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Use of a mixture of sand and water-absorbent synthetic polymer as substrate for the xenic culturing of plant-parasitic nematodes in the laboratory

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Xenic culturing of plant-parasitic nematodes on host plants was successful in sterilised natural soils in the laboratory, particularly in native soil, in which large populations of selected nematode species were observed *in situ* (Mountain, 1960; Wallace, 1963). However, there are cases when native soil cannot be used. For example, this would not be possible in our laboratory in France for the culturing of tropical nematode species, because importation of large quantities of tropical soils is prohibited for regulatory reasons and because shipping would be too expensive anyway. Moreover, tropical and temperate soils have quite different physical and chemical properties (Duchaufour, 1995) and it would not be possible to find in France soils similar to the native tropical soils. Moreover, most of the soils that are available in gardening stores are too rich in clay and organic matter to be easily handled in the laboratory.

As an alternative to native tropical soil, we tried to use pure silica sand. This substrate looked very promising because it could be obtained from quarries for the glass industry and it has several convenient features: unlimited and inexpensive supply, fine granulometry from 100 to 300 μm, dryness and cleanliness, which made sterilisation unnecessary. The addition of a mineral nutritive solution, such as Hoagland's solution, provided the mineral requirements for host plant growth. Nevertheless, during a culturing period of several weeks or months, leaves became chlorotic and the growth of the host plant was poor, compared with the growth observed on sterilised native soil. This was mainly due to the fact that watering caused the sand progressively to reach maximal compactness (Sloane, 1984). This probably reduced aeration and prohibited penetration of the hardened substrate by the roots of many host plants. Besides, water retention of pure sand is low and, when the plant has been growing for some time, water vapour loss becomes high and the plants need watering up to several times daily. Thus, pure sand was found convenient for short term (10-15 days) experiments only, e.g., determination of the invasion rate of infective J2 of *Heterodera* spp. (Reversat & Merny, 1973).

In this paper we report the use of a mixture of pure silica sand and water-absorbent synthetic polymer as substrate for the successful culturing of some tropical nematodes in our laboratory. The addition of this polymer to the silica sand balanced the drawbacks of pure sand mentioned above: progressively acquired compactness and low water content. Such water absorbents have been developed by the chemical industry since the seventies for various purposes, particularly as soil conditioners in agriculture for dry areas (De Boodt, 1990; Rognon, 1995). Most of them are based on an acrylic polymer of high molecular weight, able to gelify by rapidly absorbing up to 400 times its own weight of deionized water.

In commercial packages (*e.g.*, Graind'eau® in France, Agrosoke® in UK, Hydro Kristall® in Germany, etc.), these absorbents are conditioned as granules 1 to 2 mm in size. After absorption of deionized water, they form pieces of gel up to 10 mm in size, which could not be mixed homogeneously with sand. Consequently, the pieces of gel were first converted into a paste by sieving under pressure. For this purpose, we used a 100 ml plastic syringe with the bottom end cut out and replaced by a screen made of stainless steel wire gauze, with an aperture of 0.25 mm, solidly melted into the plastic wall of the syringe. The syringe was filled with pieces of gel and the pressure of the plunger forced the gel to pass through the screen apertures. This resulted in filaments of 0.25 mm by up to 10 mm. Since these filaments were not found to be convenient, they were put back in the same syringe and processed in the same manner to form a pasty gel. This pasty gel (consisting of 200 g of water and 2 g of

