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MYCOBIOTA OF *MICONIA CALVESCENS* AND RELATED SPECIES FROM THE NEOTROPICS, WITH PARTICULAR REFERENCE TO POTENTIAL BIOCONTROL AGENTS

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A survey of fungal pathogens of *Miconia calvescens* associated with this weed was carried out in part of its native range in Brazil and other Latin American countries aimed at finding potential biocontrol agents. *Coccodiella miconiae*, *Pseudocercospora tamonae*, *Glomerella cingulata* (= *Colletotrichum gloeosporioides* f. sp. *miconiae*), the new species *Guignardia miconiae* and *Korunomyces prostrata* were found associated with foliar diseases of this host and are described herein. Two previously undescribed spore stages of *Coccodiella miconiae* were also obtained allowing a complete description of the life-cycle of this species.

Key words: Biological control, Melastomataceae, Hawai'i, Tahiti, *Phomopsis miconiae*

INTRODUCTION

Miconia calvescens DC. (miconia) belongs to the largest genus of the Melastomataceae which contains around 1000 species. It is a neotropical genus but specially concentrated in the Andes (Renner 1993, Judd & Skean 1991). Few species in this genus are of practical importance but *M. calvescens* has become a notable exception. Once a botanical curiosity, miconia is now the most devastating plant invader in Tahiti (Meyer 1996) and Hawai'i (Gagné *et al.* 1992). Although widely distributed from Mexico to Brazil and sometimes quite common locally, particularly in disturbed forest habitats, it never forms dense populations in its native range. In some regions in South and Central America, particularly on the oriental side of the mountain ranges dividing the continents and extending from southern Mexico to Ecuador, there is a form of *M. calvescens* that has very large leaves (up to one meter long) which are green adaxially and purple abaxially (which we refer to as the highland biotype) (J.Y. Meyer 1996, K. Meyer 1998). The lowland biotype found predominantly in eastern South America and

occasionally in Central America has smaller leaves with green abaxial surfaces. Because of the attractive foliage of the highland biotype it was introduced as an ornamental in many regions of the world. In some regions this proved disastrous (Gagné *et al*. 1992, Meyer & Florence 1996, J.Y. Meyer 1996, K. Meyer 1998, Baruch *et al*. 2000). It was introduced to Tahiti in 1937 where it became naturalized and slowly invaded the native forests. It now covers two thirds of the island and forms monotypic stands in many areas (Meyer & Florence 1996). It is already present in Moorea, Raiatea and Thaa (Meyer & Florence 1996, Meyer & Malet 1997). Miconia invasion is regarded as a 'worst case' example of the effect of an invasive weed in oceanic island biodiversity (Meyer 1996). It is estimated that 70-100 native species, including 40 to 50 endemics are directly threatened by *M. calvescens* in the French Polynesia (Meyer & Florence 1996). In Queensland, *M. calvescens* was declared a noxious weed in May 1997, but its cultivation and comercialization are still allowed in other Australian states, a dangerous situation as the plant has all the attributes for becoming a serious weed in that country (Csurhes 1997, Csurhes & Edwards 1998). In Hawai'i, *M. calvescens* was introduced in the 1960s and since 1992 was included in the list of noxious invasive weeds (Medeiros *et al*. 1997, Meyer 1998). Fortunately, until now, the invasions in Hawai'i have not become as severe as those of Tahiti (Gagné *et al*. 1992) in part because of an aggressive suppression and containment program. The plant is nevertheless present on four of the main Hawaiian Islands: Hawai'i, O'ahu, Maui and Kaua'i (Meyer 1998).

A search for fungal pathogens to be used as biocontrol agents of *M. calvescens* began in June 1995 in Brazil and was extended later to Costa Rica, Dominican Republic and Ecuador. A description of the fungi collected on this host and observations regarding their biocontrol potential, based on field observations are given bellow.

MATERIALS AND METHODS

A survey of some Brazilian herbaria was made to locate known localities of *M. calvescens* in Brazil to prepare an itinerary for survey trips. Localities in the states of Minas Gerais, Espírito Santo, Bahia, Rio de Janeiro and São Paulo were selected and surveyed. *Ad hoc* collections were made in the states of Amazonas and Mato Grosso also. Additional collecting trips were made to the Dominican Republic and Costa Rica (December 1998 – Jan 1999) and Ecuador (May 2000).

Dried specimens of diseased plants were prepared using a plant press and isolates were obtained by direct or indirect isolation on V8 juice agar plates, transferred to PCA (potato-carrot agar) agar slants and maintained at 5^{o} C. The cultures were shipped to the Hawaii Department of Agriculture (HDOA) Plant Pathology Quarantine Facility in Honolulu, Hawai'i, under United States Department of Agriculture permit no. 954140 for further testing. Samples of selected biotrophic fungi were preserved on bare-root living miconia plants that were either dispatched (from Brazil and Ecuador) or hand-carried to Hawai'i (from Costa Rica).

Identifications were made using standard keys for the genera and species. Only those that appeared to have potential as biological control agents were considered further.

More detailed studies of the biology and pathology of *Coccodiella* sp. were made and are being published separately. Only an account of the taxonomy of this fungus is provided here.

Studies on the life-cycle and pathology of a fungus which was preliminarily identified as *Korunomyces* sp. were included. Initially it was thought that it might be a species of *Ceratobasidium*, a genus containing fungi that cause a similar kind of foliar blight disease. Nuclei staining and teleomorph observation are normally needed to elucidate the identity of fungi in this group. Nuclei staining (HCl-Giemsa according to Herr 1979) were performed and an attempt was made at inducing teleomorph formation with an adaptation of the method of Silveira (1966). A mycelial suspension produced on a semi-synthetic medium (Alfenas *et al.* 1991) was brushed on leaves of fresh, healthy cuttings of *M. calvescens* in Erlenmeyer flasks containing tap water. The inoculated branches were then left in a dew chamber at 26^{o}C, with a 12-hour light regime for 20 days (nine daylight lamps, 40W, suspended 1 m above cuttings). Plant parts were observed every two days for the appearance of symptoms.

Pathogenicity of *Korunomyces* sp. was evaluated by inoculating healthy detached leaves of *M. calvescens*, *Terminalia ivorensis* A. Chev., *T. catappa* L. and *Eucalyptus grandis* A. W. Hill ex Maiden with culture plugs. The fungus was cultivated in CVA (vegetable broth-agar according to Pereira *et al.* 2003). After seven days, mycelial plugs obtained from the margins of actively growing cultures were transferred to the abaxial and adaxial sides of the detached leaves (four plugs per leaf, four leaves per plant species). The leaves were then placed in humid chambers (sealed inflated plastic bags containing trays with wet cotton pads). These were left at room temperature and examined several times a day to follow symptom development.

