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Colletotrichum sp. ASSOCIATED WITH SOYBEAN AND CORN ANTHRACNOSE IN THE PAMPAS REGION OF ARGENTINA

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ABSTRACT

***Colletotrichum* species (C.) exhibit considerable genetic variability, causing diseases in different hosts and showing physiological specificity between isolates that interact with different genotypes of the same crop. The objective of this study was to identify *Colletotrichum* isolates collected from symptomatic plants of various plant species and environments in the central region of Argentina. Isolates were obtained from symptomatic stems of soybean, corn, alfalfa, and citrus plants. Fungal identity was confirmed through macro- and micromorphological analysis, as well as molecular techniques including by RAPD and sequencing of ITS1 and ITS4 fragments amplified by PCR. The identified *Colletotrichum* species included: *C. truncatum* and *C. gloeosporioides* in soybean, *C. graminicola* in corn, *C. gloeosporioides* in tangerine, and *C. truncatum* in alfalfa. These results highlight the plasticity of this pathogenic complex and the epidemiological risk posed by corn-soybean crop rotation for the development and severity of anthracnose.**

Keywords: *Collectotrichum truncatum; C. graminicola, C gloeosporiodes,* host, ITS.

INTRODUCTION

The *Glomerella* Stonem-*Colletotrichum* Corda (G/C) complex has been identified as the etiological agent of anthracnose and comprises multiple species capable of infecting a wide range of crops and fruit hosts (Jayawardena, 2016, 2021; Hyde, 2014, Damm 2014, Favaro, 2022). This generic binomial (G/C) exhibits both a sexual (teleomorph) phase, *Glomerella*, which belongs to division Ascomycota and class Sordariomycetes, and an asexual (anamorph) phase, *Colletotrichum*, traditionally classified as Deuteromycota, specifically within the group Coelomycetes (Maharachchikumbura et al., 2015, 2016; Rojo-Báez, 2017).

Zhang (2013) supports the use of the asexual name *Colletotrichum* instead of *Glomerella*, noting that *Colletotrichum* is more commonly used in applied sciences. Prior to 2012, the group was referred to as the *Glomerella/Colletotrichum* complex. However, with the adoption of the single-name nomenclature for pleomorphic fungi under the 2013 Melbourne Code of the International Code of Nomenclature for Algae, Fungi, and Plants (www.iapt-taxon.org/nomen/main.php), the name *Glomerella* is unlikely to remain in use (Crouch, 2020; Rojo-Báez, 2017; Hyde, 2009).

Knowledge of the morphological, cultural, and molecular variability of *C. truncatum* associated with soybean in South America has been crucial for effective anthracnose management (Dias, 2019). Although *C. truncatum* (Schw.) Andrus and Moore (syn. *C. dematium var. truncata*) is considered the primary causal agent of soybean

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anthracnose, other species – such as *C. coccodes*, *C. destructivum* O' Gara (teleomorph = *not observed* (Lehman & F.A Wolf)), *C. graminicola* (Ces.) (teleomorph: *Glomerella graminicola*) Wils. and *C. gloeosporioides* (Penz.) Penz and Sacc. (teleomorph = *G. cingulata* (Stonem)) – have also been reported as causal agents (Ramos et al., 2013; Hartman et al., 2015).

In the past decade, two additional species with falcate conidia, *C. chlorophyti* Chandra and Tandon, and *C. incanum* (Yang) Haudenshield and Hartman, were identified as causal agents of soybean anthracnose in the USA (Yang et al., 2014). On the other hand, *C. cereale*, a species with falcate spores originally described by Selby et al. (1909), is currently considered as a synonym of *C. graminicola* Wils., both of which are associated with corn anthracnose. Meanwhile, *C. gloeosporioides* is frequently observed in a wide range of plant species (Hyde et al., 2009a).

