

Survival of seed-borne fungi in submerged soils

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SUMMARY

By the use of the Agar Disc Method and the Buried Slide Technique it was found that the spores of most of the fungi tested showed a good germination percentage when they were placed within the non-submerged soils and poor germination when placed over the same soils. Submerged laterite and sandy soils, which are both typical rice soils, showed greater suppression of germination of fungal spores than the acid peaty soil.

INTRODUCTION

The seed normally harbours within and on its coat numerous fungi. Apart from those that are pathogenic to the seedlings, several seed-borne fungi, which are normally not parasitic on seedlings, have been investigated as organisms contributing to the deterioration of seed material in storage (Christensen & Kaufmann, 1965). In the case of low-land rice, all such fungi introduced to the seed-bed with the seed are forced to adapt themselves to a semi-aquatic habitat. To investigate the ability of these fungi to survive in submerged soil after their introduction to the seed-bed, in the absence of crops residues, a preliminary study was carried out with some fungi isolated from rice seeds.

MATERIALS AND METHODS

Three techniques were employed to find the effect of the submerged and non-submerged soils on the germination of spores and the fate of the hyphae of fungi tested.

The Agar Disc Method

In this technique developed by Jackson (1958), discs of agar about 7.5 mm in diameter by 1.5 mm in thickness are placed on a filter-paper in contact with moist unsterile soil and surface inoculated with fungal spores. After a period of incubation of forty hours, the discs are examined and the percentage germination of spores in four random fields on each of the four replicate discs recorded. Control discs are incubated on moistened filter-paper, in the absence of soil.

As a standard procedure in the experiment described here, discs were incubated for one hour at 27°C, after placing them in contact with the filter-paper, but before inoculation. The discs were prepared from agar composed of Bacto-agar 20 g. and distilled water 1 L. Bacto-peptone incorporated into this medium by Jackson (1958) has been avoided. In treatments involving submergence of soil, soils were kept submerged for fourteen days, at the end of which the water above the soil was blotted out with blotting-paper before the agar discs were placed over them.

The Buried Slide Technique

This technique was based on that devised by China (1953). Microscope slides coated with a film of 2 per cent. agar, in which are suspended spores of the fungus being examined, were buried in cement pots containing submerged and non-submerged soils. After incubation for forty hours at 27°C, the slides were removed, allowed to dry, stained and examined. Hundred spores in random microscope fields were counted, and the number which had germinated recorded.

To find the possible effect of roots of rice plants on spores germination, week-old seedlings (var. H 4) were planted with their roots in contact with the agar on the buried slide.

Old's Technique

In this technique (Old, 1965), drops of a suspension of fungal propagules are placed on the soil at certain marked places. After a period of incubation of forty hours, they were recovered by pressing adhesive tape ('Sellotape') against the soil surface. Propagules adhered to the tape which was mounted on a glass-slide with a drop of cotton-blue in lactophenol. Germination of conidia and the growth of hyphal fragments were observed.

The soils for this test were prepared in the same way as for the Agar Disc Method.

Soils

(1) Laterite soil from rice field at Peradeniya, pH 5.0; pH and E on the fourteenth day after submergence, three inches below the soil surface: 6.3 and 185 m.v., respectively.

(2) Sandy soil from rice field at Gannoruwa, pH 5.55; pH and E on the fourteenth day after submergence, three inches below soil surface: 5.95 and 215 m.v., respectively.

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(3) Peaty soil from Nuwara Eliya. pH 4.2; pH and Eh on the fourteenth day after submergence, three inches below soil surface: 5.1 and 245 m.v., respectively.

Fungi

The following fungi were used. All were isolated from surface sterilised rice seed (var. H4) plated on oat meal agar.

