The base of the Cambrian is estimated at around 600 m.y. The pre-Cambian is naturally anything over 600 m.y. The division of this long span of time (>3000-600 m.y.) into Archæas and Proterozoic is convenient and necessary. If so where should we draw the demarcation line? If we agree that orogeny and epeirogeny are continuous then the demarcation line is arbitrary and we can perhaps adopt a scheme like the one given below where the divisions are more or less equal and also mark the end of some important orogenies in India.

	Cambrian 600 m.y.	
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Upper	
Purana ==		1,000 m.y.
Proterozoic	Lower	1,600 m.y.
Archæan	Upper	2,000 m.y.
	Lower	

If on the other hand we accept the premise that orogeny is episodic we can adopt the geochronological divisions by at arrived Voitkevich* on the basis of isotope ages from the Baltic, Ukranian and from the African, Indian and Australian shields. The boundaries are at  $2650 \pm 150$  m.y.;  $1800 \pm 90$  m.y.;  $1030 \pm 50$ ;  $550 \pm 10$  m.y. Stockwell* finds good support for the above divisions from the and Vinogradov shield ages. Canadian Tugarinov* propose > 2700 m.y.  $\pm$  150, Kata Archæan;  $1900 \pm 100$  m.y. to  $2700 \pm 150$  m.y. Archæan;  $1100 \pm 100$  m.y. to  $1900 \pm 100$  m.y. Lower proterozoic;  $600 \pm 50$  m.y. to  $1100 \pm 100$ m.y. Upper proterozoic. These divisions are

not much different from what Voitkevich proposed and any one of them should be quite adequate.

Stockwell (1964) following the 'American commission on Stratigraphic Nomenclature' pleads that the actual rock of a type area be used as the basis of a definition of a unit rather than isotope dates. He illustrates this by discussing the Precambrian structural provinces of Canada. It must be confessed that it is rather very doubtful whether such a scheme can be useful in building up the geochronological divisions in India.

The present need is the recognition of the subdivisions of the pre-Cambrian period and it does not very much matter which scheme is adopted. The subdivision of the pre-Cambrian period will enable us more precisely to state the horizon of the Archæan formations. The fallacy of equating an unknown with another unknown could thus be easily avoided.

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## MYCOFLORA OF THE ROOT REGION*

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THE root region comprises the most active zone of microhabitats in the heterogeneous soil structure. Some generalizations regarding the numerical and physical stimulation of micro-organisms in the root region have been feasible¹; but little information is available on the initiation of rhizosphere effect on soil fungi, nor has qualitative studies on rhizo-

sphere fungi been possible on account of limitations in techniques. The need for newer and improved techniques has been repeatedly emphasized.¹⁻³ Apart from the tedium of examining the slides, the difficulty of identifying the fungi present limit the usefulness of the direct observation techniques⁴⁻¹⁰; it is pointed out that the micro-environment itself gets perceptibly alltered,^{11,12} in situ studies employing these methods. In the case of isolation, methods^{8,13-15} aside from other limitations it:

^{*} These references are drawn from Prof. J. Sutton's paper in Nature, 1963.

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^{*} Memoir No. 47 from the Centre for Advanced Studies in Botany.

is impossible, without further evidence, to tell which fungi on a culture plate may have arisen from hyphæ, and it is believed that fungi are present mainly as mycelium in the rhizosphere. Sphere. Sphere. Also, it remains to be demonstrated whether certain species of non-pathogenic fungi make the rhizosphere their main locus of activity.

The operations used in taking, preparing and diluting the soil samples determine to a great extent the final picture; the estimates can very easily be affected by slight changes in techniques and by method-medium interactions. The progress has been hindered due to lack of adequate techniques that would facilitate evaluation and isolation of fungi present in the root region in an active mycelial state without basically altering its environment. An agar slide technique for direct observation of fungi occurring on root-surface has been tested in this laboratory. Minimum nutrient agar medium is provided on a glass slide for initiation of growth of fungi present on the roots and inducing of spore formation by them for ready and rapid scrutiny under stereo and light microscopes. 17 Observations on root-surface fungi of Gossypium arboreum L.-strain K 6 grown on Coimbatore cotton soil, and the scope of this technique are discussed here.

