Tip Dieback of Mango (*Mangifera indica*) Caused by *Botryosphaeria ribis*

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ABSTRACT

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The conidial stage of *Botryosphaeria ribis* was isolated from diseased mango trees (*Mangifera indica*) and found to be pathogenic in mango by wound inoculation. The fungus produced dark mycelia and did not sporulate under most conditions but formed chlamydospores and immature conidial initials. In the vicinity of the inoculated stem scars, the fungus produced stromatic pycnidia, which yielded hyaline, one-celled pycnidiospores. On oatmeal agar, the dark mycelium also produced stromatic pycnidia. Hyphae were observed in sections of diseased tissues and abundant tyloses were found inside the xylem vessels. Several other fungi, including *Fusarium equiseti*, were occasionally isolated from mango hosts showing dieback symptoms, but they did not cause dieback in mango. This is the first report of *F. equiseti* in this host in the continental United States.

Additional keywords: Ascomycotina, *Botryodiplodia theobromae*, *Colletotrichum* sp., *Diplodia* sp., *Macrophoma* sp., *Oidium* sp., *Pestalotia* sp., *Physalospora* sp.

In Dade County, Florida, there are approximately 800 ha of mango, of which some 150 ha have been affected by mango tip dieback, sometimes referred to as mango decline (C. W. Campbell, *unpublished*). Typical symptoms of mango decline include terminal and marginal necroses of the leaves, which ultimately lead to the death of the leaf blade. The dieback gradually progresses to larger branches with eventual reduction in the number of secondary roots (17). However, the etiology of the disease has remained unknown since its first appearance in the late 1970s.

In Puerto Rico, dieback of mango was associated with the conidial stage of *Physalospora rhodina* Cooke. In the Jaipur district of India, the dieback was caused by *Botryodiplodia theobromae* Pat. (26). But *B. theobromae* was considered a weak pathogen that affected only weak mango trees in El Salvador, Central America (1). In Brazil, the dieback "seca el mango", with symptomatology resembling the tip dieback in Florida, was caused by *Ceratocystis fimbriata* Ellis & Halst. (syn. *Ceratostomella fimbriata* (Ellis & Halst.) J. A. Elliott) (20). Smith and Scudder (25) observed that the death of mango trees in 1949 in Florida was attributable to higher incidence of *Diplodia* sp. rather than to the poor nutritional condition of the trees. Recently, Reckhaus (19) reported a dieback causing severe damage in mango trees in the Republic of Niger. The fungus most often observed by Reckhaus (19) was *Hendersonula toruloidea* Nattras, forming arthrospores and black pycnidia containing hyaline to light green pycnidiospores. Reckhaus (19) considered that stress factors enhanced the severity of the disease.

The studies reported here were initiated to determine the etiology of mango tip dieback in Florida.

MATERIALS AND METHODS

Twigs from mango trees showing symptoms of mango tip dieback were collected and surface-sterilized with sodium hypochlorite for 3 min and rinsed three times with sterile distilled water. Sections from the xylem were dissected with a sterile scalpel and placed on potato-dextrose agar (PDA) containing 100 ppm of streptomycin sulfate. Pure cultures were produced after hyphal tip transfers to PDA, malt extract agar, cornmeal agar, oatmeal agar, water agar, or PDA supplemented with mango stem infusion.

The isolates were identified in the laboratory with taxonomic keys and references (3–5,7–9,12,13,15,16). They were also sent to the International Mycological Institute, Kew, Surrey, England, and the Division of Plant Industry of Florida for confirmation. Special emphasis was placed on the identification of stromata on inoculated seedlings. In addition, several isolates were grown in a medium with 50–200 ppm of cyclohexamide, which has been used in the identification of some species of fungi (13).

Nucellar seedlings from polyembryonic seeds of *Mangifera indica* L. 'Turpentine' and 'Peach' were grown in a growth chamber in 3.7- or 7.5-L plastic pots and kept free of insects and disease. The plants were grown in a mixture of potting soil and 50% vermiculite-peat moss containing nutrients, minor elements, and iron chelate (Fe 138). Additional nutrients were provided through weekly applications of Nutri Leaf 20-20-20.

Inoculations were made on 3- to 4-mo-old mango seedlings. All plants were of the same age and size. The plants were maintained inside a dew chamber at 30 C and 95% relative humidity (RH) for 6–8 wk. All plants were subsequently maintained inside a growth chamber at 25 C and 60–70% RH at 2,500 lux for 8–10 mo.

