Culturing of potato cyst nematodes (*Globodera*) on potato in closed containers

(Based on Foot, M.A. 1977. Laboratory rearing of potato cyst nematode: A method suitable for pathotyping and biological studies. New Zealand Journal of Zoology 4:183-186, and own experiments at ILVO)

1. Disinfect a potato tuber (a known susceptible variety) in a solution containing 5 % NaOCl solution (e.g. household bleach) for 4 minutes.
2. Rinse well with water.
3. Dry tubers, spread open at room temperature with the eyes exposed to daylight, so they can develop shoots (can take some days to some weeks)
4. Prepare nematode inoculum by soaking cysts in root exudate (potato or tomato) for one to several days.
5. Put 200 g dried clean sand + 30 ml tap water in closed container (0,5 l plastic pot, 10 cm diameter, with lid). Place the germinating potato tuber in the container with developing roots and shoots (buds) in the sand (do not cover the whole tuber).
6. Crush the cysts and collect eggs and juveniles released from the cysts in a beaker. Add about 1000 eggs and juveniles of *Globodera* spp. to the sand (at time of planting) in no more than 5 ml of water. One can also inoculate with cysts if the exact inoculum does not matter.
7. You could leave roots to grow, but not for too long before inoculation: maximum until they reach the outside of canister (Foot, 1977). Juveniles prefer growing roots to enter at the tip. Inoculating germinating tubers immediately after placing them in the sand yielded more cysts than inoculating them when roots were 1 cm or more (own tests).
8. Closed the container tightly (good fitting lid).
9. Store the closed containers in the dark at 20 °C for about 14 weeks or until new cysts have formed and turned dark brown.
10. Collect the cysts after drying roots and sand. The majority of the cysts can be picked from the roots. Cysts can also be harvested by an appropriate method (see EPPO PM 7/19).

Notes:
Use river sand (fine sand sold at construction stores). Avoid sea sand as it can contain salts, although it could be rinsed to remove the salt. The sand can be autoclaved (see Foot, 1977) or dried in an oven (100 °C, 16 h), but simply drying in open air works also, provided the sand is not contaminated (check origin). Tap water could be sterilized if contamination is expected.

Foot (1977) used 1% chlorine and soaked tubers for 30 min. Here 5% NaOCl is used and tubers are soaked for 2 - 4 min. Use seed potato tubers as these are healthier than regular ware potato tubers. When not enough tubers are available or tubers are too big, one can also use a tuber piece containing one sprout instead of a whole tuber. The piece with the eye is removed carefully from the tuber with a cork borer and left to dry before planting in the sand.

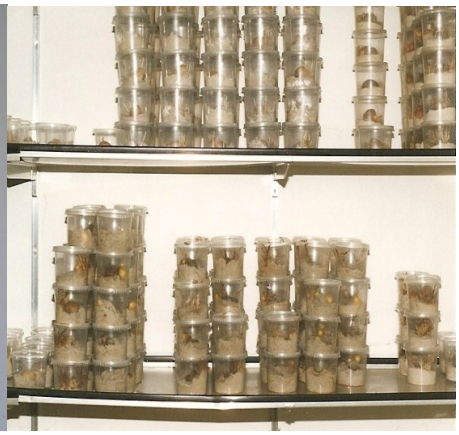
Closed container with cysts visible on potato roots.

Culturing of root-knot nematodes (*Meloidogyne* sp.) on potato in closed containers

1. Disinfect a potato tuber (a known susceptible variety) in a solution containing 5 % NaOCl solution (e.g. household bleach) for 4 minutes.
2. Rinse well with water to remove the chlorine.
3. Dry tubers, spread open at room temperature with the eyes exposed to daylight, so they can develop shoots (can take some days to some weeks)
4. Put 200 g dried clean sand + 30 ml tap water in closed container (0,5 l plastic pot, 10 cm diameter, with lid). Place the germinating potato tuber in the container with developing roots and shoots (buds) in the sand (do not cover the whole tuber).
5. Inoculation can be performed using egg masses, eggs or second-stage juveniles at time of planting or during the first two weeks (about 1000 eggs/J2 per container). Add no more than 5 ml of water to avoid rotting of the tuber.
6. Closed the container tightly (good fitting lid).
7. Store the closed containers in the dark at about 20 °C. After 8 to 14 weeks, newly formed egg masses are visible on the roots stuck to the wall of the container.
8. Take out the tuber with its roots and wash carefully in beaker with water to remove the sand, but not the egg masses. Roots can be further processed to obtain juveniles or eggs (see EPPO PM 7/19).

Notes:
Use river sand (fine sand sold at construction stores). Avoid sea sand as it can contain salts, although it could be rinsed to remove the salt. The sand can be autoclaved (see Foot, 1977) or dried in an oven (100 °C, 16 h), but simply drying in open air works also, provided the sand is not contaminated (check origin). Tap water could be sterilized if contamination is expected.

Foot (1977) used 1% chlorine and soaked tubers for 30 min. Here 5% NaOCl is used and tubers are soaked for 2 - 4 min. Use seed potato tubers as these are healthier than regular ware potato tubers. When not enough tubers are available or tubers are too big, one can also use a tuber piece containing one sprout instead of a whole tuber. The piece with the eye is removed carefully from the tuber with a cork borer and left to dry before planting in the sand.



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

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