CHAPTER 2

Literature Review

2.1 Nematodes

Nematodes may represent 80 - 90% of all multi-cellular animals in the group Ecdysozoa that can shed their exoskeleton (Jairajpuri & Ahmad, 1992; Bloemers *et al.*, 1997; Mariam, 2008). The total number of nematode species has been estimated at about 1,000,000. Over 28,000 nematode species have been described and of these over 16,000 species are parasites of insects, plants or animals (Wikipedia, 2012a).

The majority of nematodes are found within the top 10 cm of the soil, although some may live much deeper (Yeates *et al.*, 1984; Mariam, 2008). Nematodes have widely differing nutritional behaviors and therefore occupy several trophic levels in soil food webs. They can be grouped according to the type of food they consume, based on the morphology of their mouth parts. The most common groups are the plant-parasitic and free-living species that are divided into fungivorous, bacterivorous, omnivorous and predatory nematodes (Wharton, 1986; Yeates *et al.*, 1993; Mariam, 2008). Their life cycle and biology are not completely understood at present. Free-living nematodes are non-parasitic; they live in the soil and do not need plants to complete their life cycle. Their populations are usually dominant over their plant-feeding counterparts in the soils and are also commonly found inside plant roots as secondary feeders (Desaeger *et al.*, 2004).

Nematode sizes range from 0.3 mm to over 8 meters in length. University of Nebraska-Lincoln Nematology (2012) indicated that nematodes are structurally simple organisms. Adult nematodes are comprised of approximately 1,000 somatic cells, and potentially hundreds of cells associated with the reproductive system. Nematodes have been characterized as a *tube within a tube*; referring to the alimentary canal which extends from the mouth on the anterior end, to the anus located near the tail. Nematodes contain digestive, nervous, excretory, and reproductive systems, but lack a discrete circulatory or respiratory system. Reproduction is usually sexual. Males are smaller than females and often have a characteristically bent tail for holding the female for intercourse, but some nematodes are hermaphroditic keeping their self-fertilized eggs inside the uterus until they hatch. In cases of low or diminishing food supply, the juvenile will dine on their parent. Eggs produced by the female may be embryonated or unembryonated meaning their fertilized eggs may not yet be developed. A few species are known to be ovoviviparous. The eggs are protected by an outer shell and secreted by the uterus (Wikipedia, 2012a).

2.1.1 Plant-parasitic nematodes

Plant-parasitic nematodes obligatorily feed on plant tissue. They cause serious damage to many crops worldwide (Koenning *et al.*, 1999). Annual crop loss estimates caused by nematodes are roughly $80 billion worldwide. Their damages have exceeded $10 billion per year in the United States (NC State University, 2002). Members of this group have a protrusible, hollow stylet, in their mouth and use it to penetrate plant cell walls to absorb proteins and metabolites, but not all stylet bearing nematodes are plant parasites. However, they can directly affect plant health and/or be associated with disease complexes as (1) vectors (e.g. for several viruses), (2) wounding agents, (3) host conditioners, (4) resistance breakers, and (5) rhizosphere modifiers because they may cause increased root exudation affecting microbial communities and activity in the rhizosphere (Desaeger *et al.*, 2004). Each nematode species does not necessarily have the same effect on plants. Nematodes have been divided into group based on their parasitism of particular plant parts. Thus, root, stem, leaf, and seed nematodes have been distinguished (Heinz, 1988). Nematodes that feed on the surface of root cortex or epidermis such as *Helicotylenchus* and *Paratylenchus* usually have far less impact on plant productivity and vigor than vascular parasites such as *Meloidogyne* and *Heterodera* (Bernard, 1992). In addition, the host plant species and age impact infection. For example, seedlings are particularly vulnerable to damage by nematodes because their tissues are more susceptible to attack by such parasites and more conducive to nematode development than older plants (Ruehle, 1973).

(1) Morphology and anatomy

Plant nematodes are tiny, cylindrical worms usually 0.25 mm to 3 mm in length, averaging 15-35 um to 1 mm in width and taper toward the head and tail (William & Robert, 2012). Females of a few species become pear-, lemon- or kidney-shaped as they mature. Plant parasitic nematodes occupy all of the major organ systems of higher animals except respiratory and circulatory systems. The body is covered by a transparent cuticle, which bears surface marks helpful for identifying nematode species (Mid-Florida Research & Education Center, 2012)

Kris & Sodia (2002) reported that nematodes have an outer skin or cuticle that is secreted from an inner hypodermis. The muscles are attached longitudinally to the nematode’s hypodermis, causing them to move only in the dorsal ventral direction (snake-like movement). Inside the nematode from head to tail there is the alimentary canal. Between the alimentary canal and the body wall is fluid that grants pressure to maintain body shape and allow movement. At the head of a plant-parasitic nematode is a hollow mouth spear called a stylet (Figure 2.1 & 2.2).

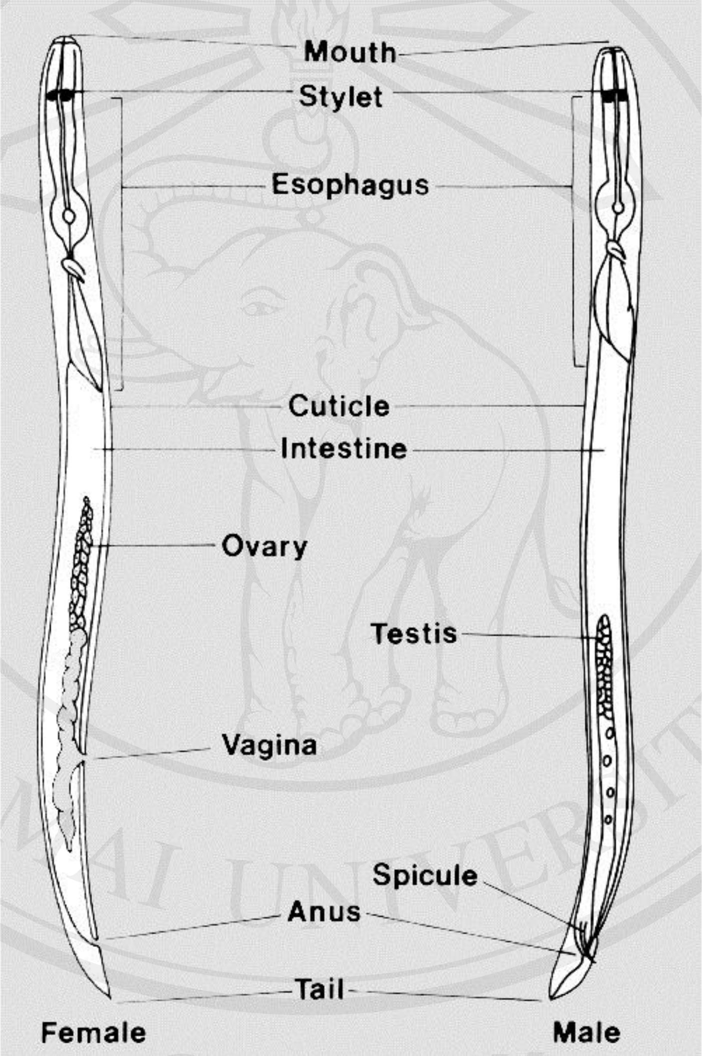
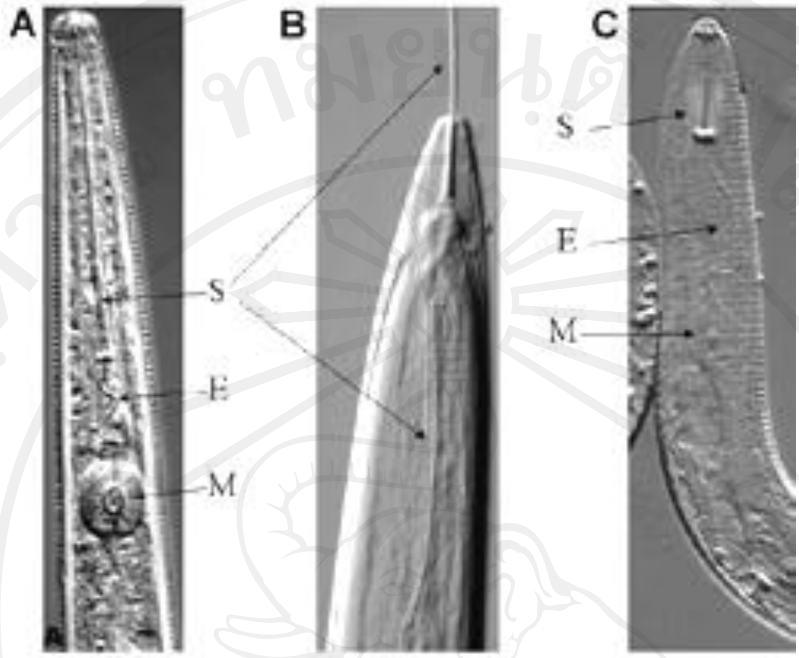


Figure 2.1 Morphological features of male and female plant parasitic nematodes.

