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INVESTIGATION ON THE BIOLOGY OF *HETERODERA SCHACHTII* IN ITALY

by
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The sugarbeet cyst nematode, *Heterodera schachtii* Schmidt, is widespread in Italy. It greatly affects sugarbeet yield because this crop often is cultivated in a short term rotation. In some instances sugarbeet is rotated with Cruciferous hosts such as cauliflower (*Brassica oleracea* L. var. *botrytis* D.C.) and broccoli (*B. oleracea* L. var. *italica* Plenk). Moreover, the nematode has a very wide host range and can reproduce on a number of wild plants. Weed control on non cultivated land is often disregarded.

The nematode has been extensively investigated in order to provide information on its biology and for better control. The number of generations per year has been shown to be variable, the variation probably reflecting different environments and management practices. Triffit (1930) stated that only one generation per growing season is possible, but Jones (1950) in England and Duggan (1959) in Ireland found 2-3 generations. In warm climates, such as in the Imperial Valley, Southern California, 5 generations per year have been demonstrated experimentally (Thomason and Fife, 1962). The number of generations per year could also be affected by the hatching behaviour of eggs in *H. schachtii* cysts. Raski (1950) found that juveniles emerge from eggs of mature females, but Jones (1956) stated that hatching of beet cyst nematode is affected by the previous crops. Poor hatching often occurs in *in vitro* tests using cysts collected in the winter and spring following a host crop. It is suggested that a maturation period is required for the cysts to became fully sensitive to the hatching stimuli. Hough and Thomason (1975) report that there was no substantial hatching during the first 10 days using newly formed cysts. Evidence of a hatching periodicity has also been shown for other cyst-forming nematodes (Oostenbrink, 1967).

There was little knowledge of the biology of *H. schachtii* under Italian conditions. Therefore investigations were undertaken in 1976-1978 to provide information on: i) the dynamics of populations in the soil and in the root of sugar beet; ii) the number of generations per growing season; iii) the emergence of juveniles from cysts.

Materials and Methods

Dynamics of the population in roots of sugar beet and soil

In 1976 six plots of 22.5 m^2 each were selected in a field in Fucino Valley (Province of L'Aquila) and sown with sugar beet cv. « Kawemono », on April 15. The plots were not irrigated. Weekly, 5 g roots and a soil sample (1.5-2 kg) composite of 20 cores, were collected from the rhizosphere of the plants in each plot from plant emergence (May 14) onwards. Roots in 150 ml of water were comminuted for 30 sec in a blender; the specimens in 3 subsamples of 5 ml each were classified according to different life stages and counted. Soil samples were thoroughly mixed, air dried and 200 ml of each sample processed by the Fenwick can and ethanol method (Seinhorst, 1974). The cysts were then counted, crushed according to Seinhorst and Den Ouden's method (1966), and the contents of eggs and juveniles counted. Soil (100 ml) from each of the plots was placed on Baermann funnels for extraction and counts made of juveniles and males recovered. The experiment was repeated in 1977 in microplots made of concrete tiles of 30 $\times$ 30 cm cross section and 50 cm long, placed 45 cm deep into the soil and filled with 45 l of soil from a field in which sugar beet had been cultivated the previous year. This soil averaged 120 eggs of *H. schachtii*/ml. Microplots were sown on April 18 with sugar beet cv. « Kawemono » and irrigated as required. Following plant emergence (May 20) soil and root samples were collected every two weeks from 4 of the microplots and processed as in 1976. Soil moisture of each sample was also determined and soil temperature at 20 cm deep continuously recorded.

Number of generations per year

Concrete microplots, as described above, were filled in 1977 with soil free of *H. schachtii* and sown with sugar beet cv. « Kawemono » on April 18. At the same time the life cycle of the nematode was monitored in an infested field cultivated with sugar beet. On July 5, the first generation was already developed in the microplots and in the field, therefore, newly formed cysts were picked up from the roots in the field and 500 of them inoculated in each of 10 noninfested microplots. In early September a second generation was completed in the inoculated microplots and cysts were collected as above. Because of lack of numbers only 100 cysts were inoculated on September 6 in each of the two noninfested microplots, to provide a third generation. In addition, newly formed cysts were also collected from infested roots in the field and 500 inoculated in each of 6 microplots.

