Nematol. medit. (1989), 17: 57-60

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STUDIES ON EMBRYONIC AND POSTEMBRYONIC DEVELOPMENT OF *APHELENCHOIDES BESSEYI*

by
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Summary. *Aphelenchoides besseyi* reached the growing point of rice seedlings between one to five days, depending on the time of inoculation. Post embryonic development was marked by multiplication of nuclei, both between and during the moults. Males developed faster and were recorded two days earlier than females. The life cycle from egg to egg was completed in 11 days.

Of the nematodes parasitizing rice, *Aphelenchoides besseyi*, Christie, the causal agent of the white tip disease of rice, is perhaps the best known. It has been extensively investigated with regard to disease development and management but so far its biology has not received much attention. Particularly, information on sequential develpment of its life stages is lacking and the results of investigations on these aspects are reported here.

Material and methods

The population of *A. besseyi* used was obtained from infected rice seeds and cultured on the fungus (*Fusarium solani*) maintained on potato dextrose agar medium. The investigations comprised the time taken by the nematode to reach the growing point of seedlings and its subsequent development i.e. egg laying, embryology and post-embryological development.

For the first part of the investigation, 5 cm diameter petri dishes containing 20 ml sterilized soil were planted with (i) diseased seed (about 16 nematodes/seed), (ii) healthy seed inoculated with 100 active nematodes per seed and (iii) three days old seedlings inoculated with 100 active nematodes. There were 25 replications in each treatment. Four replications of each treatment were washed daily and hand sections of the seedlings were cut to ascertain the progress of the nematode towards the growing point.

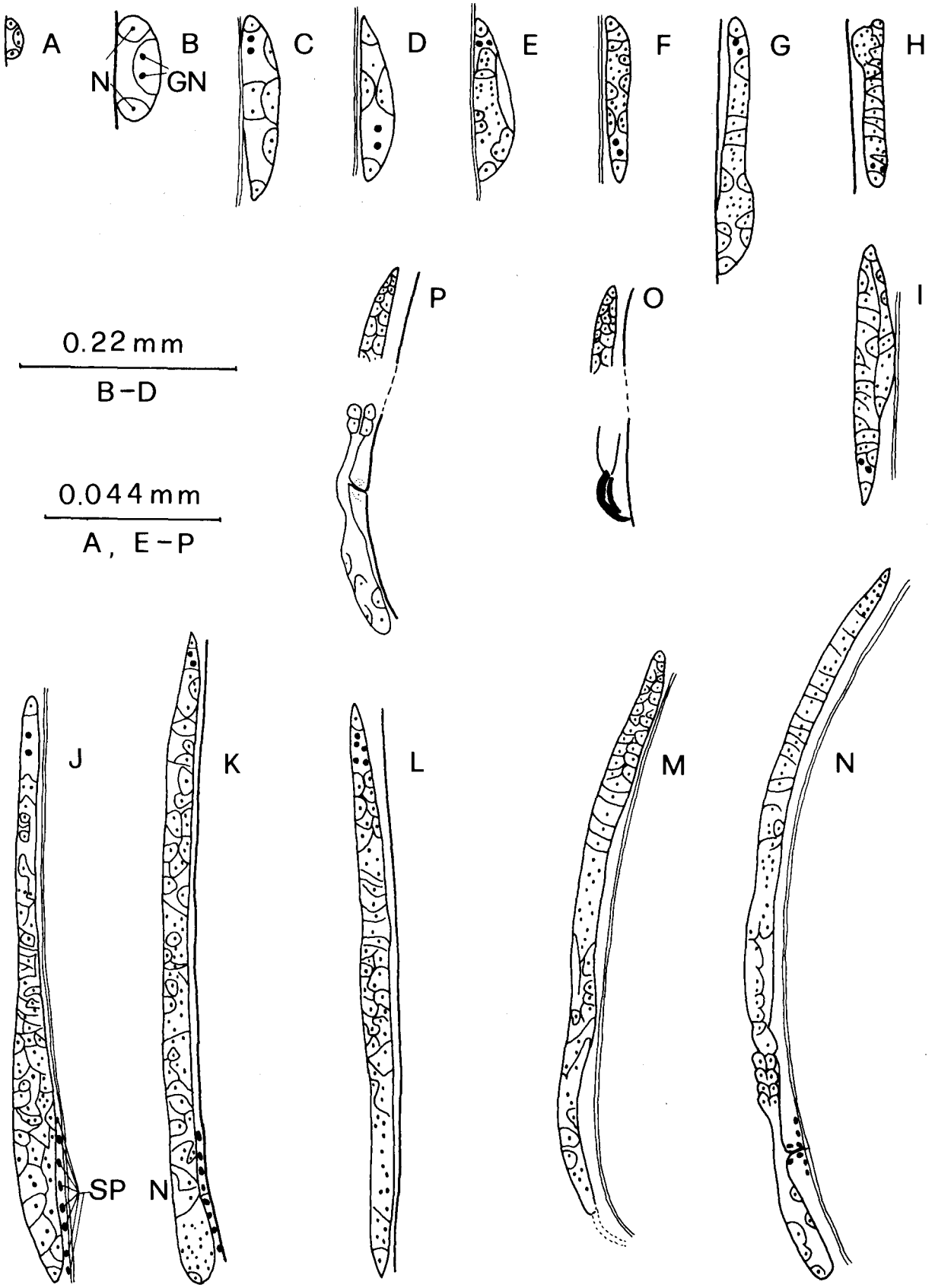
To study egg laying, embryology and postembriological development, three days old seedlings were inoculated with 100 active nematodes (4 ♀ : 1 ♂). As it had been earlier ascertained that the nematodes reach the growing point within 24 hours, each day four seedlings were harvested to record the developmental stages of the nematode. The seedlings were teased out in water to release the eggs and the developing stages of the nematode. To study embryonic development eggs were mounted singly in a drop of distilled water on a coverglass which was then inverted over the cavity of a cavity slide. The water was changed periodically. The slides were examined under an oil immersion objective of the microscope. When not required the cavity slides were stored in the dark at room temperature (25-30°C). For differentiating the post embryonic stages, the nematodes were stained either with acetic orcein or acid fuchsin to follow gonad development.

