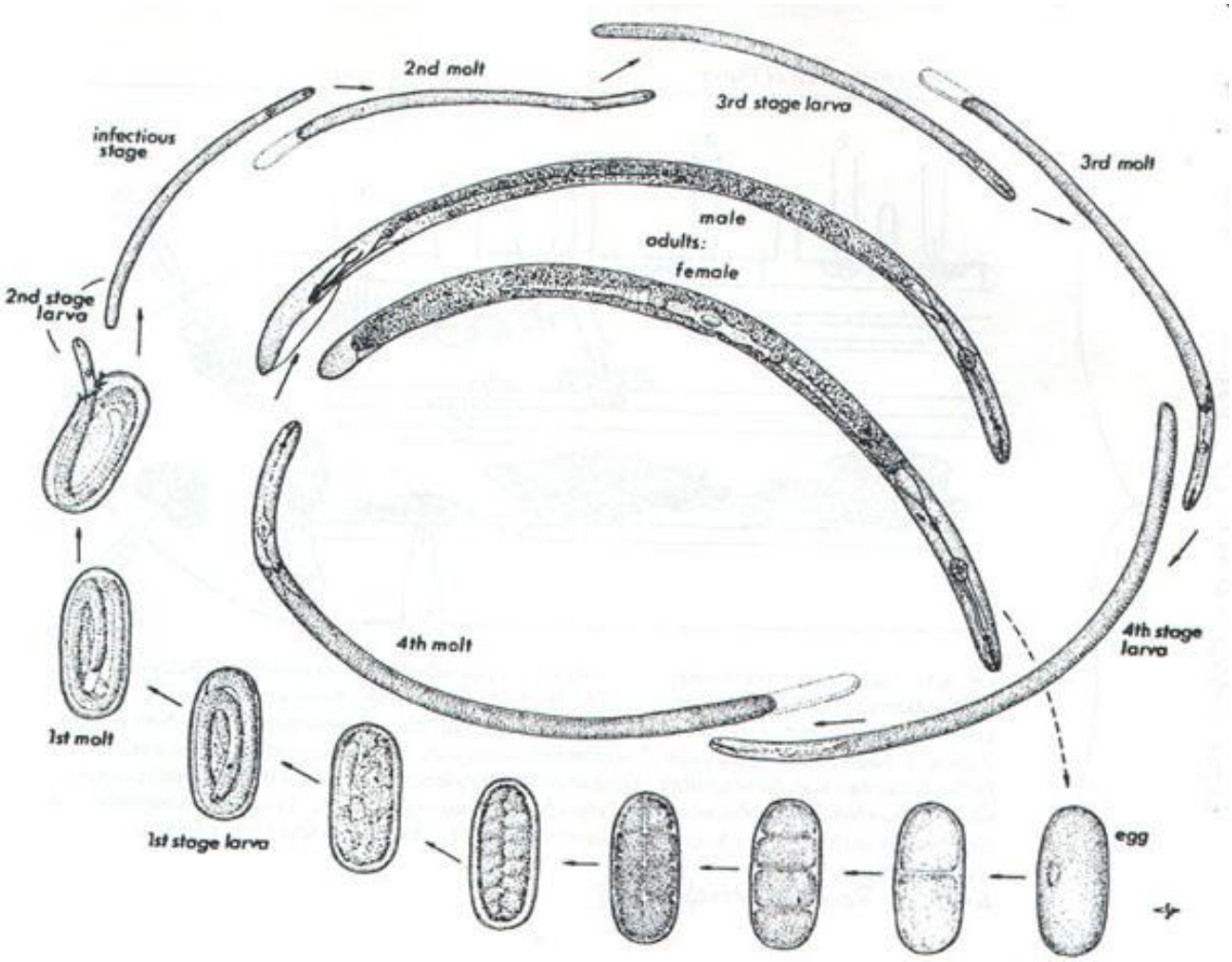
Lecture 07 - Biology of Plant Parasitic Nematodes



The life cycle of nematode has six stages. The egg state, J1 or first stage larva, J2 or second stage larva, J3 or third stage larva, J4 or fourth stage larva and the adult stage. The first four stages are the immature stages and are known as juvenile stages. The female lays eggs in soil or in plant tissues, singly or in groups as egg mass that hatch out into larvae which are almost similar to adults in appearance. The first moult occurs within the egg shell and the second stage juvenile comes out by rupturing the egg shell as J2. In case of *Xiphinema index*, the larvae are reported to emerge from the egg before the first moult. The larval cuticle is shed after each moult.

