Observations on the biology of *Xiphinema basiri* and *X. insigne*

M. Shamim JAIRAJPURI and Harish K. BAJAJ

Section of Nematology, Department of Zoology, Aligarh Muslim University, Aligarh, India.

SUMMARY

The present studies on the biology of *Xiphinema basiri* Siddiqi, 1959 and *X. insigne* Loos, 1949 include observations on reproduction, embryogenesis and the juvenile stages. The population studies on these two species have shown that *X. basiri* reproduces twice a year, in February-April and in September-October, while *X. insigne* reproduces only once in May-August. Males are extremely rare in both species and although the testes are active throughout the year, there are not enough males to impregnate the large number of females and thus it is suggested that these species are parthenogenetic. In *X. basiri* both ovaries are equally functional whereas in *X. insigne* frequency of egg production by the anterior ovary is less (1 : 60) compared with the posterior ovary. The two species take about a week for the completion of their embryonic development. Intra-uterine egg development in *X. insigne* has been reported for the first time in this genus. The duration of first stage juvenile ranges from one week to a maximum of five months ; second, third and fourth stage juveniles are present in soil throughout the year.

RÉSUMÉ

Observations sur la biologie de Xiphinema basiri *et* X. insigne.

Les observations des auteurs concernant la biologie de *Xiphinema basiri* Siddiqi, 1959 et *X. insigne* Loos, 1949 portent sur la reproduction, l'embryogenèse et les différents stades juvéniles. Des études de populations ont montré que *X. basiri* se reproduit deux fois par an (février-avril et septembre-octobre), alors que *X. insigne* ne se reproduit qu'une seule fois, entre mai et août. Les mâles sont très rares chez les deux espèces et, bien que leurs gonades soient actives tout au long de l'année, ils ne sont pas assez nombreux pour féconder toutes les femelles ; les auteurs suggèrent donc que ces espèces sont parthénogénétiques. Chez *X. basiri* les deux ovaires sont également fonctionnels alors que chez *X. insigne* la production d'œufs par l'ovaire antérieur est plus faible que par l'ovaire postérieur (1 : 60). Le développement embryonnaire dure environ une semaine chez les deux espèces. Le développement intra-utérin des œufs est signalé pour la première fois dans le genre *Xiphinema*. La durée du premier stade juvénile va d'une semaine à un maximum d'un mois ; les autres stades sont présents dans le sol toute l'année.

Egg laying in *Xiphinema* species is usually restricted to a few months in a year (Griffin & Darling, 1964 ; Flegg, 1966, 1968*b* ; Prota & Garau, 1973). There are marked differences in the mode of reproduction (Dalmasso & Younès, 1969 ; 1970) and in the duration of embryonic and juvenile developments in different species (Radewald & Raski, 1962 ; Flegg, 1968*a*, 1968*b* ; Prota & Garau, 1973) : even in a single species there may be differences in the duration of life cycle under different environmental conditions (Cohn & Mordechai, 1969). So far no comparable work has been done on the life history of *Xiphinema* species in India. In the present work the population dynamics, reproduction, embryogenesis and the juvenile stages of *X. basiri* Siddiqi, 1959 and *X. insigne* Loos, 1949 were studied.

Materials and methods

Xiphinema basiri was collected from soil around the roots of *Citrus limonia* from Jawahar Park, Lal Diggi and *X. insigne* from *Aegle marmelos* from the garden in front of Victoria Gate, Aligarh Muslim University, Aligarh. Soil samples were taken at intervals of about three weeks from around the roots of four trees : each sample consisted of ten subsamples which were taken at an equal distance from each other and at about 15 cm from the base of host plants. These samples were taken at a depth of 15-30 cm where these nematodes generally occur in largest numbers (unpubl. data). Subsequent samples were taken at a distance of at least 20 cm from the previous collection spots. When processed the samples were first mixed thoroughly. A modified Baermann's funnel technique was employed for the extraction of these nematode species.

The lengths of the ovaries and the genital branches of twenty females from each sample were measured. For this purpose the nematodes were killed in hot 4% formalin and mounted in anhydrous glycerine. The number of eggs that were present in the anterior and posterior sexual branches were also recorded separately in the two species.

The structure of female gonad was studied by making an incision near the vulva of the gravid females having developing oocytes in the hinder part of the ovary (this part appears brownish when the oocytes are present in this region) or the oviduct or uterus having eggs. The worm was placed on a glass slide coated with albumen. The body turgor pressure forces out the entire genital tract which gets adhered to the slide upon gentle heating for 4-5 minutes. These slides were first fixed in 3 : 1 alcohol-acetic acid solution for 15-20 minutes and then stained in 2% acetic orcein for 5-10 minutes. If required, the gonads were destained with 4% acetic acid before mounting in acetic orcein. The edges of the coverslip were sealed with "Glyceel" or nail-varnish. The females in the breeding as well as non-breeding seasons were studied similarly in order to compare the behaviour of the oocytes in the two seasons. The male gonad was studied similarly by teasing out the entire male genital tract from an incision near the cloaca.

Embryonic development was studied in the gravid female having fully developed eggs near the vulva. These females were left in distilled water for several days to see whether or not they would lay their eggs in water ; if they failed to deposit their eggs, these were carefully teased out. The eggs were first transferred to a drop of distilled water placed over a 22 mm square coverslip. In case of those females with some embryonic development within the body, the entire animal was placed in a drop of water on a coverslip which was then inverted over a cavity slide ; the drop of water was changed at intervals of about 24 hours, the edges of the coverslip being sealed with petroleum jelly to minimize the evaporation. Observations were made at half hourly intervals, when not under observation, the slides were placed on a moist filter paper in a petri-dish which was kept in the dark.

Observations

Structure of female genital branch (Fig. 1-3)

Xiphinema basiri

The species reproduces twice a year, firstly in late February to the middle of April and then again in October. The males are extremely rare throughout the year including the breeding season. : their role in reproduction is therefore doubtful.