Results

Compared to the treatment where healthy seeds were sown and then inoculated with nematodes, the nematode infested seed took one day longer to sprout. Further, the nematode activation was apparently delayed and it was five days before they were found at the growing point. Already germinated seed facilitated nematode entry and the nematodes were detected at the growing point 24 hours after inoculation compared with four days where ungerminated seeds were exposed to nematodes.

Eggs were recovered from the 4th day after the seedlings were inoculated with nematodes. They were unsegmented and measured 69-110 µm long and 40-66 µm wide. Embryonic development was completed within 48 hrs and moulting first stage juveniles were observed in the eggs. The embryonic development followed the same general pattern as recorded for other plant parasitic nematodes (Dasgupta *et al.*, 1970).

The 2nd stage juveniles emerged from the eggs two days after initiation of embryonic development. They did not develop any further in water, and further development was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followed in seedlings that were harvested daily. Identification of different stages was based on gonad development and length and width of the nematode body (Fig. 1, Table I). The duration of the different embryonic and postembryonic stages is given in Table II.

In the 2nd stage juvenile, che genital primordium was located at 58-69% of body length from the anterior end and consisted of two germinal and two terminal epithelial cells. At this stage, individuals destined to become males exhibited posterior migration of germinal nuclei while those which were developing into females showed the reverse.

Out of total initial inoculum, about 12% 3rd stage males were recorded on the 3rd day after initiation of egg laying. Female 3rd stage juveniles (about 28%) were observed a day later i.e. on 4th day (Table III). The gonad of 3rd stage male juveniles had 21-33 epithelial nuclei and the female juvenile 33 to 49.

TABLE I - *Measurements of post embryonic stages (n = 15) of* Aphelenchoides besseyi.

