

THE IDENTITY OF "METARRHIZIUM GLUTINOSUM" *

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(WITH 2 FIGURES)

Shortly after the beginning of the war, members of the Division of Cotton and Other Fiber Crops and Diseases, United States Department of Agriculture, in connection with work on the preservation of cotton fabrics, obtained from stored baled cotton in Washington, D. C. (4) an isolate (1334.2) representing a then unknown species of fungus, and a little later (2, 4) two additional isolates, 1334.1 and 1334.3, representing the same species, from Maryland soils. Work there, based on 1334.1 (3) and perhaps to a lesser extent (2, 4) on 1334.2 and 1334.3, soon demonstrated the species to be highly adapted to use in laboratory work pertaining to the microbiological decomposition of cotton fabrics, and later (4) 1334.2 was made the type of a new species.

After the microbiological decomposition of military fabrics in the tropics became recognized as a serious problem and a program for the development of preventive measures got under way, these cultures were widely distributed to governmental, commercial, and university laboratories in this country and throughout the British Empire. Toward the end of the war "*Metarrhizium glutinosum*," as the species was called in deterioration work, was used perhaps more than any other, not excluding the previously universally employed *Chaetomium globosum*, in the assessment of preservative treatments of fabrics. The species is now used in the Biological Laboratories of the Philadelphia Quartermaster Depot in research on the chemical nature of the fungus breakdown of cellulose, and is used elsewhere wherever similar work or assessment testing is being conducted. Its mineral nutrition, temperature, and pH relationship to cellulolytic activity were investigated recently in a Quartermaster sponsored project at the Pennsylvania State College but the results are not as yet published.

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Finally the species was introduced to the field of antibiotics when British workers (1) extracted from filtrates of a culture obtained from the U.S.D.A. an antifungal substance which they named glutinosin. Extracted along with the glutinosin but remaining in the mother liquors after crystallization of the glutinosin was another biologically active material causing a dermatitis of humans similar to that caused by poison ivy, thus necessitating the wearing of barrier creams in working with culture extracts of the mold.

Though the culture first introduced to the literature was 1334.1, it appears that 1334.2 was the one that was most generally and widely distributed, and on which most, if not all, later work in the various laboratories was based.

In view of the foregoing interest and activity, this note is presented for the purpose of calling attention to the need for further inquiry into the true identity and relationship of what is now masquerading under the name *Metarrhizium glutinosum*. The name is unfortunate from both the scientific and economic points of view, viz.: (1) it places the species as next of kin to an insect-inhabiting form to which presumably it bears no close relationship in biological activity, thus misleading workers interested in cellulolytic activity or antibiotics or other fields of endeavor, who may for obvious reasons wish to survey related forms; (2) it breaks the continuity of the literature, submerges earlier records, and stands in the way of co-ordination of effort among the various interested laboratories and individuals; and (3) the addition of new names to the literature for organisms with long prior records, and which ought to be identified, is a practice which must be more vigorously condemned in the future than it has been in the past.

What, then, is "*Metarrhizium glutinosum*" and where are to be found other species which are phylogenetically related and which might exhibit more or less similar cellulolytic, antibiotic, and other biological activity?

THE GENUS MYROTHECIUM

There is a genus *Myrothecium* which has been known—in a sketchy way, to be sure—for some 150 years. During this period it has accumulated about a dozen species. All are very inade-

quately known, but the best known and evidently the most common are what have been correctly termed by Preston (5), working in England, the three basic species: *M. inundatum* Tode ex Fr., *M. roridum* Tode ex Fr. and *M. verrucaria* (Alb. & Schw.) Ditm. ex Fr.

Dr. John Stevenson has kindly loaned the writers the *Myrothecium* folder from the general collection of the Pathology and Mycology Collections of the United States Department of Agriculture. It contains thirty-five packets, representing, according to the labeling, six species, of which twelve are said to be *M. roridum* and eleven *M. verrucaria*. Examination of the lot indicates the existence of considerable confusion in the nomenclature but that the collections do actually represent a group of at least six reasonably closely allied species. A re-sorting of these places fifteen specimens under *M. roridum* and four in *M. verrucaria*. A more complete treatment will have to await the appearance of a monograph of the genus, which is badly needed.

