

ORIGINAL ARTICLE OPEN ACCESS

Colletotrichum Species From Grape Ripe Rot Symptoms and Asymptomatic Grapevine Tissues

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Received: 21 August 2025 | **Revised:** 13 October 2025 | **Accepted:** 1 December 2025

Keywords: aetiology | endophytic lifestyle | epidemiology | etiology | *Vitis* sp.

ABSTRACT

Grape ripe rot (GRR), caused by species of the *Colletotrichum* genus, is an emerging threat to viticulture in Brazil. Significant outbreaks have been reported in the southern and northeastern grape production regions, yet little is known about the *Colletotrichum* species involved. To address this gap, we collected 182 isolates from symptomatic fruit and asymptomatic leaves, flowers and fruit of two *Vitis* species in six states of Brazil. *Colletotrichum* species were identified using ITS, *GAPDH*, *TUB2*, *CHS-1* and *ACT* gene sequences and tested for pathogenicity on injured and uninjured berries of *Vitis vinifera* ‘Thompson’ and *V. labrusca* × *V. vinifera* ‘Niagara Rosada’. Most isolates (82%) were from Paraná State. Phylogenetic analyses confirmed first reports of *C. melonis*, *C. cf. melonis*, *C. chrysophilum*, *C. hebeiense*, *C. gloeosporioides*, *C. jiangxiense* and *C. boninense* on grapevines. Other species previously reported to cause GRR in Brazil were found, including *C. nymphaeae*, *C. siamense*, *C. viniferum*, *C. theobromicola*, *C. fructicola* and *C. karstii*. All species caused disease on injured berries. *C. nymphaeae*, *C. siamense*, *C. chrysophilum* and *C. viniferum* were isolated from both symptomatic and asymptomatic grapevine tissues, with *C. nymphaeae* being the most prevalent and *C. viniferum* mainly associated with berry lesions. Seven species were exclusively detected in asymptomatic tissues, suggesting a potential endophytic lifestyle. We hypothesise that asymptomatic grapevine leaves may serve as inoculum for all *Colletotrichum* species identified in this study. These findings highlight the diversity of *Colletotrichum* in Brazilian vineyards and the need to clarify their role in GRR.

1 | Introduction

The plant genus *Vitis* includes many species that are cultivated worldwide; however, global production is largely focused on *Vitis vinifera*, which is preferred for its superior quality in wine production compared to other species such as *Vitis labrusca*, commonly used for producing less-refined table wines (Dal Santo et al. 2016; Torregrosa et al. 2015). The southern region of Brazil produces most of the country's grapes for wine production. This region is characterised by hot and humid summers (Amorim et al. 2016) that favour fungal diseases, such as grape ripe rot (GRR) (Echeverrigaray et al. 2020).

GRR is an important disease of both *V. vinifera* and *V. labrusca* worldwide, as reported in regions such as southern and northeastern Brazil, the Mid-Atlantic United States and northeast China (Cosseboom and Hu 2022; Echeverrigaray et al. 2020; Ji et al. 2021). *Colletotrichum gloeosporioides*, *C. acutatum* and *C. boninense* species complexes were found to cause GRR in *Vitis* species (Batista et al. 2023; Echeverrigaray et al. 2020; Hong et al. 2008; Lei et al. 2016). While *C. gloeosporioides* and *C. boninense* complexes were reported to be prevalent in Brazil and China (Batista et al. 2023; Echeverrigaray et al. 2020; Lei et al. 2016), the *C. acutatum* complex has been prevalent in the Mid-Atlantic United States and in New South Wales, Australia

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(Cosseboom and Hu 2022; Greer et al. 2011). GRR symptoms and signs can be observed in grape clusters during crop stage 8 fruit maturation (Lorenz et al. 1995). Initially, brown-red spots appear on the berries progressing into lesions with dark brown colour (Cannon et al. 2012). Under high humidity, fungal reproductive structures (acervuli) appear as concentric dark grey to black dots, from which a salmon-coloured spore mass can emerge (Freeman et al. 1998).

Colletotrichum spp. do not typically cause symptoms on grapevine leaves (Santos et al. 2018) but can be recovered from such tissue. For example, isolates of *C. nymphaeae* and *C. theobromicola* were obtained from symptomatic grapevine leaves (Steiner et al. 2025). The isolates were nonpathogenic on leaves but caused GRR symptoms on *V. labrusca* × *V. vinifera* ‘Niagara Rosada’ and *V. vinifera* ‘Thompson’ berries (Steiner et al. 2025). The presence of *Colletotrichum* spp. on grapevine leaves may be associated with an endophytic lifestyle of the pathogen (Steiner et al. 2025). Fungi are considered endophytic when they are detected within the tissues of asymptomatic host plant tissues (Schulz and Boyle 2006). Endophytism is a part of the life cycle of many pathogens (Wikee et al. 2013), including *Colletotrichum* spp. (Farr and Rossman 2013; Manamgoda et al. 2013; Vieira et al. 2014; Waculicz-Andrade et al. 2017). Some *Colletotrichum* spp. were isolated from asymptomatic mango and apple tissues and caused disease on inoculated fruit (Martin and Peter 2021; Vieira et al. 2014). Recent studies have also detected *Colletotrichum* spp. on asymptomatic citrus and strawberry leaves, and they were believed to play an important role in infecting flowers or fruit (Agostini and Timmer 1994; Braun and Sutton 1988). It is not known what *Colletotrichum* spp. live endophytically in grapevine tissues and whether they play a role in GRR development.

The objectives of this study were to (i) collect isolates from berries with GRR symptoms and asymptomatic flowers, leaves and fruit of several grape production regions in Paraná and other Brazilian states, (ii) identify the isolates to the species level and (iii) determine their pathogenicity on detached grape berries.

2 | Materials and Methods

2.1 | Collection and Isolation of *Colletotrichum* spp. Isolates

Colletotrichum spp. isolates were obtained from berries with GRR symptoms and asymptomatic leaves, flowers and berries of *V. vinifera* and *V. labrusca*. Samples were collected from fields in Rio Grande do Sul, Paraná, São Paulo, Distrito Federal, Pernambuco and Bahia states during the 2022 and 2023 seasons. In Paraná, a more detailed survey was conducted, covering the main macro-regions producing table grapes and grapes for juice and wine processing, with the aim of capturing greater variability associated with grapevine tissues. Isolates from GRR symptoms were obtained as described previously (Echeverrigaray et al. 2020). Berry skin tissues of approximately 5 mm in diameter were disinfected with 1% NaOCl for 1 min, washed with sterile distilled water (SDW) and dried with sterilised paper towels. The berry skin tissues were plated on potato dextrose agar (PDA) and incubated at 25°C for 4 days with a 12 h photoperiod.

