Notes brèves

IMPROVED TECHNIQUES FOR THE DETECTION OF NEMATOPHAGOUS FUNGI AND THEIR ACTIVITY AGAINST TARGET NEMATODES

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Fungal parasites are important in natural regulation of plant parasitic nematode populations. Methods for the isolation and observation of endoparasitic and nematode trapping fungi have been published (Duddington, 1955; Cooke, 1961; Barron, 1969), with some applicable for quantitative or semiquantitative evaluation of antagonistic activity (Klemmer & Nakano, 1964; Eren & Pramer, 1965; Mankau, 1975; Heintz, 1978).

All techniques however have certain disadvantages. Because the detection of slow developing endoparasites is often masked by fast growing trapping fungi it is important to test for the two groups separately.

Two standard techniques and the improvements made are outlined in this paper.

Baermann-funnel technique

The Baermann-funnel allows active nematodes passage through a filter. Specimens parasitized by endoparasitic fungi and not fully inactivated pass through the filter and parasitism is usually detected by transfer to agar plates.

Whereas, numerous examinations have been made by adding nematodes as prey to the agar plates, direct confrontation with the fungi in soil has been avoided. Since the differentiation between nematode species in field soil following parasitism is difficult an attempt was made to replace the naturally present soil populations with a target nematode marked by staining with chrysoidin. This stain was recommanded by Doliwa (1955-1956) and Kämpfe (1956) as a viability stain.

The technique allows direct detection of specific antagonists of the nematode in question.

METHOD :

1. The Baermann-funnel is replaced by a dish (Oostenbrink, 1954) that supports 100 g of soil which reduces variability obtained with other techniques using small amounts of soil.
2. Field soil is placed on the dish on two milk filters over water and active nematodes extracted for four days at room temperature.
3. The extracted nematodes are discarded from the dish and 5 000 nematodes of the target species in 10 ml of water spread evenly over the field soil on the filters. Heavy soils should be dried for one or two days before application of the target nematode. Drying reduces exposure of endoparasitic fungi with adhesive conidia to excessive moisture that could reduce attachment to the nematode cuticle.

   The dishes are not filled with water during the 48 hr incubation stage. The nematodes are stained with chrysoidin for 24 hr prior to addition.
4. The dishes are then filled with water for nematode extraction.
5. 24 hr later the nematode suspension in the dish is poured onto a 20 µm aperture sieve, washed with water, and transferred into a 15 ml graduated centrifuge tube.
6. The suspension is centrifuged for 5 mn at 2 500 rpm.
7. The supernatant is removed with a pipette and the remaining 0.3 ml mixed with 0.3 ml solution containing 100 ppm streptomycin sulphate + 200 ppm penicillin.

   The resulting suspension is spread evenly over 1.5 % water agar in a 9 cm diam. Petri dish.
8. The dishes are kept at room temperature, examination for parasitized nematodes is made immediately after addition and at two day intervals for ten days.

Differential centrifugation technique

The differential centrifugation technique in use, separates the heavier spores of the nematode-trapping fungi from the lighter spores of endoparasites. The soil fraction containing the latter is poured onto a water agar Petri dish and spread with a glass rod over 50 % of the dish surface. Nematodes are added in a water suspension. This suspension and the water from the soil extract often spreads soil particles over the Petri dish surface making observations difficult.

In addition, fungi such as Chytridiomycetes, Oomycetes and Zygomycetes are rarely detected (Barron, 1977). Furthermore, the amount of soil poured onto the agar

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surface is small which increases variation and requires the use of many replicates.

These problems can be reduced by adding larger amounts of soil to grooves cut out of the agar. The sediment of the first centrifugation step that is routinely discarded can also be used for examination of nematode-trapping fungi.

METHOD :

1. 100 g of soil are suspended in 150 ml of water.
2. The suspension is poured onto a 250 µm aperture sieve and washed with as little water as possible through the sieve into a beaker.
3. The filtrate is agitated for 10 s, then poured onto a 100 µm aperture sieve and washed through the sieve into a beaker.
4. The resulting suspension is centrifuged at 650 g for 2 mn (Dürschner, 1983).
5. The supernatant is transferred to clean centrifuge tubes while the sediment containing the spores of nematode-trapping fungi is mixed with 1 ml of water, 1-1.5 ml of the soil suspension is pipetted into a 5-6 mm wide groove cut into the middle of a water agar Petri dish (9 cm diam.) by means of two joined scalpels.
6. 3 000 chrysoidin stained nematodes are added as bait in 0.6 ml of water.
7. The supernatant of the first centrifugation step containing the spores of endoparasitic fungi is centrifuged again at 4 060 g for 30 mn.
8. The sediment is resuspended and pipetted into the grooves in water agar. Endoparasitic fungi are best observed by cutting out a cross-shaped groove which increases nematode contact with the fungal spores. In addition this allows a 2 ml soil suspension to be used per plate.
9. 3 000 nematodes are pipetted onto the soil in the grooves for the detection of endoparasites. Nematode penetration into the agar is alleviated by adding a small amount of silver sand to the grooves.
10. The plates are kept at room temperature with examination for parasitized specimens made immediately after addition and at two day intervals for two weeks.

The examination of trapping fungi requires more time and might take up to six weeks if examination is made for fungi with adhesive knobs or branches. Evaluation is also possible by transferring the agar plates to Oostenbrink dishes after the incubation period and counting the stained nematodes that have not been trapped.

Conclusions

The methods described allow detection of fungal antagonists in soil samples from the field on a target nematode without special infectivity tests. They are efficient and time saving and give an overview of the spectrum of nematophagous fungi attacking mobile nematodes in field soil. Quantitative examinations are also possible. The stain allows marking of viable target nematodes, increases accuracy, and allows the use of a selected indicator species for specific fungal antagonism.

The stain can be used for all nematode species. Optimum stain concentrations vary and must be predetermined. A 24 hr staining period and a chrysoidin concentration of 1:20 000 is recommended for *Heterodera* species, *Neoaplectana carpocapsae, Neoaplectana bibionis* and *Heterorhabditis* species, whereas, a concentration of 1 : 50 000 is sufficient for free living nematodes such as *Panagrellus redivivus* and *Caenorhabditis elegans.*

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