The attention of Preston (5) was drawn to the systematics of the genus in an attempt to identify a form actively pathogenic to the cultivated violet, *Viola tricolor*, in England. This he established as *M. roridum*, which was isolated also from tomato and snapdragon plants in England and from several species of plants in Sierra Leone, and which showed some indication of pathogenicity to several species. He recorded *M. verrucaria* from Southern Rhodesia, Cyprus, and the Sudan. The Cyprus culture was from living potato haulms and was believed to have been pathogenic. An isolate (made in 1943) from an old canvas shoe (possibly American) was said to be the first record of the species in the British Isles. It may be noted at this point that in North America *M. roridum* was reported (6) to have been the cause of a crown canker of snapdragons in a commercial greenhouse in Texas in 1933-34, killing 90 per cent of the plants, and that more recently it has received attention as a pathogen of greenhouse snapdragons in Colorado (8).

Preston appears to have established the identity of the "three basic species," previously mentioned, about as adequately as will ever be possible. The one weakness in his treatment of *M. verrucaria* is that the type, if still in existence, was not available for

his examination. Nevertheless, since his coverage of historical material and literature is good, his work is acceptable, and the species may be considered as adequately established in accordance with his treatment.

A comparison of the USDA 1334.2 culture with Preston's description and illustrations of *Myrothecium verrucaria* disclosed no detectable differences. The conclusion that the American cultures, under the name *Metarrhizium glutinosum*, were conspecific with the British cultures of *Myrothecium verrucaria* was confirmed by further comparisons as indicated on the following pages. Therefore, *Metarrhizium glutinosum* Pope should be referred to synonymy under *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fr.

MORPHOLOGICAL COMPARISON OF BRITISH AND AMERICAN CULTURES

One of the cultures cited by Preston (5, p. 168) was requested and obtained by the writers from Mr. George Smith of the London School of Hygiene and Tropical Medicine, who had originally furnished it to Preston after it had been isolated from a canvas shoe in England. The culture when received at Philadelphia was given the number PQMD 185. A detailed comparison of its microscopic characters with those of USDA 1334.2 revealed no differences whatsoever. When the two numbers were grown in parallel series on plates of potato dextrose agar (FIG. 1A, B) there likewise were no differences. A culture of *Myrothecium roridum* (PQMD 188), furnished by Dr. Stevenson after it had been isolated from tomato fruits intercepted at the Mexican-United States border by quarantine inspectors of the U.S.D.A., was grown in the same series (FIG. 1C). Its growth pattern, as indicated in the photograph, was only slightly different. This difference is of no significance in the separation of the two species since both have often been observed to sporulate in definite and sharply delimited concentric zones. The two species are very closely related as evidenced not only by their morphology but also by their occurrence, distribution, and biological activity. The best, and perhaps the only, distinguishing character is the spores, which in *M. verrucaria* are more or less ovoid with a peculiar and characteristic outline,

