New species, combinations, host associations and location records of fungi associated with hemp (Cannabis sativa)

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A 12-yr study of fungi associated with Cannabis has revealed that many taxa cited in the Cannabis literature are based on misidentifications or synonyms of species with wide host ranges (McPartland, 1992, 1994b, 1995b). New species have been discovered – Phomopsis ganjae (McPartland, 1983), Schiﬀnerula cannabis (McPartland & Hughes, 1994a), Fusarium marconii (McPartland, 1995a), and Ascochyta acuta (McPartland, 1994d). Taxonomic changes include Phoma cannabis (L. A. Kirchen) McPartl. (McPartland, 1994d) and Septoria neocannabina McPartl. (McPartland, 1995a). New host associations include Botryosphaeria obtusa, Lasiodiplodia theobromae (McPartland, 1994c), Colletotrichum dematioides, Diaporthe arctii, Leptosphaeria acuta and Leptosphaerulina trifoli (McPartland, 1995b).

In this final report of the 12-yr study, a new species is described, a combination made, five new Cannabis associations described, and three Cannabis pathogens described from new locations.

MATERIALS AND METHODS


Fruiting structures from stems and leaves were removed from host tissue, rehydrated in 3% KOH and either hand-sectioned with a razor blade or teased apart with needles under a dissecting microscope at 4×. Fungal tissue was stained with either cotton blue in lactic acid, phloxine in water or acid fuchsin in lactic acid. Root tissues with mycorrhizas were washed and plated on 1.5% water agar amended with streptomycin and tetracycline.

Genomic DNA was extracted from each isolate following the procedure of Kronland & Stanghellini (1988). Briefly, lyophilized, buffered mycelia were extracted twice with phenol–chloroform–isoamyl alcohol (25:24:1 v/v), treated with RNAase (1 mg ml⁻¹, DNase free, Sigma), extracted twice in 250 μl of chloroform–isoamyl alcohol (24:1), adjusted to 3 m sodium acetate (pH 5.0) and precipitated for 24 h in 2 vol. of 95% EtOH at −20°C. The genomic DNA pellet was washed with 80% EtOH, dried under vacuum, and dissolved in TE buffer (10 mM Tris-HCl, 1 mM Na₂EDTA). Extracted DNA was puriﬁed by electrophoresis in a 0.8% low melting agarose gel (Sea Plaque, FMC) in TBE buffer (100 mM Tris-HCl, 20 mM Na₂EDTA, 100 mM Boric acid). Escherichia coli (strain HB101) DNA was used to estimate the concentration of DNA for each isolate.

Genomic DNA was ampliﬁed with two oligonucleotide primers (LROR = 5′-ACCCGGCTGAACTTAAGC-3′ and LR7 = 5′-TACTACACCCAAGATCT-3′). LROR and LR7 are homologous to a region in 25S ribosomal DNA (rDNA) from base position 17 to 1448. PCR reactions were conducted with
AmpliTaq DNA polymerase (U.S. Biochemicals, Cleveland, OH) in 50 µl volumes. Thirty PCR cycles were conducted on an automated thermocycler (Perkin-Elmer-Cetus, Norwalk, CT). The following protocol was used: 1 min denaturation at 94º, annealing at 50º for 45 s, 50–72º gradual increase for 1 min and primer extension at 72º for 1 min. To avoid possible contamination, PCR experiments were conducted in accordance with the stringent procedures described by White et al. (1990). Also, tubes without DNA template were included in each experiment (negative control). After amplification, a 3 µl aliquot from each sample was subjected to electrophoresis. Lambda DNA digested with EcoRI/Hind III was used to determine size of PCR products. Gels were stained with ethidium bromide and photographed over a uv transilluminator to record results.

Amplified PCR products were extracted in chloroform/isooamyl alcohol (24:1), precipitated in 3 M Na acetate and 95% EtOH at −20º for 24 h, washed with 80% EtOH, dried under vacuum and resuspended in TE buffer. For restriction analysis, each PCR product was divided into equal aliquots and digested with either Hha I, Hpa II, Sau3A I, or Taq I (Promega). After digestion, samples were subjected to electrophoresis in a 4% agarose gel (NuSieve, FMC Bioproducts). × 174 DNA digested with Hae III was used as a molecular weight standard to determine size of restriction fragments. Gels were stained with ethidium bromide and photographed as described above.

RESULTS AND DISCUSSION

**Micropeltopsis cannabis** McPartl., sp. nov.  (Figs 1–3)

*Ascomata* catathecioidea, 45–130 µm diam., complanata, ampliliforma, 25–46 µm alta, Brunnea vel nigra, ostiolarata, margin integro; paries superior ex radialiter dispositus cellulis quadratis, peridia textura prismaticæ; paries basilaris similiter sed pallidior. *Ostiole* centrale, elevatum, compositum e parvis cellulis et coronam Ostiolum textura prismatica; paries basilaris similiter sed pallidior.

