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A NEW SPECIES OF *MONASCUS* FROM PINE TISSUES IN FLORIDA[1]

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During extensive investigations of sand pine [*Pinus clausa* (Chapm.) Vasey] root disease in Florida (2), a distinctive, slow growing, pigmented fungus (FIG. 1) of uncertain taxonomic affinity was repeatedly and consistently isolated from resin-soaked root tissue (1). This fungus has since been isolated from asymptomatic root and stem xylem tissues of both diseased and apparently disease-free sand pines, and in limited attempts from resin-soaked root tissues of slash (*P. elliottii* Engelm.) and longleaf (*P. palustris* Mill.) pines. In addition, the fungus has been isolated on occasion from soil impregnated with resin exuding from diseased pine roots when such soil was plated directly onto a modified malt extract/orthophenylphenol-based basidiomycete-selective medium (2). Invariably, recovery of this organism was enhanced 5- to 10-fold when pine wood chips were dipped in 95% ethanol and flamed prior to plating.

Preliminary investigations revealed that this fungus would grow on a wide variety of standard and selective fungal media with one notable exception, Czapek-Dox Agar (FIG. 2). Further cultural studies (senior author—unpubl.) suggested that the fungus has an obligate requirement for reduced nitrogen, reflecting perhaps a lack of nitrate reductase enzyme activity. Growth/temperature studies have indicated a temperature optimum near 30 C (FIG. 3). Limited inoculations of sand pines have yielded no evidence of pathogenicity.

In 1979, Dr. R. A. Samson (pers. comm.) placed this unique fungus in the genus *Monascus* van Tieghem. However, not until 1984 did a second opinion (Cannon's) provide a taxonomic consensus as to generic placement. Others (pers. comm.) had placed the fungus in various genera (and orders) of the Deuteromycetes due to 1) the misidentification of chlamydospores as conidia; 2) delayed production of ascomata; and/or 3) the early evanescence of asci characteristic of *Monascus* spp. (3, 8).

Monascus is an isolated genus, with no close relatives except for the monotypic genus *Xero-*

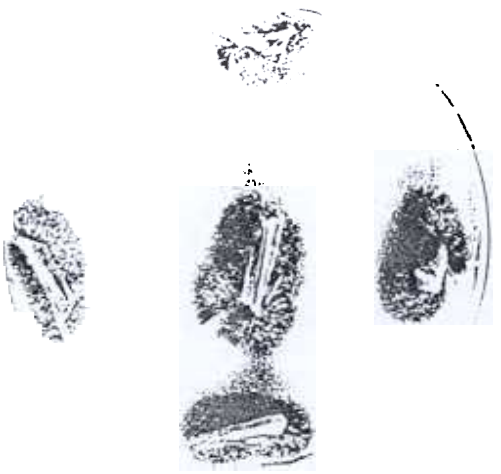

FIG. 1. *Monascus floridanus*. Colonies emerging from ethanol-flamed, resin-soaked wood chips from roots of *Pinus clausa* after 17 days on an orthophenylphenol-based, basidiomycete-selective medium.

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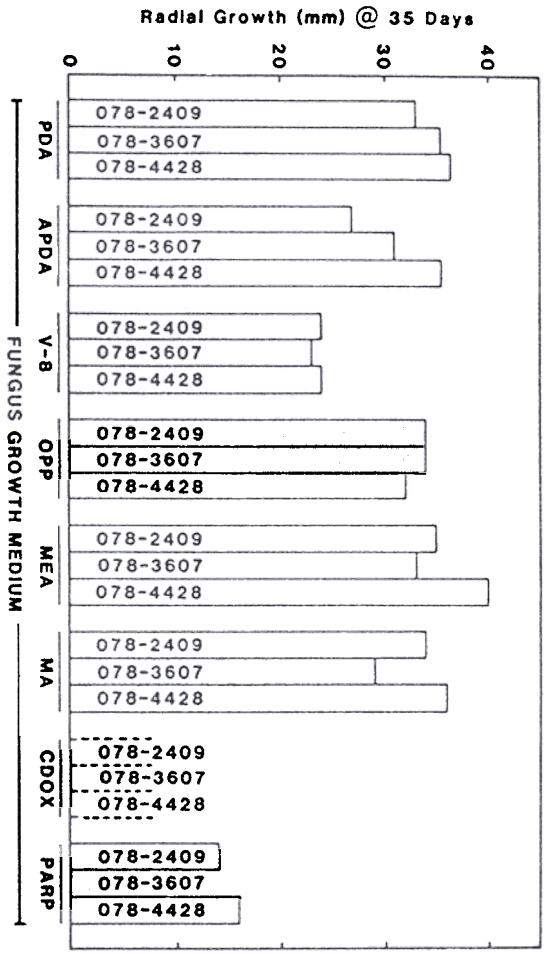


