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LIFE CYCLE OF *HETERODERA CICERI* ON CHICKPEA[1]

by
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A new species of cyst nematode, *Heterodera ciceri*, was found to cause great damage to chickpea (*Cicer arietinum* L.) in northern Syria (Greco *et al.*, 1984; Vovlas *et al.*, 1985) where this crop is one of the most important pulses. Information on the life cycle and biology of the nematode was lacking, therefore investigations were undertaken on the effect of temperature on the life cycle of *H. ciceri*, invasion of roots by juveniles, reproduction, and the rate of development of different life stages. The histopathological changes caused by the nematode on infested chickpea roots were also investigated.

Materials and Methods

A population of the nematode collected from Syria and maintained on chickpea (cv. ILC 482) was used for these studies. Second stage juveniles were obtained by incubating cysts of the nematode in chickpea root leachate at 20°C and collecting them every two days.

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The development of *H. ciceri* was investigated at 8, 10, 12, 14 or 16°C. Twenty plastic pots, containing 170 cm^3 of a sandy soil (98% sand), were sown with two pregerminated seeds of chickpea cv. ILC 482. When the plants were ten days old, each pot was inoculated with 2000 second stage juveniles of *H. ciceri*, and four pots were maintained in a growing chamber at each temperature. After 2 weeks the plants were uprooted and the nematodes extracted, counted and classified as normal second stage, enlarged second stage, or third stage juveniles.

To investigate the life cycle of the nematode pregerminated cickpea seeds were sown in plastic trays containing sandy soil infested with 20 eggs/cm^3 of *H. ciceri*. The trays were maintained in a growing chamber at 20°C (soil temperature) with 14 hrs light/day. Three days after plant emergence and then at 4-days intervals, four or five plants were uprooted and their roots weighed and comminuted (Fallis, 1943) for nematode extraction. The nematode specimens were counted and classified according to their developmental stage. When mature females were formed, some were incubated at 20°C in chickpea root leachate and others were crushed to determine embryogenic development and the duration of each phase.

The reproduction of *H. ciceri* was investigated at 15, 20 and 25°C. Twelve clay pots containing 750 cm^3 of sandy soil were sown with two chickpea seeds/pot and when the plants were two weeks old each pot was inoculated with 4000 second stage juveniles. Four pots were then transferred to growing chambers set at the different temperatures. Three months later the soil from each pot was dried, mixed, and the cysts from a 200g sample extracted by the Fenwick can and crushed to determine their egg content.

For histopathological studies infested root segments of five and 21 day old chickpea seedlings were fixed in Randolph's modified Navashin fluid for 24 hrs, dehydrated in TBA (tertiary butyl alcohol) series and embedded in paraffin. Microtome sections, 10-15 μm thick, were mounted on glass slides, stained with safranin and fast-green, mounted permanently in dummar xylene, examined microscopically, and microphotographed (Johansen, 1940).

Results

No development of *H. ciceri* occurred after two weeks at 8°C (Tab. I), but a few (3%) enlarged second stage juveniles were found at 10°C and

- *Effect of different temperatures on the development of* Heterodera ciceri *in roots of chickpea, two weeks after inoculation with second stage juveniles.*

Temperature °C	% of developmental stage								
	Normal JJ2			Enlarged JJ2			JJ3		
8	100	a	A	0	a	A	0	a	A
10	97	a	A	3	a	A	0	a	A
12	44	b	B	56	b	B	0	a	A
14	14	c	C	83	c	B	3	a	A
16	3	d	C	82	c	B	15	b	B

many more (56-83%) at the higher temperatures, suggesting that the minimum temperature for the development of the nematode is 10°C. From these findings the accumulated day degrees above 10°C, required by the nematode to reach different developmental stages, were estimated (Fig. 1) assuming that in the investigation on the life cycle of the nematode, second stage juveniles had penetrated the roots of the chickpea one day before emergence of the seedlings.

