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Department of Phytopathology, University of Kiel - 24098 Kiel, Germany

MONOXENIC CULTURE OF *XIPHINEMA INDEX* (NEMATODA: LONGIDORIDAE) ON *FICUS CARICA*

by

J. AUMANN

Summary. A method is described for establishing a monoxenic culture of *Xiphinema index* feeding on the root tips of *Ficus carica* in a sterile nutrient agar medium. It enables monoxenic cultures to be maintained for up to a year and with two nematode generations in a single Petri dish. The method could be used for biological studies on *X. index* or for screening behaviour-modifying nematicidal compounds.

Monoxenic cultures of plant-parasitic nematodes may be useful for a more effective screening of nematicides and for studying the development of biological and biotechnical control methods. Based on a method firstly described by Wyss (1977), a more detailed description of a modified method for the Petri dish culture of *Xiphinema index* Thorne *et* Allen on *Ficus carica* L. is described. It allows cultures of the nematode to be maintained for up to a year and with two generations under monoxenic conditions.

Materials and methods

Sterile agar cultures of *F. carica* were established by a surface-sterilization of seeds (obtained from commercially available, sun-dried fig fruits) for 1 h with 4% (w/v) previously paper-filtered sodium hypochlorite (Merck, Darmstadt, Germany). The seeds were then germinated in Petri dishes containing 0.8% (w/v) water agar at 25±2 °C under long-dary top-light conditions (16 h light). Under these conditions, the first seeds germinated 12-14 days later. After another two to six weeks, well-grown plants with a main root length of ca 5 cm were transferred to 9 cm diam Petri dishes containing autoclaved 0.8% (w/v) agar medium. The plates were sealed with parafilm. The agar medium had been prepared with Hoagland No. 2 nutrient solution (Hoagland and Arnon, 1938) containing Fe-EDTA (Merck) as an iron source. The Hoagland No. 2 solution was prepared as follows. Six stock solutions were prepared with glass-distilled water: solution 1 with KH_2PO_4 (136.09 g/l), solution 2 with KNO_3 (101.11 g/l), solution 3 with $Ca(NO3)_2{\bullet}4H_20$ (236.15 g/l), solution 4 with $MgSO_4{\bullet}7H_2O$ (246.48 g/l), solution 5 with H_3BO_3 (2.86 g/l), $Mn(II)Cl_2{\bullet}4H_2O$ (1.81 g/l), $ZnSO_4{\bullet}7H_2O$ (0.22 g/l), $Cu(II)SO_4{\bullet}5H_2O$ (0.08 g/l) and $H_2MoO_4{\bullet}H_2O$ (0.09 g/l), and solution 6 with Fe-EDTA (6.605 g/l). The nutrient solution was prepared with 0.1 ml of solution 1, 0.5 ml of solution 2, 0.5 ml of solution 3, 0.2 ml of solution 4, 0.1 ml of solution 5 and 0.1 ml of solution 6, to which glass-distilled water was added to give 1 litre.

Two different Petri dish systems were tested: *i*), one-chamber dishes with the nutrient agar medium covering all of the base, and *ii*), two-

chamber dishes with one light-protected chamber filled with agar in which the fig roots grew and a second light-exposed chamber containing the fig shoots. To exclude light the top, base and sides of the root-containing second chamber were covered with black paper. The other culture conditions were as described above for seed germination.

X. index was maintained in pot cultures of *F. carica* at 28±2 °C under long-day conditions (16 h light). Fig plants were grown in plastic pots filled with fine sand and fertilized monthly with Hoagland No. 2 solution to which Fe-EDTA (Merck) was added (as described above). Up to 10^4 nematodes per pot were extracted from the sand by the Baermann funnel technique. Females of *X. index* were surface-sterilized for 20 min with 0.03% (w/v) sodium azide (Merck) and then washed three times for 20 min each time with sterile, glass-distilled water. Using a fine needle, the nematodes were transferred onto the agar surface of a 9 cm diam one-chamber Petri dish at the maximum distance from the growing root tips of a single plant of *F. carica*. Active females then moved towards the root tips, whereas a significant proportion of the sterilized nematodes remained within the transfer area. When moving towards the roots, the surface-sterilized nematodes defaecated internal microorganisms which then continued to multiply in the nematode tracks on the agar surface. One day later, 10-15 females that had reached the root tips were transferred to another one- or two-chamber Petri dish in the immediate vicinity of the growing root tips of a single fig plant. One-chamber dishes were partially protected from light by covering them with 5-10 agar-containing Petri dishes whereas two-chamber dishes were covered with black paper as described above. Periods of less than one day between the first and second nematode transfer to a fig-containing Petri dish reduced the proportion of nematodes that had arrived at the root tips of the first plate and "escaped" the microorganisms in the nematode tracks, whereas a period of two days increased the risk of microorganisms reaching the sterile rhizosphere of the first Petri dish.

Results

In the one-chamber Petri dish system, egg-laying female *X. index* and the development of a second nematode generation could be observed only under reduced light-intensity conditions, i.e. with 5-10 agar plates located on the top of a monoxenic culture plate. The reduced light intensity, on the other hand, caused reduced root growth and in most cases prevented an egg-laying by the second nematode generation. This problem was solved in the two-chamber dish system where fig shoots were exposed to a higher light intensity and roots grew in low light conditions for up to one year. With this system, an egg-laying second nematode generation of females were present in several cases. Both monoxenic systems could not be maintained for more than a year because the agar medium dried below the critical point necessary for nematode activity. In the one-chamber system, water loss was exclusively through the parafilm seal, whereas water loss in the two-chamber system was mainly through evaporation from the agar-containing chamber to the chamber containing the plant shoot.

Discussion

The method described here may be applicable, in a modified form, for the long-time cultivation of other light-susceptible longidorid species under monoxenic conditions. A successful axenization of Longidoridae has firstly been described by Das and Raski (1968) who used either Aretan or dihydrostreptomycin sulfate. Wyss (1977) used sodium azide for the sterilization of *X. index* and established the first monoxenic cultures of this species on *F. carica*.

Compared with these methods, the method described here has the advantage of enabling monoxenic cultivation of *X. index* for up to one year. Long-term experiments on nematode biology or on the effect of behaviour-modifying nematicidal compounds, for instance, can be performed in a single Petri dish. Nematicidal compounds which specifically modify nematode behaviour may be overlooked with conventional screening methods. Initial experiments on the host range of *X. index* showed that, under monoxenic conditions, two nematode generations can develop on birch (*Betula pendula*) and root tip galls are induced on alder (*Alnus*) species where one generation has been observed for several weeks (unpublished results).

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