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Characteristics of the *Fusicoccum* anamorph of *Botryosphaeria ribis*, a potential biological control agent for *Melaleuca quinquenervia* in South Florida[1]

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Abstract: Eight isolates of the *Fusicoccum* anamorph of *Botryosphaeria ribis* (six from *Melaleuca quinquenervia*; two from *Rhizophora mangle*) were obtained from South Florida. Morphological characteristics of these isolates were studied on various culture media and excised leaves and stems of *M. quinquenervia* ramets. Mycelial morphology, sporulation attributes, dimension of conidiomata, and growth rate varied among isolates. Conidiophores were short and hyaline with a bulbous base arising from the lining of the stromatal locules. Fresh macroconidia were fusiform, truncate at the base, obtuse at the apex, hyaline, aseptate, but some of them developed 1–3 septa when retained in pycnidia on aged and dried culture media or germinated in water under coverslips. Regardless of septation, 1–5 germ tubes were produced from the polar as well as the lateral sides of conidia. Microconidia were rarely produced by most isolates. Microconidia did not germinate. The percentage of macroconidia germinating within 4–8 h was greatest at 25–35°C, was rare or absent at 5°C, and viability was lost at 45°C. The teleomorph stage was not observed on any growth media regardless of incubation conditions. These morphological characteristics support the characterization of the isolates as the *Fusicoccum* anamorph of *B. ribis*; also, many characteristics overlap with reported characteristics of the *Fusicoccum* and *Dothiorella* anamorphs of *B. dothidea*.

Key Words: *Botryosphaeria*, conidia, hyphal characteristics, *Melaleuca*, spore germination, taxonomy

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INTRODUCTION

Melaleuca quinquenervia (Cav.) Blake (Myrtaceae; common names: melaleuca, paperbark tree, cajeput tree), an aggressive, invasive exotic tree has become a serious weed problem in the natural ecosystems of South Florida (Crowder, 1974; Balciunas and Center, 1991). Mechanical and chemical methods of control are expensive, inefficient (Balciunas and Center, 1991; Bodle et al., 1994), and may have undesirable environmental consequences. Biological control approaches using insects and fungal pathogens hold promise of insuring ecological compatibility and economic viability (Balciunas and Center, 1991). During 1989–1990, a fungal species tentatively identified as the *Fusicoccum* anamorph of *Botryosphaeria ribis* Gross & Duggar was consistently isolated from the margins of cankers on stems of *M. quinquenervia* in the Loxahatchee National Wildlife Refuge in South Florida. This fungus is being considered as a potential biological control agent for *M. quinquenervia* (Rayachhetry 1995).

Botryosphaeria ribis (order, Pleosporales; family, Botryosphaeraceae) (Luttrell, 1973; Barr, 1987) was originally described from currants (*Ribes* sp.) in New York by Grossenbacher and Duggar (1911). *Botryosphaeria ribis* and *B. dothidea* (Moug.: Fr.) Ces. & de Not. are often treated synonymously as established by von Arx and Müller (1954) during reclassification of amerosporous pyrenomycetes, and later supported by the work of Witcher and Clayton (1963). Since then the names, *B. dothidea* to *B. ribis*, have been synonymously used by many authors (Witcher and Clayton, 1963; English et al., 1975; Spiers, 1977; Brown and Hendrix, 1981; Mass and Uecker, 1984; Pusey, 1989). However, others consider *B. ribis* and *B. dothidea* as two distinct species (Smith, 1934; Punithalingam and Holliday, 1973; Rumbos, 1987; Ramos et al., 1991).

Grossenbacher and Duggar (1911) recognized two anamorphs, a *Macrophoma*-form (pycnidial conidiomata), and a *Dothiorella*-form (stromatic conidiomata) for *B. ribis*. They also reported the occurrence of a strongly parasitic (chromogenic, purplish-pink coloration on starch paste) and a saprophytic (non-chromogenic) strain of *B. ribis*. These strains were confirmed by Shear et al. (1924) who noted that the fungus produced both microconidia and macroconidia. The occurrence of the chromogenic strain of *B.*

ribis was supported by Stevens (1926) while working on the native hosts in Cuba and the southeastern United States. However, Witcher and Clayton (1963) who considered *B. ribis* and *B. dothidea* to be synonymous, have experimentally proven that pathogenicity of *B. dothidea* was not correlated with chromogenesis in culture media.

Morphologically, no clear differences have been established for *B. ribis* and *B. dothidea.* Hyphal color in growth media has been reported to change from white to dark-gray or greenish-black for both species (Weaver, 1974; Mass and Uecker, 1984; Rumbos, 1987). A majority of macroconidia produced in uni- or multilocular pycnidial stroma are hyaline and aseptate, though 1–3 septate brown conidia are also common in both fungi (Spiers, 1977; Mass and Uecker, 1984; Rumbos, 1987). Conidia and ascospores are multinucleate but both spore types are homokaryotic, i.e., genetically similar nuclei (Wolf and Wolf, 1939).

Some authors have considered *B. ribis* to have an anamorph belonging to *Dothiorella* (Wolf and Wolf, 1939; Smith, 1934; Anahosur and Fazalnoor, 1972; Smith and Fergus, 1971), whereas others have considered it to have anamorph in *Fusicoccum* (Punithalingam and Holliday, 1973; Morgan-Jones and White, 1987). Despite the rare production of the teleomorphic stage in cultures, mycologists and plant pathologists have used the teleomorphic name, i.e., *B. ribis,* to avoid the taxonomic uncertainty of the anamorphic stages (Witcher and Clayton, 1963; English et al., 1975; Brown and Hendrix, 1981; Mass and Uecker, 1984; Rumbos, 1987; Ramos et al., 1991).

The literature on the taxonomy of *B. ribis* appears largely unsettled, especially the anamorphic stages, i.e., *Fusicoccum* vs *Dothiorella,* and their morphological characteristics. However, many mycologists and pathologists accept *Fusicoccum* as an anamorph of some species of *Botryosphaeria* Ces.& de Not., such as *B. ribis* and *B. dothidea* (Sutton, 1980; Morgan-Jones and White, 1987; Rumbos, 1987; personal communication with Punithalingam, 1990).