Developmental stage	Length (μ)	Width (μ)	Gonad length (μ)	Tail length (μ)
2nd stage juvenile	180 – 300 (220 ± 8.1)	10 – 13 (11 ± 0.1)	10 – 14 (12 ± 2.8)	18 – 21 (19 ± 0.6)
2nd moult				
Male	210 – 310 (280 ± 6.1)	12 – 15 (13.5 ± 0.2)	14 – 50 (30 ± 4.8)	17 – 21 (19.5 ± 0.8)
Female	300 – 360 (320 ± 12.1)	13 – 15 (14 ± 0.2)	14 – 60 (38 ± 3.6)	17 – 22 (20 ± 0.8)
3rd stage				
Male	300 – 430 (380 ± 5.8)	12 – 15 (13 ± 0.6)	40 – 80 (50 ± 5.6)	19 – 23 (20.6 ± 0.4)
Female	340 – 490 (420 ± 6.2)	13 – 16 (14 ± 0.5)	50 – 98 (70 ± 4.2)	22 – 27 (21 ± 0.4)
3rd moult				
Male	400 – 460 (420 ± 7.1)	13 – 15 (14 ± 0.5)	68 – 120 (90 ± 4.2)	20 – 27 (22 ± 0.5)
Female	480 – 520 (506 ± 7.4)	16 – 19 (17.2 ± 0.2)	90 – 160 (130 ± 5.1)	21 – 28 (25 ± 0.8)
4th stage				
Male	420 – 590 (480 ± 9.1)	13 – 17 (15 ± 0.6)	100 – 180 (160 ± 4.8)	25 – 30 (27 ± 0.6)
Female	500 – 600 (570 ± 8.9)	16 – 19 (17 ± 0.5)	155 – 180 (160 ± 3.2)	25 – 31 (28 ± 0.5)
4th moult				
Male	430 – 620 (490 ± 7.2)	13 – 17 (15 ± 0.2)	160 – 190 (170 ± 3.2)	31 – 34 (32 ± 0.2)
Female	550 – 700 (600 ± 6.8)	16 – 20 (18 ± 0.6)	170 – 230 (200 ± 4.1)	31 – 39 (34 ± 0.8)
Adult				
Male	430 – 620 (510 ± 6.1)	14 – 17 (15.9 ± 0.4)	180 – 200 (190 ± 2.1)	34 – 36 (35 ± 0.6)
Female	660 – 740 (700 ± 5.8)	17 – 21 (29 ± 0.2)	210 – 230 (220 ± 3.1)	36 – 42 (39 ± 0.8)

Figures in parenthesis are means and standard error of means respectively.

Fig. 1 *(Front page)* - Reproductive system of different stages of *A. besseyi*: A and B - Second stage; C - Female, second moult; D - Male, second moult; E - Female, advanced second moult; F - Male, advanced second moult; G - Female, third stage; H - Male, third stage; I - Male, third moult; J - Female, third moult; K - Female, fourth stage; L - Male, fourth stage; M - Male, fourth moult; N - Female, fourth moult; O - Anterior and posterior parts of male gonad; P - Anterior and posterior parts of female gonads; (EN = Epithelial nuclei; GN = Germinal nuclei; SPCN = Specialized ventral chord nuclei).

TABLE II - *Time required for initiation of embryonic and postembryonic stages of* A. besseyi.

Developmental stage	Time (hrs/days)
Embryonic development	(hrs.)
3 celled	8
8 celled	24
More than 8 celled	32
First stage larvae	40
Postembryonic development	(days)
2nd stage	2
3rd stage male	3
3rd stage female	4
4th stage male	5
4th stage female	7
Adult male	6
Adult female	8
Egg laying	11

Fourth stage males were present on the 5th day. Female 4th stage juveniles were recorded from the 7th day onwards with about 27% of them moulting to adult females within the next 24 hrs (Table III).

During the third moult, in female juveniles, number of specialized ventral chord nuclei increased to eight. The number of epithelial nuclei in 4th stage female juveniles increased to 68-80 and the vaginal area also became visible. Specialized ventral chord nuclei became arranged on either side of the vaginal lumen. In the male juveniles there were 41-59 epithelial nuclei and just prior to the 4th moult multiplication of germinal nuclei was initiated.

Fully developed males were recorded from the 6th day onwards as against adult females observed on the 8th day, indicating quicker development of males. The time from egg laying to adult male and female varied between six-nine days. However, it took 15 days from the time of inoculation or 11 days after initiation of egg laying, for all the juvenile stages to transform either into male or female. The life cycle from egg to egg was completed in 11 days at 28 ± 2°C.

Similar post embryonic development, characterized by continuous and general multiplication of nuclei throughout moults and different stages, has also been reported in *Ditylenchus destructor*, *D. triformis* and species of *Seinura* (Anderson and Darling, 1964; Hirschmann, 1962; Hechler and Taylor, 1966).

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TABLE III - *Per cent developmental stages of* A. besseyi.

Day (after egg laying)	Per cent developmental stage							
	Eggs (unhatched)	II	III ♂	III ♀	IV ♂	IV ♀	♂	♀
1	100							
2	62.0	38.0						
3	18.3	69.4	12.3					
4	15.7	28.2	28.1	28.0				
5	11.4	14.3	18.2	49.2	6.9			
6	3.0	10.0	12.2	50.8	20.2		3.8	
7	—	—	5.0	39.3	16.3	25.0	14.4	—
8	—	—	—	10.1	6.1	42.1	14.3	27.4
9	—	—	—	—	—	31.4	17.4	51.2
10	—	—	—	—	—	14.1	17.0	68.9
11	—	—	—	—	—	—	17.1	82.9

Accepted for publication on 17 April 1989.