The entire female genital tract is enclosed in a sheath which has nuclei whose numbers and shapes are variable in different regions. During the non-breeding season (April-September and November-January) the ovary is short and terminally connected to the oviduct and it is colourless and contains oocytes with hyaline cytoplasm. In the breeding season the hind part becomes brownish due to the presence of ripe oocytes and to the thickening of the ovarian wall. The cytoplasm of the ripe oocytes becomes refractive and granular. Depending on the size of the oocytes present in the hind part of the

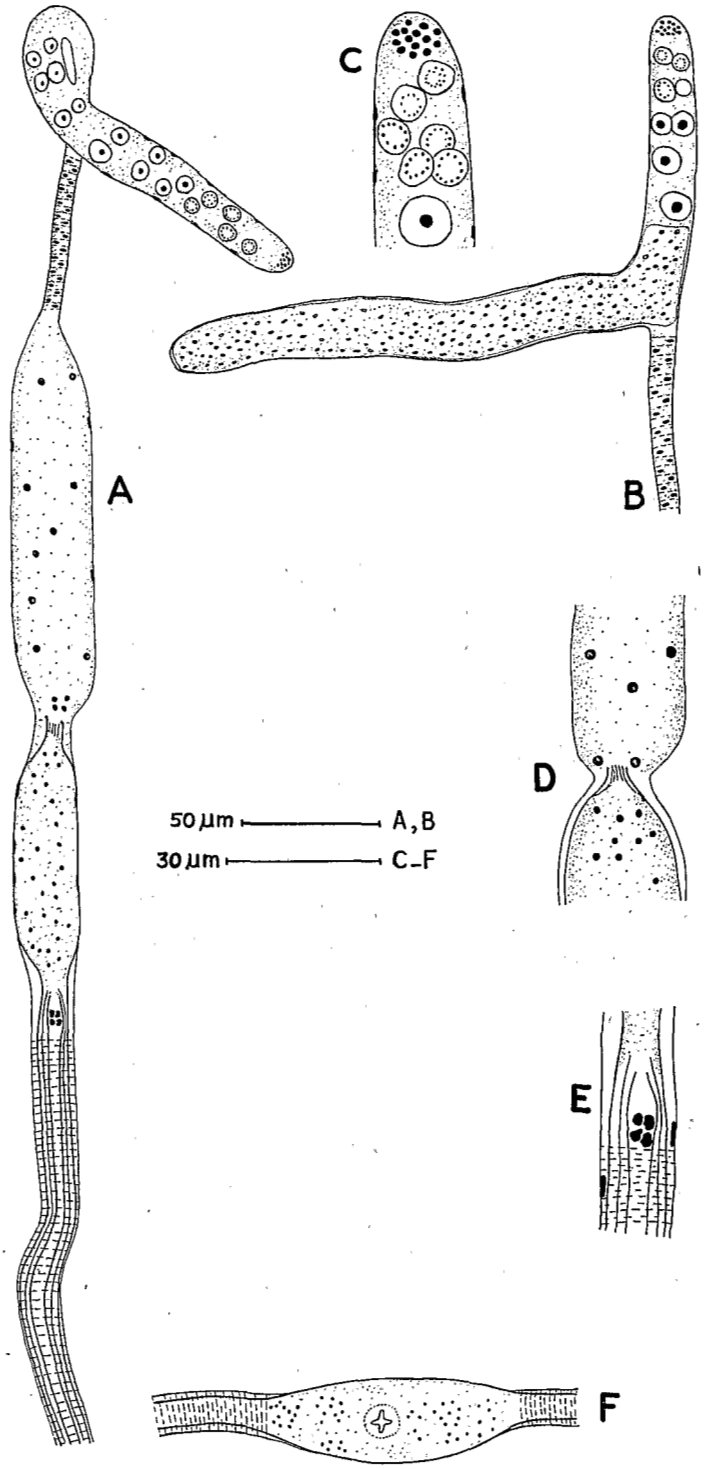

Fig. 1. Structure of female reproductive organs of *Xiphinema basiri*. A : Female sexual branch during non-breeding season ; B : Female sexual branch during breeding season ; C : Anterior end of ovary ; D : Sphincter ; E : Pseudo "Z" organ ; F : Vulval region.

ovary, the connection of the ovary with the oviduct may either be sub-terminal or median ; if the latter the extended ovary will appear as a lateral branch (Fig. 1, B). The increase in the length of the genital tract during the breeding season is probably due to the presence of the ripe oocytes in the hind part of the ovary, but the length of the genital tract when measured from the ovary-oviduct junction to the beginning of the vagina is almost the same throughout the year except when the oocytes shift to the genital tract, particularly the expanded part of the oviduct. Immediately before the beginning of the breeding season the intestinal region shows changes similar to those described in *X. americanum* by Griffin and Darling (1964).

The narrow part of the oviduct is lined by columnar epithelium with elongated nuclei arranged in double rows. The expanded part of the oviduct is thin-walled, greatly extensible, and contains sixteen lightly stained nuclei. The expanded part of the uterus is thick-walled and contains a large number of nuclei which are rounded but differ from the nuclei of the expanded part of the oviduct in staining darkly; the uterine walls and the covering sheath come closer to each other just behind the expanded part of the oviduct and form a sphincter. The sheath covering the rest of the uterus has cells arranged in a single row around the uterine wall. The inner core of the uterus shows ridges and furrows due to the arrangement of muscles of this region, the muscles in front enclosing the pieces of pseudo-Z organ and extending up to just behind the beginning of the expanded part of the uterus.

The multiplication of the germ cells takes place at the apical region of the ovary. The number of cells ranges from ten to eighteen and occupies an area of 10-15 $\times$ 4-6 µm. The cells n the next region are in an interphase stage, their cytoplasm is hyaline, nuclei indistinct and the chromatin is present towards the periphery. During the non-breeding season these cells accumulate but their cytoplasm remains hyaline. In the breeding season as the oocytes pass through the growth zone of the ovary their cytoplasm becomes granular, refractive and brownish. Both the anterior and posterior ovaries are equally functional and usually one mature oocyte in each set is present at a time.

Xiphinema insigne

This species reproduces only during May-August as evidenced by the presence of eggs in the uteri only during these months. The males are rare throughout the year including the breeding season, indicating that they may also be non-functional in reproduction.

The entire female genital tract is enveloped by a sheath which has a variable number of nuclei in different regions of the tract. The anterior ovary in this species is usually greatly reduced. The differences in the size of the two ovaries is more pronounced during the breeding season (Fig. 2) when the posterior ovary undergoes changes similar to those described for *X. basiri*. The anterior ovary usually retains the same size as in the non-breeding season, but occasionally increases in length with oocytes ripening in the growth zone, which is evident by the presence of ripe oocytes in the hind part of the ovary as well as in the expanded portion of the oviduct. The frequency of egg production by the anterior ovary is much less than by the posterior ovary (1 : 60). Histologically, the rest of the genital tract is similar to that of *X. basiri*.