In the investigation of the life cycle of the nematode, chickpea plants emerged two days after sowing pregerminated seeds. Second stage juveniles were observed in the roots five days after sowing (Fig. 1 and Tab. II). Necrotic scars were evident at penetration loci of the juveniles on the roots of the host plant (Fig. 2A). Third stage juveniles were observed nine days after sowing and were shorter and slightly more robust than second stage juveniles. Sexual dimorphism was obvious after the third moult. Both females and male fourth stage juveniles were observed about 13 days after sowing the pregerminated seeds (Fig. 1 and Tab. II). The fourth stage females lost their slender appearance and developed the typical flask shaped form; ovaries then started to develop and fill the body cavities. The fourth stage male, casting the cuticle of the third stage juvenile, grew by forming several coils within the cast cuticle. Adult females and males were observed 19 days after sowing, when 180 day degrees had accumulated (Fig. 1). The females were lemon-shaped and varied in size. Their spears were slender and the median bulbs were larger than those in the previous stages; the ovaries filled almost all the body cavity. Males were slender and wormlike in shape with well developed testes and spicules.

Second and third stage juveniles and fourth stage males developed inside the root tissues after the initial penetration of the host by the second

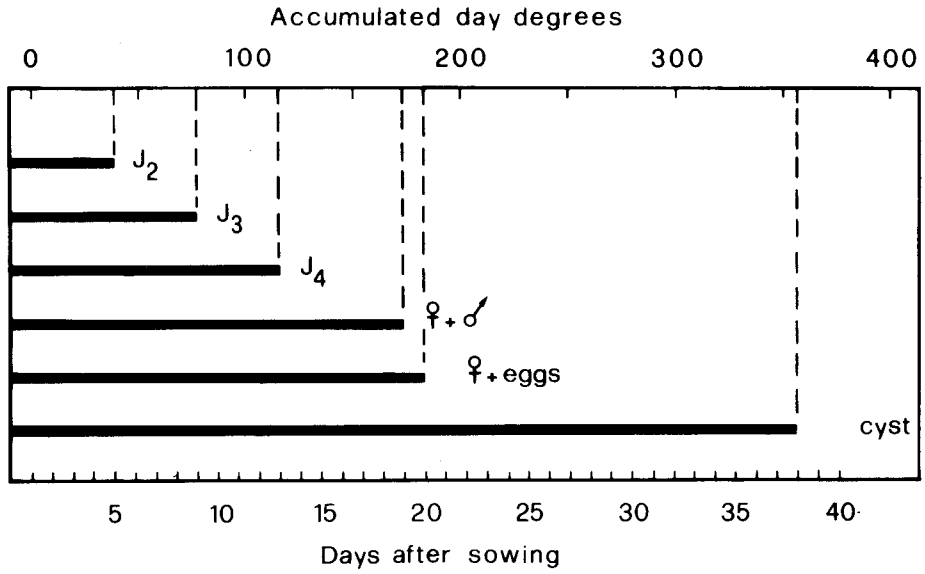

Fig. 1 - Time of appearance and day degrees required for developmental stages of *H. ciceri* in roots of chickpea seedlings grown at 20°C.

Table II - *Percent of developmental stages of* Heterodera ciceri *detected in roots of chickpea at 20°C during a period of 48 days.*

Developmental stages	Days after sowing											
	5	9	13	19	21	24	28	32	38	40	44	48
J2	100	87	60	4.0	6.0	0	0.5	3.0	0	0	0	0
J3		13	30.5	24.0	6.0	6.0	3.5	3.0	0	0	0	0
J4♀			6.5	8.0	0	0	0	0	0	0	0	0
J4♂			3.0	4.0	12	6.0	7.0	0	0	0	0	0
♀				8.0	47	47	55.0	77.5	63.5	69	7.0	45.5
♂				52.0	29	41.0	34.5	16.5	8.5	0	4.0	4.5
cyst									28.0	31	89.0	50.0

stage juveniles, but fourth stage females ruptured the cortex of the roots and were visible externally (Fig. 2B). Only the anterior part of the female remained inside the root tissue. Well developed eggs occurred within white females 20 days after sowing (190 days degrees). Later, a few females were seen to protrude a small gelatinous matrix, the egg sac, but in which

