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A NEW APPROACH TO THE PHYSIOLOGICAL HOST PLANT-PARASITE RELATIONSHIP – A TECHNIQUE IN THE FIELD OF APPLIED ENTOMOLOGY[1]

Een nieuwe benadering van het fysiologisch aspect van de verhouding tussen waardplant en dierlijke parasiet

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In connection with investigations on the influence of the physiological condition of the host plant on the population development of phytophagous insects and mites an apparatus for balance studies is described. Beans and radishes with their respective parasites, the two-spotted spider mite (*Tetranychus urticae*) and the peach aphid (*Myzus persicae*), were grown on an aerated small-scale water-culture. To the nutrient solution ^{32}P was added. Mites and aphids were measurably radioactive after the experiment. Phosphorus uptake of the bean plants was much greater per gram of fresh weight than that of the radish plants. The radioactivity of animal material accounted for 2–3% of the total radioactivity recovered from nutrient solution, plants and parasites.

INTRODUCTION

Less than ten years ago research on the feeding physiology of mites and aphids was hardly developed. Since then there have been a number of investigations about the influence of changes in the chemical composition of the leaves on the population development of insects and mites. It has nevertheless not yet been possible to acquire a useful understanding of this particular problem. This is partly due to the great diversity of the methods applied and partly to the fact that the special food requirements of the majority of parasites are as yet unknown and those known vary greatly. A comparison of the results is therefore extremely difficult and hampers an evalution of the data obtained.

Within the framework of the Dutch Study Group on Integrated Control of Pests, the authors have studied the influence of the host plant's physiological condition[2] on the population development of phytophagous insects and mites.

By changing the ion-ratio of the nutrient solution on which the plants are grown, chemical changes in the leaves are induced and differences in population development of the parasites can be evaluated. The experiments were set up as balance studies, that is to say, the whole of nutrient solution together with the plants and parasites was arranged as a closed system for certain nutrient elements. The distribution of a certain element over the three components, viz. nutrient solution, plant and phytophagous organism, is influenced by the composition of the solution, the ion uptake capacity of the plant and the food uptake and population density of the parasite.

As a closed system was being considered, the initial quantity of the test-element had to equal the sum of the quantities recovered from the three com-

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[2] This expression refers to the chemical composition of the plant tissue, which is influenced by the mineral nutrition of the plant.

ponents. Schematically this is expressed in Fig. 1, in which x, y and z represent the proportions of an element recovered from solution, plant and parasite respectively. The points A and B are hypothetical results of balance experiments with two different solutions and the same kind of host plant and parasite.

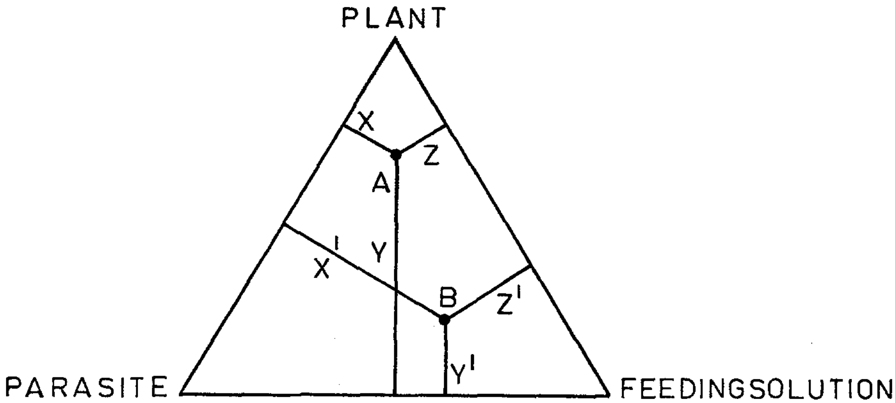

FIG. 1. Scheme of a hypothetical proportional distribution of a certain element over feeding solution, plant and parasite.

Schema van een hypothetische procentuele verdeling van een bepaald element over voedingsoplossing, plant en parasiet.

