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STUDIES ON THE BIOLOGY OF *HETERODERA GOETTINGIANA* IN SOUTHERN ITALY

by

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The pea cyst-nematode *Heterodera goettingiana* Liebscher causes considerable damage to pea (*Pisum sativum* L.) (Di Vito and Lamberti, 1976), broad bean (*Vicia faba* L.) and vetch (*Vicia sativa* L.) in Southern Italy. Other legumes of lesser agronomic importance, such as *Lathyrus cicera* L. and *Pisum arvense* L. may also harbor large numbers of the nematode on their roots (Di Vito *et al.*, 1980). In England, the nematode completes one or two generations per growing season on pea and winter beans, respectively (Jones, 1950). In Spain females and cysts were found in the roots of vetch after 58-98 days and 117-129 days, respectively (Guevara-Benitez *et al.*, 1970).

In Italy, despite the wide distribution of *H. goettingiana*, information on its biology is scanty. Therefore investigations were undertaken in 1972-1973 to determine the number of generations per year, the influence of sowing time on juvenile invasion of pea roots and on the life cycle of the nematode, and the role of eggs in egg sacs and newly formed cysts in establishing new generations in Southern Italy.

Materials and Methods

The investigations were carried out in two localities, on the sands of

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Margherita di Savoia (Province of Foggia) and at Pisticci (Province of Matera) on a sandy loam. Seven and sixteen plots of 10 m² (10×1 m) were sown at different dates (Table I) in the two localities, respectively, with pea cv. Progress 9. The roots of five plants and soil samples from the rhizosphere of the same plants were collected at weekly intervals from each plot from plant emergence onwards.

To provide information on the number of generations of *H. goettingiana* during a growing season, 30 clay pots each containing 4 dm³ of steam sterilized sand of Margherita di Savoia, were inoculated on 21 February 1973 with roots of pea plants sown at Margherita di Savoia on 11 October 1972 (locally pea is usually planted in the first half of October) to which the newly formed cysts and full egg sacs of the first generation were attached. The pots were plunged into the soil at Margherita di Savoia and two pots were sampled at weekly intervals from plant emergence, to ascertain the development of the nematode. Thirty other plots were prepared in the same way on 2 June by mixing into the soil root pieces collected from the previous pots and on which females of *H. goettingiana* were turning to cysts. Because of the summer drought these pots were irrigated twice a week.

The newly produced cysts and egg sacs at Margherita di Savoia were also used to determine whether eggs within the cysts hatch simultaneously

Table I - Sowing dates of pea cv «Progress 9».

Margherita di Savoia	Pisticci
14-09-1972	01-09-1972
11-10-1972	02-10-1972
07-11-1972	06-11-1972
25-11-1972	16-11-1972
22-12-1972	27-11-1972
06-02-1973	18-12-1972
02-03-1973	08-01-1973
	15-01-1973
	29-01-1973
	12-02-1973
	17-03-1973
	28-04-1973
	28-05-1973
	21-06-1973
	15-07-1973
	10-08-1973

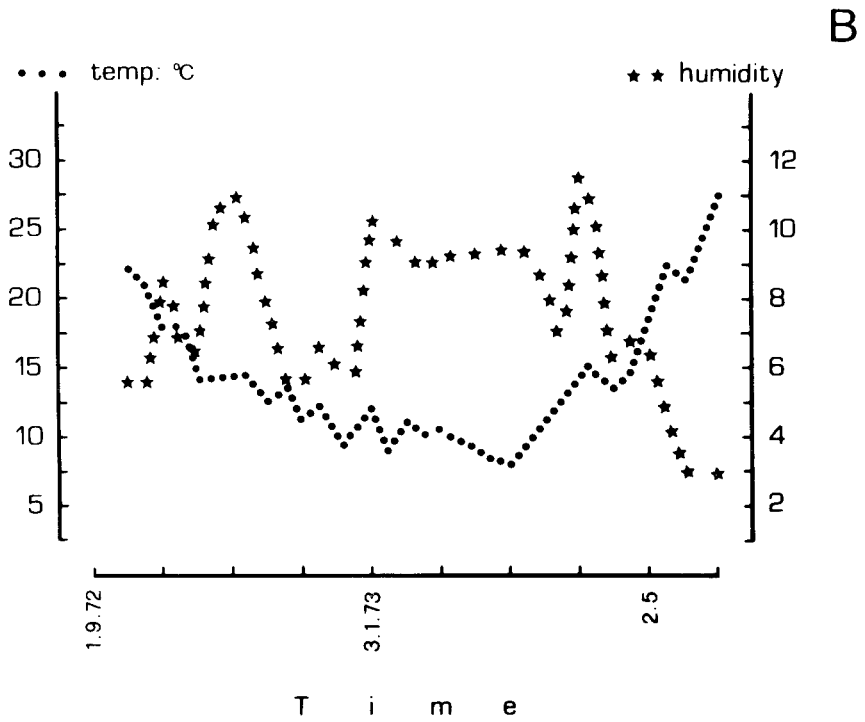
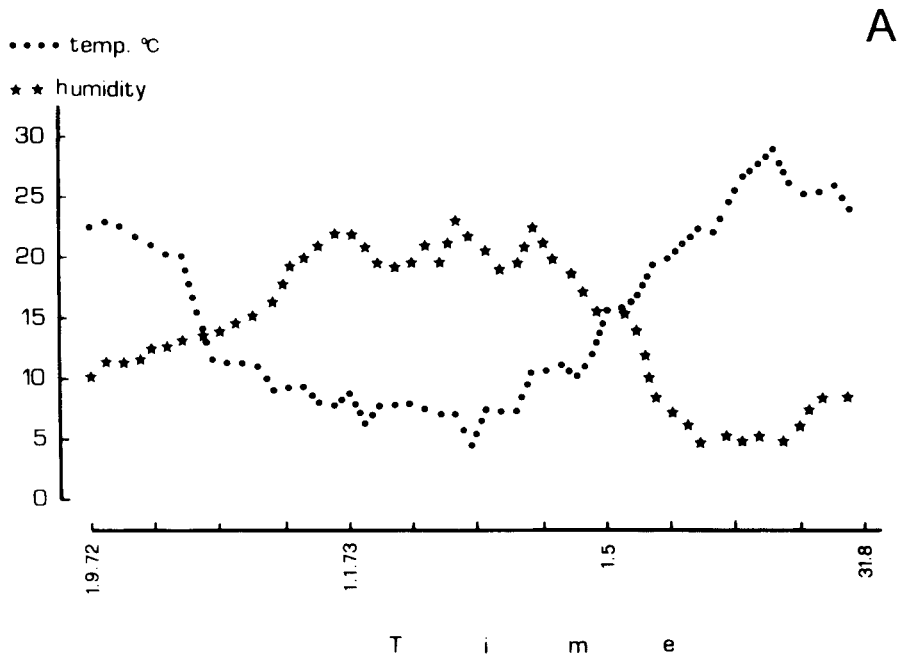