Rojo- Báez et al. (2017) described the morphology of *C. truncatum* and *C. gloeosporiodes* in a wide range of hosts. *C. truncatum* produces unicellular, hyaline conidia measuring 22.8 - 23.8 x 3 - 3. 02 µm, typically aseptate, with the central part slightly curved with parallel walls, ending abruptly in a round, truncated base. In contrast, *C. gloeosporioides* exhibits ovoid conidia measuring 13.56 - 14.24 x 4 - 4.02 µm. Colonies of *C. truncatum* are circular or irregular in shape, with entire margins, and range in color from white to salmon or gray to black. *C. gloeosporioides* shows radial mycelial growth, typically white in color, with orange conidial masses in the center of the colony. Regarding *C. graminicola* on oat, Leyva-Mir (2004) reports conidia measuring 20-30 µm in length and 4-6 µm in width.

The phenotypic variation among species of the genus *Colletotrichum* necessitates the integration of morphological, molecular and biochemical methods to accurately determine taxonomic identity (Hernandez et al., 2015; Peruzzo et al., 2017). RAPD (Random Amplified Polymorphic DNA) analysis (Sharma et al., 2018) has been successfully used to detect differences among intraspecific taxa (Gally et al., 2007). Dias et al. (2019) reported that the genetic diversity of *C. truncatum* populations, particularly among isolates collected from major soybean-producing regions of Argentina and Brazil, was effectively characterized using RAPD makers.

Echeverrigaray et al. (2019, 2020) in a preliminary study of *Colletotrichum* species in southern Brazil, six species were identified by ITS sequencing, including representatives of the gloeosporioides, acutatum and boninense clades.

However, the genetic diversity and pathogenic variability observed within *Colletotrichum* populations may require further genetic characterization using polymorphic analyses of the internal transcribed spacer (ITS) regions and other relevant molecular techniques (Coêlho et al., 2016 and Sharma et al., 2018).

In addition, Sharma et al. (2011) emphasized the importance of fungal genome sequencing and its complementary role alongside morphological characterization in the accurate identification of species.

Morphological identification is often insufficient; therefore, the combined use of molecular characterization alongside traditional diagnostics is considered an effective approach for the study of *Colletotrichum* species complexes (Hyde et al., 2009b). Among molecular markers, the ITS region of nuclear ribosomal is the most commonly used for species identification (Schoch et al., 2012).

In Argentina, the identification and validation of diseases caused by this genus in intensive crops have been achieved through the combined use of morphological and molecular characterization techniques (Favaro et al., 2022). Consequently, understanding the bio-epidemiological aspects of pathogenic populations is essential, particularly those exhibiting high genetic variability and physiological specificity, as they can interact with different genotypes of the same host and/ or different hosts of various plant species (Pioli et al., 2003a b).

The objective of this study was to identify *Colletotrichum* isolates collected from symptomatic plants of various plant species and environments in the central region of Argentina.

MATERIALS AND METHODS

Morphological characterization and identity determination of *Colletotrichum* isolates

Colletotrichum (C) isolates were obtained from samples collected from several sites and ecosystems of the agricultural Pampas region of Argentina, specifically from the provinces of Santa Fe, Buenos Aires, Entre Ríos and Córdoba (Table 1). The Pampas region accounts for approximately 30% of Argentina's land surface and more than 55% of the country's total population. The climate is temperate, with mild temperatures; summer averages range from 20 and 25°C, while winter averages 5 and 12°C, resulting in an annual mean average of 17°C. Rainfall is abundant throughout the region, decreasing towards the west (Aliaga, 2018).

Portions of infected tissues, primarily stalks exhibiting anthracnose symptoms from corn, soybean and other host species such as alfalfa and tangerine, were collected. The tissue samples were

Table 1. Host crops and geographic origin of *Collectotrichum* isolates.

Isolates	Host	Geographic origin	Coordinates
Coll 1	Corn	Pergamino (Buenos Aires)	33°53′01″S 60°34′01″O
Coll 5	Soybean	Bombal (Santa Fe)	33°27′29″S 61°19′05″O
Coll 6	Corn	Bombal (Santa Fe)	33°27′29″S 61°19′05″O
Coll 7	Alfalfa	Entre Ríos	32°06′S 59°18′O
Coll 8	Alfalfa	Entre Ríos	32°06′S 59°18′O
Coll 11	Corn	Elortondo (Santa Fe)	33°42′00″S 61°37′00″O
Coll 13	Corn	Elortondo (Santa Fe)	33°42′00″S 61°37′00″O
Coll 15	Soybean	Chilibroste (Córdoba)	32°19′59″S 62°30′47″O
Coll 16	Soybean	Banderaló (Buenos Aires)	35°00′S 63°21′O
Coll 17	Corn	Entre Ríos	32°06′S 59°18′O
Coll 23	Soybean	Entre Ríos	32°06′S 59°18′O
Coll 24	Tangerine	Helvecia (Santa Fe)	31°06′00″S 60°05′00″O
Coll 25	Soybean	Esperanza (Santa Fe)	31°26′56″S 60°55′54″O
Coll 32	Soybean	Firmat (Santa Fe)	33°27′00″S 61°29′00″O

