

SOME ASPECTS OF SOIL FUNGAL ECOLOGY*

BY K. RAMAKRISHNAN

(*University Botany Laboratory, Madras 5*)

NEARLY forty years ago Waksman (1916 *a*, 1916 *b*, 1917) demonstrated by his pioneering work that the soil which was till then considered an inert medium, was really a dynamic ecological habitat teeming with an active fungal population. Since then numerous other workers all over the world have investigated the fungal floras of various kinds of soils. Most of the literature on the fungi isolated from soils has been brought together by Gilman (1945). Many facts of synecological significance have emerged from these floristic studies; but these studies by themselves are not strictly ecological. The recent studies of Zachariah (1949), Warcup (1951) and Tresner, Backus and Curtis (1954), however, are exceptions. Many autoecological studies have also been undertaken by plant pathologists in their attempts to unravel the life processes of plant pathogenic fungi. As a result of such studies, soil-borne plant pathogenic fungi have been broadly classified as 'root inhabitants' and 'soil inhabitants' (Garrett, 1950, 1951, 1952). On the other hand, facts regarding the influence of factors of the environment on the life of the large mass of soil fungi which are not known to be pathogenic and hence are not of immediate economic value, have to be gleaned from the numerous floristic studies referred to earlier. These findings are briefly discussed below with reference to the author's own work on the synecology of the fungi of three soils in the neighbourhood of Madras.

TECHNIQUES

Many techniques have been devised for the quantitative and qualitative study of soil fungi. These could be broadly grouped as (1) direct isolation from soil by plating methods, and, (2) indirect isolation from previously colonised organic matter or artificial media. The 'dilution plate' technique of Waksman (1922) as modified by Brierley, Jewson and Brierley (1928) is a bacteriological technique, adapted for soil fungal studies. This technique has been largely used for quantitative and qualitative studies. Many workers have pointed out its limitations both for counting the numbers of fungi and for their isolation. Modifications have been suggested by Warcup (1950) who, instead of plating out soil dilutions, plated measured quantities of soil

* Paper read to the Indian Academy of Sciences at the Annual Meeting at Belgaum in December 1954 at the Symposium entitled "Soil Micro-organisms and Plant Well-being."

and demonstrated that fungi belonging to the Hymenomycetes could be made to appear in his 'soil plates'. Jensen's (1935) modification of the Rossi-Cholodny slide technique and the method described by Jones and Mollison (1949) have been claimed to give more accurate pictures of the relative abundance of fungal mycelium in the soil. These, however, because of the difficulty of operation, have not come into general use, and Waksman's technique still remains the most widely used. The technique of indirect isolation from colonized organic matter or artificial media can generally be called the 'baiting' technique, as, in all cases suitable baits are offered to the fungi for colonization. Butler (1907) was the first to use this technique, using hemp seeds as baits, for studying soil and aquatic Phycomycetes. Many later workers on the Phycomycetes like Sparrow (1943) have extensively and successfully used this technique. The 'straw burial' technique of Sadasivan (1939) as modified by Subramanian (1946) has been used in this laboratory for the study of soil-borne *Fusaria* with special reference to their occurrence and survival in soils. Zachariah's (1953) work has further helped to standardise this technique. It may be relevant to refer here to an experiment conducted by the author (Ramakrishnan, 1953) to assess the comparative merits of the 'root burial' and 'dilution plate' techniques. Fungi, as shown in the following table (Table I) were isolated by these two techniques from three soils at Vandalur, near Madras.

It is clear that neither of the techniques brought out all the fungi present in the soil. Fungi appearing on the dilution plate belonged mostly to the dry-spored group (Fungi imperfecti and Mucorales) producing enormous numbers of rather thick-walled, minute spores. These fungi are mostly saprophytes and are probably capable of withstanding microbial antagonism. These fungi probably exist in the free soil as spores most of the time. Fungi appearing on the rot bits on the other hand included strong cellulose decomposers like *Memnoniella echinata*, *Chaetomium* spp. and *Fusarium* spp. It is also interesting to note that such 'soil inhabiting' plant pathogens like *Neocosmospora vasinfecta*, *Macrophomina phaseoli* and *Fusarium solani* appeared only on the root bits. Similar results have been reported by Zachariah (1953) also. It seems reasonable to assume that these fungi do not exist in the free soil, but inside vegetable debris which they colonise, and remain there either as mycelium or in some resting stage. The finding of Venkatram (1952) that *Fusarium solani* could be induced to form abundant chlamydospores by bacterial action is of special interest in this context.

