

A Simple Method for Examination of the Vulva Area of Mature Cysts of *Heterodera* spp.¹

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To correctly speciate members of the genus *Heterodera*, the vulva area of cysts must be examined to assess the morphology of the underbridge, bullae, and fenestrae. The usual procedure (1) is to soak the cyst in water for about an hour, after which a scalpel is used to cut away the posterior part of the cyst containing the vulva area. The severed part is cleaned and trimmed, placed in absolute alcohol for a few minutes, transferred to clove oil, and finally mounted in balsam.

A new method—the totomount water agar method—was developed serendipitously while examining cysts in water agar for fungal infections. To initiate the totomount water agar method, 3–6 cysts, preferably fresh from soil or roots, or fixed specimens are placed on the surface of a 1.5-cm², 2-mm-thick block of 1.7% water agar lying on a microscope slide or on the bottom of a petri dish lid. Using a fine dissecting needle with an offset tip, a small 1-mm-deep cavity is made in the agar for each cyst. The cavity should be slightly smaller than the cyst diameter. Using the dissecting needle, each cyst is gently pushed into a cavity with the anterior end down until the posterior apex of the cyst is level with the agar surface and pointing straight up. A small drop of water is added to a 12–15-mm cover glass, after which the cover glass is inverted and gently dropped over

the embedded cysts. Immersion oil is added to the cover glass for microscopic examination. Cysts are now ready for examination with a compound microscope. Normally 50% or more of the embedded cysts provide an excellent vulva area presentation (Fig. 1). If the vulva areas should be out of alignment or focus, correction is made by removing the cover glass, orienting the cysts correctly, and adding a new cover slip with a fresh drop of water. The entire procedure takes about 10–15 minutes.

Even though it seems improbable that one can provide an excellent vulva area presentation with the light from below passing through or around the lengthwise orientation of the cyst, this method has been employed successfully for 5 years in Florida for numerous cyst identifications. Identification has been achieved with only a single cyst from a soil sample. The vulva area of young white *Heterodera* females have also been clearly observed using this

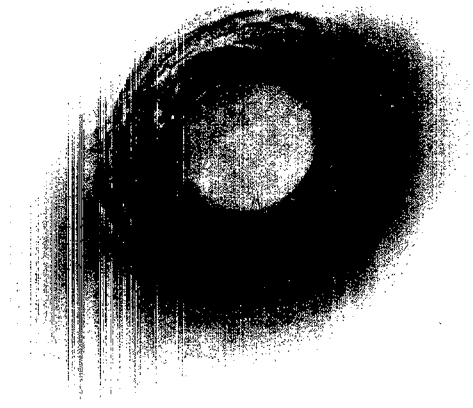


FIG. 1. Vulva area (cyst cone area) of *Heterodera cacti* prepared by the totomount water agar method.

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method. This method has been unsuccessful, however, when applied to mature swollen females of root-knot nematodes (*Meloidogyne* spp.). It seems inexplicably strange that the perineum of root-knot nematodes cannot be clearly defined using this method, whereas the fenestrae, bullae, cone pattern, underbridge, and anus of mature females and cysts of *Heterodera* sp. are clearly defined.

The principal advantages of this method are its speed, simplicity, and high success potential. After the initial identification data have been taken, the specimens can

be removed from the agar and fixed permanently or used for inoculation. If cysts are improperly oriented, with the vulva area at an angle difficult to interpret or missing from the focal plane, reorientation of the cyst is rather easy. In the older method, reorientation is difficult and sometimes impossible.

LITERATURE CITED

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