Identity of powdery mildew on *Senna* in Mexico

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Two anamorphic powdery mildews (Erysiphales) on *Senna* spp., recently found in Mexico, have been morphologically and genetically examined, as well as subjected to pathogenicity tests. Powdery mildew on *Senna occidentalis* proved to belong to the *Podosphaera xanthii* complex (*P. xanthii s. lat.*), and another collection on *S. septemtrionalis* has been identified as *Erysiphe* (*Pseudoidium*) sp.

Key words – Erysiphales – *Erysiphe* sp. – *Podosphaera xanthii* – Michoacán

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Introduction

In September and October 2011, anamorphic powdery mildew was observed and collected in commercial mango orchards in Buenavista municipality, Michoacán, Mexico, on flowering senna plants (*Senna occidentalis*) about 50 to 70 cm tall. First attempts to identify this plant disease based on morphology revealed conidia formed in true chains (catenescent, according to Braun & Cook 2012) containing fibrosin bodies, suggesting a causal agent belonging in *Podosphaera* Kunze (anamorph: *Fibroidium* (R.T.A. Cook, A.J. Inman & C. Billings) R.T.A. Cook & U. Braun). In December 2011, another anamorphic powdery mildew was found on *Senna septemtrionalis* in an adjacent commercial mango orchard in the same locality, and due to its conidia formed singly and at least partly lobed hyphal appressoria identified as *Pseudoidium* sp. (*Erysiphe* sp.). Molecular analyses based on rDNA ITS sequences and pathogenicity tests were carried out to confirm the tentative determinations.

Several powdery mildew species have been described on *Cassia*, *Chamaecrista* and *Senna* species, including *Erysiphe diffusa* (Cooke & Peck) U. Braun & S. Takam., recorded on *Senna occidentalis*, *Podosphaera cassiae* (Pandotra & Ganguly) U. Braun & S. Takam., known on *Senna occidentalis* and *S. sophera* (≡ *Senna occidentalis* var. *sophera*) in India (Jammu and Tamil Nadu), and *Pseudoidium cassiae-siameae* (J.M. Yen) U. Braun & R.T.A. Cook on *Cassia fistula*, *Senna occidentalis*, *S. siamea* and *S. tora* in Africa (Tanzania, Zambia) and Asia (India, Taiwan) [Amano 1986, Braun 1987, Braun & Cook 2012]. The genuine affinity and taxonomic status of two additional *Oidium* species described on hosts of *Cassia s. lat.* are still unknown. *Oidium cassiae-hirsutae* J.M. Yen on *Senna hirsuta* in Malaysia (Yen 1966), characterized by forming conidia in chains,

might be a synonym of *Podosphaera cassiae*, but absence or occurrence of fibrosin bodies was not mentioned in the original description, and the conidia were described to be up to 56.5 × 27.5 µm in size. Type material of this species was not available for re-examination. *Oidium cassiae-leschenaultianae* N. Ahmad, A.K. Sarbhoy, Kamal & D.K. Agarwal (Ahmad et al. 2007) on *Chamaecrista leschenaultiana* (≡ *Cassia leschenaultiana*) in India (Uttar Pradesh) is an insufficiently known, doubtful species. The broad and long conidiophores, the conidial size and germ tubes at least partly arising from a side rather suggest a species of *Podosphaera* sect. *Sphaerotheca* (Lév.) de Bary or *Golovinomyces* sect. *Depressi* (U. Braun) U. Braun, but in the original description the conidia were described as being formed singly and fibrosin bodies were not mentioned (Ahmad et al. 2007, Braun & Cook 2012).

Methods

The collections examined were mounted in distilled water when fresh or lactic acid and gently heated when dry, using oil immersion (bright field and phase contrast), but without any staining, and then examined by means of standard light microscopy (Olympus BX 50, Hamburg, Germany). 30 measurements (× 1000 magnification) of conidia and other structures were made, with the extremes given in parentheses.

