

253-60

## HUMICOLA GRISEA, A SOIL-INHABITING, CELLULOLYTIC HYPHOMYCETE

W. LAWRENCE WHITE<sup>1</sup> AND MARY H. DOWNING<sup>2</sup>

This is the second paper<sup>3</sup> of a proposed series dealing with a group of Hyphomycetous soil fungi characterized morphologically by the copious production of globose, thick-walled aleuriospores as the principal spore type, and physiologically by the fact that they are in the main among the strongest of the cellulose-decomposing fungi. The present installment is confined to a consideration of *Humicola grisea*.

### HISTORICAL AND TAXONOMIC SIGNIFICANCE OF THE SPECIES

The genus *Humicola* was established by Traaen (1914) on two species which he isolated repeatedly from Norwegian soils. His first and most frequently isolated species, *H. fuscoatra*, was characterized by spores on the order of 6-9-12  $\mu$  in diameter with walls of medium thickness and of a very dilute color; his second, *H. grisea*, by larger spores, 12-17  $\mu$  in diameter, with thicker and much more deeply colored walls. A modern investigator examining the respective type cultures, which have been maintained at the Centraalbureau since about 1912, might raise a question as to whether or not they merely represent extremes of variation within a single species. However, an adequate number of cultures has now been accumulated to demonstrate beyond all doubt that there actually are two distinct species as indicated by Traaen. The first species of Traaen, i.e., *H. fuscoatra*, is to be regarded as the generic type.

Traaen's *H. grisea* took on a peculiar and interesting significance taxonomically when Mason (1933, p. 56) speculated that it

<sup>1</sup> Farlow Herbarium, Harvard University, Cambridge, Mass., deceased.

<sup>2</sup> Pioneering Research Laboratories, U. S. Army Quartermaster Corps, Philadelphia, Pa.

<sup>3</sup> The first was published in *Mycologia* 43: 645-657. 1951.

(under the name *Monotospora Daleae* Mason) was synonymous with *Monotospora toruloides* Corda, the single species upon which Corda (1837) had founded the genus *Monotospora*. This being true, it would of course follow that *Humicola* Traaen was a synonym of *Monotospora* Corda. Actually, Mason, a few years later (1941, p. 113) discovered the paper of Traaen and expressed the opinion that Traaen's *Humicola* should be referred to synonymy under *Monotospora* Corda but not under what he called "*Monotospora* Sacc." Some years after the work of Corda, Saccardo (1886) had taken up the generic name *Monotospora* Corda in a sense that excluded Corda's *Monotospora toruloides*. Various writers have commented upon the taxonomic complications involved. Various ways have been suggested for dealing with the nomenclatural problem. That which has been basic is Mason's proposal to refer *Humicola* Traaen to synonymy under *Monotospora* Corda and to use "*Monotospora* Sacc." for the several species, probably of a miscellaneous nature, which Saccardo transferred to his (Saccardo's) *Monotospora* Corda. What Mason calls "*Monotospora* Sacc." would then, according to Mason, be conserved against *Monotospora* Corda leaving the way open for the acceptance of *Humicola* as a good genus.

Diehl (1949, p. 279) summed up Mason's point of view, and also his own, in the following words: "He [Mason] pointed out that, if *Monotospora* of Saccardo should be conserved against Corda's prior name, *Humicola* as a synonym of the discarded *Monotospora* of Corda would be legitimate with *H. fuscoatra* Traaen as the type species. Because of its convenience this should be a welcome taxonomic disposition of that hitherto debated form-genus"

The present paper deals with *Humicola*, and no detailed analysis of the early history of *Monotospora* will be attempted; nor would it be advisable on our part until we had gained a better knowledge than we now have of the species placed in *Monotospora* by Saccardo. Brief comment appears desirable, however, in order to explain our acceptance of *Humicola*. Rogers (1949, p. 459; 1950, p. 28) has, in our opinion, dealt effectively with "*Monotospora* Sacc." in stating that there simply is no such thing. Must *Humicola*

then be relegated to synonymy under *Monotospora* Corda? If Mason's contention that *Humicola grisea* is synonymous with *Monotospora toruloides* Corda is correct, the answer obviously is 'yes.' We prefer, however, to move in what appears to be a current drift toward the acceptance of *Humicola* and we believe there are reasons for doing so that are more basic than any heretofore suggested.