At the end of the experiment the quantity of a certain element in the nutrient solution was determined in the residual solution, plants and parasites. This could be done by a quantitative chemical analysis of the three components or by using the radioisotopes of the elements in question. For these balance studies the elements P, S, N, K, Ca and Mg are to be considered. N and Mg only do not have a suitable isotope and will have to be analysed chemically.

The quantities of the test-element recovered were correlated to the increase of plant and animal material during the test period. The element examined in the above mentioned manner was phosphorus. It is a very important element for both plant and parasite and it has a suitable isotope in the form of ^{32}P.

A better understanding of the chemical reactions which control, for example, the formation of energy-rich phosphates in plants and the part these phosphates play in the development of parasite populations is of great importance in studying plant-parasite relationships.

MATERIAL AND METHODS

As test plants radish, *Raphanus sativus*, and French bean, *Phaseolus vulgaris*, were chosen, with their parasites the peach aphid, *Myzus persicae*, and the two-spotted spider mite, *Tetranychus urticae*, respectively.

Available laboratory space and the permissible amount of radioactivity limited the extent of the apparatus, so a small water culture was developed allowing five plants to grow for some weeks on one litre of nutrient solution only, without changing it. The cultivation vessel was a horizontally placed perspex cylinder with a length of 80 cm and a diameter of 7 cm. In the upper part of the cylinder five holes were bored with a diameter of 2 cm in which tubes were fitted to support the plants (Fig. 2).

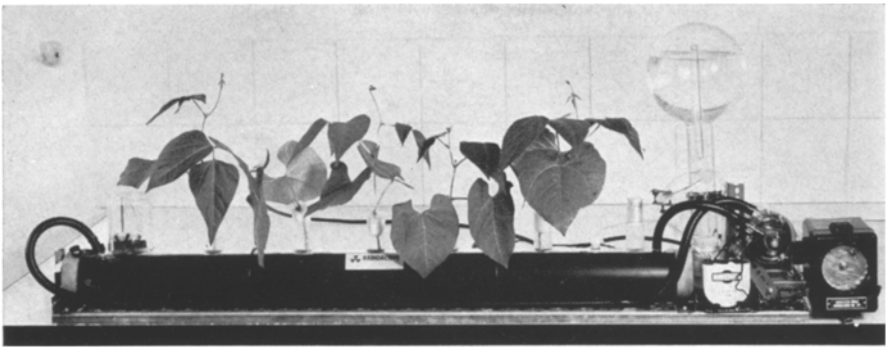

FIG. 2. Experimental set up for a small-scale waterculture.
Proefopstelling voor een waterculture op kleine schaal.

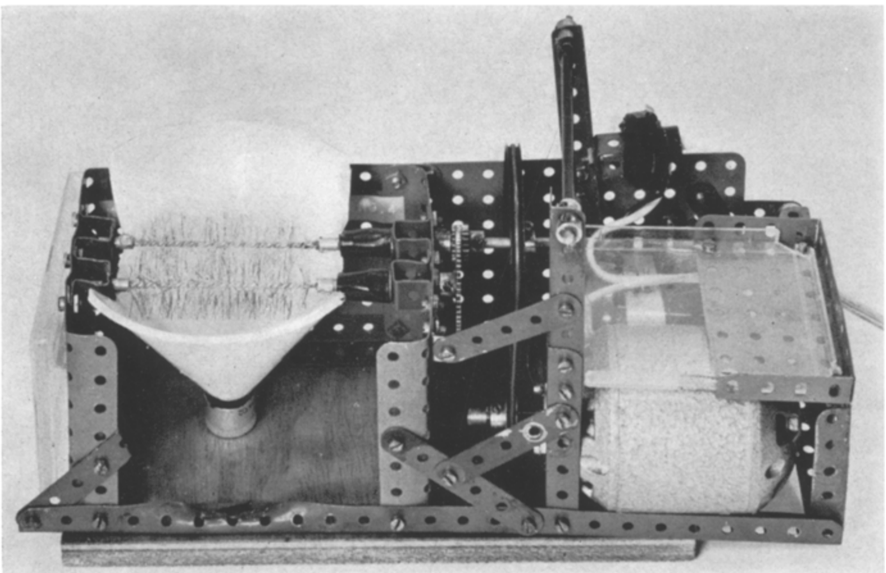

FIG. 4. Brushing machine to remove mites from bean leaves.
Borstelapparaat voor het verwijderen van mijten van bonebladeren.

