Phylogeny of some cercosporoid fungi from Citrus

Mathys C. Pretorius¹, Pedro W. Crous¹,², J. Z. (Ewald) Groenewald¹ & Uwe Braun³

¹ Department of Plant Pathology, University of Stellenbosch, P. Bag X1, Matieland 7602, South Africa
² Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
³ Martin-Luther-Universität, FB. Biologie, Institut für Geobotanik und Botanischer Garten, Neuwerk 21, D-06099 Halle (Saale), Germany


This study examines several cercosporoid species that are known to cause foliar diseases of Citrus. A cercosporoid fungus causing a new fruit and leaf spot disease on Citrus in South Africa was identified. From morphological and rDNA sequence data (ITS1, 5.8S and ITS2), it was concluded that the new disease was caused by Cercospora penzigii, belonging to the Cercospora apii species complex. It was subsequently compared with a similar organism, Pseudophaeoramularia angolensis, which is of quarantine significance to the citrus industry. The genus Pseudophaeoramularia is regarded as synonym of Pseudocercospora, and subsequently a new combination is proposed in Pseudocercospora as P. angolensis. Cercospora gigantea was shown to not represent a species of Cercospora, while Mycosphaerella citri was found to be morphologically variable, suggesting that it could represent more than one taxon. A key is also provided to the cercosporoid species occurring on Citrus.

Keywords: Cercospora, Citrus, Leaf spot, Mycosphaerella, Pseudocercospora, systematics.

A wide range of Mycosphaerella Johanson species with cercosporoid anamorphs are commonly associated with fruit and leaf spot diseases of species of Citrus L. Of these, two are regarded as being particularly serious. Greasy spot, caused by Mycosphaerella citri Whiteside (anamorph Stenella citri-grisea (F.E. Fisher) Sivan.) (Sivanesan, 1984), occurs in Florida and Texas (USA), the Caribbean, and Central and South America (Timmer & Gottwald, 2000). Phaeoramularia fruit and leaf spot, caused by Pseudophaeoramularia angolensis (T. Carvalho & O. Mendes) U. Braun, is common in sub-Saharan Africa, the Comoro Islands, and has also been reported from Yemen on the Arabian Peninsula (Seif, 2000). The most devastating effect of Phaeoramularia fruit and leaf spot is the development of fruit spots, which render the crop unmarketable. A yield loss of 50–100% is common in highly effected areas (Seif, 1995). As
Phaeoramularia fruit and leaf spot also occurs in Zimbabwe, which borders South Africa, it is of particular concern to the local citrus industry. Although the disease is presently restricted to two areas north of Harare in Zimbabwe, it has not yet spread to South Africa (Crous & al., 2000b), presumably due to unfavourable climatic conditions. This organism, however, is still regarded as of extreme phytosanitary importance.

During the course of 2000, previously unknown leaf and fruit spot disease symptoms were found associated with species of *Citrus* cultivated in Swaziland, and the Northern and Mpumalanga Provinces of South Africa. Although symptoms were not as severe as for Phaeoramularia fruit and leaf spot, the new cercosporoid disease was still regarded as a potential threat for *Citrus* cultivation. The aim of the present study, therefore, was to compare the *Cercospora* Fresen. isolates from Swaziland and South Africa to determine whether they belong to the same species, and to determine their identity. These isolates were also compared with other cercosporoid fungi occurring on *Citrus* spp., and specifically to *C. apii* Fresen., to which they were morphologically similar.