Isolates from asymptomatic leaves, flowers and berries were obtained as described previously (Borve and Stensvand 2017). Leaves were disinfected in 0.5% NaOCl for 30 s, in 70% ethanol for 1 min, and rinsed in sterile water for 2 min. Then the leaves were frozen at −18°C for 5 h and maintained at 25°C for 6 days. Flowers and berries were disinfected in 0.5% NaOCl for 30 s, in 70% ethanol for 30 s and rinsed in sterile water for 2 min, frozen at −18°C for 18 h and then incubated at 25°C for 6 days. Conidia were collected from sporulating tissue and plated on PDA. Figure S1 shows sporulated asymptomatic tissue (fruit and leaf) after incubation and symptomatic berries. Single-spore isolates were obtained from all isolated cultures as previously described (Hu et al. 2015). The monosporic isolates were preserved on filter paper in Eppendorf tubes with silica gel (Fong et al. 2000). The isolates were deposited for long-term storage at the Laboratory of Epidemiology for Integrated Management of Diseases (LEMID) at the Federal University of Paraná.

2.2 | DNA Extraction, PCR Amplification and Sequencing

Genomic DNA was extracted from all single-spore isolates as described previously (Chi et al. 2009). The rDNA internal transcribed spacer (ITS) region 1, partial glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), β -tubulin (*TUB2*), chitin synthase 1 (*CHS-1*) and actin (*ACT*) genes were amplified and sequenced using the primer pairs ITS-1F (Gardes and Bruns 1993)+ITS-4 (White et al. 1990), GDF1+GDR1 (Guerber et al. 2003), T1 (O'Donnell and Cigelnik 1997)+Bt-2b (Glass and Donaldson 1995), CHS-354R+CHS-79F (Carbone and Kohn 1999), ACT-512F+ACT-783R (Carbone and Kohn 1999), respectively (Table 1). PCRs were performed using the GoTaq Green Master Mix with a final volume of 25 μ L, containing 1 μ L of each primer at 100 nM, 1 μ L DNA template at 50–100 ng/ μ L, 12.5 μ L GoTaq Green Master Mix and 9.5 μ L nuclease-free water. The parameters for amplification of ITS, *GAPDH* and *TUB2* included an initial denaturation step of 4 min at 95°C; followed by 35 cycles of 30 s at 95°C, 30 s at 52°C (ITS), 60°C (*GAPDH*), 55°C (*TUB2*) and 30 s at 72°C; and a final extension of 3 min at 72°C. Conditions for PCR of *CHS-1* and *ACT* constituted an initial denaturation step of 5 min at 94°C; followed by 40 cycles of 30 s at 94°C, 30 s at 52°C and 30 s at 72°C. The PCR products were purified using DNA Clean and Concentrator (Zymo Research) and submitted to Sanger sequencing (Eurofins, Louisville, KY, USA).

2.3 | Phylogenetic Analysis

The DNA sequences obtained in the present study were compared with those deposited in NCBI GenBank using the BLAST tool for a preliminary identification at species complex level. Sequences from type-strains and collected isolates from *C. acutatum*, *C. boninense* and *C. gloeosporioides* species complexes were selected for use in phylogenetic analysis. Alignments of the ITS, *GAPDH*, *TUB2*, *ACT* and *CHS-1* regions were performed with MAFFT v. 7.487 (Katoh et al. 2019) and visually adjusted in using MEGA X software (Kumar et al. 2018) when necessary. Best-fit nucleotide substitution model was selected based on the corrected Akaike

TABLE 1 | Primers used for PCR amplification and sequencing.

Primer	Primer sequence (5'–3')	Amplicon length (bp)	References
ITS-1F	CTTGGTCATTTAGAGGAAGTAA	615	Gardes and Bruns (1993)
ITS-4	TCCTCCGCTTATTGATATGC		
GDF1	GCCGTCAACGACCCCTTCATTGA	308	White et al. (1990)
GDR1	GGGTGGAGTCGTACTTGAGCATGT		
T1	AACATGCGTGAGATTGTAAGT	716	Guerber et al. (2003)
Bt-2b	ACCCTCAGTGTAGTGACCCCTTGGC		
CHS-79F	TGGGGCAAGGATGCTTGGAAGAAG	299	Carbone and Kohn (1999)
CHS-354R	TGGAAGAACCATCTGTGAGAGTTG		
ACT-512F	ATGTGCAAGGCCGTTTCGC	282	
ACT-783R	TACGAGTCCTTCTGGCCCAT		

information criterion (AICc) using the ModelFinder software (Kalyaanamoorthy et al. 2017). Phylogenetic trees were produced under maximum likelihood (ML) and Bayesian inference (BI) approaches, with IQ-TREE v. 2 (Hoang et al. 2018; Minh et al. 2020) and MrBayes v. 3.2.7 (Ronquist et al. 2012) plug-ins implemented in PhyloSuite (Zhang et al. 2020) software. For the ML trees, branch support values were assessed simultaneously with the ultrafast bootstrap method and SH-aLRT branch test with 1000 pseudoreplicates. Subsequently, BI analyses were performed with two parallel runs with four chains each, using the number of generations needed to reach a standard deviation of split frequencies that was equal to or less than 0.01. Trees were sampled every 10,000 generations, with a burn-in set to discard the first 25% of the initial trees before calculating the posterior probability values. Final trees were plotted using the FigTree software (<http://tree.bio.ed.ac.uk/software/figtree/>).

2.4 | Pathogenicity Assay

Pathogenicity assays were carried out with all 182 *Colletotrichum* spp. isolates. Conidial suspensions were obtained from 14-day-old cultures on PDA. Five millilitres of SDW was transferred to each isolate plate, and using a Drigalski loop, fungal colonies were scraped to obtain the spore suspension. The suspension was filtered through sterile gauze and adjusted to 10^6 conidia/mL. Asymptomatic berries of *V. vinifera* 'Thompson' (three replicates) and *V. labrusca* × *V. vinifera* 'Niagara Rosada' (three replicates) were surface disinfected with 70% ethanol for 30 s, hypochlorite solution (1%) for 5 min and washed three times with sterile water. Berries were placed in boxes with filter paper and water, injured to a depth of 0.38 mm with a sterile needle or not injured (three replicates each) and inoculated with 5 µL of conidial suspension (10^6 conidia/mL), according to Oo and Oh (2017). Berries were inoculated with autoclaved water in control treatments. The inoculated berries were incubated at 28°C with 90% humidity. A bifactorial design was carried out in randomised blocks with three replicates per cultivar and inoculation type (with and without injury). The diameter (cm) of GRR lesion was evaluated 7 days after inoculation. Following Koch's

TABLE 2 | Tissue origin of *Colletotrichum* spp. isolates collected in the states of Rio Grande do Sul, Paraná, São Paulo, Distrito Federal, Pernambuco and Bahia, Brazil.