Collectotrichum isolates selected to validate identity by molecular techniques and subsequenly used in the inoculation trials.

surface-disinfected using 70% v/v ethanol and 0.6% v/v sodium hypochlorite, rinsed with sterile water, and dried with sterile paper towels. They disinfected samples were placed in Petri dishes containing 2% potato dextrose agar (APDA) medium, acidified with 1% lactic acid (25% v/v). Incubation was carried out in darkness at 27 ± 1 °C for 7 days, until fungal colonies developed.

The pure cultures obtained were stored at 4 °C and periodically subcultured to verify their purity and viability (Riccioni et al., 1998; Pioli et al., 2000; EMPPO, 2004; Dokken, 2007; Incremona, 2020). The G/C isolates were identified and grouped based on similarities in macromorphological characteristics of the colonies (e.g., shape, size, and pigmentation), as well as micromorphological features such as the structure of anamorph fruiting bodies (acervuli) and conidia. Identification was performed using specific taxonomic keys and references (Armstrong y col**.**, 2006; EMPPO, 2004; Manandhar and Hartman, 1999). These morphological features were observed using a stereomicroscope (40x, SWIFT) and an optical microscope (400x - 1000x, DM500, LEICA) (Incremona, 2020).

Molecular characterization and identity validation of the *Colletotrichum* isolates

Total genomic DNA was extracted from each fungal colony following the method proposed by Dellaporta et al. (1983), with slight modifications (Echeverrigaray et al., 2020). The RAPDs technique was performed as described by Sharma et al. (2018), using the following selected markers: OPA1, OPA3, OPA5, OPA6 and OPA8 (Operon Technologies, USA) to differentiate *Colletotrichum* spp. (Dias 2019) (Table 2).

Amplification products were analyzed using 6% polyacrylamide gels under denaturing conditions and/or 2% agarose gels. A Model S2 polyacrylamide gel electrophoresis kit (Life Technologies) was used. DNA LADDER 100 bp (Invitrogen™), containing 15 fragments ranging from 100 and 1,500 bp and an additional fragment of 2,072 bp, was used as molecular weight marker at a concentration of 1 µg/µL. Band visualization was performed according to the DNA Silver Staining System protocol (Promega), which involves staining polyacrylamide gels with 0.1 % (w/v) silver nitrate and 0.07 % (v/v) formaldehyde. Development was performed in a solution containing 30 g/l sodium carbonate (MERCK), 0.05 % formaldehyde, and 2.5 mg/l sodium thiosulfate until bands were clearly visible. The reaction was terminated with 10 % (v/v) acetic acid. Banding patterns were analyzed directly on the glasses using a white light transilluminator (Hernández et al., 2015). Based on the band profiles obtained for each *Colletotrichum* spp. isolate and each RAPD primer used in polyacrylamide gels, a data matrix was constructed, where the value of 1 indicated the presence and 0 the absence of a band. Scoring data were analyzed by cluster analysis and Euclidean Distance, as implemented in the Infostat software package (Hernandez et al., 2015).

Identity validation of *Colletotrichum* isolates by sequencing of PCR amplified polymorphic fragments using ITS1 and ITS4 primers

Genomic DNA was extracted from each isolate using a Wizard® Genomic DNA Extraction

Table 2. Primer sequences of different specificity used for fungal DNA amplification by PCR and detection of different *Colletotrichum* species.