Chinnayya's (1952) ingenious adaptation of the root burial technique by the use of ammonia to isolate *Coprinus* spp. indicates that this technique has not been fully exploited, and much remains to be done in this field.

TABLE I
Fungi isolated from Vandalur Soils

Fungi appearing on dilution plates	Fungi appearing on root bits	Fungi common to both techniques
<i>Cunninghamella echinulata</i> Thaxter	<i>Pythium mamillatum</i> Meurs	<i>Cunninghamella bertholletiae</i> Stadel
<i>Arthrobotrys</i> sp.	<i>Syncephalis cornu</i> Van Tiegh. & LeMonnier	<i>Mucor racemosus</i> Fres.
<i>Aspergillus sydowii</i> (B. & S.) Thom & Church	<i>Syncephalis reflexa</i> Van Tiegh.	<i>Thielavia basicola</i> Zopf.
<i>Aspergillus terreus</i> Thom	<i>Chaetomium trilaterale</i> Chivers	<i>Aspergillus niger</i> Van Tiegh.
<i>Aspergillus ustus</i> (Bainier) Thom & Church	<i>Chaetomium</i> sp.	<i>Aspergillus nidulans</i> (Eidam) Winter
<i>Aspergillus variegator</i> (B. & Br.) Thom and Raper	<i>Neocosmospora vasinfecta</i> Smith	
<i>Penicillium</i> sp.	<i>Helminthosporium microsorum</i> P. Henn.	
<i>Cladosporium herbarum</i> (Pers.) Link	<i>Helminthosporium nodulosum</i> B. & C.	
<i>Curvularia lunata</i> (Wakker) Boed.	<i>Memmoniella echinata</i> (Riv.) Gal- loway	
<i>Curvularia maculans</i> (Bancroft) Boed.	<i>Fusarium solani</i> (Mart.) App. & Wr.	
<i>Curvularia pallescens</i> Boed.	<i>Fusarium solani</i> (Mart.) App. & Wr. var. <i>minus</i> Wr.	
<i>Hormodendrum</i> sp.	<i>Macrophomina phaseoli</i> (Maubl.) Ashby	
<i>Septonema indica</i> Ramakr. sp. nov.*		

* To be described elsewhere.

Baiting techniques where artificial media are substituted for organic substrata have been described by Chesters (1948) and Evans (1954). The former demonstrated that his perforated 'immersion tubes' filled with nutrient agar could be used for isolating some rapidly growing fungi. The soil 'recolonization tube' has been used by Evans to study the rate of recolonization of sterilized soil by fungi.

An interesting technique has been described by Campbell (1951) for isolating *Phytophthora cinnamomi* Rands from infested soils. Here, holes bored in fresh apples are packed with infested soils when the fungus grew into the tissue of the fruit and then could be easily isolated in pure culture. The use of live indicator plants to demonstrate the presence of obligate parasites like *Plasmodiophora brassicae* Wor. has been described by Macfarlane (1952). In this laboratory, *Pythium aphanidermatum* (Eds.) Fitzpatrick has been consistently isolated from garden soil by a thick sowing of mustard seeds. Given optimum conditions of moisture the seedlings were invariably damped off and got covered with a pure growth of the fungus.

ECOLOGY

From techniques we may pass on to a brief consideration of the chief factors which influence the abundance and distribution of soil fungi. It is generally agreed that these factors are moisture, organic matter, reaction of the soil and the major nutrients, nitrate nitrogen, phosphorus and potassium and certain heavy metals.

Most investigators have found a direct correlation between moisture content and numbers of fungi (Waksman, 1944; Dixon, 1929 and Jasevoli, 1924). Cobb's (1932) work has shown that this correlation may not be clearly seen to the same extent in all soils. The author (Ramakrishnan, 1953) also found that moisture content and fungal numbers were directly correlated in the soil of a rice field soil at Vandalur near Madras while in two other soils no such clear correlation was observable. However, the soil with the highest moisture-holding capacity had consistently higher numbers of fungi than those with lower moisture-holding capacities. It is, perhaps, not logical to look for any unqualified correlation between any factor and fungal numbers in such a complex medium as the soil where so many conflicting influences operate.