Fig. 1 - Soil temperature and soil moisture content, referred to as % of soil dry weight, at the two localities: A) Margherita di Savoia; B) Pisticci.

with eggs contained in the egg sacs. Two series of 15 cm diam pots were filled with steam sterilized sand, to each of which were added either 25 cysts or 25 egg sacs only collected from the plots at Margherita di Savoia and planted on 26 February 1973 with pea. Emergence of second stage juveniles of *H. goettingiana* and their penetration of the roots were ascertained at weekly intervals starting from 21 March.

Soil moisture expressed as percentage of dried soil weight, was determined at each sampling date; soil temperature, at 20 cm depth, was continuously recorded during the experiments (Fig. 1).

Nematodes were extracted from 5 g aliquots of roots stained with acid fuchsin, by maceration in a blender for 30 seconds at 5000 r.p.m. The water suspension was poured on to a 710 μm sieve to remove large root particles and the nematodes were collected on 45 μm sieve. A portion of roots of each sample was shaken in 1% sodium hypochlorite water solution to release the eggs from the gelatinous matrix (Hussey and Barker, 1973). Second stage juveniles were also recovered from 50 ml aliquots of soil by Baermann funnels.

Results

In both localities, second stage juveniles of *H. goettingiana* were found in pea roots at plant emergence (Figs. 2-7) irrespective of sowing time. They then moulted to third stage juveniles in 1-3 weeks. However in the roots of pea sown at Pisticci from January 18 onwards second and third stage juveniles occurred simultaneously. Soil temperature influenced the appearance of fourth stage juveniles; they were usually observed 2-3 weeks after the second stage, but at shorter intervals during warmer periods.

Females were observed for the first time on 15-20 October on the roots of pea sown in the first half of September (Figs 2A, 5A), only one week after the fourth stage, but thereafter and through the winter months, with lower soil temperatures, longer time was required to reach the adult stage. In spring, with the rise of temperature, all developmental stages were found in the roots shortly after plant invasion. Thus, adult stages were reached in nine weeks in winter (Fig. 3B) and two weeks in spring (Fig. 7 C-F) after emergence of the peas.

Egg sacs usually protruded one week later, and eggs were laid in them after 2-3 weeks. The average content of an egg sac was 153 eggs. When females matured from late March onwards (soil temperature $> 15^{\circ}\text{C}$) egg sacs were small or absent, and when present they contained only a few eggs.