Because of the taxonomic uncertainties associated with the anamorph of *B. ribis,* the objective of this research was to establish morphological and reproductive characteristics of *Fusicoccum* isolates destined for evaluation as biological control agents for *M. quinquenervia* in South Florida.

MATERIALS AND METHODS

Isolates.—During 1989–90, six fungal isolates were obtained from the margins of stem cankers on *M. quinquenervia* trees in the Loxahatchee National Wildlife Refuge in South Florida (Palm Beach County). These isolates (referred to as BR-1 through BR-6) were tentatively identified as *B. ribis* and later confirmed by the International Mycological Institute (IMI), Kew, Surrey, England as the *Fusicoccum* anamorph of *B. ribis* (IMI #338211) though teleomorphic stages were not produced in their subcultures (personal communication with Dr. E. Punithalingam). Two other isolates of *Fusicoccum* anamorph of *B. ribis* (BR-7 and BR-8) were obtained in 1993 from necrotic tissues of the perennial, branch and stem galls of red mangrove (*Rhizophora mangle* L.) trees in coastal areas of South Florida. Monoconidial cultures of these eight isolates were used in this study.

Mycelia.—Four artificial growth media, potato-dextrose-agar (PDA) (Difco), cornmeal-agar (CMA) (Difco), mycological-agar (MA) (Difco), and 24 gm of starch (Difco) supplemented with 15 gm of agar (Difco)/liter (SA), were used to study radial growth rate. For each isolate, four petri plates per medium were inoculated with a 3-mm disk taken from the edge of a 5-day-old colony growing on acidic-PDA (3.3 ml of 50% lactic acid/liter of PDA). The plates were sealed with Parafilm (American National Can™, Greenwich, CT), randomized with respect to location on the laboratory bench and maintained at 30° C ± 1, under a 12-h diurnal cycle of fluorescent light. Radial growth of each colony was measured along four premarked radii at the time when the first colony touched the edge of its plate. The mean colony extension rates per medium, expressed in mm/day (3-day basis for PDA, CMA, and MA, and 6-day basis for SA), were used to compare isolates.

The cultures used for growth-rate assessment were further incubated under the same conditions for 21 days from the date of initiation. These plates were used to study other reproductive and morphological characteristics including sporulation behavior, mycelial color, and hyphal diameters of representative isolates.

Mycelial morphology of isolates grown in potato dextrose broth (PDB) also was studied. A drop of macerated mycelial suspension was inoculated into PDB and incubated 4 days at room temperature with continuous shaking (100 rpm). Macerated hyphae were stained with lactophenol cotton blue for measurement of hyphal diameters. Photomicrographs of hyphae were taken using a light microscope (Olympus/BMH system).

Pycnidia.—Presence or absence of pycnidia in or on the culture media was recorded. The diameters of 30 solitary and botryose (multilocular) pycnidial stroma of BR-4, BR-5, BR-7, and BR-8 were measured. These isolates were chosen because they sporulated on all media, and presented dimensions of pycnidia and

macroconidia similar to those observed in remaining isolates. Pycnidial stroma were fixed in formalin:propionic acid, infiltrated and embedded with paraffin, sectioned (8–10 μm), dewaxed through a ETOH/xylene series, and stained with Pianeze's IIIB (Rayachhetry, 1995). Numbers of locules per stroma were counted and photomicrographs were taken by light microscopy (Nikon/OPTIPHOT II or Olympus/BMH).

Fully expanded leaves of *M. quinquenervia* were wound-inoculated with a hyphal suspension of each of the eight isolates and incubated in a sterile moist-chamber to allow production of pycnidial stroma. Small stems of *M. quinquenervia* ramets were wound-inoculated with isolates of *B. ribis* and maintained in a greenhouse. Some of these inoculated stems produced pycnidia on the bark. Segments of leaves and stems with pycnidial stroma were processed, sectioned and stained by techniques described above. Morphology of pycnidia from leaves and stems were also noted and photographed. Stromatic pycnidia from leaves were also used for the study of macroconidial characteristics.

Conidia.—Macroconidial length, width, and the nuclear condition were studied using 14- to 18-day-old stromatic pycnidia from the *M. quinquenervia* leaves inoculated with each of the eight isolates. Pycnidia were placed on glass slides, in a drop of Haupt's adhesive, crushed with a cover slip, smeared, and allowed to dry for 24 h at room temperature. The macroconidia were stained with Pianeze's IIIB (Vaughan, 1914), and the number of nuclei in 100 macroconidia were recorded for each isolate.

Spore germination tests were performed using slight modifications of the procedures of Brooks and Ferrin (1994). Isolates BR-1 and BR-7 were selected to represent isolates from *M. quinquenervia* and red mangrove, respectively. Both isolates produced superficial pycnidial stroma on PDA and macroconidia could be easily extracted. These isolates were grown on PDA under a 12 h fluorescent light photoperiod at 30(±1)°C for 2 wk. Cultures containing superficial and submerged pycnidia were dried partially under sterile conditions by removing lids from the petri-plates for 5–10 minutes. Masses of macroconidia extruded from pycnidia onto the surface of PDA were washed with sterile distilled water. The resultant suspension was strained through two layers of sterile cheesecloth. One drop of spore suspension was smeared on each of three premarked strips on water agar (WA) in petri plates. The petri plates were then sealed with Parafilm. Seven culture plates of each isolate were incubated at 5, 10, 15, 20, 25, 30, 35, 40, and 45°C. One plate of each isolate per temperature treatment was removed after 1, 2, 4, 8, 12, 16, and 24 h. Removed cultures were stained immediately with cotton blue in lactophenol and 100 macroconidia per strip (total of 300 macroconidia per plate) were counted and the number of germinated macroconidia were recorded for each plate. A spore was considered germinated when the germ tube length was equal or greater than the spore length. Germination of macroconidia for each isolate per temperature was expressed as a percentage. The experiment was repeated twice using a different batch of WA and macroconidia. Photomicrographs of fungal materials were taken by light microscopy (Olympus/BMH or Nikon/OPTIPHOT II).

