A New Technique in the Artificial Culture of Nematodes

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Techniques in the artificial culture of nematodes (Heterodera species) and their infestation of plant roots have been reported by Christie of the United States Department of Agriculture, Barrons of the Alabama Experiment Station and other workers. Their work however dealt mainly with host-parasite relationships and susceptibility studies. Because of the nature of their investigations, the techniques usually consisted of inoculating sterile soil with a water suspension of nematode larvae and eggs. In other instances, plants were grown directly in nematode infested soil, or in soil inoculated by the use of chopped root galls containing larvae and eggs. These techniques, no doubt were quite adequate for the types of investigations which were conducted. However if it is desired to observe the movement of the nematode larvae and their development in relation to the plant root or many other series of examinations, an opaque material such as soil is not satisfactory. Therefore, the following technique deals with the preparation and use of a clear medium in these cultural studies.

Materials and Method

A one per cent water agar was prepared and all other organic materials such as potatoes and sugars were excluded. These organic materials usually render a medium less transparent unless special means of clarification are followed. Furthermore, these organic materials were not needed in this study, as the addition of the essential mineral elements for higher plant growth was satisfactory. The medium was then placed in small test tubes (10 x 100 mm.) The tubes were filled ½ full, plugged with cotton and sterilized. Upon cooling and solidifying, the medium was sufficiently clear to permit microscopic low power examination.

Growing Lettuce Seedlings in the Tubes

Lettuce seeds were lightly surface sterilized in a five per cent hydrogen peroxide solution for 15 minutes. This treatment eliminated most of the common surface spores and did not injure the seed embryos. The seeds were then given one washing in sterile distilled water in order to remove excess hydrogen peroxide. Next, the seeds were placed between moist sterile filter papers in petri dishes for germination. After about 72 hours or when the primary root was ¼ to ½ inches long, the tiny seedlings were transferred to the small test tubes for further growth. This transfer was accomplished by the use of sterile forceps. The seedlings were handled gently, placing one seedling in each test tube

and forcing the primary root into the surface of the medium. Further growth was soon evident as determined by the downward extension of the root system and growth of the shoot.

Nematode Inoculation of the Tubes

After about two days of seedling growth, the nematode inoculum was added. Several root-knot galls, the infested part of older lettuce roots, were cut from the plants. The surface of the galls were examined, with the aid of the binoculars, for the presence of a yellowish-brown material, the gelatinous matrix in which the eggs are imbedded. When this gelatinous matrix was found, a very small amount was placed in the depression of a well slide in a few drops of sterile distilled water. A low power microscopic examination revealed numerous nematode larvae and eggs in this suspension. A very small loopful of this suspension was used to inoculate the agar surface of each test tube. Before the inoculation was made, the loopful of suspension was examined under the microscope in order to ascertain the relative population of larvae and eggs in the film of water. The wire loop containing the suspension was held steady for microscopic examination by attaching the handle to a clamp stand and placing the loop at about the stage level of the microscope.

Results

After inoculation, the tubes were examined daily for the presence of larvae and to study their movement. This was accomplished by placing the tube horizontally on the microscope stage, and then focusing on the primary root of the lettuce seedling. Three days after inoculation, nematode larvae were observed near the seedling root tip. While gall formation was not secured, the larvae appeared to feed at the surface of the primary root near the growing point.

Contamination by fungi was of little consequence in this technique. The common molds which could be introduced in the transfer of the inoculum were observed in only a few cases, and these grew only on the surface, thus not obscuring the view of the seedling root.

Summary and Conclusions

The use of this technique in the artificial culture of nematodes should prove satisfactory in preliminary plant susceptibility studies. This technique may also be useful in a study of the movement of different nematode species and races.