

Annona squamosa, a New Host of *Ceratocystis fimbriata*

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ABSTRACT

Mango branch blight disease, caused by *Ceratocystis fimbriata*, is endemic to the municipality of São Fidelis in northern Rio de Janeiro State. In addition to mango, *C. fimbriata* was found associated with sugar apple trees (*Annona squamosa*) showing symptoms of branch blight in São Fidelis. Sugar apple and mango isolates from the same region had the same morphology and showed similar ITS-rDNA sequences. These sequences were also similar to other Brazilian isolates of *C. fimbriata* sensu stricto. Cross inoculation of such isolates obtained from diseased sugar apple and mango resulted in diseased symptoms on both plant species. This is the first record of *A. squamosa* as a host for *C. fimbriata*.

Additional keywords: Host range, sugar apple, *Mangifera indica*, pathogenic variability.

RESUMO

Annona squamosa, nova hospedeira de *Ceratocystis fimbriata*.

A seca-da-mangueira, causada pelo fungo *Ceratocystis fimbriata*, é uma doença endêmica no município de São Fidelis, no norte do Estado do Rio de Janeiro. *Ceratocystis fimbriata* foi encontrado associado a árvores de pinha (*Annona squamosa*) apresentando sintomas de seca dos ramos. Isolados obtidos de pinha e manga, originários da mesma região, apresentaram a mesma morfologia e sequências espaçadoras ITS do rDNA similares. Estas sequências foram também similares a de outros isolados brasileiros de *C. fimbriata* sensu stricto. Inoculações cruzadas dos isolados de pinha e manga resultaram no desenvolvimento de sintomas em ambas espécies de plantas. Este é o primeiro relato de *A. squamosa* como hospedeira de *C. fimbriata*.

Palavras-chave adicionais: Gama de hospedeiros, pinha, *Mangifera indica*, variabilidade patogênica.

Mango branch blight is a common disease in Brazil and is only known in this country (Ribeiro, 1997). The disease was first described in the State of Pernambuco in 1938, and it has been known in the State of São Paulo since 1940. It was hypothesized that the mango pathogen was disseminated to other states by propagation material originating from the state of São Paulo (Ribeiro, 1997). In the municipal of São Fidelis, State of Rio de Janeiro (Brazil), mango (*Mangifera indica* L.) is widely grown, and mango branch blight caused by *Ceratocystis fimbriata* Ellis & Halst. is endemic in that region. The disease is frequently seen attacking old trees of the cultivar Espada. In commercial orchards of this and other cultivars, the disease is successfully controlled by grafting onto resistant rootstocks and pruning diseased branches. Some cultivars are known to have partial resistance to mango branch blight (Ribeiro *et al.*, 1997, 1995).

Sugar apple trees (*Annona squamosa* L.) showing branch blight symptoms and wood discoloration similar to that caused by *C. fimbriata* on mango and other hosts were observed in close vicinity to diseased mango trees in São

Fidelis (Figura 1A-B). Isolations were made from diseased sugar apple and mango trees. Since sugar apple was not known as a host of *C. fimbriata*, Koch's postulates were carried out, and isolates from mango and sugar apple were compared in inoculations of the two hosts. The mango (M) and sugar apple (SA) isolates were also compared by cultural morphology and ITS-rDNA sequences (Engelbrecht *et al.*, 2005; Baker *et al.*, 2003).

Isolations from naturally infected branches and from inoculated plants were made with a modified carrot disk method (Moller & DeVay, 1968). Freshly collected samples of discolored wood were sandwiched between two carrot disks, and the disks were taped together and incubated in a moist box chamber at 25±2 °C for one week. After sporulation of the fungus on the carrot tissue, the ascospore masses formed at the tip of the perithecia were transferred to modified malt extract agar medium (MMEA, as described by Tainter, 1992). Cultures were maintained on agar slants (tubes) on MMEA at 5 °C.

