Mycosphaerella eumusae and its anamorph *Pseudocercospora eumusae* spp. nov.: causal agent of eumusae leaf spot disease of banana

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The teleomorph name, *Mycosphaerella eumusae*, and its anamorph, *Pseudocercospora eumusae*, are validated for the banana disease formerly known as Septoria leaf spot. This disease has been found on different *Musa* cultivars from tropical countries such as southern India, Sri Lanka, Thailand, Malaysia, Vietnam, Mauritius and Nigeria. It is contrasted with two similar species, namely *Mycosphaerella fijiensis* (black leaf streak or black Sigatoka disease) and *Mycosphaerella musicola* (Sigatoka disease). Although the teleomorphs of these three species are morphologically similar, they are phylogenetically distinct and can also be distinguished based upon the morphology of their anamorphs.

Keywords: Leaf spot, *Musa*, *Mycosphaerella*, *Pseudocercospora*, systematics.

A wide range of important banana leaf spot diseases are commonly associated with species of *Mycosphaerella* Johanson and its anamorphs (Carlier & al., 2000a). Of these, the most important diseases are caused by *Mycosphaerella fijiensis* Morelet [anamorph: *Pseudocercospora fijiensis* (Morelet) Deighton; black leaf streak or black Sigatoka] and *M. musicola* R. Leach [anamorph: *Pseudocercospora musae* (Zimm.) Deighton; Sigatoka disease]. In a study to determine the population dynamics and spread of *M. fijiensis*, which is spreading into new banana-growing areas and replacing *M. musicola*, Carlier & al. (2000b) came across a common, but previously undescribed disease, which they attributed to *Mycosphaerella eumusae*. As the latter pathogen has never been formally described, the aim of the present paper is to validate the name, and compare it with two similar species, *M. fijiensis* and *M. musicola*.

Materials and methods

Single ascospore cultures were discharged onto 3% water agar using the technique as explained by Crous (1998). Isolates were cultured on divided plates containing 2% malt extract agar (MEA) (Oxoid) in the one half, and carnation-leaf agar (CLA) (Crous & al., 1992) in the other. Cultures were incubated at 25°C under near-ultraviolet light to enhance sporulation. The 95% confidence intervals (range) were derived from 30 observations of structures formed on carnation leaves; the extremes are given in parentheses. Isolates are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa (STE-U), and the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands. PCR amplification and sequencing of isolates were conducted in a previous study by Carlier & al. (2000b).

Description of species

Mycosphaerella eumusae Crous & X. Mourichon, **sp. nov.** – Figs. 1–12.

