit species to recognize and defend against potential pathogens [5,16,17]. Furthermore, factors such as soil nutrition, the presence or absence of magnesium (Mg) or calcium (Ca), and geographic conditions have also been reported to influence banana susceptibility to *C. musae* [59]. Additionally, the pH of the fruit peel and the soluble solid content have been implicated in the development of anthracnose across different banana cultivars [60], highlighting that these biochemical characteristics are specific to each species.

Isolates of *Colletotrichum* sp. obtained from dwarf bananas demonstrated cross-infection capability, with pathogenicity to other fruit species, including mango, papaya, and apple, indicating their lack of specificity. This contrasts with studies that have shown that *C. musae is* almost exclusively associated with bananas [4,61,62]. However, colonization was dependent on the fruit type and its condition, particularly whether it had wounds, with mango being notably less affected. Studies have shown that mangoes contain gallotannins and resorcinols in both unripe and ripe fruit peels, with a negative correlation between the levels of these compounds and the development of anthracnose caused by *C. gloeosporioides* [63–65]. Similarly, Karunanayake et al. [66] further suggested that mango resistance to *C. gloeosporioides* is attributed to a constitutive defense system comprising antifungal resorcinols, gallotannins, and chitinases, with anthocyanins and flavonoids also associated with resistance to this pathogen [67]. Additionally, other studies have reported instances of cross-colonization of *Colletotrichum* spp. in various tropical fruits, including mango [4,18,20,61]. *C. musae* has also been shown to infect other species of fruit, such as

guava, water apple, avocado, and dragon fruit plants [68,69]; most recently, it has been reported to infect the roots and leaves of coffee and lychee trees [70,71]. The results obtained from the phylogenetic analyses corroborate the morphological characterization data of this study; the morphological features described in Section 3.1 are consistent with the descriptions of the type and epitype isolates of *C. musae* [72,73]. It is important to highlight that other *Colletotrichum* species have been reported as causal agents of banana anthracnose in Brazil, including *C. chrysophilum*, *C. tropicale*, *C. theobromicola*, and *C. siamense* [33]. Accurate and precise identification of the etiological agent is essential for the implementation of effective management strategies. However, it has been demonstrated that the presence of wounds makes the fruits more vulnerable to pathogen attack. Therefore, studies must be directed toward the search for substances that can prevent the penetration of pathogens via wounds in the integuments.

5. Conclusions

Our results show both the lack of specificity of *Colletotrichum musae* and its ability to colonize several fruit species, with greater virulence in fruits that have wounds. Care for the fruits must occur from their development in the field, avoiding the mechanical damage that may occur due to attacks by birds and insects or management practices. This highlights the care that must also be taken when harvesting, packaging, transporting, and storing fruits.

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