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## CULTURE TECHNIQUES FOR REARING SOIL ARTHROPODS\*

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Interest in soil biology has been prompted by recent investigations into the action of insecticides on plants and animals. Observations in the field must be supplemented by laboratory investigations conducted under controlled conditions. Consequently, it becomes necessary to rear and handle soil animals under artificial situations for bio-assay and life cycle studies. When large numbers of individuals are required, special problems in maintenance and manipulation arise. Relatively inexpensive and simple methods for such projects are essential and this paper describes some of those techniques which we have found expedient.

Various rearing chambers have been suggested by authors in the past, and from them we developed our own particular method. Wharton (1946) used weighing bottles fitted with ground glass stoppers and a plaster-charcoal substrate, in which ratio of plaster to charcoal was 9 to 1. Rohde (1956) described the use of Lucite cylinders set into a plaster-bottomed container. A series of these small vessels can be observed under the dissecting microscope.

Goto (1960) improved upon earlier culture methods by using a heat-resistant glass container with a plaster-charcoal base and sealed with a cork. He was able to heat-sterilize the jars, but fungi reinvaded his cultures. This problem was solved by adding a low concentration of Nipagin-M (methyl-p-hydroxybenzoate) solution to the plaster and/or the food. The food supply was yeast.

For his isolation chambers, Hale (1964) used Goto's method with 2x3/4" tubes filled with 1/2" of the top with plaster-charcoal mixture. He sealed the top with a cover slip imbedded in vaseline. Vail (1965) used essentially the same technique with tubes imbedded in a plaster-charcoal base within a fingerbowl. He reviews some of the earlier culturing techniques by other authors. Abbatiello (1965) used a plastic container similar to one described in this paper with a wire cloth bent in the lid.

More recently, Kyle and Long (1967) developed a method for rearing Collembola on filter paper suspended within vials containing sand set in a water reservoir. Algae were used as a food source in this method.

The studies being conducted on Collembola and other arthropods in our laboratory required variable numbers of individuals and, therefore, large numbers of culture containers. For ease of repetition a standardized container was adopted. At first it consisted of a small glass jar (5 1/2 cm x 5 cm) fitted with a plastic screw cap (fig. 1). A 5:1 plaster-charcoal mixture was poured into each jar to a depth of 2 centimeters. This container has the advantage of being air-tight, and water loss is held to a minimum. Brewers yeast in small amounts was supplied as food.

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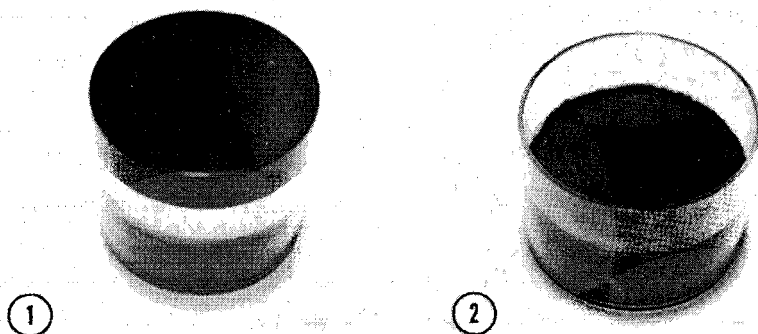


Fig. 1. Glass jar with plaster-charcoal substrate.

Fig. 2. Plastic jar with plaster-charcoal substrate.

When several hundred replications were made, the disadvantages of screw-cap jars became apparent. The constriction for the screw-cap forced the observer to tilt the jar under the microscope in order to make observations near the sidewall. Furthermore, it was necessary to remove the cap every time a check was to be made.

Later, straight sided plastic containers (5 cm x 3 3/4 cm) were obtained for general culture use (fig. 2). They were kept in controlled temperature cabinets at 60, 70 and 80 degrees Fahrenheit. The same 5:1 plaster-charcoal substrate was used, but while the mixture was still soft, a 15mm circular coverslip was pushed into the center of the surface to serve as a feeding station. The plastic containers had a clear snap-on top which allowed observations to be made without disturbing the culture. The tops were not always air-tight and some water vapor did escape. This was overcome by addition of water to the substrate once a week. A higher humidity and lower water loss could have been maintained in the cultures if a pan of water was placed in the bottom of the temperature cabinet.

