THE INFLUENCE OF HOST NUTRITION AND INTENSITY OF INFECTION ON THE SEX RATIO AND DEVELOPMENT OF MELOIDOGYNE INCognITA IN STERILE AGAR CULTURES OF EXCISED CUCUMBER ROOTS 1)

BY

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Rate of development of Meloidogyne incognita was decreased at reduced concentrations of sucrose and iron chelate. Decreasing the concentrations of vitamins and macronutrient salts resulted in an increased rate of nematode development. The concentration of the macronutrient salts also had a profound influence on the nature of the gall. Galls on a medium deficient in the macronutrient salts were much larger and less compact than those on complete nutrient. Sex-ratio of M. incognita was dependent upon host nutrition but not upon intensity of infection. At reduced sucrose concentrations, 100 percent males occurred. Rate of development was inversely proportional to the number of nematodes added to the culture and to the number of nematodes per gall.

Direct environmental influence on sex differentiation has been demonstrated conclusively in very few animals (Triantaphyllou & Hirschmann, 1965). In one case (Takeda, 1950) it has been shown that there is a relationship between rate of development and sex of a marine copepod, Tigriopus japonicus. Factors affecting development such as temperature and growth promoting or inhibiting chemicals induced significant changes in the ratio of males to females.

Apparently, in the nematode genus Meloidogyne, a comparable situation exists. Triantaphyllou (1960) and Triantaphyllou & Hirschmann (1960) suggest that the rate of development and the occurrence of males is related to the number of nematodes developing within a given volume of root and to their position within the root. When few larvae entered the root, most of them developed into females. When large numbers of larvae invaded the apical portions of the roots, larval development was retarded and a high proportion developed into males. Triantaphyllou postulated that the mechanism of this shift in sex ratio was not due to a differential death rate of males and females. He suggested that in M. incognita developing females are capable of sex reversal. By anatomical examination of developing second stage larvae he substantiated this hypothesis. Triantaphyllou confirmed previous observations (Tyler, 1933) that the occurrence of males was

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induced by environmental conditions unfavorable for female development, probably nutritional in nature, and concluded that such males resulted partly from sex-reversed females.

The nature of the environmental factors involved, however, has not been well elucidated. Bird (1960) observed that on infected tomato plants with single element deficiencies, males of *M. javanica* occurred only on those growing with reduced nitrogen. Increased ratios of male to female *Heterodera schachtii* also occurred when infected *Brassica rapa* was grown under conditions of reduced nitrogen (Kämpfe & Kerstan, 1964). Davide & Triantaphyllou (1964) applied maleic hydrazide to infected tomato plants and increased the occurrence of males of *M. incognita* and *M. javanica*.

The objective of this study was to examine in greater detail the relationship between host nutrition, intensity of infection and nematode development and sex determination.

**MATERIALS AND METHODS**

Axenized second stage larvae of *M. incognita* were aseptically introduced into sterile agar cultures of excised cucumber roots (*Cucumis sativus* var. National Pickling). The methods for these procedures have been reported as were the techniques for regulating the nutrients offered the host tissue and the intensity of infection (McClure & Viglierchio, 1966).

The concentrations of three nutrient stock solutions and sucrose were regulated as units so that final concentrations of each group were multiples or proportions of the normal concentrations. Multiplies were designated by the letter V. Thus 1.0V iron represents the normal concentration of iron, 0.1V iron one tenth of the normal concentration of iron etc.

To study the effect of nitrogen deficiency, 100 ml of the normal macronutrient stock solution were substituted with 100 ml of a stock solution made up as follows:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
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<tbody>
<tr>
<td>CaCl₂.2H₂O</td>
<td>0.871 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.184 g</td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
<td>0.340 g</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>0.250 g</td>
</tr>
<tr>
<td>Water</td>
<td>to 100 ml</td>
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</tbody>
</table>

In addition, since all the vitamins contained in the normal nutrient contained nitrogen, the entire vitamin complement was eliminated from the nitrogen deficient nutrient.

For sex determination and estimation of degree of development the cultures were harvested 17 days after inoculation. This was sufficient time for approximately 40-50 percent of the nematodes to reach maturity, but too early for the development of a second generation of infective larvae. It was also considered the best time for estimating rate of development since changes in growth rate in either direction could be detected. The staining and sexing technique described