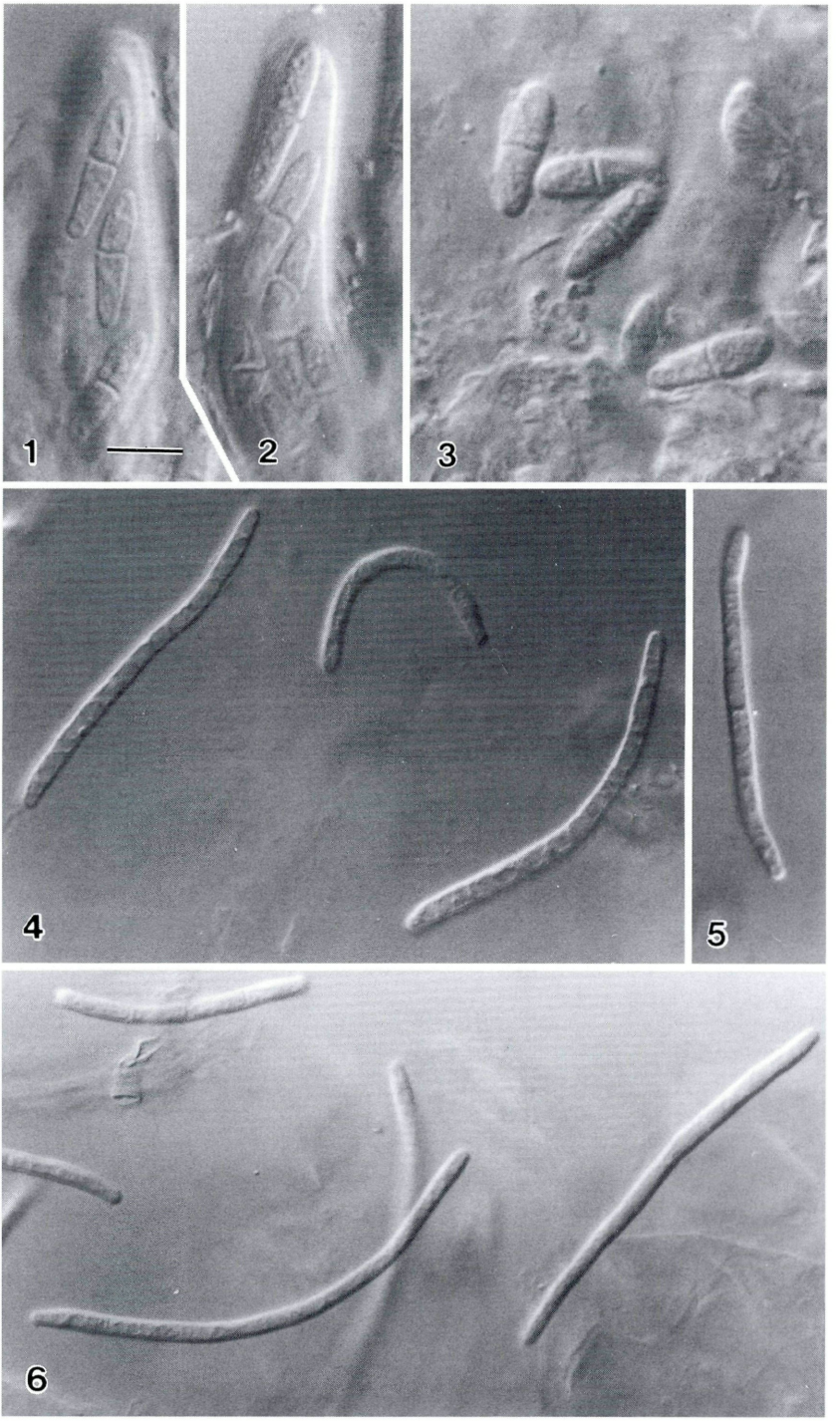
Anamorph: ***Pseudocercospora eumusae*** Crous & X. Mourichon, **sp. nov.**

Pseudothecia hypophylla, atra, subepidermalia, postea leviter erumpentia, globosa, ad 80 µm diam; paries ex 2–3 stratis texturae angularis medio brunneae compositus. Asci sine paraphysibus, fasciculati, bitunicati, subsessiles obovoidei, recti vel leviter incurvati, octo-spori, 30–50 × 9–15 µm. Ascosporae tri- ad multiseriatae, hyalinae, guttulatae, parietibus tenuibus, rectae, obovoideae, extremis obtusis, latissimae ad medium cellulae apicalis, mediano 1-septatae, (11–)12–13–(–16.5) × (3–)3.5–4(–4.5) µm.

