Mycologia, 89(2), 1997, pp. 298–303.

Botryosphaeria mamane sp. nov. associated with witches'-brooms on the endemic forest tree *Sophora chrysophylla* in Hawaii

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Abstract: *Botryosphaeria mamane* sp. nov. occurs on the leguminous forest species *Sophora chrysophylla* in Hawaii. Inoculation did not demonstrate a causal relationship, but the fungus is consistently associated with branch contortions, swellings, witches'-brooms, and eventual death of tissue. Similar to *B. ribis,* the prominently stromatic fungus is characterized by a *Fusicoccum* anamorph. Microconidia are also produced. The fungus was readily cultured vegetatively on potato dextrose agar, but produced only microconidiomata abundantly. The new species is distinguished from *B. ribis* morphologically in having larger macroconidia, asci and ascospores, but also by its association with a symptom unusual for *Botryosphaeria* and its apparent limitation to an endemic host in Hawaii, suggesting that the fungus itself may be endemic.

Key Words: *Fusicoccum,* Hawaiian fungi, mamane, native species, plant disease, systematics

INTRODUCTION

A new species of *Botryosphaeria* Ces. & de Not. has been found in Hawaii consistently associated with branch and twig infections on the endemic forest legume *Sophora chrysophylla* (Salisb.) Seem. (Fabaceae). Symptoms are produced on trees ranging in age from young and apparently vigorous to mature. *Sophora chrysophylla* forms a dominant element of subalpine vegetation in dry to mesic shrub and forest land on most of the major islands and provides critical habitat for the endemic palila (*Loxioides bailleui* Oustalet), an endangered bird of the honeycreeper group. The most prominent symptom is the formation of witches'-brooms composed of abnormally thickened, roughened, upright twigs with shortened internodes and unsuppressed lateral buds (FIGS. 1, 2). The brooms arise from contorted, spindle-shaped, or sometimes gall-like stem thickenings. Numerous black, well-developed stromata (FIG. 3), from which cirrhi of macroconidia are exuded, consistently appear erupting through split bark of dead brooms and branch swellings. The disease appears as one to several isolated brooms among otherwise normal growth, or, more rarely, trees may be predominantly broomed, with little or no normal growth visible. Affected tissue dies prematurely, leaving trees with dead broomed branches. Heavily broomed trees may be completely killed. Infected trees occur on ranchland adjacent to Hawaii Volcanoes National Park on the slopes of Mauna Loa (1220–1525 m elevation) and have been also observed within the park itself at corresponding elevations. The disease is also found in an open, dry forest on upper slopes of the northwestern portion of the island of Hawaii.

Accepted for publication November 12, 1996.

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MATERIALS AND METHODS

The size range of macroconidia was obtained by establishing the mean length and width of 100+ apparently mature, randomly selected conidia from three sources. Microconidia (25), asci (20) and expelled ascospores (20) were similarly measured. Germinability of freshly collected macroconidia and microconidia from brooms was tested by spreading them on water agar and observing germ tube development. Single-conidial isolates from germinating macroconidia were also cultured on Difco potato dextrose agar (PDA) at 22–24°C and exposed to indirect sunlight or fluorescent room lighting 12 h/d. Attempts at wound inoculation were made using both apparently healthy saplings 30–50-cm-tall transplanted to pots from the field and maintained in the greenhouse, and normal branches of trees in the field which contained brooms on other branches. Viable macroconidia extracted directly from field-collected brooms were suspended in water (approximately 1×10^3 conidia/mL). Drops of conidial suspension (approximately 0.1 mL), or mycelial fragments taken directly from PDA culture, were placed under bark flaps or into incisions made in stems and lateral buds. The wounds were wrapped with plastic tape following inoculation to prevent drying. Field inoculations were examined at 2-, 4-, 6-wk and 6-mo intervals for evidence of infection.

Fungal material was prepared for examination by crushing or sectioning stromata (at 10–40 μm) with a freezing microtome. To ascertain internal association of the fungus with broom tissue, freezing microtome sections of presporulating, newly developing broom tissue from naturally infected trees were cut. Sections were examined for autofluorescing hyphae using excitation at 400–440 nm emission wavelengths, with a Zeiss microscope equipped for epifluorescence with a 100 W DC mercury illuminator and a Zeiss 487702 filter combination. To recover the fungus from internal broom tissue, sections were cut from presporulating brooms with a sterile scalpel, plated on PDA, and maintained at room temperature (22–24°C) under fluorescent room lighting and indirect sunlight.