It is but natural that organic matter should have a great influence on the numbers of fungi in the soil. The investigations of Waksman (1916 a, 1916 b, 1917 and 1944), Ma (1933) and Jensen (1935) confirm this view. The author's studies on Vandalur soils also agree with this conclusion to a large extent.

Regarding the effect of the three major constituents of plant food, viz., nitrogen, phosphorus and potassium, as far as the author is aware there is no report in literature where the changes in these constituents in *natural soil* were correlated with their fungal numbers. There are, however, numerous reports on the effect of mineral amendments on the behaviour of soil fungi. It has been found that nitrate nitrogen, phosphorus and potassium stimulate the development of soil fungi (Waksman and Starkey, 1924). The author (Ramakrishnan, *loc. cit.*) found that in Vandalur soils the variation in nitrate nitrogen and phosphorus were directly correlated with numbers of fungi, while the effect of potassium was not clear, possibly because this factor did not appreciably vary from month to month.

Although no report on the influence of heavy metals on the general fungus flora of the soil is available, the work done in this laboratory (Sadasivan, 1951) on the influence of boron, zinc and manganese on the colonization and survival of *Fusaria* in soils, demonstrates that this aspect need further study.

Finally the influence of plant cover on the microflora of the soil should be mentioned. Recent studies on the rhizosphere microfloras of various plants (Katznelson, Lochhead and Timonin, 1948; Agnihothrudu, 1953; Chinnayya and Agnihothrudu, 1953) emphasize the importance of such studies in understanding the ecology of soil fungi. The work of Tresner *et al.* (1954) is also of great interest here.

REFERENCES

- | | |
|---|--|
| Agnihothrudu, V. | .. "Soil conditions and root diseases, VII. Rhizosphere microfloras of some important crop plants of South India," <i>Proc. Indian Acad. Sci., B</i> , 1953, 37, 1-13. |
| Brierley, W. B., Jewson, S. T. and Brierley, N. | .. "The quantitative study of soil fungi," <i>Proc. 1st internat. Congr. Soil Sci.</i> , 1928, 3, 48-71. |
| Butler, E. J. | .. "An account of the genus <i>Pythium</i> and some Chytridiaceæ," <i>Mem. Dept. Agric. India, Bot. Ser.</i> , 1907, 1, 1-160. |
| Campbell, W. A. | .. "The Occurrence of <i>Phytophthora cinnamomi</i> in the soil under pine stands in the south-east," <i>Phytopathology</i> , 1951, 41, 742-46. |
| Chesters, C. G. C. | .. "A contribution to the study of fungi in the soil," <i>Trans. Brit. mycol. Soc.</i> , 1948, 30, 352-55. |
| Chinnayya, E. J. | .. "Colonization of plant debris by <i>Coprinus</i> species in soils," <i>Nature, Lond.</i> , 1952, 170, 252. |
| ——— and Agnihothrudu, V. | .. "Rhizosphere microflora of plants growing under different ecological habitats," <i>J. Madras Univ.</i> , 1953, 23 B, 182-92. |
| Cobb, M. J. | .. "A quantitative study of the microfungi of a hemlock and deciduous forest soil," <i>Soil Sci.</i> , 1932, 33, 325-45. |