Emergence of juveniles from cysts

At the end of each generation, 5 batches of 100 newly formed cysts each were collected from roots, placed on 2 cm sieves made of a 215 μm net, immersed in 5 ml of a 3 mM $ZnCl_2$ solution, in 3 cm diameter plastic Petri dishes, which then were placed in a wide dish (Fig. 1), and incubated at 25°C. Juveniles emerging were counted after 1, 2, 4, 8 days and then weekly until 128 days. The hatching solution was renewed at each counting. Controls were 5 batches of 100 cysts each in tap water.

To investigate the hatching cysts under field conditions, 2 fields (designated A and B) were chosen in 1977. Half of each field had previously been cultivated with sugar beet and half with potatoes, and then sown with potatoes or sugar beet, respectively. One 1.5-2 kg soil samples composite of 20 cores, 25 cm deep, were collected from each crop of each field every two weeks from April 1977 to October 1977, and then monthly, until April 1978. At each sampling date, soil samples were also collected from 4 of the microplots for the study of the dynamics of the population. Four 200 ml subsamples from each of the field soil samples and only one per microplot sample, were then processed by the Fenwick can and the cysts separated from the debris and incubated at 19°C, for 1 week in tap water and thereafter as above in a 3 mM $ZnCl_2$ solution for 9 weeks. At the end of the tests the cysts were crushed according to Seinhorst and Den Ouden's method (1966) and the unhatched eggs and juveniles counted to ascertain the total number of eggs and juveniles within cysts at the beginning of the tests. Emerged juveniles were expressed as %

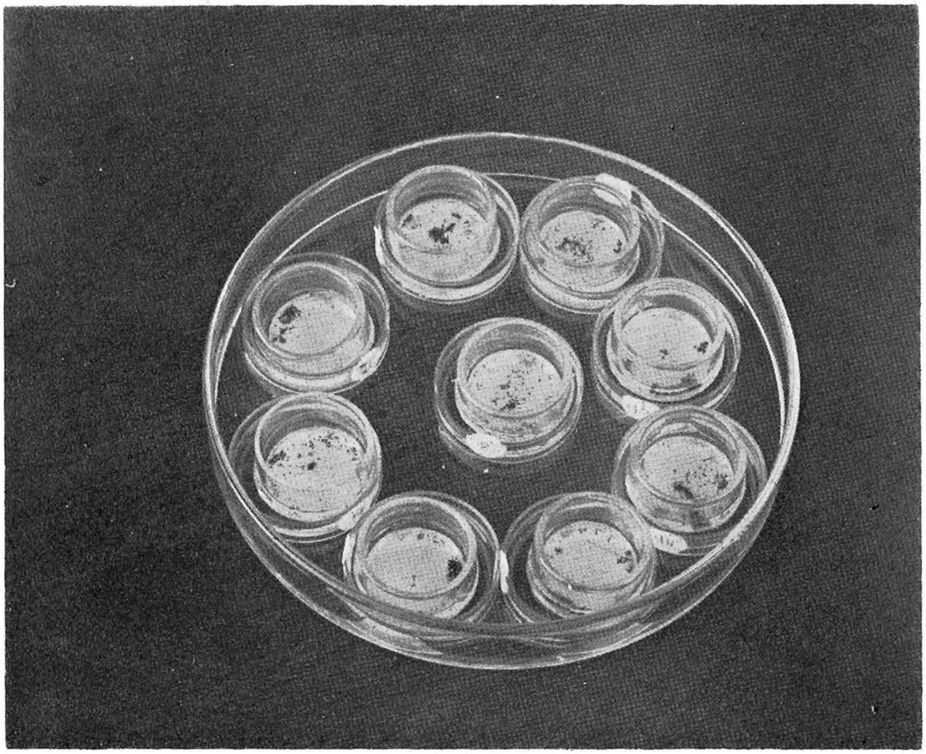

Fig. 1 - Arrangement of small sieves for hatching tests of *Heterodera schachtii.*

of the eggs and juveniles contained within the cyst at the beginning of the experiment and statistical comparison was done with the Student's t test between hatching of cysts from the sugar beet and potato crops of the same field.