Primer	Sequence	Specificity
OPA 1	(5´CAGGCCCTTC 3´)	nonspecific*
OPA 6	(5´GGTCCCTGAC 3´)	nonspecific*
OPA 3	(5’AGTCAGCCAC 3’)	*C. graminicola*
OPA 5	(5’ AGGGGTCTTG 3’)	*C. truncatum*
OPA 8	(5’GTGACGTAGG 3’)	*C. graminicola* y *C. truncatum*

*Oligonucleotides used in the characterization of *Diaporthe phaseolorum* (Hernandez, 2015; Peruzzo, 2017).

Kit (Promega, A1120). The quality control and integrity of the genomic DNA were assessed by the presence of high molecular weight bands in electrophoresis of 0.7% w/v agarose gels in 1x TAE buffer, run at 40 mA. For band visualization, gels were stained with SYBR Safe DNA Gel Stain (Invitrogen) (Hernandez et al., 2015). The ITS region of the RNAr (ITS1) from each isolate sample was amplified by PCR using the primers ITS1 (5’ TCCGTAGGTGAACCCTGCGG 3’) and ITS4 (5’ TCCTCCGCTTATTGATATGC 3’).

A fraction of the amplification product from each isolate was visualized as a single band obtained by 1% agarose gel electrophoresis and staining with SYBR Safe (Invitrogen) to validate the effectiveness of the PCR. Subsequently, the amplification products were sent to MACROGEN (Seoul, Korea) sequencing. Molecular identification was performed by comparing the obtained sequences to all fungal nucleotide sequences available in the NCBI database (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov).

RESULTS

Morphological characterization enabled the identification of the 14 *Colletotrichum* isolates obtained from four host species and various agroecological areas across the provinces of Santa Fe, Buenos Aires, Córdoba and Entre Ríos (Table 1).

The *Colletotrichum* isolates identified based on morphological makers were: a) *C. truncatum* and *C. gloeosporioides* (teleomorph *G. cingulata)* in soybean (*Glycine max*); b) *C. graminicola* (teleomorph *G. graminicola*) isolates) in corn (*Zea mays*); c) *C. truncatum* in alfalfa (*Medicago sativa*), and d) *C. gloeosporioides* (teleomorph *G. cingulata*) in tangerine (*Citrus reticulata*) (Annex 1). The *Colletotrichum* species identified in this study have also been reported in reference compendia as members of the G/C complex, which are causal agents of anthracnose in soybean and corn (Hartman et al., 2015; Nordzieke et al., 2019).

Molecular characterization of *Collectotrichum* isolates by RAPDs and identity validation by sequencing of ITS fragments

Molecular characterization by RAPDs

Ten *Colletotrichum* isolates Coll 1, Coll 6, Coll 7, Coll 8, Coll 13, Coll 15, Coll 16, Coll 24, Coll 25 and Coll 32 were selected based on the differential pathogenic behavior to interact with soybean (Annex 1). The RAPD profiles obtained from the evaluated *Colletotrichum* isolates showed band sizes ranging from 200 to 2000 pb with primer OPA 1, and from 400 to 1500 pb with primer OPA 6. A dendrogram was constructed based on the presence-absence of bands generated by the RAPD technique (Sharma et al., 2018) using OPA 1 and OPA 6 primers. The analysis revealed genetic relationships among the C*olletotrichum* isolates and validated the identity previously determined by morphological markers (Fig. 1).

The greatest distance was 20.06 Euclidian units (udE), indicating that among the ten *Colletotrichum* isolates analyzed, there was 78.9 % similarity (S), confirming that they belong to the genus *Colletotrichum*. The use of OPA 1 and OPA 6 primers enabled the definition of two Major Groups (MG_1 and MG_2), with 88.5% and 87.0% S, respectively. MG_1 is subdivided into two subgroups: $SG_{1\cdot1}$, including isolates Coll 8 and Coll 7 (from alfalfa) at a distance of 5.3 udE, with 96.7 % S; and $SG_{1\cdot2}$, including isolates Coll 16 and Coll 15 (from soybean) at a distance of 5.8 udE, with 94.2 % S. Likewise, MG_2 is subdivided into two subgroups: $SG_{2.1}$, which is further divided into two sub-subgroups ($G_{2.1.a}$ and $G_{2.1\cdot b}$), $SG_{2.2}$. $G_{2.1.a}$ includes isolates Coll 32 (from soybean) and Coll 24 (from tangerine) at a distance of 7.4 udE, with 92.6% S; while $G_{2.1\cdot b}$ includes Coll 6 and Coll 13 (from corn) at a distance of 10.5 udE, with 89.5 % S. $SG_{2.2}$ contains isolates Coll 25 (from soybean)

