

# 4

## Cyst Nematodes\*

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## 4.1. Introduction to Cyst Nematodes

The cyst nematodes are a major group of plant-parasitic nematodes and of great economic importance in many countries throughout the world. They cause considerable yield losses to many important crops, including cereals, rice, potatoes and soybean, with the most economically important species occurring within the genera *Heterodera* and *Globodera* (Table 4.1). *Heterodera* contains by far the largest number of species (Table 4.2), although several other cyst-forming species have been described within other genera (Table 4.3). Eight genera, *Heterodera* (82 species), *Globodera* (12 species), *Cactodera* (13 species), *Dolichodera* (1 species), *Paradolichodera* (1 species), *Betulodera* (1 species), *Punctodera* (4 species) and *Vittatidera* (1 species), and a total of 114 valid species are presently recognized within this nematode group (Subbotin *et al.*, 2010a,b). The classification of cyst nematodes is given in Box 4.1. Cyst nematodes were originally considered to be largely a pest of temperate regions but many cyst nematodes are now known to be present in tropical and subtropical regions (Evans and Rowe, 1998).

It is impossible to evaluate the total economic losses caused by cyst nematodes throughout the world as many environmental, biological and cultural factors cause yield losses to crops, although some specific examples of losses exist. Potato cyst nematodes (PCN) have been well studied within Europe, and overall losses are estimated at about 9% of potato production; however, in other regions of the world, or when no control strategies are employed, total losses can occur. This range could equally be applied to all major crops that are hosts to cyst nematodes.

All cyst nematodes feed within the root system of their hosts and are characterized by the tanning and drying (cutinization) of the body wall of the sedentary adult female following fertilization and production of embryonated eggs (see Section 4.2). The resultant cyst allows the succeeding generation to survive for extended periods until a suitable host is growing in the near vicinity. It is this ability to persist for many years in the soil in the absence of a host that contributes to the economic importance of this group in agricultural situations.

**Table 4.1.** Cyst nematodes of major economic importance. (Adapted from Evans and Rowe, 1998 and Subbotin *et al.*, 2010a,b.)

Genus	Species	Main crops affected	Region
<i>Globodera</i>	<i>pallida</i>	Potato, tomato, eggplant	Temperate
	<i>rostochiensis</i>	Potato, tomato, eggplant	Temperate
	<i>tabacum</i>	Tobacco, tomato	Temperate
<i>Heterodera</i>	<i>avenae</i>	Wheat, barley, oat, maize	Temperate
	<i>filipjevi</i>	Wheat, barley, oat, maize	Temperate
	<i>cajani</i>	Cowpea, pea, Phaseolus bean, pigeon pea, sesame, soybean, sweetcorn	Tropical
	<i>cruciferae</i>	Brussels sprout, broccoli, cabbage, cauliflower, radish, kohlrabi, pea, rape	Temperate
	<i>glycines</i>	Adzuki bean, broad bean, French bean, hyacinth bean, kidney bean, moth bean, mung bean, navy bean, rice bean, snap bean, soybean, blackgram, cowpea, sesame, white lupin, yellow lupin	Temperate
	<i>goettingiana</i>	Broad bean, chickpea, lentil, pea, white lupin, yellow lupin, white clover	Temperate
	<i>latipons</i>	Barley, oat, rye	Temperate
	<i>oryzicola</i>	Rice, banana and plantain	Tropical
	<i>sacchari</i>	Rice, sugarcane	Tropical
	<i>schachtii</i>	Adzuki bean, beet, broccoli, Brussels sprout, cabbage, cauliflower, celery, chickpea, chicory, Chinese cabbage, cowpea, dill, kale, kohlrabi, lentil, pea, radish, rape, rhubarb, rutabaga, spinach, tomato, turnip, yellow lupin	Temperate
	<i>sorghii</i>	Sorghum, maize, rice	Tropical
	<i>trifolii</i>	Carnation, chickpea, cucumber, gherkin, pea, pumpkin, red clover, rhubarb, spinach, squash, tomato, white clover, white lupin, zucchini	Temperate
	<i>zeae</i>	Maize, barley, rice, sorghum, wheat	Tropical