1. *Absidia corymbifera* (Cohn) Space. & Trott.
2. *A. cylindrospora* Hagem.,
3. *Apiospora montagnai* Sacc. *Arthrimum* state.,
4. *Aspergillus flavus* Link. ex Fres.,
5. *A. fumigatus* Fres.,
6. *Chaetomium dolichotrichum* Ames.,
7. *C. globosum* Kunze.,
8. *C. olivaceum* Cooke & Ellis.,
9. *Drechslera hawaiiensis* (Bugnicourt) Subramanian & Jain.,
10. *Khuskia oryzae* Hudson, *Nigrospora* state.,
11. *Nodulisporium* sp.,
12. *Phaeotrichoconis crotalariae* (Salam & Rao) Subramanian.,

RESULTS

The spore germination percentage of various fungi tested are presented in Table I. Old's technique was not successful as the recovery of spores from submerged soils was not possible in the case of most fungi.

In all non-submerged soils, the spores on buried slides showed a good germination percentage, while those on agar discs showed poor germination. Under submerged conditions there was a general decrease in the germination percentage observed by both the techniques. Among the three soils tested peaty soil alone showed instances of comparatively good germination (>50%) under submerged conditions. This too was restricted to *Aspergillus flavus*, *Chaetomium globosum*, *Absidia corymbifera* and *A. cylindrospora*. However, *C. globosum* did not show any germination with submerged sandy or laterite soils, irrespective of the technique used. In the submerged treatments, germination percentage was in the order: peaty > laterite > sandy. Increased germination percentage of spores on slides buried in submerged soils, in proximity to rice roots, was observed only in peaty soil. On slides buried in non-submerged laterite soil, five forms showed germination over 50 per cent. while on the agar discs over

the same soils, there was no instance of germination over 50 per cent. Sandy soil exhibited comparatively good germination in the non-submerged treatments.

All hyphal fragments, and germ tubes of fungi that germinated on buried slides in submerged laterite soil were seen in different stages of lysis. This was very slight in sandy soil and almost absent in peaty soil.

In all three submerged soils, the agar on regions of the buried slides in contact with rice roots, was sometimes found partially or totally decomposed.

DISCUSSION

In all non-submerged soils, spores on the buried slides showed good germination percentage unlike those on agar discs. This may be due to slightly increased carbon dioxide content within the non-submerged soil. Stover and Freiberg (1958) demonstrated that increased carbon dioxide could result in the stimulated multiplication of *Fusarium oxysporum* f. *cubense*. Newcombe (1960) substantiated this finding, but pointed out that although the fungus could tolerate and be stimulated by relatively brief exposures to carbon dioxide, its long term survival in such conditions was prejudiced.

In general, most of the fungi tested showed poor germination under submerged conditions. Soil saturation, besides restricting the oxygen supply to the fungi, may create an environment with a very high content of carbon dioxide. The effect of such high levels of carbon dioxide may be of great significance as demonstrated by Abeygunawardena & Wood (1957) in the case of *Sclerotium rolfsii*. It has been reported that even after the flood water is removed, the suppression of the mycoflora persists. The inhibition does not seem to be solely a consequence of oxygen depletion. Carbon dioxide, sulfide, or other toxic agents have been proposed as fungicidal principles generated during flooding (Nitchell & Alexander, 1962). Flooding of soil for the control of widely differing types of fungal pathogens has given encouraging results (Sewell, 1965).

Of the three soils used in this investigation, peaty soil alone is not a rice soil. Relatively good germination of some fungi in proximity to rice roots within submerged peaty soil may be due to the possible absence of most of the bacteria which may be stimulated to a considerable extent in the rhizosphere of rice.

Lysis of germ tubes and hyphal fragments were observed on slides buried within laterite soil. Generally, such lysis may be an outcome of purely internal metabolic changes (autolysis), of contact with

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enzymes of other organisms, or of exposure to toxic materials (Warcup, 1965). As Sewell (1965) says, "It is possible that while continued lack of activity of resting stages may lead to a gradual decline of numbers through senescence, there is a converse situation that reversion to activity under wrong sort of conditions may lead to an even more rapid disappearance". Chinn & Ledingham (1961) have described 'germination lysis' as a factor in the disappearance of spores of fungi from soil. Thus it is possible that fungistasis may assist in survival rather than help in the eradication of several pathogenic fungi (Dobbs & Hinson, 1953).