The Egg

The nematode eggs are oval in shape. The eggs are covered by three membranes, the external protein layer which is the secretion of uterus wall, the middle chitinous layer or the true shell secreted by egg itself and the inner lipid layer. The chitin content in the egg shell vary in different species of nematodes.

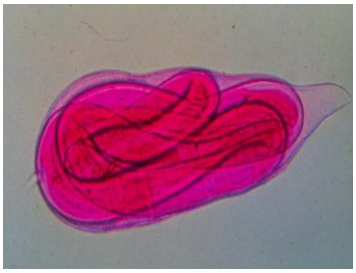
Embryonic Development

The adult female lays the eggs. The egg starts dividing by cleavage of their protoplasams to form cells. The first cleavage occur transverse to the longitudinal axis and gives two equal cells or blastomeres which are the first somatic cell (S1) and the parental germinal cell (P1). The second cleavage results in four cells which are first arranged in a T shape. This shape is achieved by the blastomere S1 dividing longitudinally and the blatomere P1 dividing transversely by P2 and S2. At last these cells get arranged in a rhomboidal shape. The transverse and longitudinal mitotic divisons of daughter cell continue. The S1 blastomere is the primary somatic cell and its two products (A & B) produce most of the nematode's ectodermal cells. The S2 blastomere produce somatic tissue and give rise to ectoderm (E), mesoderm (M) and stomodeum (St) tissues. The gonads of nematode are derived from P1. In the blastula stage the cells are so arranged as to form a fluid filled sphere bound by a single layer of the cells, while in the gastrula stage, the early embryo consists of an open mouthed sac like body with a wall of two layers of cells.

The cells A and B further divide to produce a, b, and P2 divides to give P3 and S3. The dorsal cells produced by A and B continue to divide and finally give rise to most of the hypodermis, excretory cells and nervous system. The daughter cell P2 divides into P4 and S4. These S3 and S4 are ectodermal and produce the hypodermis in the posterior region of the nematode body.

The endodermal tissue produced from the products of cell E1 and P1 divides into P5 and S5. The descendants of S5 give covers to the gonads and their ducts, while the products of P5, G1, G2 and their descendants proliferate into germ cells only.

The primary mesodermal cell M gives rise the nematode's body wall musculature and its pseudocoelomic cells, while the pharynx from St cells. During early embryonic stage these primary cells St, M and E present on the ventral surface of the embryo and are taken within the embryo by process of gastrulation. In further development the dorso ventrally



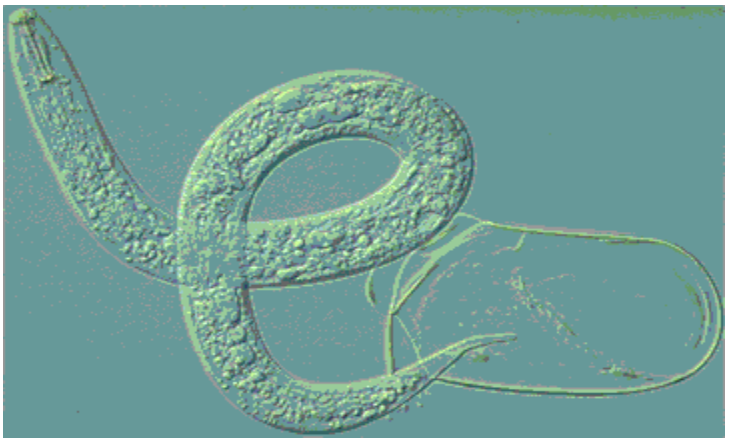
flattened embryo is changed to a cylindrical shape. The embryo starts to become worm shaped and a coiled juvenile is recognized inside the egg membrane. At last the cell constancy is reached and further cell multiplication stops in all organs except the reproductive system. The first moult take place within the egg and J2 ruptures the egg shell and hatch out. Before hatching the J1 can be seen riggling inside the egg shell. **First moult take place within the egg**

The post embryonic development in plant parasitic nematodes take place within the egg leading to the formation of juvenile which is ready to undergo first moult. In the process of post embryonic development, hatching and moulting are the important stages.

