RESEARCH NOTES

Extracting the Rice White-Tip Nematode, Aphelenchoides besseyi, with Match Sticks[1]

J. A. ADAMO, C. P. MADAMBA and T. A. CHEN[2]

Earlier laboratory studies on the vertical migration of *Aphelenchoides besseyi* Christie showed that high numbers of nematodes migrated on wooden match sticks (1). As part of an investigation into the nature of the vertical migratory behavior of aboveground forms, two experiments were designed to determine the efficiency of match-stick surface by measuring the recovery of nematodes. This report describes a new technique for extracting *A. besseyi* from fungal-nematode cultures. The method of extraction was compared to various standard methods.

In the first series of tests, a suspension of about 100 freshly isolated nematodes were mixed, by using a spatula, with 8 ml of minced 1.5% water agar in each of 25 beakers. These were divided randomly into five groups of five samples each. Nematodes of the first group were extracted by using Baermann funnels; those of the second group by sieving with standard 250-μm and 45-μm sieves; a centrifugation-sugar flotation process (2) was employed with the third group; and match sticks were used with the final two groups. Samples of the fourth and fifth groups were individually placed in Syracuse dishes. A presoaked, wet, match stick (2 x 2 x 38mm) was placed in a vertical position in each dish of agar of the fourth group. A match stick (2 x 2 x 38mm) was placed in a vertical position in each dish of the fifth group, and the upper portion of the structure was attached by 3-ply cotton string to a well containing 3-4 ml of water. The string served as a wick to carry water from the well above the culture to the stick. All nematode treatments were incubated for 18 h. In the fourth and fifth groups, the match sticks were removed and soaked in water, and the water was examined for nematodes.

Cultures of *A. besseyi* reared on *Alternaria brassicae* (Berk.) Sacc. on potato-dextrose-agar were used in the second series of experiments. The cultures were chopped and blended thoroughly, and 5 gm of the mixture were dispensed into each of 15 beakers. These were divided randomly into three groups of five samples each. The samples of the first group were placed in funnels and processed as before. The agar of each replicate of the second group was covered with water, mixed, incubated, and subjected to sieving for nematode recovery. The samples in the last group were individually placed in Syracuse dishes and supplied with match sticks connected to wells via strings as previously described. The structures were removed after 18 h and soaked in water, and the water was examined for nematodes.

In the first experiment using water agar, recoveries with standard methods of extraction and those with match sticks supplied with water from wells were statistically the same ($P = 0.05$). The greatest numbers of nematodes were recovered from samples subjected to the match sticks and sieving methods of extraction. Samples processed by sieving contained debris, whereas those recovered from match sticks were relatively free of debris. When match sticks were presoaked and not continuously supplied with moisture, very low recovery resulted.

In the second experiment, high numbers were recovered from all methods, but the greatest number was obtained by using the match-stick method. Funnels and match sticks yielded similar numbers, but much less debris was present in nematode suspensions from the match sticks.

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[2] Associate Professor, Department of Science, Ocean County College, Toms River, New Jersey 08753; Associate Professor and Chairman, Department of Zoology, University of the Philippines, Los Banos; and Professor, Department of Plant Biology, Rutgers, The State University of New Jersey, New Brunswick 08903.

One disadvantage of the match-stick technique is the requirement of postincubation soaking. Since the nematodes swim out of the sample, however, only live animals, free of media and fungal debris, are collected. Further, since all the worms are on the stick (which can be soaked in almost any volume of water), the worms can be extracted in a dilute or concentrated form.

LITERATURE CITED

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