**Materials and methods**

**Morphology**

Herbarium and type specimens were obtained from USDA U.S. National Fungus Collections, Beltsville (BPI), CABI Bioscience, Egham, England (IMI), and the Department of Plant Pathology at the University of Florida (F). Morphological observations were made on structures mounted in clear lactophenol, and descriptions were based on collections from host material. All measurements were derived from at least 30 observations of each respective structure. Cultures were obtained from freshly collected field material (*Cercospora* sp. and *P. angolensis*) by establishing colonies from single conidia on 2% malt extract agar (MEA) (Biolab, Midrand, Johannesburg). 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**

The isolation protocol of Crous & al. (2000a) was used to isolate genomic DNA from fungal mycelia grown on MEA plates. The primers ITS1 and ITS4 were used to amplify part of the nuclear rRNA
operon using the PCR conditions recommended by the authors (White & al., 1990). The amplified region included the 3’ end of the 18S (small subunit) rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS (ITS2) region and the 5’ end of the 28S (large subunit) of the rRNA gene. PCR products were separated by electrophoresis at 75 V for 1 h in a 0.8% (w/v) agarose gel in 0.5 x TAE buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.85) and visualised under UV light using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, UK) following ethidium bromide staining.

PCR products were purified by using a NucleoSpin Extract 2 in 1 Purification Kit (Macherey-Nagel GmbH, Germany). The cycle sequencing reaction of purified PCR products was carried out with an ABI PRISM BigDye Terminator v3.0 Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA, USA) following the instructions of the manufacturer. The resulting fragments were analysed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut). Isolates subjected to molecular analysis are listed in Tab. 1. The unidentified Cercospora isolates from Citrus were compared to other species of Cercospora, and to C. apii, from which they were morphologically indistinguishable.

**Phylogenetic analysis**

The nucleotide sequences generated in this study were added to a previously published data matrix (TreeBase M691, Stewart & al., 1999). Mycocentrospora acerina (R. Hartig) Deighton AY266155 served as outgroup. Sequences were assembled using the editor in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b8a (Swofford, 2000), and aligned using the CLUSTAL W software (Thompson & al., 1994). Adjustments for improvement were done manually where necessary. Phylogenetic analyses were undertaken using PAUP. Gaps were treated as a new state and all characters were unordered and weighted equally. Heuristic searches were conducted using stepwise simple addition and tree bisection and reconstruction (TBR). The robustness of the branches was evaluated by 1,000 bootstrap replications (Hillis & Bull, 1993). A second parsimony analysis was also performed for which all missing and ambiguous characters were excluded. Tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC, respectively) were also calculated. Resulting trees were printed with TreeView Version 1.6.5 (Page, 1996) and decay indices were calculated with AutoDecay Version 4.0.2 (Eriksson, 1998). Sequences were deposited at GenBank (Tab. 1), and the alignment in TreeBase (submission number SN1397).
Nucleotide differences between and within *Cercospora* species

The number and type of nucleotide differences between the 25 *Cercospora* sequences used in this study were tabulated using *C. apii* Fresen. AY266168 (TreeBase matrix M691; Stewart & al., 1999) as reference sequence. The differences within isolates of *C. penzigii* Sacc. were calculated separately. Separate counts for transversions, transitions, insertions and deletions in the ITS1, 5.8S and ITS2 regions, respectively, were made for all of the *Cercospora* sequences included in this paper.

**Results**

**Morphology**

Isolates causing the new disease on *Citrus* in Swaziland and South Africa were morphologically similar and were indistinguishable from *C. penzigii*, which is the common species of *Cercospora* occurring on this host (Chupp, 1954). They had long, fasciculate, septate, smooth, pigmented conidiophores with thickened, darkened and refractive loci. Fully developed long conidia were acicular with truncate bases, whereas young, shorter conidia were obclavate to subcylindrical with obconically subtruncate bases and darkened, thickened, refractive hila. *Cercospora apii* is a species with a wide host range and geographical distribution (Pons & Sutton, 1988), with which *C. penzigii* appears to be synonymous.

