RESEARCH NOTES

Techniques for Detecting Dactylella oviparasitica and Evaluating its Significance in Field Soils

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Dactylella oviparasitica Stirling and Mankau was first isolated from *Meloidogyne* egg masses (2) and shown to parasitize *Meloidogyne* eggs (3). The following four techniques are useful for detecting the fungus and evaluating its significance in field soils.

Examination of egg masses from the field: The gelatinous matrix of *Meloidogyne* egg masses collected from host plants in the field is partially dissolved by treatment in 1% NaOCl for about 2 minutes, and eggs are examined for parasitic fungi. Clumps of parasitized eggs are washed in sterile water and added to cornmeal agar (cornmeal infusion, 50 g; agar, 15 g; water, 1 liter), glucose-peptone agar (glucose, 10 g; peptone, 10 g; agar, 16 g; water, 1 liter) or YPSS agar (yeast extract, 4 g; K_2HPO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; soluble starch, 20 g; agar, 16 g; water, 1 liter). Inoculated media are examined daily for *D. oviparasitica.* If estimates of the number of parasitized and unparasitized eggs are desired, egg masses are macerated and eggs counted as described previously (3).

This method gives a direct indication of the number of eggs invaded by *D. oviparasitica,* but does not differentiate between parasitism of viable eggs and saprobic growth in dead eggs. Also, the disappearance of hyphae in eggs following destruction of the embryo sometimes makes it difficult to identify the original parasite.

Examination of roots from the field: About 1 g of roots are spread over the surface of one-quarter-strength cornmeal agar, and the plates are incubated at about 24 C for at least 1 mo.

A distinct succession of organisms colonizes roots and agar, similar to that observed with methods used to isolate parasites and predators of soil nematodes (1). After about 1 mo, predacious fungi begin to decline and conidia of *D. oviparasitica* can often be seen protruding from roots (Fig. 1). Conidia do not always occur on repeated samples from the same root system, but the reasons for this variability are not known. The fungus often sporulates prolifically on roots, and occasionally sporulates on agar. Under these conditions, *D. oviparasitica* is probably not nutritionally dependent on nematode eggs, but apparently grows saprophytically on roots and on cornmeal agar. If small quantities of soil are used instead of roots, *D. oviparasitica* is rarely observed. *D. spermatophaga* Drechs. also occurs on roots with this technique, and under the dissecting microscope it may be confused with *D. oviparasitica.* However, its slightly curved triseptate conidia are easily differentiated from those of *D. oviparasitica,* which are straight and 5–7 septate. *D. oviparasitica* was often isolated from peach and grape roots with this method. Single-spore isolates cultured on YPSS agar were compared with isolates recovered from parasitized eggs, and shown to be equally effective in parasitizing *Meloidogyne* eggs on agar.

Addition of egg masses to field soil: Root pieces containing egg masses from

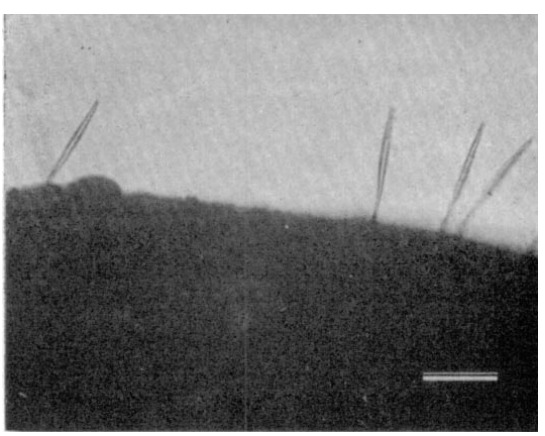

FIG. 1. *Dactylella oviparasitica* sporulating on peach roots on agar. Bar represents 50 μm.

Received for publication 9 May 1978.
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Meloidogyne-infected plants grown in the greenhouse are mixed with soil collected from the field. The soil and roots are placed in containers, and egg masses are examined 15–25 days later for *D. oviparasitica*. Fungal activity under the field environment can be evaluated by placing soil and roots in porous ceramic tubes, bags of fine nylon screening, or other materials which allow free movement of water and gases, and burying the containers in the field.

Although the presence of *D. oviparasitica* can usually be determined by this method, it is unsatisfactory if counts of parasitized eggs are used to estimate the seasonal variation in the activity of the parasite, or to compare levels of parasitism in different fields. Egg masses from greenhouse cultures usually contain large numbers of mature eggs which escape parasitism because they hatch soon after being added to soil. The number of eggs which hatch varies with different groups of egg masses and with environmental factors such as soil moisture, leaving various numbers of eggs available for parasitism.

Test of field soil in the greenhouse: Tomato seedlings are planted in field soil in pots, and second-stage *Meloidogyne* larvae are added if this soil contains few root-knot nematodes. Plants are grown at 25–27 C for about 40 days, and then egg masses are removed and eggs examined for parasitism by fungi. Since *D. oviparasitica* is associated with plant roots, incorporation of roots into the soil or the use of rhizosphere soil enhances the chances of detecting the fungus.

This technique is probably the most useful method of detecting and isolating *D. oviparasitica,* particularly if roots are incorporated into the soil, or rhizosphere soil is used. For example, about 70% of the *Meloidogyne* egg masses on tomato plants grown in rhizosphere soil from a peach orchard contained eggs parasitized by *D. oviparasitica* (unpublished data). There are indications that the number of egg masses found to contain *D. oviparasitica* by this method is related to the level of parasitism occurring naturally in that soil in the field, but further work is necessary to test this hypothesis. This method is particularly useful for detecting egg parasites in soil, because any fungus invading newly produced eggs is probably parasitic rather than saprophytic. The method is unsuitable for use in some vineyard and orchard soils because tomato seedlings will not tolerate some of the herbicides present in those soils.

LITERATURE CITED

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