TABLE I. Radial growth rates (mm/day) of cultures of isolates of the *Fusicoccum* anamorph of *B. ribis* on potato dextrose agar (PDA), cornmeal agar (CMA), mycological agar (MA), and starch agar (SA)

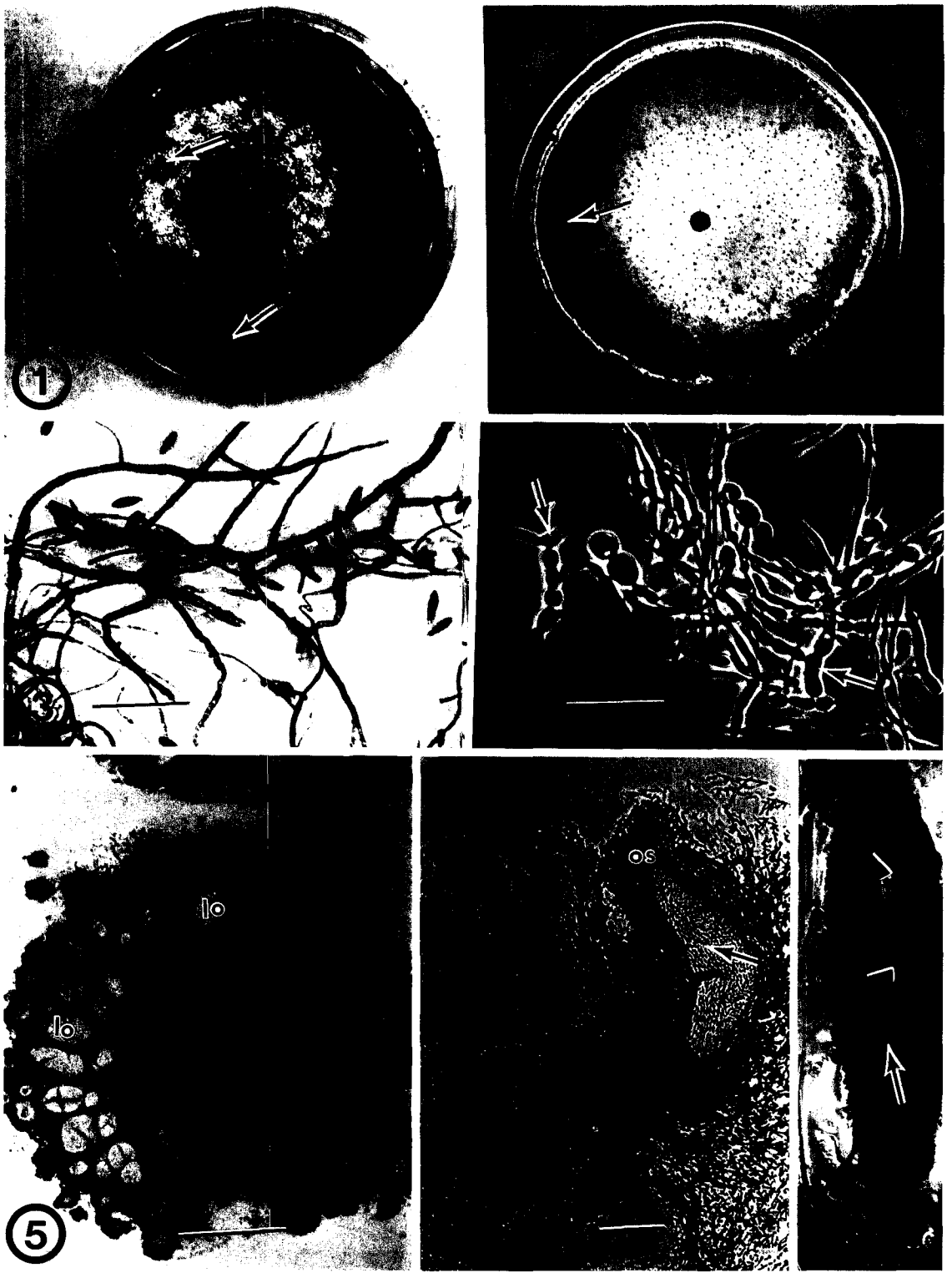
Isolate No.	Growth rates[a] (mm/day) on			
	PDA	CMA	MA	SA
BR-1	10.9A,a	9.2A,b	12.5A,a	6.8A,b
BR-2	13.8A,a	10.1A,b	14.1A,a	6.3A,c
BR-3	13.8A,a	10.4A,b	14.3A,a	4.9A,c
BR-4	12.4A,a	10.1A,b	13.0A,a	4.9A,c
BR-5	10.5A,a	9.7A,ab	10.4B,a	6.8A,b
BR-6	13.3A,a	8.3A,b	14.2A,a	5.4A,b
BR-7	11.3A,a	7.0B,b	11.1A,a	5.7A,b
BR-8	12.7A,a	9.8A,b	14.3A,a	6.0A,c

[a] Since isolate-media interaction was significant (Pr>F = 0.0001), Scheffé's multiple comparison procedure was used to locate significant differences between least square means indexed by both isolate and medium. Means followed by the same letters in upper case (comparing within columns) and lower case (comparing within rows) are not significantly different (minimum significant difference, 3.35 mm at P = 0.05).

Statistics.—The variances and means of main effects were analyzed using GLM procedure in SAS (1985). Correlation of temperature and incubation period with spore germination was tested using Pearson's method.

RESULTS

Mycelia.—The radial growth rates of the *Fusicoccum* isolates in four growth media are presented in TABLE I. In general, PDA and MA supported the most rapid growth while the slowest growth occurred on SA for most isolates. The growth rate of some isolates on a particular medium was also significantly different. Some of the isolates appeared to impart color on SA, PDA, and MA during incubation. However, light microscopic observations revealed that the colors were



FIGS. 1–7. Mycelial and pycnidial characteristics of the *Fusicoccum* anamorph of *B. ribis* on PDA, CMA, PDB, and a *M. quinquenervia* leaf. 1. Aerial hyphae imparting black color and partially submerged pycnidia (arrows) on PDA. 2. Hyphae in and on the surface of media imparting tan color and pycnidia (arrow) immersed in CMA. 3. Mycelial mat on PDA with hyphae of various diameters, bar = 50 μm. 4. Blended mycelial mat grown in PDB under continuous shaking (100 rpm). Note hyphae containing chains of chlamydospore-like cells (arrows); bar = 50 μm. 5. Cross-section through a multilocular pycnidial stroma produced on PDA. Note numerous locules (lo), bar = 500 μm. 6. Enlarged pycnidia from a portion of multilocular stroma. Note macroconidia (arrow) and an obscure ostiole (os), bar = 100 μm. 7. Pycnidial stroma on inoculated leaf, maintained in a sterile moist chamber. Note solitary (arrows) and botryose (arrow heads) pycnidia.