Source: Courtesy G. L. Schumann; Kris & Sodia (2002)



M= metacarpus

E= esophagus

S= stylet

Figure 2.2 Morphological features of plant parasitic nematodes. (A) *Belonolaimus*. (B) *Longidorus*. (C) *Helicotylenchus*. Source: Courtesy K. Lambert; Kris & Sodia (2002)

The stylet is connected to the pharynx border on the intestine and the rectum in the female nematode or the cloaca in the male. Attached to the pharynx are three - five salivary glands which produce secretions controlled from the stylet and assist the nematode in plant invasion and parasitism. The pharynx muscles are essential for food acquisition including associated secretions. The reproductive organs are located in the middle to posterior parts of the nematode. Prominent parts of female reproductive organs are ovaries and vulva, while male nematodes have one or two testes and spicules which are copulatory structures (Kris & Sodia, 2002).

Principal morphological features used in nematode identification are the presence or absence and shape of a stylet, the shape and overlap of the pharyngeal glands with the intestine, the size and shape of the nematode body at the adult stage, size of the head and tail and the number and position of ovaries in the female. However, taxonomically important characteristics for a few species may include number of lines on the nematode's cuticle or the presence or absence of pore-like sensory organs. An excellent guide to nematode identification is "Plant-Parasitic Nematodes: A Pictorial Key to Genera" by William F. Mai *et al*., 1996.

(2) Life cycle and reproduction

The life cycle of a plant-parasitic nematode has six stages consisting of egg, four juvenile stages and adult. Nematode species often have both males and females, but some plant parasitic nematodes (hermaphrodites) can reproduce asexually by parthenogenesis. Most species produce between 50 and 500 eggs per female, but some can produce more than 1,000 eggs, depending on the host plant and temperature of the habitat (William & Robert, 2012). However, larvae will not emerge from the eggs until the environment is favorable for survival (Bethney, 2012).

In many nematodes, the first molt usually occurs in the egg and it is the second-stage juvenile that hatches. The length of the life cycle varies considerably. During summer months when soil temperatures are 26 to 32^{o}C, many plant nematodes complete their life cycle in about 4 weeks (Mid-Florida Research & Education Center, 2012). They evade biotic (living organisms such as bacteria, fungi, insects, weeds, and plants) and abiotic (unfavorable temperature, water unavailability, etc.) obstacles by employing a combination of behavioral and physiological survival strategies. Interestingly, many nematodes are well adapted to abiotic stress and are capable of cryptobiosis, the ability to enter a state of suspended metabolic activity during unfavorable environmental conditions. This circumstance is one reason that some nematode species are very difficult to eradicate from a field (Kris & Sodia, 2002).

(3) Nematode dissemination

Nematodes are motile animals, but most are able to move no more than a meter through the soil within their lifetime. Vertical and horizontal movement of 50 cm in 3 days and 75 cm in 9 days, respectively, have been observed in juvenile of root knot nematode, *Meloidogyne javanica*, in soil (Prot & Netscher, 1979; Paul, 1994). However, low mobility does not mean that nematodes cannot rapidly spread from field to field. Farm equipment and even muddy shoes contaminated with nematode-infested soil are dispersal methods for nematodes. Floods, irrigation and wind transport of plant debris can disperse nematodes over long distances. Inadequate regulatory practices can allow movement of infected plants, seeds, bulbs, etc. between countries (Kris & Sodia, 2002).

(4) Nematode feeding and host-parasite relationships

Mid-Florida Research & Education Center (2012) indicated that the most important plant parasitic nematodes are soil-borne, root pathogens, but a few species feed primarily upon shoot tissues. The majority of plant parasitic nematode species are in the class Chromodorea, order Rhabditida (formerly placed in the order Tylenchida). Kris & Sodia (2002) reported seven major types of nematode feeding strategies used by plant parasitic nematodes (Table 2.1). They feed on living plant tissues using an oral stylet and inject enzymes into a host cell before moving the contents into the gut. Nematodes set up their feeding site through alteration of plant gene expression that leads to the re-differentiation of the parasitized cell (Endo, 1978;

Rosane, 2007). They secrete cellulases and pectinases into plant along the juvenile's migratory path through the root cortex. These secretions may play a role in pathogenesis through: (i) penetration and migration of second stage juveniles (J2) in the plant tissue, (ii) modification and maintenance of plant cells as feeding sites, (iii) digestion of host cell contents to facilitate nutrient acquisition by the nematodes (iv) suppression of host responses (Endo & Wergin, 1977, Hussey & Mims, 1990, Hussey, 1989; Rosane, 2007). Some nematodes do not kill the plant cells because their feeding process induces the plant cells to enlarge and grow, producing one or more nutrient-rich feeding sites. These feeding cells are formed by repeated nuclear division in the absence of cell division (giant cells) or by the incorporation of adjacent cells into a syncytium formed by the breakdown of neighboring cell walls. This condition damages the plant's root system and reduces its ability to absorb water and nutrients producing a reduction of root mass, a distortion of root structure and/or enlargement of the roots. The visible symptoms of the damage are nutrient deficiency, incipient wilt, stunting, poor yield and sometimes plant death (Kris & Sodia, 2002).

Ectoparasitic nematodes remain outside of the plant and use their stylet to feed from the cells of the plant roots (Figure 2.3). They can have extremely long stylets which assist them in feeding on nutrient rich plant cells deep within the plant root.

Table 2.1 Summary of plant parasitic nematode feeding strategies

Feeding Strategy	Example Genera	Order	Infective Stage	Resistant Stage	Notes
Ectoparasites	*Belonolaimus*	Rhabditida	J2-adult		
	Xiphenema	Dorylaimida	J2-adult		Vector viruses
	Trichodorus	Triplonchida	J2-adult		Vector viruses
	Helicotylenchus				
	Longidorus				
Semi-Endoparasites	*Rotylenchulus*	Rhabditida	J4	J4	
	Tylenchulus	Rhabditida	J2	J2	
Migratory Endoparasites	*Pratylenchus*	Rhabditida	J2-adult	1/	
	Radopholus	Rhabditida			
Sedentary Endoparasites	*Meloidogyne*	Rhabditida	J2	Egg/cyst	
	Heterodera	Rhabditida	J2		
	Naccobus	Rhabditida	J2		
Stem and Bulb Nematodes	*Bursaphelenchus*	Rhabditida	J4	J3	J4 vectored by insects
	Ditylenchus	Rhabditida	J4	J4	
Seed Gall Nematodes	*Anguina*	Rhabditida	J2	J2	
Foliar Nematodes	*Aphelenchoides*	Rhabditida	J2-adult	Adult	

1/ eggs, all juvenile stages and adults can survive the winter, but not egg-producing females.