Results

Dynamics of the population in soil and roots of sugar beet

Soil moisture never fell below 20% of soil dried weight and except in July and August remained above 30%. In 1977 soil temperatures (Fig. 2) were in the range 14-21°C from May 15 to September 20, and then fell to 10°C until the end of the experiment. Juveniles (Figs 3-4) recovered from the soil samples decreased from the begin-

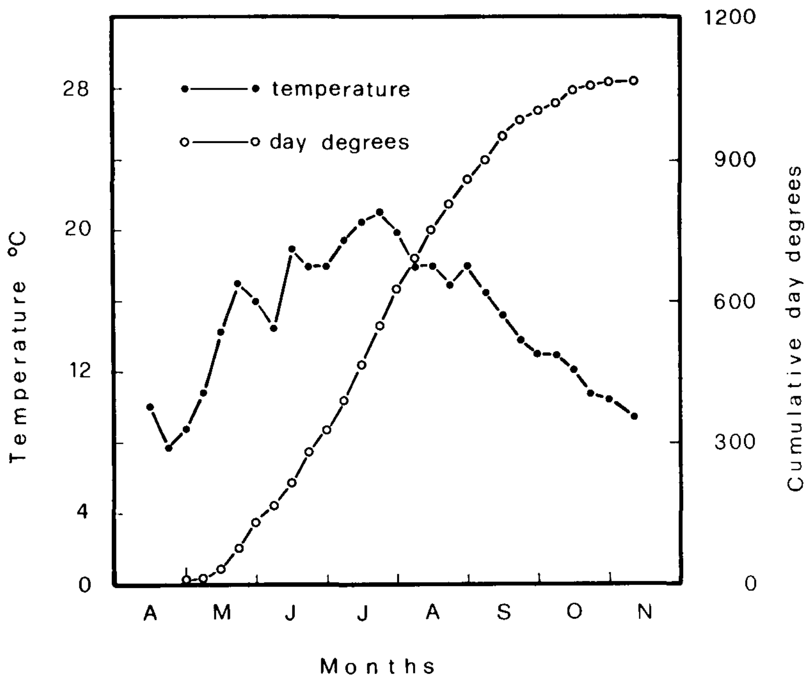

Fig. 2 - Mean temperatures recorded in 1977 at 20 cm deep and accumulated day degrees above 10°C during the experiment.

ning of the investigation until the end of June, but as found by Tacconi (1979) larger numbers were collected from July onwards. Thereafter, uniformly low numbers of juveniles were found from late autumn until April 27. Males were observed from June 1 to October 21 in 1976, and June 27 to September 6 in 1977. Eggs per cyst and eggs per ml soil observed in 1976 declined until June 16, but an increase was observed in numbers of eggs/ml soil from June 25 and, with some exception, lasted all the growing season. The average number of eggs/cyst was in the range 100-110. In 1977 the eggs/cyst and eggs/ml soil did not show increase, probably because the population level in the soil at sowing was the same as the equilibrium density shown for *H. schachtii* in the same condition (Greco *et al.*, 1982). The cysts per ml remained almost constant until the third week of June and then increased from the end of June onwards.