TABLE II. Number of pycnidial stroma per plate produced by isolates of the *Fusicoccum* anamorph of *B. ribis* on four artificial growth media within 21 days after inoculation

Growth media[a]	Isolate							
	BR-1	BR-2	BR-3	BR-4	BR-5	BR-6	BR-7	BR-8
PDA	>100	1–25	1–25	>100	50–99	N[b]	>100	>100
CMA	>100	1–25	50–99	>100	1–25	N	>100	50–99
MA	1–25	1–25	50–99	>100	26–49	1–25	50–99	50–99
SA	50–99	1–25	26–49	>100	1–25	26–49	>100	>100

[a] PDA, Potato-dextrose-agar; CMA, Cornmeal agar; MA, Mycological agar; SA, Starch agar.
[b] N = pycnidial stroma were not measured.

due to a dense network of hyphae submerged in the growth medium. Mycelial color in the center of the colony of all isolates except BR-6 changed from white to tan as early as 48 h on PDA and MA. The mycelial color of all isolates changed from white to light yellowish-green on SA within 72 h. A pigment similar to that of the mycelial color was also secreted in SA surrounding the submerged mycelial network. The mycelia of all isolates were colorless in CMA except BR-7 which turned light yellowish-green within 72 h.

Texture of cultures ranged from cottony on PDA to floccose on MA. Aerial hyphae (FIGS. 1, 2) at 21 days after incubation were relatively longer in PDA and MA than in CMA and SA. However, isolates BR-7 and BR-8 did not produce any aerial hyphae in CMA and SA. Hyphal diameter of a 21-day-old culture was 4.2 (1.8–10.2), 5.0 (1.2–10.2), 5.3 (2.8–10.2), and 5.3 (2.6–12.8) μm in CMA, MA, PDA, and SA, respectively. Short-celled inflated hyphae (FIG. 3) were abundant in all four media. Compared to CMA, MA, and PDA, SA contained hyphae with chains of chlamydospore-like cells in the intercalary positions. The majority of chlamydospore-like cells in these hyphae were guttulated. Mycelia in MA were highly melanized compared with the mycelia on the remaining three media. Most of 4-day-old hyphae grown in potato-dextrose-broth under continuous shaking consisted of chains of chlamydospore-like cells (FIG. 4) up to 18.6 μm in diameter.

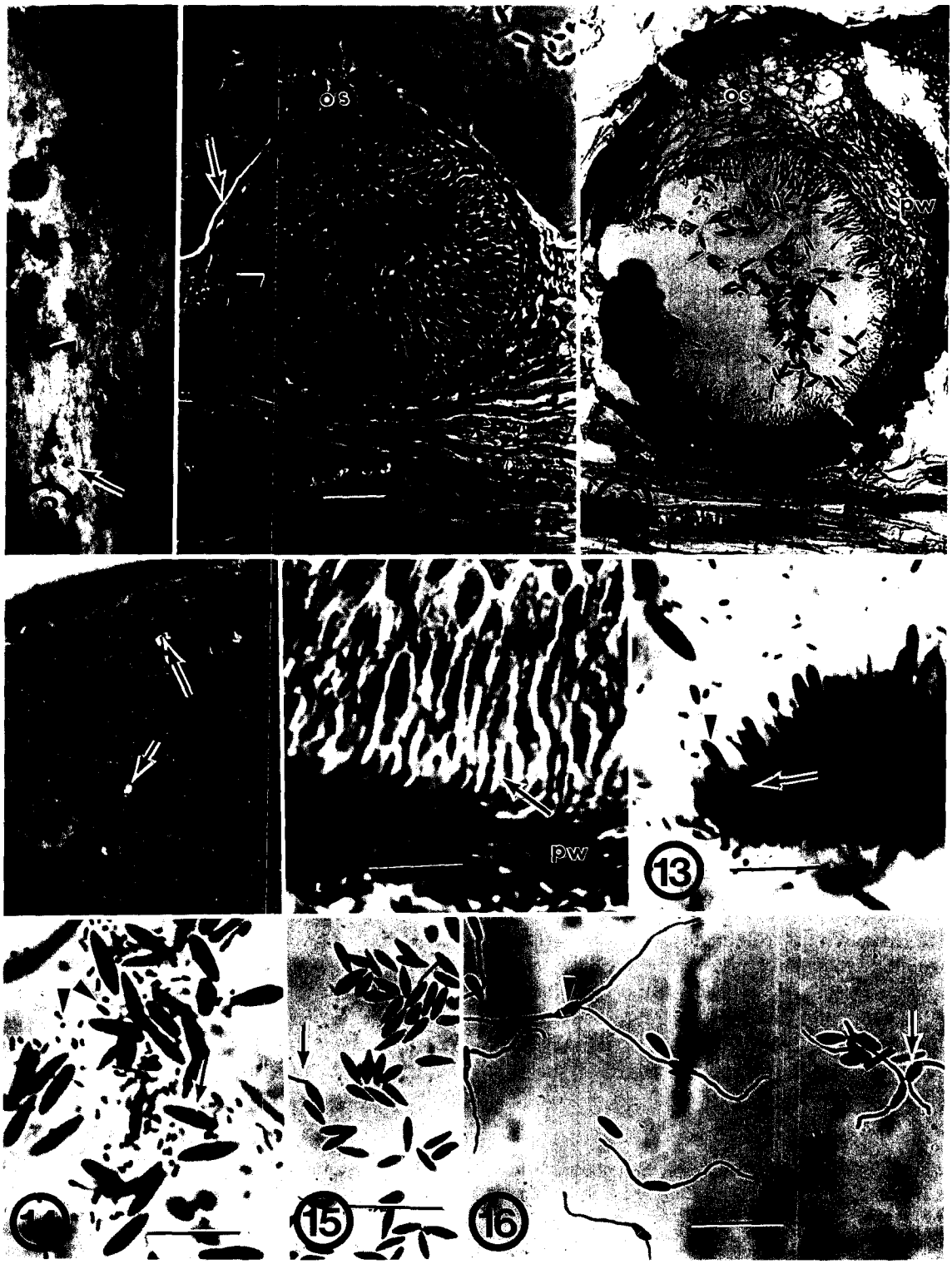
Pycnidia.—In general, production of pycnidial stroma was greatest on PDA followed by SA, CMA, and MA (TABLE II). The best and the least sporulation on all four media was achieved in isolates BR-4 and BR-6, respectively (TABLE II). In SA and CMA, the pycnidial stroma were scattered and submerged, whereas in PDA and MA, both superficial and submerged pycnidia were produced (FIGS. 1, 2). In general, the range and average diameters of pycnidial stroma varied among growth media (TABLE III). Superficial pycnidial stroma were larger in diameter than submerged pycnidia and were often covered with appendage-like hyphae. Most submerged pycnidia were solitary and unilocular, whereas most of the superficial pycnidia were multilocular (botryose), containing as many as 31 locules in BR-8 (FIGS. 5, 6). The diameter of pycnidial cavities was 124 (57–200) μm. Across isolates, the majority of 21-day-old pycnidial stroma in PDA, CMA, and SA contained conidia while in MA a majority of stroma were pseudopycnidial.