RESULTS AND DISCUSSION

Coccodiella miconiae (Duby) Hino & Katuamoto, *in* Katuamoto, K. *Journ. Jap. Bot.* 43: 282, 1968. (Figs. 1, 2, 3)

Sphaeria miconiae Duby in *Mem. soc. phys. et hist. nat. Genève* 7: 405, 1835.
Physalospora miconiae (Duby) Sacc., *Syll. Fung*. 1: 447, 1882.
Botryosphaeria miconiae (Duby) Hohnel, *Sitz-ber. Akad. Wien*. 118: 836, 1909.
Phyllachora miconiae (Duby) Sacc., *Ann. Myc*. 11: 547, 1913.
Bagnisiopsis miconiae (Duby) Petrak, *Hedwigia* 68: 275, 1928.
Coccostroma miconiae (Duby) v. Arx & Müller*, Die Gattungen der amerosporen Pyrenomyceten. Beitr. Kryptog. fl. Schw.* 11 (1): 263, 1954.

Disease (black pimple): **Lesions** on living leaves: **adaxially** initially punctiform, chlorotic becoming pale brown centrally, often raised and convex (pimple-like), sometimes concave; older lesions with a narrow well-defined chlorotic halo surrounded by diffuse chlorotic area becoming dark brown to black centrally, circular, up to 5 mm diam, coalescing in some areas of the leaves; **abaxially**, stromata initially minute and pale brown, becoming a black shinny dot set inside concavities on the leaf laminae, sometimes surrounded by narrow chlorotic halo easily seen with the naked eye, up to 3 mm diam; sometimes general foliar deformation and chlorosis resulting from severe infection.

Morphology: **Internal mycelium** intra and intercelular, branched, septate, hyaline, 2-5 μm diam. **External mycelium** absent. **Stromata** formed abaxially, erumpent, sub-spherical to pulvinate, 189-1500 μm wide, to 190- 473 μm tall, constricted at the base, 167-584 μm wide at the attachment point, isolated or aggregated, initially pale brown and having one to several spermogonia on the surface, becoming black and having several perithecial locules, walls composed of very dark *textura angularis* and internal tissue pale-brown to hyaline. **Ascomata** perithecial, embedded in the stromata, spherical, sub-spherical, sometimes having a distorted shape, 123-218 μm diam, walls composed of hyaline *textura angularis*, approximately 12-19 μm thick (but not well differentiated from the stromatal tissue). **Dehiscence** ostiolate, one ostiole per perithecium, 28.5-71 μm diam. **Hamathecium** including well-developed and abundant septate, unbranched, hyaline paraphyses (up to 75 μm long x 1 μm wide) and periphyses 20-44 x 1 μm. **Asci** unitunicate, attached to the lower part of the perithecia, cylindrical, 71-100 x 7-10 μm, thick-walled, apex round to sub-truncate, stalked, apical ring indistinct, 8-spored. **Ascospores** uniseriate, ellipsoid to sub-spherical, 7-12 x 6-8 μm, aseptate, eguttulate, hyaline becoming brown with age, smooth and relatively thick-walled, increasing in size after ejection and germinating by the formation of a vesicle of similar shape and size to the ascospores. **Spermogonia** formed early on the surface of stromata, cupulate, 42.5- 92.5 μm wide to 38-83 μm high, containing hyphoid receptive hyphae and abundant mucilaginous masses of drop-shaped 2-4 x 1-1.5 μm, hyaline, smooth-walled spermatia; darkening and becoming sterile with age changing into black horn-like projections of the stroma.
***Hemidothis* (mitosporic state)** - **Conidiomata** produced abaxially on leaves, similarly to ascomata, stromatic, multilocular, erumpent, single or forming small groups, black, shiny, having many blunt rostri, each supporting a drop of milky mucilaginous conidial mass, up to 1 mm diam; walls of dark-brown *textura angularis*, 2-5 cells, 8-26 μm thick, rough; locules spherical, ellipsoidal or irregular, 35-94 μm diam arising at different levels within the stromata; having very long, fine, septate, hyaline paraphyses that emerge through the ostiole. **Dehiscence** ostiolate, one per locule, rostrate, 19-84 μm diam. **Conidiophores** arising from the internal walls of the locules, cylindrical, tapering towards the apices, straight or flexuose, 10-41 x 1-2.5 μm, 1-2 septate, branched, hyaline, smooth. **Conidiogenous cells** terminal, enteroblastic, cylindrical, tapering towards the apices, 6.5-24.5 x 1-2 μm, hyaline, smooth. **Conidiogenous loci** minute, 0.5-1 μm. **Conidia** mucilaginous, enteroblastic, straight or curved, fusiform to falcate, 3.5-8 x 1-2 μm, apex and base rounded, aseptate (only occasionally septate), guttulate, hyaline, smooth.

Material examined: VIC 19303, Viçosa, MG, 16 March 1998; VIC 19305, São Romão (road Lumiar-Casimiro de Abreu), RJ, 24 February 1998; VIC 19306, Sana, RJ, 24 February 1998; VIC 19307, road Glicério - Vila do Grama, RJ, 24 February 1998; VIC 19308, road Frade - Glicério, RJ, 24 February 1998; VIC 19286, Estrada da Grota Funda, Rio de Janeiro, RJ, 27 December 1995; VIC 19288, Boca do Mato, Cachoeiras do Macacú, RJ, 5 February, 1996; VIC 19290, Road Rio-Petrópolis, Xerém, RJ, 24 March 1996; VIC 19291, road Dionísio -

Timóteo, MG, 30 August 1996; VIC 19292, Road Rio - São Paulo, between Barra Mansa and Arrozal, 20 September 1996; VIC 19293, road Lídice - Angra dos Reis, RJ, 20 September 1996; VIC 19294, Bosque da Barra, Barra da Tijuca, Rio de Janeiro, RJ, 30 September 1996; VIC 19295, Alto da Boa Vista, Rio de Janeiro, RJ, 30 September 1996; VIC 19296, Floresta Azul, BA, 21 November 1996; VIC 19297, Reserva Biológica de Una, Una, BA, 22 November 1996; VIC 19298, road Lajinha - Mutum, MG, 16 December 1996; VIC 19299, road BR 101, km 483, between Itabuna and Ubaitaba, BA, 19 January 1997; VIC 19300, road Ubaitaba - Maraú, BA, 19 January 1997; VIC 22202, Gutierréz Braun, Costa Rica, 3 January 1999; VIC 22203, near road Parque Areal, Costa Rica, 30 December 1998; 22204, between San Carlos and Fortune, Costa Rica, 31 December 1998; 22198, near Rio Pedra Fina, Ecuador, 10 may 2000; VIC 22205, Road Avila-Huita Cocha, Ecuador, 14 may 2000; VIC 22206, Loreto, Ecuador, 14 may 2000.