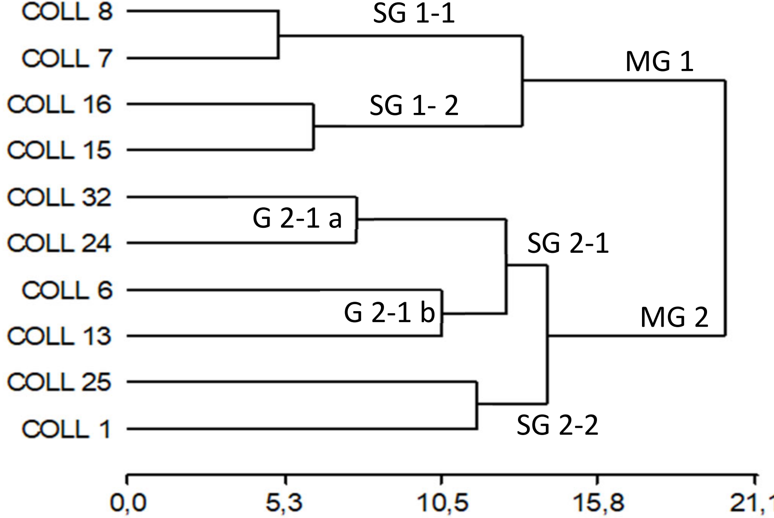

Fig. 1. Dendrogram generated from the analysis of presence-absence of bands obtained through the RAPD technique to establish relationships among *Collectotrichum* isolates (SG: subgroups; MG: major groups).

and Coll 1 (from corn) at a distance of 11 udE, with 89 % S.

Molecular identification of fungal isolates by sequencing ITS fragments

PCR products were visualized in agarose gel (Fig. 2). The identity of G/C isolates was confirmed by sequencing the ITS1 region of rDNA (Table 3), with the exception of Coll 6 and Coll 13, which could not be sequenced due to the low quality of the genomic DNA sampled.

Molecular validation of the G/C isolates was performed by comparing the obtained sequences with fungal nucleotide sequences available in the NCBI database.

DISCUSSION

The present study validated the identity of *Colletotrichum* isolates through both morphological and molecular characterization, with particular relevance to Argentina and its core agricultural region. The isolates of the G/C complex identified in the present study were obtained from four different hosts: leguminous, cereals, fruits and forage, and from different agroecological regions of the Humid Pampas, highlighting the predominant presence and variability of this pathogen. These results are of epidemiological significance, particularly related to crop rotation in agricultural-livestock production systems.

Morphological characterization confirmed the identification of *C. graminicola*. The isolates from corn initially developed colonies, which later formed a dark ring with a white central mycelium, forming a crest at maturity with a dark green, plush mycelium with reddish tints and black stromas. This differs from Sutton (1980), who described *C. graminicola* colonies as spreading with irregular margins, spongy and gray aerial mycelium, and conidial masses that were salmon-orange in color with abundant setae. For the corn host, C. *graminicola* exhibited smaller conidial sizes compared to those described by Manandhar and Hartman (1999), but the conidia had the same curved shape.

In the present study, the conidia of *C. truncatum* on soybean were curved, thin, smile-shaped, non-septate, and rounded at the ends, with a larger size than those described by Dias et al. (2019). Regarding colony color, in agreement with Dias et al. (2019), the colonies ranged from light to dark gray, with spore masses varying from orange to beige/orange.