In the first pathogenicity test, 60 mango seedlings (cv. Turpentine) were used. There were 12 seedlings for each of the four fungal isolates and 12 seedlings for control. The mango seedlings were misted with a mycelial suspension of *Botryosphaeria ribis* Gross. & Duggar and a mycelial/conidial suspension (approximately 4×10^6 conidia per milliliter) of each of the other three isolates. The leaves were mechanically wounded by sand before inoculation. The second pathogenicity test consisted of eight treatments with seven independent fungal isolates and a control. There were 12 seedlings each for *Botryosphaeria* and *Diplodia* and six seedlings each for the other five isolates and control. The PDA cultures of *Botryosphaeria* and *Diplodia* were 2–3 wk old. The third pathogenicity test consisted of two cultures each of *Botryosphaeria* and *Diplodia* of different ages and a control for a total of five treatments. There were 12 seedlings (cv. Peach) for each treatment. In the second and third tests, a 5-cm-long cut was made with a sterilized scapel under the epidermis and outer portions of the cortex about 12 cm below the apex of the stem before inoculation. The epidermal tissue of the area was swabbed with 1% sodium hypochlorite. A conidial and mycelial mixture from PDA cultures was introduced into the stem wound with a sterile spatula. The stem wound and fungus were covered with cheesecloth, which also contained

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additional inoculum. The controls were also wounded but treated with only water agar. The inoculated plants were examined for necrosis around the stem scar 2–3 wk after inoculation and then examined periodically for 5–10 mo for symptom development.

Because of the relatively large number of fungal isolates included in each pathogenicity test and limitations in space in both the dew and growth chambers, all controls were put together in one area to avoid contamination. In addition, mango seedlings from each treatment were separated into two groups and placed in different areas of the dew and growth chambers. No attempt was made for a completely randomized block experiment.

To study the upward spread of *B. ribis* in stems, a series of 2.5-cm segments of the inoculated stems were dissected at 3.5 mo after inoculation. The segments were surface-sterilized as described earlier and placed on PDA.

Cross sections, 20 μm thick, were cut from 12 healthy and six diseased mango stems with a clinical freezing microtome. The sections were dehydrated in 5–100% alcohol, stained in safranin-fast green and mounted in Permount (14). The slides were examined under the microscope for hyphae in the tissue and formation of tyloses in the xylem vessels.

RESULTS

The conidial stage of *B. ribis* (Ascomycotina, order Dothideales, family Bothyosphaereaceae) was consistently isolated from tissue of mango trees showing serious tip dieback symptoms and vascular browning during four seasons. The dark, sterile mycelia of the early isolations, which did not sporulate under ordinary conditions, were first identified by their similarities of hyphae, chlamydospores, general morphology, and colony structure. Different isolates were compared with each other by growing them side by side in different cultural media to test the confluence. Because of the lack of sporulation of *B. ribis* under ordinary conditions, early attempts to identify it in the laboratory, the International Mycological Institute, or Florida's Division of Plant Industry were unsuccessful. However, the stromatic pycnidia produced on the stem cortex of inoculated mango seedlings suggested an anamorphic state of *Botryosphaeria*. Cultures of the conidial state of the isolate were sent to the International Mycological Institute where the isolate was identified as *B. ribis*.

B. ribis induced mango tip dieback in mango cvs. Turpentine and Peach when the leaves were inoculated with fungal suspensions (Table 1) or when the fungus was applied into a stem cut (Tables 2 and 3). Inoculations of wounded tissue were much more effective.

There was a progressive dieback of twigs and branches of adult trees or in the main stem apices of mango seedlings (Fig. 1) with considerable reduction in growth. In the early stage of infection, some branches showed browning of the leaf petioles and midrib, which extended downward as the disease progressed. The terminal leaves of the branch died but remained attached to the twigs for some time. When the fungus reached the vascular system of the main stem of seedlings, a complete defoliation of apical branches occurred.

When *B. ribis* was grown on PDA or malt agar (MA) for 10 days at 20 C, its radial growth was about 40 mm. The fungus first produced a white, cottony mycelium, but the colony turned gray and finally black with age. The fungus was characterized by the formation of dark mycelia and its inability to produce conidia under most cultural conditions. After prolonged subculture on PDA, MA, cornmeal agar with mango infusion, water agar, and various light conditions, the fungus produced pigmented hyphae, swollen hyphal cells or chlamydospores, and immature conidial initials that looked like sclerotia. The hyphae in the early stage were about 1.1–1.7 μm, but the dark mycelia were 2.8–3.3 μm in diameter, septate with segments of about 20 μm long, and had occasional hyphal knots. On oatmeal agar, the dark mycelia produced stromatic pycnidia with hyaline aseptate conidia, as did the herbarium specimens at the International Mycological Institute (Herb. IMI 293744). The fungus failed to produce perithecia in culture media or on mango tissues.