**Sequence alignment**

All the *Cercospora* sequences used in the phylogenetic analysis, except for ‘*C. oryzae*’ STE-U 4303 (one nucleotide shorter) and *C. asparagi* Sacc. AF297229 (one nucleotide longer), were exactly the same length (462 bp, including 5 bp of the 3’ end of the 18S rDNA gene and 11 bp of the 5’ end of the 28S rDNA gene) when alignment gaps were excluded. The alignment contained the complete sequences of the 5.8S rRNA gene, the second ITS (ITS2) region and the 5’ end of the 28S (large subunit) of the rRNA gene. The complete ITS1 region was not included in the phylogenetic analysis of this study as the sequences of *P. angolensis* STE-U 4115, 4116 and 4118 included in the alignment did not contain the first eighteen nucleotides of the ITS1 region. For counting the nucleotide changes between the *Cercospora* species, however, the complete ITS1 was included. The manually adjusted alignments of the nucleotide sequences contained 520 sites for the data set (data not shown).
Tab. 1. - Isolates of cercosporoid species sequenced.

<table>
<thead>
<tr>
<th>Anamorph</th>
<th>Teleomorph</th>
<th>Host</th>
<th>Origin</th>
<th>Collector</th>
<th>Date isolated</th>
<th>Accession no.</th>
<th>GenBank no.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cercospora canescens</em></td>
<td>Unknown</td>
<td>Vigna</td>
<td>Free State, South Africa</td>
<td>P. S. Van Wyk</td>
<td>1995</td>
<td>STE-U 1137</td>
<td>AY260065</td>
</tr>
<tr>
<td><em>C. canescens</em></td>
<td>Unknown</td>
<td>Vigna</td>
<td>Free State, South Africa</td>
<td>P. S. Van Wyk</td>
<td>1995</td>
<td>STE-U 1138</td>
<td>AY260066</td>
</tr>
<tr>
<td>'C. oryzae'</td>
<td>'Sphaerulina oryza'</td>
<td>Oryza</td>
<td>Arkansas, U.S.A.</td>
<td>E. C. Tullis</td>
<td>–</td>
<td>STE-U 4303, IMI 303642, CBS 145.37</td>
<td>AY260064</td>
</tr>
<tr>
<td><em>C. penzigii</em></td>
<td>Unknown</td>
<td>Citrus</td>
<td>Northern Province, South Africa</td>
<td>K. Serfontein</td>
<td>2000</td>
<td>STE-U 4408</td>
<td>AY260067</td>
</tr>
<tr>
<td><em>C. penzigii</em></td>
<td>Unknown</td>
<td>Citrus</td>
<td>Northern Province, South Africa</td>
<td>K. Serfontein</td>
<td>2000</td>
<td>STE-U 4409</td>
<td>AY260068</td>
</tr>
<tr>
<td><em>C. penzigii</em></td>
<td>Unknown</td>
<td>Citrus</td>
<td>Northern Province, South Africa</td>
<td>M. C. Pretorius</td>
<td>2000</td>
<td>STE-U 4410</td>
<td>AY260070</td>
</tr>
<tr>
<td><em>C. penzigii</em></td>
<td>Unknown</td>
<td>Citrus</td>
<td>Northern Province, South Africa</td>
<td>M. C. Pretorius</td>
<td>2000</td>
<td>STE-U 4411</td>
<td>AY260071</td>
</tr>
<tr>
<td><em>C. penzigii</em></td>
<td>Unknown</td>
<td>Citrus</td>
<td>Swaziland</td>
<td>M. C. Pretorius</td>
<td>2000</td>
<td>STE-U 3946</td>
<td>AY260072</td>
</tr>
<tr>
<td><em>C. penzigii</em></td>
<td>Unknown</td>
<td>Citrus</td>
<td>Swaziland</td>
<td>M. C. Pretorius</td>
<td>2000</td>