Figure 1. Symptoms of *Coccodiella miconiae* on leaves of *Miconia calvescens*.

Figure 2. *Coccodiella miconiae*: stroma erupting through lower leaf surface (50x).

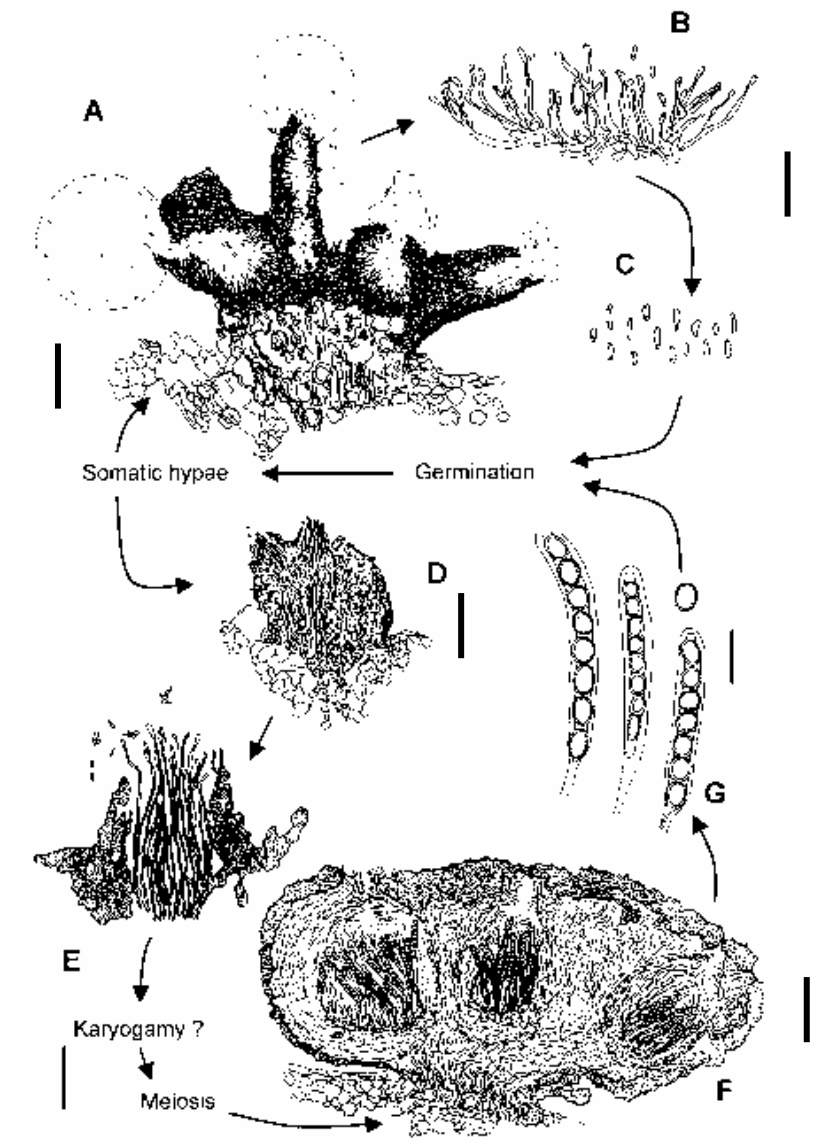

Figure 3. Proposed life cycle of *Coccodiella miconiae*. (A) Rostrate conidioma supporting drops of mucilaginous masses of conidia. (B) Conidiophores and conidiogenous cells showing conidiogenesis. (C) Conidia. (D) Young stroma erupting through leaf epidermis showing two spermogonia. (E) Spermogonium with receptive hyphae and drop-like spermatia. (F) Mature stroma with three perithecia. (spermogonia not visible in this section). (G) Asci and ascospores, (note immature ascus with hyaline ascospores). Bar for B, C, E, G= 20 μm: A, D and F = 100 μm.

The genus *Coccodiella* includes around 25 species of biotrophic fungi that are foliar pathogens of plants belonging to 10 different families. Eleven species of *Coccodiella* are parasitic on members of the Melastomataceae. Katumoto (1968) recognized that the name *Coccodiella* proposed for the genus by Hara in 1911 had priority over other names such as *Coccostroma* and *Bagnisiopsis* used by other authors in later works. He then proposed a series of new combinations but studied only material of the type species *Coccodiella arundinaria* Hara. More recently, this species was examined and redescribed by Cannon (1996). Miller & Burton (1943) studied several species of *Bagnisiopsis* (=*Coccodiella*) on the Melastomataceae. These authors observed the presence of spermogonia on the stromata in this genus for the first time but they apparently failed to observe the later development of these structures in aging stromata. Because of this and the misinterpretation of old spermogonia (and possibly also the rostrate conidiomata) as being ornamented stromata (treated as "setae-like processes" by these authors) they finally adopted their presence as important key characters. In fact, examination of fresh material of the fungus collected on *M. calvescens* having stromata in several stages of development showed quite clearly that such "setae-like processes" are either aged and dried spermogonia or rostrate conidiomata. The presence of these "ornaments" is therefore likely to be dependent on the age or life-cycle stage of the material under examination for other species as well and hence inadequate for species separation. Also, although stating that "the dimensions of ascospores are more variable than in most Ascomycetes" these authors proposed the use of spore size as critical characters for species separation in the key given in their article. These aspects associated with the high proportion of species in the genus described on *Miconia* (9 out of the 26 species of *Coccodiella* having been described from *Miconia*) suggest that some of these taxa may be in fact conspecific. Revision of the *Coccodiella* on Melastomataceae is clearly needed but outside the scope of the present work. The fungus collected during this fieldwork fits well into several overlapping species descriptions given for *Coccodiella* spp on *Miconia.* We decided nevertheless, that for the present it would be more appropriate using the name *C. miconiae* as this was proposed for a similar *Coccodiella* found growing on leaves of *M. calvescens* in Brazil. Although *C. miconiae* is not new to science, it is a poorly known fungus (as are the majority of the species in this genus) and the description given above is the first complete description of this fungus including two spore stages (spermogonial and mitosporic) which were not previously described or portrayed. A scheme of the life-cycle is given in Fig. 3. It is likely that the mitosporic stage with its slimy conidial mass functions in short distance splash dispersal while the ascospores provides the propagules for long distance wind dispersal.