Pycnidial stroma in and on *M. quinquenervia* leaves were produced within 3–14 days after inoculation. Both solitary and botryose pycnidia were observed on host tissues (FIGS. 7–10). Pycnidial stroma on both leaves and barks were either superficial or erumpent through the epidermis (FIGS. 9, 10). A majority of superficial pycnidia were covered with short erect hyphae; these erect hyphae were lacking in the erum-

TABLE III. Average diameter and range (parenthesis) of the pycnidial stroma on 2- to 4-week-old cultures of some isolates of the *Fusicoccum* anamorph of *B. ribis* on four artificial growth media

Isolate no.	Average diameter (mm)			
	PDA[a]	CMA[a]	SA[a]	MA[a]
BR-4	0.5 (0.1–1.0)	0.2 (0.1–0.5)	0.5 (0.3–1.2)	N[b]
BR-5	0.6 (0.1–1.3)	0.4 (0.1–1.0)	N	0.4 (0.1–1.0)
BR-7	0.5 (0.1–1.2)	0.2 (0.1–0.5)	0.2 (0.1–0.5)	N
BR-8	2.3 (0.1–3.8)	N	N	N

[a] PDA, Potato-dextrose-agar; CMA, Cornmeal agar; MA, Mycological agar; SA, Starch agar.
[b] N = pycnidial stroma were not measured.



FIGS. 8–16. Pycnidia and macroconidia of the *Fusicoccum* anamorph of *B. ribis* produced on artificial growth media and *M. quinquenervia* leaves and stems. 8. Pycnidial stroma erupting from dead bark. Note solitary (arrow) and botryose (arrow heads) pycnidia. 9. Section through a leaf showing a solitary pycnidium erupting through the epidermis. Note leaf cuticle (arrow), ostiole (os), and pycnidial wall (arrow head), bar = 50 μm. 10. Longitudinal section through a stem showing a solitary pycnidium erupting through the bark. Note an obscure ostiole (os), and pycnidial wall (pw) composed of textura angularis, bar = 50 μm. 11. Pycnidial stroma on a leaf oozing macroconidial cirrhi (arrows) on drying at room temperature. 12. A cross-section through a pycnidium showing pycnidial wall (pw) composed of textura angularis, and conidiophores

TABLE IV. Mean and range of dimensions (length and width) and the number of nuclei in macroconidia of isolates of the *Fusicoccum* anamorph of *B. ribis* in 14- to 18-day-old stromatic pycnidia on *M. quinquenervia* leaves[a]

	Dimensions (μm)					
	Average		Ranges		Number of nuclei	
Isolate	Length	Width	Length	Width	Average	Range
BR-1	16.8c	6.2	5.7–23.0	4.1–8.9	6.0	1.0–12.0
BR-2	16.6c	6.2	8.9–23.0	4.3–8.9	5.0	1.0–11.0
BR-3	18.4a	6.2	14.0–23.2	3.3–8.9	5.0	1.0–9.0
BR-4	17.9ab	6.1	8.9–26.8	4.1–7.9	5.0	1.0–11.0
BR-5	17.3bc	6.1	11.5–23.0	4.1–8.4	5.0	1.0–11.0
BR-6	18.0ab	6.2	7.7–23.0	3.1–8.9	4.0	1.0–8.0
BR-7	16.7c	5.3	10.2–20.4	3.8–6.4	3.0	1.0–8.0
BR-8	16.8c	5.5	9.7–21.0	3.9–6.5	4.0	1.0–7.0

[a] For each isolate, N = 100. Means with same letter(s) are not significantly different from each other at P = 0.05, according to Duncan's Multiple Range Test.

pent types. The solitary pycnidia were globose; the botryose and eustromatic ones were lobulate and often had flat tops. All types of pycnidia were ostiolate (FIG. 9); the ostioles were often obscure (FIGS. 6, 10). Superficial pycnidia produced macroconidial cirrhi (FIG. 11) following partial drying for a few minutes. The pycnidial wall consisted of heavily melanized cells of irregular textura-angularis becoming gradually loose and hyaline towards the inner lining of the cavity that produced conidiophores (FIG. 12). Conidiophores were short and unbranched with bulbous bases produced from the lining of the pycnidial cavities (FIG. 13).