<td>STE-U 3947</td>
<td>AY260073</td>
</tr>
<tr>
<td><em>C. penzigii</em></td>
<td>Unknown</td>
<td>Citrus</td>
<td>Swaziland</td>
<td>M. C. Pretorius</td>
<td>2000</td>
<td>STE-U 3945</td>
<td>AY260074</td>
</tr>
<tr>
<td><em>C. penzigii</em></td>
<td>Unknown</td>
<td>Citrus</td>
<td>Mpumalanga, South Africa</td>
<td>M. C. Pretorius</td>
<td>2000</td>
<td>STE-U 3948</td>
<td>AY260075</td>
</tr>
<tr>
<td><em>C. penzigii</em></td>
<td>Unknown</td>
<td>Citrus</td>
<td>Northern Province, South Africa</td>
<td>M. C. Pretorius</td>
<td>2000</td>
<td>STE-U 3949</td>
<td>AY260076</td>
</tr>
<tr>
<td><em>C. penzigii</em></td>
<td>Unknown</td>
<td>Citrus</td>
<td>Northern Province, South Africa</td>
<td>M. C. Pretorius</td>
<td>2000</td>
<td>STE-U 3950</td>
<td>AY260077</td>
</tr>
<tr>
<td>Anamorph</td>
<td>Teleomorph</td>
<td>Host</td>
<td>Origin</td>
<td>Collector</td>
<td>Date isolated</td>
<td>Accession no.</td>
<td>GenBank no.</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------------</td>
<td>------------</td>
<td>---------------------------------</td>
<td>------------------</td>
<td>---------------</td>
<td>---------------</td>
<td>-------------</td>
</tr>
<tr>
<td><em>C. populicola</em></td>
<td>Unknown</td>
<td><em>Populus</em></td>
<td>KwaZulu-Natal, South Africa</td>
<td>M. J. Wingfield</td>
<td>1995</td>
<td>STE-U 1051</td>
<td>AY260069</td>
</tr>
<tr>
<td><em>C. zebrina</em></td>
<td>Unknown</td>
<td><em>Trifolium pratense</em></td>
<td>Ottawa, Canada</td>
<td>K. A. Seifert</td>
<td>2000</td>
<td>STE-U 3955</td>
<td>AY260078</td>
</tr>
<tr>
<td><em>C. zebrina</em></td>
<td>Unknown</td>
<td><em>Trifolium repens</em></td>
<td>Ottawa, Canada</td>
<td>K. A. Seifert</td>
<td>2000</td>
<td>STE-U 3957</td>
<td>AY260079</td>
</tr>
<tr>
<td><em>C. zebrina</em></td>
<td>Unknown</td>
<td><em>Trifolium repens</em></td>
<td>Ottawa, Canada</td>
<td>K. A. Seifert</td>
<td>2000</td>
<td>STE-U 3958</td>
<td>AY260080</td>
</tr>
<tr>
<td><em>Pseudocercospora angolensis</em></td>
<td>Unknown</td>
<td><em>Citrus</em></td>
<td>Zimbabwe</td>
<td>M. C. Pretorius</td>
<td>2000</td>
<td>STE-U 4116</td>
<td>AY260061</td>
</tr>
<tr>
<td><em>P. angolensis</em></td>
<td>Unknown</td>
<td><em>Citrus</em></td>
<td>Zimbabwe</td>
<td>M. C. Pretorius</td>
<td>2000</td>
<td>STE-U 4115</td>
<td>AY260062</td>
</tr>
<tr>
<td><em>P. angolensis</em></td>
<td>Unknown</td>
<td><em>Citrus</em></td>
<td>Zimbabwe</td>
<td>M. C. Pretorius</td>
<td>2000</td>
<td>STE-U 4118</td>
<td>AY260063</td>
</tr>
<tr>
<td><em>Pseudocercospora</em></td>
<td><em>Mycosphaerella</em></td>
<td><em>Acacia</em></td>
<td>Venezuela</td>
<td>M. J. Wingfield</td>
<td>2000</td>
<td>STE-U 3837</td>
<td>AY260060</td>
</tr>
</tbody>
</table>
the aligned nucleotide sites for the data set, 166 characters were parsimony-informative, 127 variable characters were parsimony-uninformative and 227 were constant.