Black pimple caused by *C. miconiae* is ubiquitous on miconia. It is found throughout the year. On certain occasions the disease was almost imperceptible and very few isolated stromata per leaf were present. On other occasions it was very damaging, deforming and causing a general chlorosis of severely infected shoots. Six different mycoparasites of the stromata of *C. miconiae* was commonly observed on this fungus and in some locations they clearly play a major role in limiting the potential damage caused by black pimple.

It is difficult, nevertheless, at this stage, to explain the differences in severity observed in the field. Based on the many observations that were made

of this disease, *C. miconiae* is regarded here as one of the most promising biocontrol candidates found in the mycobiota of *M. calvescens.* It is expected that the introduction of the appropriate strain of this fungus, free from its own natural enemies (particularly its mycoparasites), will result in great impact on invasive populations of miconia.

Glomerella cingulata (Stonem.) Spaulding & Schrenk, *Science* Ser. 2 17: 751, 1903. (Fig. 4)

Meiosporic state: present in old lesions on leaves and formed in some aging cultures. mitosporic state: *Colletotrichum gloeosporioides* (Penz.) Sacc., *Atti R. Ist. Ven. Sci. Lett. Art.* ser. 6, 2, 670, 1884.

Disease (antracnose): **Lesions** on living leaf laminae and along leaf margins associated with blight-like symptoms, initiating as minute necrotic circular punctations becoming larger and roughly circular in the central part of the leaves, up to 3 cm diam and sometimes elliptical when growing on leaf veins, pale-brown in the centre, periphery dark-brown abaxially and gray-brown to totally gray adaxially, sometimes having a diffuse chlorotic halo; lesions sometimes coalescing leading to necrosis of extensive leaf area; necrotic areas easily torn and tending to fall, sometimes causing the loss of parts of the lamina leaving only a leaf vein skeleton. On one occasion, a miconia population showing widespread die-back starting at the flower buds and descending along the branches was also observed in association with this fungus (RWB 109, Ipeúna, SP).
In culture*:* **Colonies** relatively fast growing (54-86 mm diam/11 days), mycelial growth mostly within the medium having a central area of white to grayish sparse wooly aerial mycelium, where sporulation is concentrated in orange mucilagenous masses of conidia, reverse grayish-white with no pigmentation of the medium or evidence of diurnal zonation. **Ascocarp** perithecia, solitary or aggregated, spherical to subspherical, 150-309 μm diam, walls composed of brown *textura angularis*, mostly 4-5 cells thick, 8.5-25 μm, smooth. **Dehiscence** ostiolate, single, central, circular, 8.5-25.5 μm diam lined with periphyses. **Asci** unitunicate, cylindrical to clavate, 44-66 x 9-11.5 μm, rounded or slightly flattened at the apex, paraphysate, 8-spored. **Ascospores** straight fusiform to slightly curved, allantoid, 12.5-17.5 x 4.5-6 μm, aseptate, guttulate, hyaline, smooth. **Conidiomata** acervular, formed only on the host leaves abaxially on laminae and veins, setose. **Setae** straight, cylindrical, tappering towards the pointed apex, 62.5-120 μm long, 2-septate, brown, smooth. **Conidia** straight, cylindrical, apices obtuse, 9-22.5 x 3-4.5 μm, aseptate becoming one septate at germination, guttulate, hyaline, smooth. **Apressoria** very variable in shape, 8-25 x 5-17.5 μm, aseptate or sometimes septate, thick walled, dark-brown, smooth.

Material examined: VIC 19306, Sana, RJ, 24 February 1998; VIC 19307, road Glicério - Vila do Grama, RJ, 24 February 1998, VIC 19308, road Frade - Glicério, RJ, 24 February 1998; VIC 19284, road Leopoldina - Cataguases, MG, 2 May 1995; VIC 19285, Mazomba, Itaguaí, RJ, 22 December 1995; VIC 19286, Estrada da Grota Funda, Rio de Janeiro, RJ, 27 December 1995; VIC 19287,

Belvedere, Itaguaí, RJ, 27 December 1995; VIC 19288, Boca do Mato, Cachoeiras do Macacú, RJ, 5 February, 1996; VIC 19289, Cristais, Viçosa, MG, 13 March 1996; VIC 19290, Road Rio-Petrópolis, Xerém, RJ, 24 March 1996; VIC 19291, road Dionísio - Timóteo, MG, 30 August 1996; VIC 19292, Road Rio - São Paulo, between Barra Mansa and Arrozal, 20 September 1996; VIC 19293, road Lídice - Angra dos Reis, RJ, 20 September 1996; VIC 19294, Bosque da Barra, Barra da Tijuca, RJ, 30 September 1996; Alto da Boa Vista, Rio de Janeiro, RJ, 30 September 1996; VIC 19296, Floresta Azul, BA, 21 November 1996; VIC 19300, road Ubaitaba - Maraú, BA, 19 January 1997; VIC 19301, margins of Rio Jucú, ES, 17 January 1996; VIC 19302, São Joaquim, Piraí, RJ, 5 March 1997; VIC22207, road of Serrado Cantagalo, 26 November 1998; VIC 22202, Gutiérrez Braun, Costa Rica, 3 January 1999; VIC 22208, Fortuna-Lago Areal, after Tabacon, Costa Rica, 29 December 1998; VIC 22209, road Quito-Lago Agrio, Ecuador, 10 may 2000; VIC 22210, Añango, Ecuador, 12 may 2000.

Fig. 4. Anthracnose of *M. calvescens* caused by *Colletotrichum gloeosporioides* f.sp. *miconiae*.

This is a very common pathogen of *M. calvescens* in Brazil and on several occasions it was observed in the mitosporic state causing a serious foliar anthracnose. Its morphology is typical of *G. cingulata* and the data for the fungus on miconia fits unmistakeably into the description of this taxon and its *C. gloeosporioides* mitosporic stage given in the literature (Arx 1957, Mordue 1971, Sutton 1980). Although isolates were initially obtained from the lowland biotype leaf form of *M. calvescens* that is present in Brazil, tests carried out at HDOA (Quarantine Lab), based on an isolate from VIC 19284 have shown that the fungus is host-specific to *M. calvescens* and a new taxon at the *forma specialis* level was proposed (Killgore *et al.* 1999). A permit for the introduction of the fungus was granted by the Hawaiian authorities and the fungus was introduced in selected sites in the Island of Hawai'i in 1997. Later, damaging outbreaks of miconia antracnose were observed at sites unexpectedly distant from release

sites (Barreto *et al*. 2000). This represented the first example of a program involving the classical approach that led to the introduction of a fungal pathogen native from Brazil as a biocontrol agent against a weed in an alien situation (Barreto *et al.* 2001).

