Protocols for culturing Plant-Parasitic Nematodes

GREEN HOUSE CULTURES OF *MELOIDOGYNE* SPP. ON TOMATO PLANTS IN POTS

This technique can be adjusted for many nematodes on their specific host plants.
Maintenance of the host plants is time-consuming, but large quantities of inoculum can be obtained.
Watch out for contamination with other organisms, especially other nematode species of the same genus.
Also, take care to avoid contamination with certain mites, nematophagous fungi and bacteria (*Pasteuria penetrans*).

Tomato is an excellent host for many *Meloidogyne* spp., but not for all (M. *naasi, M. artiellia*).
Some tropical *Meloidogyne* spp. can also be maintained on woody plants, e.g on fig (*Ficus carica*), which require less maintenance.

Rearing of host plant (tomato):
Seedlings of a (susceptible!) cultivar of the host plant are planted in pots in a soil with a relative large proportion of sand. The latter facilitates extraction of roots and nematodes after culturing and is also a better substrate for the development of the nematode population.

Preparation of inoculum:

(i) egg masses

- pick off egg masses from infected roots and transfer one egg mass close to the roots of one plant ("single-egg mass inoculation") to initiate pure cultures
- several egg masses, even still present on roots pieces, can be added in soil close to roots of host plant

(ii) second-stage juveniles (J2)

- put pieces of infected roots with egg masses on top of Baermann funnels (under mistifier) and leave eggs to hatch or put single egg masses on mini sieves (25 µm) placed in water in a Petri dish (mini Baermann pie pan) and leave to hatch
- collect freshly hatched juveniles and infest soil in pots with host plants as soon as possible

(iii) extraction of eggs of *Meloidogyne* spp. from infected roots using sodium hypochlorite (bleach method, adapted from Hussey and Barker, 1973)

- collect roots infected with *Meloidogyne* spp. and exhibiting protruding egg masses, clean roots gently by washing over a coarse (600 µm) sieve
- cut roots in 1-2 cm long fragments and place in flask, fill flask at most half way
- prepare a sodium hypochlorite solution of 1,05 % NaOCL (if using commercial bleach with 8% NaOCl, mix 132 ml bleach + 868 ml water)
- add sufficient NaOCl solution to cover the roots and agitate for 4 minutes (use rubber or cork stopper to adequately close flask)
- pour root and NaOCL mixture over nested sieves of 250 + 75 + 26 µm and rinse with water for several minutes until smell of chlorine is gone
- rinse contents of the 26 µm sieve (containing eggs) into a beaker
- rinse roots with water to remove additional eggs and collect eggs by sieving

It is recommended to test (determine the species) some individuals of the hatched juveniles, the remainder can be inoculated on the tomato plant. Also check juveniles and egg masses regularly for infection with parasitic fungi or spores of *Pasteuria penetrans*, using a compound microscope (about 200 x).

Inoculation of the host plant:

- bring the nematode suspension to a known volume with water (not too much)
- count the number of nematodes in several subsamples so that the mean number of nematodes per ml solution can be calculated
- calculate the amount of suspension that will deliver the number of nematodes you want to add per plant (e.g. 1 nematode per g soil)
- make 3 holes around the stem of the plant, and deliver the amount of inoculum as calculated, homogenising the suspension before each infestation
- close the holes and water the plant slightly

Maintenance of the host plant:

- water plants when needed: do not leave soil to dry out, avoid very wet soil
- add a fertilizer for tomato every 1-2 weeks, or mix slow-release fertilizer (Osmocote) in the soil substrate
- if tomato: remove flowers, cut upper part regularly, keep plants about 40 cm high, bind to sticks
- control insects, mites and fungal diseases

If you have any question about this protocol, please, send e-mail to eurl.nematodes@anses.fr.

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