Low-land rice is usually sown on a bed very near saturation, and the field is flooded about five days after sowing. The introduced fungi have to face unfavourable conditions from the start. Jones & Seif el Nasr (1940) found that 'mud sowing' wheat seed controlled flag smut so efficiently that it offered an alternative to seed disinfection or the use of resistant varieties. If we may speculate on the possibilities of these fungi colonising the rice seedlings saprophytically, they would have to survive, after their introduction with the sown seed, for at least a fortnight before they could get an opportunity to colonize a dead aerial portion of an outer leaf.

It should be admitted that of the three techniques attempted, Old's technique alone could provide a study of these fungi under natural conditions. The Buried Slide Technique does not appear to be suitable for studies in submerged soil, as agar on the surface of submerged slides is sometimes decomposed, especially when it is in proximity to plant roots.

Unfortunately, there are not many techniques available for such studies under submerged conditions. Glass fibre material (Legge, 1952) has been found to help the fungi to survive for a period much longer than that shown by spores added to soil without it (Stover, 1959). Recovery of 'small' spores from submerged soil by the 'flotation method' of Ledingham & Chinn (1955), is very tedious. A better understanding of the behaviour of fungi within submerged soil is possible only if suitable and simple techniques are evolved to study them without altering the microenvironment.

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Table 1.—Mean Percentage of Germination of Spores

		Agar Disc Method						Buried Slide Techni'ue																								
		Laterite			Sandy			Peaty	Laterite			Sandy			Peaty																	
		Control	S.		N.S.		S.		N.S.	S.	S R	N.S.	S.	S R	N.S.	S.	S R	N.S.														
1.	<i>Absidia corymbifera</i>	100	..	12	..	0	..	0	..	70	..	16	..	0	..	0	..	0	..	56	..	6	..	0	..	40	..	0	..	60	..	55
2.	<i>A. cylindrospora</i>	98	..	16	..	0	..	0	..	0	..	52	..	0	..	20	..	0	..	42	..	0	..	0	..	76	..	20	..	30	..	70
3.	<i>Apiospora montagnei</i>	70	..	8	..	26	..	28	..	10	..	0	..	18	..	16	..	0	..	0	..	12	..	0	..	34	..	0	..	0	..	10
4.	<i>Aspergillus flavus</i>	98	..	0	..	10	..	0	..	60	..	0	..	80	..	8	..	0	..	50	..	0	..	10	..	70	..	0	..	74	..	40
5.	<i>A. fumigatus</i>	96	..	36	..	6	..	0	..	26	..	8	..	46	..	0	..	20	..	66	..	16	..	20	..	10	..	26	..	40	..	60
6.	<i>Chaetomium doli-</i> <i>chotrichum</i>	94	..	0	..	10	..	0	..	20	..	0	..	0	..	22	..	0	..	0	..	8	..	28	..	16	..	0	..	0	..	0
7.	<i>C. globosum</i>	100	..	0	..	0	..	0	..	0	..	70	..	30	..	0	..	0	..	90	..	0	..	0	..	44	..	0	..	0	..	30
8.	<i>C. olivaceum</i>	100	..	0	..	0	..	4	..	0	..	0	..	26	..	0	..	0	..	68	..	0	..	0	..	80	..	0	..	0	..	10
9.	<i>Drechslera hawaii-</i> <i>ensis</i>	100	..	0	..	0	..	0	..	0	..	0	..	50	..	0	..	0	..	30	..	0	..	0	..	80	..	0	..	12	..	34
10.	<i>Khuskia oryzae</i>	100	..	32	..	0	..	0	..	28	..	0	..	10	..	0	..	0	..	36	..	0	..	0	..	70	..	0	..	0	..	16
11.	<i>Nodulisporium</i>	68	..	0	..	0	..	0	..	0	..	0	..	0	..	0	..	34	..	10	..	0	..	4	..	10	..	0	..	0	..	0
12.	<i>Phaeotrichoconis</i> <i>crotalariae</i>	100	..	0	..	0	..	0	..	0	..	0	..	0	..	0	..	0	..	0	..	6	..	0	..	60	..	0	..	20	..	18

N. S.= Non submerged

S.= Submerged

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