### Foliar diseases on tea and maté in Argentina caused by *Pseudocercospora* species

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The leaf-spotting hyphomycete *Pseudocercospora theae* on tea (*Camellia sinensis*) is described from the Province Misiones in Argentina. This is the first record of this disease from Argentina. The taxonomy of *Pseudocercospora* species on *Camellia* spp. is briefly discussed, and the species concerned are keyed out. *Pseudocercospora mate* is an endemic cercosporoid hyphomycete on maté (yerba mate, *Ilex paraguariensis*) described from Argentina. This little known species is redescribed on the base of new rich specimens recently collected in Misiones. The taxonomy of this species is discussed, above all the differentiation between *P. mate* and confused Asian collections that belong to *P. naitoi*. A key to *Pseudocercospora* species on *Ilex* spp. is provided.

**Key words** – Cercosporoid hyphomycetes – Misiones – *Pseudocercospora mate* – *Pseudocercospora theae* 

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#### Introduction

Tea (Camellia sinensis (L.) Kuntze) and (yerba mate, Paraguay tea; paraguariensis A. St.-Hil.) are economically important crops in the Province Misiones of Argentina. Data on yield losses of these cultivated plants in Argentina caused by foliar diseases are old and scanty, and date back to 1983 in the case of maté (Rivera Flores 1983). Therefore, a research project on foliar diseases of these crops was carried out between 2009 and 2011. Several causal agents of foliar damages were identified, including cercosporoid hyphomycetes which were among the most important fungal pathogens due to the frequency of occurrence and initiation of premature defoliation. The leaf-spotting hyphomycete Pseudocercospora theae, found in Argentina for the first time, is described, illustrated and compared with other *Pseudocercospora* spp. on tea. The endemic *P. mate* is the cause agent of a foliar disease on maté. Based on new collections, this little known species is re-described, illustrated, compared with allied species, and a key to the species concerned is provided.

#### **Methods**

The fungal material was examined by standard light microscopy using oil immersion (bright field and phase contrast), but without any staining. Thirty measurements (× 1000 magnification) of conidia and other structures were made, with the extremes given in parentheses.

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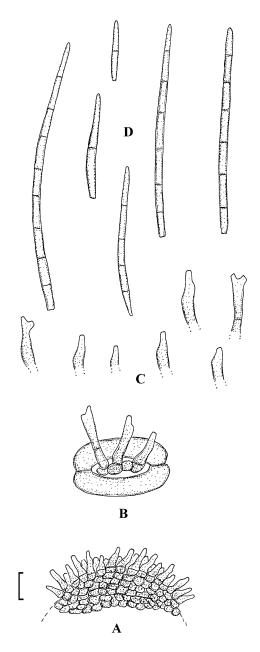
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#### **Results and discussion**

Pseudocercospora mate (Speg.) Y.L. Guo & W.X. Zhao, Acta Mycol. Sin. 8(2): 118, 1989 Figs 1–3

- ≡ *Cercosporina mate* Speg., Anales Mus. Nac. Hist. Nat. Buenos Aires 20: 426, 1910.
- = *Cercospora mate* (Speg.) Naito, Mem. Coll. Agric. Kyoto Imp. Univ. 47: 49, 1940.
- ≡ Cercospora mate (Speg.) Marchion., Direccion Invest. Sanid. Veg., A, 2: 7, 1946, comb. superfl.
- = *Pseudocercospora mate* (Speg.) U. Braun, Schlechtendalia 5: 76, 2000, comb. superfl.