FIG 2. *Monascus floridanus*. Comparative growth of three isolates from roots of *Pinus clausa* on various laboratory media (radial growth in mm at 35 days of single, non-replicated cultures of routine clinical isolates as per accession numbers indicated, Bureau of Plant Pathology, Div. of Plant Industry, Fla. Dept. Agric. & Consumer Services—Discarded). PDA = potato dextrose agar, APDA = PDA acidified with 3.3 ml of 50% lactic acid/L, V-8 = V-8 juice agar, OPP = a modified basidiomycete-selective medium *sensu* Barnard *et al.*, MEA = malt extract agar, MA = malt agar (MEA less peptone and dextrose), CDOX = Czapek-Dox agar, PARP = a modified oomycete-selective medium *sensu* Kannwischer and Mitchell.

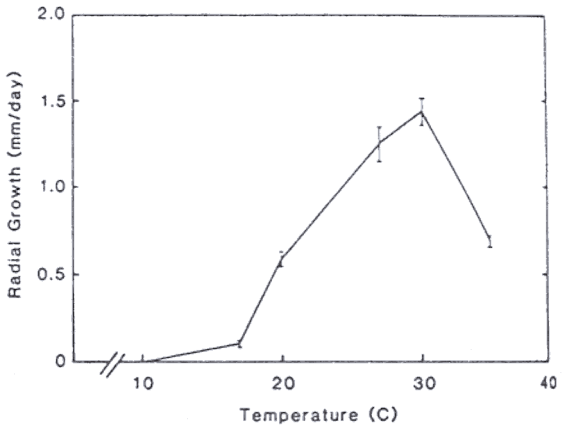


FIG. 3. *Monascus floridanus*. Radial growth as a function of temperature on acidified potato-dextrose agar. Vertical lines indicate standard deviations about the means for six replicate plates (routine clinical isolate, Bureau of Plant Pathology, Div. of Plant Industry, Fla. Dept. Agric & Consumer Services—Discarded).

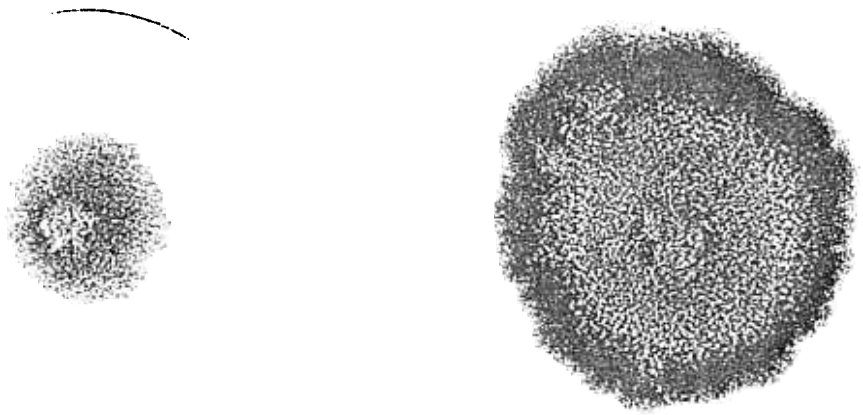
myces Fraser. In the hundred years since the genus was originally described (17), it has been associated with a wide variety of ascomycetes, but recent opinion suggests a relationship either with the Ascosphaerales (3) or the Pezizales (7, 9, 13). Modern workers concur, however, in placing *Monascus* in its own family, the Monascaceae Schröter. The genus has economic importance in several areas, particularly in the production of various fermented foods in the Orient. References to its various uses may be found in Hawksworth and Pitt (8). All species appear to be at least thermotolerant, and the genus is often an important component of silage mycofloras.

