METHODS OF CULTIVATION, ISOLATION, AND INOCULATION OF MYCOPHAGOUS NEMATODES INTO NEMATOPHAGOUS FUNGUS CULTURES

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ABSTRACT.—Instructions for the cultivation, isolation, and inoculation of mycophagous nematodes are presented. The methods utilized allowed large numbers of fungus-feeding nematodes to be reared and inoculated into nematode-trapping fungus cultures.

Aphelenchus avenae Bastian, and *Neotylenchus linfordi* Hechler, are two fungus-feeding nematode species that were used in a study of the biological relationships between nematophagous fungi and mycophagous nematodes (Monoson, 1967). Both species of nematodes have been used previously in reproductive and feeding evaluations with non-predaceous fungi (Hechler, 1962a; 1962b; Pillai, 1966).

A constant supply of *Aphelenchus avenae* and *Neotylenchus linfordi* were necessary for the investigation on the activities of five predaceous fungi that actively capture and kill living nematodes. The present paper provides information concerning the methods used in the cultivation, isolation, and inoculation of *A. avenae* and *N. linfordi* into nematophagous fungi cultures.

Cultures of *Aphelenchus avenae* and *Neotylenchus linfordi* were maintained on one-quarter-strength Difco potato-dextrose agar at room temperature. The mycophagous nematode species and their non-predaceous food source (*Pyrenochaeta terrestris* (Hansen) Gorenz, J. C. Walker, and Larsen) were transferred together every week to new one-quarter-strength Difco potato-dextrose—agar medium. Two-week-old petri-dish cultures of nematodes were scanned using a binocular microscope and areas of nematode concentrations were marked off with a marking crayon on the glass surface of the dish. From these nematode-concentration areas wedge-shaped pieces of agar were cut out and the inoculum was transferred to the new medium. The method allowed for the maintenance of nematodes in all stages of development as well as high physiological state of feeding and reproduction. All nematode transfers were made by using a positive pressure inoculation chamber in order to exclude any possible contamination of the cultures.

For the purpose of this study nematodes were grown for two weeks and then harvested for inoculation with nematophagous fungi. The contents of one petri dish usually provided sufficient nematode numbers for most inoculations.

A simple extraction apparatus was formed by placing four layers of Kimwipe tissues between two plastic funnel tops which rested in a Syracuse dish (Edwards, 1962). This apparatus and most glassware used were steam sterilized at 18 psi for 15 minutes. Nematodes were separated from the agar medium by pouring approximately 10-15 ml of sterile distilled water over the surface of nematode stock cultures. Nematodes readily moved into the surface water and were unable to reenter the agar substrate. *Pyrenochaeta terrestris* produced negligible amounts of conidia in culture and the water suspension was, therefore, essentially devoid of fungus material. The water suspension was then transferred to the extraction device by pouring the water over the Kimwipes. The apparatus was placed over the Syracuse dish and the water was allowed to filter through the Kimwipes. After one-half hour several thousand nematodes had moved down into the Syracuse dish. The process was repeated several times in order to harvest the greatest number of nematodes which were present. Nematodes collected in this manner were clean but were further washed three times in sterile distilled water by centrifugation. Nematodes cleaned by this simple method did not exhibit any bacterial or

fungal contamination when transferred to cultures containing nematophagous fungi.

Extracted nematodes were placed in a sterile measuring dispenser (distributed by Chemical Rubber Company, Cleveland, Ohio) with approximately 45 ml of sterile distilled water. The glass dispenser and measuring head had been sterilized in 70 per cent alcohol for 15 minutes and then had been thoroughly rinsed with sterile distilled water. This method eliminated the possibility of inoculation variations which may have resulted from repeated steam sterilization of the dispenser apparatus.

One-milliliter aliquots of the nematode suspension were taken and the nematodes were counted in a plastic counting dish. Subsequent 50 ml dilutions with sterile distilled water resulted in a final population of approximately 400 nematodes per ml of liquid. This final population was used either as an inoculation amount or as a base population from which all other dilutions were made.

Inoculations of nematodes into nematophagous fungus cultures were made either directly from the dispenser apparatus or from serially diluted solutions with the aid of a calibrated 1 ml duplicating pipette (distributed by E. H. Sargent and Company, Chicago, Illinois). Prior to each inoculation the liquid was swirled so that each withdrawal of liquid contained a homogeneous sample of the inoculum. One milliliter of liquid was the amount inoculated into petri dishes containing nematophagous fungi. The plates were left uncovered in a positive pressure inoculation chamber for 15 minutes so as to allow the 1 ml of liquid to evaporate. After 15 minutes sterile petri-dish lids were carefully placed over the bottom sections.

Literature Cited

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