Hatching (Ecdysis)

The term hatching is used for the emergence of the juvenile from the egg. It occurs either in response of a stimulus or stimuli from the host or take place under normal environment. In cyst forming nematodes the release of juvenile from cyst is an emergence and not hatching. Eggs have hatched within the cyst. The eggs of *Globodera rostochiensis* generally hatch in response to root exudates provided by solanaceous crops Viz., potato and tomato. After embryonic development the first stage juvenile undergoes the first moult within the egg and thus second stage juveniles are found within the egg.

After reaching a particular stage of growth and favourable hatching conditions are present, the juvenile shows vigorous movement, often causing bulging of the egg membrane as seen in case of



2nd stage juvenile cyst nematode has just hatches from an egg

Pratylenchus, Paratylenchus, Nacobbus and *Meloidogyne*. After that the juvenile makes a series of thrusts with the help of stylet on the egg shell @40-90 per minute and finally juvenile emerge out by breaking the egg shell at perforated places.

Moulting (Eclosion)

The hatched juvenile resembles the adult except for body size and gonad development. The juvenile undergoes some changes in form, particularly at the anterior and posterior ends and formation of gonads. Growth in nematodes is associated with moulting which usually occurs four times and there are five stages. After the fourth moult the nematode becomes fully grown adult. During moulting the entire cuticle including the cuticular lining of the stoma, stylet, oesophagus, vulva, cloaca, rectum, amphids, phasmids and excretory pore are shed. In most of the plant parasitic nematodes greatest growth occurs after the last moult and moulting tends to occur in the earlier half of the growth curve.

Stimulus

It is reported that the neurosecretory cells of nematodes are stimulated to produce some secretions which activate glands that produce enzymes or hormones which initiate moulting. In some cases root exudates act as a stimulus for moulting as in the case of *Paratylenchus nanus* and it acts as a stimulus to the fourth stage juvenile moult. In endoparasitic nematode, the stimulus may be more complex and may be closely associated with a increase in size of nematode, because in these nematode moulting does not occur until some growth has completed within the host. The stimulus may depend on the host, temperature, pH and the salt content of the soil. When these factors are optimal, the stimulus acts after a short exposure. Juvenile once stimulated it release the exsheathing fluid into space between the new and old cuticle which then digest the area of the sheath near the excretory pore ultimately releasing the juvenile.

The receptor

In all cases the receptor may be cuticular and hypodermal structure eg. Hemizonid. It seems to be associate with neuro secretory activity which leads to the production of an enzyme which is responsible for moulting. The juvenile becomes sluggish inactive and feed vigorously just before moulting. The old cuticle is discarded by abrasion against soil particles or any rough material. The cuticle may be shed in one piece or the anterior part may be shed separately as a cap.

Growth and development

In plant parasitic nematodes, there are four juvenile stages and an adult stage. The immature stage of the nematode called as juvenile. In case of endoparasitic nematodes, three moults occur with in the host plant. The duration of the different juvenile stages is highly variable. Gonad development starts in the first juvenile stage before hatching but the growth of

the organs is slow. The development starts with the formation of genital primordial which consists of two control germinal cells or one large cell which are bordered by two smaller somatic cells. External environment affect the structural development and physiology of the host which may influence the development of the nematode. The plant parasitic nematode fixes its feeding site in different regions of the root. *Meloidogyne* goes even up to stellar region, *Heterodera* and *R. reniformis* mostly confine to pericycle and *T. semipenetrans* penetrates cortex region.

Root – knot nematodes (*Meloidogyne* spp.)