STRUCTURE OF MALE GENITAL BRANCH (Fig. 4)

The males of *X. basiri* and *X. insigne* posess two testes, one outstretched and the other reflexed, which are connected to a common *vas deferens* which runs posteriorly and joins the rectum to form a cloaca. The entire male genital tract is enveloped in a sheath containing elongated nuclei. The multiplication of germ cells takes place in the apical region, composed of ten to twelve cells and occupies an area of 8-10 $\times$ 3.5 µm : the sperms are small, oval and without a tail. The hind part of the testes and *vas deferens* has fully formed sperms throughout the year indicating that unlike eggs the formation of sperms is a continuous process : sperms were never observed in the female genital tract.

EMBRYOGENESIS (Fig. 5)

The gravid females of *X. basiri* and *X. insigne* seldom lay eggs in water although they

may remain active for 4-5 days in this medium. The uterine eggs are usually elongate-oval or rounded-oval and measure 170-270×35-55 µm in *X. basiri* and 150-165×32-40 µm in *X. insigne*. The uterine eggs of *X. insigne* are usually colourless though some may be brownish in colour. They become dark brown when placed in distilled water. The pattern of embryogenesis in these two species is quite similar to other *Xiphinema* species (Flegg, 1968*a*). However, a brief account is provided below because of certain differences noticed in the rate of cleavage, arrangement of blastomeres, etc.

Cleavage starts after about 18-24 h of egg laying (taking out of the uterus). First and second cleavages are perpendicular to the long axis of the egg resulting in the formation of four cells which are identical in shape and size. After the second cleavage, the anterior cells divide more rapidly than the posterior cells. Due to this unequal division the cells forming

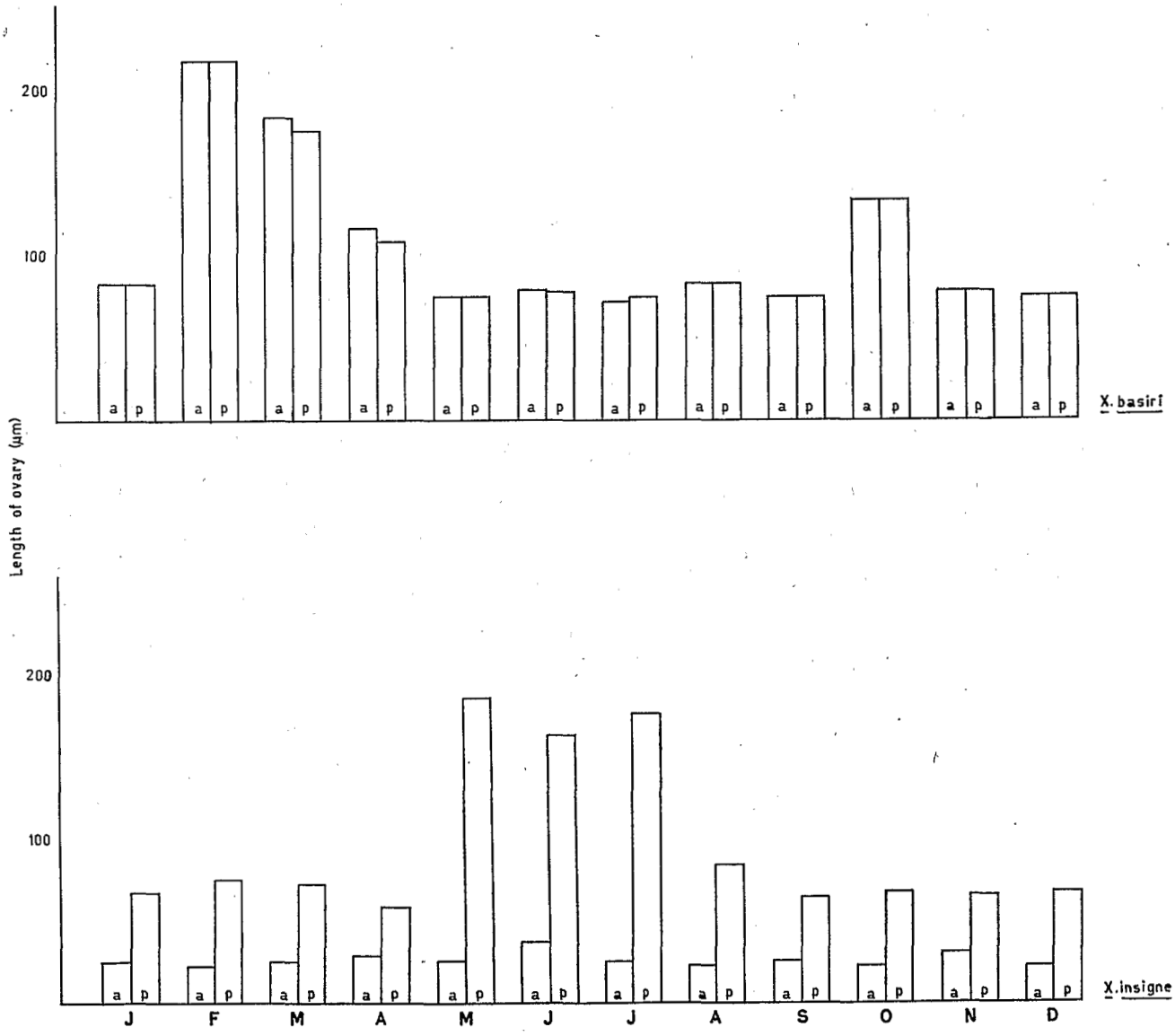

Fig. 2. Length of ovary in *Xiphinema basiri* and *X. insigne* in different months. a : Anterior ovary ; p : Posterior ovary.

the anterior part of the developing blastula are smaller whereas those of the posterior part are larger. The blastula is formed in approximately 12 h after the initiation of cell division which transforms into gastrula within the next 18 h ; within 48 h of the formation of gastrula, its anterior end becomes recurved to form the "tadpole" stage. The tip of the functional odontostyle along with "odontostyle forming cells" make their appearance about four days after the initiation of cell division. This functional odontostyle is laid down at the rate of about 4-5 μm/h and is completely formed in about 12 h ; after its completion the odontostyle contracts in size. Formation of replacement odontostyle starts several hours after the completion of functional odontostyle and is laid at the rate of 4-5 μm/h, the complete replacement odontostyle being formed within 13 h of its initiation and the odontophore is fully formed within 8 h of the beginning of replacement odontostyle formation. It takes about a week for the egg to reach the hatching stage.

