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A Study of Host-Parasite Relationships. The Potato-root Eelworm *(Heterodera rostochiensis)* in Black nightshade *(Solanum nigrum)* and Tomato.

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It is known that root diffusates from *Solanum nigrum* are capable of stimulating the emergence of larvae from cysts of the potato root eelworm, *Heterodera rostochiensis* (Russel *et al.*, 1949). Dr. D. W. Fenwick and Dr. M. T. Franklin have informed the writer that they have found the larvae of *H. rostochiensis* in the roots of black nightshade, *Solanum nigrum* but they were unable to find cysts developing. The present paper is the result of a study of the development of *H. rostochiensis* within black nightshade on the one hand and tomato on the other.

Early in September, 1951, young seedlings of black nightshade and of tomato were transplanted to pots of a three in one loam-sand mixture containing an innoculum of *Heterodera rostochiensis* cysts, collected from an infested potato plot at Rothamsted. The seedlings were roughly comparable in size, but those of *S. nigrum* were slightly the smaller. The pots were stood in a shady position out of doors until the end of September and then moved into a greenhouse as a precaution against frost-damage.

A number of plants of each species was lifted on each of four occasions after the date of transplanting; namely twelve plants at one week, twelve at two weeks, twelve at four weeks and four plants at eight weeks, and two reserve plants of *S. nigrum* and of tomato were allowed to remain in the infested soil for nineteen weeks.

After lifting, the roots were washed, fixed in formal acetic alcohol, weighed after removing surplus moisture and finally stained in hot 0.05 per cent. acid fuchsin lactophenol. Clearing and differentiation were carried out in pure phenol in which the roots were afterwards examined. Nematodes to be examined in detail were dissected from the roots, brought in stages to lactophenol and mounted in this medium.

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At the end of a week's growth in the infested soil, three of the twelve plants of black nightshade and ten of the twelve tomatoes had been invaded by the eelworm. A total of 10 larvae were found in the black nightshade and 525 in the tomatoes. Weight for weight, the tomato roots contained about twenty-four times the number of eelworms found in *S. nigrum*. (See Table III). No larvae in either host showed any signs of development at this time.

In the roots of black nightshade there was always more or less necrosis associated with the eelworm. This sometimes showed as a brownish patch on the surface of the root where the larvae had evidently entered, and sometimes as a patch deep in the cortex close to the periphery of the stele. Often the root was constricted or broken across the necrosed area and in many cases the break occurred directly across the position of the larva. The larvae appeared to be fairly evenly distributed and there were no definite swellings on the roots as there were in tomato. In the tomato roots at this stage, necrosis appeared to be less serious and was more generally confined to the surface layers. Where invasion had occurred near the root tip, brown tracks could often be seen in the piliferous layer, apparently marking the preliminary excavations of the nematode before it finally made its entry. Most of the larvae were found in groups a short distance behind the root tip and roots thus attacked almost invariably showed the beginning of lumpy swellings around the invaded parts. On the whole, at this stage, few roots were so badly necrosed that they had actually broken.

From a fortnight onward every plant of the two species examined contained larvae. However, in black nightshade they showed no signs of development at two weeks, while in tomato many had begun to thicken and a few were beginning the second moult. Since Hagemeyer (1951) discovered that the first moult of *H. rostochiensis* occurs within the egg, she thought it probable that the life cycle of this nematode resembles that of *H. schachtii*, as reported by Raski (1950). Throughout this study, therefore, the identification of the different larval stages has been based on Raski's descriptions of *H. schachtii*.

The general condition of the roots of both hosts at two weeks appeared almost unchanged, while 266 larvae were counted in the black nightshade plants and 1,759 in the tomatoes. Comparing equal weights of roots, the infestation is about four times as heavy in the tomatoes as in *S. nigrum*.