There is no overwhelming evidence that Corda's *Monotospora toruloides* is the same as Traaen's *Humicola grisea*. We submit that Corda's drawing could just as well represent *Papularia sphaerosperma* (Pers.) Höhn. Corda's organism formed a black effuse growth on a dead monocotyledonous substratum. This is the characteristic substratum and habit of growth for the *Papularia*, but not for *Humicola grisea*. The latter, insofar as has been demonstrated, is strictly a soil species or an inhabitant of humus or decaying wood in the soil. It has never been found under natural conditions in the form of a sporulating, hyphomycetous turf on the surface of any plant debris.

The microscopic morphology depicted by Corda fits the *Papularia* at least as well as the *Humicola*. The proportionate thickness of wall to size of spore by Corda is approximately correct for that of the lenticular spores of *Papularia* as they are commonly seen in face view in microscope mounts and is too great for *Humicola*. There is evidence of the lenticular shape in at least one of the spores drawn by Corda.

At any rate the two species with which the writers are principally involved, *grisea* and *fuscoatra*, are now in *Humicola*. Neither of them has a legitimate combination in *Monotospora*. Transferring them to *Monotospora* would require changes in nomenclature and add to synonymy. The "burden of proof" should fall upon the individual who makes the change. In this case we can offer neither proof of necessity for such transfers nor evidence of desirability from the point of view of phylogeny or nomenclatural stability.

Practically all the records, or possible records, of this species have come from studies in soil microbiology. It is of interest to note that of the many cultures of *Humicola* that originated from

industrial and military materials in the tropical deterioration program all were *H. fuscoatra*. This is in accord with the findings of Diehl (1949). The present writers believe that they have seen all of these cultures regardless of the laboratory in which the isolations were made.

The earliest acceptable records of *H. grisea* are those of Jensen (1912) and Dale (1912). This was just prior to Traaen's establishing the genus *Humicola*. Jensen's isolates were wrongly assumed by him to be identical with an organism that had been described by Morgan in 1895 as *Monotospora nigra* Morgan, and Dale's was wrongly presented (Dale 1912; Smith and Ramsbottom 1912) as *Basisporium gallarum* Moll.

Mason is to be credited with the sorting out of the cultures involved in the early history of *Humicola grisea*.

#### SYNONYMY

*HUMICOLA GRISEA* Traaen, *Nyt. Mag. Nat.* 52: 34. 1914.

*Monotospora Daleae* Mason, Annotated account of fungi received at the Imperial Mycological Institute, List II (Fascicle 2), p. 50, 1933.

*Melanogone puccinioides* Wollenw. and Richter, *Zentralbl. fur Bakt. Abt. 2.* 90: 76. 1934.

#### GEOGRAPHIC DISTRIBUTION, HABITAT, MATERIAL EXAMINED

*Massachusetts:* Harvard Forest I-1-2, I-1-17b, and I-6-2, isol. at Harvard Unit., 12 Oct. 1949 by Frank L. Raymond from well-drained sandy loam at point 4 inches below surface of mineral soil in 26 yr. old pine-spruce plantation, Harvard Forest, Peter-sham, Mass., Plot 26N., Prospect Hill Compartment I; Harvard Forest II-1-20, II-7-9, and II-7-10, isol. at Harvard Forest, 6 July 1950 by Frank L. Raymond from loam soil taken at line of demarcation between organic matter and mineral soil in same plot as above.

*New York:* Said by Jensen (1912) to be common in cultivated soil at Ithaca and isol. commonly in summer, winter, and fall. Jensen made the combination *Mycogone nigra* (Morgan) Jensen

on the erroneous assumption that he was dealing with *Monotospora nigra* Morgan. The latter (type specimen loaned us by Dr. G. W. Martin) is a very different thing. Jensen's description is almost certainly based on *Humicola grisea* but the specimen he cites is lost (Mason, 1933, p. 54; also personal communication from G. C. Kent, June 1951). Whether or not all of Jensen's isolates should be referred to *H. grisea* is, of course, unproven.