Source: Kris & Sodia (2002)

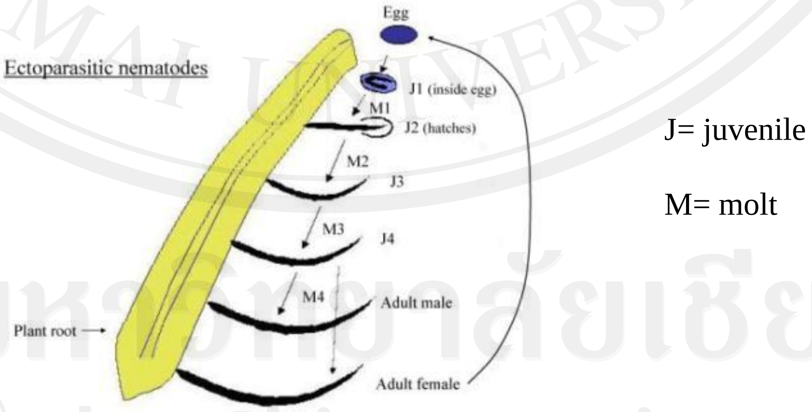
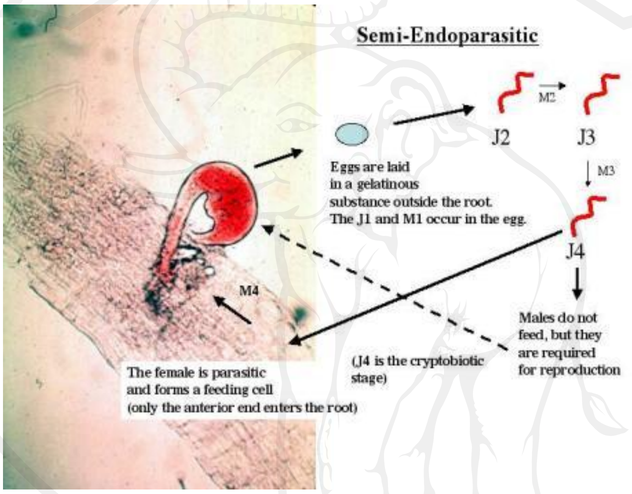


Figure 2.3 Life cycle of an ectoparasitic nematode.

Source: Courtesy K. Lambert; Kris & Sodia (2002)

Semi-endoparasitic nematodes are able to partially penetrate the plant and feed at some point in their life cycle (Figure 2.4). Usually the head of the nematode penetrates into the root and allows the nematode to form a permanent feeding cell. These nematodes swell and do not move once they have entered into the endoparasitic phase of their life cycle. A typical nematode with this life cycle is *Rotylenchulus reniformis*, the reniform (kidney-shaped) nematode.



J= juvenile

M= molt

Figure 2.4 The life cycle of *Rotylenchulus* (Reniform nematode).

Source: Courtesy K. Lambert; Kris & Sodia (2002)

Migratory endoparasitic nematodes spend much of their time migrating through root tissues destructively feeding on plant cells (Figure 2.5). These nematodes cause massive plant tissue necrosis because of their migration and feeding. When they feed from the plant, they simply suck out the plant cell cytoplasm using their stylet, killing the plant cell and moving ahead of the lesion. Because these nematodes create extensive wounds in the plant root, secondary infection by bacteria and fungi can often occur, further damaging the root system (Zunke, 1991). Examples of migratory endoparasitic nematodes are *Pratylenchus* (lesion nematode), *Radopholus* (burrowing nematodes) and *Hirschmanniella* (rice root nematode).

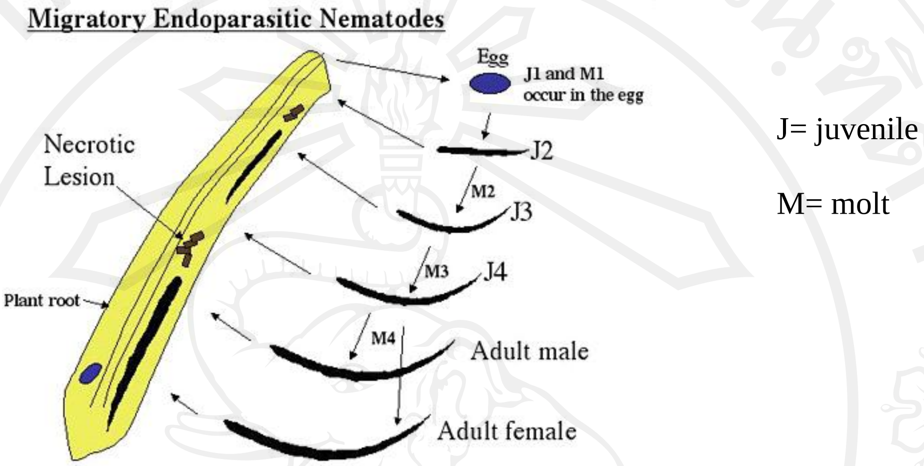
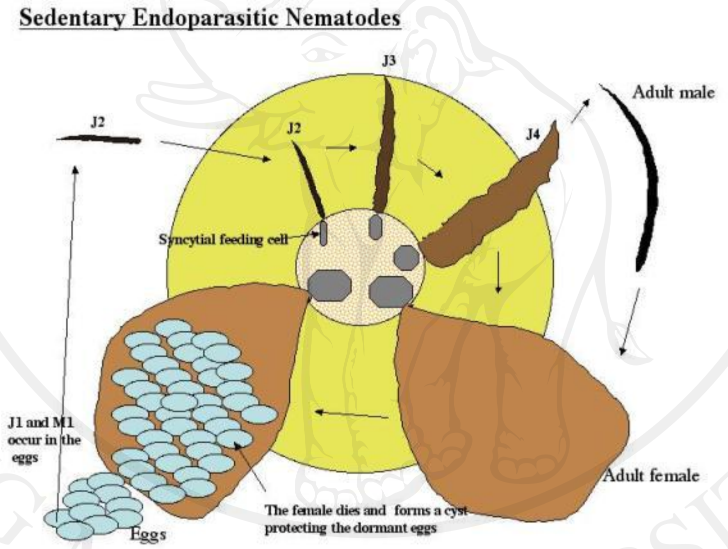


Figure 2.5 Life cycle of a typical migratory endoparasitic nematode.

Source: Courtesy K. Lambert; Kris & Sodia (2002)

Sedentary endoparasitic nematodes are among the most damaging plant pests in the world. Two important nematodes in this group are the cyst nematodes (*Heterodera* and *Globodera*) and the root-knot nematodes (*Meloidogyne*). The J2 of these nematodes invades plant near the tip of a root and migrates through the tissue to the developing vascular cells. Secretions from J2 root-knot nematodes stimulate the formation of large feeder cells (giant cells) by repeated nuclear division in the absence of cell division. Feeding cells of cyst nematodes form by the incorporation of disintegrating neighboring cells' walls forming a syncytium. The juvenile nematode rapidly becomes sedentary because their somatic muscles atrophy. They feed, enlarge and molt three times to the adult stage. The large feeding cells formed by these nematodes plug the vascular tissue of the plant making it susceptible to water stress.

Female sedentary endoparasites enlarge considerably into a saclike shape and are capable of laying large numbers of eggs. Eggs of the root knot nematode are typically laid externally in a gelatinous egg mass, but in cyst nematodes most eggs are retained inside the female body (Figure 2.6). Both types of nematode have the same basic feeding strategy, but many cyst nematodes have an obligate sexual cycle, whereas the most common species of root-knot nematodes reproduce largely by parthenogenesis.



J= juvenile

M= molt

Figure 2.6 Cross section of a soybean root depicting the life cycle of the soybean cyst nematode, *Heterodera glycines*.