Senna seeds were collected in a powdery mildew disease free area and planted on peat moss substrate to obtain healthy plants. Pathogenicity tests were carried out with four month old *Senna occidentalis* plants during October 2011. Inoculation was performed by placing infected leaves collected in the field on leaves of healthy plants previously sprayed with water. Control plants were not inoculated. Inoculated plants were covered with plastic bags for 24 hours, all plants were placed together.

DNA extraction from powdery infected leaves was done by using a Wizard Genomic DNA Purification Kit (Promega Corp., Madison, Wisconsin) following the instructions provided by the manufacturer. Amplification of the internal transcribed spacer (ITS) region including 5.8S rDNA, the 3' end of 18S rDNA and the 5' end of 28S rDNA including the variable domains D1 and D2, followed standard methods as outlined in Cunnington et al. (2003). PCR reactions were conducted with HotStarTaq DNA polymerase (HotStarTaq Master Mix Kit, Qiagen, Valencia, CA) in a thermal cycler (Mastercycler Gradient, Eppendorf). The PCR reactions were cleaned using a Wizard SV Gel and PCR Clean-up System (Promega, Corp., Madison, Wisconsin) following the manufacturer's protocol. PCR products were visualized by electrophoresis in 1.5% agarose gel in TAE buffer. Nucleotide sequences of the PCR products were obtained using direct sequencing by an external company (Macrogen Inc. Soul, Korea). For amplification of the ITS region, primers ITS5 and ITS4 (White et al. 1990) were used. Consensus sequences for the amplified region from each isolated were obtained using Pregap and Gap in the Staden Package (http://staden.sourceforge.net). Sequences were deposited in GenBank; 673 bp for *Podosphaera xanthii* on *Senna occidentalis* (JQ728480) and 755 bp for the anamorph on *S. septemtrionalis* (JQ730709). Obtained sequences were used in the BLASTN 2.2.25+ program (http://www.ncbi.nlm.nih.gov/) to determine taxon identity based on similarity between sequences expressed as percent sequence identity. Sequences were analyzed phylogenetically using Mega version 5.0. Initially, the sequences were aligned using ClustalW. Phylogenetic trees were obtained from the data by the neighbour-joining method with Tajima-Nei distance calculation, but they are not shown in this paper.

Results and Discussion

Podosphaera xanthii (Castagne) U. Braun & Shishkoff, Schlechtendalia **4**: 31, 2000 (*sensu* Braun & Cook 2012). Fig. 1

Mycelium on leaves, amphigenous, but mainly epiphyllous, forming thin white patches, subcircular to irregular, later confluent; hyphae branched, septate, thin-walled, smooth, hyphal cells 50–90 µm long and (3–)4–7(–8) µm wide, straight to sinuous, sometimes somewhat geniculate; hyphal appressoria indistinct to somewhat nipple-shaped; conidiophores arising ± centrally or usually laterally from the upper surface of the mother

Fig. 1 – *Podosphaera xanthii*, symptoms (mycelium colonies) on *Senna occidentialis*, photo based on a potted plant from pathogenicity test. – Bar = 1 cm. This picture is copyright of SP Fernández-Pavía.

cell, erect, about 120–300 µm long (with conidia), foot-cells straight, subcylindrical, (30–)40–65 × 9–12 µm, followed by 1–5 shorter cells; conidia formed in chains with crenulate outline, broadly obovoid (primary conidia) to ellipsoid-doliiform, (20–)25–35(–37) × 14–20 µm, length/width ratio 1.3–1.9, ends rounded to subtruncate, with conspicuous fibrosin bodies.

Material examined: MEXICO, Michoacán, Buenavista municipality, commercial mango orchards, on leaves of *Senna occidentalis* (*Fabaceae*, *Caesalpinioideae*), 28 September 2011, SP Fernandez-Pavia [material from pathogenicity tests] (HAL 2457 F).