Phylogenetic relationships

The aligned sequences of 37 isolates and an outgroup were subjected to maximum parsimony analysis, and only a single most parsimonious tree was obtained and evaluated with 1,000 bootstrap replications. All 25 Cercospora sequences grouped in a strongly supported clade (99% support) (Fig. 1) as did Pseudocercospora Spec. (99%) and Stenella Syd. (100%). In the main Cercospora clade, 'C. oryzae' [= Passalora jansaeana (Racib.) U. Braun] STE-U 4303 and 'C. canescens' AY266164 (TreeBase matrix M691; Stewart & al., 1999) (identifications could not be confirmed) were found outside a clade containing the rest of the Cercospora species (74%). Cercospora zebra Pass., a species with acicular to cylindrical-filiform conidia, formed a clade with a bootstrap support value of 63% within the larger Cercospora clade. Excluding all missing and ambiguous characters from the analysis did not change the topology of the tree.

Nucleotide differences between and within Cercospora species

The decrease in length for 'C. oryzae' STE-U 4303 can be ascribed to a deletion of a G at character 357, and the increase in length for C. asparagi AF297229 can be accounted for by an extra C at character 101 of the alignment (Tab. 2). Eight isolates had sequences identical to C. apii CA1 (TreeBase matrix M691; Stewart & al., 1999): C. canescens Ellis & G. Martin STE-U 1137 & 1138, C. nicotianae Ellis & Everh. AF297230, C. sorghi Ellis & Everh. f. maydis AF297232, C. beticola Sacc. AF222827, C. penzigii STE-U 4408 & 4409 and C. hayi Calp. CH6 (TreeBase matrix M691, Stewart & al. 1999). Of the remaining sixteen isolates, 'C. oryzae' STE-U 4303 and 'C. canescens' AY266164 (TreeBase matrix M691, Stewart & al. 1999) differed most from C. apii AY266168 (TreeBase matrix M691, Stewart & al. 1999), with changes at nine and three positions respectively. Eighteen changes were observed for the eleven Cercospora species studied (Tab. 2), resulting in a difference of 1.64 (18 changes over 11 species) nucleotides between species. Goodwin & al. (2001) calculated an overall mean of 1.27 differences between taxa in their Cercospora cluster, which is slightly lower than what we found. This might be ascribed to the sampling of 18 isolates representing 11 species by Goodwin & al. (2001) whereas 25 isolates representing 11
species were sampled in the present study. Within *Cercospora*, twelve transitions, four transversions and a single duplication and deletion were observed. Goodwin & al. (2001) also observed more transitions than transversions for *Cercospora* and *Mycosphaerella* based on the ITS region.
Tab. 2. - Nucleotide differences observed for Cercospora species included in this study. Base positions include spaces caused by alignment gaps.

<table>
<thead>
<tr>
<th>Species</th>
<th>ITS1</th>
<th>5.8S rRNA gene</th>
<th>ITS2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. apii CAI^5,6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. asparagi AF297229</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. canescens CCA196</td>
<td>T</td>
<td>C</td>
<td>T</td>
</tr>
<tr>
<td>C. oryzae STE-U 4303</td>
<td>C^3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. penzigii STE-U 4410</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. penzigii STE-U 4411</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. penzigii STE-U 3946</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. penzigii STE-U 3947</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. penzigii STE-U 3945</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. populicola STE-U 1051</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. penzigii STE-U 3948</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. penzigii STE-U 3949</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. penzigii STE-U 3950</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. hayi CH5^6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. zebrina STE-U 3955</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. zebrina STE-U 3957</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. zebrina STE-U 3958</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Transition.
2 Transversion.
3 Insertion / duplication of leading nucleotide.
4 Deletion.
5 Sequences identical to C. apii: C. canescens STE-U 1137, C. canescens STE-U 1138, C. nicotianae AF297230, C. sorghi f. maydis AF297232, C. beticola AF222827, C. penzigii STE-U 4408, 4409, C. hayi CH5^*.
6 Sequences obtained from TreeBase matrix M691.
Based on the ITS sequence, *C. penzigii* is distributed over three groups (Tab. 2): the first group contains two isolates (STE-U 4408 & 4409) identical to *C. apii* AY266168 (TreeBase matrix M691); the second group contains five isolates (STE-U 4410, 4411, 3946, 3947 & 3945) as well as *C. populicola* Tharp STE-U 1051, that differed from *C. apii* AY266168 (TreeBase matrix M691) at character 502; and the final group consisted of three isolates (STE-U 3948, 3949 & 3950) that contained the same two changes as in *C. hayi* AY266163 (TreeBase matrix M691). The third group has the same change at character 502 as the second group, but also an additional change at character 500. The *C. penzigii* of the first group was isolated on citrus fruit, whereas the *C. penzigii* isolates in groups two and three were isolated from leaf spots.