Guignardia miconiae C. D. S. Seixas & R. W. Barreto **sp.nov.** (Figs. 5, 6)

Ab Guignardia citricarpa *Kiely; ascomata epiphylla, 90-285 μm diametro; asci 26.5-102.0 x 24.0-31.5 μm; ascosporae ovoideus, 14.0-20.0 x 10.0-11.5 μm; appendice indistinctus; status anamorphicus* Leptodothiorella*, differens.*
Etym: on leaves of *Miconia calvescens*

Disease (punctiform tar-spot): **Lesions** on living leaves, limited to black punctations in subcircular aggregates within green tissue when young, more visible abaxially, colonized tissue becomes discolored in older lesions with a central to subcentral gray necrotic area and a dark brown to black periphery abaxially and evenly grayish-brown to gray adaxially 3-12 mm diam; irregularly distributed on the lamina and occasionally coalescing and leading to the formation of cracks in the tissue.
Morphology: **Internal mycelium** inter and intracellular, branched, septate, pale brown. **External mycelium** absent. **Ascomata** ascostromatic, epiphyllous, semi-immersed, isolate, globose to subglobose, 90-285 µm diam., walls composed of dark brown *textura angularis*, 14-74 µm thick, much thicker and melanized on the upper part, smooth. **Dehiscence** ostiolate, central, one per ascoma, 33.5-61 µm. **Interthecial filaments** absent in mature ascomata. **Asci** bitunicate, clavate, 26.5-102 x 24-31.5 µm, eight spored, pedicelate. **Ascospores** inordinate, ovoid, 14-20 x 10-11.5 µm, aseptate, guttulate, hyaline, smooth, mucilaginous sheath very tenuous rarely perceptible.
Anamorphic/spermatial stage: ***Leptodothiorella* sp.**
Conidiomata pycnidial, epiphyllous, semi-immerse, isolate, globose to subglobose, 64-145 µm diam., wall 10.5-89 µm thick, brown, smooth. **Dehiscence** as described for the ascomata. **Conidiophores** covering all the internal wall of the pycnicia, narrowly lageniform, 7.5-25.5 x 1.5-5.0 µm, 0-2 septate, unbranched, hyaline, smooth. **Conidiogenous cells** integrate, enteroblastic, cylindrical to lageniform, 6-13 x 1-4 µm. **Conidiogenous loci** one per cell, unthickened with a minute collarette. **Conidia** mucilaginous, enteroblastic, straight, dumb-bell-shaped, 4.5-5.5 x 1.0-2.0 µm, aseptate, biguttulate, hyaline, smooth.
Holotype: VIC 22211; Bosque Municipal, Águas da Prata-SP; 27/11/1998
Paratypes: VIC 22212, Águas da Prata-SP, 8 June 2001; VIC 22218, Road Vila Abhraão-Dois Rios, Ilha Grande, Angra dos Reis-RJ, 13 January 2002;

Figure 5. Tar-spot of *Miconia calvescens* caused by *Guignardia miconiae.*

There are only two species of *Guignardia* described on *Miconia*: *G. atropurpurea* Chardon and *G. punctiformis* Chardon (Chardon *et al.* 1940). Both were described from specimens collected on *Miconia* sp. from Viçosa – MG – Brazil. This work was undertaken at the type locality for both fungi but during six years of collecting diseases of *M. calvescens* in the region those fungi were never found. It is likely that these fungi have different species of *Miconia* as a host. *G. miconiae* differs from *G. punctiformis* by having larger asci (75-13-18 μm in *G. atropurpurea*) and ascospores that are of a different shape and size (long-elliptical and 17-21 x 7-8 μm). *G. punctiformis* produces smaller lesions on the host (4-6 mm diam), has wider asci (30-34 μm diam) and conidia of a different shape and size (ellipsoidal to sub-pyriform, 20-23 x 10-14 μm). No anamorphic state was described for either *G. atropurpurea* or *G. punctiformis*.

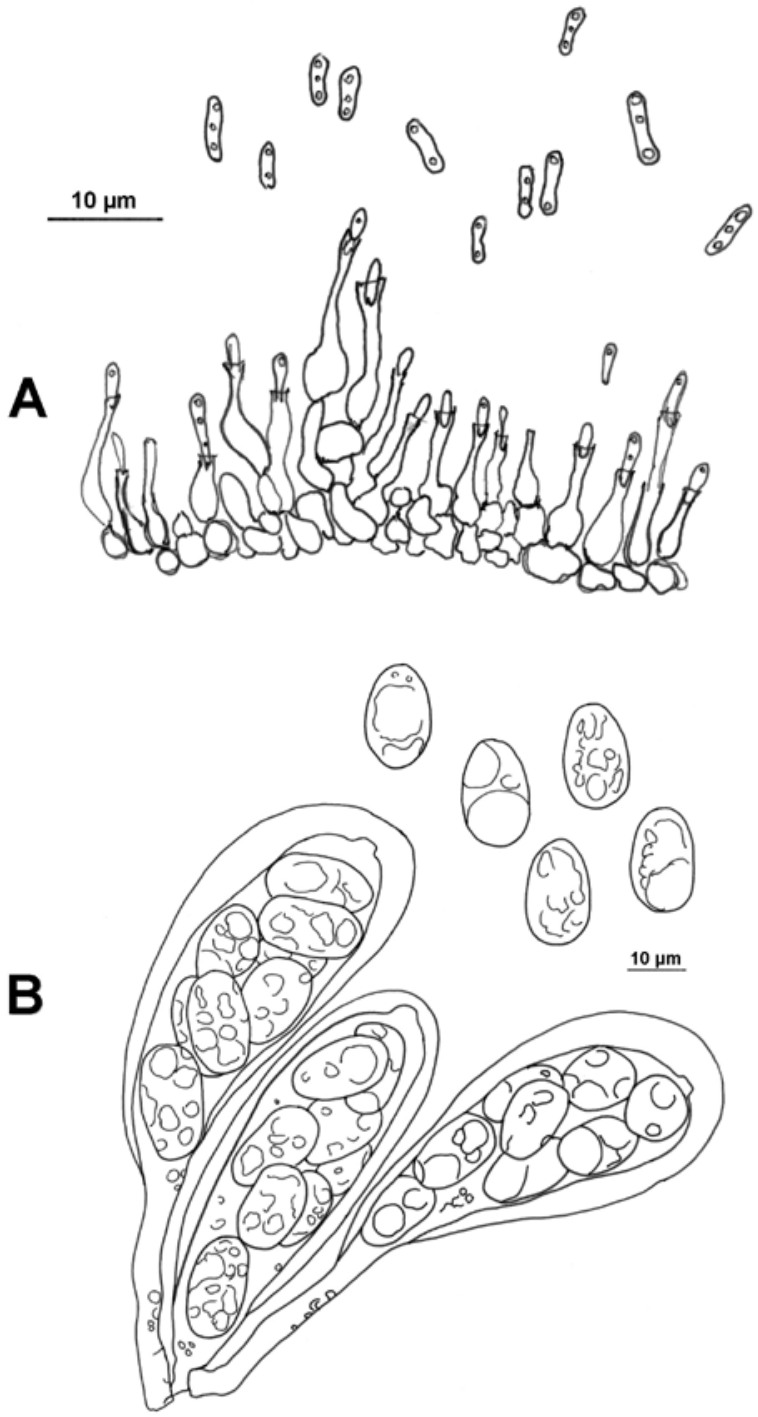

Figure 6. *Guignardia miconiae*: A. conidiophores and conidia. B. asci and ascospores.

