**Colletotrichum fructicola**, a Member of *Colletotrichum gloeosporioides sensu lato*, is the Causal Agent of Anthracnose and Soft Rot in Avocado Fruits cv. “Hass”

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**ABSTRACT**

The filamentous Ascomycota *Colletotrichum gloeosporioides sensu lato* is a fungus that has been reported worldwide as a causal agent of anthracnose disease in avocado and other crops. In Mexico, this species affects fruits from an early stage of development in the orchard until the post-harvest stage. Although fungicides are continuously applied to control *Colletotrichum* species, pericarp cankers and soft rot mesocarp in fruits are still frequently observed. Considering the lack of a precise description of the causative agent, the aim of the current study was to determine the pathogens involved in this symptomatology. Twenty-four isolates were consistently obtained from the pericarp of avocado fruits cv. “Hass” collected in the central avocado-producing area of Mexico. Morphological features such as colony growth, conidia size, and mycelial appressorium were assessed. Bayesian multilocus phylogenetic analyses were performed using amplified sequences of the internal transcribed spacer region of the nuclear ribosomal DNA; actin, chitin synthase, glyceraldehyde-3-phosphate dehydrogenase partial genes; and APn2-Mat1-2 intergenic spacer and mating type Mat1-2 partial gene from the nine selected isolates. In addition, fruits were inoculated with a conidial suspension and reproducible symptoms confirmed the presence of *Colletotrichum fructicola* in this area. This pathogenic species can now be added to those previously reported in the country, such as *C. acutatum*, *C. boninense*, *C. godetiae*, *C. gloeosporioides*, and *C. karstii*. Disease management programs to reduce the incidence of anthracnose should include *C. fructicola* to determine its response to fungicides that are routinely applied, considering that the appearance of new species is affecting the commercial quality of the fruits and shifting the original population structure.

1. Introduction

Anthracnose is a disease caused by the *Colletotrichum* species complex, an intracellular filamentous Ascomycota that is characterized by the formation of sunken spots and canker in the stems, twigs, branches, and fruits [1]. This pathogen affects many hosts worldwide such as avocado (*Persea americana*), annona (*Annona cherimola*), coffee (*Coffea arabica*), apple (*Malus domestica*), peach (*Prunus persica*), mango (*Mangifera indica*), and, more recently, tea oil (*Camellia oleifera*), among others [2–6].

*Colletotrichum gloeosporioides* has been extensively studied, and a species complex comprising 22 species along with one subspecies using multilocus phylogenetic analysis was recently described [7], and shown to be formed by 42 accepted species [8–10]. This species complex has been reported as the causal agent of anthracnose in all avocado-producing countries, and represents the most serious post-harvest disease in high rainfall growing regions [11]. Furthermore, avocado anthracnose has been reported to be caused by *C. gloeosporioides* in South Africa [12] and Israel [13], *C. fioriniae* in Australia [14], *C. aenigma* in Israel, *C. alienum* in Australia and New Zealand, *C. fructicola* in Australia [7], *C. kahawae* subsp. *ciggaro* in New Zealand, *C. queenslandicum* in Australia, and *C. siamense* in Australia and South Africa [7].

In Mexico, *C. gloeosporioides*, *C. boninense*, *C. acutatum* [15], *C. godetiae* [16], and *C. karstii* [17] have been isolated from avocado exocarp tissue with anthracnose symptoms. Nevertheless, there is no mention of *C. gloeosporioides sensu stricto* causing anthracnose in avocado in Mexico, which may
be because previous studies only assessed *C. gloeosporioides* using a single locus based on the internal transcribed spacer (ITS) region of rDNA, with no comparison to newly redefined species belonging to *C. gloeosporioides sensu lato*. Indeed, the ITS region was proposed as a fungal barcode marker [18] and has been used for *Colletotrichum* identification in several studies [15,19–21].

Nevertheless, multilocus phylogenetic analyses have clarified that the species included in the *C. gloeosporioides* complex are plant pathogenic fungi [7,22]. Therefore, the precise identification of phytopathogenic species of *C. gloeosporioides* that cause necrosis in the pericarp tissue and the soft rot mesocarp of fruits is very important [23]. This would allow for the rational and adequate use of fungicides to reduce the incidence and severity of disease [24], and provide the knowledge required for determining the sources of the primary inoculum from which the infection cycle begins in fruits [1].