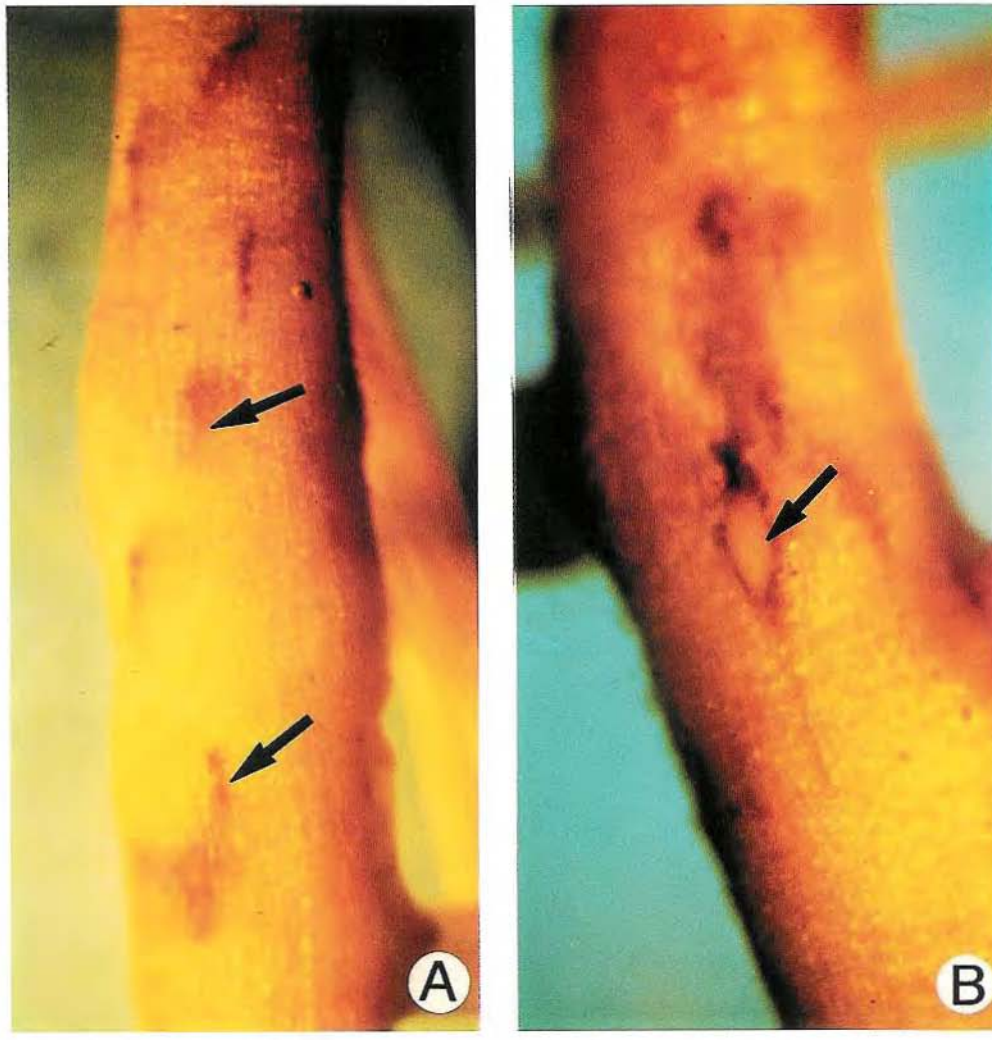

Fig. 2 - Chickpea roots infested with *H. ciceri*. A) Superficial necrotic area (arrowed) at the penetration loci of second stage juveniles. B) Necrotic symptoms and a fourth stage female (arrowed) rupturing the rhizoderm.

no eggs were laid. All the eggs were retained within the female body and an average of 256 eggs were counted per adult female, 37 days after planting. At this time (360 day degrees) 37% of the eggs were embryonated and second stage juveniles emerged from the incubated white females. The females started to change colour to become light brown. At 38 days (370 day degrees) some brown cysts were already formed.