State	Tissue origin				Total
	Endophytic			Grape rot lesion	
	Leaves	Flowers	Fruit		
Rio Grande do Sul	—	—	—	6	6
Paraná	65	3	27	55	150
São Paulo	4	—	—	—	4
Distrito Federal	8	—	—	—	8
Bahia	—	—	—	2	2
Pernambuco	3	—	—	9	12
Total	80	3	27	72	182

postulates, the pathogen was reisolated from the berries with symptoms.

3 | Results

3.1 | Collection of Isolates From Several Wine Grape Production Regions in Brazil

In total, 182 fungal isolates were obtained from 24 *V. vinifera* and *V. labrusca* cultivars grown at 32 municipalities across 52 vineyards located in the states of Rio Grande do Sul (six isolates), Paraná (150 isolates), São Paulo (four isolates), Distrito Federal (eight isolates), Pernambuco (12 isolates) and Bahia (two isolates) (Table 2, Table S1). This represented isolates from the south (156 isolates), southeast (four isolates), midwest (eight isolates) and northeast (14 isolates) regions of Brazil. The isolates were obtained from symptomatic berries (72), and asymptomatic leaves (80), flowers (3) and fruit (27).

3.2 | Isolate Collection by Region in Paraná State in Brazil

The majority (150) of isolates were obtained in the state of Paraná, in southern Brazil, representing 82% of the total number collected for this study. Isolates of *Colletotrichum* were obtained from symptomatic fruit (55) and asymptomatic leaves (65), flowers (3) and fruit (27). The percentage recovery of *Colletotrichum* from asymptomatic leaves, flowers and fruit ranged from 50% to 90% (leaves) and 0% to 5% (flowers and fruit) (Table 3).

3.3 | Identification of Isolates From Several Regions of Paraná State and Representative Isolates of Five Other Brazilian States

The 182 isolates collected for this study belonged to three different species complexes in the *Colletotrichum* genus: *C. gloeosporioides* species complex ($n=89$), *C. acutatum* species complex ($n=87$) and *C. boninense* species complex ($n=6$), totalling 13 different species (Table 4). In the *C. boninense* species complex, two species were identified: *C. boninense* (one isolate) and *C. karstii* (five isolates) (Figure 1). In the

TABLE 3 | Number of different tissues sampled and the incidence of *Colletotrichum* sporulation across the state of Paraná, Brazil.

Region	No. of tissues sampled (% <i>Colletotrichum</i> incidence)			
	Asymptomatic			Symptomatic berries
	Leaves	Flowers	Berries	
Metropolitan and coastal	196 (90)	215 (1.9)	620 (1.5)	24 (100)
South-Central	80 (65)	85 (na)	276 (0.4)	14 (100)
North/Northwest	100 (50)	75 (na)	395 (5.1)	2 (100)
Central	84 (65)	75 (1.3)	235 (na)	4 (100)
West	68 (68)	na	238 (3.4)	na
Southwest	40	na	245 (na)	14 (100)
Total (%)	568 (69)	450 (0.8)	2009 (2)	58 (100)

Abbreviation: na, not assessed.

TABLE 4 | Number of *Colletotrichum* species isolated from grapevine tissues, including asymptomatic leaves, flowers and fruit with grape ripe rot lesions.

Species complex	Species	Tissue origin				Total
		Endophytic			Grape rot lesion	
		Leaves	Flowers	Fruit		
<i>C. acutatum</i>	<i>C. nymphaeae</i>	36	3	9	26	74
	<i>C. melonis</i>	6	—	2	—	8
	<i>C. cf. melonis</i>	2	—	—	3	5
<i>C. boninense</i>	<i>C. karstii</i>	5	—	—	—	5
	<i>C. boninense</i>	1	—	—	—	1
<i>C. gloeosporioides</i>	<i>C. viniferum</i>	—	—	2	27	29
	<i>C. siamense</i>	11	—	13	6	30
	<i>C. chrysophilum</i>	9	—	—	9	18
	<i>C. fructicola</i>	3	—	—	—	3
	<i>C. hibeense</i>	2	—	—	1	3
	<i>C. jiangxiense</i>	2	—	1	—	3
	<i>C. theobromicola</i>	2	—	—	—	2
	<i>C. gloeosporioides</i>	1	—	—	—	1
	Total	80	3	27	72	182