To obtain normal growth not only a fast moving stream of nutrient solution, but also optimal aeration is imperative. Rapid exhaustion of free oxygen will influence negatively ion uptake by the roots. To take these factors into account the following provisions were made (Fig. 3). A centrifugal pump (3) with a capacity of 3 l/min circulated the solution via a tube (5) for a period of 80 seconds every 30 minutes. The sucking tube attached to the centrifugal pump reached almost to the bottom of the cylinder behind a pierced partition (7). The holes in this partition were made in such a way that when the pump was working the solution was sucked up faster than it could be supplied from the greater part of the cylinder through these holes. The pump then started to suck air which decreased its capacity. Subsequently the solution level in the aeration compartment (6) rose and the pump started to suck water again. In this manner the solution was intensively and intermittently mixed with air.

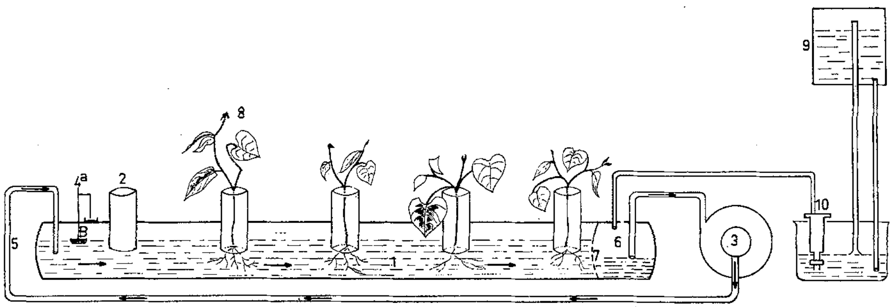

FIG. 3. Scheme of the waterculture apparatus.
Schema van het waterculture-apparaat.

1. Perspex tube/*Perspex-buis*
2. Opening for plant/*Opening voor plant*
3. Circulation pump/*Circulatiepomp*
4. Mercury switch/*Kwikschakelaar*
4b. Float/*Drijver*
5. Small tube for water circulation/*Dunne slang voor watercirculatie*
6. Aeration compartment/*Doorluchtingskamer*
7. Perforated partition/*Doorboord tussenschot*
8. Plant/*Plant*
9. Container with supply of distilled water/*Voorraadvat met gedestilleerd water*
10. Micropump connected to mercury switch/*Pompje, verbonden met kwikschakelaar*

Evaporation of water by the leaves induces undesirable changes in the concentration of the solution. To prevent this a constant level of the solution in the vessel was maintained by the following provisions: A float (4) was connected to a mercury switch (4a) which put an auxiliary pump (10) into operation if the water level dropped 4–5 %. This pump replaced the evaporated water with distilled water from the reserve tank (9). The nutrient solutions were made up according to STEINER (1961). The quantities of the various components differed but the total anion and kation concentration as well as the pH and osmotic value of the solutions were the same. In the experiments under discussion a solution of code J6 was used.

Starting the experiment ^{32}P was added to the solution as Na_2HPO_4 in the strength of 0.5 μ Ci/ml. A certain weight of aphids or mites taken from a stock population was put on the leaves. Sufficient light (800 Lux) was provided for

14 hours daily and a moisture apparatus kept the relative humidity in the plastic cage in which the whole experiment had been set up, at 60 ± 10%.

The temperature was kept at 25 ± 2°C. After two weeks the experiment was terminated. The total radioactivity was then established. It was necessary to rinse the system with a 0.2% RBS-25 solution[3] in order to include in the measurements as much as possible of the ^{32}P adsorbed on the walls of vessel and tubes.

