Foliicolous anamorphs of *Mycosphaerella* from South America

Pedro W. Crous¹, Ruth L. Benchimol², Fernando C. Albuquerque² & Acelino C. Alfenas³

¹ Department of Plant Pathology, University of Stellenbosch, P. Bag X1, Matieland 7602, South Africa
² Laboratório de Fitopatologia, Embrapa Amazônia Oriental, Caixa Postal 48, 66017-900, Belém, PA, Brazil
³ Departamento de Fitopatologia, Universidade Federal de Viçosa, 36570 Viçosa, MG, Brazil


One species of *Mycosphaerella*, and four anamorph species are newly described in the present study. *Passalora crotoniophila* comb. nov. is described from *Crotan* in Paraguay. New species from Brazil include: *Mycosphaerella bizae* sp. nov. and its *Passalora* anamorph from Bixa, *Pseudocercosporella cordifolia* sp. nov. from Cordia, *Pseudocercosporella macrospora* stat. nov. from Bertholletia, and *Pseudocercosporella xenanomincola* sp. nov. from Annona.

Keywords: *Mycosphaerella*, anamorph, *Passalora*, cercosporoid fungi, leaf spot diseases.

Although there are numerous cercosporoid fungi causing leaf spot diseases of plants in South America, these organisms have as yet been poorly studied. Furthermore, hardly any cultures have been obtained of these anamorphs, and very few have been linked to their respective *Mycosphaerella* teleomorphs by means of cultural studies. As part of a larger project aiming to establish such links, and confirming the phylogeny and host range of these fungi by means of molecular techniques (Crous & al., 1999; Crous & al., 2000), a special effort is being made to culture these fungi so that these issues can also be addressed.

In recent years several groups have attempted to revise herbarium and type specimens of many of the cercosporoids described from South America (Fons & Sutton, 1988; Inacio & al., 1996; Crous, 1998; Braun & al., 1999; Crous & al. 1999). Unfortunately, however, most of these studies have dealt with dried, preserved material only, and given the paucity and quality of material, it is unlikely that these collections could aid in molecular studies aiming at addressing the
issues outlined above. For this reason, new collections and sub-
sequent cultural and molecular studies are essential if we are to at-
tempt answering questions relating to phylogeny, teleomorph re-
lationships and host ranges of these fungi. The present study is part
of a revision of the cercosporoid species from South America, the
cultures of which will also be included in subsequent molecular
projects aimed at answering some of the questions outlined above.

Materials and methods

Specimens and isolates studied

Dilution plates were made on 2% malt-extract agar (Biolab,
Midrand, Johannesburg) (MEA) from mycelial and conidial scrapes
obtained from conidiomata sporulating on symptomatic leaves. After
24 h of incubation at 25 °C, germinated conidia were transferred to
MEA plates for a further incubation of 14 d at 25 °C in the dark. Se-
lected isolates were subcultured onto divided plates with one half
containing carnation-leaf agar (CLA) (Fisher & al., 1982; Crous & al.,
1992) and the other MEA, and incubated at 25 °C under continuous
near-ultraviolet light. Cultures were examined at weekly intervals
for sporulation. Linear growth on MEA plates was determined for
three single conidial isolates of each species afted 14 d of incubation
at 25 °C in the dark. Two perpendicular measurements were obtained
for each colony, and averages determined. Colony colours (top and
bottom) were subsequently rated on MEA using the colour charts of
Rayner (1970). Wherever possible, thirty measurements were made of
structures mounted in lactophenol, and the extremes given in par-
entheses. Type specimens were lodged at the National Collection of
Fungi in Pretoria (PREM), and ex-type cultures maintained in the
culture collection of the Department of Plant Pathology at the Uni-
versity of Stellenbosch, South Africa (STE-U).

Results and discussion

Mycosphaerella bixe Crous & R. L. Benchimol, sp. nov. - Figs. 1–3.