Source: Courtesy K. Lambert; Kris & Sodia (2002)

2.1.2 Root-knot nematodes *Meloidogyne* spp.

Scientific classification:

Kingdom: Animalia
Phylum: Nematoda
Class: Secernentea
Order: Tylenchida
Superfamily: Tylenchoidea
Family: Heteroderidae
Genus: *Meloidogyne*

Species: *Meloidogyne incognita, M. acronea*, *M. arenaria*, *M. artiellia*, *M. brevicauda*, *M. chitwoodi*, *M. coffeicola*, *M. exigua*, *M. fruglia*, *M. gajuscus*, *M. hapla, M. incognita*, *M. javanica*, *M. enterolobii* (= *M. mayaguensis*), *M. naasi*, *M. partityla* and *M. thamesi*

Root-knot nematodes (*Meloidogyne* spp.) are the most economically important nematode pests in the Pacific (Plant Protection, 2005). They are distributed worldwide, and are obligate parasites of the roots of thousands of plant species, including both monocotyledonous (pasture and grasses) and dicotyledonous (herbaceous and woody plants) crops. The genus includes more than 60 species, with some species having several races (Wikipedia, 2012b). This makes them very difficult to control because they can always survive and reproduce on other host crops including weeds. The four most common species; *M. javanica* (Javanese root-knot nematode), *M. arenaria* (peanut root-knot nematode), *M. incognita* (southern root-knot nematode), and *M. hapla* (northern root-knot nematode) are major pests worldwide.

In Thailand, *Meloidogyne incognita* and *M. javanica* are economically important nematodes of agricultural products, including imported and exported commodities. In the north-eastern and the northern parts of Thailand potato, chili, tomato, and guava plantations are especially damaged by root knot nematodes which cause 50-100% crop loss.

Root-knot nematodes cause approximately 5% of global crop loss**.** Early-season infection leads to reduced growth, a decline in quality and yield of the crop and reduced resistance to other stresses such as drought and other diseases (Makumbi-kidza & Sikora, 2000). With changing farming systems nematode damage is likely to be associated with other problems; root-knot nematodes are often components of disease complexes (Theberge, 1985). Infection of young plants may be lethal. Males do not cause disease symptoms.

Symptoms: Galls or knots on the roots and tubers are unique underground symptoms of a root-knot nematode-infected crop. Root-knot galls are distinguished as swellings from within the root whilst nodules produced by leguminous plants are swellings sticking onto the root and can be easily flicked off the root (Figure 2.7). The degree of root galling generally depends on three factors: nematode population density, *Meloidogyne* species and "race," and host plant species and even cultivar. As the density of nematodes increases in a particular field, the number of galls per plant also will increase (Makumbi-kidza & Sikora, 2000). For example, galls of tomato (*Lycopersicon esculentum*), kava (*Piper methysticum*), parsley (*Petroselinum crispum*) and papaya (*Carica papaya*) are very apparent, whereas galls in bananas

(*Musa* spp.), sweet potato (*Ipomoea batatas*) and taro (*Colocasia esculentum*) are less noticeable. Crops belonging to the grass family (Graminaceae) such as rice (*Oryza sativa*), maize (*Zea mays*) and sugarcane (*Saccharum officinarum*) also have very small galls.

Above-ground symptoms are similar to those caused by nutrient deficiency, water stress or some soil-borne diseases and include stunting or poor growth, yellowing or chlorosis, excessive wilting, reduced yield and premature death of plants. Root-knot nematodes are present in many soils, but severe infection seems to be limited to sandy and free-draining soils. Symptoms may occur in patches, or throughout infected crops (Plant Protection, 2005).

Figure 2.7 Root-knot nematode infections of (A) pawpaw, (B) parsley and (C) noni.

Source: Plant Protection (2005)

Life cycle: Root-knot nematodes can only live as parasites of plants. Microscopic, eel-shaped or vermiform larval nematodes (second-stage juveniles; J2) hatch from eggs left in the soil around the rhizosphere of the host plants. They may reinvade the host plants of their parent or migrate through the soil to find a new host root. J2 larvae do not feed during the free-living stage, but use lipids stored in the gut.

(Eisenback & Triantaphyllou, 1991). They enter and feed on growing plant roots then stop in one place within the roots. Signals from the J2 promote parenchyma cells near the head of the J2 to become multinucleate (Hussey & Grundler, 1998) and form feeding cells, generally known as giant cells, in which the J2 females become pear-shaped and produce hundreds of eggs (Sijmons *et al*., 1994) (Figure 2.8). Root-knot nematode females lay eggs into a gelatinous matrix (GM), which is produced by six rectal glands and secreted before and during egg laying (Maggenti, & Allen, 1960; Wikipedia, 2012b). The matrix initially forms a canal through the outer layers of root tissue and later surrounds the eggs, providing a barrier to water loss by maintaining a high moisture level around the eggs (Wallace, 1968; Wikipedia, 2012b). As the gelatinous matrix ages, it becomes tanned, turning from a sticky, colourless jelly to an orange-brown substance which appears layered (Bird, 1958; Wikipedia, 2012b). Eggs are either buried inside gall tissues or stand out in small groups that can barely be seen on the surface of the galls. The egg is formed as one cell, with two-cell, four-cell and eight-cell stages recognizable. Further cell division leads to the tadpole stage, with further elongation resulting in the first stage juvenile, which is roughly four times as long as the egg. The J1 stage of *M. javanica* has 558 cells (Figure 2.9). The egg shell has three layers, with the vitelline layer outermost, then a chitinous layer and a lipid layer innermost. The life span of an adult female may extend to three months, and many hundreds of eggs can be produced. Females can continue egg laying after harvest of aerial parts of the plant and the survival stage between crops is generally within the egg (Wikipedia, 2012b). Preceded by induced changes in eggshell permeability, hatching may involve physical and/or enzymatic processes in plant-parasitic nematodes (Norton & Niblack, 1991; Wikipedia, 2012b). Ammonium ions have been shown to inhibit hatching and to reduce the plant-penetration ability of *M. incognita* juveniles that do hatch (Surdiman & Webster, 1995; Wikipedia, 2012b). The life cycle from egg to mature adult takes about one month. Several generations are possible during the life of the crop, and millions of nematodes can be associated with a mature plant. Nematodes may remain in the soil for 6 months or more after an infected crop has been harvested. Usually, they decrease rapidly in number in the absence of a host plant that is, if land is left fallow, leaving a field with no plants on it for a prolonged period to starve nematodes or other pests (Plant Protection, 2005). The length of the life cycle is temperature-dependent (Madulu & Trudgill, 1994; Trudgill, 1995). Species within the *Meloidogyne* genus also have different temperature optima. In *M. javanica*, development occurs between 13 and 34°C, with optimal development at about 29°C. Root-knot nematodes exhibit a range of reproductive modes, including sexuality (amphimixis), facultative sexuality, meiotic parthenogenesis (automixis) and mitotic parthenogenesis (apomixis) (Wikipedia, 2012b).