Leaf spots subcircular to somewhat angular or irregular, 1-10 mm diam., at first dark dingy greenish or greyish green, later pale to medium brown, greyish brown, finally grey to greyish white on the upper leaf surface and brown to greyish brown below, margin indefinite or with a narrow darker border, pale to mostly dark brown, blackish brown or purple, occasionally with a diffuse yellowish halo. Caespituli amphigenous, conspicuous on the upper side, scattered, punctiform, dark brown to blackish, less conspicuous below. Mycelium internal; hyphae branched, septate, 1-4 µm wide, subhyaline to olivaceous, smooth. Stromata almost lacking to welldeveloped, epiphyllous stromata immersed, large, 40-100(-150) µm diam., circular to irregular in outline, hypophyllous stromata almost lacking or smaller, 10-50 µm diam., substomatal or occasionally intraepidermal, pale to dark olivaceous-brown, stroma cells 1.5–6 µm diam. **Conidiophores** in small, loose to large, dense fascicles, arising from stromata, on the upper side in large sporodochial conidiomata, erumpent, on the lower side in small to moderately large fascicles, emerging through stomata or occasionally erumpent through the cuticle, erect, straight unbranched, geniculate-sinuous, usually occasionally with short, peg-like terminal branchlets,  $5-40 \times 1.5-4 \mu m$ , 0-1(-2)-septate, subhyaline to pale olivaceous or olivaceousbrown, thin-walled, smooth. Conidiogenous cells integrated, terminal, or conidiophores reduced to conidiogenous cells, 5–20 µm long; conidiogenous loci (scars) inconspicuous, sometimes denticle-like, neither thickened nor darkened. Conidia solitary, narrowly obclavate-subcylindrical, long conidia sometimes subacicular-filiform, short conidia occasionally cylindrical,  $(12-)20-100 \times 1.5-4$ indistinctly 1–8-septate, um. hvaline. subhyaline or with a pale greenish or very pale olivaceous tinge, thin-walled, smooth, attenuated towards a subacute apex or tip subobtuse, base short to long obconically truncate, hilum neither thickened nor darkened, 1-1.5(-2) µm wide.



**Fig. 1** – *Pseudocercospora mate* on *Ilex paraguariensis*, based on HAL 2504 F. **A** Sporodochial fascicle of conidiophores. **B** Small fascicle of conidiophores emerging through a stoma. **C** Conidiophores. **D** Conidia. Bar: 10 μm. U. Braun del.

Material examined – **Argentina**, Misiones, Santa Anna, on leaves of *Ilex paraguariensis*, 20 June 1909, B. Zamboni (LPS, lectotype, designated by Braun 2000); Misiones, San Ignacio, on *I. paraguariensis*, August 1908, J. Torre (LPS, syntype, exhausted); Misiones, Aristóbulo del Valle, 17 Mar. 2011, M. Rybak (CTES 0566507, HAL 2504 F).



**Fig. 2** – *Pseudocercospora mate* on *Ilex paraguariensis*, based on fresh material. Epiphyllous leaf spots. Bar: 1 cm.

Cercosporina mate, described by Spegazzini (1910), was re-described by Chupp (1954) as Cercospora mate. Sutton & Pons (1980) examined syntype material of this species collected by J. Torre at San Ignacio, but without any conclusion about its generic affinity and taxonomy as this collection is devoid of any fructification, which was also confirmed by Braun (2000) who examined type collections of this species as well. However, the latter author found fungal colonies of C. mate in a second syntype collection that he

designated as lectotype. Based on inconspicuous conidiogenous loci in the lectotype material, he concluded that this species has to be reallocated to *Pseudocercospora*, but meanwhile Guo & Zhao (1989) had introduced the new combination *Pseudocercospora mate* just based on Chinese collections, i.e. without comparing the Chinese samples with type material.



**Fig. 3** – *Pseudocercospora mate* on *Ilex paraguariensis*. Lesions on leaves in a plantation after premature fall. Bar: 1 cm.

