# RESEARCH NOTES

## Anesthetization of Morphologically Similar Species with Carbon Dioxide for Separation and Culture

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Nematologists frequently encounter congeneric species in sampling for nematodes. No convenient method for the separation of morphologically similar species has been described. For *Meloidogyne* spp., inoculum consisting of hundreds of eggs can be easily obtained from a single egg mass and a pure population reared on a suitable host. The separation and culture of other plant-parasitic nematode species, such as *Paratrichodorus* spp., is often more difficult. The species may be separated by placing a single female in the rhizosphere of a host plant, but the chances of a single nematode increasing to a high population in a short time are not great. The dissecting microscope may also be used for separating species which are not very dissimilar, but obtaining high numbers of a single species is difficult, especially when the dissimilarities between species are not easily discernible. We have also tried separating nematodes which had been placed on glass slides and cooled to immobility, but their recovery rate was rapid or many specimens were killed.

We have developed a method, using vaporphase perfusion of carbon dioxide ($CO_2$), for anesthetization of nematodes. This method allowed us to start greenhouse cultures with large, pure populations of desired species.

We were concerned with the separation and culture of a mixed population of *Paratrichodorus christiei* (Allen) Siddiqi and *Paratrichodorus porosus* (Allen) Siddiqi. The nematodes were extracted from soil by Cobb's method (1) and placed in a drop of water on a glass slide. The slide was placed next to a 50-gm piece of dry ice, and both were enclosed in an inverted 2-liter beaker. The solid $CO_2$ was allowed to sublime and perfuse into the water drop. After 3 min, the slide was removed from the perfusion chamber and a coverslip was placed on the water drop and secured in three locations with paraffin. Anesthetized specimens could be examined easily under oil immersion for the presence or absence of taxonomic characters (such as vulval pores, the location of the excretory pore, and the shape of the esophagus) essential for the separation of *P. porosus* and *P. christiei*. After the species were identified, the coverslip was easily removed with a pair of jeweler's forceps and the nematodes were then placed in labeled BPI dishes filled with fresh water. After the nematodes regained mobility, several females of each species were added to pots in which alfalfa (*Medicago sativa* L. 'Moapa 69') seedlings were growing. Three months after the introduction of 50-100 *P. christiei* or *P. porosus*/pot, we were able to obtain 80,000 nematodes. Examination of these established populations confirmed that we had obtained monospecific cultures.

It is possible to bubble $CO_2$ through a nematode suspension as described by Maggenti and Viglierchio (2). Since all nematodes are relaxed, however, it is difficult to separate the live, anesthetized nematodes from those that are dead. Time would be spent examining dead nematodes which could not be used for inoculum. After a few slides are made, the suspension must be retreated with $CO_2$, but the risk of injury is increased. For these reasons, we found $CO_2$ treatment of individual slides preferable and more convenient.

Individual specimens within a species vary in tolerance to $CO_2$. Consequently, some will be killed, but most recover completely within 2-3 min after being placed in fresh water. Those which do not recover can be removed prior to inoculation.

For this technique to be useful with other nematodes, the $CO_2$ exposure and recovery times must be determined for each

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species. Nematodes must be anesthetized during the period of examination. However, they must not be exposed for a period of time which would prevent a resumption of activity.

## LITERATURE CITED

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2. MAGGENTI, A. R., and D. R. VIGLIERCHIO. 1965. Preparation of nematodes for microscopic study—Perfusion by vapor phase in killing and fixing. Hilgardia 12:435-463.