

An Evaluation of Propagules of *Cylindrocladium scoparium* in Soil by Direct Isolation

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ABSTRACT

A method which was a refinement of washing and wet-sieving techniques was developed for the direct isolation of *Cylindrocladium scoparium* from soil, and for the quantitative estimation of the relative number of microsclerotia of the fungus in soil.

Microsclerotia were first separated from most of the soil on the basis of size and density. These propagules in a measured sample were then allowed to germinate and grow on a selective medium where they could be counted. Phytopathology 60:599-601.

Cylindrocladium scoparium Morgan incites a serious root rot of numerous hosts in over 30 families. In the late 1950's and early 1960's it was responsible for serious losses of conifer transplants in several Wisconsin forest tree nurseries. It was associated with root rot in 1961 in numerous nurseries in the North Central States (1). Our subsequent studies of the movement and distribution of *C. scoparium* required, first, a simple and accurate technique for detection of the fungus in small soil samples, and, later, a method for measuring the number of propagules of the fungus in the soil. Usual procedures for direct isolation of soil fungi, such as soil dilution plates or Warcup's soil plate method, proved unsatisfactory. Bugbee & Anderson (2) detected the fungus by planting alfalfa (*Medicago sativa* L. 'Vernal') in samples of field soil in the greenhouse. We then developed the spot-plate technique (8), a modification of the bioassay used by Bugbee & Anderson. The method was useful for qualitative assays to detect the presence of *C. scoparium* in soil samples of about 0.5 cc or less. A recent paper by Morrison & French (4) reported a technique for the direct isolation on soil-dilution plates of *C. floridanum* from soil in which propagules had been concentrated by the use of soil sieves. But for quantitative studies we evolved a technique for the direct isolation and quantitative estimation of the relative number of microsclerotia of *C. scoparium* in soil (7). It was used successfully for evaluating the results of fumigation experiments for control of the fungus.

This direct isolation process is a refinement of washing and wet-sieving techniques, of which there are now many examples in the literature (3, 5). It consists essentially of two steps. First, the microsclerotia of the fungus are separated from most of the soil on the basis of size and density. Second, the propagules are allowed to germinate and grow on a selective medium. The direct isolation process (Fig. 1) was performed as follows: (i) Each sample of soil (1.0-1.5 kg) was thoroughly mixed. (ii) Three subsamples were removed from the sample; two 35-g subsamples were dried at 105 C to a constant wt for determination of the moisture content. One 400-g subsample was used in the isolation procedure. (iii) The 400-g subsample was put

into a Waring Blendor, and the jar was nearly filled with water. (iv) The blender was run at a low speed for 2 min. (v) The suspension was allowed to settle for 15 sec. (vi) The supernatant was decanted onto nested 100- and 200-mesh nematode screens. (vii) Water was added to the residue in the blender jar, and the residue was stirred into suspension. The suspension was again allowed to settle for 15 sec and then decanted onto the screens. (viii) Step vii was repeated until the supernatant was clear, usually six to nine times. (ix) The material collected on the 100-mesh screen was washed, and the water that passed through the screen was allowed to flow onto the 200-mesh screen. The washed residue from the 100-mesh screen was discarded. (x) The material collected on the 200-mesh screen was washed thoroughly, and the wash water that passed through the screen was discarded. (xi) The residue that remained on the 200-mesh screen was collected in a beaker, and was called the "inoculum concentrate". (xii) Dilute water agar (0.3% agar) was added to the inoculum concentrate in a 400-ml beaker. (xiii) The suspension was stirred with a food mixer for 10 min. (xiv) Fifteen ml of suspension were added to each of two bottles containing 200 ml of melted and cooled (46 C) NPX-medium. (xv) The volume of the remaining suspension was measured and recorded. (xvi) After being mixed, the contents of each bottle were poured into 25-30 petri plates (thus two groups of plates). (xvii) The plates were incubated at 25 C for 10 days.

A selective medium for *C. scoparium* was made by adding lactic acid (enough to produce pH 3.5) and 1,000 ppm of Union Carbide Corporation's TERGITOL Nonionic NPX (nonyl phenyl polyethylene glycol ether with 10.5 moles of ethylene oxide) to melted and partially cooled Czapek's medium containing 2.5% agar. On the NPX-medium *C. scoparium* grew slightly slower than it did on Czapek's medium, but all other organisms grew considerably slower (Fig. 2). NPX was suggested for use in plate counts by Steiner & Watson (6).