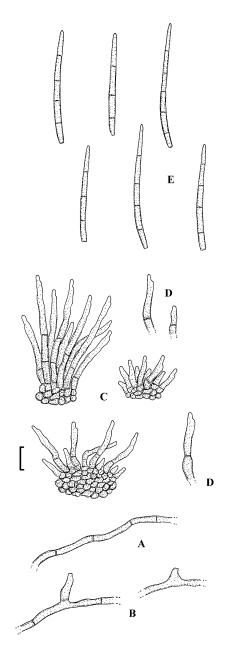
Braun (2003) compared Japanese collections of Pseudocercospora naitoi (Nakashina et al. 1999) on *Ilex chinensis* Sims (= I. oldhamii Miq.) [CUP 40367] and I. serrata Thunb. [CUP 40747] with P. mate and found obvious morphological differences. He concluded that Chinese collections on Ilex spp., misidentified as P. mate by Guo & Zhao (1989), Guo & Hsieh (1995) and Guo et al. (1998), belong to P. naitoi as well. The latter species is distinguished from *P. mate*, which is confined to Ilex paraguariensis in South America, by its olivaceous conidia, much smaller stromata and the formation superficial hyphae with solitary conidiophores which are present in Chinese as well as Japanese collections, although not described in Nakashina et al. (1999). The Asian Pseudo*cercospora* species on *Ilex* spp. is characterized as follows

*Pseudocercospora naitoi* (Togashi) C. Nakash. & Tak. Kobay., Mycoscience 40: 272, 1999

Fig. 4

≡ *Cercospora naitoi* Togashi, Trans. Sapporo Nat. Hist. Soc. 17: 101, 1942.

Pseudonym: "Cercospora mate Speg." sensu Naito, Mem. Coll. Agric. Kyoto Imp. Univ. 47: 49, 1940, non Spegazzini 1910.



**Fig. 4** – *Pseudocercospora naitoi* on *Ilex chinensis* (= *I. oldhamii*), based on CUP 40367. **A** Superficial hypha. **B** Superficial hyphae with solitary conidiophores. **C** Fascicles of conidiophores. **D** Conidiophores. **E** Conidia. Bar: 10 μm. U. Braun del.

**Leaf spots** amphigenous, subcircular to angular-irregular, sometimes confluent, 1–15 mm diam., reddish brown to blackish on the upper surface, greyish brown or grey below, margin indefinite or with a pale yellowish brown halo, sometimes spots greyish white with dark border. Caespituli amphigenous, punctiform, scattered, dark. Mycelium internal and partly also external, superficial; hyphae branched, 1.5-3 µm wide, septate, subhyaline to pale olivaceous or olivaceous-brown, thinwalled, smooth. Stromata immersed or substomatal, 10-50 µm diam., olivaceous-brown, cells 1.5-4 µm diam. Conidiophores in small to moderately large fascicles, loose to rather dense, arising from stromata, emerging through stomata or erumpent, or solitary, arising from superficial hyphae, erect, straight, cylindrical-filiform to moderately geniculate or sinuous, usually unbranched, length variable, sometimes uniformly short, sometimes short and long conidiophores mixed,  $5-50(-65) \times$ (1.5-)2-4(-5) µm, 0-3-septate, pale olivaceous or olivaceous-brown, darker in mass, thinwalled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 5-30 µm long, conidiogenous loci inconspicuous. Conidia solitary, obclavate to obclavate-cylindrical, (20-)30- $85(-100) \times (1.5-)2-3.5(-5) \mu m$ , 3-11-septate, pale olivaceous, thin-walled, smooth, apex subacute, base truncate to short obconically truncate, 1-2 µm wide, hilum unthickened, not darkened.

Naito (1940) cited Japanese collections as "Cercospora mate Speg." (he did not intend to introduce a new combination based on Cercosporina mate, but in fact did so by accident).

