

RESEARCH NOTES

Two Methods for Separating Larvae of Uniform Length from Mixed Adults and Larvae of *Panagrellus redivivus*

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When measuring growth, reproduction, or mortality of nematodes reared in nutrient media, it is sometimes advantageous to initially inoculate cultures with larval stages of uniform size. The following two methods, which can be extended to other species of nematodes, successfully separate larger or advanced stages of *Panagrellus redivivus* from uniform sized larvae.

METHOD 1: Nylon monofilament bolting cloth (Nitex®, Tobler, Ernst, and Traber, Inc., 71 Murray Street, N. Y., N. Y. 10007) with openings of 20, 25, 35, 44, 53, 64, 73, 80, and 86 μ glued with epoxy resin to 18 \times 10 mm (diameter \times depth) glass rings (#7052, A. H. Thomas Co., P. O. Box 779, Phila., Pa. 19105) were placed in watch glasses (#9850, A. H. Thomas Co.) partially filled with tap water. Only the 20, 25, and 35 μ screens proved useful for *P. redivivus*. Nematodes were placed upon the 20 μ screen and after 2–5 min larvae 564 \pm 29 μ (S.E.) long were recovered. Those nematodes remaining upon the 20 μ screen were transferred to the 25 μ screen for 15 min and a mixture of stages was recovered. Nematodes remaining upon the 25 μ screen were placed on the 35 μ screen for 30 minutes and

larvae 1371 \pm 61 μ (S.E.) long passed through and were trapped in the water. Those remaining on the 35 μ screen averaged 1757 \pm 62 μ (S.E.) in length. Ten randomly selected nematodes were measured for each determination. The 20 μ screen passed either second or mixed second and early third-stage, the 35 μ screen passed fourth-stage larvae. Increased time on the screens yielded a greater mixture of sizes; length of time is a critical factor.

METHOD 2: Glass microbeads 105–149 μ in diameter, 6 mm in depth (#1014, Microbeads Div., Cataphote Corp., Jackson, Mississippi 39205) were supported on a filter paper (#595 Schleicher & Schuell Co., Keene, New Hampshire 03431) attached to a 50 \times 10-mm plastic ring with a rubber band. This apparatus was placed in a funnel, equipped with a hose and clamp, and the funnel filled with enough water to wet the microbeads. *P. redivivus* were distributed upon the beads and large numbers of second stage larvae 432 \pm 21 μ (S.E.) long were recovered after 16 hours. Since size-classes of microbeads are available down to 44–64 μ diameters, one or more can probably be selected to segregate uniform-sized larval stages of most species of free-living or plant parasitic nematodes. The average porosity of the microbead matrix appeared to be the major factor governing the segregation of second-stage larvae. All experiments were done at room temperature, 22–23 C.

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