The root – knot nematodes are sedentary endoparasites of underground plant parts. The eggs are retained in a gelantinous matrix, which normally protrudes out of the host tissues. About 200 to 300 oval eggs are found in a single egg mass which makes its size larger than the female body.

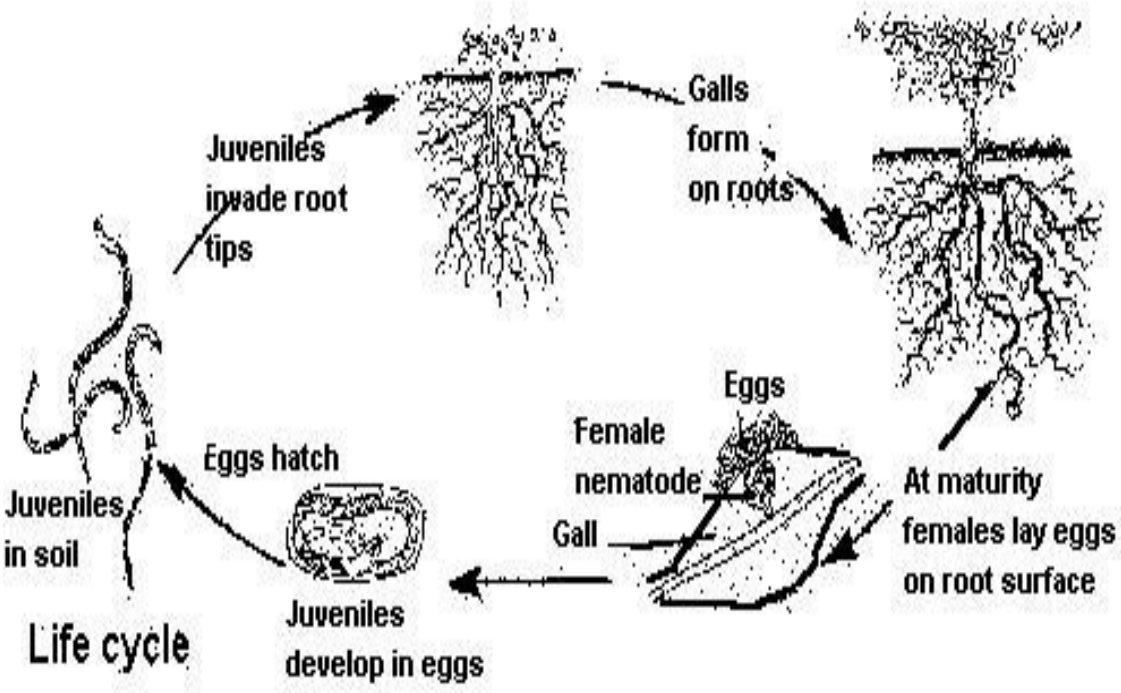
The life cycle starts from the egg usually in the one-celled stage deposited by the female. Development of the embryo starts within hour of deposition, resulting in two, four, eight cells, etc., The embryo and the first stage larva move with in the egg but not very active.

After the first moult, the second stage infective juvenile is formed within the egg. Larval hatch occurs under suitable physical condition but not depending on host root exudates or hatching factor. The emerging second stage larvae are found free in the soil. They attack new host root tissue in the region behind the root tip (meristamatic zone). The larvae which develop into females establishes feeding site in the pericycle region and become sedentary. Subsequently three moults occur and the larvae develop into females with spherical body embedded in the host tissue. The neck region is unaltered.

During feeding the larvae pierce the cell wall with secretions cause enlargement of cells in the vascular cylinder and increased cell division in the pericycle. The nematode feeding stimulates the development of a typical nurse cell system called ‘Syncytium’ or ‘Giant cell’. These cells are multinucleate which contain dense cytoplasm and enlarged nuclei with several mitochindrial and golgi bodies and are metabolically active.

The larval which develop into adult males are initially parasitic. After moulting three times they leave the host as a worm like from and come closer to the females for copulation. Parthenogenesis is reported to be common in *Meloidogyne*. For development of a mature female

it takes around 30 days which may vary depending upon the species of the host and parasite and environmental factors like temperature and soil type.