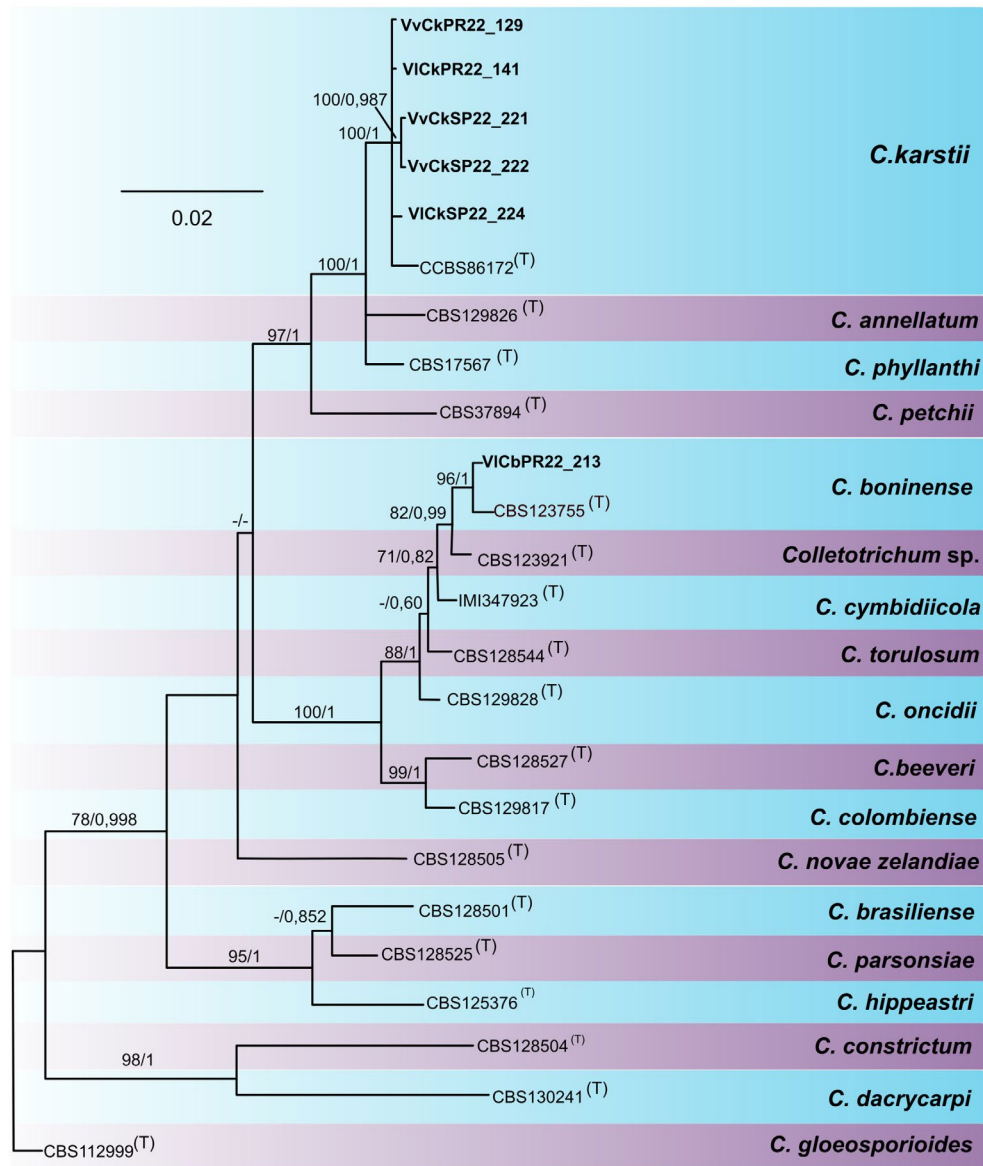


FIGURE 1 | Maximum-likelihood (ML) tree constructed with IQ-TREE using rDNA internal transcribed spacer (ITS), *GAPDH* and *TUB2* sequences from type-strains and reference strains from the *Colletotrichum boninense* species complex isolates. SYM+I+G, HKY+I and GTR were employed as the best-fit substitution models for ITS, *GAPDH* and *TUB2* alignments, respectively. ML SH-aLRT values above 70, ML ultrafast bootstrap values above 70 and Bayesian inference posterior probability values above 0.7 are presented to the left of the nodes. The tree was rooted to *Colletotrichum gloeosporioides*. Scale bar indicates the number of substitutions per nucleotide. Type strains are indicated with ^(T), and isolates obtained in this study are highlighted in bold.

C. acutatum species complex, three species were identified: *C. nymphaeae* (74 isolates), *C. melonis* (eight isolates) and *C. cf. melonis* (five isolates) (Figure 2). In the *C. gloeosporioides* complex, eight species were identified *C. chrysophilum* (18 isolates), *C. fruticola* (three isolates), *C. hebeiense* (three isolates), *C. gloeosporioides* (one isolate), *C. jiangxiense* (three isolates), *C. siamense* (30 isolates), *C. viniferum* (29 isolates) and *C. theobromicola* (two isolates) (Figure 3, Table 4).

Most isolates (150) were collected in Paraná state, where all 13 species were found. Of these isolates, most (84) were from the *C. acutatum* species complex, followed by *C. gloeosporioides* (63 isolates) and *C. boninense* species complexes (three isolates). The species *C. nymphaeae*, *C. melonis* and *C. cf. melonis* (from the *C.*

acutatum species complex) were only found in southern Brazil. Isolates of the *C. gloeosporioides* species complex were found in all six Brazilian states. Isolates of the *C. boninense* species complex were only found in the states of Paraná and São Paulo. Isolates of all 13 species were found in asymptomatic tissue.

Six *Colletotrichum* species were identified on berries with GRR. In the state of Paraná, five *Colletotrichum* species were isolated from symptomatic berries: *C. nymphaeae* (23 isolates), and *C. cf. melonis* (three isolates) of the *C. acutatum* complex; and *C. viniferum* (20 isolates), *C. chrysophilum* (six isolates) and *C. siamense* (three isolates) of the *C. gloeosporioides* complex. In the states of Bahia and Pernambuco, only species from the *C. gloeosporioides* complex were recovered, including *C. viniferum*