Here, we demonstrate that anthracnose symptoms are present in unripe avocado fruits in the field in the central part of Mexico, and provide accurate identification of the associated causal agents using morphological and phylogenetic approaches.

## 2. Material and methods

### 2.1. Sampling and fungal isolation

In 2015, isolates of *Colletotrichum* were obtained from fruits recovered in an orchard located in Molango de Escamilla, state of Hidalgo, Mexico at 1663 m above sea level. Thirty avocado fruits cv. “Hass” were washed with running tap water and surface-disinfested in 1.5% sodium hypochlorite for 5 min, rinsed three times with SDW, and then dried at room temperature. A conidial suspension (10^6 conidia/mL) was prepared from each 10-day-old monoconidial *Colletotrichum* culture by adding 5 mL of SDW to an OA plate. The fruits were inoculated with 30 μL of SDW. The inoculated samples were incubated in containers at room temperature in normal light for 10 days.

Twenty-five healthy unripe avocado fruits cv. “Hass” were washed with running tap water and surface-disinfested in 1.5% sodium hypochlorite for 5 min, rinsed three times with SDW, and then dried at room temperature. A conidial suspension (10^6 conidia/mL) was prepared from each 10-day-old monoconidial *Colletotrichum* culture by adding 5 mL of SDW to an OA plate. The fruits were inoculated using the non-wound drop inoculation method. Thirty microliters of the conidial suspension was placed onto Petri plates containing potato dextrose agar (OA: 20 g oatmeal, 20 g agar, 1 L distilled water) plates, and scraped onto the surface of the medium to induce sporulation [25]. The plates were incubated at room temperature (24–26°C) for 10 days.

### 2.2. Pathogenicity test

Mycelial discs from a five-day-old actively growing edge colony on PDA medium were transferred to oatmeal agar (OA: 20 g oatmeal, 20 g agar, 1 L distilled water) plates, and scraped onto the surface of the medium to induce sporulation [25]. The plates were incubated at room temperature (24–26°C) for 10 days.

For each *Colletotrichum* isolate, a small amount of mycelium was scraped from the surface of a 5-day-old culture (grown on PDA at 28°C) using a sterile 10-μL pipette tip, and genomic DNA was extracted using the 2% CTAB method with slight modifications [27].

The ITS regions of rDNA, including ITS1, 5.8S, and ITS2 (ITS) [28]; actin (*ACT*); chitin synthase (*CHS*) [29]; glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*); partial genes [30]; and Apn2-Mat1-2 intergenic spacer and partial mating type Mat1-2 gene (*ApMat*) [31] of nine selected *Colletotrichum* sp. obtained in this study were amplified and sequenced. The primers used in this study are shown in Table 1.

To obtain single conidium isolates, the conidia were harvested by adding 5 mL of SDW to the plates, and the concentration of the suspension was adjusted to 1 × 10^6 conidia/mL using an hemocytometer (Marienfeld, Germany). A 100-μL aliquot was spread on water agar and incubated at the same temperature as above in the dark for 24 h. A single germinating conidium was transferred to a fresh PDA plate. Twenty-four isolates were obtained and morphologically identified as *Colletotrichum* based on morphological features of the genus.

### 2.3. DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

For each *Colletotrichum* isolate, a small amount of mycelium was scraped from the surface of a 5-day-old culture (grown on PDA at 28°C) using a sterile 10-μL pipette tip, and genomic DNA was extracted using the 2% CTAB method with slight modifications [27].

The ITS regions of rDNA, including ITS1, 5.8S, and ITS2 (ITS) [28]; actin (*ACT*); chitin synthase (*CHS*) [29]; glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*); partial genes [30]; and Apn2-Mat1-2 intergenic spacer and partial mating type Mat1-2 gene (*ApMat*) [31] of nine selected *Colletotrichum* sp. obtained in this study were amplified and sequenced. The primers used in this study are shown in Table 1.