The life cycle of the root knot nematode

Cyst nematodes (Globodera spp. and Heterodera spp.)

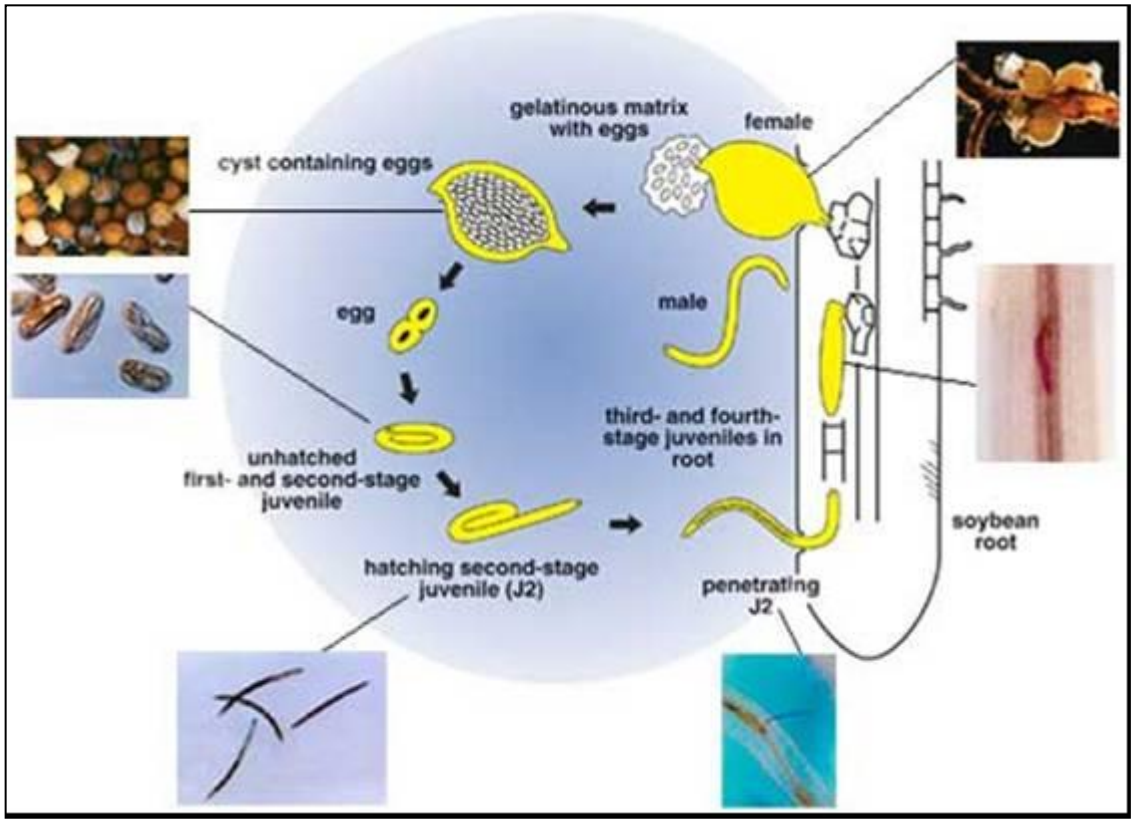
Second stages larvae usually penetrate the root just behind the growing point. These larvae grow rapidly and thee moults occur in the host. In about 5 –6 weeks after penetration, the white cysts are clearly visible which protrude from the root surface. These young cysts are packed with eggs and upon death the body wall hardens due to quinone tanning into a tough resistant brown covering known as cysts. The cysts get separated from the root and fall into soil.

Larval emergence from cysts is often in response to root exudates from a host plant. The best emergence of juveniles occurs as a result of a rise in temperature after a period of low temperature. Maximum emergence of larvae from cysts under Indian condition takes place at a temperature of 20 -22°C. The cysts continue to release eggs over a period of 3 –4 years at the rate of 50 per cent viable eggs per year. There is only one generation of the nematode in a year.