*New Jersey:* Waksman (1916) reported an isolate which he questionably labeled *Basisporium gallarum* Moll. from iron (48 per cent) soil taken at a depth of 8-10 in. in a peach orchard at Keyport. This would appear from his description to be *H. grisea*. It did not appear in several other soils. The isolate proved to be strongly active in decomposing a chemically modified cellulose. Waksman mentioned isolates of *Humicola* sp. in several additional papers but their specific identity is unknown. The writers have seen only one isolate of *Humicola* by Waksman, his No. 63, which is *H. fuscoatra* as indicated by Mason and Diehl. No data are available concerning its origin (personal communication from Waksman).

*Manitoba:* ATCC 6725 (QM 542), a culture sent (as *Monotospora Daleae*) by Dr. J. E. Machacek, then of the Dominion Laboratory of Plant Pathology, Winnipeg, to the Am. Type Cult. Col. in 1938 without detailed data. This is undoubtedly confirmatory of the reports by Bisby, James and Timonin 1933, p. 266; Bisby, Buller and Dearness 1933, p. 100; Bisby 1938, p. 121, that the species is common in soils of grasslands and wheatfields in Manitoba "but not found in other soils."

*Saskatchewan:* Isolated from roots of wheat from Indian Head (Bisby 1938, p. 121).

*Finland:* Based on the work of Vartiovaara (1935).

*Poland:* Based on work of Felsz-Karnicka (1935). Cultures not seen by us.

*Norway:* Culture obtained from C.B.S. and accessioned here as QM 993, isolated from soil in Norway by Traaen (see Traaen 1914), regarded as the type of the species. Traaen, using filter paper as an isolation medium, obtained cultures of the species from 17 samples of substrate from such habitats as decaying tree

trunks, at least some of which were coniferous, bits of leaves from various hardwoods, and samples of soil from near surface of meadow, muck, an anthill, graveyard, brook bed and potato field. It was especially common in field and meadow soil and rotten tree trunks.

*Germany*: Based on work of Wollenweber and Richter (1934). Organism made by them the basis of a new genus and species, *Melanogone puccinioides*, and later properly referred to *Humicola* by Mason (1941). Said to be an inhabitant of forest litter, decaying tree roots, and decaying structural timber at or just below ground level, specifically in roots of *Ulmus scabra-montana* killed by Dutch elm disease, forest litter of birch-pine and of spruce; not forming any conspicuous surface deposition of mold growth. A culture deposited at C.B.S. by Wollenweber was examined by us.

*England*: Culture obtained from C.B.S. and accessioned here as QM 992, isolated by Dale from soil at Woburn, England, the soil having been "continuously manured for 38 years with sulphate of ammonia" and having acquired a "distinctly acid reaction." (See Dale 1912, Smith and Ramsbottom 1912, Mason 1933, 1941, Wakefield and Bisby 1941.)

*Ceylon*: Culture cited by Mason (1933), from 'Adco,' C. H. Gadd 87. Not examined by us.

*Australia*: Culture cited by Mason (1933), from soil, Melbourne, E. McLennan. Not examined by us.

#### CHARACTERISTICS OF THE SPECIES

*Morphological*. *Humicola grisea* is known only in pure culture, there being as yet no authentic record of its ever having been identified in the form of a superficial fruiting layer on natural substrata, which is characteristic of so many of the Dematiaceae. A possible exception is the fact that Wollenweber obtained aleuriospores and phialospores on naturally infected wood which he removed from its source and exposed to another set of conditions. On agar media growth is fairly rapid and aleuriospores are formed among the underlying aerial hyphae, quickly, and usually in some abundance. FIG. 1 shows duplicate plates of four isolates grown in parallel series under room conditions on potato dextrose agar

(per liter: 20 gm dext., 20 gm agar, water from 400 gm boiled potatoes). It will be noted that the heavy, fairly compact, cottony mat in eleven days nearly reached the margin of the 9 cm petri dishes.