## 4.2. Life Cycle and Behaviour

The life cycle of cyst nematodes is shown in Fig. 4.1. After gastrulation, the embryo extends in length within the eggshell and movement begins, then folds develop in the embryo. After the first moult, the stylet forms at the anterior end of the second-stage juvenile (J2). This is the dormant stage of the life cycle and, depending on the species and environmental conditions, the J2 can remain within the protective cyst for many years (see Chapter 7 for a discussion of dormancy as a survival strategy). The eggshell containing the J2 consists of three layers in cyst nematodes: the outer lipoprotein layer derived from the vitelline layers of the fertilized oocyte, the middle chitinous layer, which provides the eggshell with its structural strength, and the innermost lipid layer, which represents the main permeability barrier. The active part of the life cycle starts when the J2 hatches out of the egg, having used its stylet to cut a slit in the eggshell; the hatching process of cyst nematodes is discussed in detail in Section 7.6.5.

**Table 4.2.** Cyst species (82) of the genus *Heterodera* Schmidt, 1871.  
(Adapted from Subbotin *et al.*, 2010b.)

Species	Main host plant family	Species	Main host plant family
<i>africana</i>	Poaceae	<i>lespedezae</i>	Fabaceae
<i>agrostis</i>	Poaceae	<i>leuceilyma</i>	Poaceae
<i>amygdali</i>	Rosaceae	<i>litoralis</i>	Amaranthaceae
<i>arenaria</i>	Poaceae	<i>longicolla</i>	Poaceae
<i>aucklandica</i>	Poaceae	<i>mani</i>	Poaceae
<i>australis</i>	Poaceae	<i>medicaginis</i>	Fabaceae
<i>avenae</i>	Poaceae	<i>mediterranea</i>	Anarcardiaceae
<i>axonopi</i>	Poaceae	<i>menthae</i>	Lamiaceae
<i>bamboosi</i>	Poaceae	<i>mothi</i>	Cyperaceae
<i>bergeniae</i>	Saxifragaceae	<i>orientalis</i>	Poaceae
<i>betae</i>	Amaranthaceae	<i>oryzae</i>	Poaceae
<i>bifenestra</i>	Poaceae	<i>oryzicola</i>	Poaceae
<i>cajani</i>	Fabaceae	<i>pakistanensis</i>	Poaceae
<i>canadensis</i>	Cyperaceae	<i>persica</i>	Apiaceae
<i>cardiolata</i>	Poaceae	<i>phragmitidis</i>	Poaceae
<i>carotae</i>	Apiaceae	<i>plantaginis</i>	Plantaginaceae
<i>ciceri</i>	Fabaceae	<i>pratensis</i>	Poaceae
<i>cireae</i>	Onagraceae	<i>raskii</i>	Cyperaceae
<i>cruciferae</i>	Brassicaceae	<i>ripae</i>	Poaceae
<i>cyperi</i>	Cyperaceae	<i>riparia</i>	Urticaceae
<i>daverti</i>	Fabaceae	<i>rosii</i>	Polygonaceae
<i>delvii</i>	Poaceae	<i>sacchari</i>	Poaceae
<i>elachista</i>	Poaceae	<i>sacchariphila</i>	Poaceae
<i>fengi</i>	Poaceae	<i>salixophila</i>	Salicaceae
<i>fici</i>	Moraceae	<b><i>schachtii</i>*</b>	Amaranthaceae
<i>filipjevi</i>	Poaceae	<i>scutellariae</i>	Lamiaceae
<i>galeopsidis</i>	Lamiaceae	<i>sinensis</i>	Poaceae
<i>gambiensis</i>	Poaceae	<i>skohensis</i>	Poaceae
<i>glycines</i>	Fabaceae	<i>sonchophila</i>	Asteraceae
<i>glycyrrhizae</i>	Fabaceae	<i>sorghii</i>	Poaceae
<i>goettingiana</i>	Fabaceae	<i>spinicauda</i>	Poaceae
<i>goldenii</i>	Poacea	<i>spiraeae</i>	Rosaceae
<i>graminis</i>	Poacea	<i>swarupi</i>	Poaceae
<i>graminophila</i>	Poacea	<i>trifolii</i>	Fabaceae
<i>hainanensis</i>	Poacea	<i>turangae</i>	Salicaceae
<i>hordecalis</i>	Poacea	<i>turcomanica</i>	Amaranthaceae
<i>humuli</i>	Cannabaceae	<i>urtica</i>	Urticaceae
<i>johanseni</i>	Brassicaceae	<i>ustinovi</i>	Poaceae
<i>kirjanovae</i>	Betulaceae	<i>uzbekistanica</i>	Salicaceae
<i>koreana</i>	Poaceae	<i>vallicola</i>	Ulmaceae
<i>latipons</i>	Poaceae	<i>zeae</i>	Poaceae

