# REPRODUCTION AND LONGEVITY OF XIPHINEMA VULGARE (NEMATODA) M.I. Coiro<sup>1</sup>, F. Lamberti<sup>1</sup>, N. Sasanelli<sup>1</sup>, L.W. Duncan<sup>2</sup> and A. Agostinelli<sup>1</sup>

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**Summary**. Development from egg to adult in a population of *Xiphinema vulgare* from Florida took ca. 274 days under Swingle citrumelo in a laboratory study carried out at  $24 \pm 2$  °C and 12 h day length. This result was equivalent to 1,216 day degrees (DD) above a minimum daily threshold temperature of 20 °C. Female longevity was slightly more than 20 months, equivalent to 2,432 DD and reproductive span ca. 13 months. Between month 3 and month 20 a single female produced a 34 progeny, equivalent to 46 DD above 20 °C per progeny. Swingle citrumelo does not seem to be a good host for *X. vulgare*.

*Xiphinema vulgare*, a monosexual species, occurs frequently in the rhizosphere of citrus in the American Tropics (Loof and Sharma, 1979; Lamberti *et al.*, 1987; Crozzoli *et al.*, 1999; Lamberti *et al.*, 2001). In Florida it is associated with decline of Swingle citrumelo rootstock on which, however, it does not seem to reproduce rapidly (Leone *et al.*, 1997). Tests under controlled conditions have indicated that *X. vulgare* can be destructive on rice and tomato (Leone *et al.*, 1999).

Since there is virtually no information on the biology of a species that should be considered a potential phatogen on various crops, studies were undertaken in controlled conditions to investigate the fecundity and the length of the life cycle of a population of *X. vulgare* from Florida, United States of America.

### MATERIAL AND METHODS

The nematodes, identified as *X. vulgare* Tarjan, were collected from the rhizosphere of declining citrus trees grafted onto Swingle citrumelo rootstock [*Citrus paradisi* Macf. x *Poncirus trifoliata* (L.) Raf.]. They were maintained for some months in clay pots planted with the same host in steamed sandy soil. When needed, specimens were extracted from soil by means of Cobb's wet sieving technique.

Young non-gravid females were hand-picked and individually placed into each of ten small (25 ml) clay pots half filled with sterilised moist white sand. To prevent nematode escape, the small pots had no drainage holes. After nematode inoculation, a newly germinated, 6 to 8 cm tall, seedling of Swingle citrumelo was planted in each small pot, and sand was added to fill it to the top.

The pots were maintained in trays filled with wet peat and each tray covered with porous cellophane, to minimize temperature and moisture fluctuations. They were maintained at  $24 \pm 2$  °C on a shelf in the laboratory and supplementary mercury vapour lamps mantained a minimum 12 h day length (Taylor and Brown, 1974).

One month later, the sand in each pot was gently removed to allow direct observation of the plant roots under a stereo-microscope. Root systems were re-examined at 60 and 90 days, minimizing nematode disturbance. Ninety days, after inoculation, females began to contain eggs in the uteri. Nematodes in each pot were then recovered at four monthly intervals until two years after their inoculation (Brown and Coiro, 1983). On each occasion progeny were counted and development stages identified and discharded. The original female in each pot, when recovered, was placed in a new pot planted with a new citrumelo seedling. The study was discontinued at the 24<sup>th</sup> month when no females or progeny were recovered from the remaining pots.

Interpolation formulae and correlations were calculated to estimate the reproductive behaviour of *X. vulgare*, compared to another tropical species, *X. ifacolum* (Coiro *et al.*, 1995).

## RESULTS

One month from the begining of the experiment, root tips exhibited necrosis and the rest of the root was discolored, indicating that the nematode had fed upon them. One month later, on root tips were observed galls; at this stage there was no indication of egg formation. The first eggs were observed in the nematode uteri three months after initiating the experiment, and at month 4 a few first and second stage juveniles were recovered (Table I). At this time, the ten females had produced a mean of four juveniles (0-8), being 50% first and 50% second developmental stages; seven females were also gravid with one or two eggs per uterus (Table II). At month 8, ten females were again recovered and they had produced a mean of 8 (3-12) juveniles and all were gravid with 2 to 6 eggs per uterus. The developmental stages recoverd were 15% J<sub>2</sub>, 43% J<sub>3</sub> and 42%