Until recently, little critical work had been carried out on the constituent species and their interrelationships. The most recent study is that of Hawksworth and Pitt (8), who particularly emphasized growth rates and colony characteristics under closely controlled temperature and nutrient regimes. These techniques had first been applied by Pitt (15, 16) to the classification of the important genus *Penicillium* Link. We therefore grew our isolates in similar conditions in order to facilitate comparisons between them and the species accepted by Hawksworth and Pitt. Three media were used: Czapek yeast extract agar (CYA), malt extract agar (MEA) and 25% glycerol nitrate agar (G25N). CYA provides a nitrogen source primarily in the form of nitrate, MEA contains nitrogen in an organic form, while G25N provides conditions of high water tension. Cultures were incubated at three temperatures, 5, 25 and 37 C. Compositions of the media may be found in Hawksworth and Pitt (8) or Pitt (16). The color chart used in the description is that of Kornerup and Wanscher (12).

TAXONOMY

Monascus floridanus P. Cannon & Barnard, *sp. nov.* FIGS. 1–11

Ab Monascus ruber van Tieghem differt quod habet crescentia multo tardior (coloniae 14–15 mm diam in agaro "Czapek yeast extract" ad temperaturam 25 C



4 5

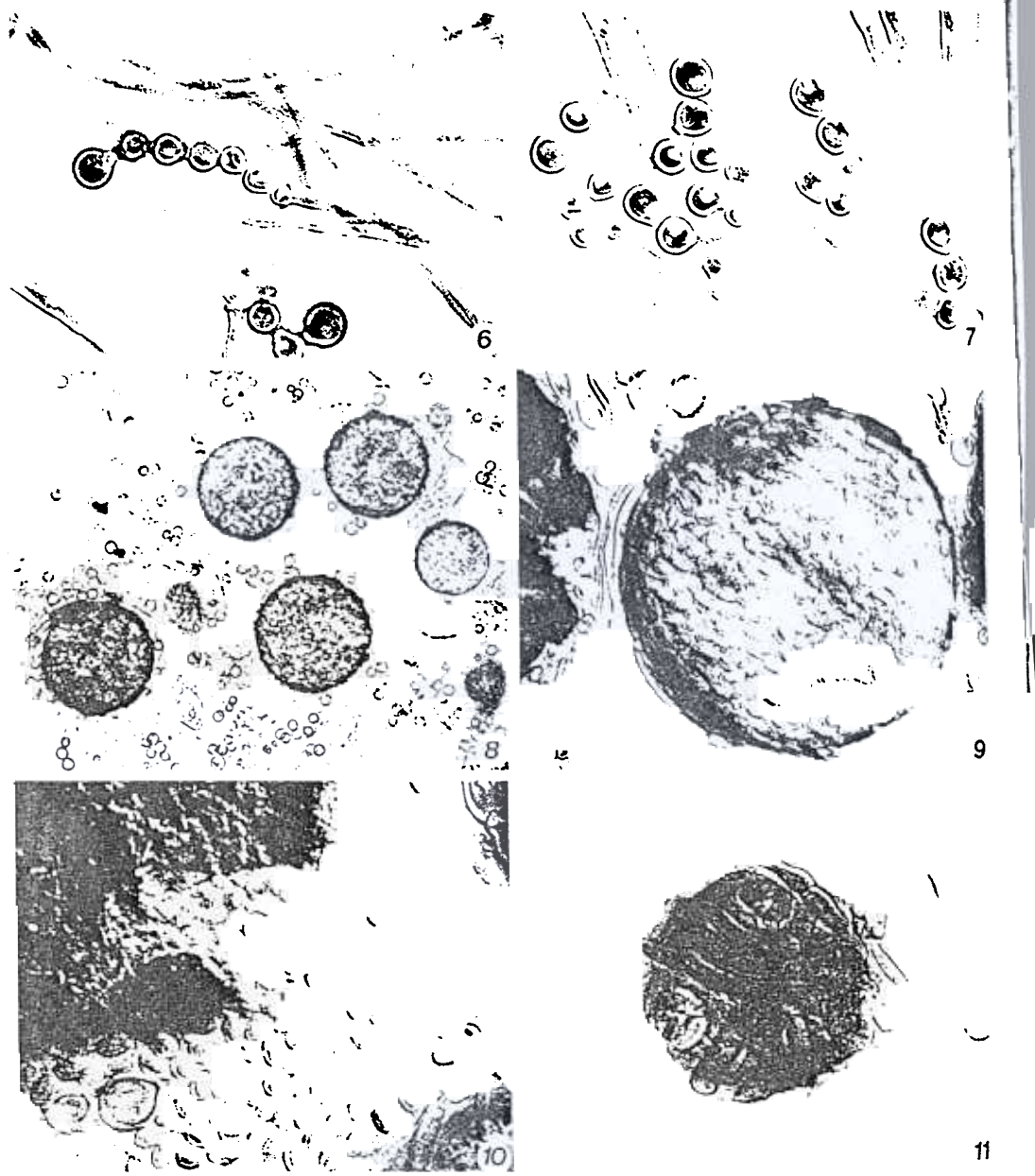
FIGS. 4, 5. *Monascus floridanus* (IMI 282587). Colonies after 30 days at 25 C, ×0.5. 4. On Czapek yeast extract agar (CYA). 5. On malt extract agar (MEA).