RESULTS

Botryosphaeria mamane Gardner, sp. nov. FIGS. 1–9

Stromata per texturam hospitis erumpentia, nigra, 0.5–1.25 mm diam, multiloculata. Loculi sphaerici ad ovatos, ostiolati, 100–200 μm diam. Ascomata, macroconidiomata et microconidiomata distincta sed saepe consociata in eodem stromate. Ascomata collo brevi, per canalem ostiolarem et non periphysatum aperientia. Asci bitunicati, clavati, 8-spori, 100–180 × 25–35 μm, cum pseudoparaphysibus filamentosis. Ascosporae aseptatae, hyalinae, contentis reticulate granulosis, ovales ad late fusiformes, 25–39 × 15–20 μm. Cellulae macroconidiogenae holoblasticae, simplices, parietem loculi uniformiter tegentia. Macroconidia primum per holoblastos, deinde per enteroblastos facta. Macroconidia hyalina, unicellularia, fusiformia, basi truncato ubi nuper formata, (19–)30–44(–55) × (7–)8–9(–10) μm. Microconidia hyalina, bacilliformia ad allantoidea, 3–9(–12) × 2–4 μm. Anamorph. *Fusicoccum* sp.

HOLOTYPUS. BISH 644614

Stromata erumpent through host tissue, black, 0.5–1.25 mm diam, multiloculate; locules spherical to ovoid, ostiolate, 100–200 μm diam. Ascomata, macro- and microconidiomata distinct but often formed in the same stroma. Ascomata with a short neck, opening through a nonperiphysate ostiolar canal. Asci bitunicate, clavate, 8-spored, 100–180 × 25–35 μm, associated with filamentous pseudoparaphyses. Ascospores aseptate, hyaline, with granular or reticulately textured contents, oval to broadly fusiform, 25–39 × 15–20 μm. Macroconidiogenous cells simple, uniformly lining locule wall. Macroconidia at first produced holoblastically, later enteroblastically. Macroconidia hyaline, 1-celled, fusiform, with truncate base when newly formed, (19–)30–44(–55) × (7–)8–9(–10) μm. Microconidia hyaline, rod-like to allantoid, 3–9(–12) × 2–4 μm.

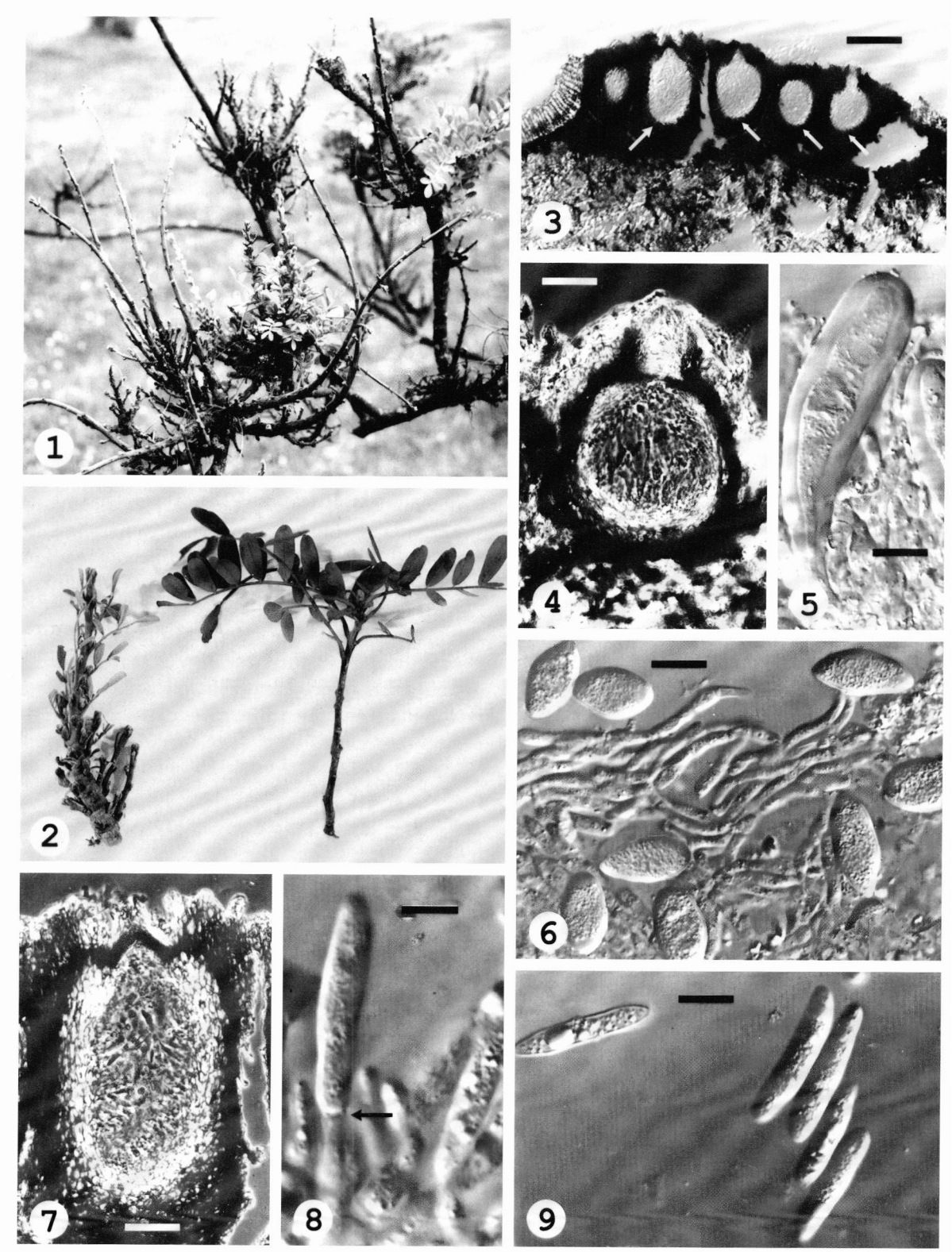
HOLOTYPE. HAWAII. HAWAII ISLAND: Hawaii Volcanoes National Park, Kipuka Ki, on bark of a swollen branch of *Sophora chrysophylla*; 1 May 1996, *D. E. Gardner* (BISH 644614, ISOTYPE: BPI 737731).