Most of the specimens, recovered from the roots (Fig. 5) at the

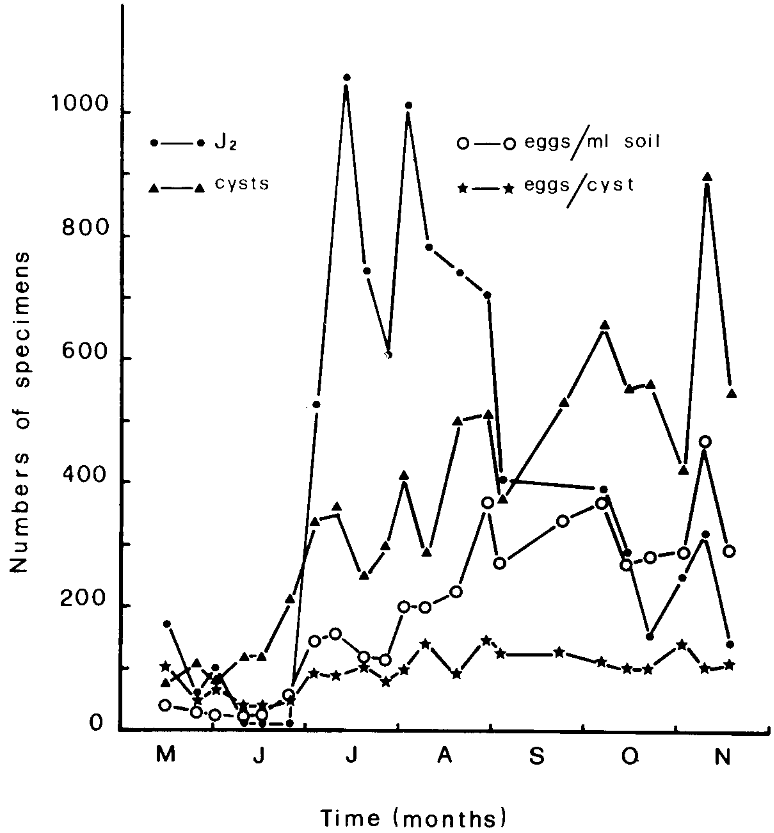

Fig. 3 - Second stage juveniles/100 ml soil, cysts/200 ml of soil, eggs/ml of soil, eggs/cysts in the soil, of *H. schachtii*, recovered in 1976.

beginning of the experiment, were second and third stage juveniles. Fourth stage juveniles and mature females usually appeared one and two weeks later, respectively. Nebel (1926) found that root invasion by juveniles was absent up to 9.5°C and poor in the range 10-14°C. Assuming that basal development temperature is 10°C, then 96 and 148 day degrees were required by the nematode to develop to fourth stage and mature females. Males were found simultaneously with fourth stage females, and cysts 3-4 weeks later (after 327 day degrees). Thereafter, all stages were found together in the roots but peaks were observed on July 12-19 for second stage juveniles and on August

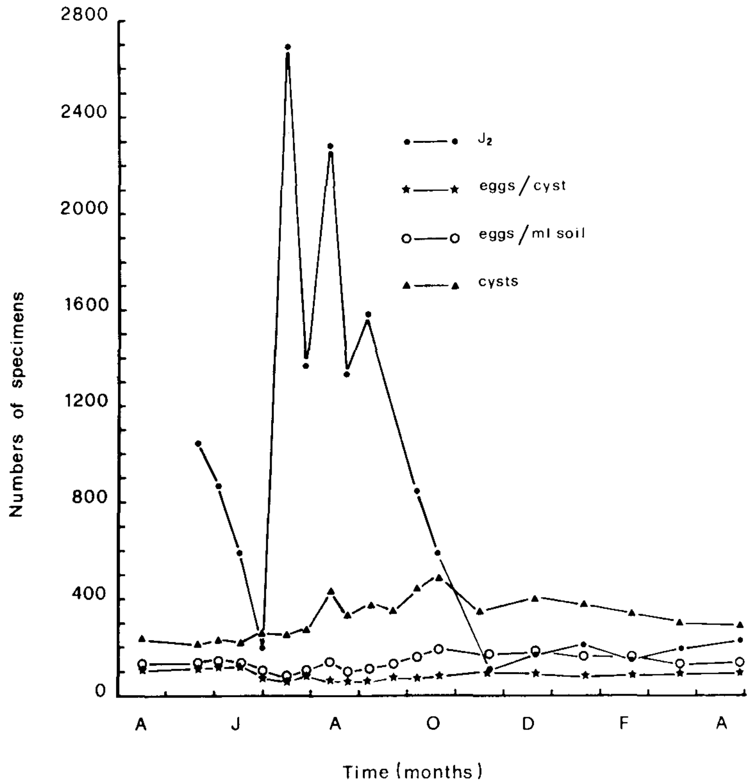

Fig. 4 - Second stage juveniles/50 ml of soil, cysts/200 ml of soil, eggs/ml of soil, eggs/cysts in the soil, of *H. schachtii*, recovered in 1977.

19-28 and early September for females and cysts, respectively, suggesting the beginning or the end of new generations.

Many females produced egg sacs but these were empty or contained few eggs (average 0.1 eggs/egg sac).