The four strains shown in FIG. 1 were used for comparison of colony characteristic mainly because they were the four most conveniently available at the time. Incidentally, they represent wide variation in age of isolate and geographic range. (A) represents a culture isolated from soil in England at least 38 years ago; (B) was isolated from soil in Norway at least 36 years ago; (C) was obtained from soil at Petersham, Massachusetts 1 year before the photo was taken; (D) was taken from a Manitoba soil about 20 years ago. These strains exhibit what most workers would consider to be a remarkable stability in culture and an equally remarkable uniformity among strains of a species. The four strains show no differences that could be put into words with the exception of (A) where the colonies exhibit a dark central area, due largely, if not entirely, to the formation of globules of liquid on and within the hyphal mat.

Growth is at first nearly white, then a very pale gray, gradually deepening and with age reaching a fairly dark gray. The color is mostly in the dark aleuriospores which develop among the hyphae. The pedicels are also noticeably dark as seen under the microscope, while the hyphae, though hyaline as seen individually, may contain a certain amount of pigment as seen in mass.

The aleuriospores (FIG. 2, A-D, and F-J) are smooth, dark, single-celled, typically globose, with an occasional one varying to obovoid or pyriform. The walls are of substantial thickness. The spores fundamentally are borne singly but it is not unusual to find two (FIG. 2, A, F) or three clinging together. Certain writers have been led to make reference to two-celled spores through the interpretation of an inflated hyphal cell adjacent to the spore (FIG. 2, G left of center, F, and I) as part of the spore. Most of the spores fall within the range of 12 to 15  $\mu$  in diameter with any strain exhibiting an occasional spore under and over this range. Detailed measurements have been made for five strains with the following averages for 70 spores:

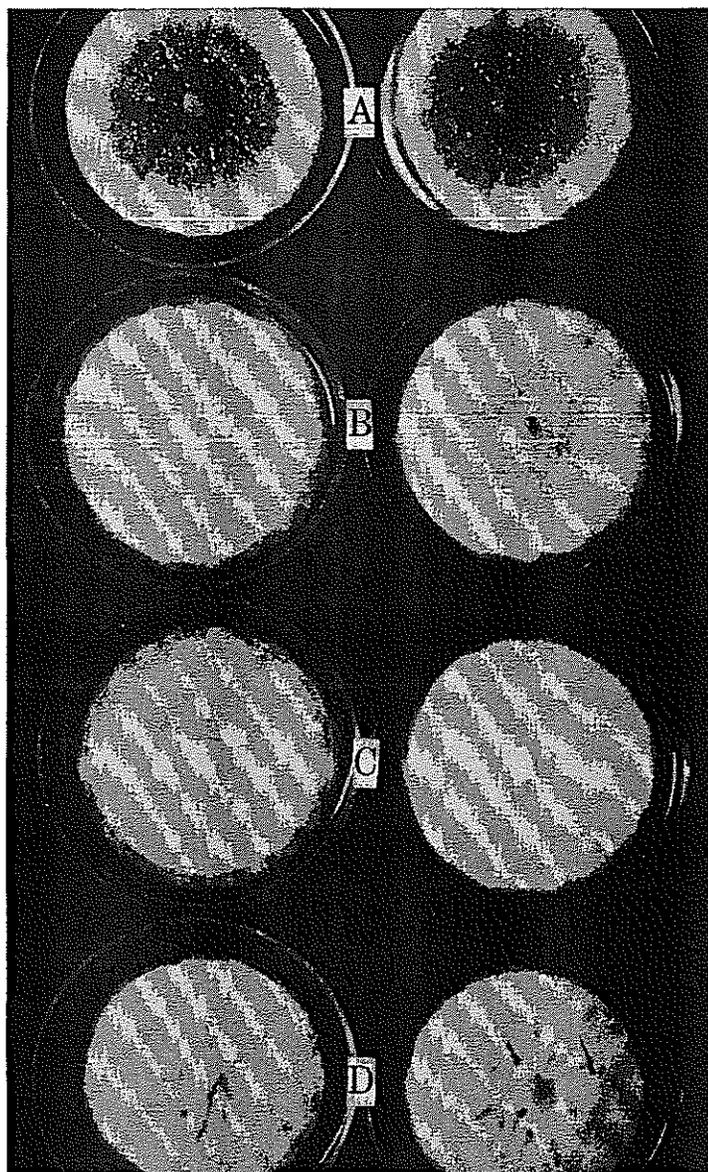


FIG. 1. Four cultures of *Humicola grisea* grown in parallel series for comparison of gross colony characteristics: A. QM 992, the culture iso-

1. QM 993, Traaen's type from 6-weeks-old colony on potato dextrose (2 percent dext.) agar, mounted in water =  $13.2 \mu$ .