Plants were inoculated by placing disks of mycelia

with perithecia and ascospore masses (from 10-day-old colonies on MMEA) into a transverse cut at the stem base. In one experiment three young plants (two months old) of mango cv. Espada and of sugar apple (cultivar unknown) were inoculated with one of two sugar apple isolates (C1672, C1592) or a control. In another experiment six older plants (two years old) of mango cv. Carlotinha and three plants (one year old) of sugar apple were inoculated with both the two sugar apple isolates, one of four mango isolates (C1558, C1588, C1658 and C1662), or a control. Additional inoculations with one sugar apple isolate (C1592) were made on one adult sugar apple plant in one domestic orchard and on a set of ten two-year-old mango plants of cv. Espada in a greenhouse. After inoculations, the inoculation site was wrapped in plastic with moistened cheesecloth to maintain the humidity. The young plants were grown in a greenhouse and planted in a 1:1 soil-sand mixture in two-liter pots and irrigated weekly with one-quarter-diluted Hoagland's solution. The older plants were similarly grown in five liters pots and fertilized with Hoagland's solution when necessary. The symptoms were evaluated 30 and 60 to 120 days after inoculation date (dai) in the first and second experiments, respectively. Recovering of the pathogen was attempted with fragments of inoculated plants removed at 5 to 10cm from the point of inoculation cut and placed between carrot disks and incubated as previously described. In the first experiment, additional isolations were made by plating surfaced-sterilized fragments onto malt extract agar medium-MMEA.

Cultures of the fungus isolated from sugar apple were identical to those isolated from mango from the same region and matched the morphology of *C. fimbriata* sensu stricto (Engelbrecht & Harrington, 2005; Harrington & Baker, 2005). *Chalara*-like endoconidia formed within one day, and perithecia and hat-shaped ascospores formed within one week on malt extract agar. Pigmented aleurioconidia (chlamydospores) were also formed. The ITS-rDNA sequence of the sugar apple isolate (C1592) was identical to that of mango isolates from the same locality and similar to those of other Brazilian isolates of *C. fimbriata* from mango and *Eucalyptus* (Baker *et al.*, 2003).

In the first experiment, the sugar apple isolates were equally pathogenic to sugar apple and mango plants. As in the inoculated young plants of Espada, the inoculated sugar apple plants (two-months old) presented extensive internal wood discoloration at the top, including new shoots. The recovery of the pathogens was consistent with the internal symptoms and was equally efficient by the carrot bait method or on modified MMED medium. The uninoculated plants showed no symptoms. Older sugar apple plants and mango cv. Carlotinha showed some resistance in the second experiment, in which external symptoms took a long time to appear when old plants were inoculated with mango or sugar apple isolates (Table 1). Inoculation of one adult plant of sugar apple in a domestic orchard gave similar results. In the adult plant, the internal discoloration was restricted to

Table 1 - Pathogenicity of six isolates of *Ceratocystis fimbriata* obtained from diseased sugar apple and mango plants, evaluated 60 days after inoculation in sugar apple plants and 120 days after inoculation in mango plants

| Isolate and host of origin | Presence of internal discoloration at a distance (cm) from the cut point | | Maximum distance (cm) from the cut point with positive recovering of the pathogen on carrots disks | |
|----------------------------|--|-----------------------|--|-----------------------|
| | sugar apple* | mango cv Carlotinha** | sugar apple* | mango cv Carlotinha** |
| C1672 (sugar apple) | 39,2(2,0) | 6(5,3) | 30(0) | - |
| C1592 (sugar apple) | 36,7(8,2) | 7,3(2,2) | 23,33(11,5) | - |
| C1558 (mango) | nt | 8,4(2,4) | nt | - |
| C1588 (mango) | nt | 13,3(1,9) | nt | - |
| C1658 (mango) | nt | 8,53(1,2) | nt | - |
| C1662 (mango) | 20,2(18) | 11(5,1) | 21,7(14,4) | - |
| Control | 0 | 0 | - | - |