Conidia.—Freshly discharged macroconidia in 3- to 7-day-old pycnidia of all isolates were fusoid, hyaline, and aseptate with truncate bases (FIG. 14). Some macroconidia from more than 2-week-old pycnidia contained 1–4 septa (FIG. 15). Development of septa or bluish-brown pigmentation or both was observed in some macroconidia retained in cavities of old pycnidia produced on growth media. Macroconidia extruded in the form of cirrhi remained hyaline and aseptate even after 12 weeks of drying at room temperature. Mean lengths of macroconidia were significantly different among isolates (Pr > F = 0.0001). The dimensions of macroconidia are presented in TABLE IV. When present, the microconidia (FIG. 14) were allantoid to shortly elongated, hyaline, and unicellular with a length and diameter of 3.7 (2.7–5.1) and 1.6 (1.3–2.5) μm, respectively.

Most of the macroconidia germinating on WA were aseptate; a few contained 1–3 septa. However, when aliquots of macroconidia from the source were placed in water under a coverslip, a majority became septate before germination (FIGS. 15, 16). Regardless of the numbers of septa, 1–4 germ tubes (majority with 2 germ tubes, 1 on each end) were produced by macroconidia maintained at 30 C for 16 h (FIGS. 15, 16). On WA, germination of macroconidia began as early as 1 h after incubation at 30–35°C (FIGS. 17, 18). After 24 h at 20–40 C, spore germination was 93–100% and 83–98%, in BR-1 and BR-7, respectively. For both isolates, the correlation (126 observations) between germination and temperature (Pr > R = 0.088) was weak and that between germination and incubation time (Pr > R = 0.0001) was strong. At a temperature range of 20–35 C, a majority of macroconidia in BR-1 and BR-7 germinated within 4 h of incubation (FIGS. 17, 18). In both isolates, spore germination was least or none at 5 and 45 C. At 45 C, the cell wall of about 5% macroconidia in both isolates lysed within 16 h of incubation. Microconidia incubated at 30 C for 16 h did not germinate either in water under a coverslip or on WA at 100 humidity.

←

(arrows), bar = 25 μm. 13. A portion of the inner wall of pycnidium squashed from a 3-day-old pycnidium. Note conidiophores with bulbous bases (arrow) bearing macroconidia (arrow head) at different stages of development, bar = 25 μm. 14. Macro- (arrow) and microconidia (arrow heads) from freshly squashed 7-day-old pycnidium. Note the microcondia are allantoid-slightly elongated and the macroconidia with truncate base and obtuse apices, bar = 25 μm. 15. Macroconidia (from 3-day-old pycnidium) fro-PDA, germinated in water. Note a mixture of aseptate and septate conidia some bearing germ tubes (arrows), bar = 50 μm. 16. Germinating aseptate (arrow) and septate (arrow head) macroconidia showing one or more germ tubes, bar = 50 μm.

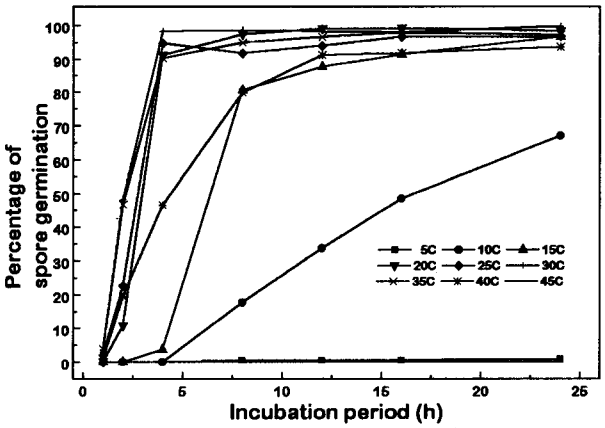


FIG. 17. Germination of the macroconidia of isolate BR-1 of the *Fusicoccum* anamorph of *B. ribis* on water agar (WA) at 100 percent ambient humidity.

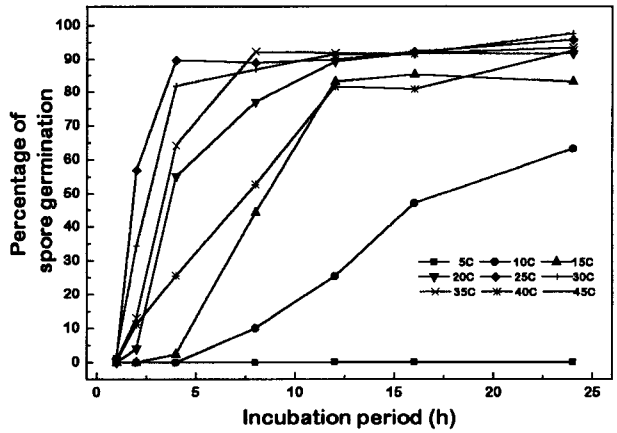


FIG. 18. Germination of the macroconidia of isolate BR-7 of the *Fusicoccum* anamorph of *B. ribis* on water agar (WA) at 100 percent ambient humidity.

DISCUSSION

Morphology of vegetative and reproductive structures observed in our studies and the published information reveal the controversy regarding anamorph-teleomorph connection between *Fusicoccum* and *B. ribis*. The controversy arises from 1) variable mycelial and conidial morphology in different growth conditions and stages, 2) rareness of sexual stages, 3) pantropic distribution, and 4) ability to cause diseases on many plant species.

Our observations regarding culture characteristics such as growth pattern, color change over time, and hyphal dimensions on PDA agree with the mycelial characteristics described for the *Fusicoccum* anamorph of *B. ribis* (Morgan-Jones and White, 1987). Also, morphology and dimensions of pycnidia, conidiophores, and fresh macroconidia from our studies agree with Wolf and Wolf (1939), Punithalingam and Holliday (1973), and Sivanesan's (1984) description for the *Fusicoccum* anamorph of *B. ribis*. The dimension of immersed and submersed pycnidia on PDA, CMA, SA, and MA were consistantly smaller than the superficial pycnidia on respective medium. Also, pycnidia on PDA were relatively larger than on CMA, MA, and SA. Based on these observations and the information from the literature (Grossenbacher and Duggar, 1911; Shear et al., 1924; Morgan-Jones and White, 1987), we suggest that the pycnidial dimensions on artificial media are influenced by the nutritional regime supporting colony development.