After 10 days' incubation, the plates were examined and the concentration of propagules in the original sample was calculated. The total number of colonies of *C. scoparium* that developed on plates poured from a single bottle of medium was determined by examining

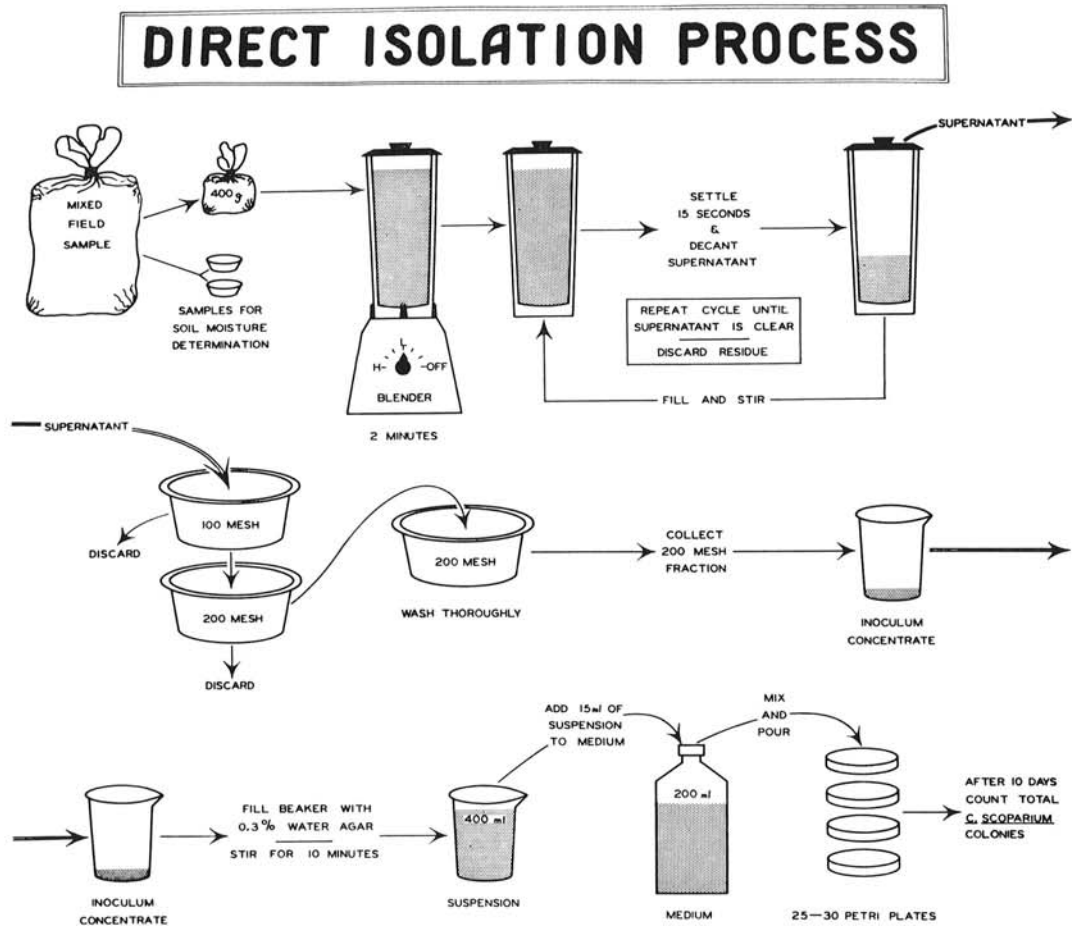


Fig. 1. The process for direct isolation of *Cylindrocladium scoparium* from soil.

each plate through the bottom (Fig. 2). The fungus was readily identified by the numerous microsclerotia that formed in the agar and imparted a characteristic dark reddish-brown color to the colony. Most colonies of *C. scoparium* sporulated to some degree on the NPX-medium; however, microsclerotia were a consistent characteristic, and were much faster to use for identification than conidia. The propagule population in each sample was calculated as follows:

$$\frac{S}{15} \times \frac{A+B}{2} \times \frac{1}{D} = p/g$$

where S = the starting amount of suspension of inoculum concentrate in water agar; A, B = number of colonies of *C. scoparium* in petri dishes poured from the two bottles of NPX-medium; D = dry wt of the process sample calculated from its starting wt of 400 g and the moisture content of the two moisture-determination samples; and p/g = propagules of *C. scoparium*/g oven-dry wt of the original sample. An example from a typical assay of a nursery soil is: S = 382 ml (step xii); A and B = 114 and 113 colonies of *C. scoparium* counted, respectively; D = 366.4 g

$$\frac{382}{15} \times \frac{113 + 114}{2} \times \frac{1}{366.4} = 7.95 \text{ p/g}$$