\*Type species.

Hatching represents the end of dormancy. Cyst nematodes exhibit diapause and quiescence, the two types of dormancy (Jones *et al.*, 1998; Chapter 7). Diapause, a state of arrested development whereby hatching does not occur until specific requirements, including a time component, have been satisfied, enables the J2 to overcome

**Table 4.3.** Cyst-forming species of genera other than *Heterodera*.  
(Adapted from Subbotin *et al.*, 2010a.)

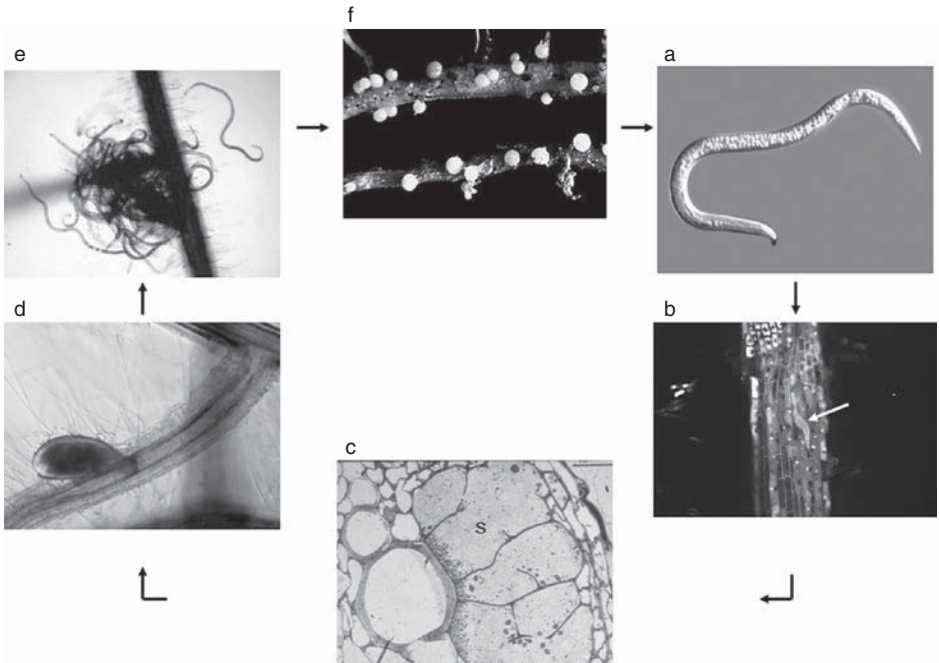
Genus (number of species)	Species	Host-plant family
<i>Betulodera</i> (1)	<b><i>betulae</i>*</b>	Betulaceae
<i>Cactodera</i> (13)	<i>acnidae</i>	Amaranthaceae
	<i>amaranthi</i>	Amaranthaceae
	<b><i>cacti</i>*</b>	Cactaceae
	<i>eremica</i>	Amaranthaceae
	<i>estonica</i>	Polygonaceae
	<i>evansi</i>	Caryophyllaceae
	<i>galinsogae</i>	Asteraceae
	<i>milleri</i>	Amaranthaceae
	<i>radicale</i>	unknown
	<i>rosae</i>	Poaceae
	<i>salina</i>	Amaranthaceae
	<i>thornei</i>	Portulacaceae
	<i>weissi</i>	Polygonaceae
<i>Dolichodera</i> (1)	<b><i>fluvialis</i>*</b>	Poaceae
<i>Globodera</i> (12)	<i>artemisiae</i>	Asteraceae
	<i>bravoae</i>	Solanaceae
	<i>capensis</i>	Unknown
	<i>ellingtonae</i>	Solanaceae
	<i>leptonepia</i>	Solanaceae
	<i>mali</i>	Rosaceae
	<i>mexicana</i>	Solanaceae
	<i>millefolii</i>	Asteraceae
	<i>pallida</i>	Solanaceae
	<b><i>rostochiensis</i>*</b>	Solanaceae
	<i>tabacum</i>	Solanaceae
<i>zelandica</i>	Onagraceae	
<i>Paradolichodera</i> (1)	<b><i>tenuissima</i>*</b>	Cyperaceae
<i>Punctodera</i> (4)	<i>chalcoensis</i>	Poaceae
	<i>matadorensis</i>	Poaceae
	<b><i>punctata</i>*</b>	Poaceae
<i>Vittatidera</i> (1)	<i>stonei</i>	Poaceae
	<b><i>zeaphila</i>*</b>	Poaceae