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**Orbilia luteola** (Roum.) McPartl., comb. nov.  (Fig. 1)


*Apothecia* superficial on stems, sessile, waxy translucent yellowish when hydrated, margin entire and round to elliptoidal, up to 0.5 mm diam. and 100 µm thick. *Excipulum* consists of hyaline thin-walled *textura globulosa*. *Asci* small, cylindrical, 8-spored, 260 × 4.5 µm. *Paraphyses* hyaline, filiform, slightly enlarged at the apex. *Ascosporae* hyaline, single-celled, fusiform, indistinctly guttulate 6.5 × 1.5 µm.


Only Dr Garonne has found this fungus on *Cannabis*, but he found enough to distribute between 60 and 100 specimens in Roumeguère’s *Fungi Gallici essicati*. Most *Orbilia* spp. occur on wood or herbaceous stems, as saprotoph. Benny, Samuelson & Kimbrough (1978) observed pockets of blue-green algae in *O. luteorubellas* (Nyl). P. Karst. They propose transferring this association to the lichens. No cyanobacterial cells were found in *O. luteola*.

**Binucleate Rhizoctonia spp.**

*Hyphae* without clamp connections, at first colourless, but rapidly becoming brown; form geometry at 45º or 90º angles from parent hyphae, constricted slightly at the branching point: a septum always forms near the base of the branch, 4–7 µm diam.

*Collection examined*: Holland: Amsterdam, leg: McPartland, on roots of *C. sativa* L., euphoriant variety – 5/1993 (BPI no. 802758, ATCC culture no. 96145).

Staining with DAPI and Safranin O demonstrated a binucleate hyphal condition. This isolate fused (followed by death of hyphal cells at the fusion point) only with binucleate *Rhizoctonia* spp. stainer strain of Ogoshi et al. (1983).

After PCR amplification of rDNA, a 1.4 kb product was obtained. No PCR products were obtained in any of the controls. After digestion of amplified rDNA with four different restriction endonucleases, the restriction phenotype of the *Cannabis* isolate was identical to the AGG tester strain and possessed all restriction fragments described by Cubeta et al. (1991).

Binucleate *Rhizoctonia* spp. are morphologically similar to *Rhizoctonia solani* J. G. Kühn, but have thinner hyphae (4–7 µm), and usually only possess two nuclei per hyphal cell. Unlike *R. solani*, binucleate *Rhizoctonia* spp. often produce
Figs 1–8. Photomicrographs of fungi associated with Cannabis. Scale bars, 38 µm. Fig. 1. Caulicolous habit of Orbilia luteola (large white apothecia) and Micropeltopsis cannabis (small black ascocarps). Fig. 2. M. cannabis, exterior aspect of ascocarp with setae. Fig. 3. M. cannabis, sectioned ascocarp with asci. Fig. 4. Curvularia cymbopogonis conidia. Fig. 5. Sphaerotheca macularis conidiophores and conidia. Fig. 6. Photomicrograph by E. G. Arzberger ca 1925, labelled ‘Endotrophic mycorrhiza on Cannabis sativa’, unknown magnification. Fig. 7. Glomus mosseae, intercellular hyphae, tight hyphal coils, and H-connections. Fig. 8. Pestalotiopsis sp., conidium.

Ceratobasidium teleomorphs. In this study, we were unable to produce the teleomorph of our strain of binucleate Rhizoctonia.

Curvularia cymbopogonis (C. W. Dodge) J. W. Groves & Skolko, Canadian Journal of Research 23:96 (1945) (Fig. 4)


Conidiophores simple, septate, brown, up to 300 µm long. Conidia acropleurogenous, smooth, straight or curved, clavate to ellipsoidal, obconical at the base with a protuberant hilum, 4 (sometimes 3) septate, middle cells dark brown with end cells paler, averaging 40–50 × 12–15 µm (slightly smaller than cited by Sivanesan, 1984).


Non-germinating seeds began sporulating with C. cymbopogonis when placed in a humidity chamber. The fungus was isolated on potato dextrose agar. Other seeds (from a fibre variety of C. sativa in Illinois) fulfilled Koch’s postulates.

Babu et al. (1977) cite another Curvularia sp. on Cannabis, C. lunata (Wakker) Boedijn. Litzenberger, Farr & Lip (1963) describe a ‘Curvularia sp.’ causing leaf spots on Cambodian Cannabis. Although they claim representative specimens were deposited at BPI, none was located.

C. cymbopogonis occurs on dicotyledons, monocotyledons and gymnosperms around the world. The fungus often causes seed and seedling blights, but also arises in leaf spots. The homothallic pseudothecia have only been seen in culture.
**Sphaerotheca macularis** (Wallr.: Fr.) Lind, *Danish Fungi*: 160 (1913) (Fig. 5)

≡ *Erysiphe macularis* (Wallr.) Fr., *Systema Mycologicum* 3: 237 (1829).

≡ *Sphaerotheca hamuli* (DC.) Burrill, *Bulletin of the Illinois State Laboratory of Natural History* 2: 400 (1887)

**Anamorph:** *Oidium* sp.

Superficial hyphae flexuous, branched, with inconspicuous appressoria, cell diam. 4–7 µm, length (37–) 64.5 (–80) µm. *Conidiophores* upright, simple, hyaline, 50–100 µm high. *Conidia* produced in chains, hyaline (turning brown with age), containing fibroso bodies (which disappear with age), ovate to barrel-shaped, single-celled, averaging 30–2 × 14–0 µm.