The isolates used in our study did not produce the teleomorphic stage in any of the tested incubation conditions. Mass and Uecker (1984) had a similar experience with the isolates of the anamorph of *B. dothidea* causing stem canker on thornless blackberry (*Rubus* spp.). According to the descriptions given by Punithalingam and Holliday (1973), the ascal and ascospore dimensions of *B. ribis* associated with *Fusicoccum* anamorph are 100–110 × 16–20 and 17–23 × 7–10 μm, respectively. The measurements given for asci and ascospores of *B. dothidea* having *Fusicoccum* anamorph are 65–140 × 16–21 and 13–35 × 6–14 μm, respectively (Pennycook and Samuels, 1985). These measurements show that the ascal and ascospore dimensions of *B. ribis* fall within the range of the dimensions of these structures in *B. dothidea.*

Though not abundant in most isolates, microconidia were produced by all isolates we studied. Our measurements of microconidia were larger than those reported by Wolf and Wolf (1939), Punithalingam and Holliday (1973), Sutton (1980), Pennycook and Samuels (1985), and Sivanesan (1984), but concur to those described by Morgan-Jones and White (1987). Based on the presence of macro- and microconidia, Morgan-Jones and White (1987) considered their isolates obtained from leaves of *Smilax* to be the *Fusicoccum* anamorph of *B. ribis.*

The separation of the anamorphs *Fusicoccum* and *Dothiorella* seems to be based on the form of conidiomata, the mode of conidiogenesis, and the morphological attributes of conidia. The criteria of enteroblastic phialidic conidiogenesis producing hyaline to light brown aseptate conidia for *Fusicoccum,* and holoblastic conidiogenesis yielding hyaline to brown and aseptate to biseptate conidia for *Dothiorella,* has been used (Barr, 1987) to differentiate these two form-genera. Sutton (1980) examined the illustrations of the type specimen of Saccardo (1886) and found holoblastic conidiogenesis for *F. aesculi.* Though Pennycook and Samuels (1985) agree with Sutton (1980), they noted that Saccardo's specimen was immature. It had been demonstrated that the

older conidiogenous cells in *Fusicoccum* are enteroblastic phialidic forming 2–3 irregularly shaped annelations (Mass and Uecker, 1984; Pennycook and Samuels, 1985).

Growth and morphology of mycelia, pycnidia, and conidia of the examined isolates of the *Fusicoccum* anamorph of *B. ribis* exhibited considerable variations. The upper range of the dimensions (TABLE IV), color, and aseptate conditions of freshly discharged macroconidia in our studies agree with the range of dimensions, color, and aseptate conditions of macroconidia reported by Weaver (1974) for an anamorph of *B. dothidea.* In our study, septation of macroconidia germinated under water and those retained in pycnidia on aged or dried culture were similar to the ones described by Mass and Uecker (1984) for isolates of the anamorph of *B. dothidea* from the cankers on thornless blackberry. Septate and pigmented conidia have been reported for the anamorphs of *B. ribis* (Wiehe, 1952; Taylor, 1958; Rumbos, 1987) and *B. dothidea* (English et al., 1975; Spiers, 1977). However, Pennycook and Samuel (1985) noted that the conidia of the *Fusicoccum* anamorph of *B. dothidea* do not become light brown or septate with age or at the time of germination. The septation of some of the older and/or germinating conidia in our studies do not agree with the description of the macroconidia of *Fusicoccum*; reportedly, they are not septate (Sutton, 1980). The uni- as well as bi-polar and sometimes lateral germ tube production by germinating macroconidia matches the description of English et al. (1975) for anamorph of *B. dothidea.* However, the majority of macroconidia that germinated on water agar at 100% ambient humidity were aseptate. These morphological variations at different environmental conditions and developmental stage may have root in their adaptive ability to a given host or microenvironment.

The macroconidia of isolates BR-1 and BR-7 germinated at 5–40 C, with an optimum range of 25–35 C. Similar temperature optima for germination of macroconidia had been reported for an anamorph of *B. dothidea* from California (Brooks and Ferrin, 1994). However, at 25–35 C, the isolates BR-1 and BR-7 needed 4 h and 8 h, respectively, to achieve 90% germination of macroconidia; the two isolates had a similar threshold for survival at high temperature (45 C).

It appears that the reported morphological characteristics of the anamorphs of *B. ribis* and *B. dothidea* overlap and become difficult to separate from one another. For example, Michailides (1991) isolated a pycnidial fungus from tissues associated with panicle and shoot blight of pistachio from California and identified it as *B. dothidea,* but he reported that the morphology of isolates fit the descriptions for the anamorph of *B. ribis.* As discussed by Morgan-Jones and White (1987), small variations in conidial dimensions of *Fusicoccum* occurs in different circumstances.

Published information and our observations of cultural, hyphal, pycnidial, and conidial characteristics indicate that the separation of the genera *Dothiorella* and *Fusicoccum* may have been based on their morphological characters observed at a certain stage of development at a given microenvironment. If these anamorphs were studied side by side at the same environment and followed through different developmental stages, one would have had difficulty in separating these genera. This opinion is supported by other authors who have discussed the anamorph-teleomorph connections of the two genera (Pennycook and Samuel, 1985; Morgan-Jones and White, 1987; Michailides, 1991). Therefore, we suggest that the taxonomic relationship of the anamorphs *Dothiorella* and *Fusicoccum* and the teleomorphs *B. ribis* and *B. dothidea* needs revision. Until this is accomplished, we consider the isolates characterized in this work as *Fusicoccum* anamorph of *B. ribis.*

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LITERATURE CITED

Anahosur, K. H. and K. Fazalnoor. 1972. Nuclear condition in the ascospores and conidia of *Botryosphaeria ribis* from India. *Caryologia* 25:327–333.

Balciunas, J. K. and T. D. Center. 1991. Biological control of *Melaleuca quinquenervia*: prospects and conflicts. Pp. 1–22. *In*: *Proceedings of the symposium on exotic pest plants.* Eds., T. D. Center, R. F. Doren, R. F. Hofstetter, R. L. Myers, and L. D. Whiteaker. Nov. 2–4, 1988, University of Miami. U.S. Dept. of Interior, National Park Service, Washington DC.