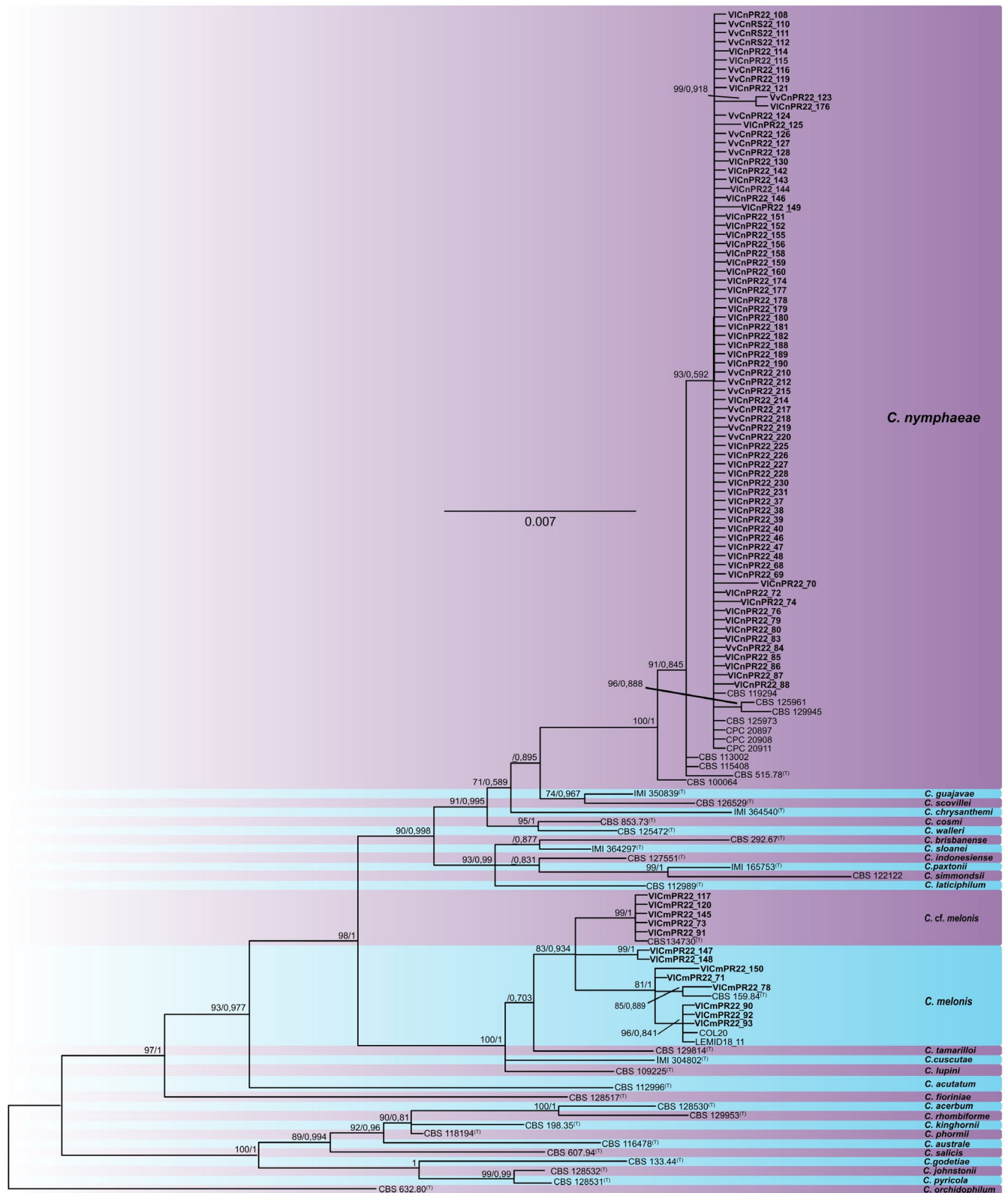


FIGURE 2 | Maximum-likelihood (ML) tree constructed with IQ-TREE using rDNA internal transcribed spacer (ITS), *GAPDH* and *TUB2* sequences from type-strains and reference strains from the *Colletotrichum acutatum* species complex isolates. SYM + I + G, HKY + I and GTR were employed as the best-fit substitution models for ITS, *GAPDH* and *TUB2* alignments, respectively. ML SH-aLRT values above 70, ML ultrafast bootstrap values above 70 and Bayesian inference posterior probability values above 0.7 are presented to the left of the nodes. The tree was rooted to *Colletotrichum orchitophilum*. Scale bar indicates the number of substitutions per nucleotide. Type strains are indicated with ^(T), and isolates obtained in this study are highlighted in bold.

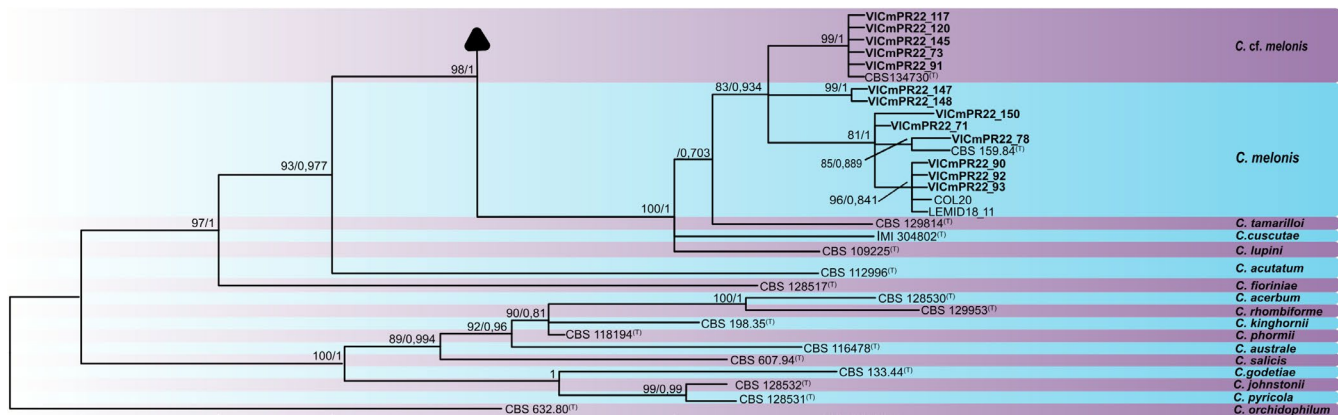


FIGURE 2 | (Continued)

(seven isolates), *C. siamense* (three isolates) and *C. chrysophilum* (one isolate). In the state of Rio Grande do Sul, species from the *C. gloeosporioides* complex, including *C. chrysophilum* (two isolates) and *C. hebeiense* (one isolate) were found, as well as *C. nymphaeae* (three isolates) from the *C. acutatum* complex.

3.4 | Pathogenicity Assays

All *Colletotrichum* species identified in this study were pathogenic on injured *V. vinifera* berries (Table 5, Figure 4). Among the 110 *Colletotrichum* isolates collected from asymptomatic tissues, 80 were pathogenic on injured *V. labrusca* × *V. vinifera* berries, and 82 were pathogenic on injured *V. vinifera* berries. This group included three *C. nymphaeae* isolates recovered from grapevine flowers, which were also pathogenic on injured berries. Of the 27 endophytic *Colletotrichum* isolates obtained from grape berries, 24 were pathogenic on injured *V. labrusca* × *V. vinifera* 'Niagara Rosada' berries and 24 were also pathogenic on injured *V. vinifera* 'Thompson' berries. Among the 72 isolates obtained from symptomatic berries, 59 were pathogenic on injured *V. labrusca* × *V. vinifera* berries and 51 on injured *V. vinifera* berries.

A total of 25 isolates of *Colletotrichum* spp. were nonpathogenic in any of the treatments. From those, 18 were from asymptomatic grapevine tissues, and seven from symptomatic berries.

4 | Discussion

This study revealed multiple *Colletotrichum* species associated with grapevines in Brazil and their endophytic nature. Thirteen *Colletotrichum* species were identified, with several isolates recovered from asymptomatic leaves, flowers and berries, suggesting that the endophytic lifestyle may be part of their disease cycle. All species caused GRR on detached berries, regardless of the tissue of origin, but a significant number require an injury for disease development. Greater variability was observed in the state of Paraná due to the higher number of isolates collected; however, seven species were also detected in the other states, confirming that variability occurs across all sampled regions. These findings underscore the epidemiological importance of

asymptomatic tissues as inoculum sources and demonstrate the complexity of GRR management in Brazilian vineyards.