PCR was performed in 15-μL reaction mixtures containing 0.18 μL of each primer, 0.18 μL of dNTPs, 0.9 U of GoTag® DNA polymerase (Promega, Madison, WI, USA), and 3 μL of DNA template (20 ng/μL). The PCR protocols were standardized
Table 1. Primer names, primer sequences, and references used in this study.

| Product name | Primer | Direction | Sequence 5'‐3' | Reference |
|--------------|--------|-----------|----------------|-----------|
| ITS          | ITSS   | Forward   | GGAGTAGAAAGATGGTAGAAACG | White et al. [28] |
| GADPH        | IT54   | Reverse   | GCCTTGACCCACTTATGATG | Templeton et al. [32] |
| ACT          | ACT12F | Forward   | AGTCGACGGCTGAGTCGAGTCGAG | Carbone and Kohn [29] |
| CHS          | ACT73R | Reverse   | TACGAGTTCTTCTTGCCTTGCCAT | Carbone and Kohn [29] |
| ApMAT        | CHS79F | Forward   | TGGGCAAGGATGCTGGAGAAGG | Rojas et al. [31] |
|              | CHS34R | Reverse   | TGGGAAACCCATGTTGAGGTG | |
|              | Apn2-Mat1-2 | Forward | GCGTLF6 | |
|              | GmMat1 F2 | Reverse | TGATGTATCCCGACTACCG | |

for each gene as follows: ITS, a first step at 95°C for 4 min, followed by 35 cycles at 95°C for 1 min, 58°C for 1 min and 72°C for 2 min, and a final step at 72°C for 10 min; ACT, a first step at 95°C for 8 min, followed by 35 cycles at 95°C for 15 s, 61°C for 20 s, 72°C for 1 min, and a final step at 72°C for 5 min; CHS, a first step at 95°C for 4 min, followed by 35 cycles at 95°C for 30 s, 58°C for 30 s, 72°C for 45 s, and a final step at 72°C for 7 min; GAPDH, a first step at 94°C for 1 min, followed by 35 cycles for 94°C for 1 min, 56°C for 75 s, and 72°C for 75 s, and a final step at 72°C for 10 min; ApMat, a first step at 95°C for 5 min, followed by 10 cycles at 95°C for 30 s, 62°C for 30 s, and 72°C for 1 min, followed by 35 cycles at 95°C for 30 s, 52°C for 30 s, and 72°C for 1 min, with a final step at 72°C for 10 min.

Amplified PCR products were cleaned using ExoSAP-IT® (Affymetrix, Santa Clara, CA, USA) and both strands were directly sequenced using BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™) in a 3130 Genetic Analyzer Sequencer (Applied Biosystems™) at Postgraduate College facilities, Mexico.

2.4. Phylogenetic reconstruction

The DNA sequences obtained from both strands were assembled to obtain consensus sequences for each isolate using BioEdit v7.0.5 [32]. Phylogenetic analysis of the Colletotrichum isolates was performed based on concatenated multi-locus datasets of ITS, ACT, CHS, GAPDH, and ApMAT sequences. Multiple sequences were aligned using the MAFFT v.7 server (http://mafft.cbrc.jp/alignment/server/) under default parameters.

Sequences of Colletotrichum species deposited in the GenBank database belonging to the Musae clade in the C. gloeosporioides species complex [7] were retrieved and included as reference species along with the sequences obtained in this study (Table 2). Concatenated alignment was performed using MESQUITE v.3.10 [33], and Bayesian inference (BI) was performed using four Markov Chains Monte Carlo (MCMC) in Mr. Bayes v.3.2.1 software [34]. Maximum-likelihood analysis was performed inraxmlGUI 1.5b2 [35]. Rapid bootstrapping with 1000 iterations was implemented using the general time reversible model with a gamma distribution.

Nucleotide substitution models were determined using MrModeltest v.2.3 [36] for each gene partition and included in the BI. Two analyses of four MCMC chains were run from random trees for 1,500,000 generations and sampled every 1000 generations. The first 25% of generated trees were discarded as the burn-in phase of each analysis and posterior probabilities were determined for the remaining trees. The phylogenetic tree was rooted with the sequence of C. gloeosporioides sensu stricto CBS 123755. Sequences derived in this study were submitted to GenBank (Table 2). DNA alignment and phylogenetic trees were deposited in TreeBase under ID study S21326.

2.5. Morphology and cultural characteristics

After phylogenetic reconstruction and identification of the pathogen, morphological analysis was performed. Conidia were harvested from the PDA plates, incubated at 28°C in the dark, and then mounted onto a microscope slide with clear 1% lactic acid. Mycelia conidial and appressorium formation were produced using a slide culture technique [37,38], and 30 of them were selected for measurement using ImageJ software (https://imagej.nih.gov/ij/) through photographs taken by Infinity 1-C2 implemented on an Olympus BX41 microscope (Tokyo, Japan). The shape and size of 50 conidia were measured with the same procedure. Colony characteristics on PDA were recorded after 7 days.