- Dixon, D. .. "The micro-organisms of cultivated and bush soils in Victoria," *Austral. J. expt. Biol. and Med. Sci.*, 1928, 5, 223-32.
- Evans, V. .. "Soil recolonization tube for studying recolonization of sterilized soil by micro-organisms," *Nature, Lond.*, 1954, 173, 1196.
- Garrett, S. D. .. "Ecology of root-inhabiting fungi," *Biol. Rev.*, 1950, 25, 220-254.
- "Ecological groups of soil fungi—a study of substrate relationships," *New Phytol.*, 1951, 50, 149-66.
- "The soil fungi as a microcosm for ecologists," *Sci. Progress*, 1952, 159, 436-50.
- Gilman J. C. .. *A Manual of Soil Fungi*, 392 pp., Iowa, The Collegiate Press, 1945.
- * Jasevoli, G. .. "Contributio all consenz degli ifomiceti terrano agrario," *Bull. Orto. Bot. R. Univ. Napoli*, 1924, 1, 217-316.
- Jensen, H. L. .. "The fungus flora of the soil," *Soil Sci.*, 1931, 31, 123-58.
- "Contributions to the microbiology of Australian soils, III. The Rossi-Cholodny method as a quantitative index of the growth of fungi in the soil with some preliminary observations on the influence of organic matter on the soil microflora," *Proc. Linn. Soc. N.S.W.*, 1935, 60, 145-54.
- Jones, P. C. T. and Mollison, J... "The estimation of the numbers of micro-organisms in soil by direct examination," *4th internat. Congr., Microbiol.*, Copenhagen, 1949, 461-62.
- Katznelson, H., Lochhead, A. G. and Timonin, M. I. "Soil micro-organisms and the rhizosphere," *Bot. Rev.*, 1948, 14, 543-587.
- *Ma, R. M. .. "A study of the Soil fungi of Peking District," *Lingnan Sci. J.*, 1933, 12, 115-18.
- Macfarlane, I. .. "Factors affecting the survival of *Plasmodiophora brassicae* Wor., in the Soil and its assessment by a host test," *Ann. appl. Biol.*, 1952, 39, 239-56.
- Ramakrishnan, K. .. "Soil conditions and fungal wilts in plants—The distribution and behaviour of fungi in soils." *Thesis* approved for the degree of Doctor of Philosophy of the Madras University, 1953, 115+xxvii pp.
- Sadasivan, T. S. .. "Succession of fungi decomposing wheat straw in different soils with special reference to *Fusarium culmorum*," *Ann. appl. Biol.*, 1939, 26, 497-508.
- "Role of trace elements in the control of root-infecting fungi," *Proc. Indian Acad. Sci.*, B, 1951, 33, 135-49.
- Sparrow, F. K. .. *Aquatic Phycomycetes exclusive of the Saprolegniaceae and Pythium*, Ann Arbor., University of Michigan Press, 1943, xx+785.
- Subramanian, C. V. .. "The saprophytic activity of *Fusarium vasinfectum* the cotton wilt pathogen in the soil, I. Colonization of cotton

* Not seen in original.

- roots buried in the soil," *J. Indian bot. Soc.*, M. O. P. Iyengar Commn. Vol., 1946, 26, 209-13.
- Tresner, J. H., Backus, M. P. and Curtis, J. T. .. "Soil microfungi in relation to the hardwood continuum in southern Wisconsin," *Mycologia*, 1954, 46, 314-33.
- Venkataram, C. S. .. "Soil bacteria and chlamydospore formation in *Fusarium solani*," *Nature, Lond.*, 1952, 170, 889.
- Warcup, J. H. .. "Soil plate method for the isolation of fungi from soil," *ibid.*, 1950, 166, 117-18.
- "The ecology of soil fungi," *Trans. Brit. mycol. Soc.*, 1951, 34, 376-99.
- Waksman, S. A. .. "Do fungi actively live in the soil and produce mycelium?" *Science*, 1916 a, 44, 320-22.
- "Soil fungi and their activities," *Soil Sci.*, 1916 b, 2, 103-55.
- "Is there a fungus flora of the soil?" *ibid.*, 1917, 3, 565-89.
- "A method for counting the numbers of fungi in the soil," *J. Bact.*, 1922, 7, 339-41.
- and Starkey, R. L. .. "Influence of soil organic matter upon the development of fungi, bacteria and actinomycetes in the soil," *Soil Sci.*, 1924, 17, 373-78.
- Zachariah, A. T. .. "Micro-ecology of the soils of cultivated fields of south India with special reference to the occurrence and physiology of *Fusaria*." *Thesis* approved for the degree of Doctor of Philosophy of the Madras University, 1949, 1-122.
- "Soil conditions and root diseases, IX. Fungal ecology of cultivated fields—Techniques," *Proc. Indian Acad. Sci.*, 1953, 38 B, 235-41.