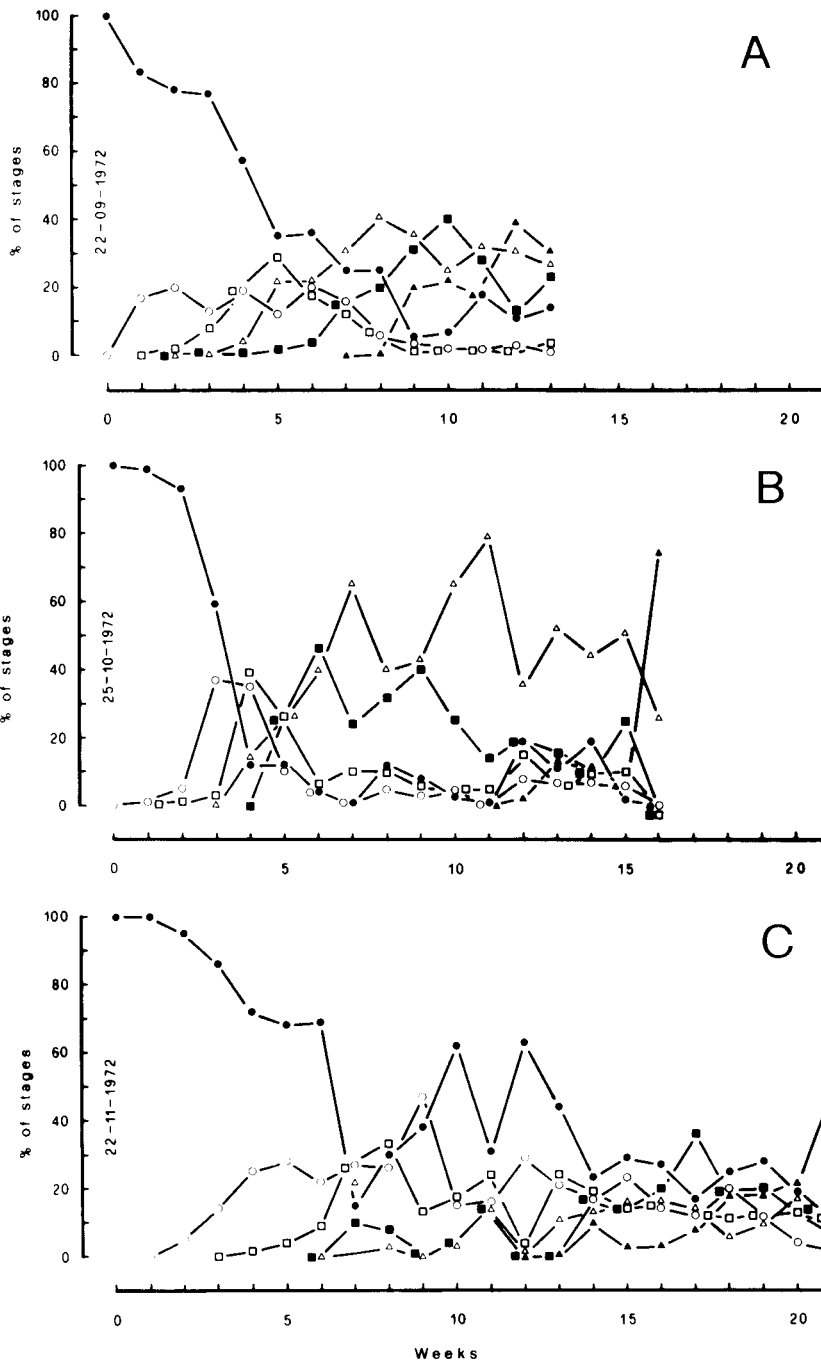


Fig. 2 - Per cent of developmental stages of *Heterodera goettingiana* recovered, at Margherita di Savoia, from 5g of roots of pea emerged on 22 September (A), 25 October (B) and 22 November 1972 (C): ● J2; ○ J3; □ J4; △ ♀; ■ ♂; ▲ cyst.

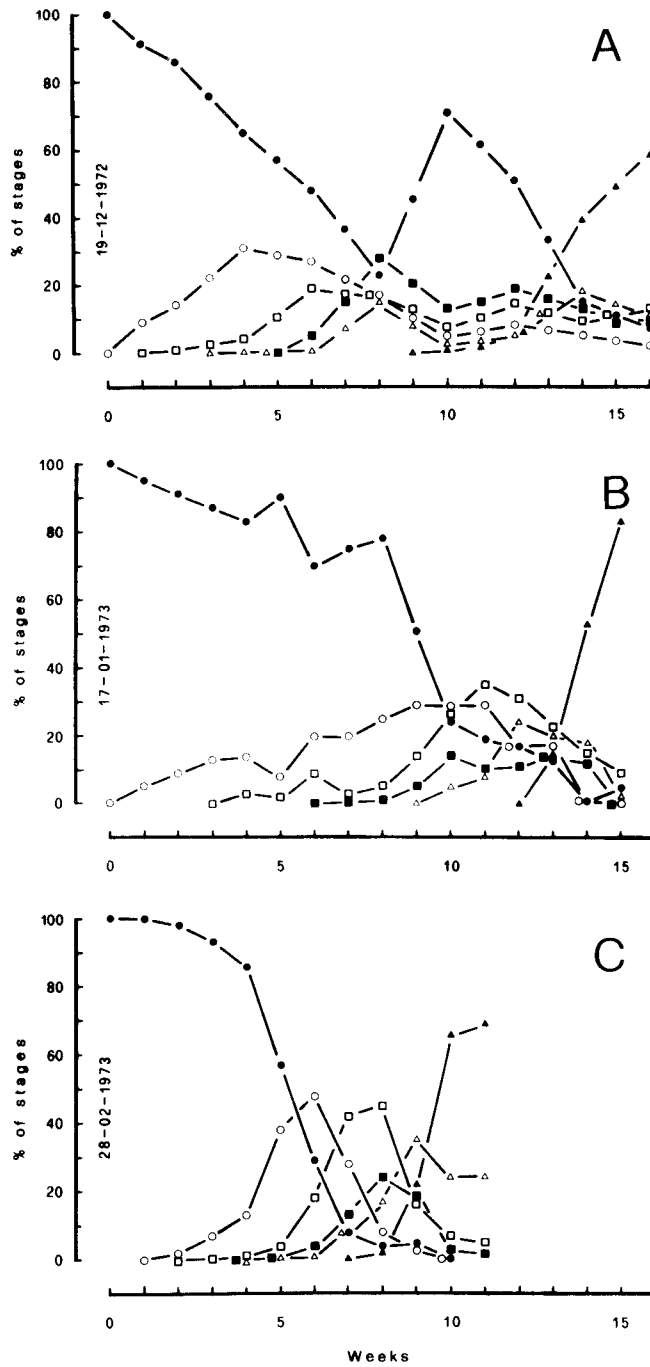


Fig. 3 - Per cent of developmental stages of *Heterodera goettingiana* recovered, at Margherita di Savoia, from 5g of roots of pea emerged on 19 December 1972 (A), 17 January (B) and 28 February 1973 (C): ● J2; ○ J3; □ J4; △ ♀; ■ ♂; ▲ cyst.