Anamorph. - Passalora sp.

Pseudothecia epiphyll, atra, subepidermalia, postes leviter erumpentia, glo-
bose, 50–80 μm diam.; paries compositus ex 2–3 stratis textureae angularis medio-
brunneae. Ascii sine paraphysibus, fasciculati, bitunicati, subsessiles obovoides ad
eilipsoidei, recti vel leviter incurvati, octospori, 25–35 × 7–10 μm. Ascosporae
multiseriatae, hyalinae, guttulatae, parietibus tenuibus, rectae, fusiforme-ellipso-
dae, extremis obtusis, latissimae ad medium cellulae apiculis, mediano 1-septatae,
8–10 × 2–3 μm. Caespituli fasciculati, amphigeni, brunnei, usque ad 50 μm lati et
70 μm alti. Conidiophora medio brunnea, levia, 1–4 septata, subcylindrica, recta ad

varie curvata, non ramosa, 20–70 × 3–4 μm. Cellulae conidiogena terminales, non ramosae, medio brunnea, leves, sympodis lifer proliferantes, 15–25 × 2.5–3.5 μm; cicatrices incrassatae, fuscatae et refractae. Conidia raro catenulata, catenis ramosis vel non ramosis, pallide brunnea, levia, angusta obclavata ad subcylindrica, apice obtusa, basis obconice truncata, recta ad leviter curvata, 3–7 septata, (35–)50–75 × (1.5–) 2.5–3.5 μm; hila incrassata, fuscata et refracta.

**Etymology.** named after its host, *Bixa*.

Leaf spots amphigenous, subcircular to irregular, 2–15 mm diam., coalescing with age, medium brown, surrounded by a red-purple border and a chlorotic zone. – *Pseudothecia* epiphyllous, black, subepidermal, becoming slightly erumpent, mostly under
conidiomatal fascicles, globose, 50–90 μm diam., apical ostiole 5–
10 μm wide; wall consisting of 2–3 layers of medium brown textura
angularis. — Asci aparyphylate, fasciculate, bitunicate, subsessile,
obovoid to ellipsoid, straight or slightly incurved, 8-spored, 25–
35 × 7–10 μm. — Ascospores multiseriate, overlapping, hyaline,
guttulate, thin-walled, straight, fusoid-ellipsoidal with obtuse ends,
widest in the middle of apical cell, medianly 1-septate or basal cell
slightly longer than apical cell, tapering towards both ends, but with
more prominent taper towards lower end, 8–10 × 2–3 μm, apical cell
4–5 μm, basal cell 4–6 μm long. — Mycelium internal and external,
light brown, consisting of septate, branched, smooth hyphae, 3–4 μm
wide. — Caespitulí fasciculate, amphiogenous, brown, up to 50 μm
wide and 70 μm high. — Conidiophores arising from superficial
mycelium, or aggregated in loose to dense fascicles arising from
the upper cells of a brown stroma up to 40 μm wide; conidiophores
medium brown, smooth, 1–4-septate, subcylindrical, straight to
variably curved, unbranched, 20–70 × 3–4 μm. — Conidiogenous
cells terminal, unbranched, medium brown, smooth, tapering to
flat-tipped apical loci, proliferating sympodially, 15–25 × 2.5–3.5 μm;
scars thickened, darkened and refractive. — Conidia rarely catenu-
late, chains branched or not, pale brown, smooth, narrowly obcla-
vate to subcylindrical, apex obtuse, base obconically truncate,
slightly curved, 3–7-septate, (25–)30–75 × (1.5–)2.5–
3.5(–4) μm; hila thickened, darkened and refractive.

Cultural characteristics. — Colonies pale mouse grey
(15°/d) (surface), and olivaceous black (27°m) (bottom), with even
margins and moderate aerial mycelium, obtaining 17 mm diam. after
14 d at 25 C in the dark.

Host. — Bixa orellana L. (Bixaceae).