3. Results

3.1. Monoconidial isolates

Original field symptoms observed in 30 avocado fruits collected in the central part of Mexico showed necrotic tissue with small dark brown spots on green-unripe fruit exocarps, which coalesced with age (Figure 1(a)). Twenty-four monoconidial isolates were obtained for further analyses.
3.2. Pathogenicity test

The pathogenicity test was performed with 24 Colletotrichum isolates on unripe fruits of avocado cv. "Hass" to confirm Koch’s postulates. The development of small circular necrotic spots was observed at 10 days after inoculation (DAI), and abundant conidia masses were observed on the surface of the avocado fruits for all isolates inoculated (Figure 1(b)). When the fruits were cut, a soft rot with dark brown discoloration in the mesocarp was observed at 10 DAI (Figure 1(c)). Setae were absent. The fungus was re-isolated from the exocarp tissue, as described above, and re-identified morphologically. All of the inoculated isolates were found to be very aggressive because the infection advanced until covering almost 50% of the inoculated fruits.

Table 2. Isolates of Colletotrichum spp. used in this study with GenBank accession numbers.

| Species          | Strain     | Host              | Country | ITS          | ACT          | CHS          | GAPDH        | ApMat        |
|------------------|------------|-------------------|---------|--------------|--------------|--------------|--------------|--------------|
| C. aenigma       | ICMP18608  | Persea americana  | Israel  | JX010244     | JX009443     | JX009774     | JX010044     | KM360143     |
| C. aeschynomenes | ICM 17673  | Aeschynomene virginica | USA  | JX010176     | JX009483     | JX009799     | JX009930     | KM360145     |
| C. alienum       | ICMP 12071 | Malus domestica    | New Zealand | JX010251    | JX009572     | JX009882     | JX010028     | KC888927     |
| C. asiaticum     | CBS 130418 | Coffea arabica     | Thailand | JF972612 | JX009584 | JX009867 | JX010053 | FR718814 |
| C. fruticola     | CPO 27.01  | P. americana       | Mexico  | KX268474     | KX355488     | KX355497     | KX687154     | KX687163     |
| C. fruticola     | CPO 27.02  | P. americana       | Mexico  | KX278718     | KX355489     | KX355496     | KX687155     | KX687164     |
| C. fruticola     | CPO 27.03  | P. americana       | Mexico  | KX278719     | KX355490     | KX355499     | KX687156     | KX687165     |
| C. fruticola     | CPO 27.04  | P. americana       | Mexico  | KX278720     | KX355491     | KX355500     | KX687157     | KX687166     |
| C. fruticola     | CPO 27.05  | P. americana       | Mexico  | KX278721     | KX355492     | KX355501     | KX687158     | KX687167     |
| C. fruticola     | CPO 27.06  | P. americana       | Mexico  | KX278722     | KX355493     | KX355502     | KX687159     | KX687168     |
| C. fruticola     | CPO 27.07  | P. americana       | Mexico  | KX278723     | KX355494     | KX355503     | KX687160     | KX687169     |
| C. fruticola     | CPO 27.08  | P. americana       | Mexico  | KX278724     | KX355495     | KX355504     | KX687161     | KX687170     |
| C. fruticola     | CPO 27.09  | P. americana       | Mexico  | KX278725     | KX355496     | KX355505     | KX687162     | KX687171     |
| C. fruticola     | ICMP 18646 | Tetragastris panamensis | Panama | JX010173     | JX009581     | JX009874     | JX010032     | JQ807839     |
| C. fruticola     | CBS 130416 | Carica arabica     | Thailand | JX010165   | FJ907426     | JX009866     | JX010033     | JQ807838     |
| C. fruticola     | GMS67 = MTCC 11679 | M. indica | India | JQ894676 | JQ894543 | JQ894614 | JQ894630 | JQ894576 |
| C. gloeosporioides | CBS 112999 | Citrus sinensis | Italy | JX010152 | JX009531 | JX009818 | JX010056 | JQ807843 |
| C. musae         | CBS 116870 | Musa sp. | USA | JX010146 | JX009433 | JX009896 | JX010050 | KC888926 |
| C. nupharicola   | CBS 470.96 | Nuphar lutea subsp. polysepala | USA | JX010187 | JX009437 | JX009835 | JX009972 | XJ145319 |
| C. queenslandicum | ICMP 1778 | Carica papaya | Australia | JX010276 | JX009447 | JX009899 | JX009934 | KC888928 |
| C. salicola      | ICMP 19053 | Salix sp. | Hungary | JX010242 | JX009562 | JX009863 | JX009916 | KC888925 |
| C. siamense      | GM018 = MTCC 11672 | M. indica | India | JQ894653 | JQ894533 | JQ894608 | JQ894624 | JQ894536 |
| C. tropicale     | ICMP 18653 | Theobroma cacao | Panama | JX010264 | JX009489 | JX009870 | JX010007 | JC9790728 |

