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A NEW METHOD FOR CULTURING PLANTS ENABLING THE OBSERVATION OF NEMATODES ON GROWING ROOTS[1])

Met een samenvatting: Een nieuwe methode voor het kweken van planten, waardoor waarneming van aaltjes op groeiende wortels mogelijk is

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INTRODUCTION

Growing plants in soil is a very unsatisfactory method for studying the behaviour of root infesting nematodes, and also of other root parasitizing organisms, as it is impossible to observe the effect of the disease agents without lifting the plants and freeing the roots from soil. This action virtually ends the experiment with the plant investigated, and the next observation has to be made on another plant.

LINFORD (1941) and others have used root observation boxes. In these, roots growing against the glass sides can be observed, but these comprise only a small proportion of the roots produced by a plant. Also the conditions for microscopic observation are rather unfavorable.

For observations during short periods GADD & LOOS (1940) and DROPKIN (1957) embedded single roots in thin layers of white sand. Sand dries out very quickly however so that this method is not suitable for observations over prolonged periods.

Plants can be grown on suitable agar substrates and MOUNTAIN (1955) even succeeded in culturing *Pratylenchus minyus* on excised corn, tobacco and red clover roots on a tissue culture medium.

Unfortunately, test tubes which are generally used for culturing plants on agar, do not present good opportunities to observe the roots and the nematodes. MOUNTAIN cultured his excised roots in petri dishes. When growing complete plants in petri dishes, the agar dries out very quickly unless it is covered in some way e.g. with a thin sheet of polythene. The method described below has been found to be far more suitable for microscopic observation than petri dishes. In this method reasonable growth of the plants, high activity of the eelworms in the culture medium and the possibility of observing the whole rootsystem at any desired moment, can be combined.

MATERIALS AND METHODS

Preparation of the culture medium

Experiments showed that agar or a similar medium would be the only material that could be prepared and handled in layers thin enough to ensure visibility of both roots and eelworms and thick enough to supply a sufficient

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amount of water and nutrients to the plant roots. However, eelworms soon become inactive in agar layers enclosed between glass plates or thin sheets of plastic.

Even when thin layers of agar were enclosed between sheets of polythene the nematodes soon ceased to move. Apparently even the amount of air diffusing through polythene did not provide sufficient aeration. However, it was noticed that the nematodes remained active in the immediate vicinity of air bubbles enclosed in the agar and it was concluded that the inactivity of the eelworms might be overcome if a sufficient amount of small air bubbles could be enclosed in the agar. This was accomplished by stirring a 4 % Hoagland agar, cooled after melting to about 35 °C, in a Waring Blendor. In order to make the knives beat more air into the agar, pieces of rubber tube were slipped over them. Probably mixers for making milk shakes would be even more suitable for the purpose.

Making thin layers in polythene bags

It was found that thin layers of this foamed agar could be enclosed in flattened polythene bags and that these provided much better conditions for observation of roots than test tubes or petri dishes. The method of preparing and using the bags is as follows.

A suitable length of polythene tube, made from polythene sheet of thickness 0.035–0.05 mm, is cut off and closed at one end by heating the edges. This makes a polythene bag into which is poured a measured quantity of well stirred agar, after which the bag is immediately flattened in a water-bath at about 35 °C between two glass plates. Small supports between the plates serve to keep them the right distance apart. The glass plates and the bag are then cooled in a second water-bath. It is necessary to work as fast as possible as otherwise the air bubbles tend to become distributed irregularly or even disappear from the agar. The open end of the bag is then closed by heating, if necessary after removing some of the agar. The agar layer should not be thinner than 0.5 mm, and its thickness should be chosen in accordance with the diameter of the roots expected to grow in it. A better distribution of the air bubbles is obtained by using a 100 ml veterinary syringe for filling the polythene bags. A bag which is closed on all sides is placed between two glass plates which are kept at the right distance by supports. The syringe is filled with foamed agar by sucking it from the mixer. Its orifice, lengthened with a sharp perspex extension piece with a 3 mm wide lumen, is then introduced into the bag through a hole in one of the corners, and the agar is pressed rapidly into the bag. The hole in the bag should give a tight fit of the polythene around the orifice of the syringe. A few tiny holes should be made in the opposite end of the bag to let air escape during the filling.