\*Type species.

environmental conditions that are unfavourable for hatch, such as extreme temperatures or drought. The extent of the diapause varies but several cyst nematodes, e.g. *Globodera rostochiensis* and *Heterodera avenae*, show obligate diapause during their first season of development. In *G. rostochiensis* and *G. pallida*, diapause is terminated in late spring, when the combination of rising soil temperature and adequate soil moisture is conducive for infection of the new potato crop. The duration of obligate diapause is affected by the photoperiod experienced by the infected plant, with unhatched J2 from plants grown under continuous light showing no obligate diapause. Facultative diapause is initiated by external factors, such as various environmental factors, from the second season onwards. Once diapause is completed, the J2 may enter into a quiescence state, which requires various environmental cues to effect further development

#### Box 4.1. Classification of the cyst nematodes.

Phylum: Nematoda Potts, 1932  
Class: Chromadorea Inglis, 1983  
Subclass: Chromadoria Pearse, 1942  
Order: Rhabditida Chitwood, 1933  
Suborder: Tylenchina Thorne, 1949  
Infraorder: Tylenchomorpha De Ley & Blaxter, 2002  
Superfamily: Tylenchoidea Örley, 1880  
Family: Hoplolaimidae Filipjev, 1934  
Subfamily: Heteroderinae Filipjev & Schuurmans Stekhoven, 1941  
Genera: *Heterodera* Schmidt, 1871 (Type Genus)  
*Globodera* Skarbilovich, 1959  
*Punctodera* Mulvey & Stone, 1976  
*Cactodera* Krall & Krall, 1978  
*Dolichodera* Mulvey & Ebsary, 1980  
*Betulodera* Sturhan, 2002  
*Paradolichodera* Sturhan, Wouts & Subbotin, 2007  
*Vittatidera* Bernard, Handoo, Powers, Donald & Heinz, 2010



**Fig. 4.1.** Life cycle of a cyst nematode. Cysts contain up to 400 eggs, each one containing a second-stage juvenile (J2). After hatch (a), the J2 moves through the soil, invades a host root (arrowed) (b) and moves through the root to establish a feeding site (syncytium) (c) on which it feeds and develops. Juveniles develop either into females, which become saccate and rupture the root (d), or to vermiform males, which leave the root, locate the female and mate (e). The female then dies to form the cyst (f).