In conclusion, the conidia of *C. truncatum* on soybean (curved, thin, non-septate, rounded at the ends, and larger in size) and *C. graminicola* on corn (smaller conidial size) differ from those described by most authors in the core agricultural region of Argentina. In this region, *C. truncatum* and *C. gloeosporioides* were identified in soybean samples collected from the provinces of Santa Fe, Córdoba, Buenos Aires and Entre Ríos. These findings align with those of Mahmodi (2013) and Cabrera et al. (2001), who also reported the presence of *C. gloeosporioides* (Penz.) Sacc in soybean plants with anthracnose, associated with *C. truncatum*. However, pathogenicity tests were only positive for the *C. truncatum truncata*

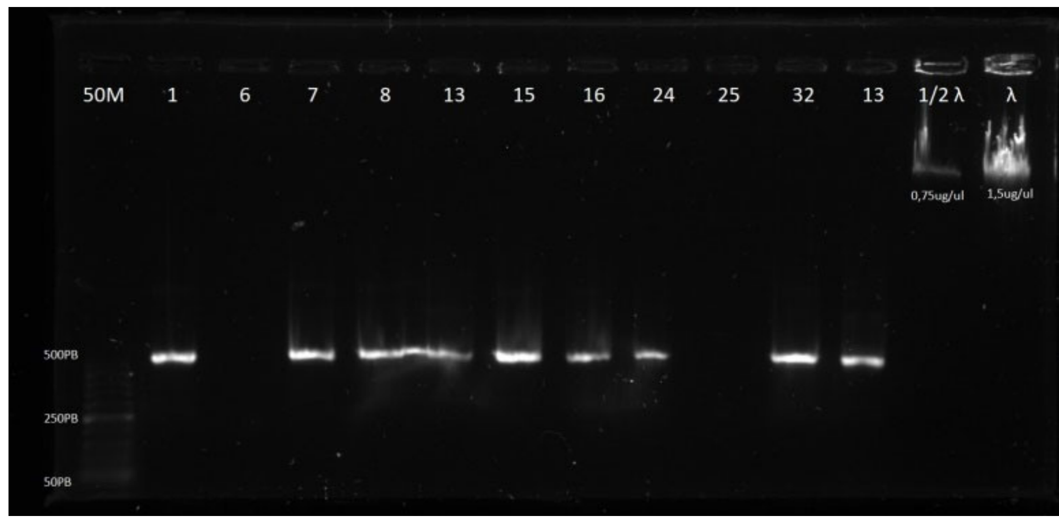

Fig. 2. PCR amplification products of the ITS1 region using ITS1 and ITS4 primers from the genomic DNA of ten isolates from the *Colletotrichum* spp. collection.

Table 3. Validation of the identity of each G/C isolate based on morphological markers, ITS molecular sequencing, and RAPD molecular characterization.

Colletotrichum isolate (Coll) and host	Identity by morphological markers	Identity validated by RAPDs	Identity by ITS1 – 4 genomic region sequencing	Score	Percentage of identity %
Coll 1 – Corn	*C. graminicola*	OP 3 and 8	No results		
Coll 6 – Corn	*C. graminicola*	OP 1, 3 and 8	No results		
Coll 7 – Alfalfa	*C. truncatum*	OP 1 and 6	*C. truncatum*	983	99.3%
Coll 8 – Alfalfa	*C. truncatum*	OP 1 and 6	*C. truncatum*	987	100%
Coll 13 – Corn	*C. graminicola*	OP 1, 3 and 8	*C. graminicola*	952	98.7%
Coll 15 – Soybean	*C. truncatum*	OP 1 and 6	*C. truncatum*	994	99.5%
Coll 16 – Soybean	*C. truncatum*	OP 1 and 6	*C. truncatum*	1002	99.3%
Coll 24 – Tangerine	*C. gloeosporioides*	OP 1 and 8	*C. gloeosporioides*	965	100%
Coll 25 – Soja	*C. truncatum*	OP 1 y 6	No results		
Coll 32 – Soybean	*C. gloeosporioides*	OP 1 and 8	*C. gloeosporioides*	1317	99.4%

Based on the data in Table 3, the genus and species identities of eight G/C isolates from different locations and hosts were validated using morphological traits, RAPD analysis, and ITS sequencing. Only three isolates (Coll 1, 6 and Coll 25) could not be validated by ITS sequencing.

strain. *C. gloeosporioides* was identified in soybean genotypes by Koch's postulates, with conidial sizes (11-16 x 4-6 μm) similar to those reported by Mahmodi et al. (2013).