Distribution. — Brazil.

Material examined. — Brazil, Amazonia, living leaves of B. orellana,
R. L. Benchimol & F. C. Albuquerque, Apr. 1999, PREM 56550 (holotype), culture
ex-type STE-U 2554.

Pseudocercospora bixae (Allesch. & F. Noack) Crous, Alfenas &
R.W. Baretto was recently re-described from Bixa orellana in Brazil
(Crous & al., 1997). P. bixae is distinguished from the Passalora
state of M. bixae by having narrower and shorter conidia (30–
130 × (2–)3–4 μm), and having unthickened conidial loci. Thus far no
species of Mycosphaerella has been described from Bixa. Although
the anamorphic connection was not established by means of single
ascospore cultures, conidiomata were clearly observed to fruit on the
subepidermal, erumpent pseudeothecia.
Passalora crotoniphila (Speg.) Crous, comb. nov. – Fig. 4.


Leaf spots hologenous, circular, 1–4 mm diam., brown throughout, or with a grey centre. – Mycelium internal and external, climbing leaf hairs, composed of smooth, branched, pale brown hyphae, 3–4 μm diam. – Caespituli amphigenous, brown, up to 50 μm wide and 40 μm high. – Conidiophores arising singly from superficial mycelium, or aggregated in dense fascicles, arising from the upper cells of a brown stroma up to 40 μm wide and 20 μm high; conidiophores pale brown, smooth, subcylindrical, 0–2-septate, straight to curved, unbranched, 10–30 x 4–5 μm. – Conidiogenous cells terminal, unbranched, pale brown, smooth, tapering rounded apices with flattened conidial loci, proliferating sympodially, 10–20 x 4–5 μm. – Conidia solitary, rarely catenulate, pale brown, smooth, obclavate, apex subobtuse to obtuse, base obconically trun-
cate, straight to slightly curved, 2–9-septate, (20–)45–70(–100) × (2.5–)3–4(–5) μm; hila unthickened.

Cultural characteristics. – No cultures are available for study.

Host. – Croton sp.

Distribution. – Paraguay.

Material examined. – Paraguay, Trinidad, on leaves of Croton sp., 1892, J. D. Anisitz no. 258 & 259 = LPS 857 (holotype).

This species was recently treated by Crous & al. (1999), and re-disposed to Pseudocercospora. As no type material could be obtained from LPS for examination, this decision was based on an examination of a Brazilian specimen (IACM 3721), which closely resembled the type description in general morphology. In the present study, the type of C. crotoniphila has been obtained and examined, and found to have smooth superficial mycelium climbing leaf hairs, and cate-nulate conidia with thickened hila, suggesting that this species would be better accommodated in Passalora. The separation of taxa in the Passalora/Mycovellosiella/Phaeoramularia-complex is not as clear as previously accepted (Crous & al., 2000), and is presently being revised (P. W. Crous, J. C. Kang & U. Braun, in prep.).

The collection treated as Pseudocercospora crotoniphila (IACM 3721) by Crous & al. (1999) is clearly a different taxon. While examining additional material from Croton collected by F. Freire in Brazil (U. Braun, pers. comm.), it was found that the material referred to as “P. crotoniphila” could be accommodated in P. tiglii (Henn.) Crous, U. Braun & Alfenas. The material collected by F. Freire had amphigenous fruiting, resembling the hypophyllous caespituli of C. tiglii (longer, looser, prominently branched conidiophores) and the epiphyllous caespituli of “P. crotoniphila” (shorter, denser conidiophores). It would seem, therefore, that IACM 3721 is only an epiphyllous form of P. tiglii, and that this collection can thus be treated as such.

Pseudocercospora cordiana Crous & R. L. Benchimol, sp. nov. – Figs. 5, 6.

