THE GROWTH OF FUNGI IN THE SOIL^{1,2}

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A few years ago, the writer published a note (6) relative to the fact that fungi exist in the soil not only in the form of spores, but also as mycelium, which indicates that these organisms are active in the soil and take a part in the soil fertility processes. A method has been suggested for the demonstration of this fact, and, by the use of this method, which is quite simple, a number of fungi have been found to exist in the soil in the form of vegetative mycelium. The majority of the organisms isolated by this method were found to belong to the *Mucorales* and were included in the genera *Mucor*, *Rhizopus* and *Zygorhynchus*. Such large groups of soil organisms as the *Penicillia*, *Aspergilli* and *Cladosporia* were not obtained at all or only in very few instances by this method. This would point to one of two considerations: either the *Mucorales* develop more rapidly out of the soil and thus crowd out the other organisms or, that the *Mucorales* and a few other fungi, such as *Trichodermae* are present in the soil in the form of vegetative mycelium, while the *Penicillia*, *Aspergilli* and *Cladosporia* are present there only in the form of spores.

When the common plate method is used for the study of soil fungi, the opposite may be found to hold true: the *Mucorales* may not develop to such an extent as the *Penicillia*, *Aspergilli* and *Cladosporia*, while such fungi as the green *Trichodermae* are obtained by both methods. The following hypothesis then suggested itself: the *Mucorales* and *Trichodermae* are always present in the soil in the form of spores and vegetative mycelium, the various representatives of these groups preferring one or another soil type or different environmental conditions; the *Penicillia*, *Aspergilli* and *Cladosporia*, which are common air inhabitants and are found abundantly above the surface of the soil, in the dust, are always present, one group or another, in the soil in the form of spores, which may germinate and produce a vegetative mycelium, when soil conditions become favorable, as in the case of addition of organic matter, proper moisture content, etc.

Since the first note was published, several reports were made by the writer (7, 8, 9), in which a study was made of the fungous flora of the soil and it was

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² This and the preceding paper appear together by mutual request of the authors.—Ed.

established without any doubt, that fungi (or molds) are permanent inhabitants of the soil and take part in important soil processes. Forms isolated in one locality may also be found in soils thousands of miles away, under entirely different environmental and soil conditions. However, the common plate method used with such convenience for the study of soil bacteria is entirely inadequate for the study of fungi, since not only the numbers but even the species of fungi developing on the common agar or gelatin plate do not give a true indication of the relative abundance and possible importance of fungi in the soil. This is due to the fact that the mycelium in the soil, however abundant, may be entirely absent, when the soil is diluted 10,000 or more times, for plating out of bacteria, while the fungi developing on the plate indicate only the spores of fungi present in the soil. These are so unevenly distributed that great variability is found not only throughout one soil sample but even on plates made from the same dilution, so that one plate may contain two or three colonies and a duplicate plate eight or ten fungus colonies.

In addition to the method suggested by the writer for the demonstration of fungus mycelium in the soil, a direct microscopic method has been suggested by Conn (2). The later investigator (1, 2), however, was unable to demonstrate any mold hyphae in the soil, which led him at first to conclude that molds are of relative insignificance in the soil. When one considers the fact that, for microscopic examinations, only a very small fragment of soil can be used and this is further diluted with water, one is not surprised that hyphae are not obtained by the microscopic method. Under these conditions any mycelium which would be present in a larger granule of soil, would be in most cases eliminated, since the mycelium is not so readily broken up into fragments as a bacterial chain. Then, mold hyphae assume in the soil a somewhat different appearance than in pure culture and might easily be mistaken, in a stained preparation, for organic matter.

The current idea about soil fungi was that these organisms are abundant only in soils of acid character and well supplied with organic matter. This is quite right, but it has also been pointed out in previous publications of the writer and others (3) that fungi are present in neutral and alkaline soils as well as in sandy soils containing very little organic matter. The following results will show that, 1, fungi are present in a mycelial stage in the soil and, 2, fungi are present abundantly not only in acid soils and those rich in organic matter, but even in nearly pure silicious sands not only in the form of spores but as abundant mycelium and also in neutral soils.