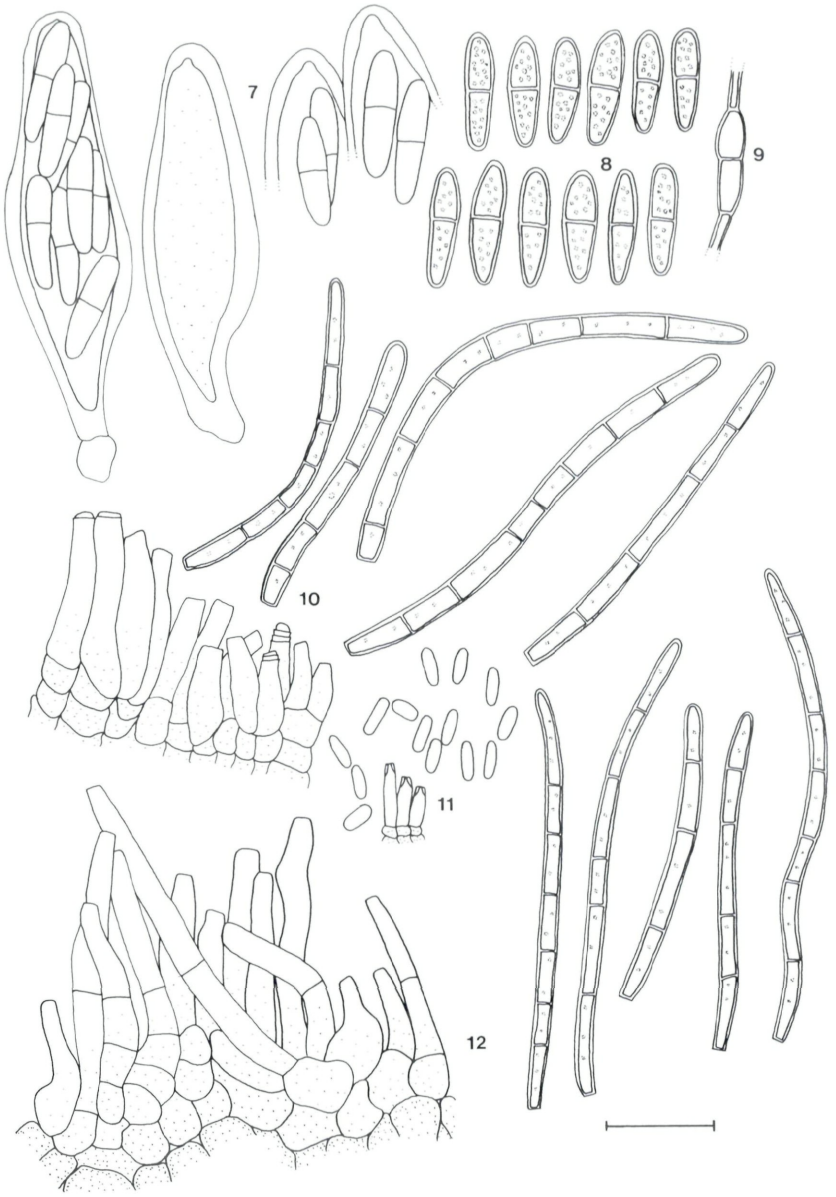
Caespituli sporodochiales, epiphylli, cani, usque ad 100 µm lati. Conidiophora hyalina, levia, 0–3-septata, subcylindrica, 10–25 × 3–5 µm. Cellulae conidiogenae terminales, hyalinae, leves, 10–20 × 3–4 µm. Conidia hyalina, levia, subcylindrica, apice obtuso, basi subtruncata, recta ad leviter curvata, 3–8-septata, (18–)30–50–(–65) × (2–)2.5–3 µm.

Etymology. – Named after its host, *Musa*.

Leaf spots amphigenous, initially visible as faint brown streaks, developing into oval or elliptical light brown lesions with grey centres and dark brown borders, coalescing to form large, brown, necrotic areas under favourable conditions. Grey spots and patches are visible in necrotic areas, and lesions are surrounded by a chlorotic yellow zone. – Pseudothecia amphigenous, predominantly hypophyllous, black, subepidermal, becoming slightly erumpent, globose, up to 80 µm diam., apical ostiole 10–15 µm wide; wall consisting of 2–3 layers of medium brown textura angularis. – Asci aparaphysate, fasciculate, bitunicate, subsessile, obovoid, straight or slightly incurved, 8-spored, 30–50 × 9–15 µm. – Ascospores tri- to



Figs. 1–6. – *Mycosphaerella eumusae* and its *Pseudocercospora eumusae* anamorph (holotype). – 1–3. Asci and ascospores in vivo. – 4, 5. Conidia in vivo. – 6. Conidia in vitro on CLA. – Bar = 10 µm.



