

## Convenient Storage of Cysts of Heterodera trifolii<sup>1</sup>

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When populations of cysts of *Heterodera* are maintained on plants or stored in soil it is necessary to screen soil each time cysts are needed, and considerable amounts of greenhouse space, maintenance effort, and soil storage space are needed to maintain a large number of populations. We decided to try to find a method of storage that would provide more convenient recovery of cysts and would require less space and maintenance.

Cysts of *Heterodera trifolii* Goffart were recovered from pots of 'Kenland' red clover (*Trifolium pratense* L.) by the procedure developed by Dunn (1). Several hundred cysts were placed in each of several 90-mm diam glass

petri dishes and covered with a thick layer of molten, cooled, 1% or 5% agar. The dishes were placed in plastic freezer bags and stored in a refrigerator at 10 C. At various time intervals thereafter, egg-laden cysts (usually 10) were picked from the agar, and placed in 7.5-cm diam pots of steamed soil with seedlings of Kenland red clover. The plants and soil were examined 2-3 mo after inoculation. Viability of the stored cysts was recognized by the presence of new cysts and immature nematodes on roots and in soil from the pots.

Cysts in 1% agar were viable after 62 days, but not after 96 days, of storage. By this time (96 days) the agar had dried to hard thin sheets in the dishes. Cysts in 5% agar were viable after 546 days, at which time the experiment was terminated because the agar was rubbery and shrunken away from the sides of the dishes and it was no longer possible to pick out individual cysts without breaking them. Large numbers of infective larvae hatched from the eggs in these cysts when pieces of the agar were put into a

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mist chamber. There was no indication of diminution of viability during the 18 -mo storage despite the development of fungal and bacterial contaminants.

This procedure provides a way of storing large numbers of cysts of *H. trifolii* for a reasonably long period of time, and particular

numbers and sizes of cysts can be collected conveniently whenever needed.

#### LITERATURE CITED

1. DUNN, R. A. 1969. Extraction of cysts of *Heterodera* species from soils by centrifugation in high density solutions. *J. Nematol.* 1:7 (Abstr.).