Number of generations per year

Microplots observations showed that the nematode may complete the first generation by June 24 (Figs 3-4-5). With inoculations done on July 5 it was possible to ascertain the end of a second generation by August 23, when a few light brown cysts were found on each of the three roots. However, more cysts (287/4 plants) were observed only

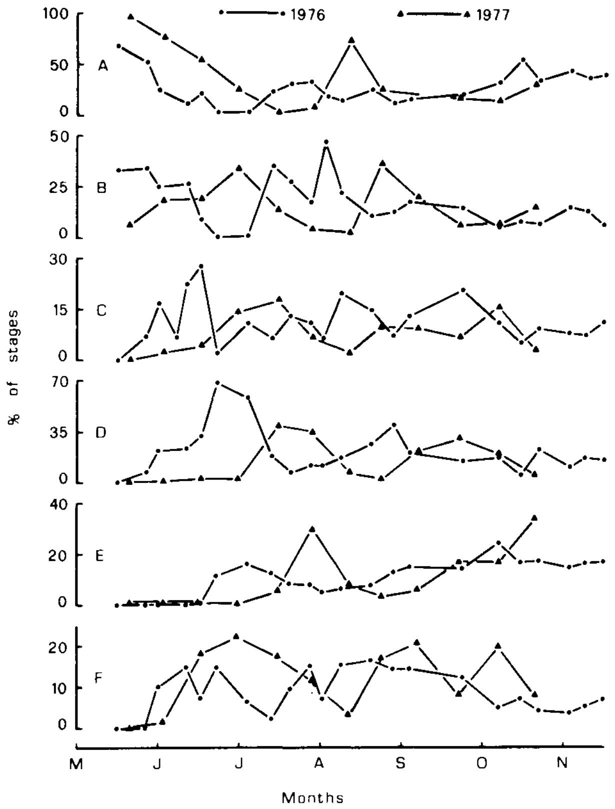

Fig. 5 - Percentage of different stages *H. schachtii* recovered from roots of sugar beet in 1976 and 1977. A) Second stage juveniles; B) Third stage juveniles; C) Fourth stage female juveniles; D) Females; E) Cysts; F) Males.

on September 5. In microplots inoculated on September 6, the cysts of the third generation appeared only on November 11.

Emergence of juveniles from cysts

Juveniles emerged promptly from cysts of the three generations (Fig. 6), and 31.4%, 24.9% and 45.9% of the eggs hatched in eight days for the first, second and third generation, respectively. The ultimate emergence was 74.7% and 54.1% after 128 days for the cysts of first and second generation, respectively, and 64.2% after 87 days for those of the third generation.

Table I - *Percentage of hatch of cysts of* Heterodera schachtii *collected from field soil sown with potato and sugar beet and from microplot soil sown with sugar beet, over a one year period, after 1, 5 and 10 weeks.*