Figure 1. Original anthracnose symptoms (a), symptoms in the pathogenicity test showing abundant sporulation on the pericarp (b) and dark soft rot in the mesocarp (c).

3.2. Pathogenicity test

The pathogenicity test was performed with 24 Colletotrichum isolates on unripe fruits of avocado cv. "Hass" to confirm Koch’s postulates. The development of small circular necrotic spots was observed at 10 days after inoculation (DAI), and abundant conidia masses were observed on the surface of the avocado fruits for all isolates inoculated (Figure 1(b)). When the fruits were cut, a soft rot with dark brown discoloration in the mesocarp was observed at 10 DAI (Figure 1(c)). Setae were absent. The fungus was re-isolated from the exocarp tissue, as described above, and re-identified morphologically. All of the inoculated isolates were found to be very aggressive because the infection advanced until covering almost 50% of the inoculated fruits.

3.3. Phylogenetic analysis

All isolates were similar in the morphological and pathogenicity tests; thus, nine isolates were selected at random for phylogenetic analysis. The combined gene alignment comprised 23 taxa and 2022 characters, including gaps (ITS: 1–507, ACT: 508–729, CHS: 730–1002, GAPDH: 1003–1266, ApMAT: 1267–1922). The best evolution models of each gene were: K80 + I for ITS, HKY for ACT, SYM + I for CHS, HKY + G for GAPDH, and HKY + I for ApMAT. The consensus tree obtained for Bayesian analysis showed that the nine isolates obtained in this study clustered with the C. fruticola clade with a posterior probability of 100%, and differed from the reference isolates. C. fruticola was separated from the most closely related species...
(C. nupharicola) with 100% support (Figure 2). All isolates analyzed in this study by a molecular approach were identical for all genes.

### 3.4. Morphology and cultural characteristics

Most of the morphological features coincided with those reported by Prihastuti et al. [39]. For all isolates, the growth of colonies on PDA media showed a dark brown color, becoming gray to dark gray at the center with age (Figure 3(a)). The aerial mycelium was gray, dense, with visible conidia masses. Acervuli and setae were absent. The reverse side was grayish with a white halo of mycelium (Figure 3(b)). Conidia were $(11.06\pm 16.14) \times (3.19\pm 4.03) \mu m (n = 50)$, one-celled, smooth-walled, hyaline, cylindrical with obtuse to slightly rounded ends; the majority had two rounded guttules (Figure 3(c)). Conidial and mycelial appressoria formed on slide cultures were $(7.68\pm 9.62) \times (5.65\pm 6.13) \mu m (n = 30)$, brown to dark brown, ovoid for conidial appressoria and slightly obtuse at the tip, pedicellate, and irregular in shape for mycelial appressoria, often containing one or more guttules (Figure 4(a,b)).

### 4. Discussion

Avocado (P. americana Mill.) is one of the most important fruit crops in Mexico because it is the main export product. In 2013, Mexico ranked first worldwide in production and export with 1,467,837 tons and 563,492 tons, respectively [40]; trades forecast that avocado exports for the marketing year 2016–2017 will increase to over 1.0 million metric tons due to expected strong international demand [41].