Multiplication of nematode is favoured by soil texture. Migration of second stage juveniles is favoured by light textured soils. The host cells close to the head region of the sedentary female being to modify and finally enlarge to form multinucleate syncytium with a thick outer boundary. The female feeds from this nurse cell system and grows. The swollen adult female protrudes out of the root tissues and later changes into brown cysts.

Although cyst nematodes induce giant cell formation, gall formation is not distinct. Each syncytium is associated with only one nematode in the case of cyst nematodes unlike the root – knot nematodes where one or more nematodes are associated with a syncytium. Nuclei is enlarged in the syncytium caused by the root – knot nematode but in cyst nematode nuclei is relatively small. The syncytia are bound by the vascular elements especially xylem which develops specific wall in growth. There is enlarged nucleoli and irregular nuclei. Abundant mitochondria, golgi bodies, protoplasts and dense endoplasmic reticulum are also found in the syncytia.

Life cycle of cyst nematode- *Heterodera*



Citrus nematode (*Tylenchulus semipenetrans*)

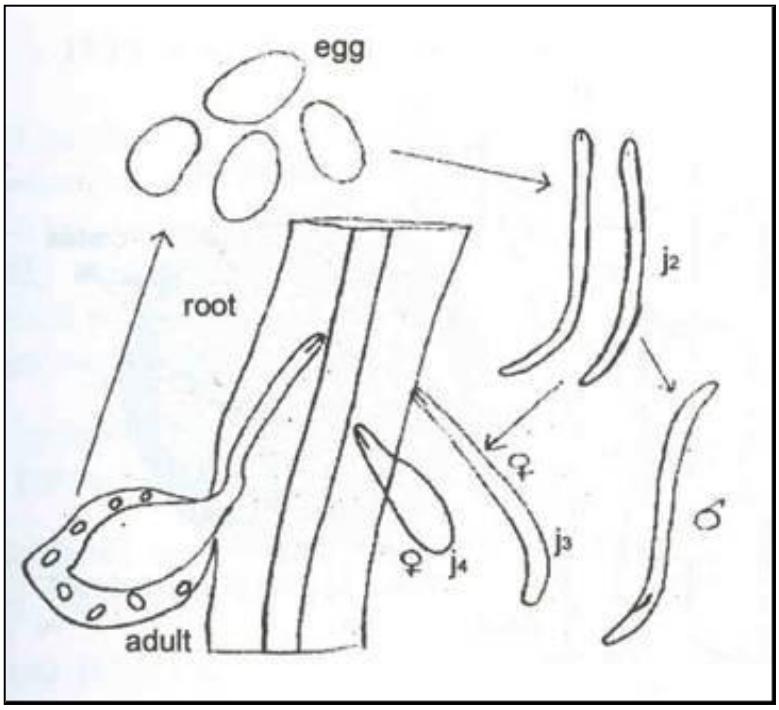
Citrus nematode is a sedentary semi – endoparasite of the Citrus root. Females are most commonly found on thick and stunted rootlets to which a layer of soil particle is clinging. These particles are held in place by a gelatinous mucus secreted by the female. The mucus and adhering soil particles protect the females and eggs deposited by them from their natural enemies. The egg laying young females can be seen in groups clinging to rootlets with their head and neck buried in the root cortex, whereas the posterior body region found outside the root surface.

Larva hatches from egg in 12 – 14 days. Mature males develop within a week after 3 moults and one moult having occurred within the egg. The long slender individuals fail to develop unless they feed on a root. The second stage female larva requires about 14 days to locate the host root and feed on epidermal cells until ready for moulting. Fourth stage females and young females are seen in about 21 days after the entry into roots. At maturity the females secrete the gelations matrix in which eggs are deposited. Egg laying occurs in about 40 days. The complete cycle from egg requires six to eight weeks at 25°C. Reproduction occurs without the help of males.

The feeding zone developed by this nematode is termed as nurse cell, which consists of uninucleate but not enlarged discrete parenchyma cells which are located in the cortex. Syncytium is not formed. This type of nurse cells system is characteristic for this nematode. Feeding of the citrus nematode in cortical cells results in necrosis. The injury does not extend to the stellar region of the root.