The reason for using a 5:1 plaster-charcoal mixture was based on egg observations. When it is necessary to count, photograph or remove eggs, the black background afforded by the substrate accentuates most arthropod eggs which are white to hyaline in color (fig. 5).

Brewer's yeast was used as a food to support the stock cultures. Most of the soil species of *Collembola* did very well on such a diet. It was added in small quantities along with a drop of water. Provided the substrate was not too wet, fungal growth was kept at a minimum.

Using these containers, various feeding experiments with *Collembola* and other arthropods were undertaken. Many soil species in culture were found to prefer dried corn leaf. It was a simple matter to collect a supply of corn leaves and store them in a freezer until needed. A cork borer was used to cut uniform discs from the leaves (fig. 3). The discs in turn were oven dried and then weighed. Once this was accomplished, the discs were placed on the coverslip feeding station within the culture containers. Various numbers of arthropods were introduced into the containers to ascertain how much



Fig. 3. Corn leaf discs cut with a cork borer, ready to be weighed and placed in the culture jars.

plant material a species could consume. At the end of a given period of time the remains of the corn leaf disc were removed, dried and weighed (fig. 4). Other experiments were then conducted by treating the discs with insecticides. The results of such experiments will be presented elsewhere. However we mention them as an example of what can be done with these relatively simple containers.

Early in our studies, manipulation of small arthropods was accomplished after anesthetizing them with ethyl ether. A hole was cut in the lid of a plastic petri dish, and a cotton plug inserted (figs. 6 & 7). Ether was dropped on the cotton and a cap was placed over the area. The cotton plug was centered over the culture container and after a short exposure, the individuals or eggs could be transferred with a camel's hair brush. Later carbon dioxide gas was used in place of the ether, and the procedure was modified accordingly. A simple glass funnel was attached to the cylinder by means of a plastic hose. The culture was then placed under the funnel and the gas introduced (fig. 8). CO<sub>2</sub> has very little long-range effect on most species and has the advantage of being safer to use in the laboratory.

The method described in this paper enabled us to rear the following species of Collembola: *Protophthora armatus* (Tullberg), *Folsomia fimetaria* (L.), *Proisotoma minuta* (Tullberg), *Isotoma albella* Packard, *Hypogastrura armatus*



Fig. 4. Corn leaf disc after one week exposure to *Folsomia fimetaria* (L.) in a culture jar. The remains can be removed and weighed.

(Nicolet), *Tomocerus flavescens* (Tullberg), *Lepidocyrtus violaceus* (Geofroy), *Orchesella hexfasciata* Harvey, *Pseudosinella violenta* (Folsom), and *Neanura muscorum* Templeton.

Other arthropods maintained were Entotrophi, Pseudoscorpions, Isopoda, and Diatopoda. The techniques involved with these orders varied somewhat from those used for Collembola. The Entotrophi lived adequately in the containers with the addition of yeast, as well as some fragments of leaves for shelter. Cool temperatures (around 70 F.) seem to be adequate to maintain the cultures. The isopods and diplopods are best kept in moist containers with corn leaves and a small quantity of chalk scrapings. Two species of Isopods were successfully cultured through their life cycles; *Cylisticus convexus* (DeGeer) and *Tracheoniscus rathki* (Brandt). When their juveniles first hatched, yeast was added as a dietary supplement. The Pseudoscorpions were easily reared by the addition of Collembola to their containers. These provided an adequate diet.

Further modifications of the above techniques are presently under way and the numbers of species in culture are being increased.