Caespituli fasciculati, epiphylli, grisei, usque ad 70 μm lati et 35 μm alti. Conidiophora aggregata in densis fasciculis ex orientibus ex cellulis superioribus stromatis brunnel usque ad 45 μm lati et 35 μm alti; conidiophora pallide brunnea, levia, 0–2-septata, subcylindrica, recta ad sinuata, non ramosa, 15–25 × 2.5–4 μm. – Cellulæ conidiogenæ terminales, non ramosæ, pallide brunneæ, leves, in locis aplanatæ decrescentes, sympodialiter proliferantes, 7–15 × 2–3 μm. – Conidia
solitaria, pullide brunnea, levia, subcylindrica, apice anguste subobtuso, basis longa obconice truncata, varie curvata, raro recta, 1-3(-5)-septata, (30-)40-60 x 2-3 µm; hila inconspicua.

Etymology. — named after its host, Cordia.

Leaf spots hologenous, irregular to subcircular, 2-10 mm diam., dark brown. — Mycelium predominantly internal, pale brown, consisting of branched, smooth hyphae, 2-3 µm wide. — Caespituli fasciculate, epiphyllous, grey, up to 70 µm wide and 35 µm high. — Conidiophores aggregated in dense fascicles arising from the upper cells of a brown stroma up to 45 µm wide and 35 µm high; conidiophores pale brown, smooth, 0-2-septate, subcylindrical, straight to sinuous, unbranched, 15-25 x 2.5-4 µm. — Conidiogenous cells terminal, unbranched, pale brown, smooth, tapering to flat-tipped loci, proliferating sympodially, 7-15 x 2-3 µm. — Conidia solitary, pale brown, smooth, subcylindrical, apex narrowly subobtuse, base long obconically truncate, variously curved, rarely straight, 1-3(-5)-septate, (30-)40-60 x 2-3 µm; hila unthickened.

Cultural characteristics. — Colonies pale mouse grey (15°"d) (surface), and olivaceous black (27°"m) (bottom), with even margins and moderate aerial mycelium, obtaining 25 mm diam. after 14 d at 25 C in the dark.

Host. — Cordia goeldiana Huber (Boraginaceae).

Distribution. — Brazil.


Three cercosporoid taxa have been described from Cordia, namely Mycovelllosiella cordiicola (J. Yen) R. K. Verma & Kamal from Singapore, Stenella myxa (Syd.) J. Yen, Kar & Das from India, and Cercospora cordiae Chupp from Venezuela. The latter species was omitted from the monograph of Cercospora (Chupp, 1954), but was published in the series Monographs of the University of Puerto Rico (Chupp, 1934). It was described as not being associated with definite leaf spots, having hypophyllous fruiting, with superficial mycelium climbing leaf hairs, no fascicles, cylindrical to sometimes obclavate 1-septate conidia, with rounded bases and blunt apices, 12-20 x 2.5-4 µm, thus being clearly distinct from P. cordiana.
Leaf spots hologenous, confined by leaf veins, spots stretching in bands from midrib to margin, 3–10 mm diam., brown with dark brown margins. – Mycelium internal and external, medium brown, consisting of branched, smooth to finely verruculose hyphae, 3–4 μm wide. – Caespituli sporodochial, mainly epiphyllous, dark brown to black, up to 100 μm wide and 50 μm high. – Conidiophores aggregated in dense fascicles arising from the upper cells of a brown stroma up to 90 μm wide; conidiophores pale brown, smooth, 0–2(–4)-septate, subcylindrical, straight to slightly curved, unbranched, 10–45 × 4–5 μm. – Conidiogenous cells terminal, unbranched, pale brown, smooth, tapering to flat-tipped loci, proliferating sympodially, occasionally percurrently, 10–20 × 4–6 μm. – Conidia solitary, pale brown, smooth, shape variable, mostly subcylindrical to obclavate, apex obtuse, base truncate to obconically truncate, straight to slightly curved, (1–)3–6-septate, (17–) 30–50(–65) × (3–)4–5 μm; hila unthickened.