Guignardia miconiae was rare, being found only at two very distantly located sites (Ilha Grande in the state of Rio de Janeiro and Águas da Prata in the state of São Paulo). It has a typical hemibiotrophic habit, starting to produce its fruiting structures in green tissue that gradually yellows and become necrotic. In a recent visit to Ilha Grande damage observed to individual leaves was significant. Several attempts at isolating this fungus with different methods were made but without success. This was unexpected as fungi in this genus are known to grow well in culture. An attempt to inoculate *M. calvescens* with a suspension obtained by squashing fresh ascomata of material from Ilha Grande also failed to yield symptoms. For the moment it is difficult to evaluate the biocontrol potential for this fungus.

Korunomyces prostratus C.D. S. Seixas & R.W. Barreto **sp. nov.** (Figs. 7, 8)

Ab Korunomyces terminaliae *Hodges & Ferreira; propaguliphora prostrata, haud distinctus ab hyphae, 3-4 usque 5-8 μm (ad propagula base) diametro, propagula elementis 4.0-10.0 μm diametro, propagula elementis terminalibus 7-13 μm longum, differens.*
Etym.: reference to the predominant formation of prostrate propagulophores and propagules.

Disease (leaf blight): *Lesions* necrotic, initially circular with a central grayish-brown centre and a brown periphery, becoming irregular with age with concentric dark brown peripheral rings often resulting in a scale-like pattern, often surrounded by a yellowish halo, coalescing and leading to an extensive leaf-blight; older parts of the lesions tend to crack leaving irregular holes on the leaves.

Morphology: **External mycelium** amphigenous, branched, septate, initially hyaline becoming yellow or orange later. **Internal mycelium** indistinct. **Propagulophores** often difficult to distinguish from ordinary hyphae, cylindrical, simple, length indeterminate, individual cells 11-27 x 3-4 µm, diam. below propagules 5-8 µm, hyaline, smooth, point of rupture indistinct or absent. **Propagules** subglobose to irregular when mature, formed on the apex of usually prostrate hyphae or occasionally on erect propagulophores, multicellular, formed of primary branches with an initial dichotomous branching pattern becoming dendritic later, 69-273 x 64-272 µm, branch elements 4-10 µm diam., terminal elements 4-5 x 7-13 µm, initally hyaline becoming orange when mature, smooth. **In culture**: relatively fast-growing (3.2-6.5 cm diam after 13 days); colonies of cottony-wooly aerial mycelium showing marked diurnal zonation on PDA, dark orange with a dark-orange reverse; flattened aerial mycelium centrally surrounded by an area of sparse aerial mycelium and strongly radial superficial growth on CVA, white, cream to pale orange, reverse as for the surface; on PDA many germinated propagules observed at or near the surface of the medium, rarely along or at the apices of aerial hyphae; sterile.
Holotype: VIC 22213 Ilha Grande-RJ, 04/01/2000
Paratypes: VIC 22198, near Rio Pedra Fina, Ecuador, 10 may 2000; VIC 22219, National Park Napo-Galeras, Ecuador, 14 may 2000; VIC 22218, road Vila Abhraão-Dois Rios, Ilha Grande, Angra dos Reis-RJ, 13 January 2002.

Figure 7. Foliage blight of *M. calvescens* caused by *Korunomyces prostratus.*

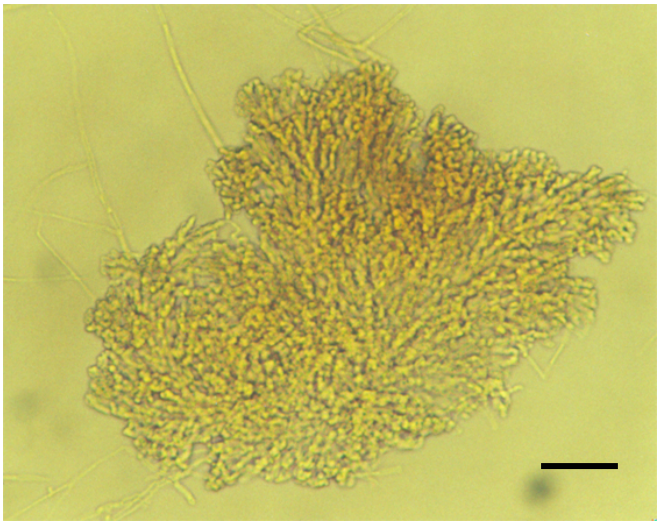

Figure 8. Propagule of *Korunomyces prostratus.* (Bar=50μm)

The genus *Korunomyces* was, until now, monotypic. *K. terminaliae* was proposed by Hodges & Ferreira (1981) for a fungus causing a leaf and stem blight on *Terminalia ivorensis* A. Chev. These authors discussed the similarity of this fungus with members of *Cristulariella*, *Papulaspora* and *Aegerita* and concluded that the fungus on *T. ivorensis* deserved the status of a new genus. Nothing has been published on this genus since then. The fungus found on *M. calvescens* is similar to *K. terminaliae* but morphological differences are regarded here as sufficient to place it as separate species. Perhaps the most significant difference between the two species is the clear achene-like form and probable dispersal function of the combination of propagule-propagulophore in *K. terminliae* and the predominantly prostrate condition of propagules of *K. prostratus.* In the new species these structures are probably not functional as dispersal units and appear to work as infection pads instead. Dispersion in this species is probably dependent on propagule elements or some spore stage that was not observed during the present work. Observations of HCl-Giemsa microscopic mounts revealed that *K. prostratus* is multinucleate.

