RESEARCH NOTES

Journal of Nematology 16(3):341–342. 1984.

Preparing Nematode Permanent Mounts with Adhesive Tapes[1]

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Keywords: permanent mount, method, adhesive tape.

Nematode specimens for taxonomical work generally are mounted permanently in glycerol between two coverslips in a Cobbs aluminum slide or on a glass slide under a thin coverslip. In both cases, glass fibers of suitable diameter are used to keep the coverslip from pressing on the specimens. For larger nematodes, pieces of nickel-chrome wire can be used instead of the glass fibers (1).

These types of supporting material are cumbersome to use. In addition, specimens mounted with only these supports can slip away from the center of the mount, get under the ringing substance, and be obstructed for microscopical observation.

A paraffin ring of suitable thickness can be used not only to support the coverslip but also to confine the nematodes in the desired position on the slide (1). We have encountered difficulties, however, in providing a paraffin ring of correct thickness and in containing the specimens within the ring once the coverslip is in place.

In our efforts to improve the method, we found transparent adhesive tape to be a suitable supporting material. This paper reports the mounting technique using transparent adhesive tape.

Polyester base adhesive transparent tape (tape no. 8535 of 3M Co., or similar quality tape) was adhered to a paraffin paper and a 9-mm-d circular disc removed from the tape (Fig. 1B) with a punch (Fig. 1A). The tape was then trimmed with scissors to a ring with a rim of approximately 2 mm (Fig. 1C). This ring was then removed from the paraffin paper and adhered to a good quality clean microscope slide. The adhesive tape ring was pressed against the slide with a glass rod to ensure its firm adherence. A drop of dehydrated glycerol was then placed in the round well created by the tape, onto which glycerin-impregnated nematodes were transferred. After properly arranging the nematode specimens at the bottom of the glycerol, a 22- × 22-mm coverslip was carefully placed over the well. Volume of the glycerol drop should be adjusted in such a way that it overflows the well slightly upon placement of the coverslip, thus leaving no air bubble in the well. The space around the tape was further filled with glycerol through capillary action. To complete the mounting, the margin of the coverslip was sealed with Canada balsam or other suitable ringing substances (Fig. 1D). The glycerol in the space between the tape well and the margin of the coverslip prevented direct contact between the adhesive tape and the ringing substance whose solvent might react with the tape adhesive.

With a thickness of approximately 45–50 μm (personal correspondence with 3M do Brasil Ltda.), the tape served as an effective support of the coverslip for mounting the majority of plant parasitic nematodes, eliminating the need for glass fibers. In addition, the tape ring confined the specimens in the central well, preventing them from slipping to the margin and being obstructed by the ringing substance.

Experience indicated that upon place-

Received for publication 19 April 1983.

[1] Supported in part by Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq, Brazil. The authors thank 3M do Brasil Ltda. for furnishing the adhesive tapes used in this study.

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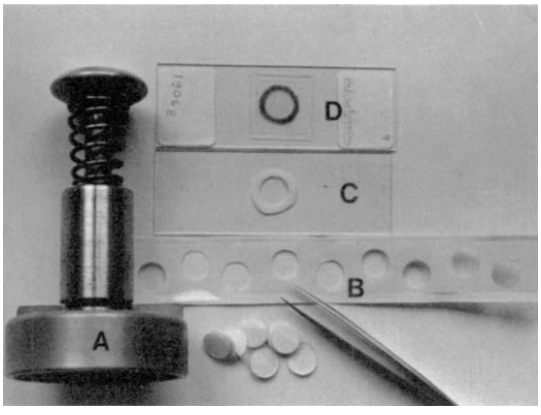

FIG. 1. Equipment, materials, and procedures used in making permanent nematode mounts. A punch (A) was used to perforate 9-mm holes in an adhesive tape (B) adhered to a paraffin paper strip to facilitate the operation. The adhesive tape, still adhered to the paraffin strip, was then trimmed to rings with rims of about 2 mm (C). The adhesive tape ring was then separated from the paraffin paper and firmly adhered to a clean glass slide. Nematodes were mounted in the well created by the tape ring and covered with a coverslip which was then ringed with Canada balsam or other suitable material (D). The adhesive tape ring was specially colored for photographing.

ment of the coverslip, specimens tended to slip away from tape wells whose diameters were less than 5 mm. Since smaller wells facilitate microscopic localization of the nematodes, experiments were conducted to find out the optimum well size for mounting and microscopic observation.

Given numbers of glycerine impregnated *Ditylenchus dipsaci* were mounted in wells of various sizes, and the frequency of nematode expulsion from the wells upon placement of coverslip was evaluated. The results (Table 1) showed that risk of nematode expulsion was significantly increased for the wells smaller than 9.5 mm in diameter, especially those mounted with 20 nematodes per well. We therefore recommend wells of 9–10 mm for routine operation.

To adapt the method for nematodes with larger diameters, two layers of the tape can be superimposed before the circular well is prepared, or a thicker tape may be used. Females of *Cynipanquina danthonia* Maggenti, Hart, & Paxman, 1973 with ca. 100-μm diameters, were mounted on slides

TABLE 1. Probability of glycerine-impregnated *Ditylenchus dipsaci* being expelled from adhesive tape wells of various sizes, upon placement of coverslips for permanent mountings.

Well diameter (mm)	Nematodes expelled*			
	10 nemas/well		20 nemas/well	
	Range	Average	Range	Average
5.0	0–10	3.3	0–20	9.2
7.0	0–10	3.3	0–15	7.5
8.0	0	0	0–10	4.2
9.5	0–10	1.6	0–5	2.5
11.5	0	0	0	0
13.0	0	0	0	0
15.0	0	0	0	0
16.0	0	0	0	0

* Results of six replicates.

prepared with two superimposed tapes. Measurements of the nematodes made with a camera lucida before and after mounting showed that the sizes of the nematodes were not modified.

A cellophane base adhesive tape (tape no. 600 of 3M Co.) of similar thickness was much easier to work with than the one with a polyester base because it could be more easily cut. It was cautioned, however, that the cellophane might be dilated by the glycerol during prolonged preservation (personal correspondence, 3M do Brasil Ltda). Our comparative experiments, nevertheless, have not yet showed any difference between the two types of tapes 28 months after mounting.

When using this method, we encountered some difficulties with extremely small nematodes such as *Paratylenchus* spp. and *Gracilacus* spp. These nematodes tended to float in the medium thus limiting their observation under oil immersion. Also, the method should not be used for temporary mounts involving formaldehyde solution, since formaldehyde quickly dilates the tape ring.

LITERATURE CITED

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