post 7 dies), ascosporae parviores (3.5–4.5 μm long.). Anamorph ad Basipetosporae rubrae Cole & Kendrick similis, sed conidia parviores (4–9 μm long.).

Mycelium abundant, hyphae frequently and irregularly branched; of uneven width, hyaline to pale brown, smooth-walled except for very old hyphae which are sometimes slightly roughened, usually 2–5 μm wide. *Conidia* usually terminal but sometimes intercalary. Terminal conidia are formed by swelling of the apex of the fertile hypha. This portion then becomes thicker-walled and a septum is formed below the swollen portion to delimit the conidium. The process is repeated, forming an unbranched chain of conidia while the fertile hypha becomes progressively shorter. Under normal conditions, secession occurs shortly after delimitation so the chains are rarely long. [See Kendrick (**11**), Cole and Samson (5) and Minter *et al.* (**14**) under *Basipetospora rubra* for discussions of the development of a similar fungus.] Terminal conidia 4–9 × 3.5–9 μm, globose to obovoid or obpyriform, pale brown, very thick-walled, secession scar usually very prominent, 1.5–2 μm diam, usually protruding for about 0.5 μm from the body of the conidium. Intercalary conidia similar in length but narrower, 2.5–6 μm wide, often rather irregular in shape. *Ascomata* cleistothecia, arising singly from dark brown hyphae which are somewhat irregular in form and more frequently septate than those of the vegetative mycelium. Cleistothecia globose, 22–58 μm diam, wall 3–6 μm thick, composed of dark brown relatively thick-walled ramifying hyphae 2–3 μm diam. *Asci* evanescent at an early stage, the ascomatal cavity completely filled with released spores at maturity. *Ascospores* maturing simultaneously, 3.5–4.5 × (2–)2.5–3 μm, ellipsoidal, rather thick-walled, hyaline, smooth-walled.