Based on conidia with fibrosin bodies formed in genuine chains and indistinct to nipple-shaped hyphal appressoria, the powdery mildew anamorph found on *Senna occidentalis* in Mexico can be unambiguously referred to the genus *Podosphaera* (Braun & Cook 2012), which has been additionally proven by molecular sequence analyses. A blast search based on the sequence retrieved from powdery mildew on this host showed a close similarity or identity with other sequences of species of *Podosphaera* sect. *Sphaerotheca* subsect. *Magnicellulatae* (U. Braun) U. Braun & S. Takam. The following deposited sequences have a coincidence of 100%: GQ927253.1 (deposited as *Podosphaera phaseoli* (Z.Y. Zhao) U. Braun & S. Takam., obtained from material on *Vigna unguiculata*, China), FJ625796.1 (as *P. balsaminae* (Wallr.) U. Braun & S. Takam., obtained from material on *Impatiens balsamina*, China), AF011319.1 (as *Sphaerotheca fusca* (Fr.) U. Braun & Shishkoff, obtained from material on *Cucurbita pepo*, USA, California), AB046985.1 (as *P. xanthii*, obtained from material on *Verbena* ×*hybrida* from the USA, New York) and AB040339.1 (as *Podosphaera* sp., obtained from material on *Saintpaulia* sp. from Australia). Additional sequences agree with 99%: AB040318.1 (as *P. balsaminae*, obtained from material on *Impatiens noli-tangere* from Japan), AB525914.1 (as *P. fusca*, obtained from material on *Calendula officinalis*, Argentina), AB040320.1 (as *P. pseudofusca* (U. Braun) U. Braun & S. Takam., obtained from material on *Fatoua villosa*, Japan), EF050036.1 (as *P. xanthii*, obtained from material on *Physalis angulata*, Taiwan), AB040308.1 (as *Podosphaera* sp., obtained from material on *Hibiscus mutabilis* from Japan).

All sequences with a coincidence of 100% belong to *Podosphaera xanthii* as recently circumscribed by Braun & Cook (2012), who revised the whole complex of *Podosphaera* sect. *Sphaerotheca* subsect. *Magnicellulatae*. *P. xanthii* is characterized by having large chasmothecia, about 80–110 µm diam., and asci with large terminal oculi, usually 15–25 µm diam., and is known to be pathogenic on a wide range of hosts belonging to different plant families. Plurivorous races, able to infect a wider range of hosts, are involved. *Podosphaera phaseoli* has been reduced to synonym with *P. xanthii* (Braun & Cook 2012), and *Podosphaera* on *Impatiens balsamina* belongs to *P. xanthii* as well, i.e. it is not conspecific with *P. balsaminae* which is confined to *Impatiens noli-tangere* mainly in Europe (Ito & Takamatsu 2010, Braun & Cook 2012). The name *Sphaerotheca fusca* (Fr.) S. Blumer (≡ *Podosphaera fusca*) has previously been applied in a very broad sense, including the current *P. xanthii* (Braun 1987). The true *P. fusca* is, however, confined to *Doronicum* species as hosts and characterized by having small chasmothecia, 65–90 µm diam., with very long appendages, asci with small terminal oculi, diameter < 15 µm, and forms brown secondary mycelium (Braun & Cook 2012).

The results of sequence analyses indicate that the Mexican *Senna occidentalis* powdery mildew belongs to *P. xanthii*. The infection has probably been caused by a plurivorous race of this species. The identification of this powdery mildew is supported by the morphology of its anamorph, which agrees perfectly with characteristics of conidial states of the latter species. However, anamorphs of species within *Podosphaera* sect. *Sphaerotheca* subsect. *Magnicellulatae* are little differentiated and usually not distinctive. The anamorph of the Indian *Podosphaera cassiae* (Braun 1987, Braun & Cook 2012) is very similar, but without sequence data retrieved from Asian samples of the latter species, it is not yet possible to elucidate relations between the two species.