There are some additional species of *Pseudocercospora* on *Ilex*, viz. the North American *P. ilicis* (Ellis) U. Braun (Chupp 1954: 53, Braun 2003: 85 and 95, Fig. 9), *P. ilicis-micrococcae* Goh & W.H. Hsieh (Hsieh & Goh 1990: 26 and 28, Fig. 14) endemic in Taiwan on *Ilex maximowicziana* Loes. and *I. micrococca* Maxim., the North American *P. pulvinula* (Cooke & Ellis) U. Braun (Chupp 1954: 54, Braun 2003: 86 and 95, Fig. 10), and the South American *P. yerbae* (Speg.) U. Braun (Chupp 1954: 55, Braun 2000: 74 and 75, Fig. 19). The following key contains all

*Pseudocercospora* species known to be leafspotting pathogens on *Ilex* spp.:

# Key to the species of *Pseudocercospora* on *Ilex* spp.

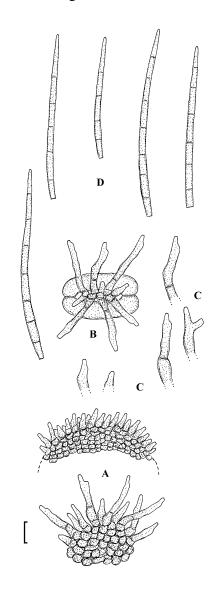
- 1\* Caespituli less conspicuous, stromata either lacking or smaller, 10–50 μm diam.; on *Ilex* spp. in Asia, Europe or North America .... 4

- 4. Conidiophores long and often branched, 45–155  $\mu$ m; on *Ilex* spp., Asia (Taiwan)

5\* Conidiophores longer and wider, (20–)30–90(–125) × 4–6 μm, wall somewhat thickened; conidia somewhat wider, 2–5 μm, hila (1.5–)2(–2.5) μm wide; superficial hyphae and solitary conidiophores lacking; on *Ilex* spp., North America ............ *P. ilicis* 

Pseudocercospora theae (Cavara) Deighton, Trans. Brit. Mycol. Soc. 88: 390, 1987 Fig. 5

- *Septoria theae* Cavara, Rev. Mycol. 11(44): 190, 1889.
- ≡ *Cercoseptoria theae* (Cavara) Curzi, Boll. Staz. Patol. Veg. Roma, Ser. 2, 9: 385, 1929.



**Fig. 5** – *Pseudocercospora theae* on *Camellia sinensis*, based on HAL 2505 F. **A** Sporodochial fascicles of conidiophores. **B** Small fascicle of conidiophores emerging through a stoma. **C** Conidiophores. **D** Conidia. Bar: 10 μm. U. Braun del.



**Fig. 6** – *Pseudocercospora theae* on *Camellia sinensis*, based on fresh material. Epiphyllous leaf spots. Bar: 1 cm.

Leaf spots amphigenous, circular to somewhat angular-irregular, 0.5-3 mm diam., ochraceous, pale to medium dark brown, later centre becoming greyish brown, grey to greyish white, with narrow raised rim followed by a dark marginal line or halo. Caespituli amphigenous, punctiform, scattered, greyish brown to blackish. Mycelium internal, hyphae 1-2.5 µm wide, hyaline to pale olivaceous. Stromata lacking to well-developed, 10–60 um diam., substomatal or intraepidermal (on the upper leaf side immersed, larger and welldeveloped, substomatal; lacking or smaller below). Conidiophores in small, loose to rich and dense fascicles (large fascicles with large stromata sporodochial), arising from internal hyphae or stromata, erumpent or emerging through stomata, erect, subcylindrical to strongly geniculate-sinuous, unbranched, rarely with short terminal branchlets,  $5-40 \times 1.5-4$ μm, 0–2-septate, pale olivaceous, thin-walled, smooth; conidiogenous cells integrated, terminal or conidiophores reduced to conidiogenous cells, 5-25 µm long; conidiogenous loci inconspicuous, neither thickened nor darkened.