 $J_4$ , confirming that X. vulgare has four juvenile stages (Lamberti et al., 2001). Reproduction was most intense between months 8 and 12. At the end of the first year, the same females were recovered and all were still gravid (1-4 eggs / uterus) and active. They had all produced progeny, a mean of 19 juveniles (5-34), comprised of 7%  $J_1$ , 30%  $J_2$ , 33%  $J_3$  and 30%  $J_4$  developmental stages. At month 16, nine females were recovered; only six were gravid (1-2 eggs/uterus). They had produced a mean of 3 juveniles (2-5), being 44%  $J_{2}$ , 46% J<sub>3</sub> and 10% J<sub>4</sub>. Twenty months after the begining of the experiment, two J<sub>1</sub>, two J<sub>2</sub> and two J<sub>3</sub> were recovered from six females, whereas the remaining females were inactive and had translucent body. No progeny nor live females were recovered at the completion of the second year.

### DISCUSSION

Considering the 365 days of a year, and then 30.4 days/month, it is estimated that development from egg to adult of the Florida population of *X. vulgare* takes ca. 274 days, equivalent to 1,094 day degrees (DD) [274 x (Experimental Temperature – Basal Temperature)] above a minimum daily threshold temperature of 20 °C. It can be hypotesized that the difference between the third month (when the first eggs are observed) and the twelfth month (when the certain females occur) is the necessary time lapse (nine months) for the completion of the cycle of *X. vulgare* from egg to female, having observed fourth stage juveniles (Table II) eight months after inoculation; they would have certainly reached the adult stage if they had been in the

Table I. Total reproductive capacity of individual female of Xiphinema vulgare on Swingle citrumelo.

1 9) /Repl. Pot n°	Number of juveniles									
				Months						
	4	8	12	16	20	24	Total			
1	4	16	22	22	22	22	22			
2	0	12	29	31	31	31	31			
3	1	9	25	29	29	29	29			
4	5	14	37	42	42	42	42			
5	4	13	31	35	36	36	36			
6	8	16	48	52	53	53	53			
7	6	13	47	50	51	51	51			
8	3	9	26	29	30	30	30			
9	4	7	12	15	16	16	16			
10	4	11	30	33	34	34	34			
Total	39	120	307	338	344	344	344			
Mean	4	12	31	34	34	34	34			

**Table II.** Mean number of developmental stages of *Xiphinema vulgare* at four month intervals, from individual females on Swingle citrumelo.

Month	Mean n° eggs/uteri	Mean n° developmental stages					
	·	J1	J2	J3	J4		
4	1.25	2.00	2.00	-			
8	3.50	0.00	1.25	3.50	3.40		
12	2.00	1.40	5.60	6.20	5.50		
16	0.60	0.00	1.60	1.70	0.30		
20	0.00	0.33	0.33	0.33	0.00		
24	0.00	0.00	0.00	0.00	0.00		

pots for 12 months. This high basal temperature was selected because of the soil temperature occurring in central Florida from where the nematode population was obtained.

The longevity of females was slightly more than 20 months, which is equivalent to 2,432 DD and their reproductive span was ca. 13 months, equivalent to 1,581 DD.

Between month 3 and month 20, females of *X. vulgare* produced a mean total progeny of 34, equivalent to one progeny for 46 DD above 20 °C. The number of progeny produced per female was probably underestimated as some losses could have occurred during the extraction procedure and eggs were not recovered. Assuming losses of 10% (Brown, personal cominication) the estimated total reproductive capacity of an individual female *X. vulgare* may be calculated as 40 individuals.

To describe the reproductive behaviour of *X. vulgare*, data concerning its reproductive capacity and longevity were tested using five models interpolation formulae: 1) linear ( $y = mx \pm b$ ); 2) power ( $y = bx^m$ ); 3) exponential ( $y = be^{mx}$ ); 4) logarithmic ( $y = m \ln x \pm b$ ) and 5) sigmoid  $y = a + b/[1+e^{-(x-c)/d}]$ .

The best fit to the data on mean cumulative total reproductive capacity and observation times was obtained by the sigmoid equation for which the coefficient for *X. vulgare* was r = 0.998 with a statistical significance of P=0.01 (Fig. 1). The relationship between progeny recorded at four month intervals and mean number of

eggs in the uteri (Table II) may be represented by the linear equation from which laying rate can be calculated (Fig. 2). Laying rate is the ratio between progenies recovered and mean number of eggs observed in the uteri in the previous period of observation.