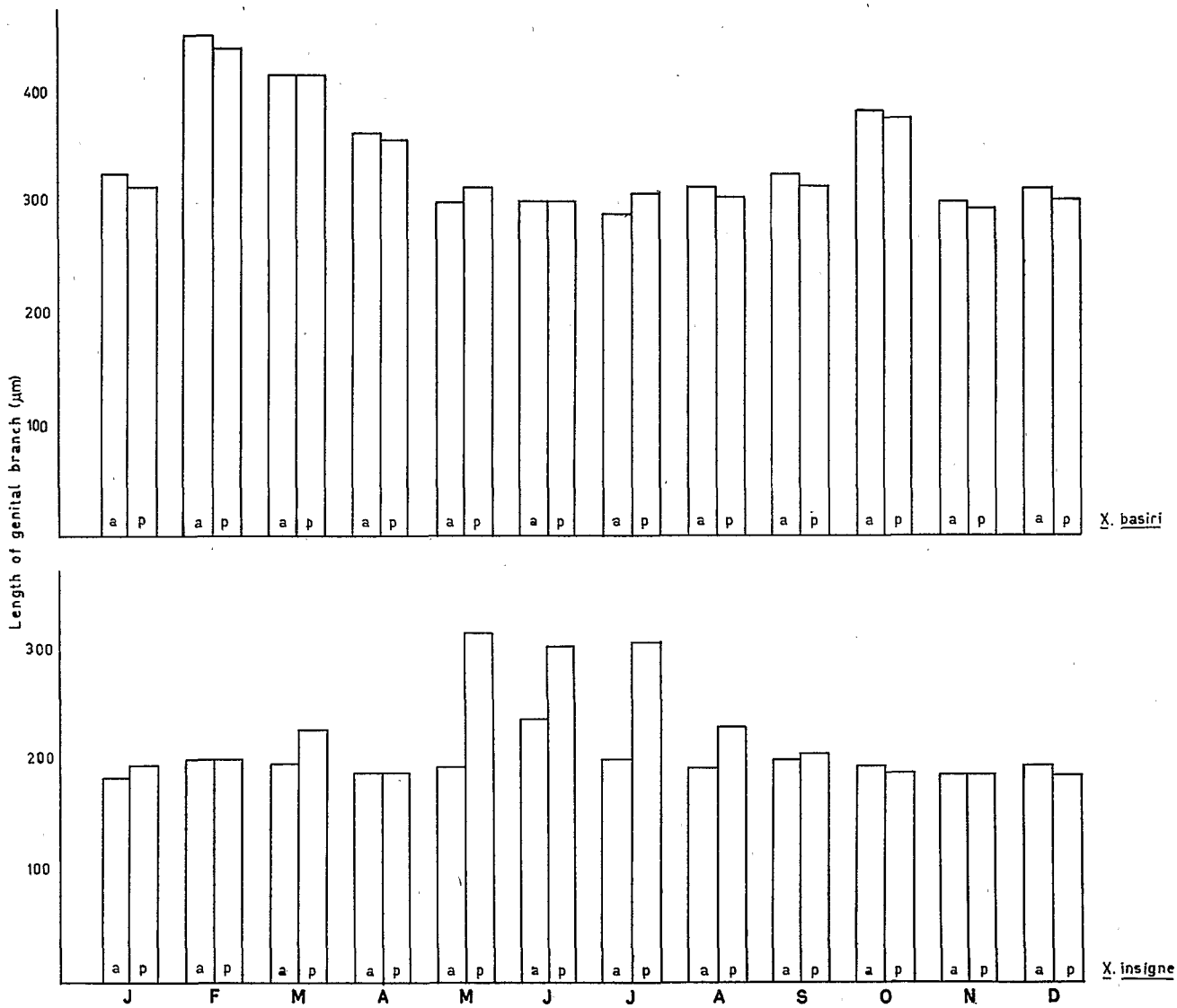

Fig. 3. Length of genital tract in *Xiphinema basiri* and *X. insigne* in different months. a : Anterior sexual branch ; p : Posterior sexual branch.

Intra-uterine egg development (Fig. 6)

Gravid females of *X. insigne* with well developed eggs in their uteri when kept in distilled water for 4-5 days become very sluggish and fail to lay eggs. Development then starts within the eggs in the uteri. The intra-uterine egg development which was observed in six females is here reported for the first time in *Xiphinema*. The embryonic development of the eggs inside the uterus is very similar to that described above. It was also observed that the mother dies once the developing juvenile reaches three fold stage.

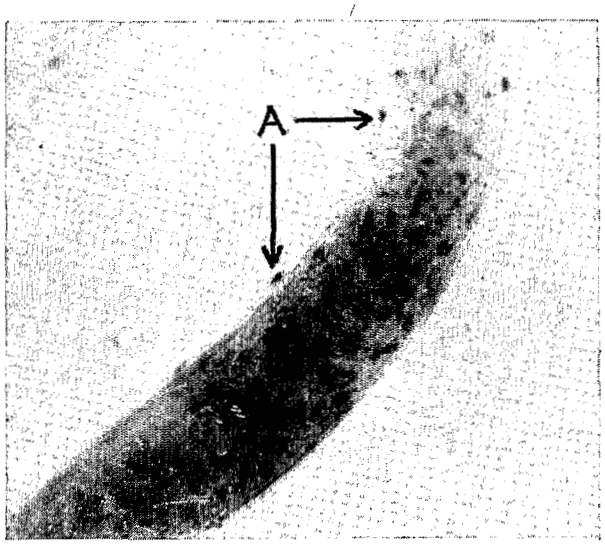

Fig. 4. Growth zone of testis of *Xiphinema basiri* : A : Nuclei of enveloping sheath.

THE JUVENILE STAGES

The various juvenile stages can easily be distinguished from each other as well as from the adults on the following basis :

The adults can be distinguished from the juveniles in having only the functional odontostyle, while the juveniles possess a functional as well as a replacement odontostyle. The first stage juveniles are distinct from the other juvenile stages in having the tip of the replacement odontostyle enclosed within the odontophore. The second, third and fourth stage juveniles can be distinguished from each other on the basis of lengths of functional and replacement odontostyles. The tail shapes also differ in the various stages. The position of guiding ring from anterior extremity, the ratios c and c' are quite characteristic for each juvenile stage and can be useful in differentiation. The number of cells forming the genital primordium itself can be used to separate the various stages : four cells in J_2, ten in J_3 and 30-48 in J_4 ; they were not observed in J_1 but are perhaps less than four, i.e. one or two.

POPULATION FLUCTUATIONS (Fig. 7 & 8)

Changes in populations of adult females, juveniles and the occurrence of eggs in the uteri are presented in Figures 7 and 8. The numbers of second, third and fourth stage juveniles were grouped for each month.