Second and third stage juveniles were observed during a period of about 20-24 days after sowing (Tab. II). Many second stage juveniles developed into the third stage within a short period. Fourth stage males persisted longer than fourth stage females; the latter were observed for a period of six days while fourth stage males were seen for 15 days. At the end of the experiment, 48 days after sowing, half of the nematode specimens within the roots were white females and the others were brown cysts. At this time the males had moved into the soil.

Observations on the embryogenesis of *H. ciceri* revealed that the first cleavage of the single celled egg was equatorial resulting in two blastomeres of equal size within a period of two days (Figs. 3-4). All time periods mentioned hereafter start with the egg laying stage. Both second and third egg divisions were transversal. The second and the following divisions up to the multicellular egg stage were completed within a period of seven days. The gastrula stage and the stage of embryo with two flexures were observed after ten and twelve days, respectively. The first stage juveniles appeared in about 15 days and the second stage in about 17 days. Some juveniles emerged one day after they were formed. Therefore, the average time required for the emergence of second stage juveniles was about 18 days at 20°C, close to that reported by Vovlas and Inserra (1983) for *H. mediterranea*, which was 15-18 days at 24° ± 2°C.

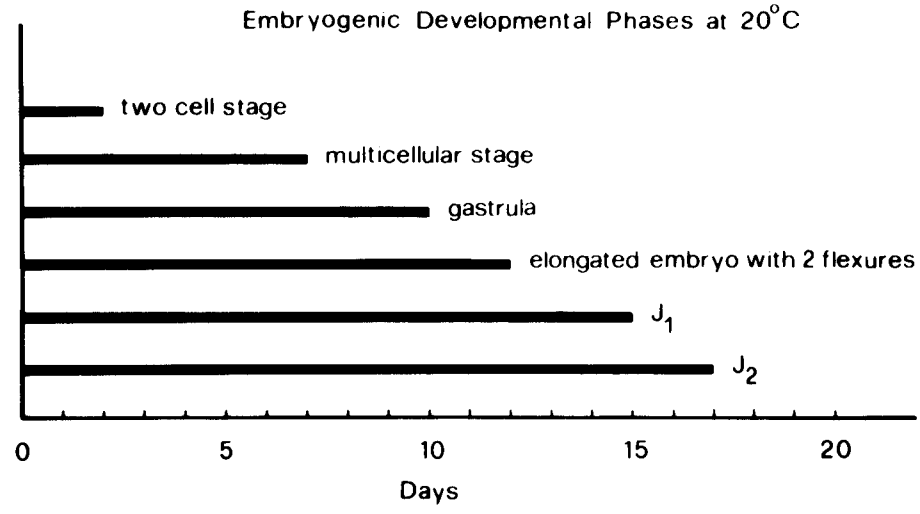

Fig. 3 - Phases of embryogenic development of newly formed eggs of *H. ciceri*, within the adult females, on root of chickpea grown at 20°C.