At four weeks one larva only out of a total of 917 in the roots of *S. nigrum* was found to be developing. This had just started the second moult and appeared normal in size and internal structure. Even more

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FIG. 1

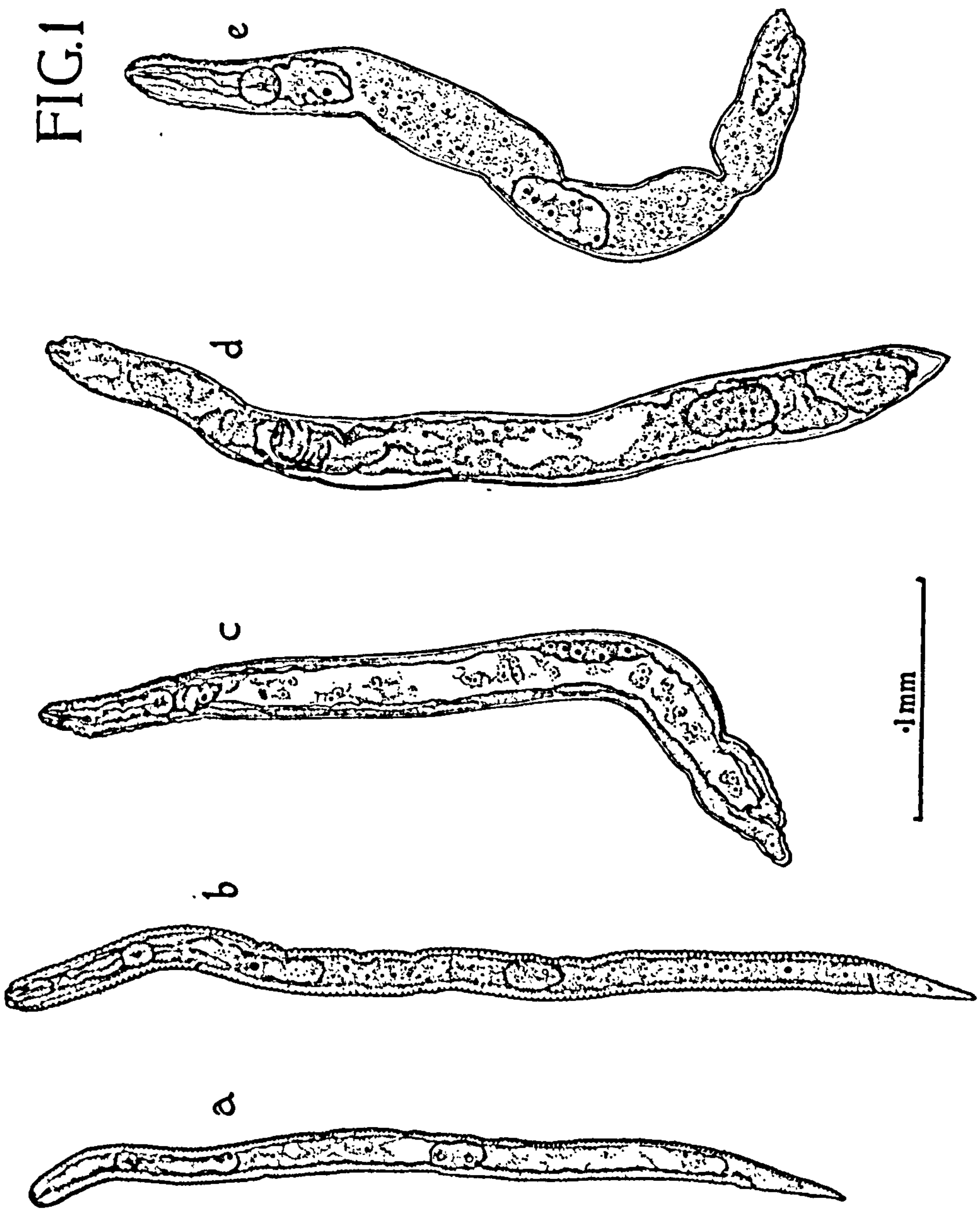

Fig. 1.—a. Degenerating second stage larva from a plant of *Solanum nigrum* grown for eight weeks in infested soil. b. Healthy second stage larva from tomato. c and d. Degenerating third stage larvae from a plant of *Solanum nigrum* grown for eight weeks in infested soil. e. Healthy early third stage larva from tomato.