Sampling dates	WEEKS 1					5					10				
	A Potato	A Beet	B Potato	B Beet	Microplots	A Potato	A Beet	B Potato	B Beet	Microplots	A Potato	A Beet	B Potato	B Beet	Microplots
21.04.1977	12.29	9.66			9.79	60.46	41.44			58.20	70.06	52.27			73.79
18.05	7.72	6.53				50.41	48.78				59.73	58.25			
24.05	7.69	5.31	8.21	7.04	18.87	54.92	41.85	51.35	59.18	50.01	65.02	46.83	59.98	69.86	59.55
07.06	3.02	6.99	7.87	5.24	8.91	45.13	33.86	40.43	42.07	52.73	54.77	37.82	48.77	47.76	68.05
21.06	7.44	11.08	4.12	12.28	14.15	50.32	39.82	46.88	48.18	49.53	61.43	49.49	59.32	58.36	60.29
02.07	4.69	17.91	3.47	13.75	13.84	46.86	45.96	53.63	54.86	51.60	57.55	54.00	67.08	61.07	66.78
18.07	2.66	3.22	2.24	5.55	10.99	50.36	50.96	43.66	25.77	38.43	64.39	65.11	59.53	36.28	50.52
01.08	4.44	6.54	1.87	4.76	9.44	46.83	56.44	30.90	39.25	33.01	60.38	74.81	48.99	49.36	43.40
19.08	2.84	5.60	3.55	4.07	9.47	29.29	34.60	28.70	26.02	35.91	43.37	56.46	43.83	39.62	49.79
26.08	1.49	3.75	4.05	8.18	7.83	26.55	36.41	28.79	23.99	50.94	38.27	55.89	40.86	33.85	66.19
15.09	3.93	4.81	3.03	2.60	2.04	29.18	36.73	20.64	31.75	29.57	43.53	54.56	32.79	47.55	45.57
27.09	3.66	4.72	4.55	5.49	2.94	47.81	50.88	42.77	47.53	54.52	73.77	76.24	69.57	66.48	82.76
10.10	6.69	10.02	2.67	8.55	11.56	32.45	45.91	28.71	38.39	45.15	48.30	61.10	45.46	55.98	67.88
25.10	5.50	4.49	2.40	4.28	6.88	33.06	35.68	27.28	38.80	45.23	47.27	54.99	42.85	52.94	67.98
22.11	1.00	6.00	1.78	0.80	2.00	39.76	57.11	33.12	44.20	41.26	53.39	71.25	42.68	54.54	59.11
20.12	1.52	4.10	5.06	0.39	5.50	33.99	52.99	41.69	43.44	52.30	43.31	63.13	50.88	51.52	65.49
21.01.1978	0.52	0.94	1.97	0.31	0.75	24.51	37.99	25.22	18.78	38.18	34.89	51.83	35.30	24.63	55.42
22.02	2.97	3.69	0.04	0.04	6.03	37.75	56.36	34.10	17.15	54.37	48.31	69.41	45.31	25.66	67.30
20.03	5.97	4.19	0.02	0.10	5.88	39.74	47.34	23.23	18.95	50.19	48.01	55.79	30.54	26.62	61.61
27.04	8.56	4.94	6.92	2.05	4.87	37.29	42.86	26.96	32.64	50.87	46.62	50.28	32.83	38.78	59.66

The hatching trend of cysts collected every two weeks or monthly, during the first weeks in tap water (Tab. I) showed that in both fields, in May and early June, juveniles emerged more plentifully from cysts recovered from soil under a potato than a sugar beet crop. However, it appeared that when the crops were established, significantly more juveniles emerged from cysts collected in the field cultivated with sugar beet than with potato. This may be due to the occurrence of newly formed cysts and/or because cysts were stimulated to hatch in the soil by sugar beet root diffusates. The same hatching trend was observed in fields A and B after 5 and 10 weeks of the hatching test, while the differences between the emerged juveniles from cysts of the same field cultivated with sugar beet or potato were not statistically significant when compared by the Student's *t* test. The field B remained flooded in February and March 1978 and, probably because of lack of oxygen, emergence was negligible during the first week in tap water but it recovered when the cysts were transferred to a 3 mM $ZnCl_2$ solution. The hatching of cysts from the microplots showed the same trend as those from the field cultivated with sugar beet.

Discussion

Under laboratory conditions newly formed cysts hatched readily and large numbers of second stage juveniles were collected from roots of sugar beet and field soil during the first half of July. Oostenbrink (1967) found that juveniles emerge more readily from cysts collected during May and August than during December to April. Our experiments indicated that juveniles emerged from cysts collected throughout the year and no periodicity was observed. Nevertheless, it appeared that in the absence of a host, cysts hatched more readily in the spring than in the winter (Table I). When the host crop was present many juveniles emerged during the first week in a hatch test conducted in water using cysts collected during June to October. The sugarbeet crop was well developed at this time and root diffusates may have stimulated hatching of the eggs. This information is useful for deciding the best period of the year to test the effectiveness of nematicides using a hatching test with cysts taken from treated plots. Greco *et al.* (1982) have shown that hatching test periods of one and two months were required when the treatments were done in April and October, respectively.