The author's method used for demonstrating fungi is, briefly, as follows: a clump of soil, the size of a large pea, taken out carefully from the soil sample with as little contamination from the air as possible, is placed in the center of a sterile plate, into which 10-12 cc. of a sterile nutrient agar, favorable for the development of fungi, has been placed; the soil is slightly pressed into the agar, so as to be surrounded by the nutrient medium. The plates are incubated at 25-30°C. At the end of 24-26 hours' incubation, the plates are

examined. Mold hyphae are then found to radiate out of the clump of soil into the surrounding medium. This is based upon the fact that the fungi present in the soil in the form of mycelium will grow at once into the medium, before the spores can germinate and develop hyphae. When a small piece of agar containing the growing mycelium, preferably a tip of a growing hyphae as far from the clump of soil as possible, is transferred upon a sterile slant of agar practically a pure culture of the particular fungus may be obtained.

One of the most common soil fungi found in the soil by the above method is a species of *Zygorhynchus*, closely related (5) to *Zygorhynchus vuilleminii* (Namys). A somewhat different species has been commonly found in northern soils obtained from Alberta (Canada) and Alaska. This fungus has been found by the direct method in practically all the soils examined, independent of the fact whether the soil is rich or poor in organic matter, acid, neutral or alkaline in reaction. Of special interest is the fact that this organism is found abundantly in subsoils, particularly sandy subsoils. Invariably a pure culture of this organism has been obtained by placing a clump of sandy subsoil, even as far as thirty inches deep, upon the plate.

Recently a sample of soil was received from Lakewood, N. J. It was obtained from an excavation and was stated to be present there in large quantities; its character was so unusual that an analysis was asked for. The soil belongs to the Lakewood series of sandy soils described in the soil Survey of the Freehold area (4), and is nearly a pure white sand, so typical of the New Jersey pine barrens. This particular sample, which was obtained in a sand excavation and was stated to represent a "considerable quantity" was found to be clumped together by a sort of a fine cottony mass penetrating throughout the soil. The soil had a reaction equivalent to pH 6.2 and contained, by the Kjeldahl method, 0.0123 per cent nitrogen.

This soil was examined for its content of microorganisms. The common plate method gave, in a 10,000 dilution, two or three bacterial colonies and two or three actinomycetes, per plate. A 1000 dilution gave fifteen to thirty bacterial colonies, six to ten actinomyces colonies, and one or two colonies of a green *Penicillium*. The direct method revealed the puzzle. A pure culture of the common soil *Zygorhynchus* was readily obtained and this was found to be the organism, whose mycelium penetrated the sand to such an extent that it held it in a compact mass. This organism which is found abundantly in the poor sandy subsoils of the Sassafras series of New Jersey was found to grow to such an extent in this barren sand as to hold the sand together compactly. What rôle this organism plays in the soil is not yet known. It either decomposes the traces of organic matter present in the soil, making the nitrogen and minerals available for the scrub pines, or, as in the case of the subsoils, it merely thrives in this medium, practically free from other competing microorganisms, on the minerals washed down from the surface soil by the drainage waters. This fact, of course, would not point to any great importance of fungi in the soil, but tends to indicate that they may become readily active under favorable circumstances.

Another interesting instance of the activity of fungi in the soil is found in the following illustration. A farmer located a few miles from the Experiment Station observed the fact that, by mixing soil, undecomposed organic matter, such as clover, alfalfa, etc., and non-nitrogenous mineral fertilizer, then adding the proper amount of water, a good growth of molds takes place. When this mixture was turned over every two or three days, the molds decomposed the organic matter so rapidly that, in seven days, the whole mass was quite similar to leafmold, and compared favorable with prepared organic fertilizers for certain greenhouse plants. The farmer went so far as to apply for a patent on this process. When this mixture of soil, organic matter and mineral fertilizer was examined, it was found that a few species of *Mucor* (*M. plumbeus*, etc.), were chiefly responsible for the decomposition of the organic matter and for the artificial formation of the leafmold. The *Mucors* grew to a height of one to two inches above the surface of the mixture.

However, to be able to determine the actual number of fungi in the soil, as represented both by the numbers of spores and pieces of mycelium, a new procedure has been developed. This was a result of a series of studies on the variability of numbers of microorganisms in the soil as determined by the plate method (10). It was found that, when the numbers of fungi are determined on the plates prepared for the count of bacteria and actinomycetes, the variability is so great, due to the high dilution of the soil and, therefore, to the small numbers of fungi obtained, that the probable error obtained even from as many as 50 plates prepared from one soil is so large as to make the results worthless. Where low dilutions of the soil are employed, so many bacteria will develop on the plate as to actually crowd out a good many fungi and make the count entirely unreliable.