Cysts of the first generation occurred during the first half of November (Figs 2A, 5A) some 45-55 days after penetration of the roots of early sown peas by the second stage juveniles, but 12-15 weeks with winter sowings (Figs 2C, 5C, 6A-B). Dark-brown cysts contained 265 eggs each, mostly embryonated. Males were found within the roots simultaneously with the females. No root invasion occurred at Pisticci on peas sown from May 28 to August 10.

Second stage juveniles were recovered from field soil until mid May both at Margherita di Savoia (Table II) and Pisticci (Table III), the largest numbers being present throughout April. No juveniles were recovered later.

In investigating the occurrence of a second generation, the roots of peas sown in pots on 21 February, contained both second and third stage juveniles by 21 March, fourth stages by 5 April, females and males by 11 April and cysts by 26 April (Fig. 8A). In this case only two females, out of 3066, protruded egg sacs in which no eggs were laid.

In pots sown on June 6, investigating the possible occurrence of a third generation of *H. goettingiana*, no juveniles were found in the roots.

Finally, more nematodes were collected from the roots of peas grown in pots and inoculated with egg sacs than with cysts, despite the larger numbers of eggs contained within the cysts (Fig. 8B).

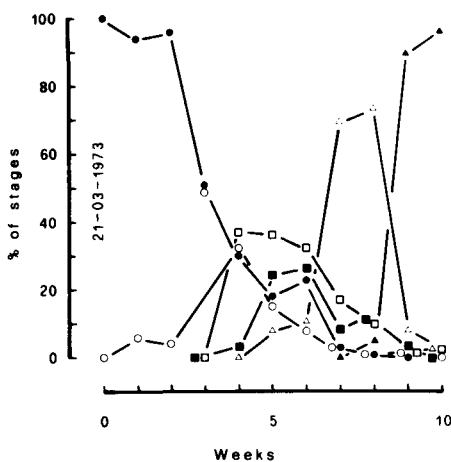


Fig. 4 - Per cent of developmental stages of *Heterodera goettingiana* recovered, at Margherita di Savoia, from 5g of roots of pea emerged on 21 March 1973: ● J2; ○ J3; □ J4; △ ♀; ■ ♂; ▲ cyst.