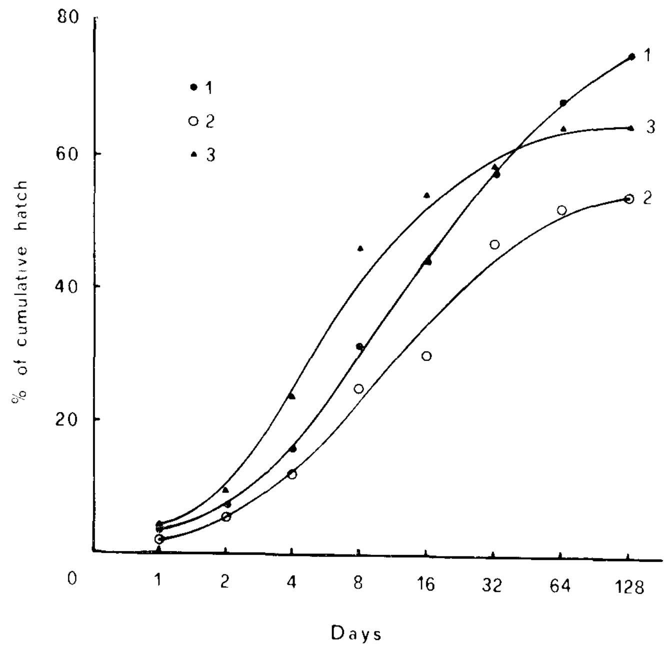

Fig. 6 - Emergence of juveniles of *H. schachtii* from cysts of the three generations, at 25°C.

Heterodera schachtii can infest sugar beet feeder roots throughout the growing season. White females and cysts may be found on the roots of sugar beet at the beginnig and the end of June, respectively. Eggs in the egg sacs were negligible, therefore their effect on the number of generations of the nematode was discounted.

According to Raski (1950) coiled juveniles inside the eggs occur five days before the cyst, thus in the microplot experiment reported here the first generation of the nematode would be completed by June 24, after 294 day degrees (basal development temperature, 10°C). Cysts were found on the roots of sugar beet by June 29, when 327 day degrees had accumulated. These findings agree with those of Raski (1950) who found that 289 and 350 day degrees were required respectively. Larger numbers of juveniles were collected from the soil ten days after the appearance of the first cyst (July 3, 1976 and July 14, 1977), when many females had developed to brown cysts. Therefore, a second generation of *H. schachtii* was probably completed by the first decade of August, by which time 710 day degrees had accumulated. Many juveniles would have emerged from newly formed cysts by August 15, to start a third generation, which would be completed by early October, after 1050 day degrees. In the present study the inoculation of the microplots may have delayed invasion of the sugar beet feeder roots by *H. schachtii* juveniles. Sufficient day degrees would have accumulated to allow the second generation to develop by August 22, assuming that juveniles invaded the roots 4-5 days after the inoculation of newly formed cysts. This prediction agrees with the microplot results as only a few cysts were found in the roots of sugar beet on August 23. In the Fucino area sugar beet is usually sown during the first half of April. Assuming that the plants emerge and are invaded by juveniles at the end of April or early May, some specimens could develop 3 generations per year by September 1, by which time 882 day degrees could accumulate.

Many white females were present on sugar beet feeder roots at harvest and according to Steele (1972) they may survive in the post harvest soil and proceed with their development if soil temperatures are still suitable.

The three generations of *H. schachtii* found in these investigations are the same as those reported by Jones (1950) in England and Duggan (1959) in Ireland. The results obtained in our experiments can probably be extrapolated to encompass Central and Northern Italy where cultural practices for sugar beet production and climate are generally similar to ours. In Southern Italy, sugar beet may be sown either in October or February, and harvested in July or August, respectively. In some instances spring sugar beet follows shortly after winter crops, such as broccoli and cauliflower, and many weed species including several Cruciferous spp. are present from autumn to spring. Under such circumstances the nematode may complete more than three generations as occurs in warm climates e.g. California (Thomason and Fife, 1962).

SUMMARY

Investigations were carried out in Italy during 1976-1978 to provide information on the biology of *Heterodera schachtii*. Hatching tests showed that juveniles emerged readily from newly formed cysts of each generation, and no periodicity was observed among the cysts collected from fields and microplots over a one year period. More juveniles emerged from cysts collected in the spring from potato crops and in the summer onwards from sugar beet crops, during the first week when water was used. The nematodes invaded sugar beet feeder roots throughout the growing season and many juveniles were found in the roots and soil from July until October, when the cysts of the first and second generation hatched. Three generations were recorded in the microplots, the first being completed by June 24 after 294 day degrees, the second by August 22 after 820 days degrees, and the third by the first week of November after 1095 day degrees. Extrapolating the generation time day degrees requirement under field conditions, 3 generations may be completed by September 1 when 882 day degrees have accumulated. However, in most instances 3 generations would be completed by the beginning of October after 1050 day degrees.

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