Our results revealed large species diversity in Brazilian grapevines. We report for the first time the occurrence of *C. melonis*, *C. cf. melonis*, *C. chrysophilum*, *C. hebeiense*, *C. gloeosporioides* and *C. boninense* in grapevine tissues in Brazil. Additionally, this is the first report of *C. jiangxiense* in the country, a species initially described on *Camellia sinensis* in China (Liu et al. 2015) and later reported as an endophyte in *Dendrobium* spp. (Ma et al. 2018). Other species identified in this study, including *C. nymphaeae*, *C. siamense*, *C. viniferum*, *C. theobromicola*, *C. fructicola* and *C. karstii*, had already been associated with GRR in Brazil (Batista et al. 2023; Echeverrigaray et al. 2020). Species from the *C. acutatum* and *C. gloeosporioides* complexes have been reported in Korea (Hong et al. 2008; Oo and Oh 2017), China (Lei et al. 2016; Yan et al. 2015; Yin et al. 2025), the United States (Daykin and Milholland 1984), India (Chowdappa et al. 2009; Sawant et al. 2012a, 2012b), Australia (Greer et al. 2011) and Brazil (Batista et al. 2023; Echeverrigaray et al. 2020). However, the species diversity reported in those studies was lower than that identified in the present work. Although the *C. boninense* complex has been reported in association with GRR symptoms in Brazil (Echeverrigaray et al. 2020), it is more commonly associated with anthracnose on other hosts, including olives (Schena et al. 2014) and avocados (Silva-Rojas and Ávila-Quezada 2011), among others (Damm et al. 2012). Finally, *C. orchidearum* complex has also been associated with GRR in China (Lei et al. 2016).

Colletotrichum nymphaeae was the most prevalent species in our study. Previous studies have already reported its association with GRR and anthracnose of other fruits in Brazil and China (Echeverrigaray et al. 2020; Guginski-Piva et al. 2018; Liu et al. 2016; Santos et al. 2018). This species from the *C. acutatum* complex was found exclusively in our collection from southern Brazil, especially in Paraná. However, the distribution of this species within Brazil is not clear. The geographic limit in occurrence of this species identified in our study may be due to sampling bias, or it may indeed be a species with greater prevalence in Paraná state. In contrast, species from the *C. gloeosporioides* complex were widely distributed and represented the only species found in the northeast and central-west regions.

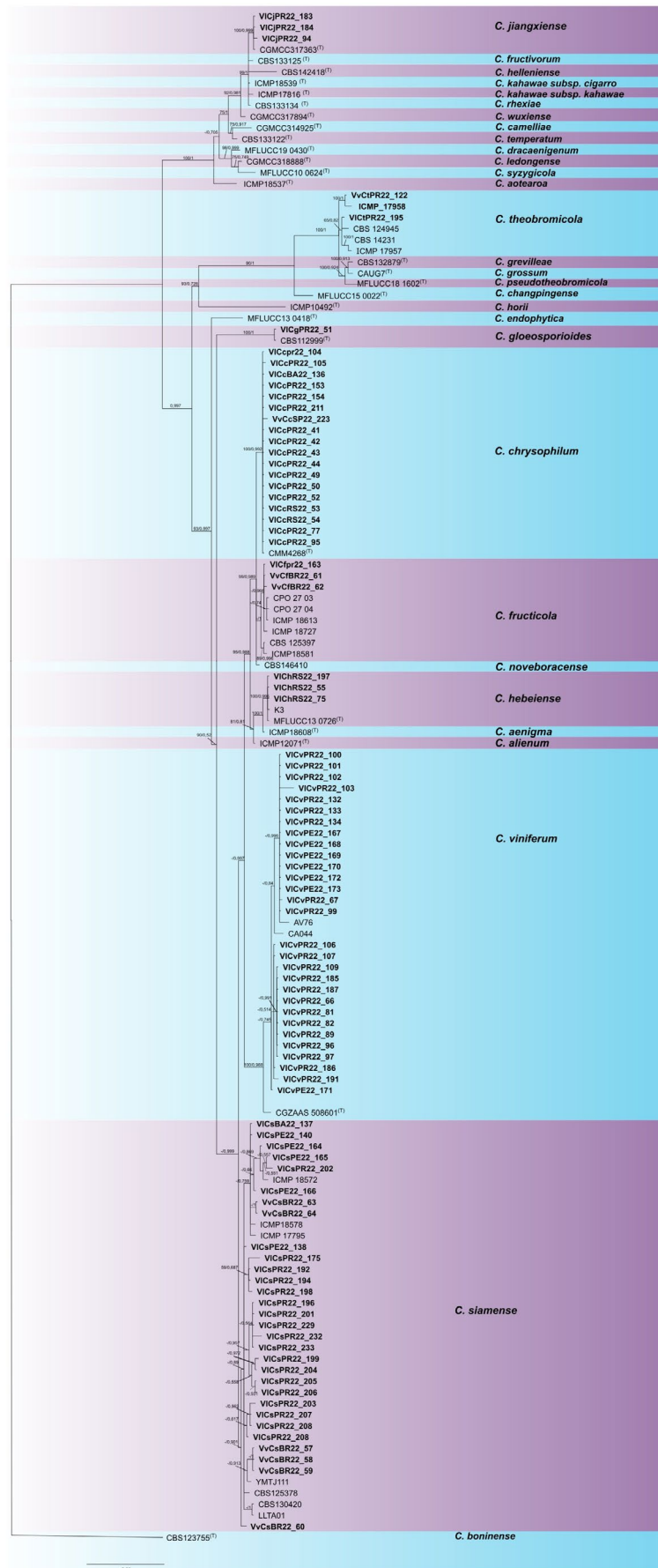


FIGURE 3 | Legend on next page.

FIGURE 3 | Maximum-likelihood (ML) tree constructed with IQ-TREE using rDNA internal transcribed spacer (ITS), *GAPDH*, *TUB2*, *ACT* and *CHS-1* sequences from type-strains and reference strains from *Colletotrichum gloeosporioides* species complex isolates. SYM + I + G, HKY + I and GTR were employed as the best-fit substitution models for ITS, *GAPDH*, *TUB2*, *ACT* and *CHS-1* alignments, respectively. ML SH-aLRT values above 70, ML ultrafast bootstrap values above 70 and Bayesian inference posterior probability values above 0.7 are presented to the left of the nodes. The tree was rooted to *Colletotrichum boninense*. Scale bar indicates the number of substitutions per nucleotide. Type strains are indicated with ^(T), and isolates obtained in this study are highlighted in bold.

TABLE 5 | Pathogenicity of isolates of *Colletotrichum* species in berries of *Vitis labrusca* × *V. vinifera* 'Niagara Rosada' and *V. vinifera* 'Thompson', with and without injury.