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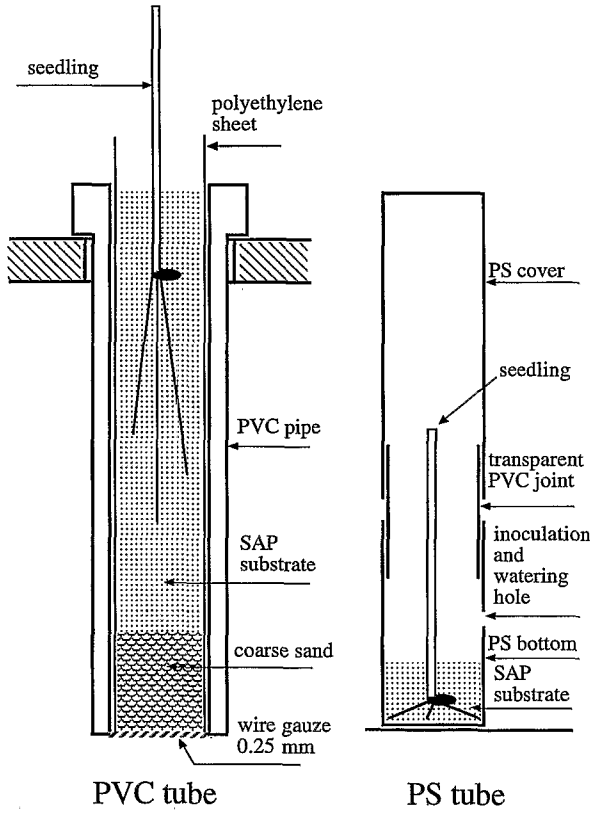

Fig. 1. *PVC tube and PS tube: two devices for the xenic culturing of plant-parasitic nematodes on host plants growing on SAP substrate (Sand + Absorbent Polymer) and Hoagland's mineral nutritive solution (PVC tube: inner diameter 26 mm, length 205 mm; PS tube, polystyrene transparent tubes: diameter 30 mm, bottom length 70 mm, cover length 100 mm).*

polymer Grain d'eau®) was then intimately mixed with sand (2200 g). The dry and clean silica sand used, from the Fontainebleau quarries in France, had the following granulometric characteristics: over 0.3 mm: 5%; from 0.3 to 0.2 mm: 35%; from 0.2 to 0.1 mm: 42%; from 0.1 to 0.05 mm: 15%; under 0.05 mm: 5%. The homogeneous mixture was allowed to dry at room temperature. After a few days, 2202 g of SAP (Sand + Absorbent Polymer) substrate were ready to be used immediately or to be stored for months.

The SAP substrate was used in two devices (Fig. 1) for the xenic culturing of plant-parasitic nematodes under 12/24 h fluorescent lighting, with about 120 $mmol \cdot m^{-2} \cdot s^{-1}$ of PAR (Photosynthetically Active Radiations) (Pontailler, 1990) at the substrate level, in a room at 25-30°C.

The first device (Fig. 1, PVC tube) was made of a piece of PVC pipe (Reversat & Destombes, 1998) with an effective volume of 100 ml. This tube was lined with a sheet of transparent polyethylene and 20 ml of coarse sand (granulometry: 74% between 0.5 and 1 mm and 26% between 1 and 2 mm) was added at the bottom to improve drainage. Then the tube was filled, in several stages, with dry SAP substrate and an excess of deionized water, which allowed the optimal swelling of the polymer. Immediately after the tubes were made, or the next day, one seedling of the host plant was replanted into the wet SAP substrate.

The tubes were watered with deionized water three times weekly and 10 ml of 1/1 Hoagland's solution was added twice weekly just after watering. Excess water was drained off. It must be noted that the Hoagland's solution formula with iron as Fe-EDTA was used successfully with most of the tested host plants, e.g., *Vigna radiata*, sorghum, and pearl millet, but not with rice, as it causes rice leaves to become chlorotic. With rice, it is necessary to use another form of iron, Fe-EDDHA, to obtain healthy plants. Moreover, since the reaction of the polymer in contact with a mineral solution was not the same as with pure water (Djabourov & Guenet, 1995), particles of hydrated polymer shrank somewhat, which caused the level of the wet SAP substrate to fall slightly in the tube (from 5 to 10%). This drop was offset by the addition of dry SAP substrate. Another effect of the shrinkage was the development of air pores in the wet SAP substrate, which were visible to the naked eye by pulling out the cylinder of SAP substrate within its transparent sheet of polyethylene. This porosity of the SAP substrate appeared to be very appropriate for the growth of roots and the movement of nematodes, by increasing the aeration of the wet SAP substrate. Due to the suction effect, the content of free water in the wet SAP substrate, outside the particles of gel, increased from the top to the bottom of the tube and the introduction of coarse sand at the bottom of the tube during the filling stage made drainage easier. Some experiments showed that bigger tubes can be used (6 cm in diameter, 25 cm in length, 600 ml in volume), providing the bore of the drainage part at the bottom of the tube, at the level of the melted screen, was the same as the bore of the tube itself.