Figs. 7–12. – *Mycosphaerella eumusae* and its *Pseudocercospora eumusae* anamorph (holotype). – 7. Asci, showing apical chamber. – 8. Ascospores. – 9. Germinating ascospore on leaf surface. – 10. Conidiophores and conidia in vivo. – 11. Spermatophores and spermatia. – 12. Conidiophores and conidia in vitro. – Bar = 10 µm.

multiseriate, overlapping, hyaline, guttulate, thick-walled, straight, obovoid 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, (11–)12–13(–16.5) × (3–)3.5–4(–4.5) µm. – Spermogonia predominantly hypophyllous, subepidermal, substomatal, globose, dark brown, up to 75 m diam. – Spermatia hyaline, rod-shaped, 3–6 × 1–2 µm. – Mycelium internal, pale brown, consisting of septate, branched, smooth hyphae, 1.5–2.5 µm wide. – Caespituli sporodochial, subepidermal, substomatal, predominantly epiphyllous, grey, up to 100 µm wide. – Conidiophores aggregated in dense fascicles arising from the upper cells of a brown stroma up to 70 µm wide; conidiophores subcylindrical, smooth, hyaline or pale brown below, 0–3-septate, straight to geniculate-sinuous, unbranched or branched below, 10–25 × 3–5 µm. – Conidiogenous cells terminal, unbranched, hyaline, smooth, tapering to flat-tipped apical loci, proliferating sympodially, or 1–4 times percurrently near the apex, 10–20 × 3–4 µm; scars inconspicuous. – Conidia solitary, subhyaline to pale olivaceous, thick-walled, smooth, subcylindrical, apex obtuse, base subtruncate, straight to variously curved, 3–8-septate, (18–)30–50–(–65) × (2–)2.5–3 µm; hila inconspicuous.

Ascospore germination after 24 h on water agar. Ascospores germinate predominantly from one end, with germ tubes growing parallel to the long axis of the spore (Carlier & al., 2000b). Ascospores remain hyaline, but become slightly constricted at the median septum.

Cultural characteristics. – Colonies pale olivaceous grey (23''''d) to rosy vinaceous (7''d) (surface), and brown vinaceous (5'''m) (bottom) (Rayner, 1970), with even margins and moderate aerial mycelium, obtaining 10 mm diam. after 2 mo at 25 °C in the dark.

Hosts. – *Musa* spp. (Musaceae).

Distribution. – Southern India, Sri Lanka, Thailand, Malaysia, Vietnam, Mauritius, Nigeria.

Specimens examined. – RÉUNION, on leaves of *Musa* sp., J. Carlier, 2001, PREM 57314 (holotype of teleomorph), PREM 57315 (holotype of anamorph) cultures ex-type (CIRAD 1156, 1157 = STE-U 4579, 4580). INDIA, Kannara, on leaves of *Musa* cv. Grande Naine AAA, J. Carlier, 1995, CIRAD 535 = STE-U 4557. MALAYSIA, Johor State, on leaves of *Musa* cv. Pisang Kapas AAB, J. Carlier, 1993, CIRAD 458 = STE-U 4578. MAURITIUS, on leaves of *Musa* cv. Grande Naine AAA, J. Carlier, 1997, CIRAD 744 = STE-U 4559. NIGERIA, on leaves of *Musa* sp., J. Carlier, CIRAD 1088 = STE-U 4560. SRI LANKA, Gannoruwa, on leaves of *Musa* cv. Cavendish AAA, J. Carlier, 1995, CIRAD 554 = STE-U 4558. THAILAND, Tha Yang, on leaves of *Musa* cv. Kluai Hom Tong AAA, J. Carlier, 1994, CIRAD 487 = STE-U 4561. VIETNAM, Mekong Delta, on leaves of *Musa* cv. Pisang Mas AAA, J. Carlier, 1995, CIRAD 670 = STE-U 4562.