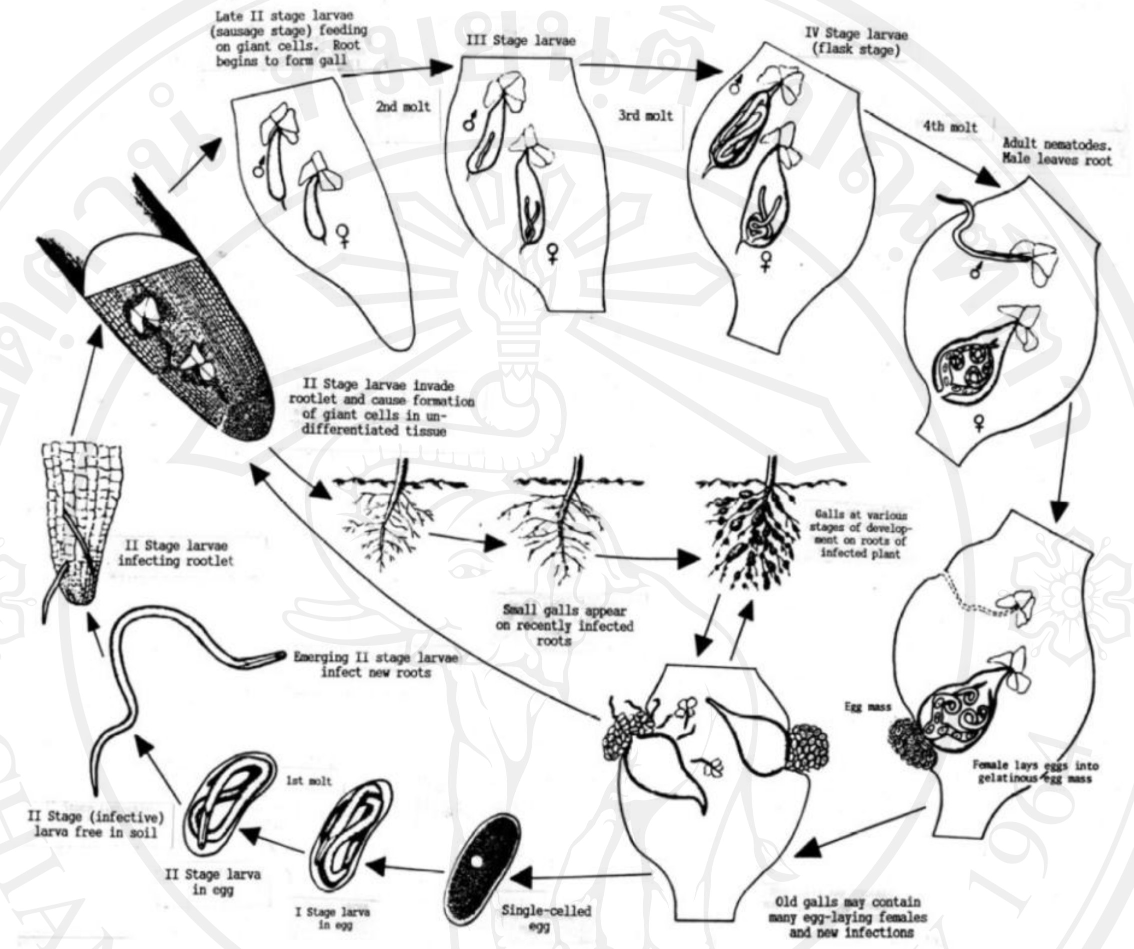


Figure 2.8 Disease cycle and epidemiology of root knot nematode.

Source: Agrios (2005)

Egg stage

J2 infective stage

Root

Figure 2.9 Development stages of *Meloidogyne* spp.

Source: Nematology 100: Lecture 15 (2012)

Host ranges: The host range of root-knot nematodes is very extensive. Many vegetables (Figure 2.10), flowering-plant, dry crops, bedding plants, shrubs and trees including weeds are susceptible (Mary, 2011). Some of the most susceptible crops in the Pacific Island countries and territories (PICT) are listed under the Host List section (Table 2.2). In Thailand, vegetable crop includes *Solanaceae* (tomato, tobacco, eggplant, chili, potato, ginger, pepper, etc.), *Cucurbitaceae* (cucumber, pumpkin, zucchini, melon, etc.) and *Cruciferae* (lettuce, cabbage, etc.) including carrot, guava, papaya, banana and some species of dry crop are reported host ranges of root-knot nematodes.

Table 2.2 The most susceptible crops of root-knot nematodes.

Common names	Botanical name
Eggplant	*Solanum melongena*
Tomato	*Lycopersicon esculentum*
Ginger	*Zingiber officinale*
Bele, aibika, island cabbage	*Abelmoschus esculentus*
Noni, nonu, nono, non,	*Morinda citrifolia*
Parsley	*Petroselinum crispum*
Carrot	*Daucus carota*
Kava, Ava, Yagona	*Piper methysticum*
Cucumber	*Cucumis sativus*
Bananas	*Musa* spp.
Lettuce	*Lactuca sativa*
Melon	*Citrullus* sp.
Okra	*Hibiscus esculentus*
Papaya	*Carica papaya*
Sweet potato	*Ipomoea batatas*
Yams	*Dioscorea* spp.
Tobacco	*Nicotiana tabacum*
Beans	*Phaseoulus* spp.
Black pepper	*Piper nigrum*
Cowpea	*Vigna sinensis*
Gourds	*Lagenaria siceraria*

Source: Plant Protection (2005)

Figure 2.10 Root-knot nematode damage to carrot roots (left) and potato (right)

Source: University of Illinois (1993)

2.1.3 Principles of nematode management

Because root-knot nematodes have a very wide host range more than 2,000 plants worldwide, they are amongst the most difficult plant parasitic nematodes to control. Identification of species is essential for effective nematode management. Root-knot disease can become severe when the natural balance of soil microorganisms is upset by such practices as mono-cropping, short rotations and repeated growing of susceptible crops without any fallow periods (Plant Protection, 2005).

The most reliable practices are preventive control; including sanitation and reduction of existing infestations through fallowing, crop rotation, soil solarization and use of succeeding crops that are non-hosts. However, these methods are primarily suitable for annual plants or to help young woody plants establish. Providing optimal conditions for plant growth including sufficient irrigation and soil amendments makes plants more tolerant to nematode infestation (UC Statewide IPM Program, 2012). At the same time, awareness of how root-knot nematodes spread to new areas is very important because this will help growers prevent their movement and will save on costly control methods (Plant Protection, 2005). There are many situations for which no safe, effective chemical nematicide is available. Most chemical nematicides are relatively toxic, so they are hazardous to people, pets, and other animals if handled carelessly. Integrated nematode management programs will often help keep nematodes below damaging levels and improve effectiveness of nematicides if they are available and must be used (William & Robert, 2012). The general practices for removing and controlling nematodes are shown as follows:

(1) Sanitation: To prevent the spread of nematodes, avoid moving reproductive plant parts, tools or equipment and soil from infested areas. In some cases, application of hot water treatment at a suitable temperature can kill nematodes in infected planting material (Plant Protection, 2005).

(2) Land management and cultural practices: Making the soil less favorable for nematode growth and reproduction such as by removing plant residue, especially root systems as soon as a crop is finished. This is very important in the case of root-knot nematode. Rotation to non-host crops or poor host crops may allow natural mortality factors to reduce nematode numbers (William & Robert, 2012). A possible crop rotation would be: susceptible > poor host > poor host > non-host or resistant host > susceptible. Good agronomic practices that give a rapid start and good crop growth may help overcome harmful effects of nematodes. Proper watering, drainage, fertility and weed control will be helpful (Plant Protection, 2005). William & Robert (2012) reported flooding with pathogen-free water at a high level for several weeks may sometimes be used to help reduce numbers of nematode pests. In addition, fallowing may be used and accompanied by regular cultivation to prevent growth of weeds and to expose new portions of the soil and nematodes to the effects of drying and heating. The amendments which include peat, manure, and compost are useful for reducing the effect of nematodes to plant crops that are water-stressed and increasing the water and nutrient holding capacity of the soil, especially in sandy soils. Marigolds (*Tagetes* spp.) are nematode-suppressive plants. French marigolds (*Tagetes patula* L.), including varieties Nemagold, Petite Blanc, Queen Sophia, and Tangerine, are the most effective in suppressing root knot and lesion nematodes (UC Statewide IPM Program, 2012).

(3) Resistant or tolerant varieties: The best ways to manage nematodes is to use varieties and rootstocks that are resistant to nematode injury (UC Statewide IPM Program, 2012), but each of these has resistance that is effective against only one, two or at most three species of nematodes (William & Robert, 2012). There are many varieties of crops in a region with some tolerance to root-knot nematodes, so growers can observe their crop varieties and select the ones showing tolerance whilst avoiding the planting of those that are very susceptible (Plant Protection, 2005).

(4) Chemical control: Nematicides are poisonous to man and animals, including the environment and water supplies so people using them must understand and follow the instructions on the pesticide labels. Some of the nematicides that can be used to kill root-knot nematodes and other nematodes in the soil are: Dazomet, a granular formulation that can be used for seedbed treatment. When incorporated in moist soil dazomet releases methyl isocyanate gas, which kills nematodes. Fenamiphos, another granular formulation that can be used before or at planting or in established crops. Oxamyl in a liquid formulation is supplied with an application gun that makes application into the soil safer (Plant Protection, 2005). Nematicides sometimes can be very profitable when used correctly in appropriate situations. However, their effects are almost universally short-lived, so they should be used in conjunction with other practices that minimize nematode re-infestation of a planting site (William & Robert, 2012).