2. QM992, Dale's culture, grown in parallel series with the preceding, also mounted in water =  $13.2 \mu$ .

3. Harvard Forest I-1-2, in parallel series with the two preceding =  $14 \mu$ .

4. Harvard Forest II-1-20, from a potato dextrose agar (2 percent dext.) stock slant of unknown age, probably from refrigerated storage =  $12.8 \mu$ .

5. ATCC 6725 (QM 542), grown in parallel with 1-3 above =  $12.4 \mu$ .

These measurements agree closely with those recorded by Traaen, Bisby, and others, and are recorded only for the purpose of showing that there is no great amount of variation among strains.

The variations in microscopic characters described above are variations within a strain. No noteworthy variation has been found among strains in the course of routine examinations and comparison of photomicrographs. In accompanying plates the spores of the recent isolate Harvard Forest I-1-2 (FIG. 2, G-J) may be compared with those of Traaen's type culture (FIG. 2, A-C).

Traaen, Wollenweber, and Mason all found phialospores and phialides in their cultures in addition to the aleuriospores. These were inconspicuous, however, and were encountered only under certain conditions. Traaen noted their development as a whitish covering on old colonies grown on filter paper and mineral salts. Mason found them in all the strains with which he worked, but only on potato dextrose agar, and in all but one isolate they were always in old cultures after the period of active aleuriospore formation. Wollenweber produced them on the surface of naturally

lated by Dale prior to 1912 from soil in England; B. QM 993, Traaen's type culture isolated from Norwegian soil prior to 1914; C. Harvard Forest I-1-2 from soil at Harvard Forest, Petersham, Mass. in 1949; D. ATCC 6725 isolated from soil in Manitoba about 1930. Colonies started by transplanting from stock cultures a small piece of agar with fungus. Grown on potato dextrose agar (per liter: 20 gm dext., 20 gm agar, water from 400 gm boiled potatoes), 35 cc medium per dish, room conditions, 11 days. Magnif. approx.  $\frac{1}{2}$ . Photo by Frank White.

