Mycoscience (2006) 47:388–390
DOI 10.1007/s10267-006-0319-3

NOTE

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First report of *Mycopappus alni* in Japan: species identification of the pathogenic fungus of a frosty mildew disease in *Crataegus chlorosarca*

Received: May 11, 2006 / Accepted: August 11, 2006

Abstract A leaf disease similar to frosty mildew disease caused by *Mycopappus* species was detected on the leaves of *Crataegus chlorosarca* in Tomakomai, Hokkaido Prefecture. From morphological observations and gene analyses of rDNA-ITS, the fungus was identified as *M. alni*, which causes leaf blight disease on *Alnus* spp., *Betula* spp., and a *Pyrus* sp. in North America and Turkey. This is the first report of *M. alni* in Japan and *Crataegus* as its new host genus.

Key words *Crataegus chlorosarca* · Frosty mildew · *Mycopappus alni* · Taxonomy

Mycopappus is an anamorphic fungal group established by Redhead and White in 1985, and four species are now known in the world (Redhead and White 1985; Wei et al. 1998; Suto and Kawai 2000). Of these, *M. aesculi* Wei C.Z., Y. Harada & Katum. and *M. quercus* Y. Suto & M. Kawai are known in Japan (Wei et al. 1998; Suto and Kawai 2000). In 2002, a leaf disease similar to frosty mildew disease caused by *Mycopappus* species was detected on the leaves of *Crataegus chlorosarca* Maxim. in Tomakomai, Hokkaido, northern Japan (Figs. 1, 2). From morphological observations and gene analyses, the fungus was identified as *M. alni* (Dearn. & Barthol.) Redhead & G.P. White, the type species of the genus *Mycopappus*, which causes leaf blight disease on *Alnus* spp., *Betula* spp., and a *Pyrus* sp. in North America and Turkey (Redhead and White 1985; Braun et al. 2000).

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Mycopappus alni (Dearn. & Barthol.) Redhead & G.P. White, Can. J. Bot. 63:1430, 1985.
≡*Cercosporella alni* Dearn. & Barthol., Mycologia 9:362, 1917 Figs. 1–9

Leaf spots variable in shape, subcircular to irregular, alternating broad pale brown zone and narrow olivaceous brown necrotic zone, lesions often confluent with margin pale green or dark brown. Propagules conspicuous, epiphyllous on leaf spots or near the spots, numerous, scattered to aggregated, white to pale brown to naked eye, conical when filaments agglutinated, or moplike in form when spread, 140–420 µm (mean 202.2 µm, SD 59.6, $n = 20$) wide, 175–550 µm (mean 407.3 µm, SD 93.1, $n = 20$) high, erumpent from the cuticle, easily abscised.

Propagule composed of stromatic base and long or short filamentous appendages. Stromatic base dichotomously branched, 30–75 µm (mean 52.4 µm, SD 12.1, $n = 20$) wide, 20–50 µm (mean 32.6 µm, SD 7.9, $n = 20$) high, interwoven cells, giving rise to numerous long or short filamentous hyphae. Long-type appendage (filamentous hyphae) cylindrical, with obtuse apex, 187.5–450 × 3.5–5.5 µm (mean 323.1 × 4.5 µm, SD 73.0 × 0.6, $n = 20$), 2–8-septate (mean 5-septate, SD 1.84, $n = 20$). Short-type appendage (claviform hyphae) cylindrical to clavate, 33–117.5 × 5–10.5 µm (mean 67.3 × 8.4 µm, SD 21.9 × 1.6, $n = 20$), aseptate or 1–3(–4)-septate (mean 2.4-septate, SD 1.0, $n = 20$). Both appendage types were intermingling, and claviform hyphae might be immature filamentous hyphae. Sclerotia elongate, blackish, with a white medulla containing residues of host plant tissue, 3–15 × 0.3–1 mm (mean 6.3 × 0.6 mm, SD 3.2 × 0.2, $n = 20$), on the midrib or primary veins of the leaf, usually formed on fallen leaves at the time of low temperature (approximately 10°C).