Paratypes. HAWAII. HAWAII ISLAND: Kapapala Ranch, on bark of swollen branches and twigs from witches'-brooms on *S. chrysophylla;* 1 Apr. 1996, *D. E. Gardner* (BPI 737732, BPI 737733).

Etymology. "Mamane" is the common Hawaiian name for *S. chrysophylla.*

Pathology.—Sporulation of *B. mamane* occurred on recently dead tissue of witches'-brooms. Many of the brooms on approximately 30 infected trees examined were composed of living tissue and were therefore too young for sporulation to occur. On the other hand, spores were no longer present in stromata of older, long-dead brooms. No evidence was found that seasonality influenced the formation of brooms, or the production of macroconidiomata, microconidiomata, or ascomata. Macroconidiomata were found most often in sporulating tissue, but associated microconidiomata and ascomata also were frequently observed. The ostiolar neck of the ascoma was not periphysate, but was formed of thin-walled, nonpigmented cells giving a striated appearance resembling that described for *B. xanthocephala* (H. & P. Sydow & Butler) Theissen, a species also with a *Fusicoccum* anamorph (Samuels and Singh, 1986). Microconidia were formed in the same locule as macroconidia, as well as their production in microconidiomata. Predominance of one spore form over another may have been correlated with stage of disease development, but no attempt to establish such a correlation was made in this study. Ascospores and macroconidia germinated but microconidia did not. Germination rate for ascospores was not quantified, but was approximately 70% for macroconidia. Cultures from macroconidia produced colonies of rapidly growing medium to dark gray mycelium, becoming darker with age on PDA. Individual hyphae were darkly pigmented and were coarse, measuring up to 7 μm in width. Well-developed stromata were produced in some older (to 6 wk) cultures exposed to sunlight, but these produced predominantly microconidia, which were exuded in cirrhi. The teleomorph was not found in PDA culture. Macroconidia were observed in culture only rarely and sparsely but appeared similar in dimensions and morphology to those observed in host tissue.