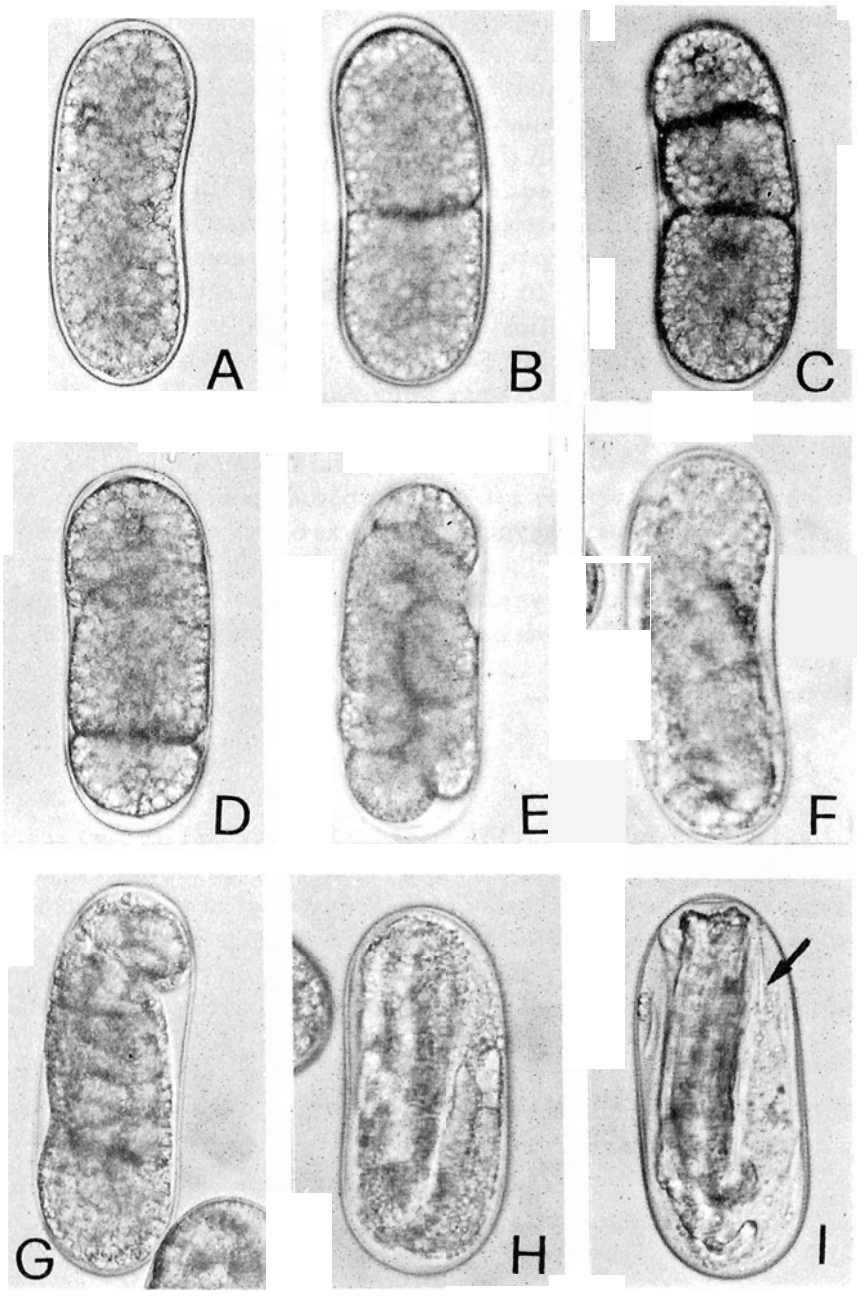

Fig. 4 - Embryogenic development of *H. ciceri*. A) Egg at one-cell stage. B) Two-cell stage. C) Three-cell stage. D) Four-cell stage. E) Multicellar-cell stage. F) Gastrula. G) Elongated embryo with two flexures. H) First stage juvenile. I) Second stage juvenile with developed stylet (arrowed). (Mean dimension of embryonated eggs 50×134 μm).

The reproduction of *H. ciceri* was similar at all tested temperatures (Tab. III) even though significantly more cysts were found at 15 and 25°C. Nevertheless, it appeared that larger nematode population densities may occur at 20 and 25°C, because by the end of the experiment many more eggs were contained within the cysts formed at these temperatures.