Cultural descriptions.—CYA, 25 C, 7 days: Colonies 14–15 mm diam, sometimes irregular in outline due to variable growth rates, plane or almost so, sparse, surface texture delicately floccose; margins fimbriate to feathery; mycelium hyaline to white at first, becoming Orange White (5B2-3) with age; exudate absent; soluble pigments not produced; reverse similarly colored to the obverse (cf. FIG. 4). *MEA, 25 C, 7 days:* Colonies 16.5–18.5 mm diam, regular in outline, often strongly domed, surface texture strongly floccose to lanose, pale brown at the margin, with a narrow band of white mycelium behind this, the central portion Dull Green (29E3), the aerial mycelium tinged with brown at the center; exudate absent; soluble pigments not produced; reverse Greenish Grey (26E2), paler and more yellow towards the center (cf. FIG. 5). *G25N, 25 C, 77 days:* Colonies 3–4.5(–6) mm diam, regular in outline, plane to shallowly domed, surface texture strongly floccose. Mycelium Yellowish Grey (4B2-3) at the edge, with a band of white mycelium behind, the center Olive (3E-F3-4); exudate absent; soluble pigments not produced; reverse Olive Brown (4D-E4), paler at the edge. *CYA, 5 C, 7 days:* No growth. *CYA, 37 C, 7 days:* Colonies 4–7 mm diam, often rather irregular in outline, plane to slightly domed, surface texture strongly floccose; margins slightly fimbriate; mycelium white to Yellowish White (3A2); exudate absent; soluble pigments not produced; reverse similarly colored to the obverse. Optimum temperature for growth is about 30 C.

Of the three species accepted in *Monascus* in the most recent revision (**8**), *M. floridanus* seems



FIGS. 6–11. *Monascus floridanus* (IMI 282587); 6, 7. Developing conidia in film culture, ×850. 6. Weakly adhering chain of conidia, oldest at apex of chain. 7. Free (seceded) conidia; note truncated basal scars. 8. Ascomata in various stages of maturity, ×210. 9. Mature ascoma showing mass of ascospores within, ×670. 10. Broken ascoma (top left) exuding mass of free ascospores. Note two conidia at lower left, ×1070. 11. Ascomatal initial showing wide, brown, thick-walled hyphae making up the ascoma wall, ×850. (All photos–Nomarski DIC microscopy.)

to be most closely related to *M. ruber* van Tieghem, the type species. It has a number of features in common with this species, including the brown ascomatal walls, and conidia, the absence of exudates and soluble pigments, and general morphological features of the colonies. However, it is much slower growing (colonies 14–15 mm diam on CYA at 25 C after 7 days, as opposed to 20–

32 mm for *M. ruber*), and has smaller conidia (4–9 μm long compared with 10–18 μm for *M. ruber*). In addition, it is easily distinguished from the three other species of *Monascus* by the small size of its ascospores (3.5–4.5 μm in length). In *M. pilosus* K. Sato *ex* D. Hawksw. & Pitt they measure 5–8.5 μm in length, in *M. purpureus* Went they are 5.5–7 μm long, and in *M. ruber* their length is 5–7.5 μm.

The enzymic activity of *M. floridanus* has been compared with that of other species of *Monascus* by Bridge and Hawksworth (4). They showed that all four species could be reliably distinguished using inoculations of conidial suspensions onto API ZYM strips. According to their results, *M. floridanus* was the only species to exhibit trypsinase activity, and was the only one of the four not to show valine arylamidase activity.