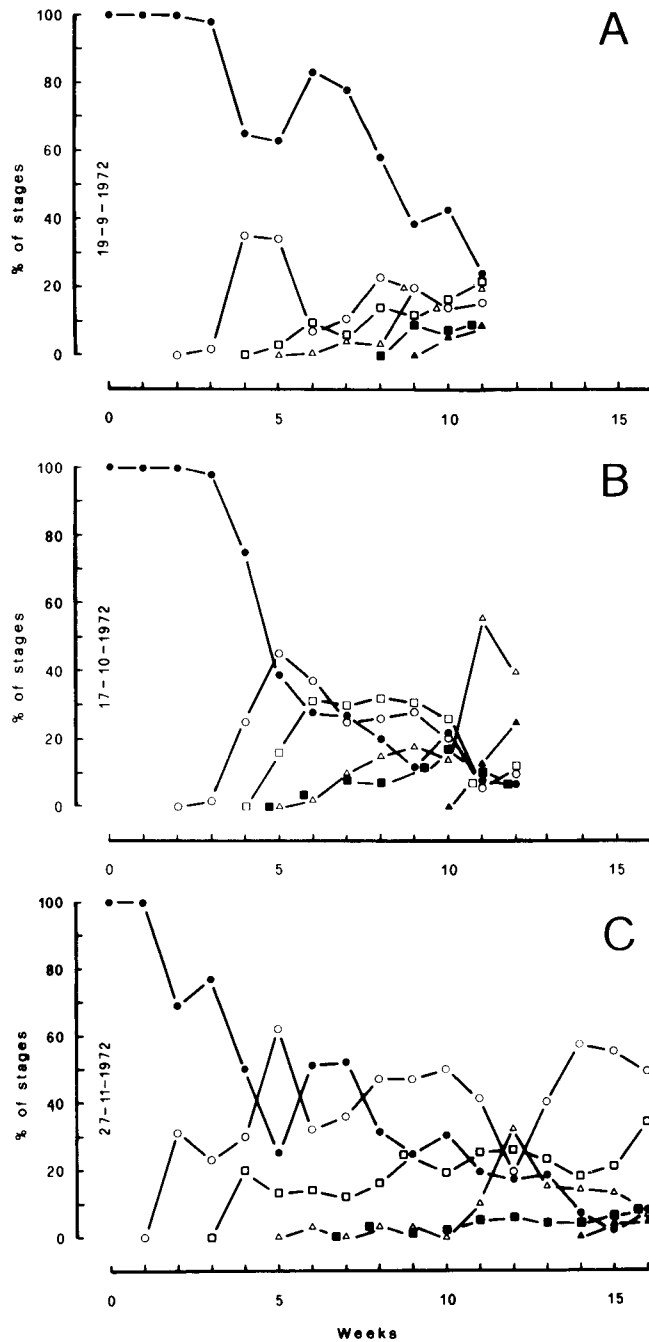


Fig. 5 - Per cent of developmental stages of *Heterodera goettingiana* recovered, at Pisticci, from 5g of roots of pea emerged on 19 September (A), 17 October (B) and 27 November 1972 (C): ● J2; ○ J3; □ J4; △ ♀; ■ ♂; ▲ cyst.

Table II - Numbers of second stage juveniles collected from 50 ml of soil at Margherita di Savoia.

Emergence of plants	Weeks after plant emergence																				Last sampling			
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		20	21	
22-09-72	29	155	122	130	148	341	193	124	130	62	289	22	87	27										19-12-72
25-10-72	67	106	102	160	150	239	956	349	168	365	100	49	39	194	86	11								07-02-73
22-11-72	37	56	22	22	126	119	25	163	401	339	336	25	342	420	77	85	14	30	78	7	9	197	19-04-73	
19-12-72	40	39	15	16	39	52	64	91	111	70	101	152	176	230	288	71	9							11-04-73
17-01-73	75	49	70	14	123	81	40	86	62	122	74	52	58	58	35	75								02-05-73
28-02-73	13	11	47	20	35	65	39	98	8	1	1													16-05-73
21-03-73	7	45	72	20	62	36	160	7	35	8	1													30-05-73

Table III - Numbers of second stage juveniles collected from 50 ml of soil at Pisticci.

Emergence of plants	Weeks after plant emergence																Last sampling	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		16
19-09-72	2	3	2	31	106	58	212	36	19	28	66	98						04-12-72
17-10-72	7	60	15	60	28	134	130	208	117	105	87	52						15-01-73
27-11-72	34	64	63	34	24	73	109	167	103	269	183	319	131	196	54	135		26-03-73
11-12-72	90	33	8	83	84	142	137	162	173	212	175	251	496	235	78	60		09-04-73
27-12-72	19	109	53	134	109	234	260	904	635	396	508	562	182	104	17	36	17	30-04-73
29-01-73	50	74	187	122	140	285	201	207	312	245	103	140	68	8	0			08-05-73
24-02-73	99	103	93	205	402	421	245	161	50	7	0	0						14-05-73
09-03-73	153	118	256	276	624	120	81	2	42	0								14-05-73
02-04-73	175	522	164	18	1	0	0	0										21-05-73
09-04-73	240	162	290	10	0	0	0											21-05-73
30-04-73	8	37	0	0														21-05-73
08-05-73	0	0	0	0	0	0												11-06-73