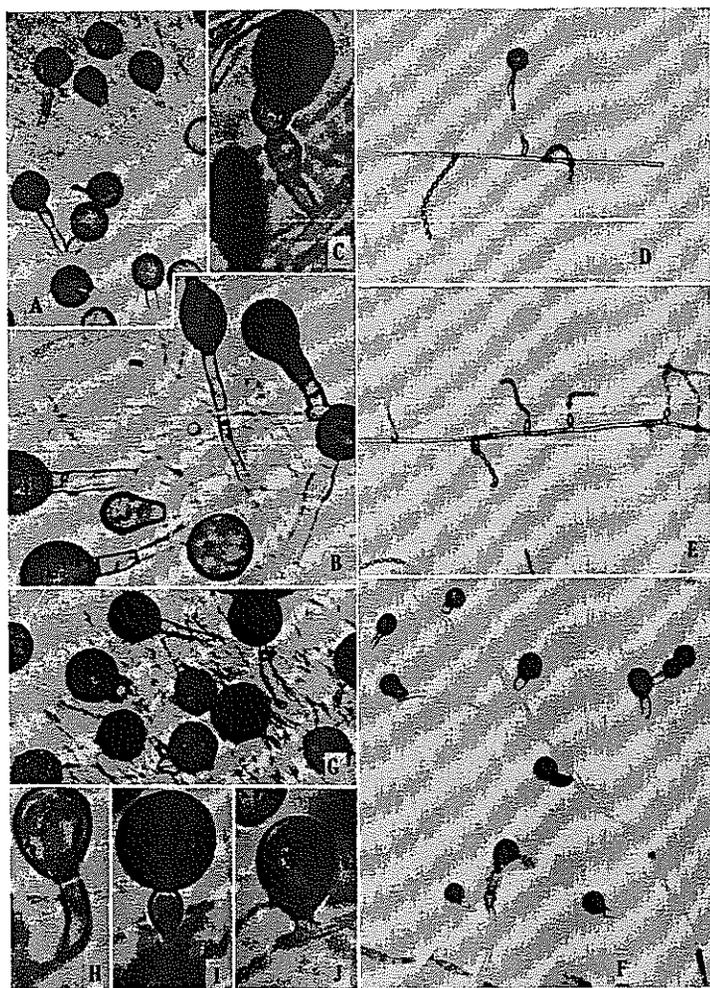


FIG. 2. Photomicrographs of *Humicola grisea*. A-F. QM 993 from plate cultures of Traaen's type: A,  $\times 500$ , B,  $\times 775$ , and C,  $\times 1000$ , aleuriospores of the culture on peptone-glucose (0.5-1 percent) agar after 12 days under room conditions, mounted in lacto-phenol, unstained; A and B from growing culture, photomicrograph by Leo Kaplan; C from the same culture after it had been dried down as an herbarium specimen, photomicrograph by Paul Brown. D, E, F, all  $\times 260$ . Photomicrographs by the junior author, taken directly from a culture on mineral salts agar containing fragments of corn stalk and leaves of *Phragmites communis* after 12 days at

infested wood, possibly by placing the pieces in a moist chamber. The phialides appear as single, elongate, tapering cells about  $8-16 \times 2-3 \mu$ , seated laterally on a hypha. They extrude small, hyaline, obovoid spores about  $3 \times 2 \mu$ , in chains or in balls. In the present study phialides have again been demonstrated in QM 993, a culture from Traaen's type grown on fragments of corn stalk and on leaves of *Phragmites communis* in mineral salt agar ( $30^\circ \text{C}$ , 12 days) (FIG. 2, D, E). Under the same conditions, no phialides were found in the six other cultures examined.

*Physiological.* The species appears to be characterized by a physiological stability comparable to the morphological. This at least is true of its cellulolytic ability. In a previous paper (see footnote, first page), a table was presented comparing the cellulolytic activity of numerous isolates of four allied species (*Humicola grisea*, *H. fuscoatra*, *Coccospora agricola*, and *Monotospora lanuginosa*). With the exception of *M. lanuginosa*, all exhibited strong cellulolytic properties.

The cellulolytic activity of these isolates did not appear to be affected by the length of time that they had been maintained on artificial media containing a sugar as the carbon source. The minimum periods under which the isolates under test were kept on such media:

	QM 992—38 years
	QM 993—36 years
	ATCC 6725 = QM 542—20 years
Harvard Forest I-1-2	= QM 994—1 year
Harvard Forest I-6-2	= QM 995—1 year
Harvard Forest I-1-17b	= QM 996—1 year

$30^\circ \text{C}$ , no mount; D shows aleuriospore and phialospores produced from same basal hypha; E illustrates phialospore production, and F shows branching hyphae bearing aleuriospores. G-J. Photomicrographs by Paul Brown of aleuriospores from Harvard Forest I-1-2 culture: G,  $\times 500$ . From potato dextrose agar mounted in lacto-phenol, unstained; H,  $\times 1000$ . Spore on an elongate, cylindrical hyphal branch from an unknown medium, mounted in lacto-phenol, stained with fast green; I,  $\times 1000$ . Spore seated on an inflated hyphal cell, mounted in lacto-phenol, stained with cotton blue; J,  $\times 1000$ . Spore originating directly from the side of a hypha, mounted in lacto-phenol, stained with fast green.

In an attempt to round out the present study, excerpts from Traaen's work (1914) are presented covering the conditions for growth:

Temperature: 5–30° C; optimum 20–25° C.

Carbon Sources: Good: Glucose, sucrose, fructose, inulin, starch, xylan, pectin. Poor: Glycerol, maltose, mannitol.

Nitrogen Sources: Good:  $\text{NH}_4\text{NO}_3$ ,  $\text{KNO}_3$ ,  $(\text{NH}_4)_2\text{HPO}_4$ , alanine, leucine, tyrosine, urea, glycocholl, arginine, guanidine, nucleic acid, sodium humate. Poor:  $\text{NH}_4\text{Cl}$ ,  $(\text{NH}_4)_2\text{SO}_4$ , creatine.