Attempted inoculation of either potted *S. chrysophylla,* or trees in the field did not produce apparent infection. Therefore, a direct causal association of the *Botryosphaeria* with witches'-broom formation on *S. chrysophylla* was not shown. However, the fungus was consistently recovered in pure culture from sections of internal, presporulating broom tissue plated on PDA. Robust hyphae, possibly those of the *Botryosphaeria* species, also were observed in broom sec-



FIGS. 1–9. *Botryosphaeria mamane* on *Sophora chrysophylla*. 1. Newly developing, presporulating witches'-brooms on a young, previously vigorous tree. 2. Comparison of a thickened, abnormally proliferating branch from a presporulating broom (left) with a normal branch (right). 3. Section through a multilocular stroma, showing association of a macroconidioma (large arrow) together with ascomata (small arrows). Bar = 200 μm. 4. Young ascoma. Bar = 50 μm. 5. Immature ascus. Bar = 20 μm. 6. Ascospores and pseudoparaphyses. Bar = 16 μm. 7. Young macroconidioma. Bar = 50 μm. 8. Macroconidiogenous cell with enteroblastically developing conidium. Arrow indicates periclinal thickening. Bar = 10 μm. 9. Macroconidia. Bar = 16 μm.

tions, indicating intimate association of a fungus with symptomatic tissue.

DISCUSSION

The genus *Botryosphaeria* comprises saprophytic or facultatively parasitic fungi usually associated with bark cankers (von Arx and Müller, 1954), but species causing foliar infection also are included (Barr, 1972; Sivanesan, 1984). *Botryosphaeria ribis* Grossenb. & Duggar, while typically a dieback and canker causing pathogen on a wide range of woody hosts, also causes postharvest fruit rots of apple, avocado, kiwifruit, and lemon (Punithalingam and Holliday, 1973; Pennycook and Samuels, 1985). Gardner and Hodges (1988) described *B. pipturi* Gardner & Hodges in Hawaii, where it causes leaf spots on the endemic understory species *Pipturus hawaiensis* Lévl. (Urticaceae). Association of *B. mamane* with witches'-brooms is unique in the genus. Low concentrations of macroconidia or ineffective inoculation approaches may account for the lack of symptom reproduction. Since broom formation appears to involve hyperplastic bud tissue, further attempts at bud inoculation, together with production of abundant macroconidia in culture, eventually may prove successful. Production of microconidiomata, macroconidiomata, and ascomata in single spore cultures of ascospores and macroconidia would prove the connection among these spore states reported for *B. mamane.* However, their occurrence in the same stroma is considered compelling evidence that they are produced by the same fungus (FIG. 3).

The brooming symptom reported here is reminiscent of infections on other species in warmer regions caused by *Sphaeropsis tumefaciens* Hedges, a fungus usually associated with gall formation on woody hosts (Holliday and Punithalingam, 1970; Sinclair et al., 1987). Galls of *S. tumefaciens* occasionally produce a proliferation of shoots resulting in a witches'-broom (Marlatt and Ridings, 1979). This fungus earlier was reported on citrus in Hawaii (Moreau, 1947), but current confirmation of its occurrence in Hawaii is lacking (Raabe et al, 1981; Farr et al., 1989). Furthermore, *B. mamane* is readily distinguished from *S. tumefaciens* by close comparison of characteristics of the fungi themselves (ref. Hedges, 1911).

Anamorphs of *Botryosphaeria* have been placed in the genera *Botryodiplodia* (Sacc.) Sacc., *Dothiorella* Sacc., *Diplodia* Fr., *Macrophoma* (Sacc.) Berl. & Vogl., and *Sphaeropsis* Sacc. (Sivanesan, 1984; Hanlin, 1990), with links to *Fusicoccum* Cda. anamorphs being less common. However, *B. berengeriana* de Not., and *B. dothidea* (Moug.: Fr.) Ces. & de Not., which are accepted by some authors as synonyms of *B. ribis* (Sutton, 1980; Sivanesan, 1984), are considered to have *Fusicoccum* anamorphs (Sutton, 1980; Pennycook and Samuels, 1985). The anamorph of *B. ribis,* in its broad sense, was referred to as *F. aesculi* Cda., but Morgan-Jones and White (1987) later questioned this designation, noting that *F. aesculi* was not known to have a microconidial state as they observed among collections of *B. ribis* from Alabama. They referred to the anamorph merely as the *Fusicoccum* state of *B. ribis* and described macroconidia as fusiform or somewhat clavate, hyaline, aseptate, smooth, straight, obtuse at the apex, truncate at the base, 14–23 × 3–4.5 μm. Microconidia were described as oblong, narrowly ellipsoid or cylindrical, rod-like, aseptate, smooth, hyaline, 3–7 × 1–1.5 μm (Morgan-Jones and White, 1987). Macroconidia of *F. aesculi* were described similarly to those from Alabama, but measured 18–25 × 4–4.5 μm (Sutton, 1980).