Barr, M. E. 1987. *Prodromus to class Loculoascomycetes.* Hamilton I. Newell Inc., Amherst, Massachusetts. 168 pp.

Bodle, M. J., A. P. Ferriter, and D. D. Thayer. 1994. The biology, distribution, and ecological consequences of *Melaleuca quinquenervia* in the Everglades. Pp. 341–355. *In*: *The Everglades, the ecosystem and its restoration.* Eds., S. M. Davis and J. C. Ogden. St. Lucie Press, Delray Beach, Florida.

Brooks, F. E. and D. M. Ferrin. 1994. Branch dieback of southern California chaparral vegetation caused by *Botryosphaeria dothidea. Phytopathology* 84:78–83.

Brown, E. A. and F. F. Hendrix. 1981. Pathogenicity and

histopathology of *Botryosphaeria dothidea* on apple stems. *Phytopathology* 71:375–379.

Crowder, J. P. 1974. Exotic pest plants of South Florida. *A study appendix to the South Florida ecological study.* Bureau of Sport Fisheries and Wildlife. U.S. Dept. of Interior, Washington DC. 49 pp.

English, H., J. R. Davis, and J. E. DeVay. 1975. Relationship of *Botryosphaeria dothidea* and *Hendersonula toruloidea* to a canker disease of almond. *Phytopathology* 65:114–122.

Grossenbacher, J. G. and B. M. Duggar. 1911. A contribution to the life history, parasitism, and biology of *Botryosphaeria ribis. New York Agric. Exp. Stn. Geneva Tech. Bull.* 18:114–188.

Luttrell, E. S. 1973. Loculoascomycetes. Pp.135–219. *In: The fungi.* Vol. IVA. Eds., G. C. Ainsworth, F. K. Sparrow, and A. S. Sussman. Academic Press, New York.

Mass, J. L. and F. A. Uecker. 1984. *Botryosphaeria dothidea* cane canker of thornless blackberry. *Plant Dis.* 68:720–726.

Michailides, T. J. 1991. Pathogenicity, distribution, sources of inoculum, and infection courts of *Botryosphaeria dothidea* on pistachio. *Phytopathology* 81:566–573.

Morgan-Jones, G. and J. F. White Jr. 1987. Notes on Coelomycetes. II. Concerning the *Fusicoccum* anamorph of *Botryosphaeria ribis. Mycotaxon* 30:117–125.

Pennycook, S. R. and G. J. Samuels. 1985. *Botryosphaeria* and Fusicoccum species associated with ripe fruit rot of *Actinidia deliciosa* (Kiwifruit) in New Zealand. *Mycotaxon* 24:445–458.

Punithalingam, E., and P. Holliday. 1973. *Botryosphaeria ribis. Description of Pathogenic Fungi and Bacteria, No. 395.* Commonw. Mycol. Inst. and Assoc. Appl. Biol., Kew, Surrey, England. 2 pp.

Pusey, P. L. 1989. Availability and dispersal of ascospores and conidia of *Botryosphaeria* in peach orchards. *Phytopathology* 79:635–639.

Ramos, L. J., S. P. Lara, R. T. McMillan, Jr., and K. R. Narayanan. 1991. Tip dieback of mango (*Mangifera indica*) caused by *Botryosphaeria ribis. Plant Dis.* 75:315–318.

Rayachhetry, M. B. 1995. Evaluating the pathogenicity of *Botryosphaeria ribis* Gross. & Duggar on *Melaleuca quinquenervia* (Cav.) Blake in South Florida. Ph.D. Dissertation, Univ. of Florida, Gainesville, Florida. 152 pp.

Rumbos, C. 1987. Twig and branch dieback of walnut trees induced by *Botryosphaeria ribis. Pl. Pathol.* 36:602–605.

SAS Institute Inc. 1985. *SAS User's Guide: Statistics.* Ver. 5 ed. SAS Inst. Inc., Cary NC, 584 pp.

Saccardo, P. A. 1886. *Fungi Italici Sphaeropsideae no.* 1459.

Shear, C. L., N. E. Stevens, and M. S. Wilcox. 1924. *Botryosphaeria* and *Physalospora* on currant and apple. *J. Agric. Res.* 28:589–598.

Sivanesan, A. 1984. *The bitunicate Ascomycetes and their anamorphs.* J. Cramer, In der A.R. Gantner Verlag Kommanditgesellschaft, Vaduz. 701 pp.

Smith, O. C. 1934. Inoculations showing the wide host range of *Botryosphaeria ribis. J. Agric. Res.* 49:467–476.

Smith, D. H. and C. L. Fergus. 1971. Effect of light intensity on sporulation of *Botryosphaeria ribis. Mycopathol. Mycol. Appl.* 45:311–315.

Spiers, A. G. 1977. *Botryosphaeria dothidea* infection of *Salix* species in New Zealand. *Pl. Dis. Rep*orter 61:664–667.

Stevens, N. E. 1926. Occurrence of the currant cane blight fungus on numerous hosts in the southern states. *Mycologia* 18:278–282.

Sutton, B. C. 1980. *Coelomycetes.* Commonwealth Mycological Institute, Surrey. 696 pp.

Taylor, J. 1958. Stem canker and related blueberry diseases. *North Carolina Agric. Exp. Sta. Techn. Publ. 132.* 24 pp.

Vaughan, R. E. 1914. A method for the differential staining of fungous and host cells. *Annals of the Missouri Botanical Garden* 1:241–242.

von Arx, A. J. and E. Müller. 1954. Die Gattungen der amerosporen Pyrenomyceten. *Beitr. Kryptogamenfl. Schweiz* 11:1–434.

Weaver, D. J. 1974. A gummosis disease of peach trees caused by *Botryosphaeria dothidea. Phytopathology* 64: 1429–1432.

Wiehe, P. O. 1952. Life cycle of *Botryosphaeria ribis* on *Aleurites montana. Phytopathology* 42:521–525.

Witcher, W. and C. N. Clayton. 1963. Blueberry stem blight caused by *Botryosphaeria dothidea* (*B. ribis*). *Phytopathology* 53:705–712.

Wolf, F. T. and F. A. Wolf. 1939. A study of *Botryosphaeria ribis* on willow. *Mycologia* 31:217–227.