#### SUMMARY

1. *Humicola grisea* is a member of the Dematiaceae characterized mainly by the copious production on agar media of dark, globose, thick-walled aleuriospores 12–15  $\mu$  in diameter, and by its strong cellulose-decomposing capacity in pure culture tests.

2. It is an inhabitant of cultivated and forest soils and of decaying wood and other coniferous and hardwood forest debris in or on the soil, and is not known to form any surface deposition of mold growth in nature such as is characteristic of many of the Dematiaceae. Available data indicate occurrence in a great range of soil types, especially those rich in organic matter.

3. It has been isolated from widely separated parts of the world but mainly from parts of North America and Europe having cold winters. It appears to be common where adequate studies have been made.

4. Strains of the species exhibit a remarkable stability and uniformity in pure culture.

#### BIBLIOGRAPHY

1. Bisby, G. R., N. James and M. Timonin. 1933. Fungi isolated from Manitoba soil by the plate method. *Can. Journ. Res.* 8: 253–275, ill.
2. —, A. H. R. Buller and John Dearness. 1933. Additions to the fungus flora of Manitoba II. *Rep. Canadian Pl. Dis. Survey* 13: 93–102.
3. —. 1938. The fungi of Manitoba and Saskatchewan. 189 pp. National Research Council of Canada, Ottawa.
4. Corda, A. C. I. 1837. *Icones fungorum* 1: 1–32, ill. (Pragae).
5. Dale, Elizabeth. 1912. On the fungi of the soil. *Ann. Myc.* 10: 452–477, ill.

6. Diehl, W. W. 1949. Concerning the identity of Iterson's cellulolytic *Mycogone*. *Mycologia* 41: 277–279.
7. Felsz-Karnicka, Halina. 1935. Rozklad cellulozy w glebach kwasnych. *Pamiętnik Państwowego Instytutu Naukowego Gospodárstwa Wiejskiego w Pulawach* 16: 1–48, ill.
8. Jensen, C. N. 1912. Fungous flora of the soil. New York (Cornell) Agr. Exp. Sta. Bull. 315: 414–505, ill.
9. Mason, E. W. 1927. On species of the genus *Nigrospora* Zimmermann recorded on monocotyledons. *Trans. Brit. Mycol. Soc.* 12: 152–165, ill.
10. —. 1933. Annotated account of fungi received at the Imperial Mycological Institute. List II (Fasc. 2). [Imp. Myc. Inst. Mycol. Papers. 3.] 67 pp., ill.
11. —. 1941. Annotated account of fungi received at the Imperial Mycological Institute. List II (Fasc. 3—Special Part). [Imp. Myc. Inst. Mycol. Papers. 5.] 103–144, ill.
12. Rogers, D. P. 1949. Nomina conservanda proposita and nomina confusa—Fungi. *Farlowia* 3: 425–493.
13. —. 1950. Nomina conservanda proposita and nomina confusa—Fungi. Supplement. *Farlowia* 4: 15–43.
14. Saccardo, P. A. 1866. *Monotospora* Sacc. *In Sylloge Fungorum* 4: 807 pp. Padova.
15. Smith, A. Lorrain and J. Ramsbottom. 1912. New or rare microfungi. *Brit. Mycol. Soc. Trans.* 4: 165–185.
16. Traaen, A. E. 1914. Untersuchungen über Bodenpilze aus Norwegen. *Nyt. Mag. Naturv.* 52: 19–121, ill.
17. Vartiavaara, U. 1935. Suomen Maataloustieteellisen Seuran Julkaisija. *Acta Agralia Fennica* 32: 107 pp.
18. Wakefield, E. M. and G. R. Bisby. 1941. List of Hyphomycetes recorded for Britain. *Brit. Myc. Soc. Trans.* 25: 49–126.
19. Waksman, S. A. 1916. Soil fungi and their activities. *Soil Sci.* 2: 103–156, ill.
20. Wollenweber, H. W. and H. Richter. 1934. *Melanogone*, eine neue Gattung der Dematiaceen. *Zentralbl. für Bakt., Paras. und Infekt. Abt. 2.* 90: 74–76, ill.