Soybean Seed Lot Contamination by *Melanopsichium pennsylvanicum* Smut Galls

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ABSTRACT

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Smut galls were found in a soybean (*Glycine max* 'Williams') seed lot harvested from a field near Jerseyville, IL in 1977. The galls were determined to be smut-infected inflorescences of smartweed (*Polygonum pennsylvanicum*). The smut fungus was identified as *Melanopsichium pennsylvanicum;* this fungus previously had been identified as the causal agent of a smut of soybean, *M. missouriense.* The Jerseyville specimen was compared to herbarium specimens of *M. pennsylvanicum* on *P. pennsylvanicum* from the Illinois State Natural History Survey (ILLS), Urbana, and the type specimen of *M. missouriense* obtained from the National Fungus Collections, Beltsville, MD. It was compared also to fresh material from smartweed plants wound-inoculated with *M. pennsylvanicum* chlamydospores from the Jerseyville specimen. Gall and chlamydospore morphology was similar for all specimens. All wound-inoculated smartweed plants developed galls. Symptoms did not develop on soybean plants inoculated with chlamydospores of *M. pennsylvanicum.* Scanning electron micrographs of chlamydospores showed variation in the wall ornamentation of some chlamydospores in the ILLS specimen. Based on the above comparisons, we consider *M. pennsylvanicum* and *M. missouriense* to be synonymous.

Additional key words: Sclerotinia sclerotiorum, seed-borne pathogens, soil peds.

A new smut fungus of soybean (*Glycine max* [L.] Merr.), *Melanopsichium missouriense* Whitehead and Thirumalachar, was named in 1960 from galls found in nine lots of four soybean cultivars collected during the 1954 through 1958 growing seasons in Missouri (6). The smut galls were believed to be partially composed of malformed soybean pods, and where infection was observed to be partial, remnants of soybean seeds. In 1969, similar galls were observed associated with soybean seed lots from Missouri and Tennessee and were described as resembling "soil peds" (2). There are no reports in the literature of a smut disease on soybeans grown under field conditions (5). The purpose of these studies was to determine the relationship of the smut galls collected in Illinois from soybean seed lots with other herbarium specimens and disease samples.

MATERIALS AND METHODS

A soybean (cultivar Williams) seed lot containing seeds of smartweed (*Polygonum pennsylvanicum* L.) and hard, reddish-brown galls of *M. pennsylvanicum* similar to those described by Whitehead and Thirumalachar (6) and Gerdemann and Chamberlain (2) was obtained from a soybean field near Jerseyville, IL in 1977. These galls were compared with those from Whitehead and Thirumalachar's original material obtained from the National Fungus Collections, Beltsville, MD (BPI); with smut galls caused by *M. pennsylvanicum* on smartweed, collected in Illinois, from the Illinois State Natural History Survey (ILLS) Herbarium, Urbana; and with fresh galls from smartweed plants wound-inoculated with a suspension of chlamydospores from the Jerseyville specimen, by light microscope studies of thin sections (7–10 μm) made with a Hooker microtome. Galls from each of the BPI, ILLS, and Jerseyville specimens were crushed with a mortar and pestle and mixed with lactophenol and azure A (4) for light microscope studies of the chlamydospores. Galls of the BPI and Jerseyville specimens were examined with a binocular microscope for remnants of host tissues.

Chlamydospores of the Jerseyville specimen were placed in sterile double-distilled water, either nonamended or amended with soybean seed extract plus glucose (SEGB), to observe chlamydospore germination. The SEGB mixture was prepared by autoclaving (1.06 kg/cm^2 for 15 min) 10 soybean seeds in 10 ml of a 1% glucose solution prepared with distilled water. Chlamydospores from the BPI, ILLS, and Jerseyville specimens were compared for wall ornamentation with those from wound-inoculated smartweed plants by means of a scanning electron microscope at the Center for Electron Microscopy, University of Illinois, Urbana. Mounts for use in the scanning electron microscope were prepared by scratching chlamydospores from locules of each specimen and placing them on separate scanning electron microscope pegs coated with Scotch No. 850-silver polyester film tape (3-M Company, St. Paul, MN).