By the use of special acid media, on which no bacteria and actinomycetes will develop, and low dilutions (about 1000), the numbers of fungi can be determined quite accurately. Of the acid media two can be suggested:

1. Raisin agar, prepared by heating 60 gm. of raisins in 1000 cc. of tap water for 1 hour, then adding 25 gm. of agar, dissolving, adjusting the reaction to pH 4.0, filtering, tubing and sterilizing as usual.

2. Synthetic agar: 10 gm. dextrose, 5 gm. peptone, 1 gm. KH_2PO_4 , 0.5 gm. MgSO_4 , 1000 cc. of water. Enough 1.0 *N* acid (sulfuric or phosphoric) is added to make the reaction equivalent to pH 3.6–3.8. This will require about 5–7 cc. 1.0*N* acid per liter of medium. Twenty-five gm. of agar are then added and dissolved by boiling. The medium is then filtered through cotton, tubed and sterilized as usual. The reaction of the medium after sterilization should be pH 4.0.

By the use of the two acid media and a low dilution of soil (2–0.5 per cent of that used for counting of bacteria), plates can be obtained containing only fungus colonies. The number of fungus colonies per plate should be between 30 and 100. Incubate 48–72 hours at 25°C. Where the majority of the colonies are of the same species, there is ground for suspicion of air blown spores, rather than soil forms.

By the use of this method, not only were the numbers of fungi present in the soil found to be lower, but the results were more definite and less variable. Even more important, a definite correlation has been found between soil treatment, soil reaction and numbers of fungi in the soil, as indicated in the following table.

TABLE 1
Influence of soil treatment upon numbers of fungi as determined by the plate method

SOIL FERTILIZATION	REACTION	NUMBERS OF FUNGI PER GRAM OF SOIL
	<i>pH</i>	
Minerals only	5.6	37,300
Heavily manured	5.8	73,000
Sodium nitrate	5.8	46,000
Ammonium sulfate	4.0	110,000
Minerals and lime	6.6	26,200
Ammonium sulfate and lime	6.2	39,100

Manure and acid fertilizers (ammonium sulfate) stimulate an increase in the numbers of fungi. By making the reaction less acid, lime results in a great decrease in the numbers of fungi. Further information on this method and more extensive results will be published later.

REFERENCES

- (1) CONN, H. J. 1916 Relative importance of fungi and bacteria in soil. *In Science*, n. s., v. 44, p. 857-8.
- (2) CONN, H. J. 1918 The microscopic study of bacteria and fungi in soil. N. Y. (Geneva) Agr. Exp. Sta. Tech. Bull. 64.
- (3) DALE, E. 1912-1914 On the fungi of the soil. *In Ann. Mycol.*, v. 10, p. 452-477; v. 12, p. 33-62.
- (4) JENNINGS, H., DICKEY, J. B. R., AND LEE L. L. 1916 Soil Survey of the Freehold Area, New Jersey. U. S. Dept. Agr. Bur. Soils, Field Operations, 1913.
- (5) NAMYSLOWSKI, B. 1910 *Zygorhynchus Vuilleminii*, une nouvelle mucorinée, isolée du sol et cultivée. *In Ann. Mycol.*, v. 8, p. 152-155.
- (6) WAKSMAN, S. A. 1916 Do fungi live and produce mycelium in the soil? *In Science*, n. s., v. 44, p. 320-322.
- (7) WAKSMAN, S. A. 1916 Soil fungi and their activities. *In Soil Sci.*, v. 2, p. 103-155.
- (8) WAKSMAN, S. A. 1917 Is there any fungus flora of the soil? *In Soil Sci.* v. 3, p. 565-589.
- (9) WAKSMAN, S. A. 1918 The importance of mold action in the soil. *In Soil Sci.*, v. 6, p. 137-155.
- (10) WAKSMAN, S. A. 1922 Microbiological analysis of soil as an index of soil fertility. I. The mathematical interpretation of numbers of microorganisms in the soil *In Soil Sci.*, v. 14, p. 81-101.