One of the main problems faced by producers to obtain fruits with high commercial quality is the incidence of fungal diseases. Anthracnose caused by
different species of Colletotrichum represents one of the challenges within production systems. Through amplification of ITS sequences, C. gloeosporioides, C. acutatum, and C. boninense were initially described to be associated with avocado anthracnose fruits in the states of Michoacan and Morelos, Mexico, respectively [15,42]. Furthermore, it has been reported that C. godetiae and C. karstii, which belong to the C. acutatum and C. boninense complexes, affect avocado in the state of Morelos, Mexico [16,17]. In the present study, C. fructicola, a member of the C. gloeosporioides species complex, was found to be a causal agent of anthracnose in avocado fruits collected in the state of Hidalgo, in the central part of Mexico, using morphological and multilocus phylogenetic analyses. Nevertheless, variation in colony morphology (colony color, pigment, sporulation, perfect state, and conidia size) has been reported [43] and sterile isolates of C. fructicola have been encountered [3], suggesting high genetic variation.

Colletotrichum fructicola was proposed as a crop pathogen for the first time by Prihastuti et al. [39] using a polyphasic approach, affecting coffee berries in Thailand. Since then, it has been reported to cause anthracnose, thus representing an important fungal pathogen in several plantations worldwide [6]. Colletotrichum fructicola was the dominant and most aggressive species associated with bitter rot of apple in Uruguay [44] and strawberry in Japan [45]. In China, C. fructicola was reported to cause anthracnose of sandy pear (Pyrus pyrifolia), an economically significant disease in southern China [43], and was the principal fungus of the C. gloeosporioides complex causing citrus anthracnose [46]. Furthermore, C. gloeosporioides, previously reported to cause anthracnose in different crops worldwide, was then reclassified. For example, C. fructicola is a causal agent of strawberry anthracnose in Korea, which was previously reported as C. gloeosporioides [47].

Colletotrichum gloeosporioides sensu stricto as a causal agent of avocado fruits anthracnose has only been reported recently in Turkey [48]. In addition, C. gloeosporioides sensu stricto was not present in tropical fruits tested in Laos and Thailand [49]. Diverse studies reported C. gloeosporioides as a pathogen causing anthracnose in avocado fruits using analyses of the ITS region, in which the isolates were
grouped in different clades, suggesting that different species were present [12,15]. Nevertheless, other genes and statistical approaches are needed to determine robust phylogenetic relationships and provide the true species delimitations [23,49]. For example, the intergenic sequence of apn2 and Mat1-2 (ApMat) can improve resolution for the definition of the Colletotrichum species complex compared to other commonly used loci [2,50], and ApMat and GS loci can help to resolve species identification in the C. gloeosporioides complex affecting Camellia [51].

It is possible that C. fructicola is amply distributed in other crops and that diverse species can cause the same disease in avocado because of an ample range host and the high diversity of this genus, which have not been previously investigated in Mexico. However, special attention should be given to this species, because even though anthracnose symptoms in the field were not very evident (Figure 1(a)), when the fruits were placed in favorable environmental conditions, the fungus was able to colonize almost the entire fruit in a short time (Figure 1(b)) accompanied by mesocarp soft rot (Figure 1(c)). This observation further indicates the potential aggressiveness of these isolates and the risk that can be introduced in other areas where C. fructicola has not yet been reported to date.

Further research focused on determining the distribution of Colletotrichum species is needed for implementation of specific management strategies based on fungicide applications in each avocado production region, because variation in sensitivity can occur as well as the emergence of fungicide-resistant isolates [52,53]. Furthermore, the emergence of more aggressive species of Colletotrichum highlights the need for regular surveys, particularly when taking into account the fact that most commercial groves are cv. “Hass” monocultures, the effects of climate change, and the adaptation of pathogens to new varieties and ecological niches.

In conclusion, the present results obtained by molecular and morphological techniques as well as pathogenicity tests suggest that C. fructicola is the causal agent of anthracnose and soft rot in avocado fruits cv. “Hass” in the central part of Mexico. This finding is very important because the state of Hidalgo is close to Michoacan, the main avocado-producing state in Mexico, and thus C. fructicola

Figure 5. Distribution of the Colletotrichum species complex in the main avocado-producing areas in Mexico. Image created by the authors. Map source: INEGI, Mapa Digital de México.
could spread into areas where only \textit{C. gloeosporioides}, \textit{C. acutatum}, and \textit{C. boninense} have been reported to date (Figure 5). Moreover, this species should be included in sensitivity to fungicides tests aimed at maintaining the high quality of avocado fruits, which is essential for the exportation trade.

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\textbf{Disclosure statement}

The authors declare no conflict of interest related to any part of the research.

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