Xiphinema basiri

There are two peaks in the total population, the first in April-May and the second in October-December. Adult males are rare throughout the year and usually constitute less than 1% of the total adult population. Adult females constitute 50% of the total population in the periods January-March and September-October, these peaks in the female population indicating that many fourth stage juveniles moult to adult stage in the months preceding the breeding season. The female population decreases at the end of the breeding season suggesting that a number of older females die after egg-laying. First stage juveniles appear in March-June and then in October-November, their appearance in March and October demonstrating the short embryonic period of this species under natural conditions. First stage juveniles are most numerous in May when they constitute about 17% of the total population. The peaks in the populations of the other juvenile stages occur subsequent to the appearance of first stage juveniles in the soil. The life-span of first stage juveniles is quite variable, ranging from a week to a maximum of four months : the presence of second, third and fourth stage juveniles throughout the year indicates that the life span of these juveniles is variable and may be quite long.

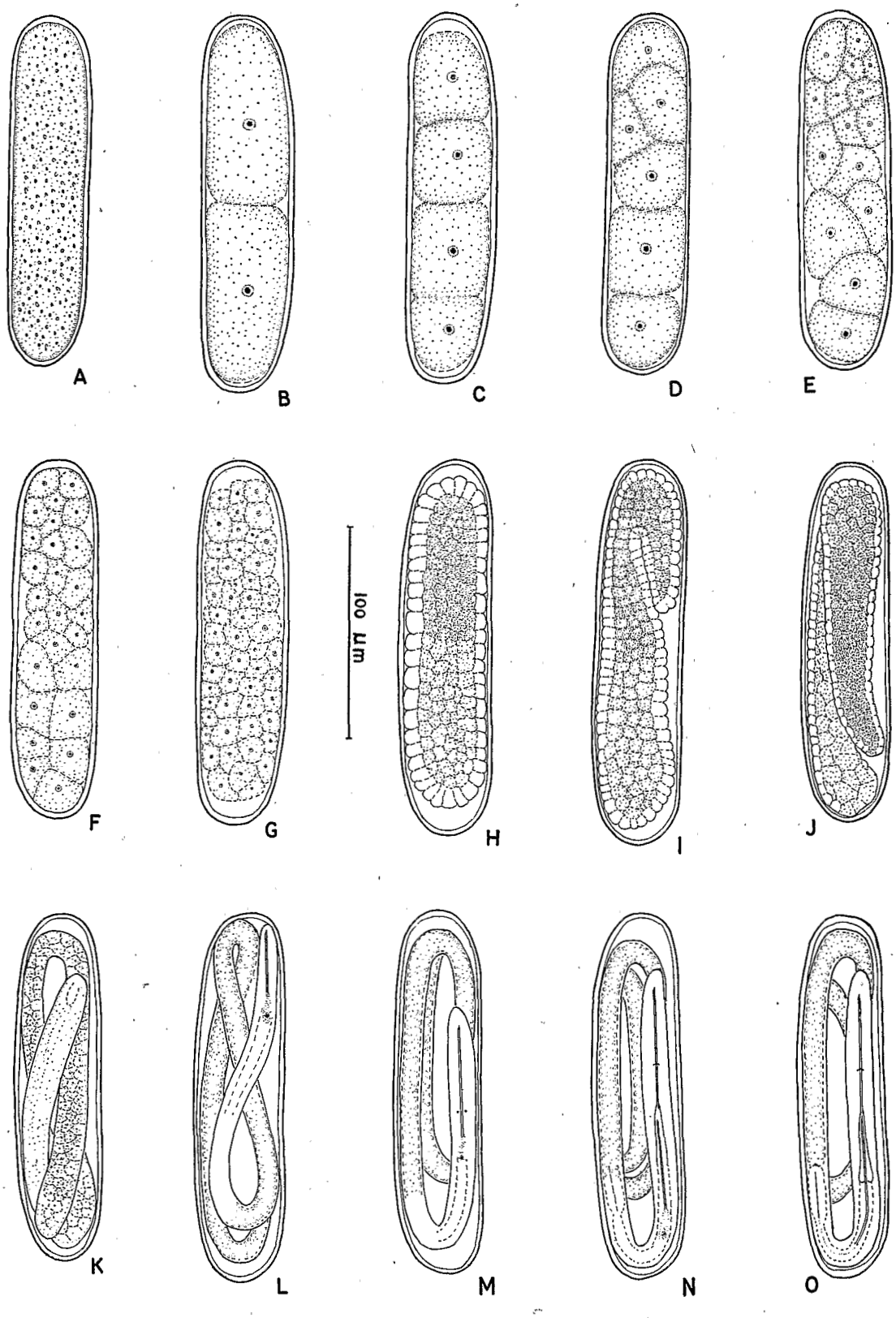

Fig. 5. Embryogenesis in *Xiphinema basiri*. A : 1-celled stage ; B : 2-celled stage ; C : 3-4-celled stage ; D : 6-celled stage ; E : 16-celled stage ; F : 28-celled stage ; G : Blastula stage ; H : Gastrula stage ; I : "Tadpole" stage ; J : One folded juvenile ; K : Two folded juvenile ; L-M : Juvenile with functional odontostyle (developing) ; N : Juvenile with developing replacement odonstyle ; O : Juvenile just before hatching.

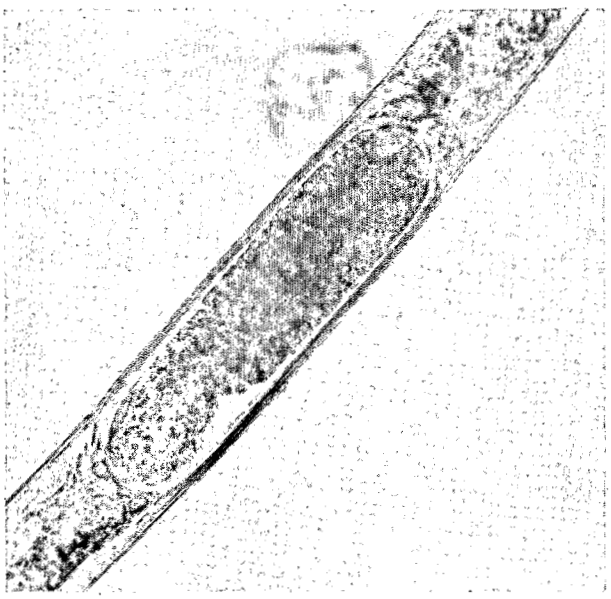

Fig. 6. Intra-uterine egg development in *Xiphinema insigne*.