Coll 13, morphologically identified as *C. graminicola*, exhibited two types of conidia: some falcate and others smaller and oval. These forms have been recorded in Argentina, as described by Nordzieke et al. (2019) and Sukno et al, (2008), who described this specie associated with corn. While both types of conidia were observed, the falcate form was associated with acervuli on the surfaces of lesions and plays a role disease dispersal, whereas the oval conidia were found inside stems and leaves. Molecular analysis confirmed that this isolate corresponded to morphologically validated *C. graminicola.*

In isolates from alfalfa obtained from the Entre Ríos region, *C. truncatum* was identified, in contrast to Damm et al. (2014), who considered *C. destructivum* as a pathogen of alfalfa. The presence of *C. truncatum* on alfalfa was confirmed morphologically and molecularly in this study.

Our findings are consistent with those

of Weir and Damm (2012), who reported C. gloeosporioides in citrus. The presence of C. gloeosporioides was confirmed morphologically and molecularly in *Citrus reticulata*, which is considered to be saprophytic in the field.

Regarding the molecular characterization of the isolates, preliminary results from the analysis using 5 oligonucleotides (OPA primers) showed that the 9 *Colletotrichum* isolates are closely related (> 96udE).

Electrophoretic analysis of PCR amplification with OPA 1 and OPA 6 primers revealed a band profiles ranging from 200 and 2,000 bp. combined analysis of both primers allowed the differentiation of two closely related groups of isolates, which, were unrelated in terms of morphology, host, or environment.

Sequencing of PCR amplification products using ITS primers allowed confirming the molecular identity of several isolates, validating their corresponding morphological identity. For instance, Coll 7 and Coll 8 from alfalfa host confirmed the presence of *C. truncatum*. This finding contrasts with reports of Frayssinet et al. (2008), Latunde-Dada et al. (2001), and Damm et al. (2014,) who identified *C. destructivum* as the only pathogen affecting alfalfa and soybean.

Ramos et al. (2013) reported the presence of *C. truncatum* and *C. destructivum* in soybean in Argentina using the Amplified Fragment Length Polymorphism (AFLP) technique. However, the results obtained by RAPD and ITS analyses, in the present study, showed that Coll 15, Coll 16 and Coll 32 isolates obtained from soybean corresponded to *C. truncatum* and *C. gloeosporioides*. Primer 3 differentiated these isolates from others, while primer 8, which was specific for *C. truncatum* and *C. graminicola,* separated both species in corn and soybean. Although genetic identity could not be confirmed by ITs sequences due to the poor quality of the DNA, the morphological characterization and RAPD similarity percentages supported the identification of these three strains as *C. graminicola*. Primers 1, 6 and 8 provided significant information for differentiating *C. truncatum* and *C. gloeosporioides* in soybean and mandarin crops. In agreement with Echeverrigaray (2020) the confirmation of Koch's postulates from lesions with the pathogen was performed by ITS sequencing.

The *Colletotrichum* isolates were validated by sequencing of the ITs 1-4 region of rDNA, except for Coll 1, Coll 6 and Coll 25, which could not be sequenced due to the low quality of the genomic DNA extracted. Although genetic identity could not be confirmed by sequences of ITs, we endorsed them with the morphological characterization performed and by the percentage of similarity obtained by RAPDs, confirming as *C. graminicola* in strains Coll 1 and 6 and *C. truncatum* in Coll25.

According to Dias et al. (2019), the RAPDs technique is a valuable tool to describe the genetic diversity of *Colletotrichum* added to the morphological characterization. However, technological advances have allowed advances in new identification techniques such as multilocus for the separation of *Colletotrichum* species (Astolfi, 2022 and Zhang, 2024).

In agreement with Dias et al. (2019), the phenotypic plasticity of *Colletotrichum* and the limitations of identifying it solely through morphological characters highlight the need for an integrated approach that combines morphological and biochemical methods for accurate taxonomy. According to Dias et al. (2019), the molecular marker system (RAPDs) was able to establish a high degree of similarity between and within the populations (isolates) of *Colletotrichum* spp and the species *C. truncatum* and *C. gloesosporioides*, which would allow guiding the search and determination of sources of resistance in *Glycine max* germplasm.