Table 1. The pathogenicity of four species of fungi on mango seedlings (*Mangifera indica* 'Turpentine')

Fungi[a]	Plants[b] (no.)	Plants with dieback (no.)	Foliage necrosis after 15 days (%)
R4 *Botryosphaeria*	12	6	33
R5 *Fusarium* sp.	12	0	25
R3 *Curvularia* sp.	12	0	20
R6 *Chalaropsis* like	12	0	15
Control	12	0	0

[a] Cultures of *Botryosphaeria ribis* potato-dextrose agar cultures were 4 wk old. R4 and R5 fungi were isolated from mango cv. Tommy Atkins.
[b] Wounded leaves of mango seedlings were inoculated by misting; plants were maintained for 4 wk in a dew chamber under artificial light.

Table 2. Pathogenicity of seven species of fungi on mango seedlings (*Mangifera indica* 'Peach')

Fungi	Plants inoculated[a] (no.)	Plants with dieback (no.)	Mean necrosis around inoculated stem wound after 3 wk (mm)
R15 *Botryosphaeria*	12	6	8.4
R33 *Diplodia* sp.	12	2	4.6
R22 *Macrophoma* sp.	6	0	6.6
R23 *Alternaria* sp.	6	0	4.0
R100 *Pestalotia* sp.	6	0	2.6
R26 *Fusarium equiseti*	6	0	4.0
R17 *Stigmina* sp.	6	0	3.0
Control	6	0	0

[a] Wounded stems were inoculated with mixtures of conidia and/or mycelia of each fungus from 2- to 3-wk-old cultures on potato-dextrose agar.

Table 3. Comparative pathogenicity of *Botryosphaeria ribis* and *Diplodia* sp. and the effects of age of fungal cultures on the tip dieback symptoms in seedlings of mango (*Mangifera indica* 'Peach')

Fungi	Age of colony (days)	Plants inoculated[a] (no.)	Plants with dieback (no.)	Mean necrosis around inoculated stem wounds after 3 wk (mm)
R15 *Botryosphaeria*	10–14	12	10	8.4
R15 *Botryosphaeria*	47	12	1	3.4
R33 *Diplodia* sp.	14	12	2	4.6
R33 *Diplodia* sp.	47	12	1	3.0
Control		12	0	0

[a] Wounded stems were inoculated with mixtures of conidia or mycelia of each fungus.

Stromatic pycnidia were produced on the stem cortex of mango seedlings near the inoculation stem scar, which yielded abundant one-celled hyaline pycnidiospores (7.2 × 16.8 μm) (Fig. 2B). The conidia produced by this fungus matched the description of the pycnidial stage of *B. ribis* given by Grossenbacher and Duggar (11) (E. Punithalingam, Commonwealth Mycological Institute, *personal communication*).

The age of the *B. ribis* culture affected pathogenicity. It was evident that the incidence and severity of dieback increased considerably when the inoculum from a 10- to 14-day-old culture was used instead of a 47-day-old culture (Table 3).

The upward progress of the infection of *B. ribis* along the stem of mango cv. Peach was studied for several months. The fungus moved at a rate of about 3.5 cm per month. This slow growth may explain the 5–10 mo required to develop full stem dieback or blight symptoms in the apices and subapices of inoculated seedlings, while early symptoms are observed after only a few weeks.

Stems of inoculated mango seedlings showed necrotic xylem vessels, colonized by hyphae, and the formation of dark inclusions inside the xylem vessels (Fig. 2D), as well as abundant production of tyloses (Fig. 2C). No hyphae or tyloses were observed in the uninfected controls. The presence of necrotic vessels, hyphae, and tyloses may bring about a dysfunction of the xylem elements with a restriction in the flow of water and minerals.

A second isolate, which produced less severe dieback, was occasionally found in tissues of infected mango trees. It produced pycnidia and two-celled, dark conidia. The isolate was identified as *Diplodia* sp. (N. E. El-Gholl, Division of Plant Industry, FL; *personal communication*). Even when the perfect state of the *Diplodia* sp. was also observed on the stem cortex of inoculated mango seedlings, it was not possible to determine beyond a reasonable doubt if the asci were of *Botryosphaeria* on the basis of morphology.