This investigation demonstrates that *X. vulgare* requires a relatively long time to complete its life cycle, in contrast with *X. ifacolum* (8 weeks from egg to adult) another tropical species that is widespread in Africa (Coiro *et al.*, 1995).

The data concerning longevity and reproductive capacity of *X. vulgare* and *X. ifacolum* were comparatively tested with the above mentioned interpolation formulae. The best fit to the data on mean cumulative total reproductive capacity and DD was observed with the logarithmic equation (Fig. 3) which indicated that reproduction in the two species is quite a bit different, and in particular in *X. vulgare* the eggs hatch after a longer period of feeding by the adult females.

Populations of *X. index* required from 21 to 90 DD for individual egg production (Brown and Coiro, 1985) and their reproductive capacity was affected by the host plant. Possibly, reproductive capacity of *X. vulgare* would be enhanced by a better host than Swingle citrumelo, which during nematode infestation might undergo root biochemical changes that suppress nematode reproduction, as occurs in the relationship between olive and *X. index* (Sasanelli *et al.*, 1999; Ridolfi *et al.*, 2001).

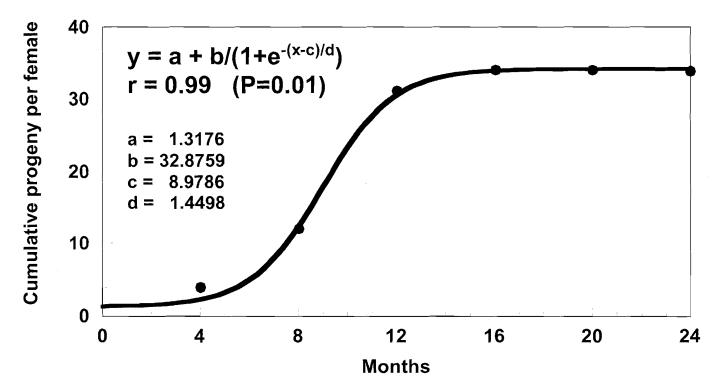
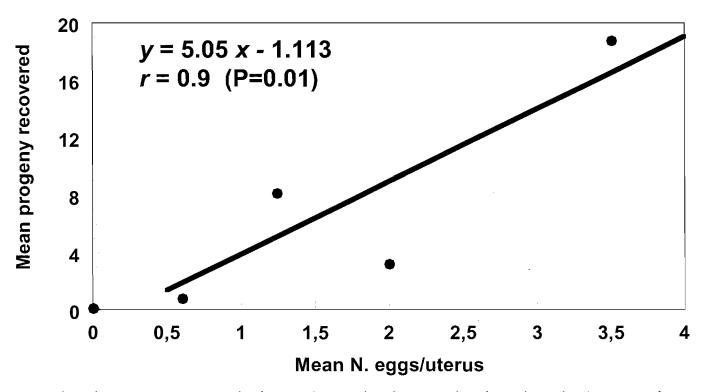
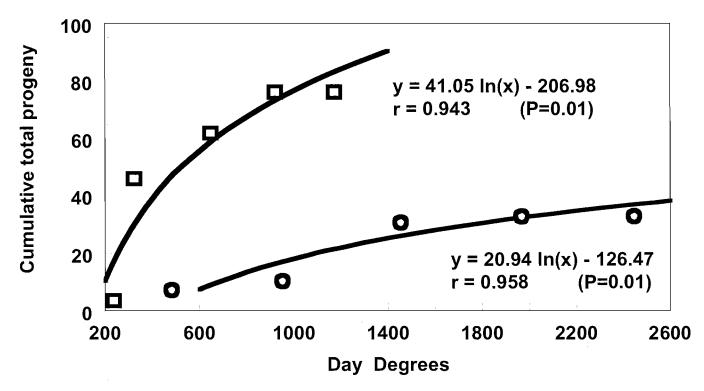


Fig. 1. Relation between cumulative total progeny production and observation times for Xiphinema vulgare.



**Fig. 2.** Relation between progeny recovered at four months intervals and mean number of eggs observed in the previous observation in the uteri of *X. vulgare*.



**Fig. 3.** Relation between cumulative total progeny and day degrees for *Xiphinema ifacolum*  $\Box$  [y = 41.05 ln (x) – 206.98; r = 0.943] and *X. vulgare* **O** [y = 20.94 ln (x) – 126.47; r = 0.958].

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