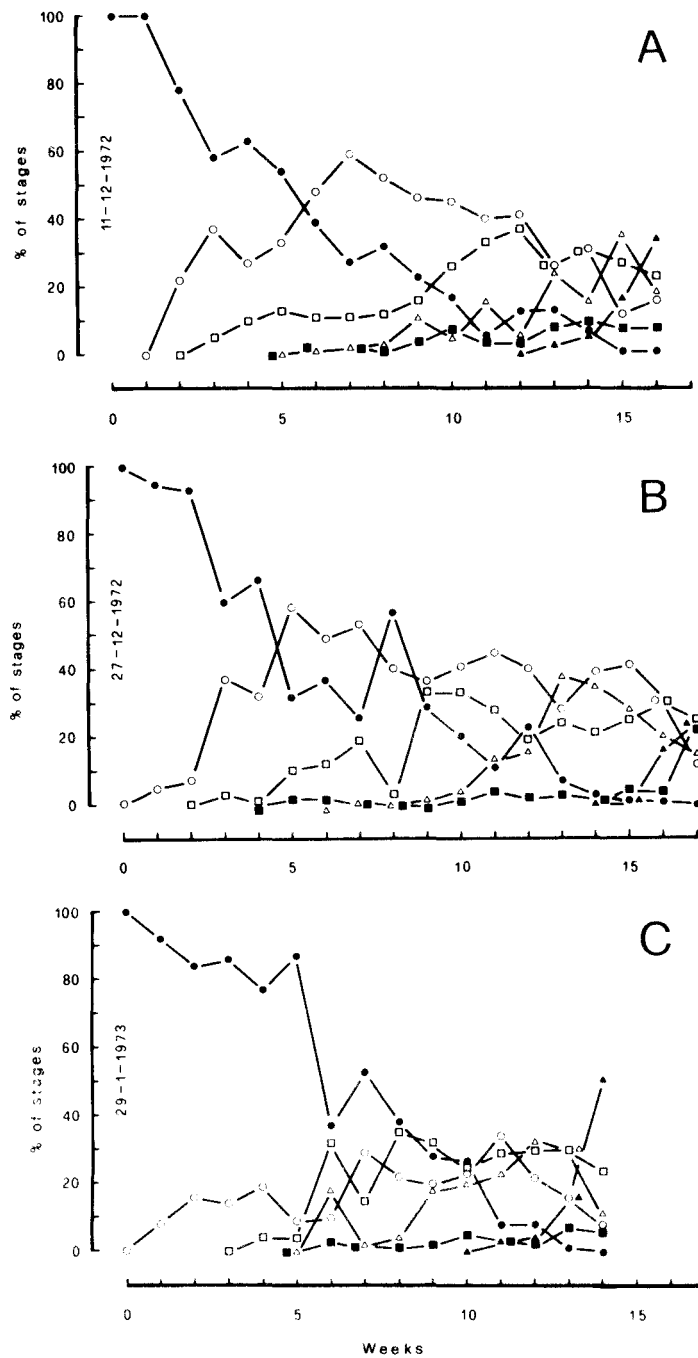


Fig. 6 - Per cent of developmental stages of *Heterodera goettingiana* recovered, at Pisticci, from 5g of roots of pea emerged on 11 (A) and 27 (B) December 1972 and 29 January 1973 (C): ● J2; ○ J3; □ J4; △ ♀; ■ ♂; ▲ cyst.

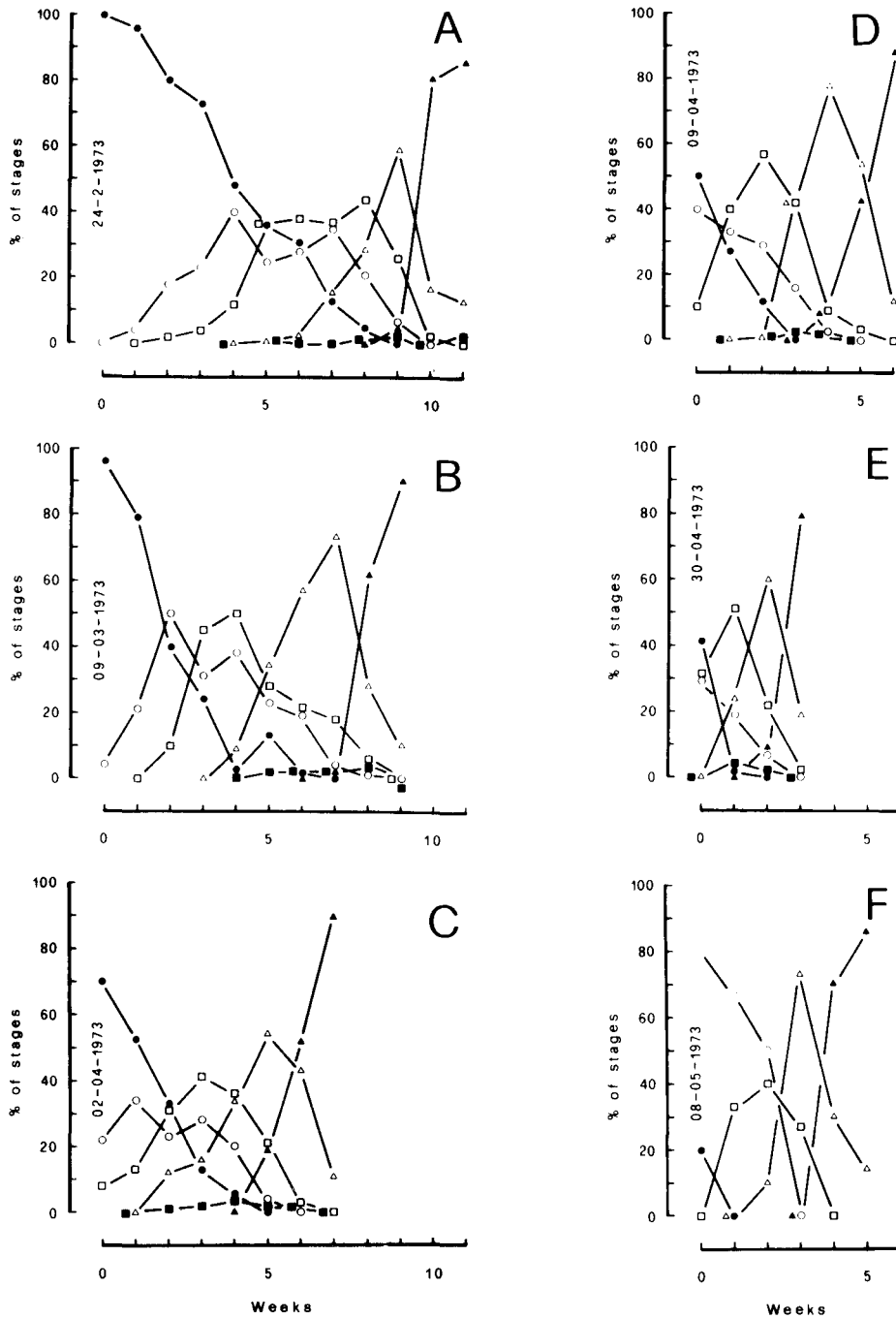


Fig. 7 - Per cent of developmental stages of *Heterodera goettingiana* recovered, at Pisticci, from 5g of roots of pea emerged on 24 February (A), 9 March (B), 2 April (C), 9 April (D), 30 April (E) and 8 May 1973 (F): ● J2; ○ J3; □ J4; △ ♀; ■ ♂; ▲ cyst.