roots than before were necrosed and broken and almost the only growing points to be seen were on young roots at the distal end of the system and on new laterals just beneath the crown. The tomatoes also showed greater necrosis than before, especially in heavily attacked parts; many roots were broken off and more patches of deep necrosis were evident. 2,609 *H. rostochiensis* were counted in the tomatoes at this stage, representing just less than twice the number in an equal weight of *S. nigrum* root. The female larvae demonstrated all stages of development up to the advanced third stage (Fig. 2, g) being flask-shaped with elongated ovaries. The males were even more advanced and one which was dissected out was just ready to burst from the fourth larval cuticle as a fully developed adult. (Fig. 2, i.)

At eight weeks, the black nightshade plants were in a more healthy condition than the tomatoes which were stunted and their roots reduced and badly swollen. The nematodes in *S. nigrum* were very scattered and the older roots which had survived attack sometimes showed broad, shallow surface lesions, often with an undeveloped and shrivelled second stage larva in the bottom of them. Out of 2,700 *H. rostochiensis* counted in the four *S. nigrum* plants at this stage, only 12 were found which showed any signs of development. However, some late second stage larvae were almost certainly missed owing to the difficulty of identifying those which lay deep in the roots. Two of the developing forms found were larvae in their second moult, while eight others were third stage larvae which had begun to swell. Three of these showed the first signs of elongation of the genital primordium and were probably males. Structural degeneration was very marked in six of the third stage larvae and the only developing form found in another smaller experiment on *S. nigrum* was an extremely degenerate larva which was probably in the third moult, although the gonad was almost undeveloped. This was found in a plant which had grown for seven weeks in infested soil. All these larvae had little or no food reserves in the intestinal region, which appeared quite void and the structure of the subcuticular layers was, in parts, badly broken down. This degeneration appeared to start in irregular patches in the body-wall musculature between the rectal and oesophageal regions. The patches evidently enlarge and run together in later degenerative stages, leaving only irregular strands of tissue beneath the cuticle. (Fig. 1, c.) These degenerate early third stage larvae were measured and compared with five comparable stages dissected from tomato. The measurements with their means are given in Table I.

