DISCUSSION

The above observations on *Xiphinema basiri* and *X. insigne* show that these species 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. This clearly indicates that the breeding season of *X. basiri* is during those months when the climatic conditions are moderate while that of *X. insigne* during warmer months of the year. This difference in the breeding periods of the two species is not dependent upon their host plants or periodicity of their root growth as was presumed by Cotten (1973) for *X. index*. The two species studied by the present author were never observed to reproduce at the same time on a common host plant. The reproduction in *X. basiri* is strikingly similar to that of *X. index* which also reproduces twice a year with majority of the eggs appearing in the uteri in spring and to a lesser extent in autumn (cf. Prota & Garau, 1973). The reproduction in *X. insigne* on the other hand is like *X. diversicaudatum* (cf. Flegg, 1968b) which reproduces once a year.

Thus from the above account it is clear that *Xiphinema* spp. reproduce only either once or twice a year. The continuous reproduction reported in some species of *Xiphinema* (e.g., *X. index* and *X. brevicolle*) by Cohn & Mordechai (1969) under cultural conditions is most likely due to an almost constant optimum temperature (20-23°C) throughout the experiment. It is also possible
that this temperature range may be favourable for the reproduction
of species like X. index, X. brevicolle but not for X. diversicaudatum
X. italicae and X. mediterraneum. It also becomes apparent from
the present observations that X. basiri and X. insigne produce
eggs in different seasons of the year when the temperature and
humidity of the soil are quite different (avg. soil humidity 40% and temp. 20°C for X. basiri and humidity 60% and temp. 30°C for
X. insigne). This difference may be chiefly responsible for the
once or twice yearly reproduction in these species. Undoubtedly,
the 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

As mentioned above 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 (1973b) hypothesis that the monodelphic species of Xiphinema
possibly have evolved from the didelphic forms by the reduction
or loss of the anterior ovary. This species represents first step
in this direction where the anterior ovary is reduced in size and
in egg producing capacity. The reduction of the anterior ovary
has perhaps been along the following line:

i) X. elongatum (both ovaries equally developed, V=40).

ii) X. insigne (anterior ovary reduced both in size and function
    V=23)
iii) *X. orbum* (entire anterior sexual branch greatly reduced, \( V = 28 \)),

iv) *X. simillimum* (anterior ovary lacking),

v) *X. chambersi* (anterior sexual branch lacking).

A cellular membrane surrounding the female gonads in *X. italicae*, *X. mediterraneum* and *X. index* was noticed by Grimaldi & 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 has been reported here for the first time in this genus.

The gametogenesis in *X. basiri* and *X. insigne* is almost similar to that described by Dalmasso & Younes (1969 & '70) for *X. index* but differs from that of *X. mediterraneum* in number of chromosomes, colour of the ovaries, thickness of the ovarian walls and piling up of the oocytes in the non-breeding season (*X. mediterraneum* has 5 chromosomes, ovaries are thick-walled and brownish in colour, oocytes do not pile up during non-breeding season, and the cessation of reproduction is more marked). In most cases the anterior ovary of *X. insigne* behaves the same way as it does during the non-breeding season which again suggests its rather low egg production capacity. The increase in the length of the two ovaries (only posterior in *X. insigne*) is in accordance with the observations of Flegg (1967) in *X. vuittenezi*, but there is no increase in the length of the genital tract (excluding ovaries) which is not in agreement with Flegg. The changes that occur in the digestive tract of these species in the breeding
period are the same as those described by Griffin & Darling (1964) in *X. americanum*.

The few males that were collected have well developed sperms in their testes as well as in the vas deferens throughout the year. In no case the male gonads seemed 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 (1974a). Since the females were never found impregnated with sperms, it shows that the males do not play any role in the reproduction and the species propagate by parthenogenesis. The number of chromosomes is 20 in both *X. basiri* and *X. insigne* and this supports Dalmasso & Younes's (l.c.) view that haploid number of chromosomes is 5 in *Xiphinema*. Thus both these species are tetraploid as is *X. index* (cf. Dalmasso & Younes, l.c.).

Since both these species reproduce by parthenogenesis like many other species of *Xiphinema* particularly of *americanum*-group in which the males are also rare, the variability within a single species of these groups is obvious (Lima, 1968; Tarjan, 1969; Cohn & Sher, 1972, and Chapter II). 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 deal of 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. Mutation being a continuous process, one mutation
after the other may get established in different lines of descent. This to some extent explains the variability among populations of *X. americanum* from the world collected by Tarjan (l.c.) which show similarities as well as variations in certain characters. Thus parthenogenesis is responsible for producing marked intra-specific variations which are quite evident in *Xiphinema* species.