Young seedlings are planted through holes made in the polythene in the top of the bag. These holes should be kept small to prevent drying out of the agar surrounding the seedlings. Nematodes and other organisms can be introduced through cuts at suitable places. The latter holes can be closed afterwards with scotch tape. It is necessary to see that the roots of the seedlings come in close contact with the agar.

It is possible of course to work under completely sterile conditions, but for most purposes it is sufficient and much easier to mix some streptomycin sul-

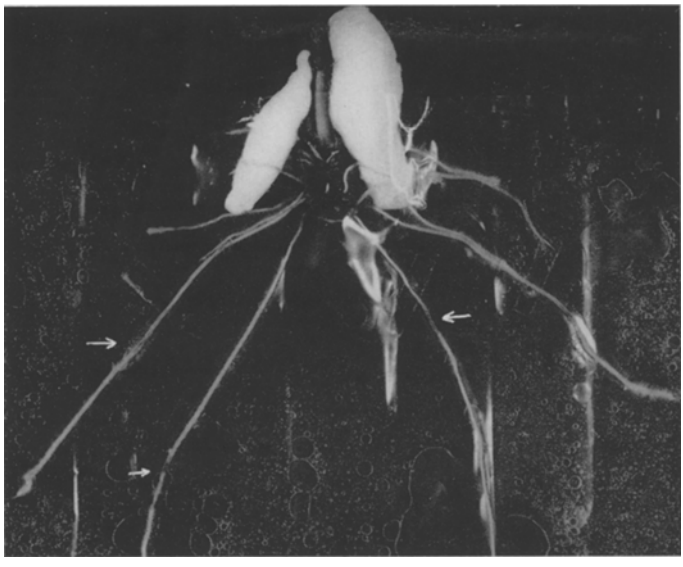

FIG. 1. Rooted potato sprout grown in agar with lesions caused by *Heterodera rostochiensis* (near point of arrows).
Bewortelde aardappelspruiten, gekweekt in agar, met lesies veroorzaakt door Heterodera rostochiensis.

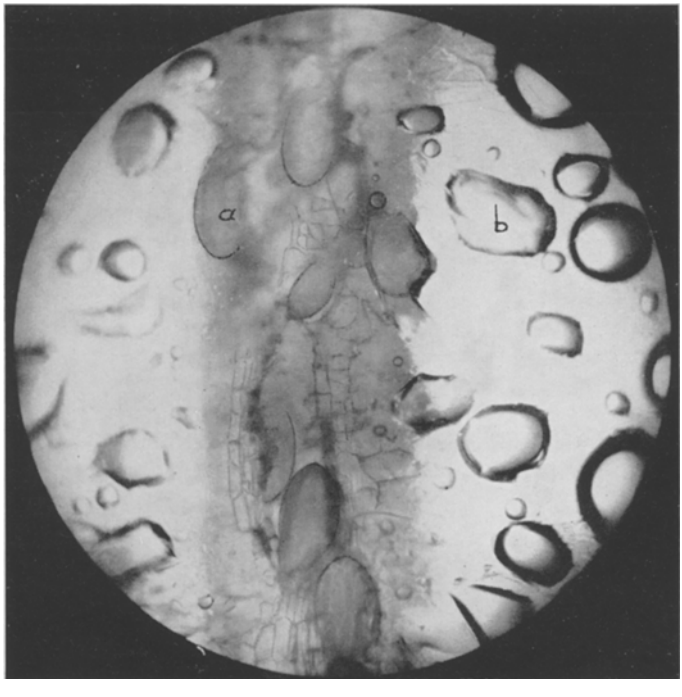

FIG. 2. Young females of *Heterodera rostochiensis* on a potato root growing in agar. a. young female. b. air bubbles in the agar.
Jonge wijfjes van Heterodera rostochiensis *op een aardappelwortel gekweekt in agar. a. jong wijfje. b. luchtbellen in de agar.*