Species	Number of isolates	Origin	Number of isolates by tissue	Pathogenic				Not pathogenic
				<i>V. labrusca</i> × <i>V. vinifera</i>		<i>V. vinifera</i>		
				With injury	Without injury	With injury	Without injury	
<i>C. nymphaeae</i>	74	Asymptomatic tissue	48	42	27	44	13	2
		Symptomatic berries	26	24	9	16	8	0
<i>C. siamense</i>	30	Asymptomatic tissue	24	18	7	18	5	3
		Symptomatic berries	6	6	3	6	3	0
<i>C. viniferum</i>	29	Asymptomatic tissue	2	2	2	2	2	0
		Symptomatic berries	27	24	14	23	16	2
<i>C. chrysophilum</i>	18	Asymptomatic tissue	9	5	4	3	3	3
		Symptomatic berries	9	5	1	3	0	4
<i>C. melonis</i>	8	Asymptomatic tissue	8	6	2	5	0	2
		Symptomatic berries	0	0	0	0	0	0
<i>C. cf. melonis</i>	5	Asymptomatic tissue	2	0	0	2	1	0
		Symptomatic berries	3	0	0	3	2	0
<i>C. karstii</i>	5	Asymptomatic tissue	5	2	1	1	1	3
		Symptomatic berries	0	0	0	0	0	0
<i>C. fructicola</i>	3	Asymptomatic tissue	3	1	1	2	0	2
		Symptomatic berries	0	0	0	0	0	0
<i>C. jiangxiense</i>	3	Asymptomatic tissue	3	2	1	1	0	1
		Symptomatic berries	0	0	0	0	0	0
<i>C. hibeense</i>	3	Asymptomatic tissue	2	1	0	1	0	1
		Symptomatic berries	1	0	0	0	0	1
<i>C. theobromicola</i>	2	Asymptomatic tissue	2	0	0	1	0	1
		Symptomatic berries	0	0	0	0	0	0
<i>C. gloeosporioides</i>	1	Asymptomatic tissue	1	1	0	1	0	0
		Symptomatic berries	0	0	0	0	0	0
<i>C. boninense</i>	1	Asymptomatic tissue	1	0	0	1	1	0
		Symptomatic berries	0	0	0	0	0	0
Total	182		182	139	72	133	55	25

Colletotrichum species were frequently recovered from asymptomatic grapevine tissues, confirming their endophytic behaviour. This report confirms *Colletotrichum* spp. as endophytes in grapevines, with a higher frequency in leaves, corroborating previous studies that had suggested this possibility (Steiner et al. 2025). This aligns with reports of endophytic

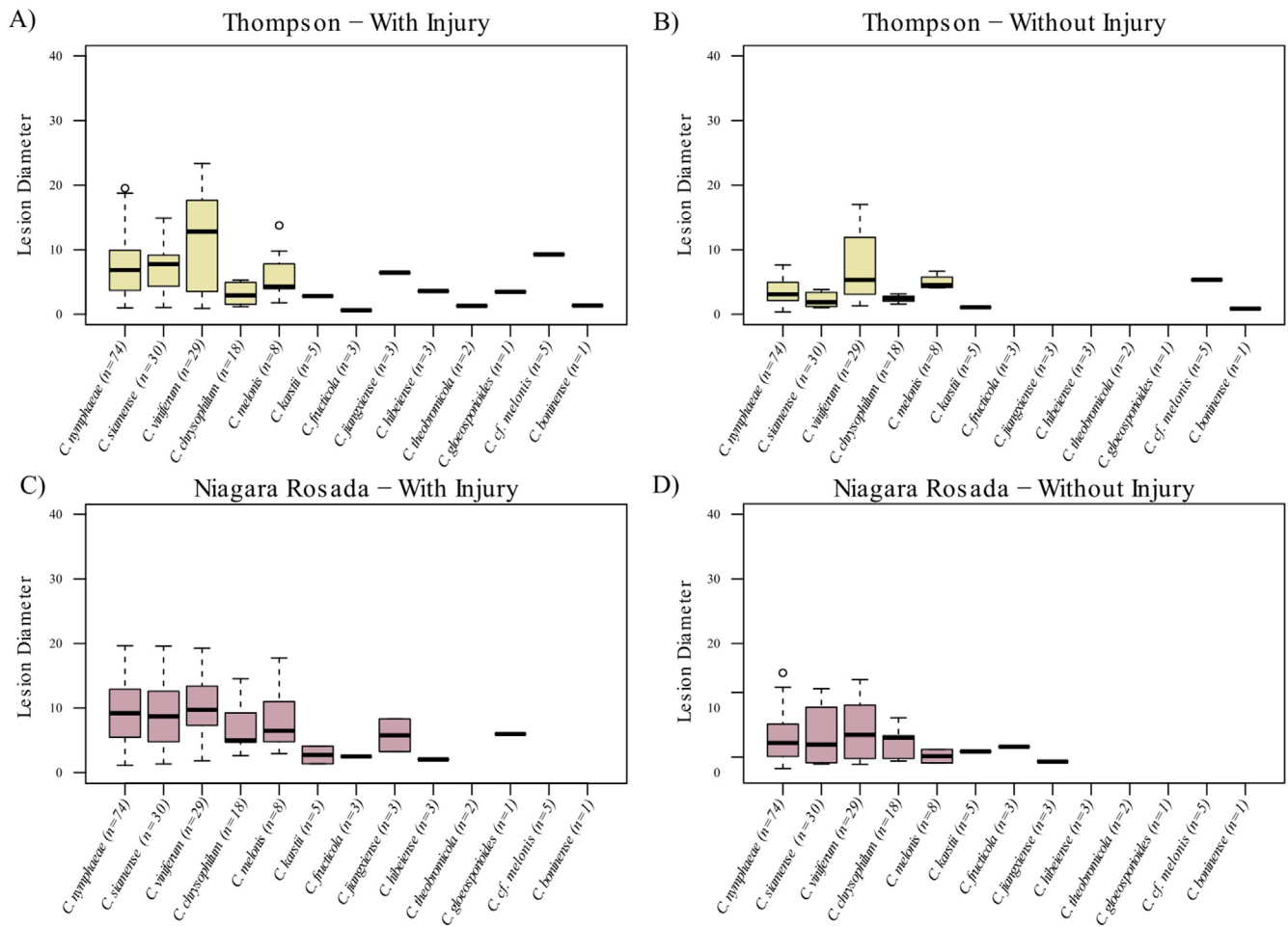


FIGURE 4 | Variation of the average diameter of lesions on grape berries caused by 182 *Colletotrichum* spp. isolates. (A) *Vitis vinifera* ‘Thompson’ with injury. (B) *V. vinifera* ‘Thompson’ without injury. (C) *Vitis labrusca* × *V. vinifera* ‘Niagara Rosada’ with injury and (D) *V. labrusca* × *V. vinifera* ‘Niagara Rosada’ without injury. Analysis of the data was performed using R software v. 2023.12.1 + 402 (R Core Team 2023).