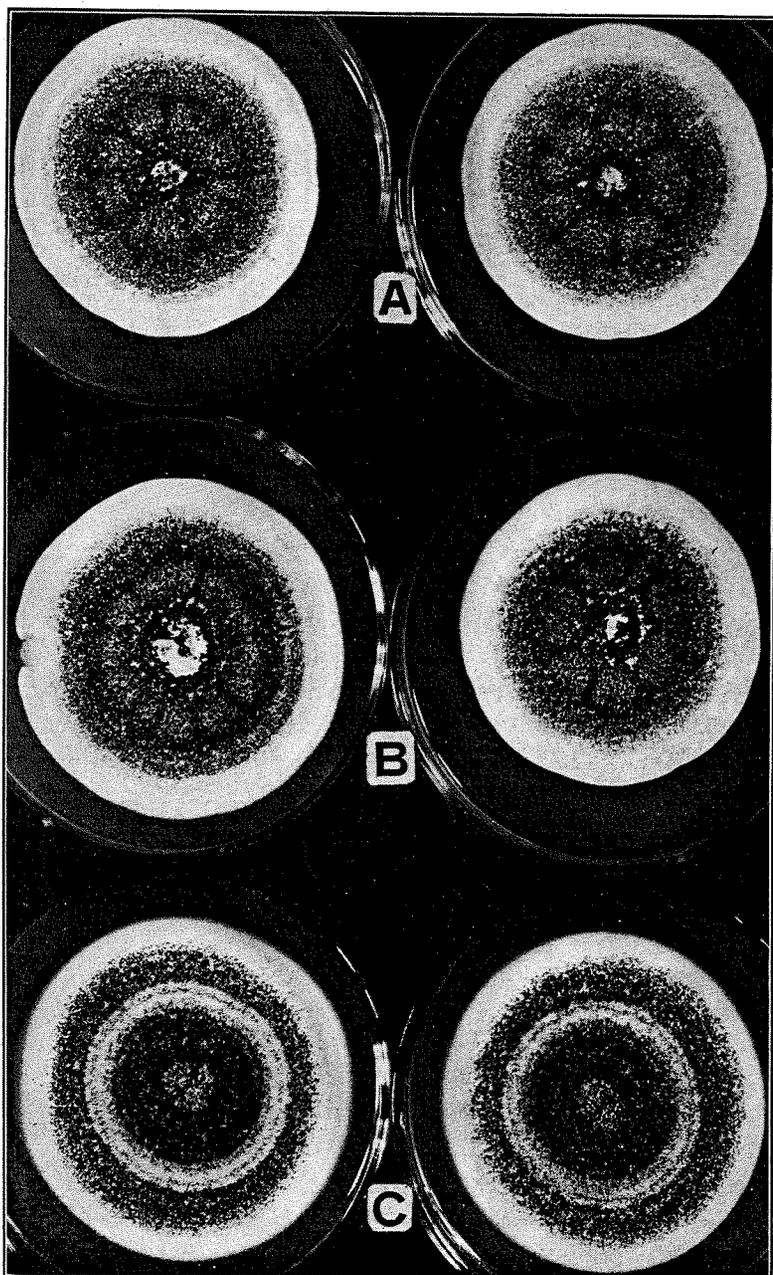


FIG. 1. *Myrothecium verrucaria* (A, B) and *M. roridum* (C) on potato dextrose agar.

whereas in *M. roridum* they are narrow and cylindrical. The microscopic characters of *M. verrucaria* were adequately described and illustrated by both Preston and Pope, and of *M. roridum* by Preston.

More recently the remaining USDA cultures of "*Metarrhizium glutinosum*," 1334.1 and 1334.3, were supplied by Dr. Paul Marsh and the additional cultures cited by Preston were obtained from him. They exhibit no noteworthy variation and all represent the same species. Preston's cultures of *Myrothecium roridum* were also made available.

CELLULOLYTIC ACTIVITY IN PURE CULTURE ASSAY

The cellulolytic activity of Cultures PQMD 185 and USDA 1334.2 representing *Myrothecium verrucaria* and PQMD 188 representing *Myrothecium roridum* was compared as follows:

Strips of 3.3 oz., relatively size-free, bleached cotton sheeting were raveled to a width of exactly 1 inch, cut to six-inch lengths and placed 1 each in 150 × 25 mm. test tubes each containing 25 ml. Greathouse Formula A mineral salts solution (3) with lower half of strip submerged, and autoclaved 20 min. at 15 lbs. pressure. Inoculum was prepared as follows: Each of the three organisms was grown on 2 per cent potato dextrose agar slants for 17 days. Formula A solution was placed in the tubes, the spores gently dislodged by agitation with the tip of a pipette, and the suspensions emptied into flasks containing glass beads and shaken. The spore counts were adjusted to approximately 22,000,000 per ml. for each of the three cultures to be tested. Two ml. of the resulting suspension was pipetted evenly over the exposed portion of each strip. Incubation was in a room set at approximately 85° F. and 80 per cent relative humidity. At harvest the strips were removed from the tubes, washed in 95 per cent alcohol, rinsed in tap water, dried in the laboratory atmosphere, then conditioned 24 hours in an atmosphere containing 65 per cent relative humidity, temperature 75° F., and broken on the motor driven Scott tensile strength tester with a three inch space between the jaws. Each figure in the following table represents pounds breaking strength retained, based on an average of ten replicates. The decline in