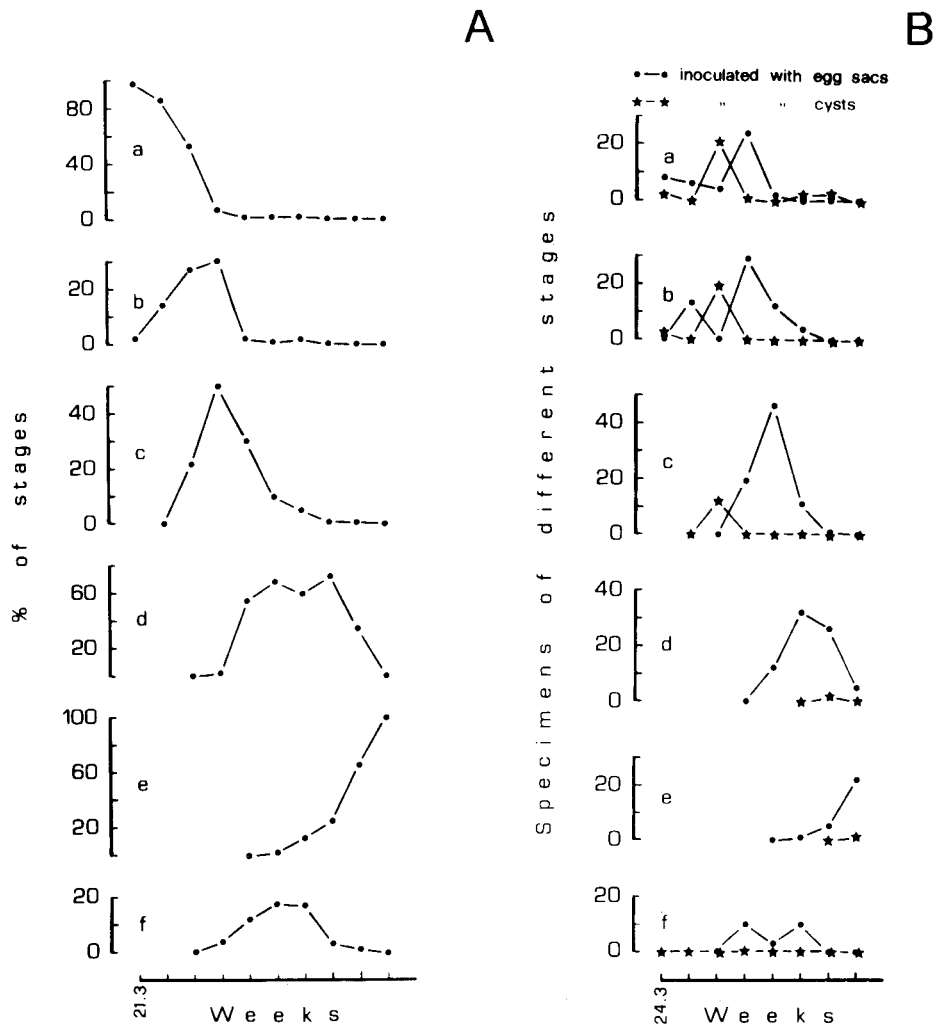


Fig. 8 - Per cent of developmental stages of *Heterodera goettingiana* recovered, at Margherita di Savoia, from 5g of roots of pea sown in pots on (A) 21 February and inoculated with pea roots infested with cysts and egg sacs of the first generation of the nematode or (B) on 26 February and inoculated with either 25 newly formed cysts or 25 egg sacs of the nematode: a J2; b J3; c J4; d ♀; e cyst; f ♂.

Discussion and Conclusion

Field and pot experiments indicate that the life cycle of *H. goettingiana* (Fig. 9) is greatly affected by soil temperature and soil moisture. In autumn and winter, with low temperature and high soil moisture, all developmental stages of the nematode persisted longer, while in spring they had shorter life spans. Development from second stage juveniles to cysts required up to 15 weeks in autumn and winter, compared with a much shorter time in the spring, in one case only three weeks. On several occasions it was observed that when soil temperatures rose, fourth stage females soon reached the cyst stage but they were small with a thin cuticle and contained only a few eggs. When late spring and summer soil temperatures were above 25°C (Fig. 1) no specimens were recovered from the pea roots, even though plots and pots were irrigated, because the high soil temperature inhibited the emergence of second stage juveniles from the eggs (Greco, unpublished).

Guevara-Benitez *et al.* (1970) in Spain and Beane and Perry (1984) in England, did not report the occurrence of eggs within the egg sacs of *H. goettingiana*, but Jones (1950) stated that a variable number of them (possibly affected by drought) is sometimes present. In our investigations too, maturation of females and egg laying were greatly affected by environmental conditions. Large egg sacs were formed in autumn and winter when the soil temperatures were in the range 10-12°C; but from late spring onwards soil temperatures were above 14-15°C and the egg sacs were absent or small, containing few eggs, and females developed to cysts earlier, probably in response to unsuitable soil conditions. These data agree with field observations, since egg sacs are commonly found through early April in cool and rainy years.