There was no difference between the number of changes in the ITS1 and ITS2 regions of the *Cercospora* sequences (5 changes each between the eleven species). However, eight changes in the sequence of the 5.8S gene were observed among the eleven species. All eight changes occurred in ‘*C. oryzae*’ STE-U 4303 and ‘*C. canescens*’ AY266164 (TreeBase matrix M691), whereas no changes were observed for this region in the rest of the *Cercospora* isolates. Goodwin & al. (2001) also reported a very small difference in the number of changes between the ITS1 and ITS2 region, but found no changes in the 5.8S gene. As ‘*C. oryzae*’ STE-U 4303 and ‘*C. canescens*’ AY266164 (TreeBase matrix M691) clustered outside the main *Cercospora* clade, it appears that they are not part of the *C. apii* complex, and that the *Cercospora* isolates in the main clade (74 % bootstrap support) represent *C. apii sensu lato*.

**Treatment of species**

*Cercospora gigantea* F. E. Fisher, Phytopathology 51: 300. 1961. – Fig. 2.

**Hosts and distribution.** – *Citrus sinensis* Pers., *C. paradisi* Macfad. (Rutaceae), USA (FL).

**Specimen examined.** – USA, Florida, Orange County, Winter Park, on grapefruit leaves, F. Fisher, 28 May 1957, F-46419 (holotype).

*Cercospora gigantea* was described as having straight, fasciculate conidiophores with broad, 3–12-septate, brown conidia with rounded apices and beveled bases, 80–180 × 6–8 μm (Fisher, 1961). Although the specimen is in a poor condition, a few conidia fitting this description were found. However, the conidia are distoseptate with darkened, thickened hila, and resemble those of *Corynespora citricola* M. B. Ellis (Ellis, 1971). The poor quality of the type specimen, however, made it impossible to resolve this issue.
Leaf spots amphigenous, circular to irregular, 2–30 mm diam.,
pale to dark brown, margin raised on lower surface, medium brown,
surrounded by a chlorotic zone. – Caespituli chiefly hypophyllous,
fascicles dense to loose and divergent; more compact with shorter
conidiophores on epiphyllous surface. – Stromata medium to dark
brown, erumpent, up to 70 μm diam.; fascicles grey (compared to
brown tufts of *P. angolensis*). – Mycelium internal, pale brown,
consisting of septate, branched, smooth hyphae, 3–4 μm. – Con-
idiophores in loose to dense fascicles, arising from stromata,
straight to geniculate-sinuous, subcylindrical, unbranched, 20–
300 × 4–6.5 μm, multi-septate, pale to medium brown, smooth. –
Conidiogenous cells terminal, pale brown, smooth, tapering
to a subobtuse or swollen apex, 20–60 × 3–5 μm; scars thickened,
darkened and refractive. – Conidia solitary, long, fully developed
conidia acicular, short conidia obclavate or subcylindrical, 50–
300 × 2.5–5 μm, multi-septate, hyaline, apex obtuse to subacute to
subobtuse, base truncate in acicular conidia or long obconically
subtruncate in obclavate-cylindrical conidia, hilum thickened, darkened and refractive; secondary conidia arising via microcyclic conidiation hyaline, subcylindrical to acicular or obclavate, 1-3-
septate, 15–35 × 2–3.5 µm, with thickened, darkened and refractive hila.