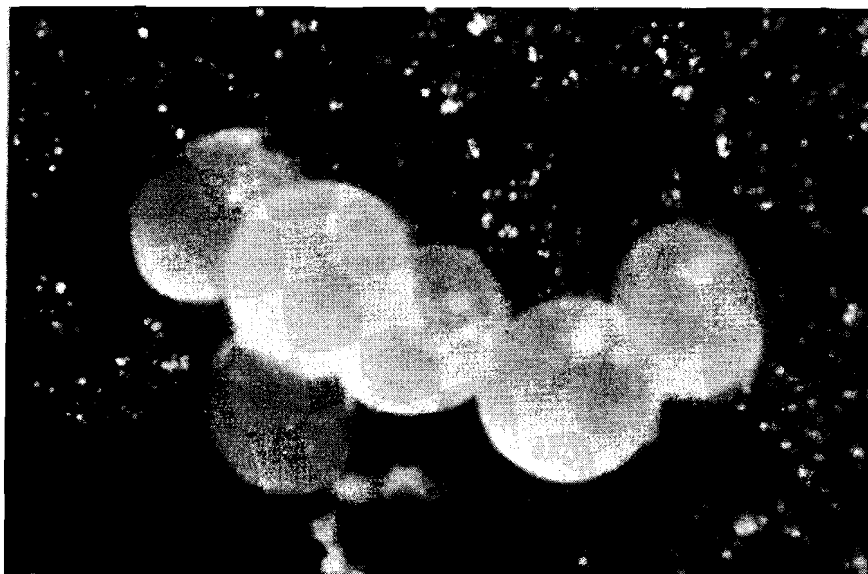


Fig. 5. *Folsomia* eggs photographed on the plaster-charcoal substrate.

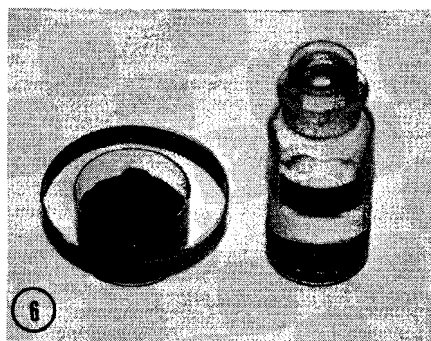


Fig. 6. Petri dish placed over culture jar ready to receive ether. Cap in place after ether has been put on cotton wad.

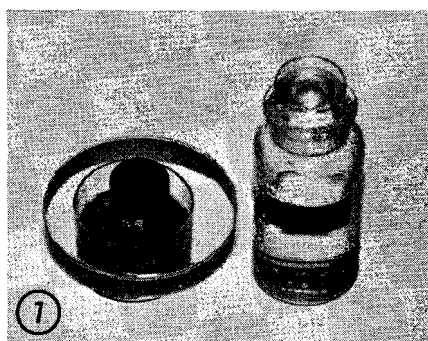


Fig. 7.

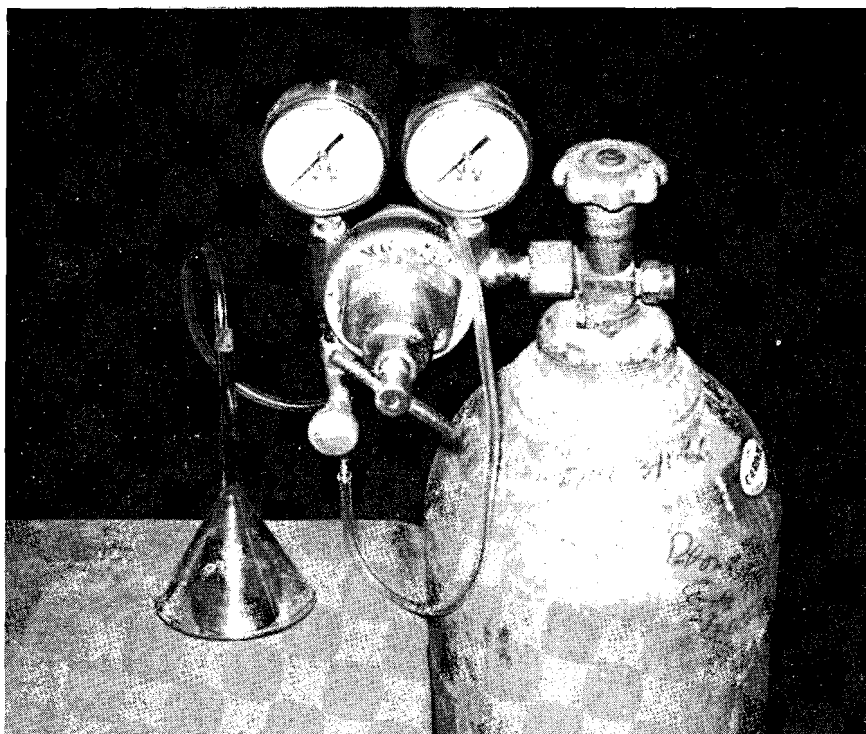


Fig. 8. CO<sub>2</sub> technique with culture jar in place.

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