(5) Biological control: Natural enemies for the control of root-knot nematodes are now being developed as commercial products in many developed countries (Plant Protection, 2005). Many different bacteria and fungi that are natural enemies of nematodes have been isolated from nematode populations. Most of them are recognized as promising for biological control of one species or quite specific in terms of which nematodes they will attack (William & Robert, 2012). The bacterium *Pasteuria penetrans* is one such natural enemy that has been developed into a commercial biological nematicide for controlling root-knot nematodes (Plant Protection, 2005). Nordbring-Hertz *et al*. (2002) stated the early biological control experiments to control plant-parasitic nematodes concentrated on using nematode-trapping fungi, e.g. *Arthrobotrys* or *Monacrosporium* species, and later shifted towards endoparasitic fungi, e.g. *Hirsutella rhossoliensis* and *Drechmeria coniospora*, and egg-parasitic fungi, e.g. *Verticillium chlamydosporium*.

2.2 Nematophagous fungi

Nematophagous fungi or trapping fungi are found in most fungal taxa including Ascomycetes, and their hyphomycete anamorphs, Basidiomycetes, Zygomycetes, Chytridiomycetes and Oomycetes (Figure 2.11) (78 Steps Health Journal, 2012). They are micro-fungi that can capture, kill and digest nematodes. They use special mycelial structures, the so-called traps, or spores to trap vermiform nematodes or hyphal tips to attack nematode eggs and cysts before penetration of the nematode cuticle, invasion and digestion. There are a number of reasons for an interest these fungi as bio-control agents against plant- and animal-parasitic nematodes (Nordbring-Hertz *et al*., 2006).

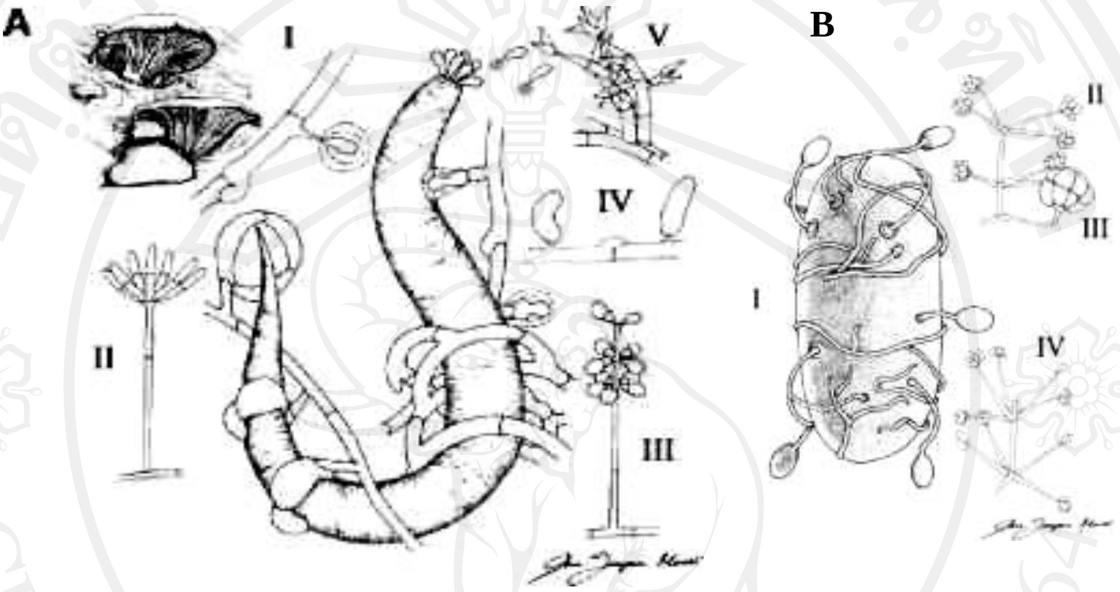


Figure 2.11 Biology of nematophagous fungi. Vermiform (motile) nematode (A) displaying infection structures: (I) toxin-producing fungus (*Pleurotus* sp.), nematode-trapping fungi (II) *Drechslerella* sp. (III) *Arthrobotrys* sp., (IV) *Nematoctonus* sp. and (V) endoparasitic *Drechmeria* sp.. Nematode (sedentary) egg (B) (similar features can be found in egg masses, females and cysts) displaying infection structures: penetrating hyphae and appressoria of egg-parasitic fungi (I), conidia (II) and chlamydospores (III) of *Pochonia* sp., and conidia of *Lecanicillium* sp. (IV).

Source: 78 Steps Health Journal (2012)

Nematophagous (nematode-destroying) fungi comprise more than 200 species of taxonomically diverse fungi that all share the ability to attack living nematodes (juveniles, adults and eggs) and use them as nutrients. The fungi differ in their saprophytic/parasitic ability. The ability to capture nematodes is connected with a specific developmental phase of the fungal mycelium (Table 2.3).

Table 2.3 Typical infection structures of some nematophagous fungi

Infection structure	Species	Taxonomic classification
Adhesive nets	*Arthrobotrys oligospora* *A. conoides* *A. musiformis* *A. superba* *Duddingtonia flagrans*	Deuteromycetes
Adhesive branches	*Monacrosporium gephyropagum*	Deuteromycetes
Adhesive knobs	*M. ellipsosporum* *M. haptotylum*	Deuteromycetes
Constricting rings	*A. dactyloides* *A. brochopaga*	Deuteromycetes
Adhesive knobs and adhesive spores	*Nematoctonus concurrens*	Basidiomycetes
Adhesive spores	*N. leiosporus*	Basidiomycetes
	Drechmeria coniospora *Hirsutella rhossoliensis*	Deuteromycetes
Ingested spores	*Harposporium anguillulae*	Deuteromycetes
Zoospores	*Catenaria anguillulae*	Chytridiomycetes
	Haptoglossa dickii	Oomycetes
Adhesive hyphae	*Stylopage hadra* *Cystopage cladospora*	Zygomycetes
Toxic droplets	*Pleurotus ostreatus*	Basidiomycetes
Appressoria	*Pochonia chlamydosporia*	Deuteromycetes

Source: (Nordbring-Hertz *et al.*, 2006).

2.2.1 Diversity of infection structures

The type of nematode-trapping structures formed depends on species or even strains of species as well as on biotic and abiotic environmental conditions. The most important biotic factor is living nematodes, which not only induce the formation of trapping structures by touching the mycelium but also serve as a food source for the fungi after invasion. Trapping structures of other fungi, such as branches, knobs and constricting rings, may be formed spontaneously, indicating the greater need of these fungi for nematodes as nutrient source. The endoparasites and the spontaneous trap-formers present a high parasitic ability. Traps may be formed directly on germination of conidia (spores) to form the so-called conidial traps. This developmental pattern occurs in practically all trap-forming species when conidia are allowed to germinate in natural substrates, such as cow dung or rhizosphere soil (Persmark & Nordbring-Hertz, 1997; Nordbring-Hertz *et al*., 2006).

Endoparasitic fungi: These fungi use their spores to infect nematodes. The spores adhere to the nematode cuticle or, in some species, are ingested together with food. The endoparasitic fungi are obligate parasites of nematodes and spend their entire vegetative lives inside infected nematodes. Examples of these fungi are *Catenaria anguillulae*, *Drechmeria coniospora* and *Nematoctonus concurrens* (American Society for Microbiology, 2010).

Egg-parasitic fungi: The fungi that parasitize the nonmotile stages of nematodes, i.e. eggs, use a different strategy. For example, Hyphae of *Pochonia chlamydospora* and other fungi grow towards the eggs and appressoria are formed on the hyphal tips which penetrate the eggshell. The fungi then digest the contents of the egg, both immature and mature (containing juveniles) eggs (Nordbring-Hertz *et al*., 2006).

2.2.2 Taxonomy and evolution of nematophagous fungi

The taxonomic position of some of nematophagous fungi species has been clarified by the discovery of the corresponding sexual stages of the fungus (Pfister, 1997; Nordbring-Hertz *et al*., 2006). For example, the sexual stages (teleomorphs) of a number of *Arthrobotrys*, *Monacrosporium* and *Dactylella* species (anamorphs) have been identified as *Orbilia* spp. belonging to the discomycetes (Ascomycetes).