Conidia solitary, acicular, i.e. with truncate base and gradually attenuated towards a subacute tip, to narrowly obclavate, i.e. base obconically truncate and apex obtuse to subacute, short conidia occasionally subcylindrical,  $30-90 \times 1.5-4 \mu m$ , (1-)3-9-septate, hyaline, subhyaline to very pale olivaceous, thin-walled, smooth,  $1.5-2(-2.5) \mu m$  wide at the truncate base, hila unthickened, not darkened.

Material examined – **Argentina**, Misiones, Aristóbulo del Valle, on *Camellia sinensis*, 17 Mar. 2011, M. Rybak (CTES 0566508, HAL 2505 F)

This is the first record of Pseudocercospora theae from Argentina. This species is also known from Africa (Malawi, Mauritius and Tanzania), Asia (Hong Kong, Java, Nepal and Taiwan) and Europe (Italy). Deighton (1983) briefly described and discussed this species under the name Cercoseptoria theae, mainly based on Cavara's original description, and reallocated it later to Pseudocercospora (Deighton 1987). Hsieh & Goh (1990: 327 and 328, Fig. 248) described and illustrated material of P. theae from Taiwan. P. theae is morphologically close to and confusable with P. ocellata. Several additional cercosporoid hyphomycete species are known as pathogens of Camellia spp. and have often been confused:

**Pseudocercospora camelliae** (Deighton) U. Braun (Braun 1993: 282)

≡ *Mycocentrospora camelliae* Deighton (Deighton 1983: 6).

On *Camellia* spp. (ornamental species), North America (USA, Georgia), New Zealand.

**Pseudocercospora camelliicola** U. Braun & C.F. Hill (Braun & Hill 2002: 222)

- ≡ Stigmina theae Deighton (1983: 4), non Pseudocercospora theae (Cavara) Deighton, 1987.
- ≡ *Cercostigmina theae* (Deighton) U. Braun (Braun 1993: 108).

On *Camellia* cf. *japonica* L., *C. sinensis*, *Camellia* sp. (ornamental species), Africa (Malawi and Mauritius), Asia (Nepal and Taiwan), New Zealand [Deighton (1983), Braun & Hill (2002), Kirschner et al. (2009)]. Described in detail and illustrated from Taiwan by Kirschner et al. (2009).

**Pseudocercospora** javanica Deighton (Deighton 1983: 5).

On *Camellia sinensis* var. *assamica* (J.W. Mast.) Kitam., Asia (Java).

**Pseudocercospora ocellata** (Deighton) Deighton (Deighton 1987: 390)

- ≡ *Cercoseptoria ocellata* Deighton (Deighton 1983: 2).
- ≡ *Cercospora theae* Breda de Haan (Breda de Haan 1900: 11), non *Pseudocercospora theae* (Cavara) Deighton, 1987.

On *Camellia sinensis*, Africa (Nigeria and Tanzania), Asia (India, Indonesia, Japan and Sri Lanka) [Crous & Braun (2003)]. Recorded from India by Kamal (2010) and from Japan by Kobayashi (2007).

# Key to the species of *Pseudocercospora* on *Camellia* spp.

- 1\* Stromata well-developed; superficial mycelium and solitary conidiophores lacking .. 2
- 2. Conidiomata sporodochial, composed of large immersed stromata, 50–120 μm diam., and numerous densely arranged conidiophores (stromata sometimes becoming spermogonia or pycnidia with a single locule); conidiogenous cells unilocal, determinate or percurrently proliferating, annellations not very conspicuous; conidia ± acicular, 50–170(–190) × 2.5–4 μm; on *Camellia* spp. in Africa, Asia and New Zealand

- 3\* Conidiophores longer, 25–85 μm, up to 5-septate, occasionally branched; conidial

- 4. Leaf spots small, up to about 2 mm diam., evident as typical eye-spots (at first black or purplish, later with pale center surrounded by a narrow swollen purplish brown margin); conidia acicular-filiform, base truncate, about 50–80 × 2–4 μm; on *Camellia sinensis*, Africa and Asia

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