Inoculation studies. Galls from the Jerseyville seed lot were crushed with a mortar and pestle to release chlamydospores and mixed at approximately one gall:10,000 parts of steamed greenhouse soil. The gall:soil mixture was placed in a greenhouse flat and planted with smartweed and Williams soybean seeds from the Jerseyville soybean seed lot in alternate rows.

Beginning when plants were 4–6 wk old, three seedlings each of soybean and smartweed were wound-inoculated weekly for 6 wk with a chlamydospore suspension in SEGB by means of a hypodermic needle and syringe. Soybean seedlings initially were inoculated at the cotyledonary node, then on the first trifoliolate leaf, and (as the plants matured) on the stem above the cotyledonary node and finally on immature pods. Smartweed seedlings were wound-inoculated at each node beginning at the fourth node above the ground, and at random on leaves and flowers. All plants were examined daily and notes were taken weekly for the development of smut galls. Stems of both plant species and pod tissues of soybeans, above and below the points of inoculation as well as crown, root-tissues, and flowers of both plant species were examined for the develop-

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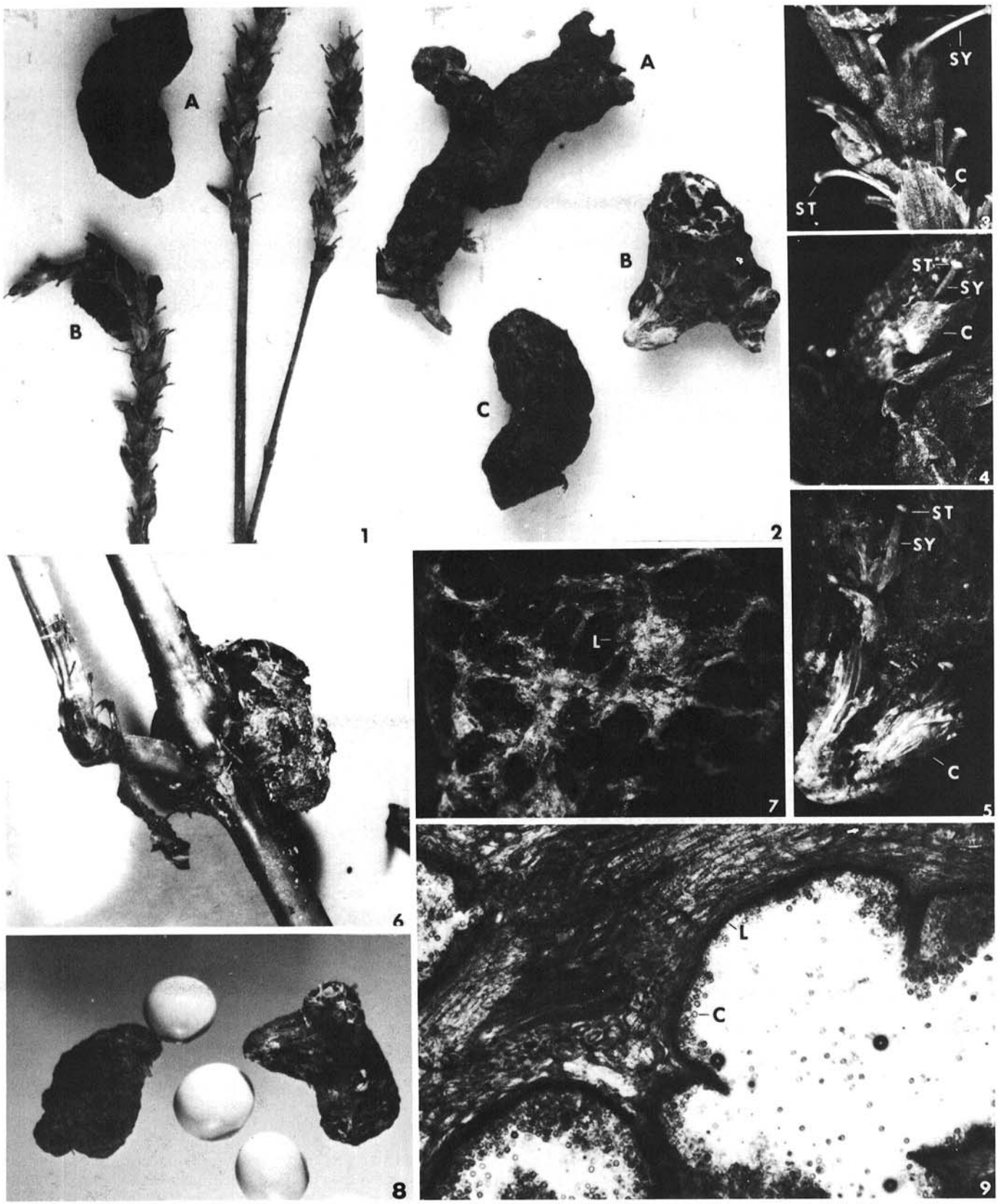