Other fungi were occasionally isolated from mango hosts showing dieback. Even though most of them were also present in asymptomatic plant tissues, they were included in the pathogenicity test (Tables 1 and 2). None of these fungi were able to induce dieback, but several showed some degree of pathogenicity. They included *Pestalotia* sp., *Oidium* sp., *Curvularia* sp., *Colletotrichum* sp., *Macrophoma* sp., *Fusarium equiseti* (Corda) Sacc., and others. *Macrophoma* sp. produced large necrosis around the inoculation scar. From this group of fungi isolated from mango trees, *F. equiseti* is reported here for the first time on this host in the continental United States.

DISCUSSION

This is the first report of *B. ribis* associated with mango tip dieback and its related symptomatology in Florida. Previously, it was reported in the Hawaiian Islands in 1929 (28). The

Fig. 1. Typical progressive apical dieback of mango plants 6 mo after stem wound inoculation with the conidial stage of *Botryosphaeria ribis*. The necrotic leaves at the tip of the stem progressively die but can remain attached to the stem for some time.

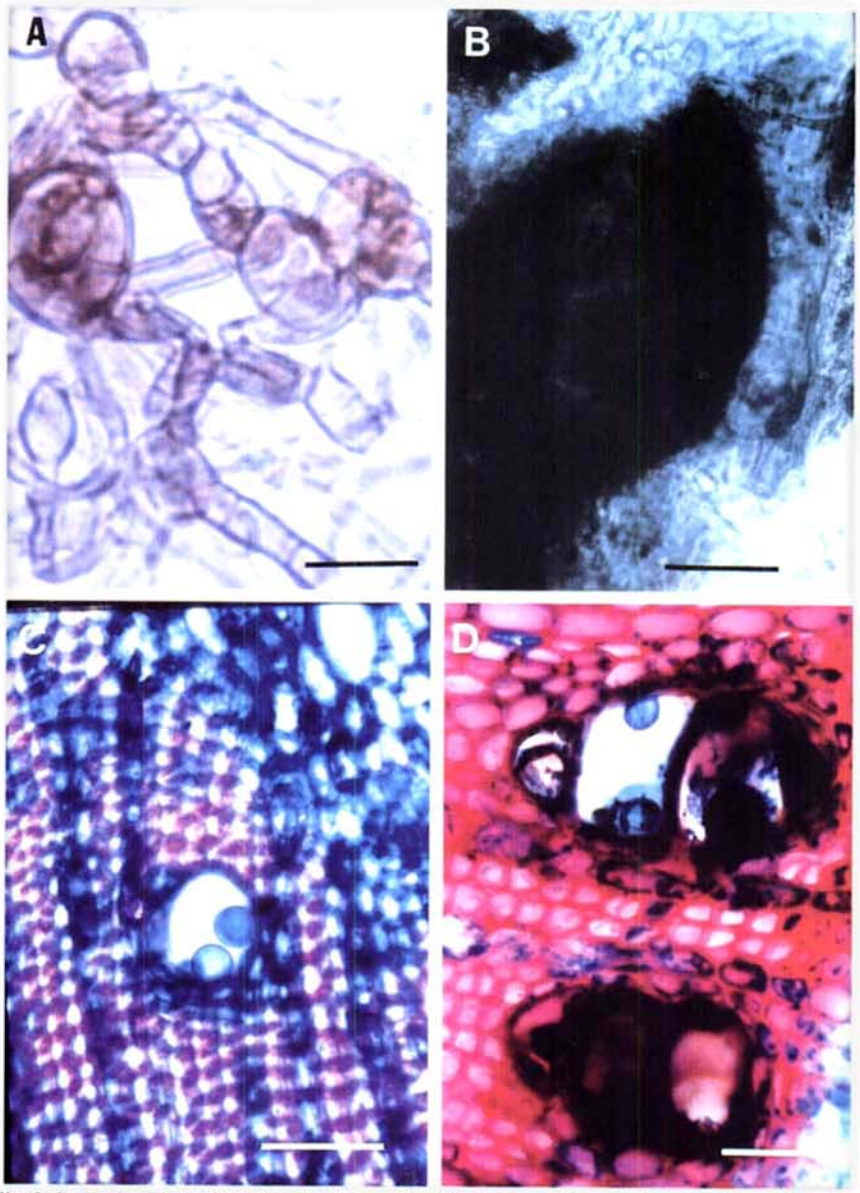

Fig. 2. Some morphological characteristics of conidial state of *Botryosphaeria ribis* and sections of infected mango (*Mangifera indica*) tissues. **(A)** Pigmented and swollen hyphal cells or chlamydospores of *B. ribis* grown on cornmeal agar. Scale bar = 8 μm. **(B)** Dark globose stromatic pycnidium of *B. ribis* and one-celled hyaline pycnidiospores. Scale bar = 20 μm. **(C)** Cross section of stem tissue of mango infected plant showing xylem vessels with tyloses. Scale bar = 44 μm. **(D)** Cross section of stem tissue of mango infected plant showing necrosis of the xylem vessels, the formation of dark inclusions, and the presence of fungal hyphae. Scale bar = 20 μm.

