Direct Microscopy for Study and Count of Soil Protozoa

It is generally accepted that only direct microscopy is able to give a reliable estimate of the total numbers of micro-organisms present in soil. Suitable direct techniques have been developed for counting the bacteria, fungi[1] and algae[2]. No adequate technique is available for the protozoa. A recently developed culture technique[3] is a considerable improvement, but tends to be selective and is time-consuming in operation.

The direct examination of protozoa in soil has been made difficult by their low numbers and by the lack of a suitable differential staining technique. The following method overcomes these difficulties.

A known amount of 1 : 5 soil : water suspension is spread on a slide and fixed wet with osmic or formalin vapour. After drying, it is stained with erythrosin, washed, and then counterstained with methyl green. The preparation is mounted in immersion oil.

Colloidal particles in the soil appear green, protozoa pink with a purple nucleus. Flagella and cilia are readily visible. Contrast is sufficient to enable effortless counting with a low-power objective lens. The selective influence of cultural methods is eliminated.

The technique is of general use for soil studies. Bacteria, fungi and algae are stained purple and may be picked out quite readily against the green background of soil particles.

The details of this method will be published elsewhere.

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The Sorghum Midge Diapause

The sorghum midge, *Contarinia sorghicola* (Coq.), is an important pest of grain sorghums in Queensland, where its incidence and intensity of attack vary considerably from season to season and from early to late crops. Activity commences about two weeks after wet weather and high humidity, and crops flowering at the time may suffer severely. In central Queensland, where the rainy season is concentrated in late January and February, the mid-season crops are often damaged whereas those flowering in March and early April escape the pest. Farther south the wet season is not so well defined, although the peak often coincides with late flowering. In these districts the midge is seldom a pest of early and mid-season crops.

Laboratory experiments have demonstrated that larval diapause is broken and midge will emerge after the infested material is thoroughly wetted and incubated at relative humidities of 94–100 per cent, at temperatures of 25°–30° C. Emergence commences a fortnight after wetting, and may continue for a further four weeks. Under these conditions midge and its parasite, *Eupelmus australiensis* Gir., have emerged in appreciable numbers even from infested grain which had been stored for four years.

These laboratory findings are being correlated experimentally with midge behaviour in the field, and a full account will be published at a later date.

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Internal Infection of Tomato Seed by *Didymella lycopersici* Kleb.

Previous studies have indicated the importance of infected seed as a source of the stem and fruit rot disease of tomatoes caused by *Didymella lycopersici* Kleb. Hickman[1] reported 25–34 per cent infection of an outdoor crop grown from seed, a proportion of which was infected. Schoevers[2] demonstrated the presence of mycelium and pycnidia of the fungus on the seed coat and radicle of germinating seed, and Ogilvie[3] observed the fungus on the cotyledons and hypocotyl of tomato seedlings.

These studies were concerned with the location and effect of the fungus after germination had occurred. There appears to be little information, however, on the way in which the seed becomes infected and the location of the fungus on or in the ungerminated seed. Since such information is likely to be of value in determining the best methods of obtaining clean seed, a study has been made of the matter in the course of research now being conducted on the disease.

Sections of naturally infected fruit (var. Stonor's Money-Maker) bearing black lesions at the calyx end approximately ½–1 in. in diameter showed that the fungus had grown down the placenta and invested many of the seeds. Sections of the seeds revealed the presence of numerous hyphæ and pycnidia within the tissue of the funicle and also a more extensive internal invasion of some seeds. In these, the fungus had grown beneath the outer seed coat as far as the distal end of the seed and had formed numerous pycnidia. The main site of fungal growth was within the gaps which form between the layers of the seed coat as it matures. No fungal hyphæ were seen in the embryo. Dried seed from infected fruit also contained hyphæ and pycnidia.

Following these observations on naturally infected material, ripe tomato fruits (var. Radio) were artificially infected at the calyx end with a spore suspension from an isolate of *Didymella* known to be pathogenic to tomato stems. After thirty days, seed

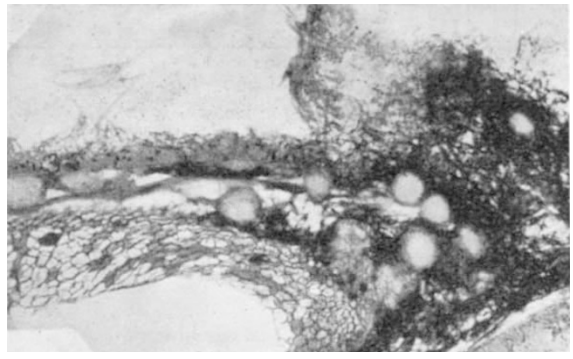

Fig. 1. Section of tomato seed (var. Radio) infected with *Didymella lycopersici* Kleb. showing hyphæ and pycnidia of the fungus in the funicle region and beneath the outer hairy seed coat. × 45