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

R. M. RIEDEL, J. G. FOSTER, and W. F. MAI[1]

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 × 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.

Received for publication 7 February 1972.

[1]Assistant Professor, Plant Pathology Department, Ohio Agricultural Research and Development Center, Columbus, Ohio 43210; Laboratory Technician and Professor, Plant Pathology Department, Cornell University, Ithaca, New York 14850, respectively.

Approved for publication as Journal Article 8-72 of the Ohio Agricultural Research and Development Center, Wooster, Ohio 44691.

LITERATURE CITED

1. KRUSBERG, L. R. 1961. Studies on the culturing and parasitism of plant-parasitic nematodes, in particular *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi* on alfalfa tissues. Nematologica 6:181-200.
2. RIEDEL, R. M., and J. G. FOSTER. 1970.

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