Fig. 1–9. Galls of *Melanopsichium pennsylvanicum* on *Polygonum pennsylvanicum* or mixed with a soybean (*Glycine max*) seed lot: **1,** Gall from the Whitehead and Thirumalachar type specimen (W & T) (A), and galls on *P. pennsylvanicum* inoculated with spores of the Jerseyville, IL specimen of *P. pennsylvanicum* (B) (×4). **2,** Galls from the Illinois State Natural History Survey (ILLS) herbarium specimen No. 7115.1 (A), Jerseyville, Illinois seed lot specimen (B), and the W & T specimen (C) (all × 4). **3,** Flower parts from the ILLS specimen (stigma [ST], style [SY], and calyx [C]) (×4). **4,** Flower parts of gall from the W & T specimen (stigma [ST], style [CY], and calyx [C]) (×4). **5,** Flower parts in gall from the Jerseyville specimen (stigma [ST], style [SY], and calyx [C]) (× 4). **6,** Gall from *P. pennsylvanicum* plants wound-inoculated with chlamydospores of the Jerseyville specimen (×4). **7,** Cross-section of gall from Jerseyville specimen showing locules (L) (×20). **8,** Soybean seeds compared with galls of *M. pennsylvanicum* (×4). **9,** Cross-section of a gall showing locules (L) and chlamydospores (C) (×75).

ment of smut galls.

Ten other smartweed plants at the flowering stage growing in the same greenhouse were wound-inoculated above the fourth node at several points along the stem as described previously.

RESULTS

Galls from the Jerseyville soybean seed lot were similar in color, size, and shape to those in the Whitehead and Thirumalachar and ILLS collections. They ranged from 0.25 to 0.85 cm in diameter and 0.50 to 1.65 cm in length (Fig. 1–4). Similarity of galls to soybean pod tissue as described by Whitehead and Thirumalachar (6) was not observed. However, the galls from both the Whitehead and Thirumalachar and Jerseyville specimens contained recognizable parts (stigma, style, and calyx) of smartweed flowers (Fig. 1,3−5).

Galls of the four specimens possessed locules (Fig. 7,9) containing clusters of chlamydospores (Fig. 9,10) indistinguishable from those described for *M. missouriense* (6) or *M. pennsylvanicum* (1,7). Chlamydospore morphologies of the Jerseyville, Whitehead and Thirumalachar, the ILLS specimen, and those from wound-