Cultural characteristics. – Colonies pale mouse grey (15“d”/d) (surface), and olivaceous black (27“m”) (bottom), with even margins and moderate aerial mycelium, obtaining 23 mm diam. after 14 d at 25°C in the dark.

Host. – Hancornia speciosa Gomes (Apocynaceae).

Distribution. – Brazil.

Material examined. – Brazil, Para, Belém, living leaves of H. speciosa, A. C. Allenas, 28 Apr. 1999, PREM 36593, culture STE-U 2558.

The morphology of the present collection correlates well with that of the holotype specimen. As cultures were obtained in the present study, a full cultural description is also provided. Furlanetto & Dianese (1999) discuss the morphological similarities of P. luzardii to other species in the Apocynaceae.

**Pseudocercospora macrospora** (Bat. & Peres) Crous & R. L. Benchimol, stat. nov. – Figs. 8, 9.


Leaf spots hologenous, circular to irregular, 2–15 mm diam., medium brown, surrounded by a chlorotic margin. – Mycelium
mostly internal, medium brown, consisting of branched, smooth hyphae, 2–3 \( \mu \text{m} \) wide. – Caespituli sporodochial, initially sub-epidermal, becoming erumpent at maturity, amphigenous, medium brown, up to 150 \( \mu \text{m} \) wide and 70 \( \mu \text{m} \) high. – Conidiophores aggregated in dense fascicles arising from the upper cells of a brown stroma up to 120 \( \mu \text{m} \) wide; conidiophores pale brown, smooth, 0–1-septate, subcylindrical, straight to variously curved, unbranched, 15–35 \( \times \) 4–6 \( \mu \text{m} \). – Conidiogenous cells terminal, unbranched,
paler brown, smooth, tapering to flat-tipped loci, proliferating sympodially, 5–10 × 3–4 μm. Conidia solitary, pale brown, smooth, guttulate, thick-walled, subcylindrical, apex obtuse, base truncate, straight to curved, 5–10-septate, (40–)50–75–(90) × 3–4 μm; hila unthickened.

Cultural characteristics. Colonies mouse grey (15""""11") (surface), and olivaceous black (27""""m) (bottom), with slightly irre-
gular margins and moderate aerial mycelium, reaching 17 mm diam. after 14 d at 25 C in the dark.

Host. – Bertholletia excelsa H.B.K. (Lecythidaceae).

Distribution. – Brazil.


Two cercosporoid species are known from Bertholletia in Brazil, namely C. bertholletiae Albuquerque and C. bertholletiae var. macrospora Bat. & Peres (Albuquerque, 1960; Batista & al., 1965). These two taxa are chiefly distinguished by C. bertholletiae having distinctly obclavate conidia, 28-64 × 4 μm (Albuquerque, 1960), while those of C. bertholletiae var. macrospora are cylindrical-obclavate, thick-walled, with unthickened, truncate bases, 35.5-85 × 3-3.5 μm. Although attempts to obtain the type specimen have proven unsuccessful, the description and illustration of the latter species (Batista & al., 1965) clearly resemble the specimen obtained in the present study. The pigmented conidia and unthickened hila determine that this collection would be better accommodated in Pseudocercospora.

Pseudocercospora xenoannonicola Crous & R. L. Benchimol, sp. nov. – Fig. 10.

Caespituli fasciculati, praecipue hypophylli, grisei ad medio brunnei, usque ad 60 μm lati et 35 μm alti. Conidiophora aggregata in densis fasciculis exorienti- bus ex cellulis superioribus stromatis brunnei usque ad 30 μm lati; conidiophora medio brunnea, levia, 0-2-septata, subcylindrica, recta ad geniculata sinuosa, non ramosa vel supra ramosa, 10-30 × 3-4 μm. Cellulae conidiogenae terminalis, non ramosae, pallide brunneae, leves, in loca plana decrescentes, sympodialiter proliferantes, 7-20 × 3-4 μm. Conidia solitaria, pallide brunnea, levia, anguste obclavata ad subcylindrica, apice obtuso ad subobtuso, unicum acro, basis anguste obconice truncata, recta ad curvata, 5-7-septata, (30-)50-80(-100) × 2-3 μm; hila inconspicua.