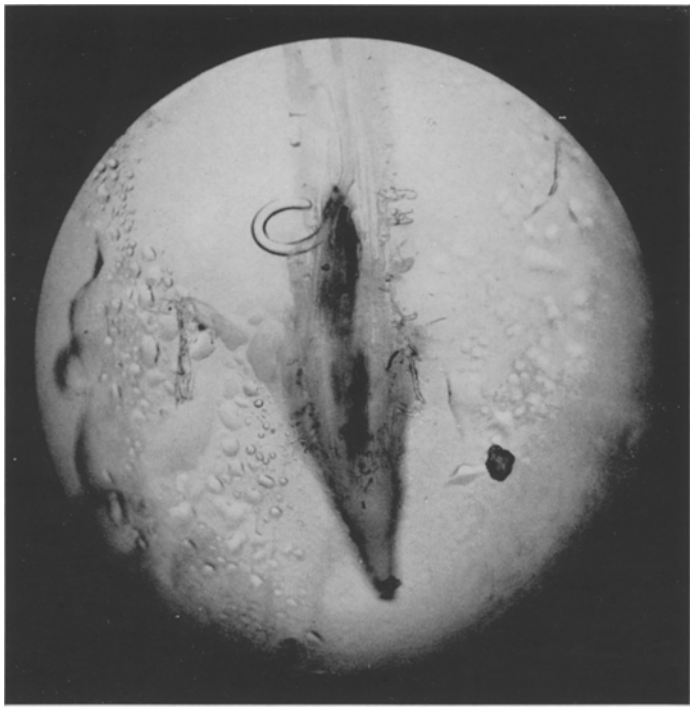

FIG. 3. Roottip of carrot with lesions caused by *Hoplolaimus uniformis*.
Worteltop van peen met lesies veroorzaakt door Hoplolaimus uniformis.

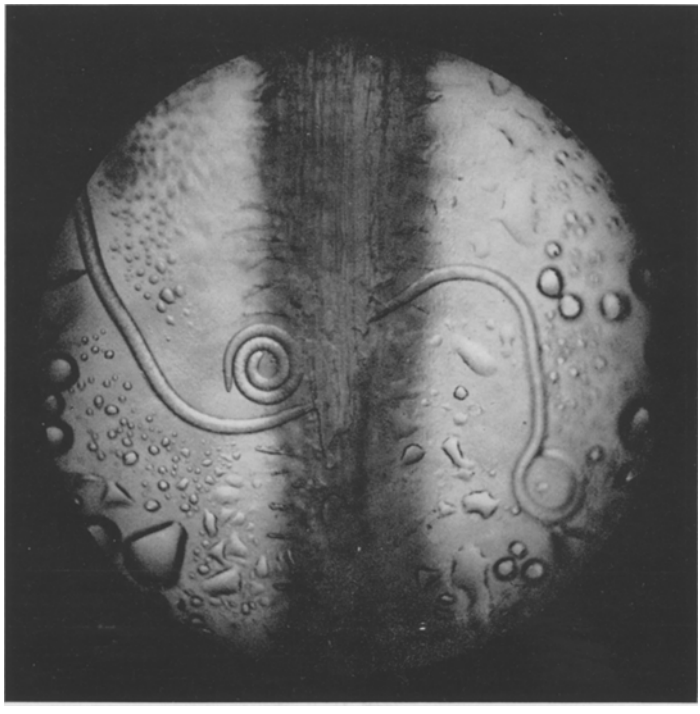

FIG. 4. *Hoplolaimus uniformis* on a root of carrot in agar.
Hoplolaimus uniformis *op een wortel van peen gekweekt in agar.*

phate into the agar to a concentration of about 0.01 %. If the bags, the agar and the seedlings are kept reasonably clean, the streptomycin sulphate will check growth of bacteria almost completely.

After planting seedlings in the bags the latter are placed between a folded sheet of stiff paper, so that the developing stems and leaves stick out at the top, but the roots are growing in darkness. This also keeps algae from developing in the agar.