Korunomyces prostratus was found in several different locations in Brazil (states of Rio de Janeiro, São Paulo, Minas Gerais) and also in Costa Rica and Ecuador associated with the highland biotype of miconia. Leaf blight was often very damaging to the affected leaves but the number of diseased leaves per plant was rarely high. Neither symptoms nor a perfect stage of the fungus were obtained after inoculation of mycelial suspensions on leaves. Inoculating detached leaves showed that *K. prostratus* is (under such conditions) capable of causing necrosis on leaves of *M. calvescens*, *T. ivorensis* and *E. grandis* but not of *T. catappa.* There appears to be a partial overlap of the host-range of the two species of *Korunomyces. K. terminaliae* was capable of infecting three species of *Terminalia* but not *Eucalyptus grandis* (Hodges & Ferreira, 1981). This result also indicates that considerable care needs to be taken regarding the elucidation of the host-range of *K. prostratus* before its introduction as a classical biocontrol agent is considered. Nevertheless, if safety requirements are met and an adequate procedure of manipulating this fungus is developed, this fungus may prove useful as a bicontrol agent.

Pseudocercospora tamoneae (Chupp) U. Braun & Castañeda, *Cryptogamic Botany* 2/3: 294 (1991) (Figs. 9, 10)

Cercospora tamoneae *Chupp, A monograph of the fungus genus* Cercospora*, p. 383, Ithaca: Published by the author, 1954.*

Disease (leaf-spot): **Lesions** on living leaves, irregular, vein delimited, brown surrounded by a diffuse, chlorotic halo, up to 13 x 7 mm.
Morphology: **Internal mycelium** intra- and intercellular, 2 μm diam., branched, septate, pale-brown, smooth. **External mycelium** hypophyllous, poorly developed, septate, pale-brown, smooth. **Stromata** erumpent, initially subglobose, becoming cylindrical, 24-43.5 x 25-57 μm, composed of grayish-brown cells. **Conidiophores** amphiginous, either densely fasciculate (often more than 20 per fasciculum) or produced singly on external mycelium, cylindrical,

straight to slightly sinuose, 15-57 x 2-4 μm, 0-4 septate, unbranched, pale-brown, smooth. **Conidiogenous cells** terminal and intercallary, integrated, holoblastic, proliferating sympodially, cylindrical, straight to slightly sinuose, 1-2 geniculate, 5-19.5 x 3-4 μm, pale-brown, smooth. **Conidiogenous loci** flattened, 2-3 μm wide, unthickened, not darkened. **Conidia** dry, isolate, holoblastic, subcylindrical tappering towards the apices, often slightly curved but sometimes straight, 53-90 x 3-5 μm, apex rounded, base subtruncate, 2-3 μm, 6-11 septate, scar unthickened and not darkened, guttulate, pale-brown, smooth.

Material examined: VIC 19294, on *Miconia jucunda* (DC.) Triana, Bosque da Barra, Barra da Tijuca, RJ, 30 September 1996; VIC 22214, on *Miconia* sp., Chapada dos Guimarães, MT, 11 October 1998; VIC 22215, on *Miconia* sp., Parintins, AM, 30 August 1997.

Figure 9. Symptoms of leaf spots caused by *Pseudocercospora tamonae* in *Miconia calvescens*.

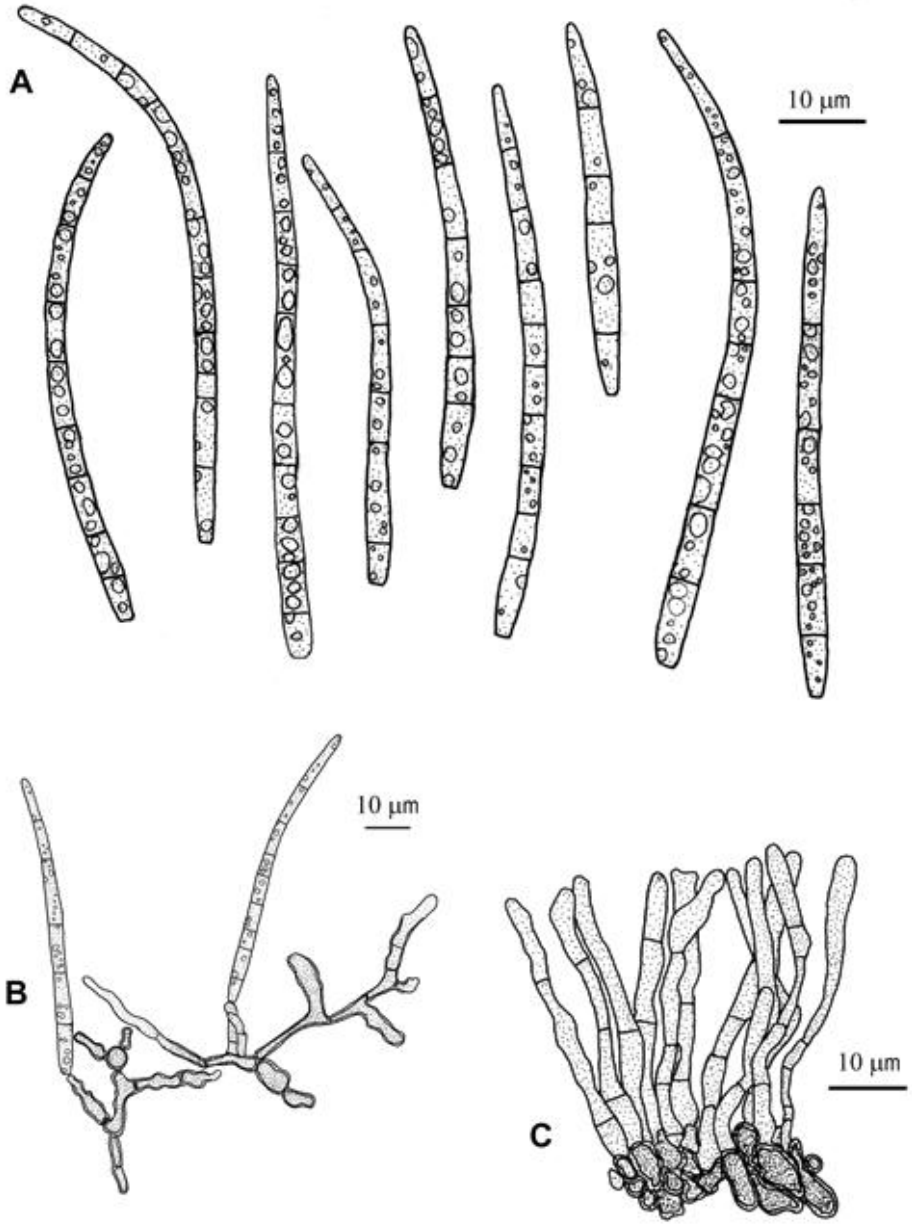

Figure 10. *Pseudocercospora tamonae*: A. conidia. B. conidiophores on external mycelium. C. conidiophore fascicle.