Pathogenicity tests were carried out with four month old senna plants during October. Inoculation was performed by placing infected leaves collected in the field on leaves of healthy plants previously sprayed with water. Control plants were not inoculated. Inoculated plants were covered with plastic bags for 24 hours. Powdery mildew symptoms began to develop eight days after inoculation. All leaves were covered with mycelium, conidia were formed 18 days after inoculation, and defoliation was observed after one month. Non-inoculated plants remained healthy throughout the experiment.

***Erysiphe* sp. (*Pseudoidium* sp.)** Fig. 2

Mycelium amphigenous, mainly epiphyllous, effuse or in irregular thin patches, white to grayish white; hyphae straight to somewhat flexuous, branched at ± right angles, septate, thin-walled, hyaline, smooth, 2.5–8 µm wide; hyphal appressoria sparingly developed, almost nipple-shaped to lobed, 3–6 µm diam.; conidiophores arising ± centrally to laterally from the upper surface of mother cells, erect, about 20–60 µm long, width somewhat increasing from base to top, basal septum at the junction with the supporting hypha or only slightly elevated (up to 5 µm), foot-cells straight, occasionally slightly curved-sinuous, subcylindrical, 14–30 × 6–9 µm, followed by 1–2 cells that are shorter than the foot-cells, about as long or somewhat longer, following cells about as wide as the foot-cells or often somewhat wider, 8–12 µm, forming conidia singly; conidia ellipsoid-ovoid, subcylindrical-doliiform, 22–38 × 14–20 µm, apex of primary conidia rounded, subtruncate in secondary conidia, base always truncate or subtruncate.

Material examined: MEXICO, Michoacán, Buenavista municipality, commercial mango orchards, on leaves of *Senna septemtrionalis* (*Fabaceae*, *Caesalpinioideae*), 12 December 2011, SP Fernandez-Pavia (HAL 2478 F).

Symptoms and micro-morphological characters of Mexican specimens on *Senna septemtrionalis* resemble those of the mainly North to South American *Erysiphe diffusa*, but the anamorph of the latter species is still insufficiently known (Braun & Cook 2012). Furthermore, *E. diffusa* is undoubtedly heterogeneous and represents a complex of different races or even cryptic species. The Mexican samples on *S. septemtrionalis* differ from the anamorph of *E. diffusa* in having distinct arrangements of conidiophore cells (in

