

It will also be of interest to determine the extent to which the results of the present investigation are applicable to other rhizomatous species.

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¹ Hoagland, D. R., and Arnon, D. I., *Circ. Calif. Agric. Expt. Sta.*, 347 (1939).

² Palmer, J. H., *Nature*, 174, 84 (1954).

A Semi-quantitative Method of Counting Nematode-trapping Fungi in Soil

THE usual method of observing nematode-trapping fungi in soil, as outlined by Duddington¹, consists of sprinkling soil in a Petri dish, dispersing in cool but still liquid agar medium, and then examining the whole dish for the trapping structures of these fungi after several days of incubation. Because of the uneven size and distribution of soil particles, and the large area to be examined, this method is difficult and unreliable as a means of estimating numbers of trapping fungi present. However, as outlined here, an adaptation of this method can be used to obtain semi-quantitative estimates.

As a first step in this method, each soil sample is reduced to a slurry or mud of even consistency by mixing in a Waring blender with an approximately equal volume of water. A 0.5-ml. aliquot of each slurry is then pipetted into an empty Petri dish and dispersed in 30 ml. cooled but still liquid 3 per cent water agar. Long narrow strips (3 mm × 60 mm are convenient) of the hardened soil-agar mixtures are cut from these dishes by means of two scalpels tied together with blades parallel. These strips are transferred by spatula to the centres of empty Petri dishes, and the plates are then flooded with 30 ml. of 'Difco' corn meal agar adjusted to 10 per cent of the specified concentration of nutrients. Finally, 1-ml. volumes of a water suspension of saprophytic nematodes are pipetted over the soil-agar strips. The dishes are then set aside to incubate.

Several species of saprophytic nematodes from soil can be grown in Petri dishes in an agar medium supporting good growth of common soil bacteria on which the nematodes feed². The nematodes can later be extracted from this medium by a modified Baermann funnel³ and standardized to approximately 1,000 nematodes per ml. of water.

After several days of incubation, the soil-agar dishes are examined for the presence of trapping structures in hyphae which have grown out from the soil-agar strips. Provided these examinations are made in areas close by and parallel to the soil-agar strips, and before extensive branching of hyphae has occurred, it is generally possible to distinguish between hyphae originating from separate loci within the strips. Such hyphae bearing one or more trapping structures are each given a count of one. The low nutrient content of the corn meal agar is a distinct aid in these examinations, both in reducing the extensiveness of branching in hyphae of trapping fungi, and in reducing growth of contaminating fungi.

By standardizing the procedure described here, counts per dish can be converted to counts per gram of soil. They are then directly comparable to other microbial counts obtained by conventional soil-dilution plating techniques.

We have obtained estimates of nematode-trapping fungi in soil from a replicated field-plot experiment in which soil-treatment variables consisting of plant trash incorpora-

tion and nematocide fumigation both significantly affected populations of these fungi.

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¹ Duddington, C. L., *Trans. Brit. Mycol. Soc.*, 38, 97 (1955).

² Dougherty, E. C., and Calhoun, H. G., *Proc. Helm. Soc. Wash.*, 15, 55 (1949).

³ Anderson, E. J., and Yanagihara, T., *Phytopath.*, 45, 238 (1955).

Induction of Phototaxis in *Chlamydomonas snowiae* by Indolyl-3-acetic Acid and Ethylenediamine Tetraacetic Acid

IN a previous report it was shown that acetic acid and certain other substances could prevent a phototactic response of cells of *Chlamydomonas* without impairing their motility¹. It was of interest to see whether the reverse action could also be obtained, that is, to induce phototaxis in motile cells which do not respond to light. It will be shown that indolyl-3-acetic acid (IAA) and ethylenediamine tetraacetic acid (EDTA) induce such responses.

The cultures of *Chlamydomonas snowiae* were grown and handled, and phototaxis was measured as previously described¹⁻³. The medium containing the algae was adjusted to the required pH with hydrochloric acid or potassium hydroxide. In the controls phototaxis was then assayed while to the experiments a suitable amount of the reagent was added and the pH checked again.

The effect of IAA on the phototactic response of a fully motile culture of *Chlamydomonas snowiae* is shown in Table 1. The response to IAA is seen to be dependent on concentration and pH. Clearly IAA is effective in the non-ionized form, presumably because it is this form which penetrates into the cell. IAA at concentrations between 10⁻³ and 10⁻⁴ M was able to induce phototaxis at pH 6.0 in about 45 out of 50 replicates of the experiments. In a few cases, about 5-10 per cent of the replicates, it failed to induce phototaxis in motile culture, apparently because lack of phototactic response was due to other causes. Analogues of IAA, indole butyric acid and indole propionic acid as well as naphthalene acetic acid and 2,4-dichlorophenoxyacetic acids were also effective, but higher concentrations were required and the induction of response was less consistent than with IAA.

Table 1. INDUCTION OF PHOTOTACTIC RESPONSE BY IAA AT VARIOUS CONCENTRATIONS AND VARIOUS pH

| pH | 5.4 | 5.6 | 5.8 | 6.1 | 6.3 |
|--------------------|-----|-----|-----|-----|-----|
| IAA conc. M | | | | | |
| 0 (control) | - | - | - | - | - |
| 10 ⁻⁴ | +++ | +++ | +++ | +++ | +++ |
| 2.10 ⁻⁵ | ++ | ++ | ++ | + | - |
| 10 ⁻³ | +++ | ++ | ++ | - | - |
| 10 ⁻⁶ | ± | ± | - | - | - |

-, no response. +, positive response. +++, very strong response.

Not only did IAA induce phototaxis, but it was also able to reverse to a considerable extent the inhibitory action of acetic acid on the phototactic response. The phototactic response of a culture responding well to light was inhibited by the addition of acetic acid at pH values between 6.0 and 5.2. Addition of 10⁻⁴ M IAA did not reverse the inhibitory action. However, when the IAA concentration was raised to 10⁻³ M, then phototaxis was fully restored between pH 5.8 and 5.4 and almost fully at pH 6.0 and pH 5.2. IAA did not reverse the action of detergents such as 'Tween 80' on phototaxis. Reversal of inhibitory effects could also not be obtained consistently by the IAA analogues.

Other compounds were tested for their ability to induce phototactic response in motile, non-responding cultures of