

## Direct Observation of Fungal Activities on Soil

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Supported in part by a grant from McIntire-Stennis Cooperative Forestry Research Program. Published with approval of the Director, Hawaii Agricultural Experiment Station, Journal Series Paper No. 1246.

### ABSTRACT

Quiescence of fungal spores and lysis of fungal mycelia, two of the general reactions of fungi to soil, were studied directly on soil with a vertical-illumination microscope. The invasion of papaya roots by germ tubes from germinating sporangia of *Phytophthora palmivora* on soil was observed in situ with the same technique. *Phytopathology* 61: 437-438.

*Additional key words:* fungistasis, interaction.

A major limiting factor in investigation of fungal activities in soil has been the lack of adequate techniques (11). Indirect methods involving the interposition of a semipermeable membrane between fungal propagules and the soil have been used (1, 5). Improvements were made by using cellophane tape (12) and plastic film (9) to remove fungal propagules from the soil surface for observation. These methods, however, are not suitable for all species and soils. Detailed surface structure of microorganisms growing on soil was observed with the stereoscan electron microscope (6), but the technique causes dehydration and distortion of the organisms. Recently, a metallurgical reflected-light microscope was used to observe microorganisms on soil (4). The technique has a limited application because the soil sample must be observed from the bottom through a coverslip, and the interpretation of the observed results is complicated by the stimulatory effect of the glass surface on microbial growth (3).

Direct observation of microbial activities on soil with a vertical-illumination microscope greatly reduces the disadvantage of the reflected-light microscope technique. The principle of these two types of microscopes is similar.

The Zeiss Universal microscope with a Model II C vertical illuminator and a graduated mechanical stage was used in this study. Zeiss 10 $\times$  and 40 $\times$  Planachromat objectives were used for large spores such as sporangia of *Phytophthora palmivora* Butler and conidia of *Alternaria solani* (Ell. & G. Martin) Sor., and an 80 $\times$  Epiplan objective for small spores such as conidia of *Trichoderma viride* Fr. and *Penicillium frequentans* Westling.

Stony sandy loam from a papaya field was sifted and stored in polyethylene containers. The soil was adjusted to about 50% moisture (w/w), and ground in a mortar.

No grinding is necessary for soils with high clay content. Approximately 4 g of soil was placed on a glass slide, compressed, and the surface smoothed with a spatula to give a final soil mass of about 35  $\times$  20  $\times$  4 mm. Each slide was kept in a moist chamber consisting of a petri dish containing moist filter papers.

Conidia of the following fungi were obtained from cultures on potato-dextrose agar: *Alternaria solani*; *Helminthosporium stenospilum* Drechsler; *Glomerella cingulata* (Ston.) Spauld. & Schrenk; *Mucor ramanianus* Möller; *Penicillium frequentans*; and *Trichoderma viride*. Sporangia of *P. palmivora* were produced under fluorescent light on V-8 juice agar (2). Conidia and ascospores of *Neurospora tetrasperma* Shear & Dodge were obtained from cultures grown on nutrient agar (per liter: 10 g maltose, 4 g yeast extract, 4 g glucose, 20 g agar). Ascospores were separated from conidia by sedimentation in a column of distilled water, and heat activated at 58 C for 20 min before use. For germination tests, 100 spores were counted for each treatment. All experiments were done at least twice.

Quiescence of fungal propagules is one of the general reactions of fungi to soil (5, 11). This phenomenon, which is commonly called soil fungistasis, was studied directly on soil with the technique described. Suspensions of conidia of *A. solani*, *G. cingulata*, *M. ramanianus*, *N. tetrasperma*, *P. frequentans*, and *T. viride* were each added to a smoothed surface of soil on the glass slide, and incubated at 24 C. Spores on water agar or potato-dextrose agar were used as controls. Spore germination was observed directly on soil with the vertical-illumination microscope after various periods of incubation. None of the fungal spores germinated on soil after 16 hr, while more than 90% of them germinated on nutrient media (Fig. 1-A). *Neurospora tetrasperma* ascospores which are insensitive to soil fungistasis (7) were also tested, and approx 98% germinated on soil. Therefore, sensitivity of spores to soil fungistasis as observed in situ was similar to that observed after removal from soil (7).