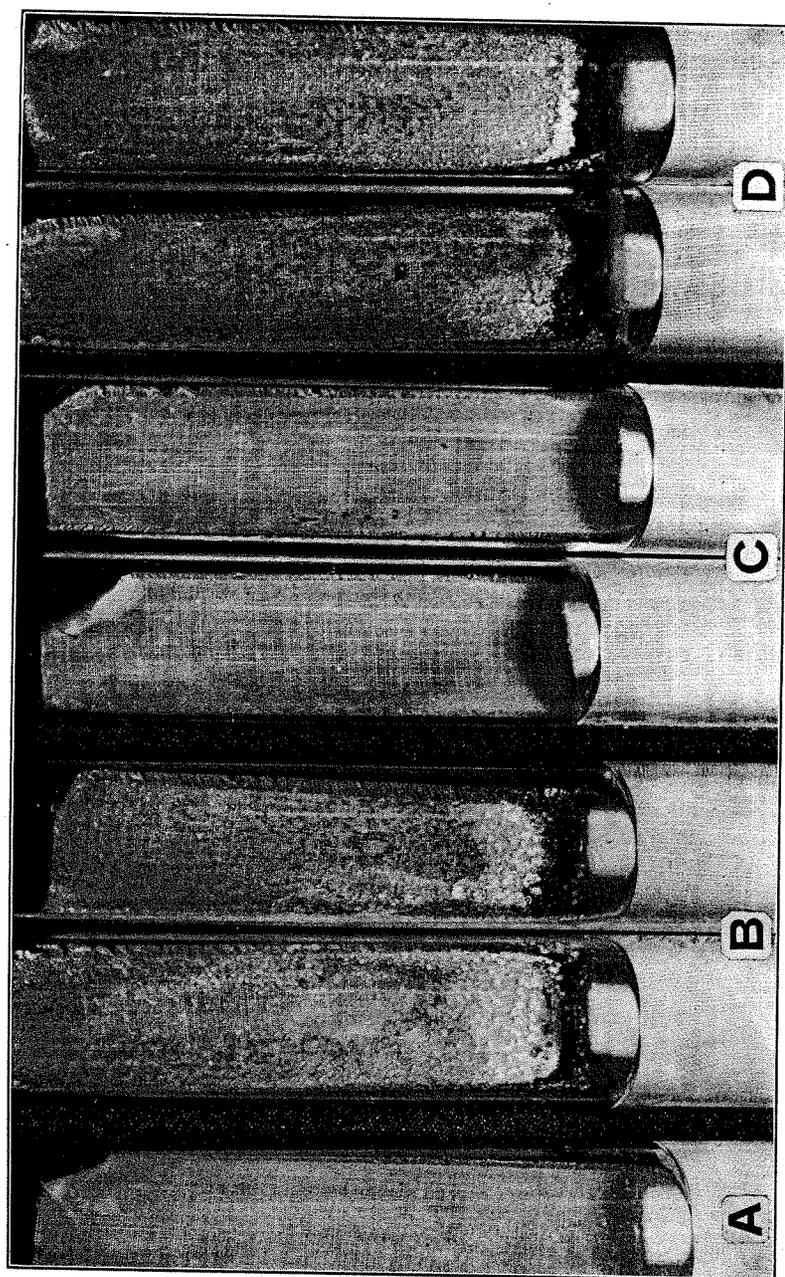


FIG. 2.

 TABLE I
 CELLULOLYTIC ACTIVITY OF MYROTHECIUM VERRUCARIA AND *M. RORIDUM*

Organism	Lbs. tensile strength retained		
	40 hours	64 hours	90 hours
PQMD 185 <i>M. verrucaria</i>	28.0	11.4	3.0
PQMD 188 <i>M. roridum</i>	40.7	30.5	14.8
USDA 1334.2 <i>M. verrucaria</i>	28.9	13.5	3.1
Uninoculated control	40.6	40.2	41.2

tensile strength of the cloth is taken as a measure of the cellulolytic activity of the organisms.

