A Simplified Medium for Monoxenic Culture of Pratylenchus penetrans and Ditylenchus dipsaci

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Present methods of establishing and maintaining monoxenic cultures of plant parasitic nematodes are laborious. Use of complex, multicomponent nutrient media for production of callused host tissues is a factor contributing to the inefficiency of these methods. For example, the most commonly used of these media contains 19 constituents (1). This note reports maintenance of Pratylenchus penetrans and an onion race of Ditylenchus dipsaci on callus tissue produced with simplified nutrient agar.

Week-old, sterile onion (Allium cepa 'Aristocrat') and alfalfa seedlings (Medicago sativa 'Ranger') produced by previously described methods (2) were cultured for 2 weeks on nutrient medium (20 g sucrose, 5 g yeast extract [Difco Laboratories, Detroit, Mich.], 2 mg 2,4-dichlorophenoxyacetic acid, 10 g Difco-Bacto agar, 1000 ml distilled water) in 25 X 150 mm tubes. Thereafter, onion and alfalfa callus tissues were inoculated with D. dipsaci and P. penetrans, respectively. Cultures were maintained in the dark at 23 C. D. dipsaci were extracted from each of ten 8-week-old cultures with modified Baermann funnels. Nematodes in three 1-ml aliquots were counted. Numbers of P. penetrans in 10-week-old cultures were determined similarly.

Populations of D. dipsaci produced by these methods ranged from 500 to 23,300 nematodes/culture tube. The average population was 10,500/tube. Culture tubes infested with P. penetrans contained an average of 20,460 nematodes. Populations ranged from 8,800 to 40,000/tube. These tests have been repeated with similar results.

Populations of D. dipsaci and P. penetrans reared in our laboratories by standard procedure on Krusberg's medium (1) average approximately 20,000 and 36,000/tube, respectively (2). Thus, reproduction of these nematodes on simplified medium, although lower, compares well with the average rates attained with previous methods. Because of the time savings inherent in its production, the simplified medium affords a useful culture substrate in teaching and research applications where maximum population development is not required.

Moreover, since maximum populations developed on the simplified medium were comparable to the best reproduction rates expected with Krusberg's medium, further development of the simplified medium could result in a simple culture substrate capable of supporting excellent nematode reproduction.

LITERATURE CITED

Monoxenic culture of *Ditylenchus dipsaci* and *Pratylenchus penetrans* with modified Krusberg’s and White’s media. Plant Dis. Reptr. 54: 251-254.

A Mechanical Sieving Apparatus for Nematode Extraction

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Jenkins’ rapid centrifugation-flotation technique (1) is being used to assay irrigated soils of southern Alberta for plant-parasitic nematodes. The survey size and number of soil samples required make it desirable to minimize the man-hour input and tedium involved in processing samples.

The need for assistance in sieving the samples has been removed by the construction and use of the apparatus described herein. The apparatus consists of an iron plate, 33 X 33 X 0.6 cm, fitted with three upright iron rods, each 44 cm long (Fig. 1). Two aluminum rods clamped across the upright rods near the top make the stand rigid. One jaw-type clamp attached to each upright rod at the same height supports the sieve above the receiving pail. The clamp nearest the motor is mounted with the single adjustable jaw on the underside. A variable-speed, electric stirring motor fitted with a short crank bar is clamped in position on one of the uprights. A sieve of the desired mesh size is clamped in position. The height of the motor is adjusted so that the rotating crank barely touches the top of the sieve. A pail is placed under the sieve. The motor is switched on and the screw on the underside of the clamp nearest the motor is adjusted until the rotating crank strikes the top rim of the sieve. The soil sample, suspended in water, is then poured onto the sieve by the operator. Motor speed can be adjusted to promote the optimum flow of water and suspended soil through the screen and into the receiving pail.

**FIG. 1.** Mechanical sieving apparatus used for separating nematodes from aqueous suspensions of soil.

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