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FIG. 2

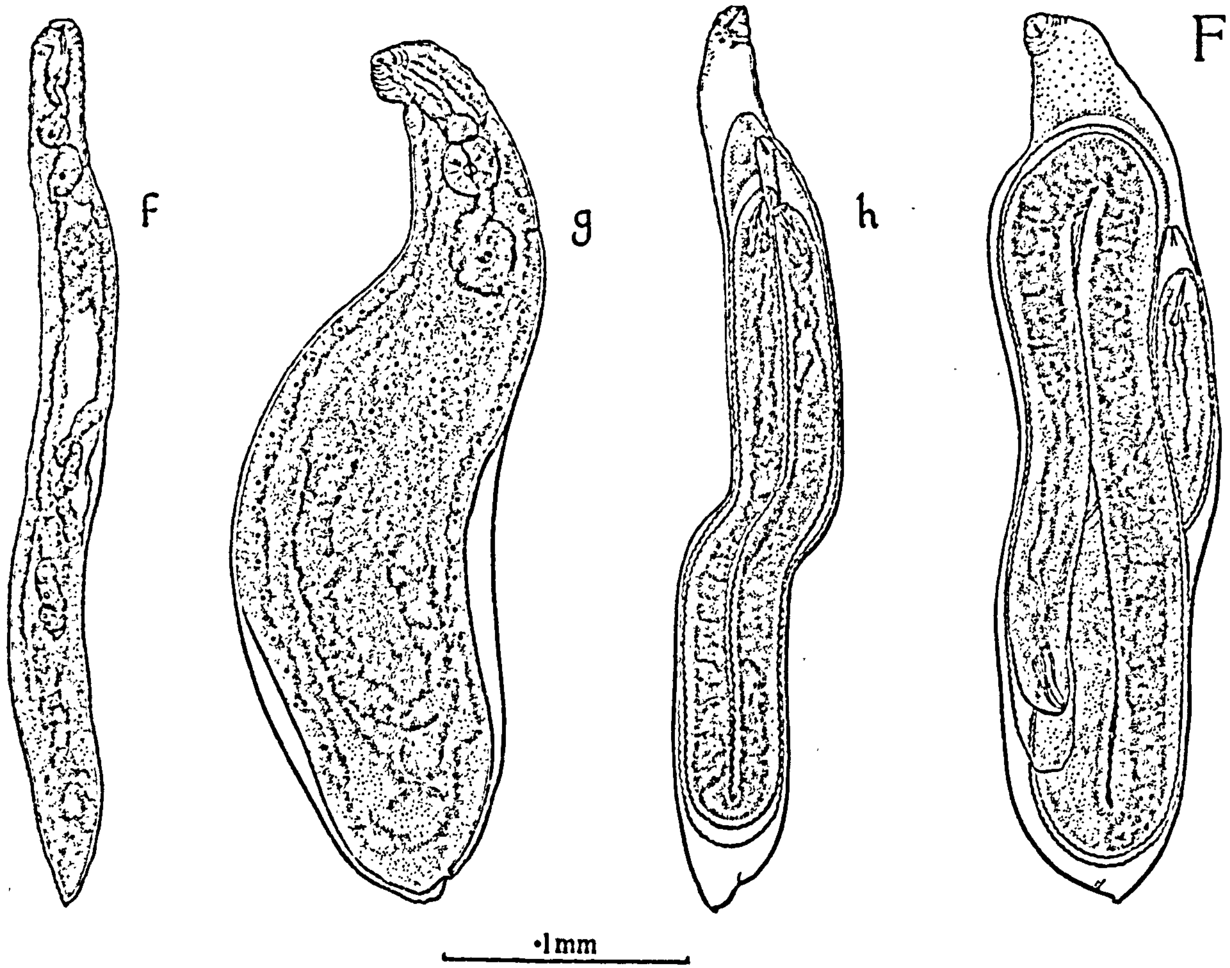

Fig. 2.—f. Degenerating third stage larva from a completely necrosed root of tomato. g. Healthy third stage female larva from a tomato plant grown for four weeks in infested soil. The third moult has just begun. h. Male larva undergoing the final moult. Dissected from a plant of *Solanum nigrum* grown for eight weeks in infested soil. i. Male larva undergoing the final moult. Dissected from a tomato plant grown for four weeks in infested soil.

Probably both of the apparently healthy third stage larvae from black nightshade were males, the more advanced one having a testis 0.197 mm. long but not yet reflexed at the anterior end. One larva had apparently begun the third moult, but it was shrivelled and distorted inside the third larval cuticle and the gonad was little developed. (Fig. 1, d.) The most advanced stage found in *S. nigrum* was an apparently healthy and nearly fully developed male, still enclosed by the third and fourth stage cuticles, but with well-developed spicules, gubernaculum and testis. (Fig. 2, h.) It seems significant that the three most healthy and advanced forms found in black nightshade were males: in a favourable host it is they which normally reach maturity first. However, the fifth stage male from *S. nigrum* was small compared with similar stages dissected from tomato. It measured 0.558 mm. long and 0.021 mm. in diameter and the third stage cuticle which ensheathed it was 0.89 mm. long by 0.48 mm. diameter. Seven similar stages taken at random from tomato averaged 0.872 mm. in length and 0.031 mm. in width. These ranged from 0.985 to 0.766 mm. long and 0.033 to 0.028 mm. in width.

TABLE I.