It will be noted from the table that under the conditions of the test the capacity of the two strains of *M. verrucaria* to break down cellulose was approximately equal whereas that of the single strain of *M. roridum* was somewhat weaker. The appearance of the three cultures on the fabric test strips after ninety hours incubation is shown (FIG. 2). Here again little or no difference is noted between the two strains of *M. verrucaria* (FIG. 2B, D). Mycelial development and sporulation is apparent over all of the exposed portions of the strips but is particularly heavy at and just above the surface of the liquid. In the case of *M. roridum* (FIG. 2C) visual growth was comparatively light and confined mostly to a rather scanty mycelial development on and just above the surface of the liquid, with little or no sporulation. In both strains of *M. verrucaria*, the cloth became intensely yellow just above the surface of the liquid and more faintly so progressively further up. In *M. roridum* a similar pigmentation was present but much less intense. As is typical of fungi tested by this method, cellulolytic action is greatest immediately above the surface of the liquid.

After the remaining cultures cited by Preston under *M. verrucaria* and *M. roridum* were received, these were also subjected to cellulolytic assay. The latter set of tests, although much less exact than the first, was sufficient to indicate that strong cellulolytic

action in pure culture is a characteristic common to both species. Of approximately one hundred species of fungi surveyed in this laboratory during the past two years, none has been found to exhibit stronger action. A few approach it, but the vast majority act more slowly, and of course many are wholly non-cellulolytic.

OCCURRENCE ON FABRICS AND PLANT MATERIALS IN THE FIELD

A preliminary examination of herbarium material from the U. S. Department of Agriculture, and to a lesser extent from other sources, together with a review of literature records, a few of which were cited on previous pages, indicates that both *M. verrucaria* and *M. roridum* are fairly common and of widespread occurrence in both North America and Europe and in the tropics. *M. verrucaria* appears to be the less common of the two species. Most of the specimens from the U.S.D.A. are on plant debris. However, some furnish additional evidence that both, but especially *M. roridum*, are capable of invading the living tissues of a wide range of herbaceous economic plants. One, for example, taken in Virginia in 1937 shows numerous sporodochia of *M. verrucaria* on a large necrotic area on a tomato fruit. The sporodochia of *M. roridum* are abundant in concentric rings of leaf spots of *Hibiscus* sp. from Honduras, and of *Citrus maxima* from Puerto Rico, presenting unmistakable evidence that *M. roridum* is the causal agent of the disease.

Among several thousand cultures at hand, made from deteriorated cotton fabrics and related military and industrial materials, mostly from tropical exposures, these two species appear only a few times. In a few instances *M. roridum* has been found sporulating on the fabric, but in no case has *M. verrucaria* been seen on the several hundred molded samples which have been thoroughly examined. As pointed out recently (7) by the senior author, *M. verrucaria*, despite its strong cellulolytic activity in pure culture laboratory tests and its special adaptability to laboratory experimental work, appears to be of little significance as a destroyer of cotton fabrics in the field.

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EXPLANATION OF FIGURES

FIG. 1. Species of *Myrothecium* after two weeks on potato dextrose agar (20 g. dextrose per liter), 35 cc. medium in each dish, under room conditions.—A, PQMD 185, *M. verrucaria*, isolated from an old canvas shoe in England and recorded by Preston as *M. verrucaria*;—B, USDA 1334.2, *M. verrucaria*, isolated from stored baled cotton in Washington, D. C. and made by Pope the type of a new species, *Metarrhizium glutinosum*;—C, PQMD 188, *M. roridum*, isolated from tomatoes which had been intercepted at the Mexican border by quarantine inspectors of the U. S. Dept. of Agriculture. Magnification approx. 3%. (By Photographic Dept., Philadelphia Quartermaster Depot.)

FIG. 2. Species of *Myrothecium* after 90 hours incubation on bleached cotton sheeting in the presence of Greathouse Formula A mineral salts solution in a room set at 85° F. and 80 per cent relative humidity—A, Control;—B, PQMD 185, *M. verrucaria*;—C, PQMD 188, *M. roridum*;—D, USDA 1334.2, *M. verrucaria*. Magnification $\times \frac{1}{10}$. (By Photographic Dept., Philadelphia Quartermaster Depot.)