Colonies grown at 20°C in the dark, on potato dextrose agar (PDA; Difco, Becton Dickinson, Detroit, MI, USA), 6.5 cm in diameter in 5 days, covering the whole agar surface (9 cm in diameter) in 10 days, felty, pale brown (C5Y20M70K30) (Anonymous 2002) in 14 days; on malt extract agar (MEA; malt extract 20 g, peptone 1 g, glucose 20 g, agar 15 g, distilled water 1000 ml), 5 cm in diameter in 5

Figs. 1–9. *Mycopappus alni*. **1** Frosty mildew symptom on *Crataegus chlorosarca* at Tomakomai, Hokkaido Prefecture. **2** Zonate spots and propagules on diseased leaves of *Crataegus chlorosarca*. **3** Propagules on diseased leaf surface. **4** Vertical section of a propagule. **5** Propagule. **6** Sclerotia forming on leaf midrib and veins (*arrows*). **7** Transverse section of sclerotium. **8** Melanized cortex and white medulla of sclerotium. **9** Colony appearance on PDA in 14 days at 20°C in the dark. **2**, **4**, **5** TOFO-F26; **3** TOFO-F72; **6–8** TOFO-F77, **9** culture FB29. *Bars* **2** 2 cm; **3** 500 µm; **4** 50 µm; **5** 100 µm; **6** 2.5 cm; **7** 200 µm; **8** 25 µm

days; felty, white to light yellow (C0Y0M30K0) in 14 days. Microconidia produced on culture media after 1 month, globose to subglobose, 1.9–3.5 µm in diameter (mean 2.87 µm in diameter, SD 0.29, $n = 30$).

Materials examined: propagules and sclerotia on leaves of *C. chlorosarca*, at Hokkaido University, Tomakomai Experimental Forest, Tomakomai, Hokkaido Pref., Sept. 3, 2002, Y. Harada, HHUF 27537 (Herbarium of Hirosaki University, Fungi) (propagules and sclerotia); Sept. 2, 2003, Y. Harada, HHUF 28119 (propagules); Aug. 8, 2004, Y. Harada, TOFO-F26 (propagules, isolate FB28 and FB29); TOFO-F72 (propagules); TOFO-F77 (sclerotia).

The internal transcribed spacer (ITS) region of ribosomal DNA (ITS1/5.85 rDNA/ITS2) of *M. alni* (North American isolate, CBS 893.85; Japanese isolate, FB28 and FB29) were sequenced and used for homology analysis. Cultured mycelia of each isolates were homogenized, and DNA was extracted from the homogenate using the

DNeasy Plant Mini Kit (Qiagen, Tokyo, Japan), according to the manufacturer's instructions. The region of rDNA-ITS was amplified by polymerase chain reaction (PCR) from the DNA with a primer pair ITS5/ITS4 (White et al. 1990) in Platinum PCR SuperMix (Invitrogen, Carlsbad, CA, USA). The regimen for PCR amplification consisted of prior heat denaturation at 94°C for 1 min 25 s, 35 amplification cycles of treatments at 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, and the final primer extension at 72°C for10 min. PCR products were purified as described by Ogata et al. (2000) and then used for sequence analysis. The nucleotide sequencing was performed using a Thermo Sequenase Primer Cycle Sequencing Kit (Amersham Biosciences, Piscataway, NJ, USA), according to the manufacturer's instructions. Products of sequencing reactions were analyzed with a SQ-5500L DNA Sequencer (Hitachi Electronics Engineering, Tokyo, Japan). ITS sequences of Japanese isolates (FB28 and FB29, DDBJ/EMBL/GenBank accession no. AB254189 and AB254190) were distinct from the North American isolate (CBS 893.85, DDBJ/EMBL/GenBank accession no. AB254177) in two nucleotides in ITS2, which showed 99.6% homology.

Sclerotial production by *M. alni* on culture media was reported by Redhead and White (1985) but had never been discovered in the field. It should be noteworthy that we found sclerotia of *M. alni* produced on diseased leaves in the field, thus suggesting the possibility that the genus *Mycopappus* might belong to the Sclerotiniaceae.

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