Evolution: The parasitic habit of nematode-trapping fungi has evolved among cellulolytic or lignolytic fungi as a response to nutrient deficiencies in nitrogen-limiting habitats (Barron, 1992). Nordbring-Hertz *et al*. (2006) reported many nematode-trapping deuteromycetes are indeed good saprophytes and can utilize cellulose and other polysaccharides as carbon sources. Noticeably, the saprophytic ability varies among nematode-trapping fungi and is correlated with their parasitic activity. Species with high parasitic activity grow more slowly and have more special nutrient requirements than species with low parasitic activity. Thus, it appears that over evolutionary time, the more specialized parasitic species have lost some of the activity of the enzymes involved in saprophytic metabolism. The fact that several of the identified teleomorphs of nematode-trapping deuteromycetes are wood decomposers also supports the hypothesis that nematode-trapping fungi have evolved from cellulolytic or lignolytic fungi. The phylogenetic relationships of other nematophagous fungi including the endoparasites, is still virtually unknown. A different evolutionary history is expected within the endoparasitic fungi, which are generally more dependent on nematodes.

2.2.3 Molecular phylogeny

The fact that species of nematode-trapping fungi are found in all major groups of fungi indicates that nematode parasitism has evolved independently several times. Moreover, molecular methods such as ribosomal DNA (rDNA) sequences offer new possibilities to examine the evolutionary origin and the relationships of nematode trapping fungi in more detail. In the past, identification was based solely on the morphology of the conidia and conidiophores, but Scholler *et al.* (1999) reclassified the *Orbiliaceae* genera based on the ITS1, ITS2, and 5.8S rDNA sequences, demonstrating that trapping devices are more informative than other morphological structures. Korabecna (2007) showed that the PCR amplification and subsequent restriction analysis of the ribosomal region spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA is a powerful method for species identification and epidemiological tracing in medical mycology. Liou & Tzean, 1997; Ahŕen *et al*., 1998; Nordbring-Hertz *et al*., 2006 using the 18S rDNA region have recently shown that a number of the common species of nematode-trapping fungi, including species of the genera *Arthrobotrys*, *Dactylaria* and *Monacrosporium*, form a monophyletic group (clade) (Figure 2.12). Notably, the phylogenetic patterns within this clade were not concordant with the morphology of the conidia and the conidiophores according to traditional classification but rather with the morphology of the infection structures. Accordingly, Scholler *et al*. (1999); Nordbring-Hertz *et al*. (2006) suggested that predatory anamorphic Orbiliaceae fungi should be divided into four genera:

Arthrobotrys forming adhesive networks, *Drechslerella* forming constricting rings, *Dactylellina* forming stalked adhesive knobs and *Gamsylella* species producing adhesive columns and unstalked knobs.

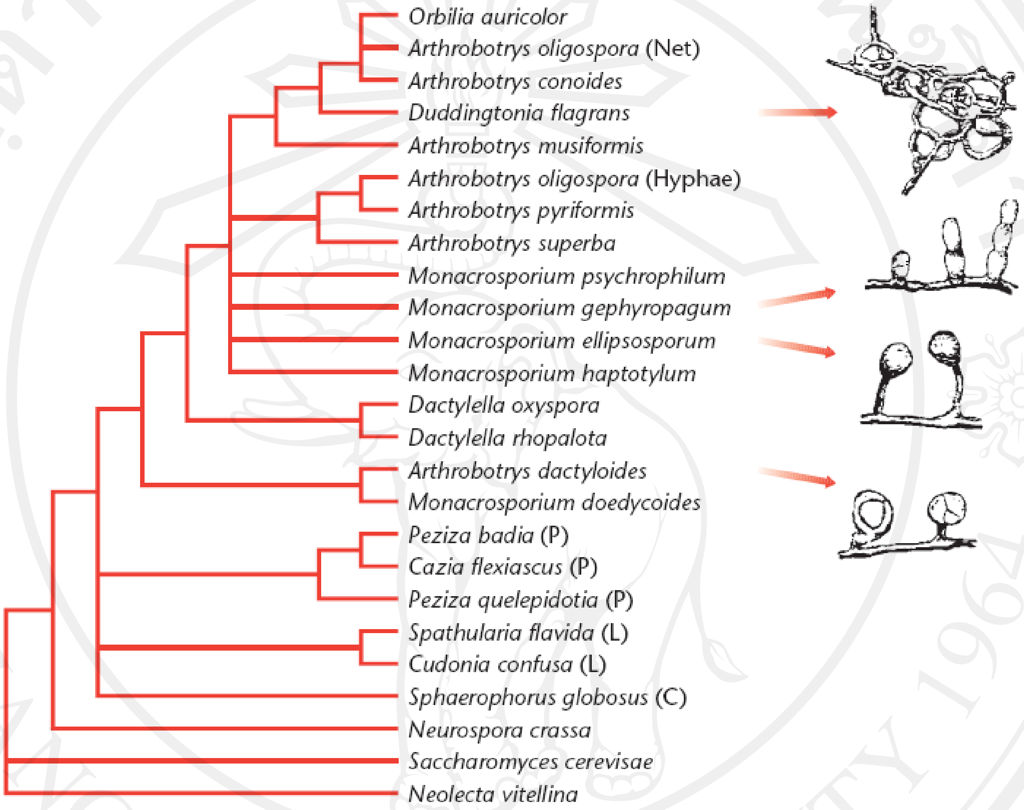


Figure 2.12 A phylogenetic tree based on the sequences of 18S rDNA showing the relationships among the nematode-trapping fungi and the position of this clade among species from the Pezizales (P), Leotiales (L) and Calciales (C). *Neolecta vitellina* was used as an outgroup for the analyses. Note that the phylogenetic pattern is concordant with the structure of the trapping devices. *Orbilia auricolor* is the teleomorph (sexual stage) of *A. oligospora*. after Ahŕen *et al*. (1998).

Source: Nordbring-Hertz *et al*. (2006)

2.2.4 Ecology of nematophagous fungi

Nematophagous fungi have been found in all regions of the world and are especially abundant in soils rich in organic material. In agricultural soils in temperate regions the nematode trapping fungi follow a seasonal variation, with highest densities and number of species in late summer and autumn, possibly due to the higher soil temperature and increased input of organic debris. Jerzy & Aneta (2007) reported the optimum temperature for growth and spore production ranged between 24 and 28^{o}C but, the best growth of *Lecanicillium psalliotae* was at 30^{o}C.

The fungi are most frequent in the upper 20 cm of the soil and appear to be almost absent below 40 cm (Persmark *et al*., 1996; Nordbring-Hertz *et al*., 2006). A strict correlation between number of propagules of nematophagous fungi and number of nematodes is difficult to obtain, although in some soils a correlation exists between number of species of nematophagous fungi and the number of nematodes. This raises the question whether the parasitism of nematophagous fungi can regulate the population size of soil nematodes. Pesticides may be affecting the subsistence of these fungi especially their growth and sporulation. For example, carbendazim caused 99% inhibition of radial mycelial growth of *Arthrobotrys oligospora* in Petri dishes at all concentrations tested (10-40 μg a. i. ml^{-1}) in comparison to non-treated controls. mancozeb caused 43% and 23% inhibition at 250 and 500 μg a. i. ml^{-1} respectively and 99% inhibition at a concentration of 1,000 μg a. i. ml^{-1} and above, *in vitro*. In addition, Goltapeh *et al.* (2008) indicated diflubenzuro and malathion at 10-40 μg a. i. ml^{-1} caused 30-41% and 24-54% inhibition, respectively while formalin (0.5-2.0% v/v) inhibited growth of *A. oligospora* completely.