The population of the citrus nematode is closely related to the stage of decline of the trees. The nematode infestation is severe in sandy loam soil.

Life cycle of citrus nematode



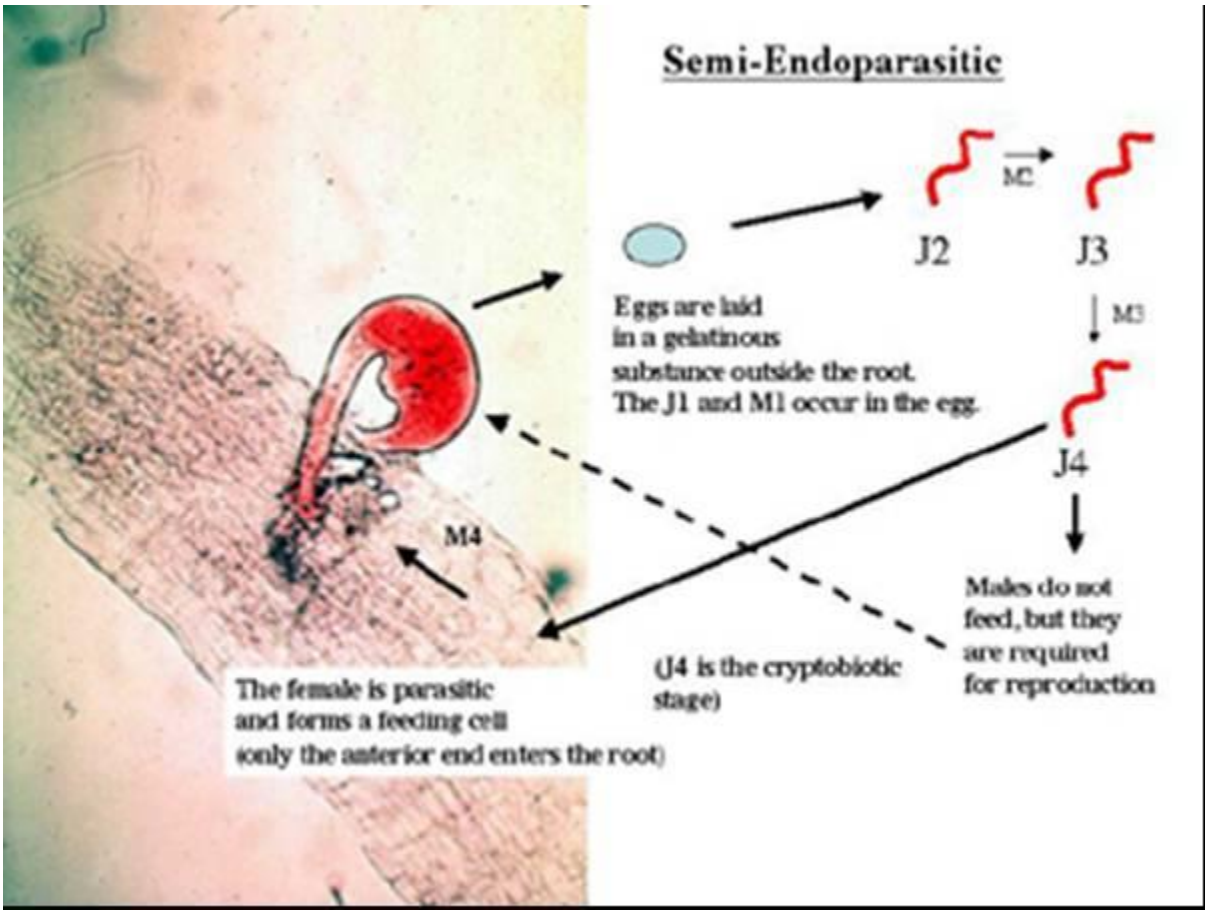
Reniform Nematode (*Rotylenchulus reniformis*)

The adult female is an obligate, sendentary, semi-endoparasite of roots while the males are non – parasitic. The species is bisexual and reproduction is by amphimixis.

