Characterization of *Colletotrichum gloeosporioides*, Causal Agent of Anthracnose in Soursop (*Annona muricata*) in Valle del Cauca, Colombia

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Introduction

Tropical fruit crops comprise one of the better options of Colombian agriculture. National and international demand of soursop has been growing at 3.8% per year, and the potential for developing a profitable postharvest industry is high.

Anthracnose generates losses of up to 90% in traditionally grown soursop crops. The pathogen attacks the leaves, branches, flowers, and fruits, producing black fruit rot, especially during the rainy season.

Flowers **Leaves** **Branches**
Symptoms characteristic of Antracnosis in soursop.

Materials and Methods

Cultures of the pathogens. Affected tissue samples were obtained from farms with different levels of disease incidence. Isolates of *Colletotrichum* sp. were obtained by either incubating tissue samples in a humidity chamber or plating in culture medium. After obtaining monosporic isolates, sporulation was stimulated by scraping mycelia in the same petri dish and incubating for 2 or 3 days.

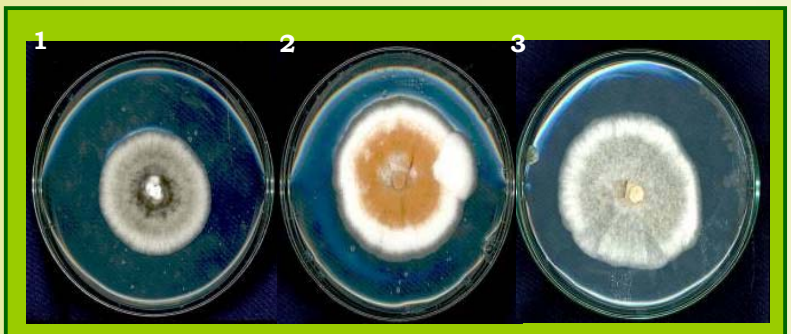
Morphological characterization. On PDA the variables "colony diameter" and "mycelium color" of 54 isolates were measured.

Pathogenicity. A greenhouse trial was conducted and small trees were inoculated. These trees were three-month-old scions from the canopy of a tree of the soursop cultivar Elita, grafted on a sexually-produced individual, indigenous to the region. Each isolate was inoculated onto four trees by spraying with a spore suspension at 1 x 10^7/mL. Plants were incubated in humid conditions at 27°C-29°C. A severity scale was designed to evaluate the disease, taking into account the appearance of lesions on the stem. The first evaluation was carried out at 72 h; the second, 10 days after the first evaluation; and the third, 20 days afterward.

Genetic characterization. Fungal mycelium was frozen and DNA extracted using a modified version of the Mahuku protocol (2004). The ITS (internal transcribed spacer) region was amplified through PCR, using universal primers from the conserved region of the rDNA gene. Eight different enzymes were evaluated in this study. Polymorphism obtained with the different random amplified microsatellite (RAM) primers on seven *Colletotrichum* isolates was evaluated to then amplify the 56 isolates with the most polymorphic primer.

Results and Discussion

- There was a correlation of -0.84 for the relationship between growth characteristics and pathogenicity. Slow growing isolates presenting gray mycelium, limited sporulation, and undulating relief (*in vitro*) were found to be highly pathogenic.



Types of colonies of *Colletotrichum* isolates recovered on medium PDA containing streptomycin (300 mg/L).

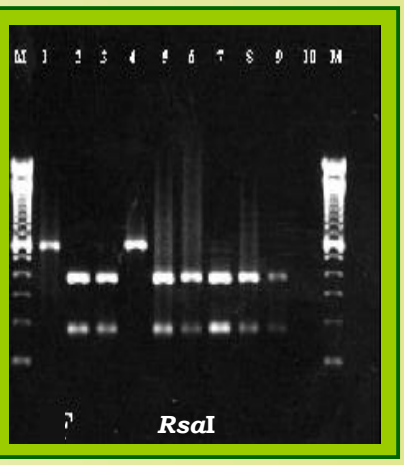
- Specific DNA amplification with primers for *C. gloeosporioides* (50 isolates) and *C. acutatum* (4 isolates) generated 450-bp and 490-bp fragments, respectively, confirming the species identity.

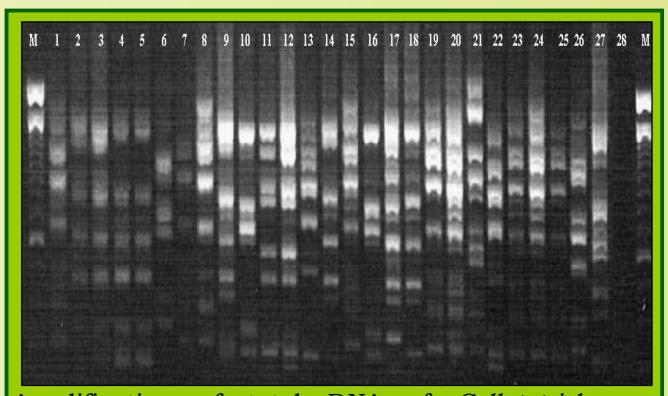
Amplified ITS region of ribosomal DNA from *Colletotrichum* spp. isolates. Lanes 1 and 7: *Phaeoisariopsis griseola* (bean); 2-5 and 8-11, *Colletotrichum spp.* (soursop).

- The combination of universal primers ITS1 and ITS4 generated a 580-bp fragment, which when digested by *Hae* III and *Rsa* I, yielded DNA fragment patterns that could be clustered into two groups that corresponded to each of species of the fungus.

- The analysis of RAM indicated a high degree of variation in the pathogen population.

Digestion of the product amplified with primers ITS 1 and ITS 4 from 10 *Colletotrichum* isolates with the restriction enzymes *Msp*1, *Alu*1, *Hae*III, *Dra*I, *Hind*III, and *Rsa*I. M = 100-bp marker.





Amplification of total DNA of *Colletotrichum* isolates, using the primer CA (RAM).

Conclusion

- These results illustrate the phenotypic and genetic diversity of *Colletotrichum* spp. and confirms that *Colletotrichum gloeosporioides* and *C. acutatum* are associated with anthracnose in soursop.

- Spraying without wounding allowed infection under similar conditions, which resemble those of a rainy season. The findings confirm observations that higher incidence of the disease occurs during heavy rains and in crops with high-density planting.

References

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