of the life cycle. In temperate regions this usually occurs with an increase in soil temperature together with specific hatching stimuli produced by the host root system, termed root diffusate or root exudate. Whilst all species hatch in large numbers in response to appropriate host root diffusates, cyst nematodes can be classified into three broad categories based on their hatching responses to water: (i) low J2 water hatch (*G. rostochiensis*, *G. pallida*, *H. cruciferae*, *H. carotae*, *H. goettingiana*, *H. humuli*); (ii) moderate J2 water hatch (*H. trifolii*, *H. galeopsidis*, *H. glycinis*); and (iii) high J2 water hatch (*H. schachtii*, *H. avenae*).

Cyst nematodes exhibit considerable variation in optimum temperature for hatch; for example, *G. pallida* is adapted to lower temperatures than *G. rostochiensis* (16°C and 20°C, respectively). Low optimum temperatures for hatching are characteristic of cyst nematodes that can invade during winter or early spring, such as *H. cruciferae*. As expected, nematodes adapted to warmer climates exhibit higher temperature optima, e.g. 30°C for *H. zaeae*. Soil type can also affect rates of hatch. In general, coarse-textured soils favour hatching and subsequent invasion of root systems, providing suitable conditions for aeration and nematode migration. Maximum hatch usually occurs in soil at field capacity, whilst drought and waterlogging inhibit hatch.

Once hatched out of the eggshell, the J2 then leaves the cyst via either of the natural openings of the cyst, i.e. the fenestral region or the neck where the female's head has broken away. The J2 released into the soil will begin to search for a suitable host, relying primarily on gradients of chemicals released by the host's root system (see Chapter 8). As a survival strategy not all juveniles hatch out at the same time. A proportion of J2 are retained either within the cyst body and/or in external egg masses. *Globodera* species do not produce egg sacs but occasionally exude a small droplet of moisture, whilst egg sac production varies between *Heterodera* species and in individual species according to environmental conditions, e.g. *H. glycinis* produces more eggs in egg masses under favourable conditions.

The J2 enters the root system of its host, usually directly behind the growing root tip, and then migrates to the pericycle and proceeds to select a suitable cell with which to form a feeding site (see Chapter 9). The hollow mouth stylet pierces a cell wall, being careful not to bridge the plasmalemma until a feeding tube is formed. Saliva from the pharyngeal glands is then injected and the cell contents are withdrawn into the nematodes by the action of the pharyngeal pump. The feeding tube acts as a particle filter to stop large molecules being ingested. This specific interaction induces enlargement of root cells and breakdown of their walls to form a large syncytial 'transfer cell' with dense, granular cytoplasm. The transfer cell develops cell-wall ingrowths adjacent to the conducting tissue, which greatly increase the internal surface area, facilitating the passage of nutrients into the syncytium. Provided that the J2 are able to stimulate the host plant to induce and maintain syncytia of sufficient size to receive all the nutrients they require, juveniles develop into both male and female adults. This stage of the life cycle will take approximately 7 days depending on the temperature, and the second moult to third-stage juvenile (J3) will then take place. The J3 has a well-developed genital primordial and rectum; the male has a single testis and the female has paired ovaries. The female at this point is about 0.4 mm long and its shape is becoming globular to facilitate the rapid growth of the developing ovaries. At the fourth moult, the female ruptures the root cortex and the formation of the vulva gives access to the reproductive system, which is being taken over by the formation of eggs.



Males develop at a similar rate in the same root as the females. They too emerge at the fourth moult but are still wrapped in the third-stage cuticle on emergence. Males are non-feeding, free-living and live for only a short time in the soil. The males are attracted to females, which exude sex pheromones (see Chapter 8) and may be the subject of multiple mating. After mating, the embryos develop within the egg sac as far as the formation of the J2 while still within the female's body. As indicated earlier, the female then dies and her cuticle tans to form a tough protective cyst containing several hundred embryonated eggs, the number depending on species and prevailing environmental conditions. In some of the *Heterodera* species an egg sac is exuded outside the cone region of the cyst. Eventually the cysts become detached from the roots as the plant dies and remain dormant in the soil until the next suitable host grows in the vicinity (Turner and Evans, 1998).