The growing seedlings are sprayed frequently with a fine mist of water containing a trace of ferric chloride or sulphate, and now and then with a nutrient solution to keep the plants from drawing too heavily on the relatively small supply of nutrients and water in the polythene bag.

Nematodes should be transferred to the agar with as little water as possible as they have considerably difficulty in leaving drops or films of water on agar. Selected specimens are therefore brought into a drop of water in a watchglass covered with a thin layer of paraffin wax (SEINHORST, 1945). The wax prevents the spreading of the water. Excess water can then be removed with a micropipette, after which the nematodes are picked up with a splinter of bamboo or sharpened bristle mounted on a needle.

INOCULATION EXPERIMENTS

Potatoes. Small plants were grown from sprouts. When these had developed roots about 5 cm. long, *Heterodera rostochiensis* larvae were placed in the agar at a distance of 5–10 mm from the roots. The larvae moved quickly away from the inoculation spot mostly in the direction of the root tips. Within a day after inoculation many larvae were seen entering the roots. Two days later lesions appeared on the roots between the root tip and the place of inoculation. This was most probably due to the large number of larvae entering a small portion of the root. With smaller numbers damage to the tissues was not so apparent in the first stage of attack (see fig. 1). Four weeks after inoculation females were seen bursting out of the root tissue (fig. 2) and many males were moving around, both along the roots and in the agar.

Carrots. Carrot seedlings having roots about 5 cm in length were inoculated with *Hoplolaimus uniformis.* Within a day after inoculation these nematodes were seen puncturing cortex cells with the stylet. Some animals were seen with the anterior part of the body temporarily embedded in the tissue. Within a few days after inoculation lesions appeared on the roots (fig. 3, 4).

Wheat. Most specimens of *Pratylenchus pratensis* brought into the neighbourhood of wheat roots had disappeared from the agar within a few days. They could not be seen in the roots by direct microscopical investigation, but after staining with lactophenol cotton blue their presence in the roots could be demonstrated. No lesions or other changes in the root tissue were observed in this case.

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SUMMARY

A method for growing plants in thin layers of agar is described. The agar contains a large amount of air bubbles and is enclosed between two sheets of polythene.

The method can be used for the observation of nematodes and other root parasites requiring a well aerated medium while attacking growing roots.

SAMENVATTING

Bij het bestuderen van het gedrag van aaltjes en andere wortelparasieten op levende plantewortels worden ernstige moeilijkheden ondervonden, doordat in grond gekweekte planten onvoldoende mogelijkheden bieden voor waarnemingen onder de microscoop. Een kunstmatige voedingsbodem als agar garandeert in veel gevallen geen voldoende luchttoevoer voor plantewortels en parasieten en droogt in een dunne laag aangebracht snel uit. Teneinde deze moeilijkheden te omzeilen werd een methode ontworpen, waarbij een tot schuim geslagen 4 % Hoagland-agar bij ongeveer 35 °C in een zak van polytheen wordt gebracht, waarna deze snel tussen twee glasplaten tot een gewenste dikte kan worden platgeperst. De plastic zak wordt door verhitting van de bovenrand dichtgesmolten, waarna via kleine gaatjes kiemplanten en inoculum kunnen worden ingebracht. De gaatjes worden desgewenst gesloten met een strookje doorzichtig plakband.

De schuimagar wordt vervaardigd in een Waring Blendor waarvan de met stukjes rubberslang omwikkelde messen een extra toevoer van lucht in de agar waarborgen. Toevoeging van een hoeveelheid streptomycinesulfaat-oplossing tot 100 d.p.m. verhindert de ontwikkeling van bacteriën. De aaltjes blijven in dit goed geaëreerde medium voldoende actief.

Het aardappelcystenaaltje *Heterodera rostochiensis* kon bijv. in zijn volledige ontwikkelingscyclus worden geobserveerd. Ook de aantasting van peen door *Hoplolaimus uniformis* kon goed worden waargenomen.

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