When plants were old enough, each tube was inoculated with nematodes, as in other methods for the culture of plant-parasitic nematodes. After the required time of development, nematodes were recovered from the soil by the two-flask technique of Seinhorst (1955) and from the roots in a mistifier (Seinhorst, 1950).

This device and the SAP substrate allowed the successful culture of several tropical species of plant-parasitic nematodes: *Heterodera sacchari*, *Meloidogyne graminicola*, and *Pratylenchus zeae* on rice, *Hoplolaimus pararobustus*, *Pratylenchus brachyurus*, and *Meloidogyne javanica* on *Vigna radiata*, *Hemicycliophora oostenbrinki* on *Sorghum vulgare*, *Scutellonema cavenessi*, and *Helicotylenchus dihystera* on pearl millet. At the present time, however, the reproduction of *Xiphinema parasetariae* on *V. radiata*, which is considered to be a good host for this species, is low compared with the rate obtained in native soil. With this large nematode species, the SAP substrate probably needs a different type of sand with a more appropriate granulometry.

The second device consisted of transparent polystyrene containers with only 10 g of SAP substrate (Fig. 1, PS tube). It was found convenient for rice seedlings only. Containers were made of two transparent polystyrene tubes (diameter: 30 mm; lengths: 70 mm for the bottom tube and 100 mm for the cover tube), joined together with a piece of rolled transparent PVC sheet. A hole drilled in the wall of the bottom tube allowed the inoculation of nematodes with pipettes and watering. One 5-day-old rice seedling was planted in the bottom PS tube with 10 g dry SAP substrate and 2.5 ml deionized water and the PS cover tube was adjusted. One week later, 100 J2 of *H. sacchari* in 0.1 ml of water were inoculated through the lateral hole of the bottom tube. Tubes were then moderately watered three times a week, just to keep the SAP substrate wet but not flooded. Each week, the watering was done twice with deionized water and once with 1/1 Hoagland's solution. Five and a half weeks later, rice plants had four or five green leaves rolled at the top of the cover tube. Their roots had numerous light brown cysts.

Since 1992, no development of micro-organisms, such as fungi or bacteria, able to interfere with the development of the host plant or with the reproduction of the nematodes was ever observed in wet SAP substrate. This immunity of acrylic polymers to current consumers of organic matter in soils is known and used as a selling point by the manufacturers of these products.

However, this SAP substrate has one drawback: a fine sleeve of sand remains attached to many roots, despite the washing of the root system in water after the final recovery. Particles of sand stuck to particles of gel, which were themselves penetrated by root hair and thus fastened onto roots. This allowed root observation, for hand-picking of cysts for example, or treatment of roots in the mistifier, but prevented a precise weighing of roots after an experiment and added an extra parameter for the determination of the root system biomass. For instance the organic matter content of roots could be measured easily with the chemical oxygen demand (Reversat, 1981) without interference of sand.

To conclude, SAP substrate proved to be very efficient for the culturing of several tropical nematode species in the laboratory. It could probably also be used for the culturing of some temperate nematode species. The method can be adapted by increasing the proportion of polymer in sand from the present value of less than 0.1% to higher values. The material used was inexpensive, needed no sterilisation, and the final product of the nematode recovery was free of interfering impurities. Brown cysts of *H. sacchari* were clearly visible against this white and transparent background and very easy to count or collect. Moreover the SAP substrate was chemically pure since the sand and the polymer contained only trace amounts of minerals able to solubilize in water. Thus, changing the formula of the added mineral nutritive solution would make it possible to study the effects of deficiencies or excesses of selected minerals on the host-nematode relationships.

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