*/** = averages and standard deviation between parenthesis calculated from six* and three** replicates, respectively
 (nt) = not tested
 (-) = no recovery of the pathogen

radial sectors, and no wilt or blight occurred until 135 days after inoculation. No insect attack (by ambrosia beetles, Coleoptera: Scolytidae) was observed on the inoculated or uninoculated sugar apple or on Carlotinha mango plants, even in the proximity of heavily infested and diseased Espada mango plants in greenhouse experiments. In some sugar apple plants, longitudinal extension of the lesions was observed, but recovery of the pathogen was infrequent at a distance beyond the inoculation site (Table 1). The pathogen was not recovered from any of the Carlotinha mango plants at 120 days after inoculation. For comparison, a similar inoculation with one isolate from mango on ten 2-year-old plants of mango cv. Espada was followed by heavy attacks by ambrosia beetles, which resulted in 100% plant mortality at 45 to 60 days after inoculation. The mango cv. Carlotinha presented a high level of resistance to infection compared with mango cv. Espada in greenhouse experiments.

Research done in São Paulo State, Brazil, had confirmed the resistance of some cultivars of mango and the existence of pathogenic variability among *C. fimbriata* isolates from mango trees (Ribeiro *et al.*, 1995, 1986, 1984, Rosseto *et al.*, 1996, 1980). However, in São Paulo State mango cv. Espada is considered resistant to *C. fimbriata* infection from naturally infested soil. The same cultivar is susceptible when branches are inoculated under some experimental conditions, and severe symptoms and high rates of mortality can occur (Ribeiro *et al.*, 1986). In the north of Rio de Janeiro, old mango trees of cv. Espada are frequently attacked and killed by the blight, specially when the disease symptoms appear at the stem base.

Although *C. fimbriata* may be native to the Atlantic coastal regions of Brazil and is known on many hosts, including *Colocasia esculenta* (L.) Schott. (taro), *Eucalyptus*

spp., *Ficus carica* L., and *Gmelina arborea* Roxb., recent work suggests that isolates from these hosts represent a single species, though there are indications of some host specialization (Baker *et al.*, 2003; Harrington *et al.*, 2005; Thorpe, 2004). Most Brazilian isolates of *C. fimbriata* appear to be pathogenic to young mango plants, and the present study shows that mango isolates from the São Fidelis region are pathogenic to sugar apple.

Sugar apple plants naturally lose their leaves in the cold and dry season. At 60 days after inoculation the leaves of the older plants were yellowing in the second experiment (Fig 1D). However, the inoculated older plants lost all the leaves, while control plants had green leaves still attached to them 120 days after inoculation. According to Silveira *et al.* (2001), the disease on sugar apple plants does not appear economically important, occurring only occasionally, mainly on stressed and abandoned plants. Transmission from mango to sugar apple through infested tools was not investigated.



FIG. 1 - Internal wood discoloration caused by *C. fimbriata*. A. Symptoms on mango; B. Symptoms in sugar apple branches. Note the holes in bark caused by ambrosia beetles on mango (arrows) and the absence of holes in sugar apple branches; C. Internal wood discoloration of the apical portion of one inoculated stem of a young sugar apple plant; D. Right: Healthy (uninoculated control), Left: diseased (inoculated), one year old sugar apple plants. Inserts are showing details of internal discoloration in the stems of inoculated plants (arrows). E. Colonies of *C. fimbriata* (C1592) on carrot disks for recovering the pathogen from stem pieces of inoculated young sugar apple plants; F. New shoot emerging from a one year old sugar apple plant and internal wood discoloration (arrow) 60 days after inoculation.

Ambrosia beetle holes are rarely found on diseased sugar apple trees, though they are frequently present on diseased mango trees, and ambrosia beetle frass expelled from diseased trees could contain propagules of *C. fimbriata* (Iton, 1960). The diseased sugar apple trees had pruning wounds near the discolored xylem tissue, and it is possible that fresh pruning wounds were infected by infested pruning equipment, by spores carried by insects, or by ambrosia beetle frass carried by wind. However, all of these possibilities still need more investigation.

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