

A Quantitative Technique for the Extraction of Soil-Inhabiting Mites (Acarina) and Springtails (Collembola) Associated with Pod Rot Disease of Peanut

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ABSTRACT

A technique is described for rapid extraction of certain soil microfauna associated with the pod rot disease complex of peanut. It is suggested that greater consideration be given to all soil microfauna in studies of the ecology of soilborne pathogens.

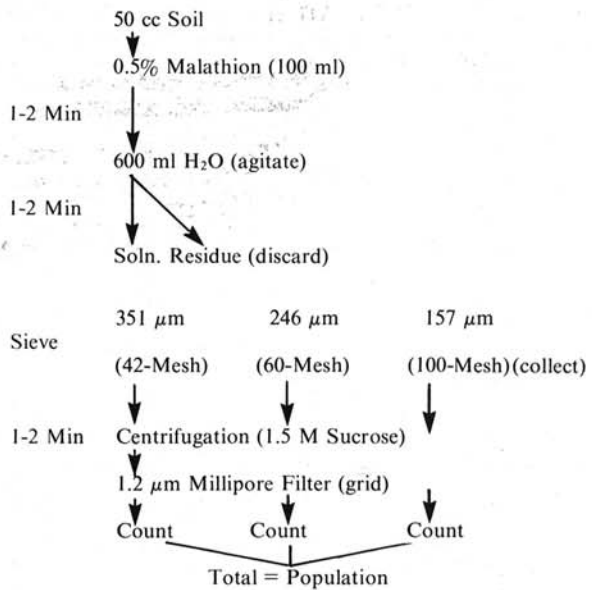
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Although numerous soilborne fungi have consistently been found in association with rotting pods of peanut (*Arachis hypogaea* L.) (3), adequate control of this important disease complex has not been achieved. It is generally accepted that plant-parasitic nematodes play an important role in many disease complexes (6). Control of plant-parasitic nematodes in problem fields is necessary to profitably grow peanuts. However, nematode control by itself failed to alter pod rot incidence in repeated tests.

Several recent reports have indicated that various types of mites can be involved in initiation of disease. The mite *Siteroptes reniformis* Krantz has been reported to serve as a vector of *Nigrospora oryzae* (Berk. & Br.) Petch in the initiation of *Nigrospora* lint rot of cotton (4). A mutualistic form of symbiosis between these organisms was indicated. It has also been reported that astigmatid mites were found in association with subterranean parts of peanut plants, and in certain circumstances they penetrated the pods and disseminated *Aspergillus flavus* Link spores in the process (1). Moreover, in a recent report, peanuts from Virginia fields were found to have microscopic damage which was detectable only by staining procedures. These small wounds apparently provided a mode of entry into the pod for *A. flavus* (5). In order to investigate the possible role of these and other soil microfauna in peanut pod rot, a technique based on certain sugar flotation-sieving methods used for extracting nematodes (2) was developed to facilitate their identification and enumeration.

Four 100-cc soil samples were collected from each plot or treatment, samples were mixed thoroughly, and a 50-cc sub-sample was placed in a 1.0-liter beaker in the following extraction procedure. One hundred ml of 0.5% Malathion® (O,O-dimethyl phosphorodithioate diethylmercaptosuccinate) solution was added to immobilize certain animals, the soil slurry was agitated and allowed to stand for 1-2 min, and 600 ml of H₂O were added (Fig. 1). The soil suspension was agitated, allowed

Fig. 1. Procedure for extraction of soil microfauna.



to settle for ca. 1 min, and the aqueous suspension was poured through a series of 15-cm diam 30, 42, 60 and 100-mesh sieves (589, 351, 246, and 157 μm openings, respectively) and washed gently with ca. 300 ml tap water. Residues collected on the 42-, 60-, and 100-mesh sieves were washed into 50-cc Nalgene centrifuge tubes with 5-10 ml H₂O; 30 ml of 1.5 M sucrose solution were added and tubes centrifuged for 1 min at ca. 450 g in an International Clinical Centrifuge, Model CL. The sucrose solution was then decanted into a 300-ml Millipore filter funnel, suction applied, and 20-30 ml H₂O were added to collect substances on a 47-mm diam 1.2 μm gridded Millipore filter. Filters containing substances collected on the 42-, 60-, and 100-mesh sieves were placed in 9-cm diam petri dishes for counting, using a stereoscopic microscope. Filters were often placed in a refrigerator (4 C) for temporary storage, or to immobilize active animals. Tentative identification of orders or families were made, and specimens were preserved in 95% isopropyl alcohol for further identification.

To test quantitative aspects and reproducibility of this procedure, 20 astigmatid mites were added to 50-cc soil samples, the soil was thoroughly mixed and microfauna were extracted as previously described. Recovery of mites averaged 85% with a range of 65-100% in five tests.

Although it has not been determined whether soil mites or springtails serve as vectors or provide modes of entry for fungal pathogens, in this investigation they were found within sound pods showing microscopic damage. Moreover, elimination of soil mites from field soil by applying certain acaricides has contributed to control of pod rot in repeated tests. Further studies on the role of soil microfauna in the pod rot disease complex are in progress. Sufficient evidence is available, however, to suggest that greater consideration should be given to soil microfauna in studies of the ecology of soilborne pathogens.

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