As it is not very easy to get an accurate measurement of the total radioactivity of plants by counting leaf discs, the plants were homogenized in a Waring blendor for 5 minutes. To each 25 g of plant material 100 ml of water were added. On a heating-plate the plants were digested with 10 ml concentrated H_2SO_4 (96%) for one hour. While still boiling, 20 ml H_2O_2 (30%) were added to the digest. It was neutralized carefully with concentrated NaOH (50%). The fluid was then made up with water to a known volume. Three 0.5 ml samples of the solution were pipetted on aluminium planchettes, which, after evaporation, were counted with a thin window GM-tube. The same procedure was followed when sampling the radioactivity of nutrient solution and animal material collected from the plants.

To collect all mites and their products, such as eggs, egg shells, larval skins, faeces and webs, a modified brushing machine was constructed with soft brushes to avoid damage to the tender bean and radish leaves (Fig. 4). The honeydew excreted by the aphids was collected on filterpaper placed under the leaves during the experiment. These papers were digested in the same way as the plants.

DISCUSSION

Due to the excellent growing conditions, the plants grew extremely well. Multiplication of the parasites was better than usual. The radioactivity of adult females was more than 100 c.p.m. per mite and about 1500 c.p.m. per aphid. It was surprising that of the totally recovered radioactivity no less than 2–3% was present in the mites as well as in the aphids.

It was imperative that the total amount of radioactivity recovered from solution, plants and parasites approached the theoretical value calculated from the initial amount of radioactivity. Although adsorption of the isotope to the walls of the vessel and tubes, mistakes in sampling, salt-formation on the planchettes during evaporation, and imperfections in the methods of collecting the parasites influenced eventual recovery, a percentage of more than 90% was obtained. In order to compare the results of various experiments accurately, it is necessary that the percentage recovered should not only be as high as possible, but also that it should have approximately the same value in all cases.

An impression of the effects a parasite population has on the host plant can be obtained from a control experiment under the same conditions but without infesting the plants with parasites. It is our intention to use a number of nutrient solutions made up according to STEINER (1961) to ascertain differences in the distribution of some essential elements under different nutritional conditions of plant and parasite.

[3] Detergent, especially suited to remove absorbed radioactive material.

Changes in the distribution of a test element in solution, plant and parasite using different nutrient solutions, could supply information about the extent to which the roots take up certain ions, about the quantities of food ingested by the parasites, and about the reaction of the parasites in the form of changes in population development. Results of experiments carried out in this way, which are based on quantitative data obtained from a closed system, could be the starting-point for investigating the biochemical reactions which play a part in the tissue formation of plant and animal.

SAMENVATTING

In verband met het onderzoek naar de invloed van de fysiologische toestand van de waardplant op de populatieontwikkeling van fytofage insekten en mijten wordt een proefopstelling voor balansstudies beschreven (fig. 2). Bone- en radijsplanten, met hun respectievelijke parasieten het kasspint (*Tetranychus urticae*) en de groene perzikluis *(Myzus persicae)*, werden gekweekt op een geaereerde kleine waterculture (fig. 3).

In fig. 1 wordt schematisch weergegeven hoe, bij gebruik van twee verschillende voedingsoplossingen, de procentuele verdeling zou kunnen zijn van een bepaald element over voedingsoplossing, planten en parasieten. ^{32}P werd aan de voedingsoplossing toegevoegd. De mijten en bladluizen, welke van de planten verwijderd werden door middel van een speciaal aangepast borstelapparaat, waren na de proef meetbaar radioactief (fig. 4).

De radioactiviteit van het dierlijke materiaal bedroeg 2–3% van de totale radioactiviteit teruggevonden in voedingsoplossing, planten en parasieten.

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REFERENCE

STEINER, A. A., – 1961. A universal method for preparing nutrient solutions of a certain desired composition. Pl. Soil 15: 134–154.