The direct isolation process gave a relative population count. The blending step breaks up soil particles and pieces of organic matter so that most spores and hyphal fragments are washed through the 200-mesh screen. This procedure reduces the total number of other organisms in the plates, thus making it easier to count colonies of *C. scoparium*. When a sample was not blended, 95% of the propagules of *C. scoparium* were collected on the 200-mesh screen, with the remainder collected on the 100- and 325-mesh screens. When a similar sample was blended, nearly twice as many propagules were detected; approximately 50% of the propagules detected were collected on the 200-mesh screen with the remainder collected on the 325-mesh screen. The propagules that passed through the 200-mesh screen after blending were apparently small fragments broken off the normal propagules. Comparisons of pairs of subsamples (one blended, one unblended) showed that blending did not reduce the number of

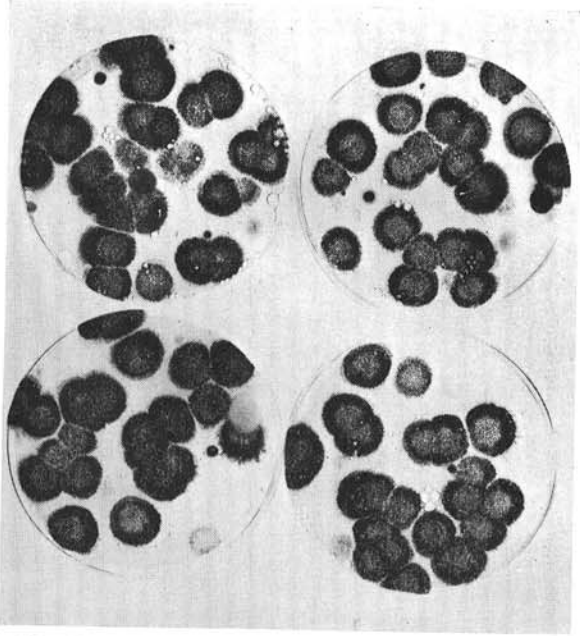


Fig. 2. Plates from the direct isolation process as viewed from the bottom. The dark masses are microsclerotia formed by the 10-day-old colonies of *Cylindrocladium scoparium*.

C. scoparium propagules collected on the 200-mesh screen. Tests of several samples of the same thoroughly

mixed lot of soil yielded consistently a similar number of propagules.

Most soil samples processed have yielded from 10-25 p/g; however, numbers as high as 60 p/g were detected.

LITERATURE CITED

1. BUGBEE, W. M., & N. A. ANDERSON. 1963. Host range and distribution of *Cylindrocladium scoparium* in the North Central States. *Plant Dis. Repr.* 47:512-515.
2. BUGBEE, W. M., & N. A. ANDERSON. 1963. Infection of spruce seedlings by *Cylindrocladium scoparium*. *Phytopathology* 55:1267-1271.
3. MC KAIN, A. H., O. V. HOLTZMANN, & E. E. TRUJILLO. 1967. Concentration of *Phytophthora cinnamomi* chlamydospores by soil sieving. *Phytopathology* 57:1134-1135.
4. MORRISON, R. H., & D. W. FRENCH. 1969. Direct isolation of *Cylindrocladium floridanum* from soil. *Plant Dis. Repr.* 53:367-369.
5. PARKINSON, D., & S. T. WILLIAMS. 1961. A method for isolating fungi from soil microhabitats. *Plant & Soil* 13:347-355.
6. STEINER, G. W., & R. D. WATSON. 1965. Use of surfactants in the soil dilution and plate count method. *Phytopathology* 55:728-730.
7. THIES, W. G. 1969. The biology and control of *Cylindrocladium scoparium* in Wisconsin State Forestry Nurseries. Ph.D. Thesis, Univ. Wis. 93 p. plus 21 p. suppl.
8. THIES, W. G., & R. F. PATTON. 1966. Spot-plate technique for the bioassay of *Cylindrocladium scoparium*. *Phytopathology* 56:1116-1117.