The species has an unusual life cycle. Although a newly hatched second stage larva have well developed stylet, they do not feed. They soon pass through three super imposed moults to become young females and adult males. The young females force their way through cells of root cortex until they partially or completely become embedded in this tissues. During the process they feed on cortical cells. Three days after feeding, a slight swelling of the posterior body is seed and eight days later eggs are deposited in a gelatinous matrix outside the root tissue. When these eggs are placed in water they promptly begin to hatch. The life cycle is completed in about 25 days provided the young females have found the host immediately. The nematode as a semi-endoparasite of sedentary nature induces a specialized nurse cell systems for continuous food supply. The system involves wall expansion of several cells at the feeding site, partial wall dissolution, fusion of neighboring cell protoplasts and finally establishment of a multinucleate syncytium. These syncytia are mostly confined to the pericycle. Other pericycle cells are metabolically stimulated but they remain discrete and uninucleate.

The young infective females destroy the exterior cortical cells o roots and the damage increase when the nematode moves towards the phloem.

Life cycle of Reniform Nematode



Burrowing Nematode (*Radopholus similis*)

Females and all juvenile stages are infective. Males are non-parasitic and morphologically degenerate (without stylet). Penetration occurs mostly near the root tip. The nematode penetrates within 24 hours and the cells around the site of penetration becomes brown. After entering the roots, the nematodes occupy intercellular position in the cortical parenchyma where they feed on the cytoplasm of nearby cells causing cavities which coalesce to form tunnels. Nematodes do not enter the stellar portions of the root. The nematode completes its life cycle within 24 – 30 days at a temperature range of 21 - 32°C. Females lay eggs within infested tissues with an average of 4 –5 eggs for two weeks. Eggs hatch after 8 – 10 days and the

juvenile stages are completed in 10 –13 days. A low soil temperature, adequate soil moisture and availability of fresh tender roots help in the build up of population.

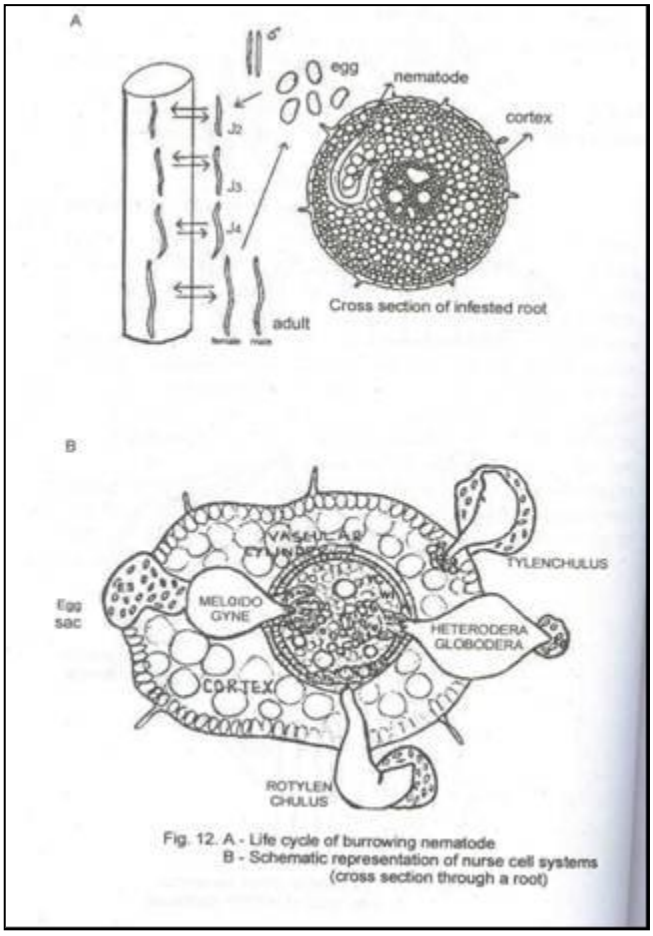


Fig. 12. A - Life cycle of burrowing nematode
B - Schematic representation of nurse cell systems (cross section through a root)

Life cycle of burrowing nematode