The anamorph of this powdery mildew was previously described (McPartland, 1983). No teleomorph developed. Recent attention has focused on the taxonomic significance of *Erysiphe* anamorphs. ‘Imperfect keys’ for powdery mildews have gained a measure of reliability. Using a key by Boeseiwinkel (1980), the *Oidium* sp. is easily identified as *S. macularis*.


**Glomus mosseae** (T. H. Nicolson & Gerd.) Ger. & Trappe, *Mycolological Memoirs* 5: 40 (1974) (Fig. 7)

*External hyphae* grow in septate, stolon-like strands along surfaces of roots and extend into soil. External hyphae contiguous with internal hyphae, via penetration points in endodermis. *Internal hyphae* in cortex grow longitudinally in roots, rarely radially or circumferentially, inter- and intracellular, septate, narrower than external hyphae, but always > 1 µm diam., uncommonly producing H-connections, hyphal loops, tight hyphal coils and vesicles; neither arbuscules nor sporulating structures seen.


Feeder roots from a naturalized stand of hemp were harvested, sectioned and stained. They revealed mycorrhizal hyphae, but no sporulating structures. Abbott & Robson (1978) devised a key to VA mycorrhizal fungi based on infection morphology, in the absence of sporulating structures. The characteristics described above are typical for *Glomus* spp., and closely resemble *G. mosseae* (Carling & Brown, 1982).

Mosse (1961) goes on to describe spore morphology. Her *Endogone* sp. spores resemble those of *G. mosseae*, according to Trappe’s (1982) synoptic key. Mosse’s *Cannabis* fungus also colonized apple, clover, onion, strawberry and tomato; *G. mosseae* occurs on these hosts (Gerdemann & Trappe, 1974).

Evidence of VA mycorrhizal association with *C. sativa* proceeds Mosse’s study. Archives at BPI contain a glass-plate negative labelled ‘Endotrophic mycorrhiza on *Cannabis sativa*’. The undated photomicrograph (of unknown magnification) was taken by E. G. Arzberger, who conducted his research in the 1920s and 1930s. It is reproduced in Fig. 6.

**Pestalotiopsis sp.** (Fig. 8)

Acrevuli epiphyllous, circular to oval in outline, dark brown, up to 280–480 µm diam. *Conidiophores* cylindrical, septate, occasionally branched, hyaline, up to 10 µm in length. *Conidiogenous cells* holoblastic, annellidic, hyaline, cylindrical. *Conidia* fusiform, 4-septate, smooth, averaging 25 × 5–6 µm; basal cell hyaline, with a simple hyaline appendage averaging ± 6–µm in length; three median cells umber to olivaceous brown, thick-walled, slightly collapsed between the septa; apical cell hyaline, conical, with appendages. *Appendages* (setulae) tubular, flexuous, with three or less commonly two branches, averaging 17–1 µm in length.

**Collection examined.** Nepal: 2 km north of Pokhara, leg: McPartland, on leaves and stems of *Cannabis indica* euphoriant variety – 10.1986 (BPI no. 802681).

Using keys in Guba (1961), the fungus can be placed in section Quinqueloculatae, Non-spathulatae, Vesicolorae, Umberae-Olivea. It could be any one of 40 fungi in this section. Of the species described by Nag Raj (1993) this *Pestalotiopsis* sp. resembles *Pestalotiopsis karstenii* (Sacc. & Syd.) Steyaert, except *P. karstenii* lacks basal appendages. Paulsen (unpublished 1971 report, Kansas State University) isolated a *Pestalotia* sp. from a naturalized stand of fibre-variety *Cannabis* near Lawrence, but the fungus was not described and no voucher specimens retained.

**Expanded geographic ranges**

**Pseudoperonospora cannabina** (G. H. Otth) Curzi is one of two fungi causing downy mildew on *Cannabis*. A distribution map published by the Commonwealth Mycological Institute (1971) includes Europe, Kazakhstan, Pakistan, India and Japan. Waterhouse & Brothers (1981) expand the range to other parts of the former U.S.S.R. and China. A collection of *P. cannabina* on wild hemp in Illinois (Hanna City) expands the fungus range to the Western Hemisphere.

**Septoria neocannabina** has previously been reported in New York (Peck, 1884). The taxon *Septoria cannabina* var. *microspora* Briosi & Cavara is identical to *S. neocannabina* (McPartland, 1995 a). This synonymy expands the range of *S. neocannabina* to the Eastern Hemisphere.

The anamorph of *Gibberella zeae* (Schwein.: Fr.) Petch, *Fusarium graminearum* Schwabe, has previously been described from hemp in Germany (Wollenweber & Reinking, 1935) and Romania (Ceapiou, 1958). A collection of *F. graminearum* collected from wild hemp near Hanna City, Illinois, expands...
the geographic range on this host to the Western Hemisphere. The Illinois strain is homothallic and readily produces perithecia. A culture is deposited at the Fusarium Research Center (accession number R-8965), The Pennsylvania State University.

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REFERENCES

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