Most plant-parasitic nematodes attack plant roots so many nematode trapping fungi have been found to occur more frequently in the rhizospheres of plants, especially leguminous plants, e.g. soybean and pea, than in root-free soil. This effect could possibly be due to increased or changed plant root exudation. Evaluate whether trapping structures and consequently trapping of nematodes are actually more abundant in rhizosphere. Sobita & Anamika (2011) reported that amending the soil with application of mass cultures of *Arthrobotrys oligospora* and *Dactylaria eudermata* reduced the number of root galls by 86.9% and 81.1% and of females by 94.2% and 91.7%, respectively. The mass culture of these fungi increased the plant growth: shoot length by 41.9% and 38.8%, root length by 44.6% and 41.8%, fresh weight of shoots by 61.1% and 58.7%, and fresh weight of roots by 24.3% and 22.5%, respectively over non-treated, nematode-infested soil. The better performance of *A. oligospora* may be attributed to better colonization and establishment than *D. eudermata* and may be due to better tolerance by the fungus to soil fungistasis.

Interactions with other fungi and plants: Nematode-trapping fungi such as *Arthrobotrys oligospora* attack their host fungi as mycoparasites by coiling of the hyphae of the nematode-trapping fungi around the host hyphae, which results in disintegration of the host cell cytoplasm without penetration of the host. It was shown that nutrient transfer took place between the nematode-trapping fungus *A. oligospora* and its host *Rhizoctonia solani* using radioactive phosphorous tracing (Olsson & Persson, 1994). *A. oligospora*, *P. chlamydosporia* and other nematophagous fungi have the capacity to colonize plant roots (Bordallo *et al*., 2002; Nordbring-Hertz *et al*., 2006). The fungi grow inter- and intracellularly and form appressoria when penetrating plant cell walls of epidermis and cortex cells, but never enter vascular tissues. Histochemical stains show plant defense reactions, e.g. papillae, lignitubers and other cell wall appositions induced by nematophagous fungi, but these never prevented root colonization. The growth of the nematophagous fungi in plant roots is endophytic, i.e. the host remains asymptomatic. Endophytic growth of *P. chlamydosporia* in barley and wheat roots appeared to increase plant growth and reduce growth of the plant parasitic take-all fungus *Gaeumannomyces graminis* var. *tritici* (Monfort *et al*., 2005; Nordbring-Hertz *et al*., 2006). Mycoparasitism and plant endophytism may be important issues for extension of the biological control potential of the nematophagous fungi.

Biological control: There are two general ways of applying biological controls of nematodes using nematophagous fungi: addition of large amounts of fungi to the soil; or stimulation of the activity of the existing fungi using various amendments. In general,V-8 juice agar (VA), cornmeal agar and potato dextrose agar were good media for growth, and malt extract agar, VA and yeast dextrose agar were good for sporulation of *Hirsutella rhossiliensis* (Liu & Chen, 2002). Débora *et al*. (2003) reported that the use of solid state fermentation may provide effective formulations of fungi that are employed in the control of nematodes. For example, bio-compost with coffee husk showed a reduction in the number of nematodes that reached 80% while bio-compost with cassava bagasse had 60%, and bio-compost with defatted soybean cake had 100%. However, the performances of these biological control agents have varied and, so far, no commercial products are available. There is a renewed interest in using nematode-trapping fungi, partly due to an increased knowledge on the biology of these fungi and partly due to better methods of formulating and applying fungal bio-control agents to soil. One way to improve the control potential of nematophagous fungi would be to use genetic engineering to increase the pathogenicity and survival of the introduced fungus. Using genetic transformation, it was possible to generate mutants of the nematode-trapping fungus *A. oligospora* that over-expressed a protease gene (PII). Mutants containing additional copies of the PII gene developed a higher number of infection structures and had an increased speed of capturing and killing nematodes (A˚hman *et al*., 2002). Nevertheless, Bourne *et al*. (1996) and Nordbring-Hertz *et al*. (2006) suggested that rhizosphere colonization is necessary for successful establishment, and therefore screening for rhizosphere-competent strains of nematophagous fungi is of paramount importance.

2.2.5 Nematode-fungus interaction mechanisms

Recognition and host specificity: There are recognition events in the cell–cell communication at several steps of the interaction between fungus and nematode, which might elicit a defined biochemical, physiological or morphological response. Nematodes are attracted to the mycelia of the fungi in which they may induce trap formation and they are attracted even more to fully developing traps and spores. This is followed by a *short-range* or contact communication: adhesion. This step may involve an interaction between a carbohydrate-binding protein (lectin) in the fungus and a carbohydrate receptor on the nematode. Recognition of the host is probably also important for the subsequent steps of the infection, including penetration of the nematode cuticle (Figure 2.13) (Nordbring-Hertz *et al*., 2006).

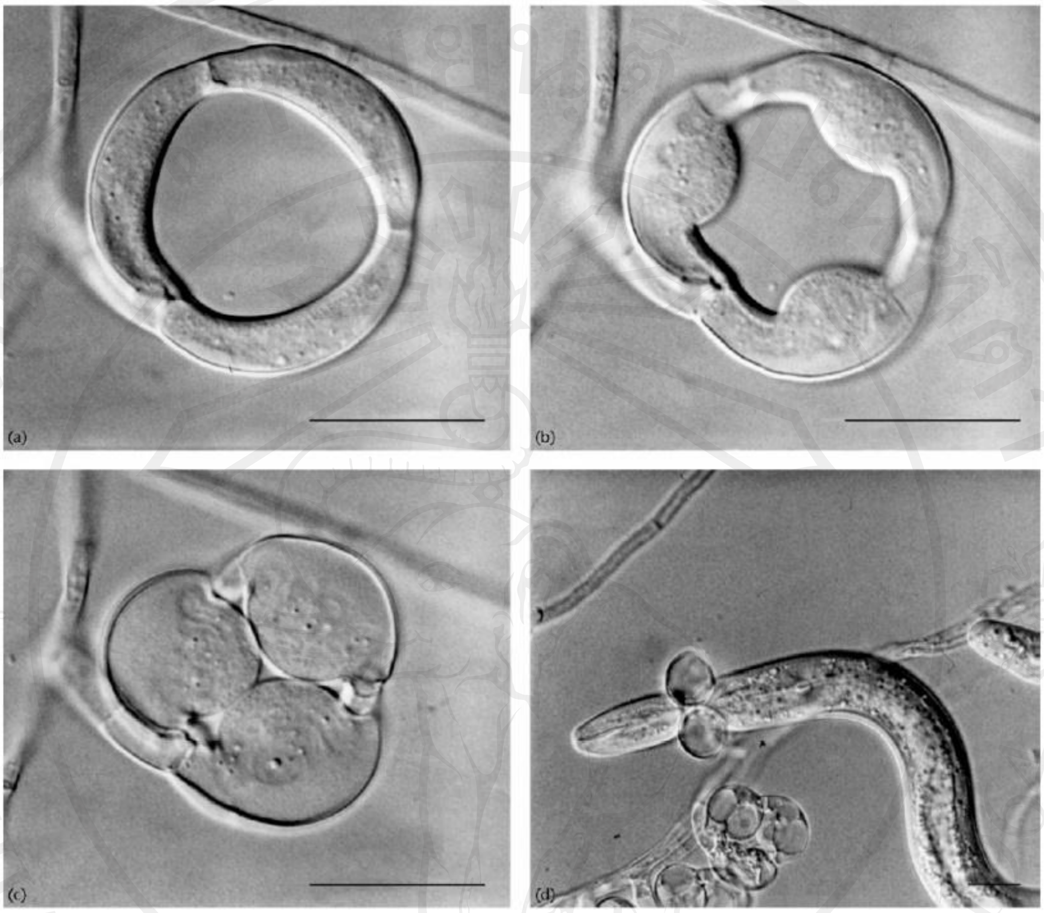


Figure 2.13 Trapping mechanism of constricting rings of *Arthrobotrys brochopaga*. (a–c) Closure of a ring triggered by applying heat to the trap. The closure is rapid (0.1 s), irreversible and is accompanied by a large increase in cell volume leading to an almost complete closure of the aperture of the trap. Bars, 5 mm. (d) Nematode firmly captured in a ring. Bar, 10 mm.

Source: Nordbring-Hertz *et al*. (2006)