The embryonic developments in *X. basiri* and *X. insigne* described above are almost similar to that described by Flegg (1968a) for *X. diversicaudatum, X. mediterraneum* and *X. vittenezi* except for the slight differences. The longer time taken for the initiation of cleavage (18 hours-3 days) by eggs of different species of this genus may be due to the fact that eggs when taken out of the body by incision or when laid are at the anaphase stage of the first meiotic division (Dalmasso & Younes, 1970) and no polar bodies are formed till this time. Evidently, the eggs undergo formation of these bodies first before the initiation of cell division. The present observations markedly differ with those of Flegg (l.c.) in the arrangement of the blastomeres after the second cleavage. In these species after the 4-celled stage all the cells do not divide simultaneously but only the anterior 2 cells first divide resulting in the formation of a 6-celled stage in which the anterior 4 cells are smaller than the posterior 2 cells which are larger. Also, after this stage there appears marked differentiation in the size of anterior and posterior cells. The relative duration of the early cleavage (up to 6-celled stage), gastrula etc., are similar to other species of *Xiphinema* (cf. Flegg, 1968a).
The formation of odontostyle is strikingly similar to that described by Coomans & De Coninck (1963) during moulting. In *X. basiri* and *X. insigne* the odontostyles also contract and shorten as do the developing odontostyles during moulting process. Such contraction was not reported by Flegg (1968a). The replacement odontostyle which starts its formation just posterior to the base of functional odontostyle does not start its formation immediately after the complete formation of functional odontostyle as reported by Flegg (l.c.). These observations support the hypothesis that the replacement odontostyle during moulting process also starts its development quite late after the replacement of odontostyle. The formation of odontophore and spear guiding ring are also similar to the formations of these structures during moulting process, i.e., these are formed when the replacement odontostyle is about 1/4th formed. The rate of formations of odontostyles in these two species is considerably higher being 4-5 um/hour against 1-2 um/hour in the species studied by Flegg (l.c.). This even more strongly supports the hypothesis of Coomans & De Coninck (l.c.) that the spear forming gland cell is of great metabolic activity.

Though hatching could not be studied in these species, the active and quiescence periods of the juveniles are similar to that of other species of *Xiphinema* (Radewald & Raski, 1962, Flegg, 1968a). There is marked increase in the flexibility of egg shell membranes after the odontostyle formation and a fluid was some times seen coming out of the mouth of the developing juvenile. This suggests
that the hatching is principally same as in other *Xiphinema* spp. (Radewald & Raski, l.c.; Flegg, l.c.) and is by the pressure of lip region against the egg shell wall, since in no case spear protusion was seen. The fluid that was seen coming out of the mouth of the developing juvenile may contain proteolytic enzymes which act when the movement of the developing juvenile has helped in emulsifying the lipoid membrane of the egg shell along with an osmotic intake of water to generate pressure against the shell wall as is suggested by Wilson (1958) for the animal parasitic nematodes.

The total embryonic period in these species is markedly shorter than those described by Flegg for other *Xiphinema* species. His explanation that the differences in the embryonic period of different species is due to difference in their body size seems to have little justification since *X. basiri* is avg. 3.0 mm long and *X. insigne* is avg. 2.2 mm long and are larger than *X. mediterraneum* but take only about a week to develop to the hatching stage. The temperature too is not responsible for this difference since embryonic development was carried at about the same temperature at which Flegg carried out his studies. Instead, the embryonic period of these species is similar to that of *X. index* (Radewald & Raski, l.c.). These similarities and differences in the duration of embryonic development may be inborn or may be due to differences in the life span as well as due to differences in the rate of juvenile development. The life cycle is 12-16 months in *X. index* (cf. Prota & Garau, l.c.) against 2-3 years required only for the juveniles to reach the adult stage in *X. diversicaudatum*, *X. mediterraneum* and *X. vuittenezi* (cf. Flegg, 1968b).
The temperature of $30^\circ C$ was shown to be lethal to the developing eggs of *X. diversicaudatum* by Flegg (1969). Though no direct effect of temperature on the development of *X. basiri* or *X. insigne* was studied, but there is clear evidence to show that $30^\circ C$ is not lethal at least for the eggs of *X. insigne* since during the breeding season of this species (May-August) the temperature is usually much higher than $30^\circ C$ and there is also marked increase in the number of first stage juveniles in this season (Fig. 26).

*Endotokia matricida* seen in the sluggish females of *X. insigne* reflects the adaptation of this species to reproduce under unfavourable conditions. This also shows that it is not only the age factor which is responsible for this intra-uterine development. Instead the factors which adversely affect the activity of the females may induce this development. In soil, it is quite possible that there may be quite a number of females with developing embryos within their uteri but they go undetected due to failure of our usual technique to isolate sluggish nematodes.

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* remain in the soil for only a few months which is quite in contrast to its prolonged occurrence in *X. diversicaudatum*, *X. mediterraneum* and *X. vuittenezi* (Flegg, 1968b). In these species the juveniles are present throughout the year and contribute up to 40% of total population.