Heterodera ciceri induced cellular alterations in cortical, endodermal, pericyclic, and vascular parenchyma tissues of infested chickpea roots (Fig. 5). The second stage juveniles penetrated the primary roots and settled near the central cylinder. In the early stage of infection (3-5 days after penetration) cellular modifications were observed in the vicinity of the head of the nematode (Fig. 5B). Syncytial development was restricted primarily to pericyclic and adjacent vascular tissues when the nematode feeding site was in the pericycle, but cortical feeding sites were also observed. Thick cell walls, cell-wall dissolution and fragmentation in syncytial cells were evident five days after the nematode penetration (Fig. 5B, C, D). The cytoplasm of active syncytia cells was dense, granulated, and contained numerous hypertrophied nuclei and nucleoli (Fig. 5F). Thirty days after penetration, secondary root compression of xylem elements and disorder of stelar structure were common in infested chickpea roots (Fig. 5E, F).

Conclusions

The results show that the life cycle of *H. ciceri* and its host reaction are similar, in their general patterns, to those observed for other cyst forming nematodes. The times required for second stage or third stage juveniles to develop respectively to third and fourth stage juveniles, was similar to those reported for *H. schachtii* (Raski, 1950) at 19.3°C and *H. mediterranea* at 24-30°C (Vovlas and Inserra, 1983). Fourth stage juveniles of

Table III - *Reproduction of* Heterodera ciceri *on chickpea at different temperatures.*

Temperature °C	Cyst/200g of soil			Eggs/200g of soil	
15	67	a	b	887	a
20	57		b	3050	a
25	83	a		3790	a