Others have described fungi belonging to the Eurotiales from wood tissues which have certain similarities to our organism. Von Arx and Nilsson (18) described *Xylogone sphaerospora* from stored pulpwood chips in Sweden and Crooks (6) described *Mycogala marginata* from *Eucalyptus marginata* Donn *ex* Smith in Australia. Both of these fungi produce cleistothecial ascomata with asci which are evanescent at maturity. However, the ascomata of our fungus are considerably smaller (22–58 μm) than those of either of these two fungi (50–90 μm and 50–150 μm, respectively). In addition, the anamorphs of these two fungi are completely different from that of the new species of *Monascus*. *Xylogone sphaerospora* produces thick-walled hyphae which fragment irregularly into two- to four-celled units (18). *Mycogala marginata* was described as having two different anamorphs, one very similar to that of Xylogone; "oidia—superficially resembling the endoconidia of *Thielavia basicola* Zopf" (6), a reference in fact to the conidia of the unrelated *Thielaviopsis basicola* (Berk. & Br.) Ferraris. The other anamorph described is of unremarkable "chlamydospores," structures similar to which might be found in cultures of almost any ascomycete grown under the right conditions, and which are quite different from the well-organized chains of resting-spores found in *Monascus*.

TYPE: U.S.A.: Florida: Santa Rosa Co., isol. *ex* resin-soaked roots of *Pinus clausa* Vasey *ex* Sargent; 1982; *E. L. Barnard DOF 49* (IMI 282587—holotype, FLAS F54662—isotype).

OTHER CULTURE EXAMINED: U.S.A.: Florida: Marion Co., isol. *ex Pinus elliottii* Engelm.; April 26, 1982; *E. L. Barnard DOF54* (IMI 282588).

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TELEOMORPH OF *SPHAEROTHECA FULIGINEA* ON CUCURBITS IN NORTH CAROLINA[1]

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Ballantyne (2) indicated no reports of the telemorph of *Sphaerotheca fuliginea* (Schlecht. : Fr.) Poll. on cucurbit species in North America although the anamorph was reported from numerous locations in the United States (2, 5, 10–12) and Canada (8). Since Ballantyne's study in 1975, perithecia of *S. fuliginea* were reported on *Cucurbita pepo* var. *melopepo* Alef. cvs. Summer Squash, Zucchini Dark Green and Ambassador in the Imperial Valley, California (7) and on *Cucumis sativus* L. cvs. Harliton Seedless, Burpee Hybrid, Highmark II, and Marketmore grown in glasshouses in Ontario, Canada (4). Perithecia also have been reported for the first time on a variety of cucurbit species from India (6), Saudi Arabia (1) and New Zealand (3).

In late September and October, 1986, samples of cucurbit species with powdery mildew from a demonstration field study at the North Carolina State University Mt. Horticultural Research Station, Henderson Co., and in early November from a commercial field in Richmond Co., North Carolina, were received. Examination of the samples revealed perithecia which were identified as *S. fuliginea*. As this represents only the third report of perithecia of *S. fuliginea* in North America and the first occurrence on several cucurbit species and/or cultivars, details of the perithecia are provided.

Perithecia were observed only on the underside of leaves of the following plants: *Cucumis melo* L. cv. Ambrosia, *Cucurbita maxima* Duchn. cvs. Show King, Big Max, and Waltham Butter, *C. pepo* cvs. Vegetable Spaghetti and Zucchini, and *Lagenaria siceraria* (Mal.) Standl. cvs. Birdhouse, Clemson's Club, Dipper, and Hercules Club. Perithecia (FIGS. 2, 3) were (63.1–)95.9 (–121.0) μm with peridial cells (15.8–)21.3(–32.6) μm (FIG. 3), one ascus/perithecium (FIG. 4), asci (20.0–)49.4(–65.0) × (32.5–)68.4(–87.5) μm. No mature ascospores were observed, but immature ascospores were noted in a small percentage of asci. Hyphoid appendages (FIG. 2) were characteristically septate and brown from their origin on the perithecium, fading to hyaline. Perithecial morphology agreed with previously published descriptions of *S. fuliginea* (8, 9). Perithecia were found primarily on senescent leaves or necrotic areas of leaves, although green leaves were heavily colonized.

The anamorph of *S. fuliginea* was found on: *Cucumis sativus* cv. Carolina, *Cucurbita maxima* cv. Blue Hubbard, and *C. pepo* cvs. Jack O'Lan-

[1] The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named, nor criticism of similar ones not mentioned.