Xiphinema insigne

These are most abundant in June-September when populations may exceed 1,650 per dm^3 of soil. After September, their population gradually declines, becoming lowest in the months of March and April. Adult females are present throughout the year but are numerous in March-May, when they constitute about 60% of the total population. This increase in the population of females in the months preceding the breeding season (May-August) may be due to the fact that many fourth stage juveniles moult to become adults. The sharp decline in their population after May is presumably due to the death of "old" females. The first stage juveniles appear in the soil in May and can be found until October ; they are most numerous between July to September, as about 20% of the total population. The appearance of the first stage juveniles in May confirms, under natural conditions, the short embryonic period of this species. Second, third and fourth stage juveniles are at their peak in July-October and lowest in March-May, the increase in the population of these stages coinciding with the appearance of the first stage juveniles in the soil, indicating that the life-span of the first stage juveniles is rather variable, from a week to a maximum of five months.

Results and discussion

Xiphinema basiri and *X. insigne* have different egg laying seasons. *X. basiri* reproduces twice a year : first in February-April and then in October, while *X. insigne* reproduces only once in May-August. The breeding season of *X. basiri* falls during those months when the climatic conditions are moderate (mean seasonal temperature 20 °C), while that of *X. insigne* is during the hotter months of the year (mean seasonal temperature 30 °C). This difference in the breeding periods of the two species is not dependent upon their host plants or periodicity of their root growth, as presumed by Cotten (1973) for *X. index*, as they were never observed to reproduce at the same time on a common host plant. Reproduction in *X. basiri* is strikingly similar to that of *X. index* which also reproduces twice a year with the majority of the eggs appearing in the uteri in spring and to a lesser extent in autumn (Prota & Garau, 1973). The reproduction in *X. insigne* on the other hand is like *X. diversicaudatum* (Flegg, 1968*b*) which reproduces once-yearly.

It seems most probable from this and other studies that *Xiphinema* spp. in natural conditions have either one or two periods of reproduction each year. The continuous reproduction reported in some species of *Xiphinema* (e.g., *X. index* and *X. brevicolle*) by Cohn and Mordechai (1969) under cultural conditions is most likely due to the almost constant optimum temperature (20-23 °C) maintained throughout the experiment. It is also apparent from our observations that *X. basiri* and *X. insigne* produce eggs at times of the year when the temperature of the soil is quite different. Temperature may not be the only cause for this difference since large populations of *X. insigne* also occur at high altitudes in India where the temperature is much lower. Most probably, certain neurosecretory hormone(s) may play an important role in activating the ovaries to produce eggs as has been reported in some insects and mammals (Raven, 1961).

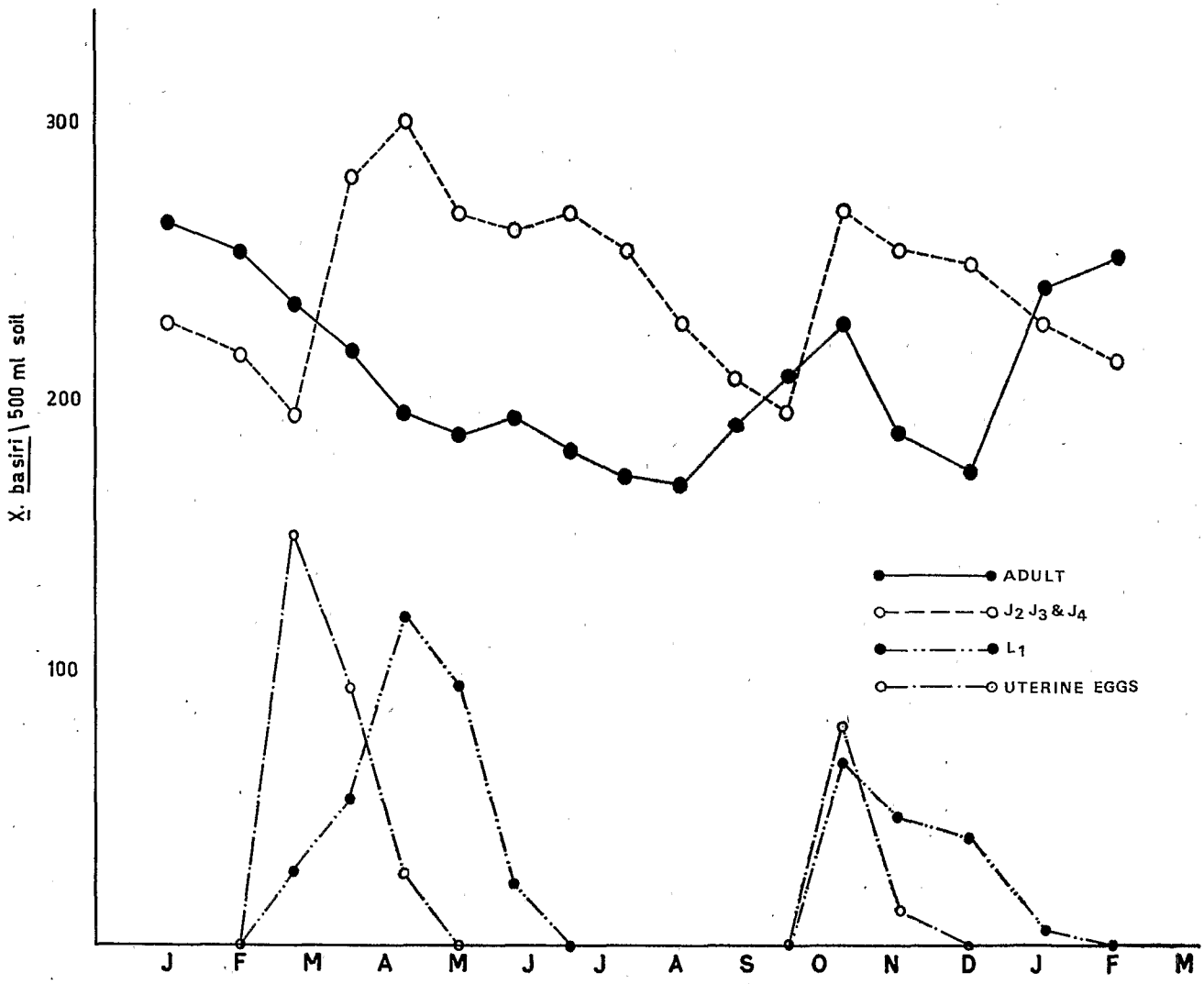

Fig. 7. Population structure of *Xiphinema basiri* in different months.