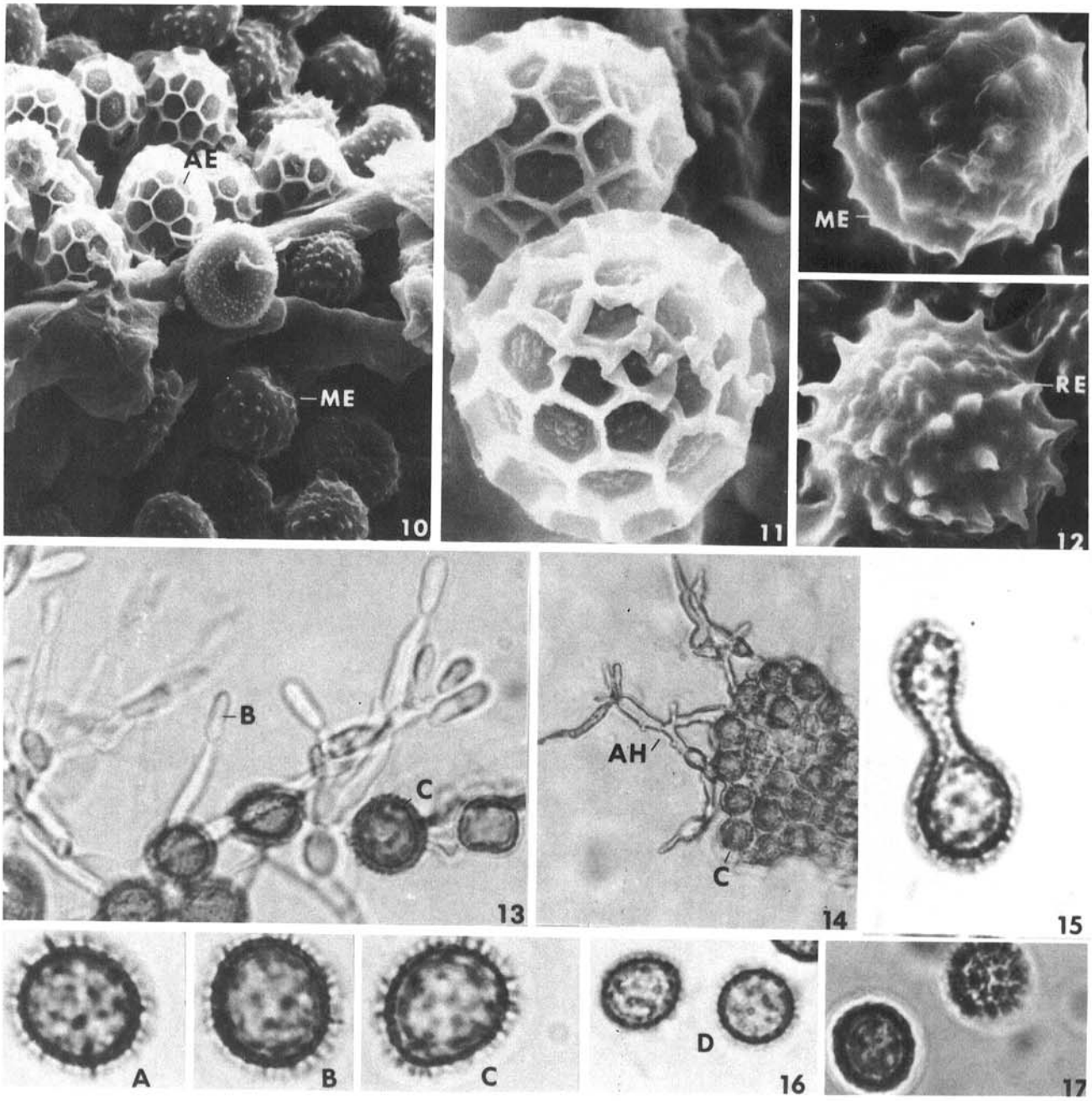

Fig. 10–17. Scanning electron microphotographs (10−12) and light photomicrographs (13−17) of chlamydospores of *Melanopsichium pennsylvanicum* from *Polygonum pennsylvanicum:* **10,** Alveolate-echinulate (AE) and moderately echinulate (ME) chlamydospores from galls of Illinois State Natural History Survey Herbarium (ILLS) specimen No. 7115.1 (×1,400). **11,** Alveolate-echinulate chlamydospores (×4,000). **12,** Moderately echinulate chlamydospores (ME) from the Whitehead and Thirumalachar type specimen (W & T) and roughly echinulate chlamydospores (RE) form *P. pennsylvanicum* plants wound inoculated with chlamydospores from Jerseyville, Illinois specimen, and similar to Jerseyville specimen chlamydospores (×4,000). **13,** Germination of chlamydospores (C) and production of basidiospores (B) from the Jerseyville specimen in soybean extract glucose broth (×1,200). **14,** Chlamydospores from the Jerseyville specimen germination (C) and anastomosing of hyphae (AH) to form a dikaryon (×600). **15,** Chlamydospore from the Jerseyville specimen budding in doubled distilled water (×1,400). **16,** Chlamydospore of the echinulate type from the W & T specimen (A) (×1,400); ILLS specimen (B) (×1,400); Jerseyville specimen (C) (×1,400); and wound-inoculated *P. pennsylvanicum* specimen (D) (×1,200). **17,** Chlamydospores of the alveolate-echinulate type from the ILLS specimen (×1,200).