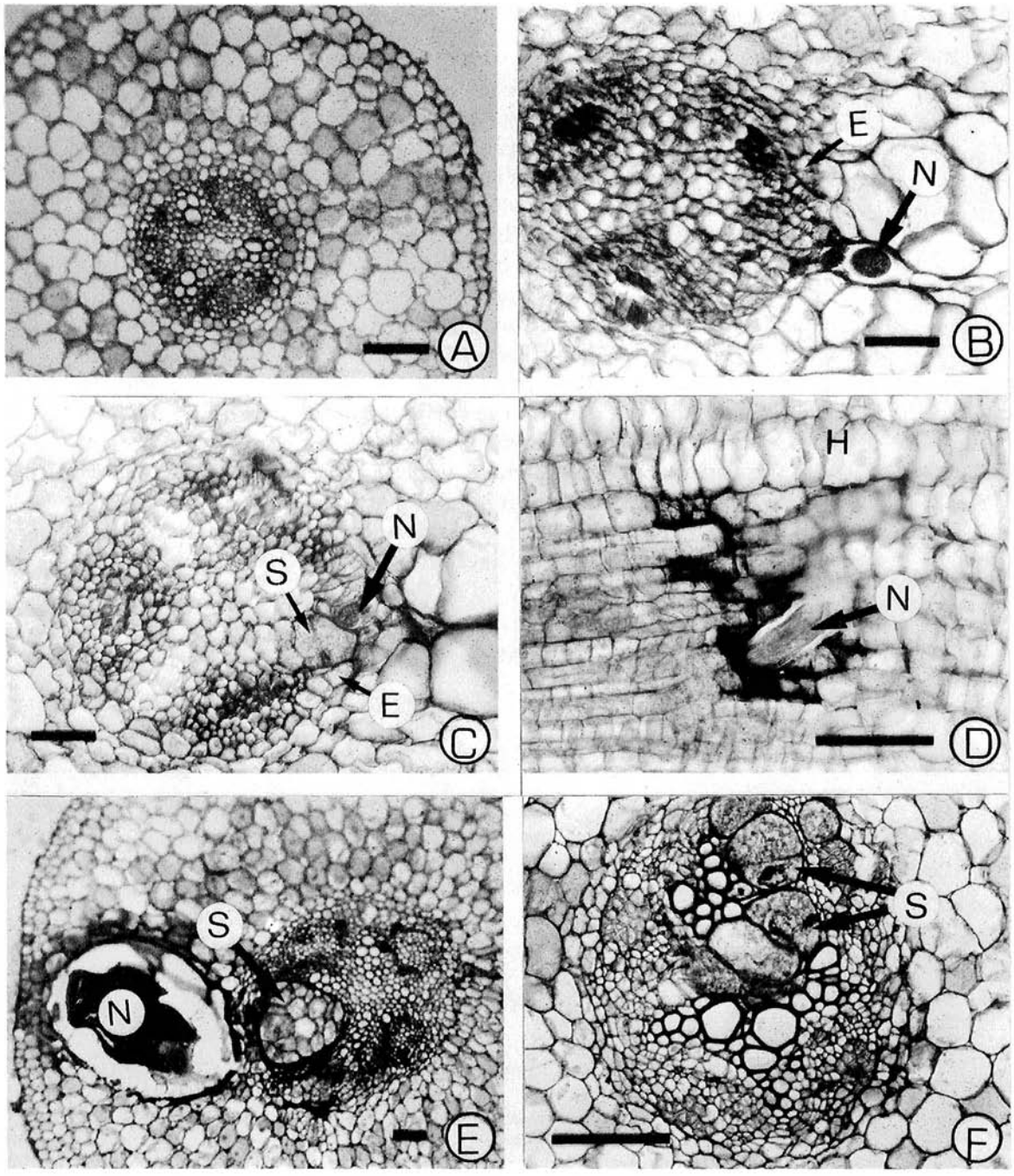

Fig. 5 - Normal and infested roots of chickpea showing penetration, establishment of infection and anatomical changes induced by *H. ciceri*. A) Young primary root cross section. B, C) Cross sections of primary roots with juveniles (N) in contact with endodermis. Note the hypertrophied cells forming a small stelar syncytium (S). D) Longitudinal section showing cortical lesions around the nematode (N) body and hypertrophied syncytium-initial cells (H). E, F) Cross section of 21 day old infested chickpea roots showing stelar syncytia (S) and disorder of stelar structures: N = nematode body. (Scale bar = 50 μm).

H. schachtii and *H. mediterranea* were observed after 15 and 18-21 days, respectively, compared to the 13 days for *H. ciceri*, at the above temperatures. Female *H. ciceri* with embryonated eggs had developed by 340 day degrees and cysts by 370 day degrees close to 290 and 340 day degrees reported for the same stages of development in *H. schachtii* (Raski, 1950; Greco *et. al.*, 1982), but less than 416 and 519 day degrees required by *Globodera rostochiensis* in Cyprus (Philis, 1980) to reach these stages on autumn and spring planted potato crops.

It is noteworthy that *H. ciceri* can invade roots of chickpea even at 8°C, indicating that in the field root invasion may occur in the winter. However, at 10°C egg hatch is poor (Kaloshian *et al.*, 1986) and juvenile development slow. Therefore, the life cycle of the nematode would proceed slowly in the winter, but early in the spring massive invasion of the host plant roots and rapid development of the nematodes may be expected. Later in the spring low soil moisture, which is usual in the dry areas of middle east countries, would limit hatching of eggs, migration of juveniles and invasion of the host roots. Therefore only one generation of the nematode per growing season is likely to occur.

SUMMARY

Investigations on the life cycle of *Heterodera ciceri* at 20°C revealed that coiled second stage juveniles were found inside newly formed eggs 17 days after they were produced. Females, females containing embryonated eggs, and cysts, were observed on the roots of chickpea 19, 36 and 38 days after sowing, equivalent to an accumulation of 180, 350 and 370 day degrees, respectively, above the 10°C basal temperature. The largest nematode populations were obtained at 20-25°C. Syncytia formation and disorder of the root stelar structures are the main anatomical changes induced by the parasite. It is inferred that only one generation of the nematode per growing season of the host crop would be completed under field conditions.

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