THE UNKNOWN HATCHING AGENT FOR THE
POTATO ROOT-EELWORM
(Some Purification Aspects)

BY

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INTRODUCTION

It is well known that potato root-diffusate shows activity in hatching potato root-eelworm. A number of years were spent in purifying these diffusates, with the ultimate aim of isolating the hatching factor. Although some progress was made in purifying the crude diffusate, methods have not been satisfactory, owing to the many impurities to be eliminated from the crude preparation. Later, a simultaneous set of experiments were started from a different angle. MASSEY and NEAL showed, that an active substance is present in tomato leaves. This substance may be obtained by floating small disks from tomato leaves on distilled water. After one or two days the water shows hatching activity. The hatching factor seems to diffuse from the punches into the water. We repeated their experiments with potato leaves and also with other parts of the potato plant and were able to confirm their results. So far no hatching activity could be discovered in parts of potato tuber. The reason, we were interested in the active substance(s) of other parts from the potato plant is, that perhaps here lies a possibility of solving the problem of the hatching agent from a general viewpoint. A resemblance between these active substances seems possible.

In order to examine the active substance from leaves and stems we collected an amount of crude material by extracting the pulverized plants (except the tuber) with 50 p.c. alcohol. (This method is described in the second part). This way, a second raw "starting-material" was added to our first root-diffusate preparations. Other methods of purification were necessary to get information about the nature of the hatching agent. The methods used and the results obtained are described in the third part; the results are discussed in part four.
DESCRIPTION OF THE METHODS FOR COLLECTING CRUDE MATERIAL FROM ROOT-DIFFUSATE AND LEAVES (AND STEMS) FROM THE POTATO PLANT

1. Collecting of the dry crude material from root-diffusate.

Root-diffusate is filtered by passing a layer of charcoal. After two or three days the column is extracted with a mixture of benzene, acetone and water. After evaporating the organic solvents in a thin-layer-evaporator the residue is dried in an exsiccator at reduced pressure. The dry material is then stored in sealed tubes at 0-5°C. This is a modification of the method used by Hurst and Calam (1931-1939) and is therefore called the "classic" method.

2. Collecting of the dry crude material from leaves and stems.

The plants are ground (except the tubers) and mixed with 10 to 20 parts of approximately 50 percent alcohol. After 2 to 4 days the extract is filtrated. The alcohol is removed by evaporation. Chlorophyll can easily be removed by shaking with one third of volume ethylacetate. Although ethylacetate takes up some of the active material, the activity of the watery solution has been increased after chlorophyll removal. The ethylacetate still present in the water is evaporated and the watery solution is concentrated in a thin-layer-evaporator and afterwards dried in an exsiccator at reduced pressure. The dry material is stored in sealed tubes at 0-5°C.

DESCRIPTION OF PURIFICATION METHODS.

1. Methods used with root-diffusate.

a) Adsorption of the active substance on charcoal (norite) columns followed by elution with organic solvents, or organic solvents mixtures with water.

Result: Unsatisfactory, due to the poor adsorption of norite.

b) Extraction of the active substance from watery solutions by means of organic solvents slightly soluble or insoluble in water.

Result: Of several organic solvents, ethylacetate proved to be the best extracting liquid. On a bigger scale however, it is worthless because the solubility of the active agent in this solvent is still very poor.


A. Removal of high molecular organic material and part of the inorganic substances (see fig. 1).

A concentrated solution of leaf-extract or root-diffusate in water is