Of the species of *Botryosphaeria* described by Sivanesan (1984), only *B. berengeriana* was associated with a *Fusicoccum* anamorph. According to von Arx and Müller (1954), *B. berengeriana* is a synonym of *B. ribis.* This consideration, together with the morphology of its teleomorph, suggest that *B. mamane* is most nearly comparable to *B. ribis.* Recently Rayachhetry et al. (1996) provided a thorough review and discussion of the species concept of *B. ribis.* Macroconidia of this species were mostly hyaline and aseptate, but 1–4 septate, pigmented older conidia also were common. These authors questioned the distinction between *Fusicoccum* and *Dothiorella,* suggesting that morphological variation may be dependent on the developmental state of macroconidia. Failure of macroconidia of *B. mamane* to be produced reliably in culture in the present study precluded critical comparison among developmental states, but mature macroconidia from infected tissue agreed closely with those described for *F. aesculi* (i.e., hyaline, fusiform, aseptate, truncate base) (Sutton, 1980; Pennycook and Samuels, 1985), except for their larger dimensions. Rayachhetry et al. (1996) described *B. ribis* in agar with consistent, but usually not abundant, production of microconidia among isolates studied. *Botryosphaeria mamane* produced microconidia readily both in culture and in infected tissue, with abundant macroconidiomata and ascomata also forming on the host.

Although macroconidia of *Fusicoccum* are often large (Sutton, 1980), those of *B. mamane* are unusually so [range (19–)30–44(–55) × (7–)8–9(–10) μm] and distinguish this species from *B. ribis.* The size range among the eight *Fusicoccum* isolates of the study of Rayachhetry et al. (1996) may be summa-

rized as 5.7–26.8 × 3.1–8.9 μm. Punithalingam and Holliday (1973) and Sivanesan (1984) gave the measurements of the macroconidia of *B. ribis* as 17–25 × 5–7 μm, and those of microconidia 2–3 × 1 μm. Although similar in morphology, asci (100–180 × 25–35 μm), ascospores (25–39 × 15–20 μm), and microconidia [3–9(–12) × 2–4 μm] of *B. mamane* also are larger than those reported for *B. ribis*. Sivanesan (1984) reported measurements of asci and ascospores of *B. ribis* as 100–110 × 16–20 μm and 17–23(–28) × 7–12 μm, respectively. Measurements reported by Punithalingam and Holliday (1973) agree closely, with a slight variation for ascospores (17–23 × 7–10 μm).

With the exception of *B. pipturi, B. ribis* and its synonyms and anamorphs have accounted for all previous reports of *Botryosphaeria* from Hawaii. In earlier surveys of Hawaiian fungi, Stevens and Shear (1929) noted the scarcity of *Botryosphaeria* and allied genera in the islands relative to their abundance in southern regions of the continental United States. Nevertheless, they recorded *Botryosphaeria,* as *B. ribis chromogena* Shear et al., on a number of hosts representing a variety of families. This list has since been increased, in accordance with the wide known host range of *B. ribis* (Raabe et al., 1981). Hodges (1983) reported the *Dothiorella* anamorph of *B. dothidea* associated with a virulent blue-stain canker disease in plantations of slash (*Pinus elliottii* Engelm.) and loblolly (*P. taeda* L.) pines in Hawaii. The teleomorph was not observed in the study. Kliejunas (1976) described a canker of Norfolk Island pine [*Araucaria heterophylla* (Salisb.) Franco] associated with *Botryodiplodia theobromae* Pat. and *Dothiorella* sp., the latter of which was identified as the anamorph of *B. ribis*. The teleomorph of the *Dothiorella* was not produced on infected tissue or in culture.

With some exceptions, the above-listed hosts of *B. ribis* in Hawaii are introductions to the islands. The apparent confinement of *B. mamane* to an endemic Hawaiian host suggests that this fungus itself may be endemic, and further distinguishes this species from previously described species of *Botryosphaeria.*

ACKNOWLEDGMENTS

The field and greenhouse assistance of Steven Bergfeld, Brion Duffy, Rob Anderson, and the Resource Management Division of Hawaii Volcanoes National Park is acknowledged. I thank David Webb for help with histological procedures, and appreciate Gary Samuels and James Plaskowitz of the USDA Systematic Botany and Mycology Laboratory for assistance in interpreting the fungus and for the micrographs. I thank Charles Hodges for his review and suggestions for the manuscript, and Angela Piper and Karla McDermid for the Latin description. The work was supported by National Biological Service project funding.

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