Three species of *Mycosphaerella* are now known to cause Sigatoka-like symptoms, namely *M. fijiensis*, *M. musicola* and *M. eumusae*. *M. fijiensis* can be distinguished from the latter two species by its symtomatology and morphology. Fouré (1987) identified six main stages in symptom expression, the most characteristic being stage two, when the streaks on the upper surface changes from brown to black, but the underside remains brown, from where the streaks coalesce and form brown spots, which often remain black on the upper surface. The final stage, stage six, is represented by grey leaf spots with dark borders. On growing plants streaks are usually present on the third, fourth and fifth leaves, and streaks and spots on the fifth and older leaves (Carlier & al., 2000a). Symptoms of *M. musicola* first become visible as light green specks which develop into streaks that elongate to form elliptical spots surrounded by water-soaked halos. Spots turn brown, and later grey. The leaf tissue surrounding the spots turns yellow, forming a border around the grey spot. The yellow halo is particularly characteristic of this disease. *Mycosphaerella eumusae* is associated with symptoms similar to those of *M. fijiensis* and *M. musicola*. Initial symptoms are brown streaks that expand to form large brown spots. These spots darken, and later become grey with a dark brown border. Mature spots are generally larger than those of *M. fijiensis* and *M. musicola*.

Although the *Mycosphaerella* states of all three species are largely similar (Carlier & al., 2000b), their anamorphs are distinct. *Pseudocercospora fijiensis*, the anamorph of *M. fijiensis*, is distinguishable from the other two taxa by having predominantly hypophyllous fascicles that consist of pale brown, 0–5-septate, straight to geniculate, occasionally branched, subcylindric conidiophores, 16.5–62.5 × 4–7 µm. Conidiogenous cells are up to 25 µm long, 2–4 µm wide at the apex, and have 1–3 minutely thickened scars. Conidia are subhyaline, obclavate to cylindric-obclavate, have an obclavate basal cell, (1–)5–7(–10)-septate, 10–120 × 2.5–5 µm, with hila that are slightly thickened and darkened (not refractive) along the rim (Pons, 1987; Carlier & al., 2000a).

Pseudocercospora musae, the anamorph of *M. musicola*, is characterized by having amphigenous sporodochia that form on dark brown substomatal stromata. Conidiophores are pale brown, aseptate, unbranched, straight, mostly reduced to ampulliform conidiogenous cells, 5–25 µm in length, lacking any visible scars. Conidia are smooth, pale olivaceous, cylindrical to cylindric-obclavate, straight to curved, (0–)2–5(–8)-septate, 10–80 × 2–6 µm, and have subtruncate to obclavate ends without any visible scars (Carlier & al., 2000a). Guo & Hsieh (1995) illustrate Chinese collections to have dense fascicles, with conidiophores reduced to olivaceous-brown conidiogenous cells with rounded apices, 6.5–22 × 2.5–4 µm. Conidia

are described as being obclavate-cylindrical, pale olivaceous with an obconically truncate base, 3–10-septate, 30–100 × 2.5–4 µm. In his illustration of the holotype collection of *P. musae* (IMI 107272), Pons (1989) clearly showed conidia to be pale olivaceous, obclavate to subcylindrical, and to have obconically truncate basal cells, and conidiophores to be reduced to doliiform or ampulliform conidiogenous cells.

Pseudocercospora eumusae, the anamorph of *M. eumusae*, is characterized by having predominantly epiphyllous sporodochia that form on dark brown substomatal stromata. Conidiophores are subhyaline to pale olivaceous, becoming pale brown at the base, subcylindrical, 0–3-septate, 10–25 × 3–5 µm, with conidiogenous cells terminating in truncate ends. Although sporodochia of *M. eumusae* develop in a similar fashion to those of *M. musicola*, the conidiophores are much longer and more septate in the former. Conidia of *P. eumusae* are subhyaline to pale olivaceous, subcylindrical, (18–)30–50(–65) × (2–)2.5–3 µm, 3–8-septate, and have subtruncate ends without any visible scars. Conidia can be distinguished from those of *M. musicola* by their more cylindrical shape, subtruncate ends, and shorter dimensions.