In *X. insigne* the anterior ovary is much reduced and is occasionally functional. Its frequency of egg production is 1 : 60 as compared to the posterior ovary. This difference in the activity of the two ovaries supports Southey's (1973) hypothesis that species of *Xiphinema* with one ovary possibly have evolved from those with two ovaries by the reduction or loss of the anterior ovary. This species *(X. insigne)* represents the first step in this direction where the anterior ovary is reduced in size and in egg producing capacity.

A cellular membrane surrounding the female gonads in *X. italiae*, *X. mediterraneum* and *X. index* was noticed by Grimaldi de Zio and Morone (1974). Such a membrane also surrounds the female gonads of *X. basiri* and *X. insigne* and appears to be of uniform occurrence in *Xiphinema* spp. The fact that a similar sheath surrounds the male gonad also is here being reported for the first time in this genus.

The behaviour of the reproductive organs during breeding and non-breeding seasons is similar to that described by Dalmasso and Younès (1969, 1970) for *X. index* but differs from that of *X. mediterraneum* in the colour

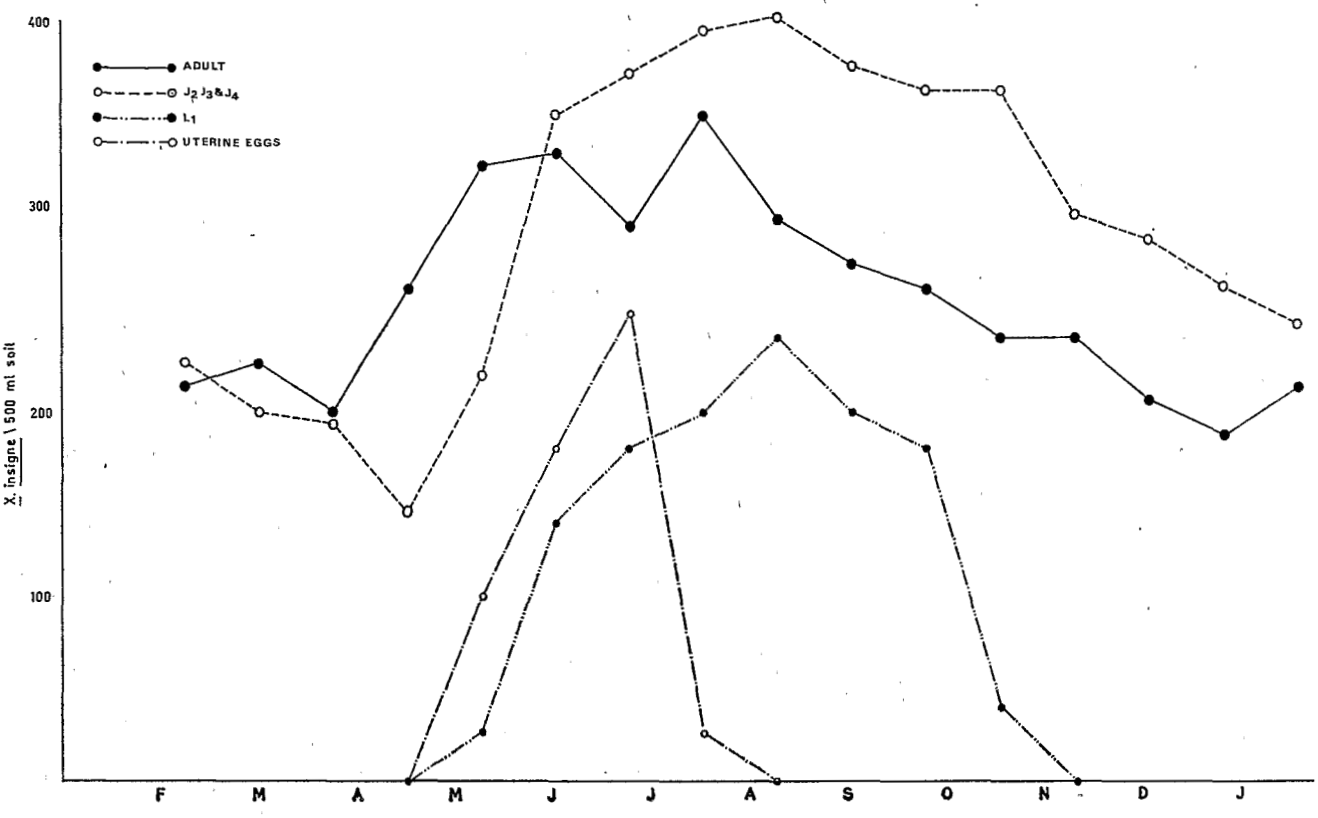

Fig. 8. Population structure of *Xiphinema insigne* in different months.

of the ovaries, thickness of the ovarian walls and piling up of the oocytes in the non-breeding season (ovaries are thickwalled and brownish in colour, oocytes do not pile up during the non-breeding season and the cessation of production is more marked). The anterior ovary of *X. insigne* is the same as it is during the non-breeding season, indicating a rather low egg production. The increase in the length of the two ovaries (only posterior in *X. insigne*) is in accordance with the observations of Flegg (1967) on *X. vuittenezi*, but there is no increase in the length of the genital tract (excluding ovaries) which is not in agreement with Flegg's findings.

The few males that were collected had well developed sperms in their testes as well as in the *vas deferens* throughout the year. In no case did the male gonads seem to be non-functional or incapable of sperm production which was the case with the males of *X. americanum* and *X. brevicolle*, studied by Heyns (1974). Since impregnated females were never found, it appears that males do not play any role in reproduction and that the species are parthenogenetic. This parthenogenetic mode of reproduction may be mainly responsible for producing marked intraspecific variations which are quite evident in *Xiphinema* spp. (e.g. Tarjan, 1969 in *X. americanum*; Bajaj & Jairajpuri, 1977 in *X. insigne*). This tends to support the view expressed by White (1954) that parthenogenetic species that have been in existence for some time are likely to show a greater variability, which may not necessarily be correlated with their geographical distribution. Any mutation in a parthenogenetic species remains confined to that population as exchange of genes is not possible.