Algeria, Argentina, Azerbaijan, Bhutan, Caucasus, China, Cuba, Dominican Republic, India, Italy, Japan, Mexico, Papua New Guinea, Senegal, South Africa, Swaziland, USA (FL, MS, TX), Venezuela.


*Cercospora penzigii* is morphologically similar to other cercosporoid species that are commonly referred to as part of the *Cercospora apii*-complex. This suggests that *C. penzigii* could have a wide host range (other than Rutaceae) and distribution. Morphologically this is a highly variable taxon with regards to conidiophore length, arrangement of scars on the conidiogenous cells (*in vitro* vs. *in vivo*), conidium length, shape, basal cell taper and fascicle morphology.

As shown in the present study, numerous *Cercospora* species are indistinguishable from the *C. apii* complex based on morphology and ITS sequence data (Fig. 1). It is tempting to reduce them all to synonymy with *C. apii*, as inoculation studies have also shown many of these taxa to exhibit cross-pathogenicity between hosts (Johnston & Valleau, 1949; Berger & Hanson, 1963; Kaiser & Lukezic, 1965), but as we presently only have one molecular data set at our disposal, we will refrain from doing this step formally until a multi-locus DNA data set has been established for the *Cercospora* complex surrounding *C. apii*. ITS sequence data is a valuable tool for species
identification, but insufficient as sole data set on which to base specie
eys synonymies. Additional data sets are therefore presently being
generated to address host specificity in Cercospora.

Mycosphaerella citri Whiteside, Phytopathology 62: 263. 1972. – Fig. 4.

Anamorph: Stenella citri-grisea (F.E. Fisher) Sivan., In Sivanesan,

This species was treated in detail by Sivanesan (1984, pp. 226–228).

Hosts and distribution. – Species of Aeglopsis Swingle, Citrus, Fortunella Swingle, Murraya L., Poncirus Rafin. (Rutaceae).

Brazil, Costa Rica, Cuba, Dominican Republic, El-Salvador, Gabon, Haiti, Hong Kong, Japan, Puerto Rico, Surinam, Taiwan, Thailand, USA (FL, HI, TX), Venezuela, Virgin Islands.


A similar disease, also known as greasy spot, has been observed on Citrus in Australia (Timmer & Gottwald, 2000). Examination of a voucher specimen (Australia, Nambour, on leaves of Citrus latifolia, R. Thomas, BRIP 14527, 13 Jun. 1984, IMI 290702) found ascospores to be similar in size (10–12 × 2.5–3 μm) to those of M. citri, guttulate and fusiform, widest in the middle of the apical cell, and not con-
stricted at the median septum. Symptoms vary, however, in being small, hypophyllous black specks surrounded by chlorotic halos (Timmer & Gottwald, 2000). It appears, therefore, that the Australian species represents yet another distinct species on Citrus. A further species of Mycosphaerella known to occur on Citrus in Japan is *M. horii* Hara (Timmer & Gottwald, 2000). After numerous attempts, however, we were unable to obtain any type material. Ascospores were reported to be 9–12.5 × 2.5–3 μm (Corlett, 1991).

Conidiophore fascicles of *S. citri-grisea* tend to be predominantly associated with spermatogonia or pseudothecia. Specimen BPI 439371 showed a lot of variation regarding ascospore size, with ascospores being up to 15 μm long and 4 μm wide. The anamorph is also highly variable, suggesting that this may, in fact, be a species complex. In some collections there is abundant superficial mycelium (BPI 420196), and short, narrow conidia, while in others conidia are borne on fascicles, and are long, wide and flexuous. Cultures and molecular studies would be required, however, to resolve the variation observed within *S. citri-grisea*.
**Pseudocercospora angolensis** (T. Carvalho & O. Mendes) Crous & U. Braun, **comb. nov.** – Fig. 5.


