

# Extraction of Eggs of *Pratylenchus penetrans* from Alfalfa Callus and Relationship between Age of Culture and Yield of Eggs

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Studies of nematode life cycles depend on a source of uniform inoculum of known developmental stage. The egg masses of *Meloidogyne* spp. and eggs in cysts of *Heterodera* spp. have provided such inocula, but similar sources of inoculum are lacking in most groups of plant-parasitic nematodes. The purpose of this paper is to describe the procedure I have used to obtain large numbers of eggs of *Pratylenchus penetrans* (Cobb) from alfalfa (*Medicago sativa* L.) callus tissues.

Callus tissue was obtained (approximately 2 g/culture) from 10 cultures maintained as described by Riedel and Foster (3). The tissue was comminuted for 30 sec with 200 ml of 30% sucrose solution (30 g sucrose/70 ml water) in a food blender. The resulting suspension was poured into a 250-ml round-bottom centrifuge bottle and centrifuged approximately 8 min at 1500 g in a swinging-bucket head. The supernatant was poured through a 325-mesh screen to remove coarse plant debris, then through a mesh with 15- $\mu$  openings (Nitex<sup>®</sup> nylon mesh; Tobler, Ernst & Traber, Inc., 420 Sawmill River Road, P.O. Box 112, Elmsford, N.Y. 10523). Eggs of *P. penetrans*, approximately 24  $\mu$   $\times$  61  $\mu$  (2), were caught on this mesh and rinsed to remove the sucrose. The residue on the mesh, including eggs, many vermiform *P. penetrans*, and callus tissue debris, was washed into a beaker with 25 ml or less of water. This suspension was then layered on 20 ml of 30% sucrose solution contained in a 50-ml centrifuge tube by holding the tube as near horizontal as possible while the suspension was carefully poured down its side. In the resultant preparation, the aqueous suspension and underlying sucrose solution remained as separate phases with a distinct interface between them. After centrifugation for 4 min at about 1500 g (full speed in an IEC Clinical centrifuge), the eggs and vermiform nematodes

were concentrated in a band at the interface and the callus debris was deposited at the bottom of the tube. An aspirator bottle was used to remove the eggs from the interface. They were again collected and rinsed on the 15- $\mu$  mesh.

I freed the egg suspension of most vermiform nematodes by diluting it to 100-150 ml and sieving it repeatedly through a 325-mesh screen. Remaining nematodes were removed by pipette or pick with the aid of a stereoscopic microscope. Eggs were placed on a 15- $\mu$  mesh screen in deionized water, and second-stage juveniles which hatched were collected from the bottom of the dish.

During the development of the egg extraction procedure, a test was conducted to determine the effect of culture age on yield of viable eggs. Cultures incubated at 20-24 C for 6, 9 and 11 weeks from date of subculturing (infestation of sterile alfalfa callus tissue with nematode-infested callus from a previously established culture) were processed individually. The mean numbers of eggs recovered from four cultures of each age were 12,700, 9400 and 5100, respectively. The mean numbers of juveniles recovered from the hatching units after 26 hr of incubation at room temperature were 1068, 608 and 583, respectively. In another experiment, the greatest yields of eggs were from cultures aged 6 weeks, and cultures 10, 29 and 49 weeks from infestation yielded far fewer eggs per culture.

The technique can be adapted easily to extract eggs of other species cultured *in vitro*. I have also used the above procedure, with direct centrifugation of soil (1) substituted for the steps preceding the first collection on the 15- $\mu$  mesh, to extract eggs of *Paratylenchus* sp. and *Pratylenchus penetrans* from field soil.

## LITERATURE CITED

1. DUNN, R. A. 1971. Comparison of two centrifugal flotation techniques with Seinhorst elutriation for efficiency of nematode extraction from soil. *J. Nematol.* 3:308-309.
2. HUNG, C. L. and W. R. JENKINS. 1969. Oogenesis and embryology of two plant parasitic nematodes, *Pratylenchus penetrans* and *P. zeae*.

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J. Nematol. 1:352-356.

3. 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. Rep.  
54:251-254.