Lysis of fungal mycelia, the other general reaction of fungi to soil (8, 10), was observed sequentially directly with the present technique. Conidia of *H. stenospilum* were germinated in potato-dextrose broth at 24 C. Young hyphae were washed twice with sterile distilled water by centrifugation and placed on the smoothed surface of soil on the glass slide. The same hyphae were observed daily for 4 days. Lysis of *H. stenospilum* hyphae occurred after 3 days at 24 C in a moist chamber. The hyphal tip of *H. stenospilum* lysed at a faster rate than the portion of hypha near the spore. A print was left on the soil surface after the disappearance of the hyphae.

This technique also was used to examine the interaction between vigorously growing roots of papaya and the pathogen, *Phytophthora palmivora* (14), previously identified as *P. parasitica* (13). Sporangia of *P. palmivora* were added to smoothed surfaces of soil on glass slides. Two glass slides were placed 2 cm apart and 1.5 cm away from the edge of a large petri dish (150  $\times$  20 mm). A 3-week-old papaya seedling was carefully re-

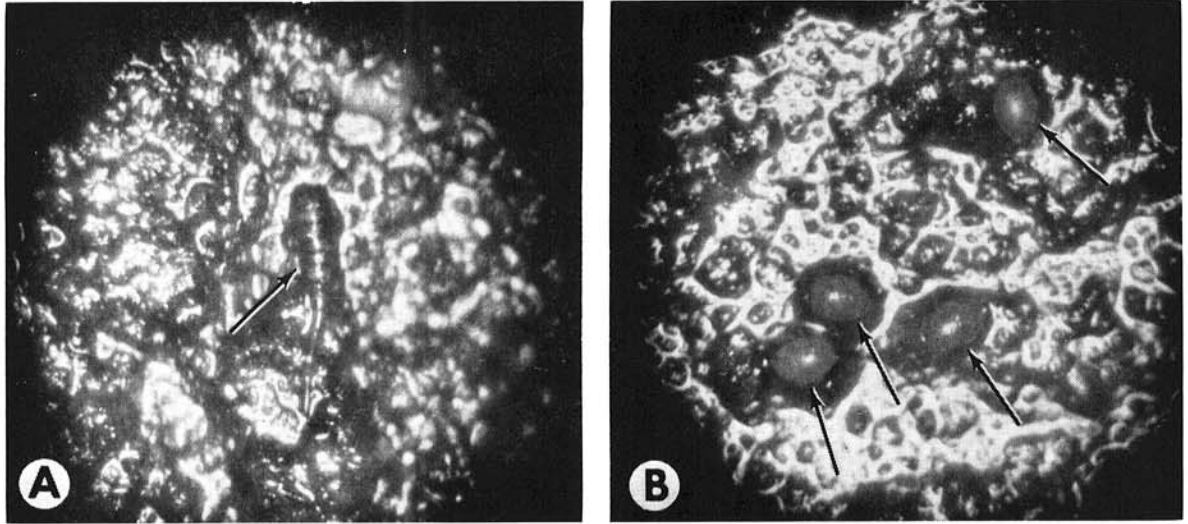


Fig. 1. A) Conidium of *Alternaria solani* (arrow) on soil after 16 hr at 24 C. ( $\times 375$ ) B) Sporangia of *Phytophthora palmivora* (arrows) on soil after 12 hr. ( $\times 375$ )

moved from the soil and washed by dipping roots in tap water to remove soil particles. The seedling was placed between the two slides with roots resting on soil surfaces. The petri dish was covered and incubated at 24 C with 200 ft-c of fluorescent light. Sporangia on soil without papaya roots were used as controls. After incubation, the cover was removed and the petri dish placed on the stage of the microscope. The interaction between plant roots and sporangia was observed directly on soil with the vertical-illumination microscope. In the absence of papaya roots, most of the sporangia of *P. palmivora* on soil failed to germinate either directly by the production of germ tubes or indirectly by the release of zoospores (Fig. 1-B). However, some of the sporangia germinated indirectly within 2 hr. Zoospores were observed moving on the soil surface and inside sporangia. Encystment of zoospores was observed within 12 hr. In the vicinity of papaya roots, many sporangia were stimulated to produce germ tubes, presumably by exudates from papaya roots (11). The invasion of papaya roots by germ tubes from sporangia was also observed directly on soil for the first time.

This technique enables us to study sequentially in situ the activities of fungi and the interaction between fungi and plant roots on soil.

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