Juveniles emerged promptly from egg sacs, but a substantial hatch occurs of the eggs within newly formed cysts only after two months (Greco, unpublished). In southern Italy vetch and broad bean are usually sown in October-November and harvested in April-June. However, the pea crop is sown in early autumn or winter and harvested in March-May. Therefore considering that egg laying in the egg sacs take place 50-60 days after root invasion, it can be assumed that in a growing season of the above crops, the nematode would complete only one generation from cyst to cyst on late sown crops, but on early sown crops and if environmental conditions are suitable for the formation of egg sacs, two-three generations per growing season may occur.

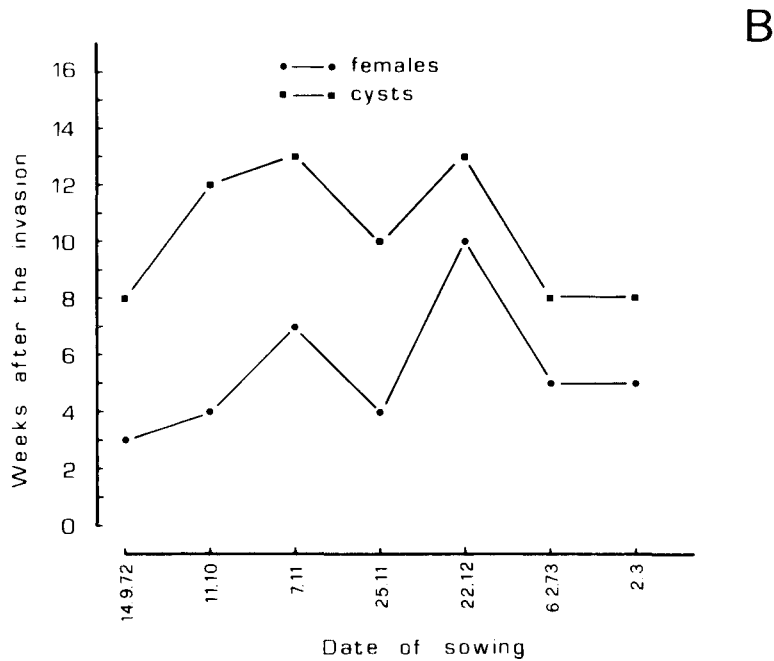
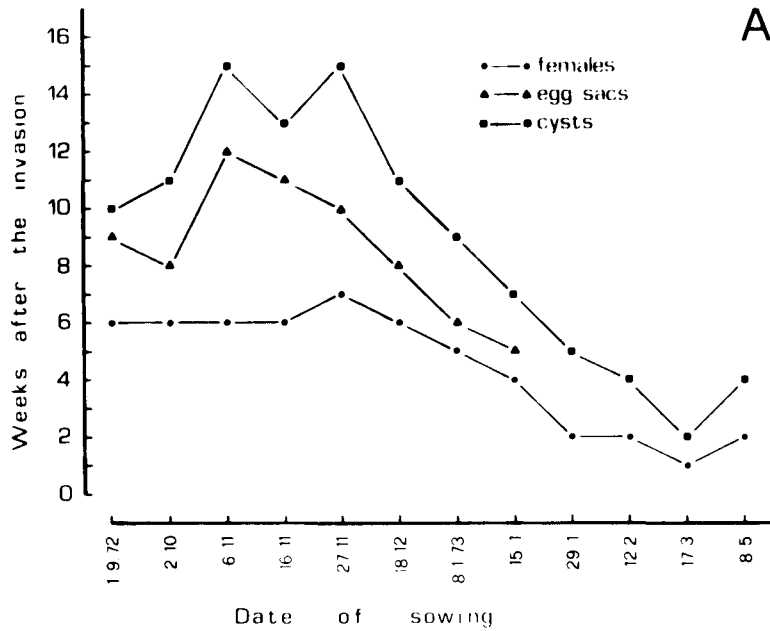


Fig. 9 - Numbers of weeks required by *Heterodera goettingiana* to reach female and cyst stages and to lay eggs in the egg sacs, on root of pea sown at different dates at Pisticci (A) and at Margherita di Savoia (B). (No record of egg sac protrusion was taken at the latter locality).

Considerable growth reduction of pea plants occurs at population densities larger than 4.4 eggs of *H. goettingiana*/ml of soil (Di Vito *et al.*, 1978). Chemical control has been proved to be effective against the pea cyst nematode (Di Vito *et al.*, 1976; Whitehead *et al.*, 1979) but it is expensive. Crop rotation, instead, is suggested to reduce the nematode population to non-damaging levels. The number of generations completed by the nematode in a growing season would greatly affect its rate of reproduction and therefore the length of the rotation required to limit the yield losses of host crops. Late autumn and winter sowings would allow only one generation of the nematode to be completed and therefore they are recommended for infested areas in southern Italy.

S U M M A R Y

Investigations were undertaken to study the life cycle of *Heterodera goettingiana* on pea in Southern Italy. The time required by the nematode to complete a generation was up to 16 weeks in winter and as short as 3 weeks in spring indicating that it was greatly affected by soil temperature. In late spring and throughout the summer, when soil temperature was above 25°C, there was no root invasion by the nematode nor second stage juveniles were found in the soil. Egg sacs were well formed and filled of eggs (up to 153/egg sac) in autumn and winter, but they were small with few eggs or absent in spring. More specimens were found in the roots of pea inoculated with egg sacs than in those inoculated with newly formed cysts. *H. goettingiana* usually completes one generation per year, from cyst to cyst, but 2-3 generations per year may occur if egg sacs are produced.

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