conidial stage of *B. ribis* appeared to be a very aggressive pathogen, whereas the *Diplodia* sp. isolate appeared to be a weak pathogen. While some *Diplodia* spp. have been included under *Physalospora* by some authors (26,30), new mycological publications based on the most recent revisions have listed *Diplodia* as an anamorphic state of *Botryosphaeria* (12,16). On the other hand, there is disagreement about delimitation of the species within the genus (12). *Botryosphaeria* usually occurs in the anamorphic state (12). Several determinations of *Physalospora* and *Botryospheria* have been based on their anamorphic states (26,28).

The primary organism causing mango dieback in southern Florida was identified as the conidial state of *B. ribis* (syn. *B. dothidea* (Moug.:Fr.) Ces. & DeNot.). A second isolate identified as *Diplodia* sp., which is a weak pathogen, was also able to induce tip dieback in mango, but as mentioned previously, *Diplodia* itself is considered an anamorphic state of *Botryosphaeria* (12).

The organism causing mango tip dieback in southern Florida, *B. ribis*, is apparently different from those reported elsewhere as causing mango dieback and showing related symptomatology. Other causal agents of mango dieback, such as *P. rhodina* (2), *C. fimbriata* (20), *B. theobromae* (29), *Macrophoma* (10), and *H. toruloidea* (19), have been associated with some types of dieback in Puerto Rico, Brazil, India, and Nigeria. From these, *Botryodiplodia*, *Diplodia*, and *Macrophoma* can be considered as part of the anamorphic state complex of *Botryosphaeria* (12).

B. ribis has a very wide host range and has been reported from 34 genera in 20 plant families, but its pathogenicity varies from host to host (22). This fungus is thought to occur only in the Americas, where it causes a dieback in currants (*Ribes* sp.) and dieback and canker diseases on *Cercis*, *Forsythia*, *Rhus*, *Platanus*, and *Tilia*. It affects cypress, *Eucalyptus*, and other trees in Argentina (18) and has been reported in *Poinciana* sp. and *Quercus* sp. in Florida (24).

B. ribis has not been reported on mango in Florida or the continental United States. However, it was reported in this host as early as 1929 in the Hawaiian Islands (28). It has also been reported in avocado (*Persea americana* L.) in Cuba (27). These isolates were also pathogenic on currant. Many of the *Diplodia* stages are widely distributed in the tropics growing as wound parasites or secondary organisms in numerous tropical and subtropical hosts, including *Theobroma cacao* L., *Hevea brasilensis* (Willd. ex Adr. Juss.) Müll. Arg., *Ananas comosus* (L.) Merr., *Carica papaya* L., *Coffea arabica* L., *Cocos nucifera* L., *Musa* × *paradisiaca* L., mango, *Chrysophyllum cainito* L., *Citrus choronia*, and others (2).

The source of infection of mango tip dieback remains unclear, but it is likely that the major source of inoculum is the pycnidiospores from pycnidia on the dead bark of twigs, which may remain on the trees during the growing season; new infection continues to develop as long as the bark remains on diseased twigs. In apple, viable spores of the pathogen *B. ribis* and *Physalospora obtusa* (Schwein.) Cooke were extruded from pycnidia throughout a 6-yr period, and mature pycnidia with viable pycnidiospores were produced within 58 days after the first symptom was noted (6).

The pathogen, *B. ribis*, invades the vascular system of the mango trees. The avenue of penetration and infections of unwounded stems or twigs is unknown.

Stress predisposition of mango trees, attributable to mineral deficienicies such as iron, zinc, and manganese, has been related to the infection causing mango tip dieback in Florida (21). Studies with *B. dothidea* in dogwood (*Cornus stolonifera* Michx.) (23) indicated that water stress predisposes young vascular tissues near the cambium to the attack by nonaggressive pathogens and freezing stress predisposes older wood rings of the stem but not the young vascular system.

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