Nineteen cercosporoid fungi have been described on the Melastomataceae: *Cercospora aciotidis* Chupp; *Cercostigmina curta* (Syd.) Braun; *Pseudocercospora dissotidis* (Chupp & Doidge) Crous & U. Braun; *Pseudocercospora erythrogena* (G.F. Atk.) U. Braun; *Pseudocercospora gracilenta* (H. Syd.) Deighton; *Pseudocercospora leandrae* (Syd.) U. Braun; *Cercospora melastomatis* Patouillard; *Pseudocercospora melastomobia* Chupp; *Pseudocercospora melastomombin* (W. Yamam.) Deighton; *Cercospora miconiae* Fragoso & Ciferri; *Cercospora miconiicola* Chupp; *Pseudocercospora mirandensis* (Chupp) R.F. Castañeda & U. Braun; *Cercospora monochaeti* Chupp & Muller; *Cercospora monochaeticola* Chupp; *Pseudocercospora tamonae* (Chupp) U. Braun & R.F. Castañeda; *Pseudocercospora oxysporae* (A.K. Kar & M. Mandal) Deighton; *Pseudocercospora osbeckiae* (Kaapor, Lal & Munjal) Kamal; *Cercospora tibouchinae* Viégas; *Ramularia microlepiae* F. Stevens. Braun has recently reexamined many of those species but some still await a reevaluation under the new concepts for the taxa in this group (Braun 1995, 1998). In addition to those taxa, there is a record of a "*Cercospora*" on phylloplane of *Metrosideros polymorpha* Gaud. from Hawai'i (Baker *et al.* 1979). Confirmation of this record and elucidation of the identity of the fungus, despite the relevance for this work, is not possible as no materials appear to have been deposited by the authors in a herbarium. Among the species listed above, six were recorded on hosts belonging to the genus *Miconia* (Chupp 1953, Farr *et al* 1989, Viégas 1961): *P. erythrogena*, *C. melastomatis*, *C. miconiae*, *C. miconicola*, *P. mirandensis* and *P. tamonae*. Type material of some relevant species was obtained for comparison with the material from Brazil and original descriptions were studied. The cercosporoids specimens obtained from miconia in Brazil fit well within the descriptions and is very similar to the type specimen of *P. tamonae* and was therefore recognized as belonging to this taxon.

The degree of damage caused by *P. tamonae* was variable. In one instance it caused minor disease (angular leaf-spots) on a single plant of *M. jucunda.* In another situation, it caused a severe disease of foliage of *Miconia* sp. at Chapada dos Guimarães. Another isolate of this fungus from Parintins was sent to HDOA Quarantine Lab for further study. Tests undertaken in Hawai'i indicated that *P. tamonae* is capable of causing a severe disease on the Hawaiian biotype of *M. calvescens.* Unfortunately it transpired that this fungus appears to have a wide host-range attacking *Psidium cattleianum* Sabine and other plant species. It also appears to be unable complete its cycle on the local biotype of *M. calvescens.* So far, under the experimental conditions it was unable to sporulate on the inoculated plants.

Additional fungi on *Miconia*

Phomopsis sp.

Lesions on living leaves, circular, elliptical or irregular, initially grayish-green, becoming gray and finally whitish, surrounded by a dark green narrow rim that

becomes pale brown and slightly raised with age; necrotic tissue in older lesions easily torn and sometimes associated with central stromata remains (often sterile or insect eaten) sometimes together with stromata of *Coccodiella miconiae,* 1-5 mm diam*.* **Internal mycelium** inter- and intracellular, 2-3.5 μm diam., ramified, septate, hyaline. **External mycelium** absent. **Conidiomata** eustromatic, immersed, separate, variable in shape but often subglobose (cupuliform when viewed in section), 292-459 μm high and 417-709 μm wide, upper part of wall of dark brown *textura angularis*, lower part pale-brown to hyaline and thinner, 3-9 cells 9,5-18,5 μm thick, smooth. **Dehiscence** ostiolate, single, central, papillate, aproximately 80 μm diam. **Conidiophores** originating from the walls, lining all the interior of the conidiomata, cylindrical, tapering towards the apices, 17.5-26.5 x 1.5-2 μm, 2-5 septate, unbranched or with one or few branches, hyaline, smooth. **Conidiogenous cells** terminal, integrated, enteroblastic, phialidic, and associated minute periclinal thickening, cylindrical tappering towards the apices, 4.5-13 x 2 μm, hyaline, smooth. **Conidiogenous loci** minute, terminal, unthickened, not darkened. **Conidia** in mucilagenous groups, of two kinds: α conidia, ellipsoid to fusiform, 4.5-9.5 x 2-2.5 μm, aseptate, hyaline, smooth, guttulate (often with two guttules); β conidia, filiform, hamate or sigmoid, 9.5-19 x 1 μm, aseptate, hyaline, smooth, eguttulate.

Material examined: all specime on *Miconia prasina* (Sw.) DC - VIC 19303, Xerém, road Rio - Petrópolis, RJ, 15 September 1997; VIC 19304, Frade, RJ, 24 February 1998; VIC 19305, São Romão (road Lumiar-Casimiro de Abreu), RJ, 24 February 1998; Reserva Biológica de Una, Una, BA, 22 November 1996; VIC 19298, road Lajinha - Mutum, MG, 16 December 1996.

Phomopsis is considered a difficult genus in taxonomic terms because of its size (Uecker 1988 listed over 800 species) and the considerable morphological similarity between the species. No species belonging to *Phomopsis* has been recorded in association with *Miconia* spp. nor to any other member of the Melastomataceae. The lack of host-specificity in this genus coupled with the lack of major morphologically distinct features discourages the recognition of a separate taxon for the fungus on miconia*.* The pathological status of *Phomopsis* sp. on miconia is doubtful. Level of damage observed in the field was limited and it is possible that this may not be a pathogen but a secondary invader or even an endophyte. That, coupled with the fact that this fungus only occurred on *M. prasina* suggest that this fungus is of limited relevance to biocontrol.

***Pythium* sp.**

Disease (crown and root-rot): **Lesions** - general and sudden wilt of aerial parts accompanied by rot of plant base and roots.

Material examined: VIC 22216, Boca do Mato, Cachoeira do Macacú-RJ, 26 may 1998.

This straminipilous fungus was readily isolated from necrotic tissues but little attention was given to this fungus. This disease was found only once attacking two neighbouring *M. calvescens* plants, besides, pathogenic species of *Pythium*

are well known as having wide host-ranges and hence are inappropriate for classical biological control.

Other fungi on *Miconia* sp.

In addition to the above fungi, specimens of *Corticium* sp., *Phyllachora* sp., *Melanconium* sp., *Myrothecium* sp., and *Pestalotiopsis* sp. were collected on *M. calvescens* or other species of *Miconia.* None of these was regarded as being of interest for biocontrol and for the moment no further study was regarded as necessary on these fungi.

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