Etymology. – resembling P. annonicola, but morphologically distinct.

Leaf spots hologenous, subcircular to irregular, 7-40 mm diam., initially chlorotic yellow, becoming grey to pale brown in centre, dark brown in outer region, surrounded by a chlorotic margin. – Mycelium internal and external, pale brown, consisting of branched, smooth hyphae, 2-3 μm wide. – Caespituli fasciculate, mainly hypophyllous, grey to medium brown, up to 60 μm wide and
35 μm high. Conidiophores aggregated in dense fascicles arising from the upper cells of a brown stroma up to 30 μm wide; conidiophores medium-brown, smooth, 0–2-septate, subcylindrical, straight to genulate-sinuous, unbranched or branched above, 10–30 × 3–4 μm. Conidiogenous cells terminal, unbranched, pale brown, smooth, tapering to flat-tipped loci, proliferating sympodially, 7–20 × 3–4 μm. Conidia solitary, pale brown, smooth, narrowly obclavate to subcylindrical, apex obtuse to subobtuse, never acute, base narrowly obconically truncate, straight to curved, 5–7-septate, (20–)50–80(–100) × 2–3 μm; hila unthickened.

Cultural characteristics. Colonies olivaceous grey (25°5°b) (surface), and olivaceous black (27°7°m) (bottom), sectoring, with uneven, smooth margins and sparse aerial mycelium, obtaining 18–20 mm diam. after 14 d at 25°C in the dark.


Distribution. Brazil.


Several Cercospora species with pigmented conidia have been reported from Annonaceae. Two of these have subsequently been treated and placed in genera other than Pseudocercospora, namely Stenella annonaceae (Henn.) U. Braun and Stigmia oblecta (Syd.) U. Braun. Other species such as Cercospora ananae Muller & Chupp (50–150 × 5–6 μm) and Pseudocercospora scitula (Syd.) Deighton (50–110 × 5–8 μm), have conidia much wider than those of P. xenoannonicola. Three species, however, have conidia of similar dimensions, namely Pseudocercospora annonicola Goh & Hsieh (20–75 × 2.5–3 μm), Cercospora annonificii Bat. & Peres (23–241 × 3.5–5 μm), and Cercospora caracasensis Chupp & Muller (15–75 × 2.5–5 μm). P. annonicola is clearly distinct from P. xenoannonicola by having much smaller leaf spots (1–8 mm diam.), and conidia that have acute apices. C. annonificii has much longer, multisepate, verrucose conidia, and is illustrated by Batista & Peres (1964) to have thickened conidial hila, suggesting that it is probably a species of Stenella. C. caracasensis has wider conidia, angular leaf spots, and mostly epiphyllous fruiting.

None of the cultures of cercosporoid species studied here proved to be fertile on MEA or CLA. Given this fact, as well as their rather nondescript grey colonies, these cultures proved to be of limited value in providing additional features for species separation. However,
as reported by Crous (1998), this is not the case with all anamorphs of *Mycosphaerella*. Furthermore, the added advantage of molecular studies still provide sufficient motivation to obtain cultures of these fungi for future studies aiming to clarify their host range and phylogeny. In the present study we have also refrained from describing the *Passalora* anamorph of *Mycosphaerella bicae* as new. The description was, however, included as part of the holomorph. The reason for this is partly to move towards a system of integrating anamorph and teleomorph names, and partly because molecular data (Crous & al., 2000) have shown *Mycosphaerella* to be monophyletic, including these cercosporoid anamorphs.

References


(Manuscript accepted 28th June 2000)