The method is simple and inexpensive. Five centimeter pieces of roots freed from adhering soil paricles and surface-dried on sterile filterpaper, were placed on thin layer of Martin's agar with rose bengal and streptomycin on glass slides. In case of plants raised on clayey soils, it was also necessary to wash the roots with a fine jet of sterile water to remove adhering soil particles and remove the moisture with sterile filter-paper prior to plating to cut down the bacterial development to a negligible level. Slides were incubated in sterile, moist, Petri plates for 30-36 hr. at 25° C. The slides were examined unstained, or stained after air-drying and fixing. Mycelia and sporophores with spores were observed growing on the agar smear and on to the glass surface, as well as on the upper surfaces of the root pieces. Identification is facilitated as it is possible to observe on the slides the pattern of attachment of spores to sporophores. Where necessary, fungi could be conveniently isolated for species identification and confirmation. The root pieces were seldom found overcrowded; invariably pure clusters of individual forms occurred. Also, there were regions where no fungus appeared. Incidentally, it was found that if the root was plated before the agar

medium set thereby getting coated with agar, sterile mycelia of a phycomycete (unidentified) predominated to the exclusion of most other fungi. With longer incubations, only Mucor spp. and Rhizopus spp. emerged through the dense covering of the hyaline hyphæ in such samples. In slides, where root pieces were plated without thoroughly removing the moisture following washing, the surface of agar along the length of the root was overridden with bacteria, but from the upper surfaces of the root grew pure colonies of several fungi, but with shorter sporophores.

Fusarium solani (Mart.) App. et Wr. and an unidentified phycomycete, and occasionally Rhizopus oryzæ were observed on root-tips including the root-cap (Fig. 1) of cotton seedling roots. Four genera of fungi, viz., Alternaria, Aspergillus, Cunnighamella and Fusarium, have also been recorded at root-tip regions of Crotalaria juncea L. using this technique.17 These observations are of significance in revealing initiation of rhizosphere effect as the root strikes the soil. As the roots mature, the mycoflora becomes stabilized. The rhizosphere effects of root-tip regions have to be viewed against two facts: root exudation at this region is particularly important18 19; secondly, the root-tip is continuously getting in contact with fresh soil. Previously, investigators²⁰ ²¹ failed to observe any fungus at root-tips of various plants. In fact, Parkinson,1 concludes 'that the root-tip region of the actively growing root remains uncolonized during the whole period of active growth'.

Fusarium solani and the unidentified phycomycete were observed on cotton roots throughout the period of observation of 60 days from the date of sowing. As the root system grew, F. solani became progressively dominant and the phycomycete less frequent. After 45 days, F. solani was the most dominant fungus on the root system. The other fungi observed were Aspergillus flavus Link., A. luchuensis Inui., A. nidulans (Eidam.) Winter, Chætomium olivaceum Cook and Ellis., Choanephora sp., Cladosporium epiphyllum Persoon, Mucor hicmalis Wehmer., Penicillium spp., Rhizopus oryzæ Went and Gerlings and Trichoderma viride Pers. ex Fries. The frequency of occurrence of these fungi was less on the roots of 60-day-old plants

It is difficult to say at this stage whether some fungi are suppressed and what the forms are. It also restricts its use to the study of root-surface fungi, and those that invade the root-tissues. It provides scope for development