The time taken to complete the life cycle, from egg to egg, of a cyst nematode varies depending upon the co-evolution of the species with its host range and the environmental conditions. Typical life cycles are completed in about 30 days but this may be reduced in warmer climates; for example, *H. oryzae* completes a life cycle in 23 days at 27°C, *H. glycines* 21 days at 25°C, whilst the temperate species *H. trifolii* requires 31 days at 20°C but 45 days at 15.5°C.

The number of generations per year also varies between cyst nematode species. Generally, as the soil temperature increases so does the number of generations, up to an upper threshold for each species. Under standard field conditions most temperate species of cyst nematodes will complete one or two generations, corresponding to the natural life cycle of its host combined with the length of the optimal temperature range. However, in tropical regions where favourable environmental conditions are more constant throughout the year, multiple generations are usual, with up to 11 generations being reported for *H. oryzae*.

### 4.3. General Morphology of the Subfamily Heteroderinae

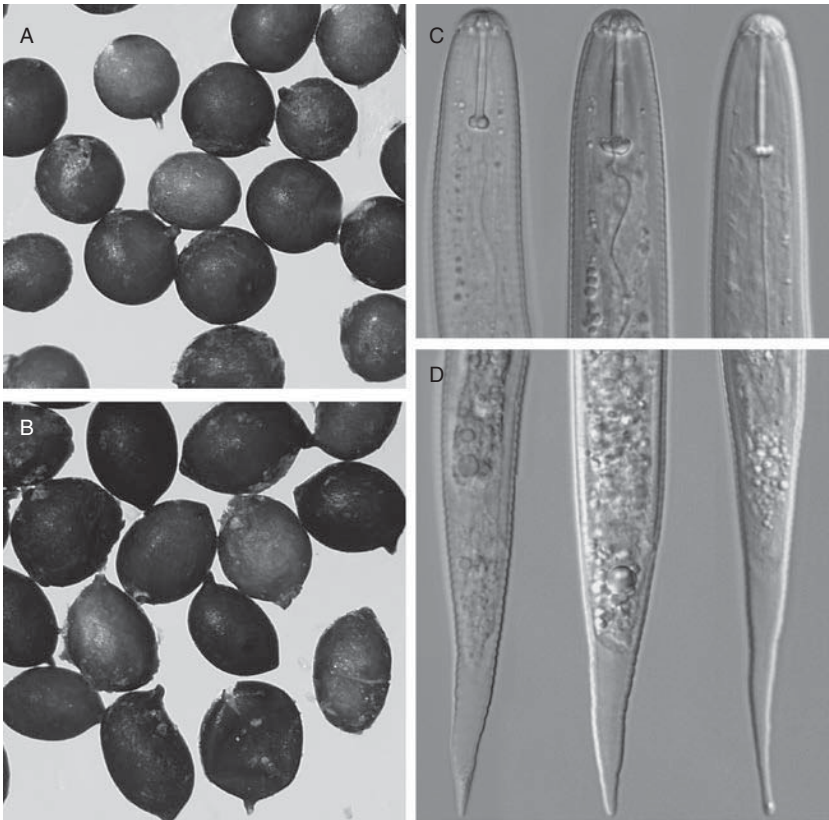
Nematode species of the subfamily Heteroderinae have similar gross morphology and are often distinguished from each other only by small details. The adult female (or cyst) and J2 are of the greatest importance in diagnosis as they are the stages most often found in soil extracts. Morphological identification using only the juveniles is not reliable and should be avoided, although they are the stage most likely to be obtained in soil extracts. If only juveniles are found in the soil it should be sampled again for females or cysts.