The results obtained in the present work provide relevant information on the stages of parasitism and survival of the biological cycle of the pathogen *Colletotrichum* and the physiological-genetic responses of the hosts and the epidemiological importance of Anthracnose affecting soybean and maize crops, especially relevant for our region. Future research should consider agricultural practices and crop rotation as part of cultural management and incorporate genetic tools such as Multi-locus phylogenetic analyses (Astolfi, 2022 and Zhang, 2024) for the identification and study of *Colletotrichum* species diversity,including more soybean and corn isolates from different regions of the country and observe their development under field conditions.

CONCLUSIONS

The morphological and molecular identities of three *Colletotrichum* species associated with relevant crops from central Argentina were determined. The identified species were *C. truncatum* and *C. gloeospoiroides* in soybean, *C. graminicola* in corn, *C. gloeospoiroides* in tangerine and *C. truncatum* in alfalfa. The conidia of *C. truncatum* in soybean and *C. graminicola* in corn differ from those described by most authors in the core region of Argentina.

In Coll 13 from maize, two types of conidia were identified: falcate and oval, consistent with the findings of Sukno (2008). The falcate conidia were associated with acervuli on the lesion

surfaces and played a role in the spread of the disease, while the oval conidia were found on stems and leaves.

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The author reports that, due to involuntary omission, the participation of Dr. Rosanna Pioli (Doctoral Thesis Director), Dr. Carlos Cairo (Co-Director) and Dr. Facundo Hernández, in the preparation and writing of the published manuscript, was not adequately included. For this reason, their contributions are detailed below:

Dr. Rosanna Pioli and Dr. Carlos Cairo wrote the Framework Project that provided the Objectives, Hypotheses, Techniques and Methodological Criteria of the published manuscript. Results: Dr Rosanna Pioli participated in the macro and micromorphological identification of the fungal collection. Dr. Rosana. Pioli and Dr. Cairo co-participated in the molecular characterization using RAPDs and Dr. Facundo Hernández implemented and wrote the molecular validation using ITS and the sequencing process. They also participated in the analysis and interpretation of Results, writing of the Discussion and integration of the manuscript. Table 1: Dr. Incremona (author) and Dr. Pioli. Table 2: Dr. Pioli. Figure 1: Dr. Incremona (author) and Dr. Pioli. Figure 2: Dr. Hernandez. Annex of images (structures and measurements): Dr. Incremona (author) and Dr. Pioli.

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Annex 1. Macro and micro morphological characteristics of the 14 *Collectotrichum* isolates (Coll: Code) from different hosts and agro-ecological areas.

	C. graminicola	*C. truncatum*	*C. graminicola*	*C. truncatum*	*C. truncatum*	*C. graminicola*	*C. graminicola*
Coll	**Coll 1**	**Coll 5**	**Coll 6**	**Coll 7**	**Coll 8**	**Coll 11**	**Coll 13**
Conidial shape Conidial size	curved, sickle and wider 21.36– 23.36µm. 5.05 µm	sickle and thin, resembling a smile 21.79µm 2.75µm	curve and straight (boomerang) 18.44- 20.12µm 3.3 µm	thin, slightly curved, non septate 27.6 µm/3.8 µm	curved, unlike a smile 8.7 – 9.9 µm 5.3 µm	sickle with fine tipped ends 30.8 µm	falcate and small ovoid, gutulae 24.1/3.25 µm
Colonies							
Acervuli							
Conidio							

Coll	Coll15	Coll 16	Coll 17	Coll 23	Coll 24	Coll 25	Coll 32
Conidial size	*C. truncatum* smile -like elongated 30.3µm	*C. truncatum* hemispheric or truncate end 21.1/ 2.7 µm	*C. graminicola* slightly curved 21.3 /1 a 1.5µm	*C. gloeosporioides* ellipsoid with central cell rounded ends 11.82/2.7 µm	*C. gloeosporioides* small short divided in middle 10.72/ 5 µm	*C. truncatum* Smile - shape with thin ends 25 µm	*C. gloeosporioides* rod- like ellipsoid elongated rounded ends 17 µm
Colonies							
Acervuli				Image not available		Image not available	Image not available
Conidia							

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