This species was treated in detail by Kirk (1986).

**Host range and distribution.** – *Citrus sinensis, Citrus spp.* (Rutaceae).


*Cercospora angolensis* was originally described as having hyaline, subclavate conidia (De Carvalho & Mendes, 1953). For this reason it was seen as distinct from a *Phaeoisariopsis* Ferraris species causing a severe disease on *Citrus* in Nigeria (Emechebe, 1980). Kirk (1986) found this to be the same organism, and placed the fungus in *Phaeoramaria* Munt.–Cvetk. as *P. angolensis* based on its conspicuous, slightly pigmented scars, and pale brown catenulate conidia. Braun & Mel’nik (1997) established the genus *Pseudophaeoramaria* U. Braun for species with unthickened or only very slightly thickened, but somewhat darkened-refractive scars (intermediate between *Pseudocercospora* and *Phaeoramaria*) and hence proposed the combination *Pseudophaeoramaria angolensis*. In a later molecular study, however, Crous & al. (2001) reported that genera with such scars as in *Paracercospora* and *Pseudophaeoramaria* belong in *Pseudocercospora* (Fig. 1).
Fig. 5. – Conidiophores, conidiogenous cells and conidia of *Pseudocercospora angolensis* (IMI 176562). – Bars = 10 μm.

Because this has again been confirmed in the present study, a new combination for *Cercospora angolensis* is herewith proposed in *Pseudocercospora*.
Key to cercosporoid species on *Citrus*¹

1. Conidia hyaline, acicular or obclavate to subcylindrical, 50–300 × 2.5–5 μm, multisepitate, with thickened, darkened, refractive hila .......................... *Cercospora penzigii* (= *C. apii* s.lat.)

1*. Conidia pigmented .............................................. 2

2. Conidia and superficial mycelium verruculose; conidia pale oliveaceous, subcylindrical to narrowly obclavate, catenulate, hila thickened, darkened, refractive, 6–50 × 2–4.5 μm, (0–)3–6(–9)-septate .......................... *Stenella citri-grisea* (*M. citri*)²

2*. Conidia and superficial mycelium smooth .......................... 3

3. Conidial hila and scars inconspicuous, or minutely thickened .......................... 4

3*. Conidial hila and scars prominently thickened, darkened and refractive .......................................................... 5

4. Conidia solitary, narrowly obclavate, base narrowly subtruncate, 3–4 μm wide, scars inconspicuous; occurring on leaves only .......................... *Pseudocercospora citri* Crous & U. Braun³

4*. Conidia solitary or catenulate, cylindrical to obclavate, base truncate, 4–5(–6.5 μm) wide, scars inconspicuous or minutely thickened; occurring on leaves and fruit .......................................................... *Pseudocercospora angolensis*

5. Conidia 1–6-septate, 28–60 × 1.5–2(–2.5) μm .......................... *Passalora citrigena* Crous & U. Braun (*Mycosphaerella citrigena* Crous & U. Braun)¹

5*. Conidia 0–1(–3)-septate, 18–35 × 4–5 μm .... *Passalora citricola*¹

¹ For additional species on *Citrus* and Rutaceae see Crous & Braun (2003).

² Regarded as a species complex.

³ Described in Braun & al. (2003).

Acknowledgments

The authors gratefully acknowledge the assistance of the curators of BPI, F and IMI for making materials available for study. The National Research Foundation (NRF) is also thanked for financial support provided to P. W. Crous, while M. C. Pretorius acknowledges support from the South African Citrus Growers Association. Dr K. A. Seifert (Agriculture & Agri-Food, Canada) is thanked for collecting several cercosporoids included in this study.

References


303


(Manuscript accepted 16th June 2003)