With the description of *Pseudocercospora eumusae* as anamorph of *M. eumusae*, the question remains as to how this anamorph could have been confused as a species of *Septoria* Sacc. To understand this situation, one has to look at its unique mode of development. Conidiophores are arranged in dense sporodochia that develop in the substomatal cavities, mainly on the upper leaf surface. These are mingled with developing spermatogonia. Young sporodochia are subepidermal and substomatal, and initially produce conidia that appear to be exuding from a subepidermal, substomatal pycnidium. In section, however, the subepidermal and substomatal structure is seen to be a sporodochium, not a pycnidium. As more stromatal tissue is formed, conidiophores become erumpent, and sporodochia burst through the epidermis, almost appearing acervular, but in fact being subepidermal sporodochia. Conidia and conidiophores are also hyaline to pale olivaceous, thus resembling those of other species accommodated in *Septoria*. Recent molecular data have shown, however, that most anamorph form genera have evolved several times in *Mycosphaerella* (Crous & al., 2001). Within *Mycosphaerella*, therefore, the anamorph state is generally not phylogenetically informative. However, this state still represents the part of the holomorph that is morphologically the most informative in distinguishing different taxa.

In spite of the morphological differences that exist among these taxa, it is obvious that *M. eumusae* is morphologically very similar to *M. musicola*. Furthermore, literature suggests that these two patho-

gens have commonly been confused in the past, thereby also disputing the value of much of the published literature on this disease complex. Leaf spot symptoms attributed to *M. eumusae* first came to attention after a survey conducted in Southeast Asia during 1992–1995 (Carlier & al., 2000b). For reasons explained above, the anamorph of *M. eumusae* was initially regarded as being a species of *Septoria*, hence the name Septoria leaf spot disease (Carlier & al., 2000a, b). The naming of diseases after fungal genera is problematic, as these are bound to change as new data becomes available, creating confusion as to what the causal organism may be, or what the disease should be called. A similar example is black Sigatoka, where the anamorph was initially described in *Cercospora* Fresen., later transferred into *Pseudocercospora* Speg. due to its pigmented conidia, and later again to *Paracercospora* Deighton, due to the minutely thickened spore scars (Deighton, 1979). Subsequent to this, however, molecular data have proven that these minutely thickened scars are not phylogenetically important in the cercosporoids (Stewart & al., 1999), and that *Paracercospora* should be merged back into *Pseudocercospora* (Crous & al., 2000, 2001). The anamorph of *M. fijiensis*, therefore, is now correctly referred to as *Pseudocercospora fijiensis*. For this reason, it is firstly preferable to refer to the fungus based on its teleomorph name where this is known, and secondly preferable to name diseases based on some feature other than the fungus itself. The naming of diseases based on symptoms is also problematic. For instance, Black Goo disease or Phaeoacremonium young vine decline of grapevines was initially linked to several species of *Phaeoacremonium* W. Gams & al. (Crous & al., 1996). The main pathogen was, however, recently placed in a separate genus, *Phaeomoniella* Crous & W. Gams (Crous & Gams, 2000; Groenewald & al., 2000). Several additional fungi have now been linked to Black Goo symptoms, while the fungal disease name *Phaeoacremonium*, is also not suitable for the disease. The final solution, therefore, was to name the disease after its founder, Petri, hence Petri disease. This brings us to the problem of naming the leaf spot disease caused by *M. eumusae*. For the sake of consistency, therefore, we herewith propose the name eumusae leaf spot for the disease formerly known as Septoria leaf spot of banana (Carlier & al., 2000b).

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