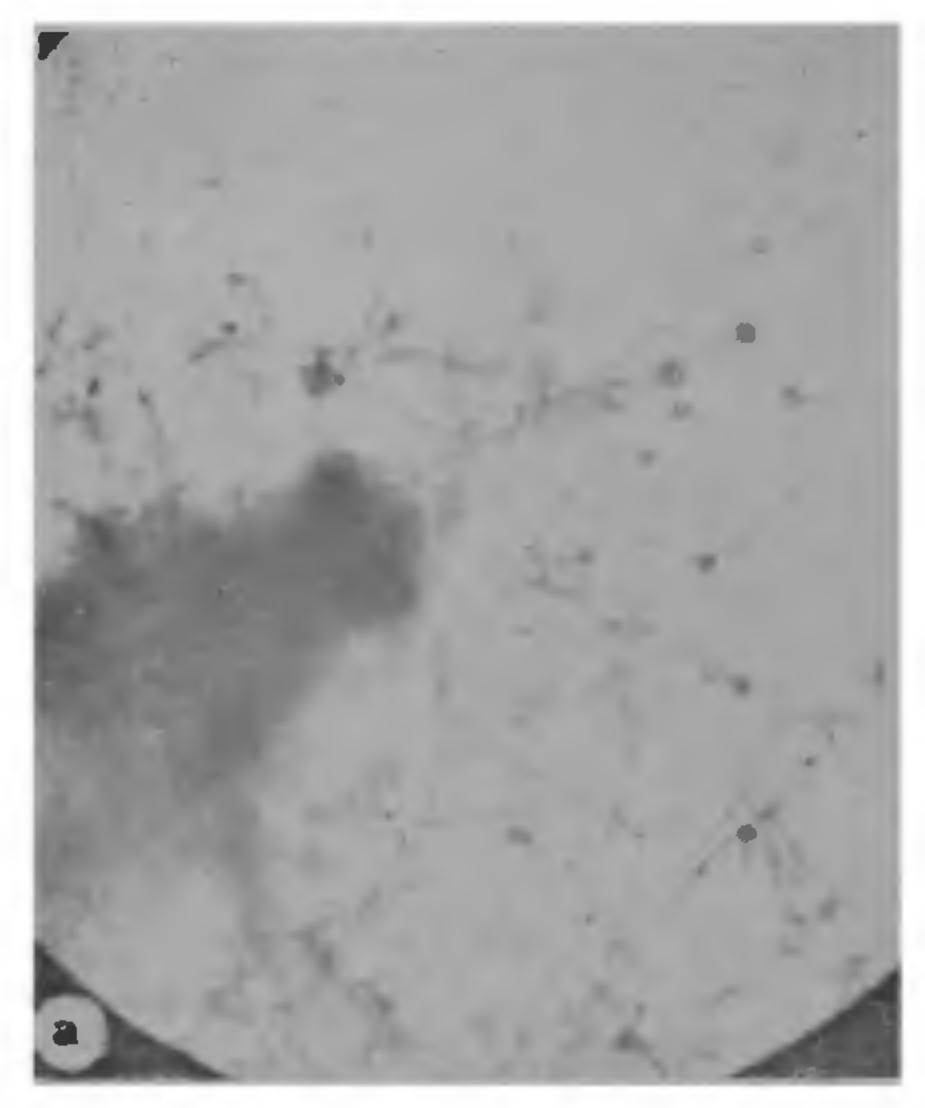




FIG. 1. a, Fusarium solani and b, An unidentified phycomycete growing from root-tips of cotton—K 6 seedling,  $\times$  360.

of fungi without exerting much selectivity between heavily- and sparsely-sporulating forms. It affords opportunity for the mycelia to grow and produce spores at the same time avoiding chances of spores lodged on roots from germinating and growing through the vegetative phase to produce reproductive structures in the limited period of incubation. Several genera of fungi, not normally found in dilution plates in parallel studies, were observed on the roots by this technique.¹⁷ For screening fungi that invade the root-tissues, the seedling roots were surface-sterilized with 1 in 14 aqueous calcium hypochlorite solution; such samples required incubation for longer than 36 hr. The results were significant in revealing the presence of species of Aspergillus, Choanephora, Cladosporium and Fusarium inside the tissues of healthy cotton roots. However, there appears to be some residual inhibitory effect of the surface-sterilizing agent, which needs to be elucidated.

It has been found that this technique could be adapted to suit specific requirements with judicious choices of medium, pre-treatment of root samples, and standardization of incubation time and temperature.

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