The embryonic development of *X. basiri* and *X. insigne* is similar to that of *X. diversicaudatum*, *X. mediterraneum* and *X. vuittenezi* except for the arrangement of the blastomeres after the second cleavage. In *X. basiri* and

X. insigne after the four-celled stage all the cells do not divide simultaneously but only the anterior two cells divide first resulting in the formation of a six-celled stage in which the anterior four cells are obviously smaller than the posterior two cells. Later on this marked difference in the size of anterior and posterior cells persists. The formation of odontostyle is strikingly similar to that described by Coomans and De Coninck (1963) during moulting. In *X. basiri* and *X. insigne* the odontostyle also contracts and shortens as do the developing odontostyles during the moulting process. Such contractions were not reported by Flegg (1968*a*). The replacement odontostyle which is initiated just posterior to the base of the functional odontostyle does not start its formation immediately after the completion of the functional odontostyle as reported by Flegg (1968*a*). The rate of formation of odontostyles in these species is considerably higher, being 4-5 μm/h as against 1-2 μm in the species studied by Flegg (1968*a*). This even more strongly supports the hypothesis of Coomans and De Coninck (1963) that the spear forming gland cell is of great metabolic activity.

The total embryonic period in these species is markedly shorter than that described by Flegg (1968*a*) for other *Xiphinema* spp. His explanation that the differences in the embryonic period of different species is due to differences in their body size seems to have little justification since the average length of female *X. basiri* is 3.0 mm and that of *X. insigne* is 2.2 mm, both being larger than *X. mediterraneum*. Temperature does not seem to be a factor as the embryonic development of *X. basiri* was done at the same temperature at which Flegg carried out his studies. The embryonic periods of *X. basiri* and *X. insigne* are the same as those for *X. index* (Radewald & Raski, 1962). These similarities and differences in the duration of the embryonic development may be inborn or may be due to the differences in their life span as well as due to differences in the rate of juvenile development.

The temperature of 30 °C was shown to be lethal to the developing eggs of *X. diversicaudatum* by Flegg (1969) and, although no direct effect of temperature on the development of *X. basiri* and *X. insigne* was studied, it is probable that 30 °C is not lethal to eggs of *X. insigne* as the temperature during the breeding season of this species (May-August) is usually higher than 30 °C.

The intra-uterine egg development observed in sluggish females of *X. insigne* suggests that this species may reproduce in this way under unfavourable conditions and is not just the effect of age.

The distinguishing characters of the various juvenile stages are similar to those described by Coomans (1965) in *X. basilgoodeyi*. The first stage juveniles of *X. basiri* and *X. insigne* are found in soil for only a few months strongly contrasting with the results of Flegg (1968*b*) for *X. diversicaudatum*, *X. mediterraneum* and *X. vuittenezi*.

Acknowledgements

The authors wish to thank Professor S. Mashhood Alam, Head of the Department of Zoology, for providing the necessary laboratory facilities and Dr. J. J. M. Flegg of East Malling Research Station, Maidstone, Kent, England for going through the manuscript and for his valuable suggestions. The junior author also thanks the CSIR, New Delhi, for the award of a Junior Research Fellowship.

References

Bajaj, H. K. & Jairajpuri, M. S. (1977). Variability within *Xiphinema insigne* populations from India. *Nematologica*, 23 : 33-46.

Cohn, E. & Mordechai, M. (1969). Investigations on the life cycles and host preferences of some species of *Xiphinema* and *Longidorus* under controlled conditions. *Nematologica*, 15 : 295-302.

Coomans, A. (1965). *Xiphinema basilgoodeyi* n. sp. with observations on its larval stages (Nematoda : Dorylaimina). *Nematologica*, 10 : 581-593.

Coomans, A. & De Coninck, L. (1963). Observations on spear formation in *Xiphinema*. *Nematologica*, 9 : 587-601.

Cotten, J. (1973). Feeding behaviour and reproduction of *Xiphinema index* on some herbaceous test plants. *Nematologica*, 19 : 516-520.

Dalmasso, A. & Younès, T. (1969). Ovogenèse et embryogenèse chez *Xiphinema index* (Nematoda : Dorylaimidae). *Ann. Zool. Ecol. anim.*, 1 : 265-272.

Dalmasso, A. & Younès, T. (1970). Étude de la gamétogenèse chez *Xiphinema mediterraneum*. *Nematologica*, 16 : 51-54.

FLEGG, J. J. M. (1966). Once yearly reproduction in *Xiphinema vuittenezi*. *Nature, Lond.*, 212 : 741.

FLEGG, J. J. M. (1967). Genital tract development in *Xiphinema vuittenezi*. *Nematologica*, 13 : 640-641.

FLEGG, J. J. M. (1968 *a*). Embryogenetic studies on some *Xiphinema* and *Longidorus* species. *Nematologica*, 14 : 137-145.

FLEGG, J. J. M. (1968 *b*). Life-cycle studies of some *Xiphinema* and *Longidorus* species in south eastern England. *Nematologica*, 14 : 197-210.

FLEGG, J. J. M. (1969). The effect of temperature on the embryogeny of *Xiphinema diversicaudatum*. *Nematologica*, 15 : 285-286.

GRIFFIN, G. D. & DARLING, H. M. (1964). An ecological study of *Xiphinema americanum* Cobb in an ornamental spruce nursery. *Nematologica*, 10 : 471-479.

GRIMALDI DE ZIO, S. & MORONE DE LUCIA, M. R. (1974). Presenza di un involcro alle gonadi di femmine di Longidoridae (Nematoda : Dorylaimoidea). *Nematol. medit.*, 2 : 77-80.

Accepté pour publication le 13 mars 1978.

HEYNS, J. (1974). The genus *Xiphinema* in South Africa I. *X. americanum* group (Nematoda : Dorylaimida). *Phytophylactica*, 6 : 157-164.

PROTA, U. & GARAU, R. (1973). Indagini sulla biologia di *Xiphinema index* Thorne *et* Allen in vigneti Sardi. *Nematol. medit.*, 1 : 36-54.

RADEWALD, J. D. & RASKI, D. J. (1962). A study on the life cycle of *Xiphinema index* (Abstr.). *Phytopathology*, 52 : 748.

RAVEN, C. P. (1961). *Oogenesis. The Storage of Developmental Information.* Pergen Press, Oxford, London, New York, Paris, 274 p.

SOUTHEY, J. F. (1973). Terminology of the female reproductive organs in nematodes with special reference to *Xiphinema* species. *Nematologica*, 19 : 405.

TARJAN, A. C. (1969). Variation within *Xiphinema americanum* group (Nematoda : Longidoridae). *Nematologica*, 15 : 241-252.

WHITE, M. J. D. (1954). *Animal Cytology and Evolution.* Cambridge Univ. Press : 27-30.