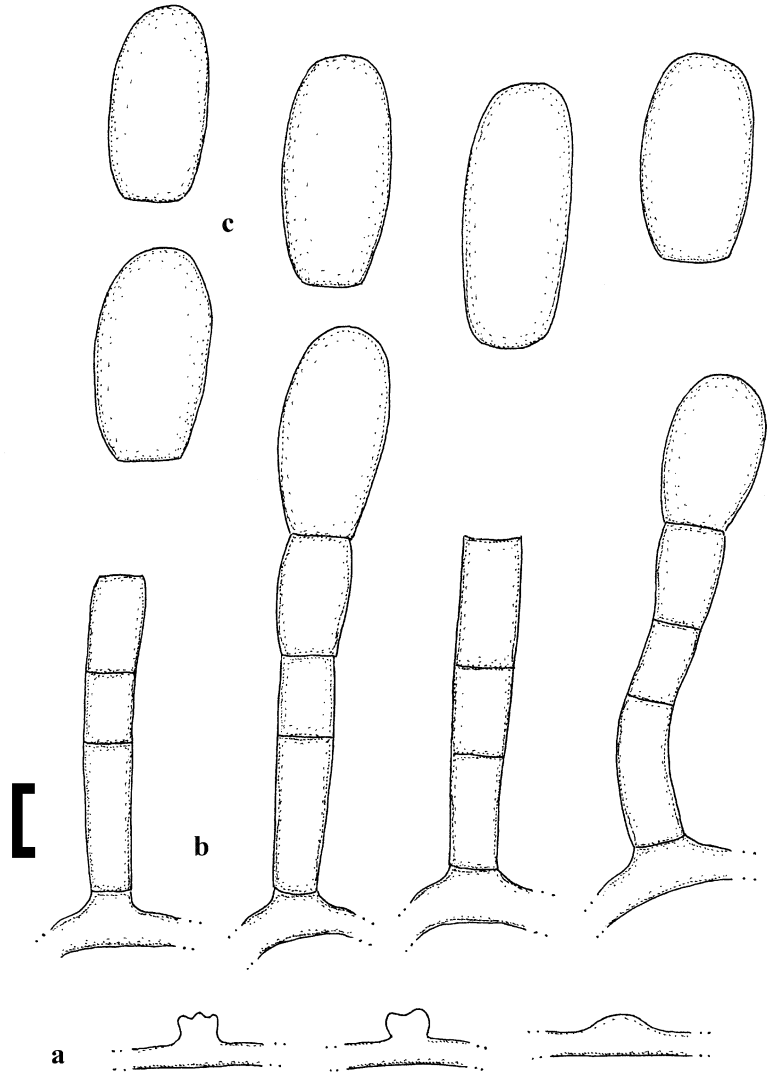

Fig. 2 – *Erysiphe* sp (*Pseudoidium* sp.) on *Senna septemtrionalis*, based on HAL 2478 F. **a** Appressoria. **b** Conidiophores. **c** Conidia. – Bar = 10 µm. U. Braun del.

the latter species foot-cells are followed by shorter cells) and rather short conidiophores mostly distinctly increasing in width from base to top (in *E. diffusa* longer subcylindrical conidiophores). *Pseudoidium cassiae-siameae* (≡ *Oidium cassiae-siameae* J.M. Yen, = *O. cassiae-torae* N. Ahmad, A.K. Sarbhoy, Kamal & D.K. Agarwal), which is known from Africa and Asia on *Cassia* and *Senna* spp. (Braun 1987, Ahmad *et al.* 2007, Hosagoudar and Agarwal 2009, Braun & Cook 2012), is similar, but differs in having much longer conidiophores, 50–100 µm, and longer conidiophore foot-cells, 25–60 µm, which are followed by shorter cells. *Erysiphe pisi* DC., which is also a complex heterogeneous species, may have conidiophores with similar cell arrangements, but they are much longer and not distinctly increasing towards the apex. However, exact host ranges of *E. diffusa*, *E. pisi*, *P. cassiae-siameae* as well as other powdery mildew species on legumes are insufficiently known. There is a lot of confusion around the taxonomy and nomenclature of species of *Erysiphe* DC. sect. *Erysiphe* and sect. *Microsphaera* (Lév.) U. Braun & Shishkoff on legumes. Determinations to be found in herbaria and in literature are often wrong, above all when exclusively based on anamorph material. It is possible that various records of *Oidium* sp., *Erysiphe communis* auct. and *E. polygoni* DC. on different species of *Cassia s. lat.* (Amano 1986) belong to *E. diffusa* or *P. cassiae-*

siameae. *Senna occidentalis* is listed as host of *E. diffusa* in Braun & Cook (2012). Based on morphology, an unambiguous determination of the anamorph on *Senna septemtrionalis* is not yet possible, i.e. it can only be assigned to *Erysiphe* (*Pseudoidium* sp.).

A blast search based on the sequence retrieved from powdery mildew on *Senna septemtrionalis* revealed several very similar sequences, all derived from powdery mildew material on legumes, with 99% coincidence: EF196675.1 (as *Erysiphe diffusa*, on *Glycine max*), GU570595.1 (as *Erysiphe polygoni*, on *Phaseolus vulgaris*), AY739109.2 (as *Erysiphe* sp., on *Phaseolus vulgaris*), AB522715.1 (as *Oidium* sp., on *Crotalaria* sp.) and HQ444198.1 (as uncultured *Oidium* clone, on *Phaseolus vulgaris*). These data are not sufficient for a final determination of the Mexican anamorph collection on *Senna septemtrionalis*. ITS data are in this complicated complex of *Erysiphe* species on legumes not sufficient. Additional sequence data based on teleomorph material of *E. diffusa* and other taxa involved or chasmothecia developed on *S. septemtrionalis* are necessary for a final identification.

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