		Length in mm.	Diameter in mm.
Degenerated early third stage larvae from *S. nigrum*.	1	0.422	0.034
	2	0.361	0.027
	3	0.354	0.032
	4	0.350	0.025
	5	0.346	0.032
	Mean	0.366	0.030
Healthy early third stage larvae from tomato.	1	0.465	0.041
	2	0.425	0.038
	3	0.375	0.032
	4	0.357	0.027
	5	0.354	0.036
	Mean	0.395	0.035

Many second stage larvae in *S. nigrum* were indistinguishable from those in tomato, but others had the same empty appearance characteristic of the third stage larvae already described and it seems likely that these forms represented individuals which were unable to develop. (Fig. 1, a.)

The four tomatoes grown for eight weeks in infested soil had numerous mature cysts protruding from the roots and a total of 1,147 *H. rostochiensis* was counted. The tomato roots were the most stunted and their larval contents still represent a density of infestation nearly

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twice that in black nightshade. Only some of the larvae in seriously necrosed parts of the tomatoes showed the same kind of structural degeneration which was apparent in the nematodes from black nightshade. (Fig. 2, f.) However, none was found which was in quite such an advanced state of deterioration.

TABLE II.

The number of *H. rostochiensis* larvae in Black Nightshade and Tomato in relation to weight of roots and to duration of exposure to attack.

	BLACK NIGHTSHADE. Duration of exposure to attack. Weeks.				TOMATO. Duration of exposure to attack. Weeks.			
	1	2	4	8	1	2	4	8
No. of plants	12	12	12	4	12	12	12	4
Total weight of roots examined. gms.	0.25	0.43	1.47	3.65	0.55	0.68	2.21	0.85
Total count of *H. rostochiensis*	10	266	917	2,700	525	1,759	2,609	1,147
No. of *H. rostochiensis* per 0.1 gm. root	4.0	61.9	62.4	74.0	95.5	258.6	118.0	134.9

TABLE III.

Relative density of larval population ; Tomato/Black Nightshade per unit weight of root.

Duration of exposure to attack. Weeks.			
1	2	4	8
23.9	4.2	1.9	1.8

The tomato and black nightshade plants remaining in the infested soil for nineteen weeks were all found to be practically free from infestation. The distal roots of the tomatoes were still swollen, but only two or three *H. rostochiensis* were found within each plant. The upper roots were well grown and much thickened and had apparently never been attacked. No trace of any infestation could be found in the plants of *S. nigrum*. This may be partly explained by the fact that the plants had undergone little active growth during November, December and January and that in consequence an active cyst-hatching agent was not being produced (Russel *et al.*, 1949.) Moreover, during November the plants had accidently been allowed to become very dry and

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though they recovered, this may have destroyed many of the larvae in the soil which had previously hatched, but not yet invaded the roots. Many of the nematodes in the tomato plants had probably matured and fallen from the roots as cysts, while the larvae which had invaded black nightshade must have died in the necrosed roots, some of which probably broke from the plant. In others new cortex may have been produced beneath the necrosed areas, thus bringing the nematodes to the exterior in shallow surface lesions resembling those found in the eight week plants.

Summary.

Larvae of *Heterodera rostochiensis* have penetrated *S. nigrum*, quickly caused necrosis in the invaded roots and usually themselves suffered degeneration, which it is believed has led to their death. In these cases the region of the intestine apparently becomes devoid of food reserves and the subcuticular layers of the body wall degenerate into irregular strands of tissue. Only of the order of 0.5 per cent. completed the second moult in eight weeks, while the same stage was reached in tomatoes in a little over two weeks. Many larvae apparently do not develop at all. Tomato roots react to invasion by becoming swollen, and necrosis is less in evidence than in black nightshade.

In black nightshade few, if any, larvae reached maturity, but males evidently reached a more advanced stage than females. In tomatoes males reached maturity in four weeks, or just over and females in less than eight weeks. The tomatoes were more readily invaded than the black nightshade plants and the density of the invading eelworm population was about twenty-four times that of black nightshade after one week, about four times after two weeks and twice that of black nightshade at four weeks and at eight weeks.

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