Colletotrichum spp. in tomatoes (Silva Santos et al. 2022), apples (Gelain et al. 2023; Martin and Peter 2021), mango (Vieira et al. 2014), coffee (Prihastuti et al. 2009), cacao (Rojas et al. 2010), grasses (Manamgoda et al. 2013) and forest species (Cannon and Simmons 2002). In our study, all species collected were identified in asymptomatic tissues; however, 23 isolates (from *C. karstii*, *C. boninense*, *C. fruticola*, *C. gloeosporioides*, *C. melonis*, *C. jiangxiense* and *C. theobromicola*) were exclusively detected in asymptomatic organs, either leaves or berries, suggesting a potential endophytic lifestyle. An endophytic lifestyle may offer a survival advantage, allowing the fungus to persist in host tissues without triggering plant defences (Arnold et al. 2003).

Although 110 isolates were detected in asymptomatic tissues, only 58 isolates could cause disease in uninjured berries, considering both *Vitis* species tested. This suggests that many of these fungi may function primarily as endophytes, with limited or no pathogenic potential under the conditions tested. However, external factors such as mechanical injury or postharvest stress may favour the expression of symptoms and shift the fungal behaviour toward pathogenicity. A well-documented example is *C. fruticola*, a member of the *C. gloeosporioides* complex, which can colonise living tissues during a biotrophic, symptomless

phase and transition to a necrotrophic phase during fruit ripening, when rot becomes evident (Zakaria 2025). Furthermore, during infection of *V. amurensis* by *C. gloeosporioides*, the plant responds by accumulating proanthocyanidins and anthocyanins, which play important roles in defence and may hinder symptom development (Yu et al. 2023).

Both genetic and genomic differences among *Colletotrichum* isolates, *Colletotrichum* species or even within a species complex may influence their interaction with their hosts and play key roles in overcoming host defences. For example, a comparative genomic and transcriptomic study involving the closely related *C. tofieldiae* and *C. incanum* species and their host *Arabidopsis* revealed genomic signatures associated with evolutionary transitions from pathogenic to beneficial lifestyles (Hacquard et al. 2016). Furthermore, comparative genomic analyses focusing on *Colletotrichum* species from the *C. acutatum* species complex describe the role of contraction of gene families of CAZymes, proteolytic enzymes and lineage-specific effector protein candidates (LSECs) in host-specific species, suggesting that species with broad host ranges may rely on CAZymes and proteases for host colonisation, instead of LSECs (Baroncelli et al. 2016). In this sense, differences in the genetic and genomic repertoire, as well as patterns of gene expression, between *Colletotrichum*

isolates may explain why some isolates behave as endophytes under the conditions evaluated, and why mechanical injuries may trigger shifts in behaviour toward pathogenicity. Overall, future studies combining comparative genomic and transcriptomic approaches of the isolates identified here would contribute to a better understanding of the molecular basis underlying the lifestyle transitions in the *Colletotrichum*–*Vitis* interactions.

An unexpected result in our study was the low frequency of *Colletotrichum* in grapevine flowers. Endophytic fungi are known to colonise flowers during bloom and remain latent until fruit maturity (Tadych et al. 2012; Zuckerman 1958). Our findings contrast with this expectation, highlighting the need for further studies to clarify the colonisation dynamics in grapevine flowers. *Colletotrichum* infection of apple flowers is common and has been associated with the progression of bitter rot and *Colletotrichum* fruit spot symptoms during the growing season (Castellar et al. 2023; Gelain et al. 2023; Hamada et al. 2020). Similarly, this latent infection route via flowers has already been well documented for *Botrytis cinerea*, which can be detected in strawberry flowers and later develops into grey mould on fruit (Bristow et al. 1986).

Interestingly, the species associated with GRR symptoms in berries, including *C. nymphaeae*, *C. siamense*, *C. chrysophilum* and *C. viniferum*, were also found in asymptomatic leaves. Similar dynamics have been observed in apple (Borve and Stensvand 2017). These findings challenge the traditional notion that mummified fruits are the sole inoculum source (Dowling et al. 2020), adding complexity to GRR management strategies. Pathogenicity tests revealed that both endophytic and GRR-associated isolates could cause disease symptoms. Furthermore, our data did not show significant interspecific differences in virulence among grape cultivars, consistent with Yan et al. (2015). In addition, our data are consistent with the results of a study in China, in which the wounded treatment more frequently led to the development of GRR compared to the unwounded treatment (Yin et al. 2025). In our study, *C. viniferum* showed the most variation in lesion diameter, echoing findings from China (Yan et al. 2015). Therefore, this dual presence suggests that endophytic infections in leaves may contribute to fruit disease. To fully validate this hypothesis, we recommend further investigation to determine whether conidia produced on leaves during the ripening period can lead to berry infection. This would help clarify the epidemiological link between latent *Colletotrichum* in leaves and the increase in GRR symptoms.

The species diversity of *Colletotrichum* spp. and their ability to colonise endophytically on grapevine can be a challenge for disease management. We identified species from three major complexes, *C. acutatum*, *C. gloeosporioides* and *C. boninense*, raising concerns about proper fungicide choices. This is particularly relevant given that species within the *C. gloeosporioides* complex have shown tolerance to fludioxonil (Schnabel et al. 2021), while species from the *C. acutatum* complex are inherently sensitive to thiophanate-methyl (Peres et al. 2004). Our results highlight the importance of studying the sensitivity of isolates from different species to fungicides and reinforce the need for further epidemiological studies to determine how endophytic spores are released from asymptomatic tissues and infect berries, particularly under Brazilian vineyard conditions.

Author Contributions

Daniel Ricardo Maass Steiner: methodology, conceptualization, investigation, writing – original draft, data curation. **Camila Iavorski Zela:** investigation, methodology, visualisation. **Debora de Oliveira Franco:** investigation, validation, methodology. **Jhulia Gelain:** investigation, methodology, visualisation, writing – original draft, validation. **Desirré Alexia Lourenço Petters-Vandresen:** investigation, methodology, formal analysis, validation. **Guido Schnabel:** conceptualization, writing – review and editing, investigation, resources, supervision, funding acquisition. **Louise Larissa May De Mío:** conceptualization, investigation, writing – review and editing, project administration, resources, supervision, funding acquisition, data curation.

Acknowledgements

We are grateful to the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq) for financial support grant no. 403918/2021-9 and scholarship to Daniel Ricardo Maass Steiner grant no. 423476/2018-1. The Article Processing Charge for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614).

Funding

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico, 403918/2021-9, 423476/2018-1.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** *Colletotrichum* sporulation in different grape tissues. (A, B) Grape leaf and berry with sporulation of *Colletotrichum* spp. after overnight freezing. (C) Grape ripe rot symptoms in the field. **Table S1:** Isolate number, *Colletotrichum* complex and species, state, tissue, isolate origin (endophytic or from grape ripe rot symptoms) and GenBank accession number. **Table S2:** ppa70115-sup-0003-TableS2.docx. *Colletotrichum* isolates types and non-types used in phylogenetic analysis.