inoculated smartweed plants were similar (Fig. 10–12). Chlamydospores were subglobose to globose and smoothly to roughly echinulate with some spores appearing to be alveolate-echinulate (Fig. 9–17). Galls from the ILLS specimens contained two distinct chlamydospore types, the alveolate-echinulate type and some with alveolar reticulations (Fig. 10,11,16,17).

Chlamydospores from the Jerseyville specimen budded (Fig. 5) and germinated (Fig. 13,14) in distilled water and SEGB, more rapidly in SEGB. Germ tubes were observed to fuse and to form a dikaryon in both media (Fig. 14). Chlamydospores in SEGB germinated within 3 wk and produced basidiospores laterally and terminally on septate promycelia (Fig. 13).

No gall formation was found upon or within tissues from wound-inoculated soybean plants. All smartweed plants that were wound-inoculated during the 3rd and 4th wk produced galls on stems above the point of inoculation (Fig. 6). No gall formation occurred on the roots or crowns of either soybean or smartweed plants grown in soil infested with *M. pennsylvanicum*.

DISCUSSION

The galls and chlamydospores from the two herbarium specimens, and from the Jerseyville and inoculated plants (Fig. 1,2,6) were not different from *M. pennsylvanicum* as described on smartweed from Illinois and Kansas (3). Galls showed no evidence of a soybean origin but did contain flower parts of smartweed. Remnants of soybean pods and immature seeds reported by Whitehead and Thirumalachar (6) were not observed. Considering that a smut disease has not been reported in nature on soybean (5) and no disease developed on soybean plants wound-inoculated with chlamydospores of *M. pennsylvanicum*, we believe that the smut reported on soybean was a misinterpretation of contamination of soybean seed lots. We believe that galls found in the Jerseyville specimen were the same as reported in other soybean seed lots (2,6) and all specimens should be properly identified as *M. pennsylvanicum*, causal agent of smartweed smut.

The galls of *M. pennsylvanicum* have the appearance of "soil peds" (2) or sclerotia of *Sclerotinia sclerotiorum* (5) and may be misinterpreted as such without careful observations (Fig. 8). The galls can be carried in soybean seed lots and the fungus could become indigenous in areas where *P. pennsylvanicum* is established. Smartweed seeds also were found in the Jerseyville seed lot; thus, seeds of the host and galls of the pathogen might be shipped to areas where neither occurs naturally. It is possible that the galls could be harvested from the *Polygonum* plants and deposited in the soybean seed lot causing a reduction in seed lot quality.

Two chlamydospore types were found in the ILLS specimen of *M. pennsylvanicum* on smartweed when studied by light and scanning electron microscopy (Fig. 10), one being similar to the spores of the other three specimens (Fig. 10,12). We feel that the two chlamydospore types may represent developmental stages in the formation of a mature chlamydospore, or possibly represent two developing sori from different smuts in the same host, rather than more than one species of *Melanopsichium* in the same gall on the same host.

Based on the comparison of the type species of *M. missouriense* with the Jerseyville and ILLS specimens of *M. pennsylvanicum*, we believe that the two fungi: *Melanopsichium pennsylvanicum* Hirsch., Notas Museo La Plata 6:149 (1941) and *Melanopsichium missouriense* Whitehead and Thirumalachar, Mycologia 52:191-192 (1960), are synonymous.

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