Nematologica 12 (1966): 225-236. E. J. Brill, Leiden

ANALYSIS OF HOST-PARASITE RELATIONSHIPS OF ROOT-KNOT NEMATODES BY SINGLE-LARVA INOCULATIONS OF EXCISED TOMATO ROOTS

BY

VICTOR H. DROPKIN AND WILLIAM R. BOONE

Nematology Investigations, Crops Protection Research Branch, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland

This report describes a bacteria-free culture unit to be used in large numbers for detailed analysis of host-parasite relationships of *Meloidogyne* sp. The unit consists of an excised tomato root growing in agar medium in a test tube to which one nematode larva has been added. Almost every nematode which penetrates induces a gall; a high proportion of these galls have eggs externally after 3 weeks of incubation at 28° C. Galls appear from the 1st to the 9th day, eggs from the 18th to the 26th day, and larvae from the 25th to the 32nd day after inoculation. Infectivity of *Meloidogyne incognita acrita* was 53%. Variations in nutrient composition had no marked effects except that high potassium stimulated egg production without affecting incidence of gall formation; high concentrations of iodine increased brown discoloration of galls. Addition of juice from a resistant plant (*Cornus florida*) depressed the infectivity of *M. incognita acrita* for tomato roots. A resistant variety of tomato, Nemared, maintained its resistance to *Meloidogyne* in excised root culture.

Progress in understanding the host-parasite relationships of plant-parasitic nematodes depends on the development of adequate analytical techniques. We set out to produce a standard culture unit in quantity for analysis of the host-parasite relationship of root-knot nematodes.

Several investigators have maintained *Meloidogyne* sp. on root or seedling cultures *in vitro*. Using single-larva and multiple infections, Tyler (1933) found that the rate of growth of roots as well as the number of nematodes within a root profoundly influenced nematode development. Sayre (1958) demonstrated effects of auxin and kinetin on nematode life cycles in root cultures in liquid media. He also collected larvae aseptically from such cultures. Peacock (1959) investigated methods of isolating nematodes free from associated microorganisms, and showed that resistance to *Meloidogyne* persisted through many passages of a clone of roots. Sandstedt & Schuster (1963) developed methods for mass cultures in Petri dishes as part of a study of the effect of *Meloidogyne* on growth substances in infected plant tissues.

The ideal culture unit should be: 1) easily assembled; 2) easily observed; 3) reproducible. The life cycle of *Meloidogyne* lends itself to this approach. Each larva reveals its presence within a root by the gall surrounding it. The percentage of roots which develop galls after inoculation with single larvae measures larval

infectivity. A high proportion of such larvae develop into females which lay eggs external to the root. The frequency of egg masses is therefore a measure of the success of the nematode's life cycle under experimental conditions. Ten-fold magnification suffices for observation of both galls and eggs.

OUTLINE OF PROCEDURE

Stock Cultures

Seeds of *Lycopersicon esculentum* 'Pan America' are washed overnight in tapwater, then shaken for 5 to 10 minutes in 2% NaOCl solution (40% commercial chlorox), rinsed in sterile water, and distributed evenly on Petri plates of 1.5% agar in water. When roots are 1-2 cm long, they are cut at the hypocotyl and transferred single to sterile, modified White's medium in Petri dishes [1]) (Sandstedt & Schuster, 1963). Each plate is sealed with a rubber band. Sterile eggs are added when side roots are plentiful. Once established, roots fill the entire surface of the agar. The second generation of nematodes supplies abundant egg masses. Subcultures are routinely made from uncontaminated cultures incubated at 28° C for 60 days.

To obtain sterile eggs to initiate cultures we use the following procedure modified from Loewenberg, Sullivan & Schuster (1960):

1) Egg masses collected from roots are shaken for 5 minutes in 10 ml of 0.25% NaOCl and centrifuged at low speed to remove clumps of eggs and particles of soil.
2) The supernatant, which contains free eggs, is decanted into sterile water and concentrated by centrifugation. The eggs are transferred aseptically with a minimum of water to excised roots in Petri dishes.

Contaminations occur in this procedure, but uncontaminated galls transferred to fresh plates will give rise to populations of nematodes free of microorganisms.

Single larva inoculations

Excised roots, produced as described for stock cultures, are transferred to agar slants in 18 × 150 mm test tubes with plastic caps. On the same day, we move egg masses from stock cultures to sterile water in small dishes. The following day, newly hatched larvae are transferred to a drop of water in a dry, sterile, plastic Petri dish. We transfer the individual larvae by micropipette in very small drops of water to the root tips. The entire operation is performed under 10 × magnification in a transfer room. Micropipettes are sterilized with 70% ethanol. All cultures are incubated at 28° C.

---

[1]) The medium contains the following, expressed in mg/liter: $MgSO_4 \cdot 7\ H_2O$, 72.0; $Ca(NO_3)_2 \cdot 4H_2O$, 144.0; KNO_3, 80.0; NH_4NO_3, 400; KH_2PO_4, 38.0; KCl, 65.0; $ZnSO_4 \cdot 7H_2O$, 2.7; $MnSO_4 \cdot H_2O$, 4.9; H_3BO_3, 1.6; KI, 0.75; nicotinic acid, 0.5; pyridoxine hydrochloride, 0.5; thiamine hydrochloride, 0.1; glycine, 2.0; sucrose, 20,000; and agar, 1500. Iron was added as Versenol-F, Dow Chemical, Midland, Michigan. 43.0 (= 3 p.p.m.).