**Mature females.** The mature females are swollen into a spherical, sub-spherical or lemon shape to contain the developing ovaries (as well as the developing eggs) within the body cavity. Within some specimens, J2 can also be found. An egg sac may be extruded from the body but this depends on the species. In the mature female of the cyst nematode genera, annulations are restricted to the head region. The stylet and pharynx are strongly developed, with a prominent median bulb, and lie in the anterior part of the body, which forms a 'neck'. Posterior to the excretory pore, the swelling of the body is greatly developed and the excretory pore lies at the base of the neck. In most of the genera, the vulva is at the opposite pole of the body to the neck. The vulval slit runs transversely.

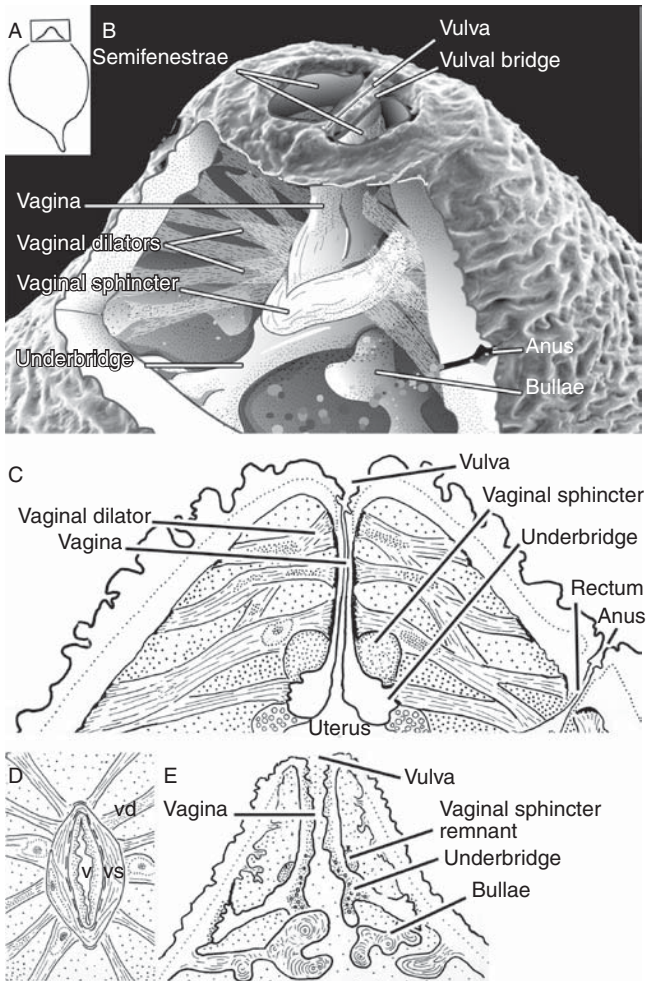


**Cysts.** Cysts are formed by the polyphenol oxidase tanning of the female cuticle, and they retain the female shape (Fig. 4.2A and B). The surface of the cyst is covered by a pattern of ridges derived from the pattern on the female cuticle. A thin-walled area surrounds the vulva and the cuticle can be lost, forming an opening, the fenestra (see Fig. 4.3 for the basic structures of terminal regions used for diagnosis). The fenestration (presence or absence; shape) is used in the diagnosis of genera (Fig. 4.4). Other measurements from the cysts are used in diagnosis of genera and species (Fig. 4.5).

**Eggs.** Eggs of most species fall within a similar size range and similar length:breadth ratios, i.e. length (L) = 86–134  $\mu\text{m}$ ; width (W) = 36–52  $\mu\text{m}$ ; L/W ratio = 2.1:2.6. In general, eggs are usually unornamented and are not a reliable stage for diagnostic purposes. However, the eggs from species of *Cactodera* can be used for diagnostic purposes; in some species, such as *C. cacti* and *C. milleri*, the eggshell is covered with small punctuations that resemble microvilli, whereas other members of the group, such as *C. weissii* and *C. amaranthi*, have smooth cuticles.

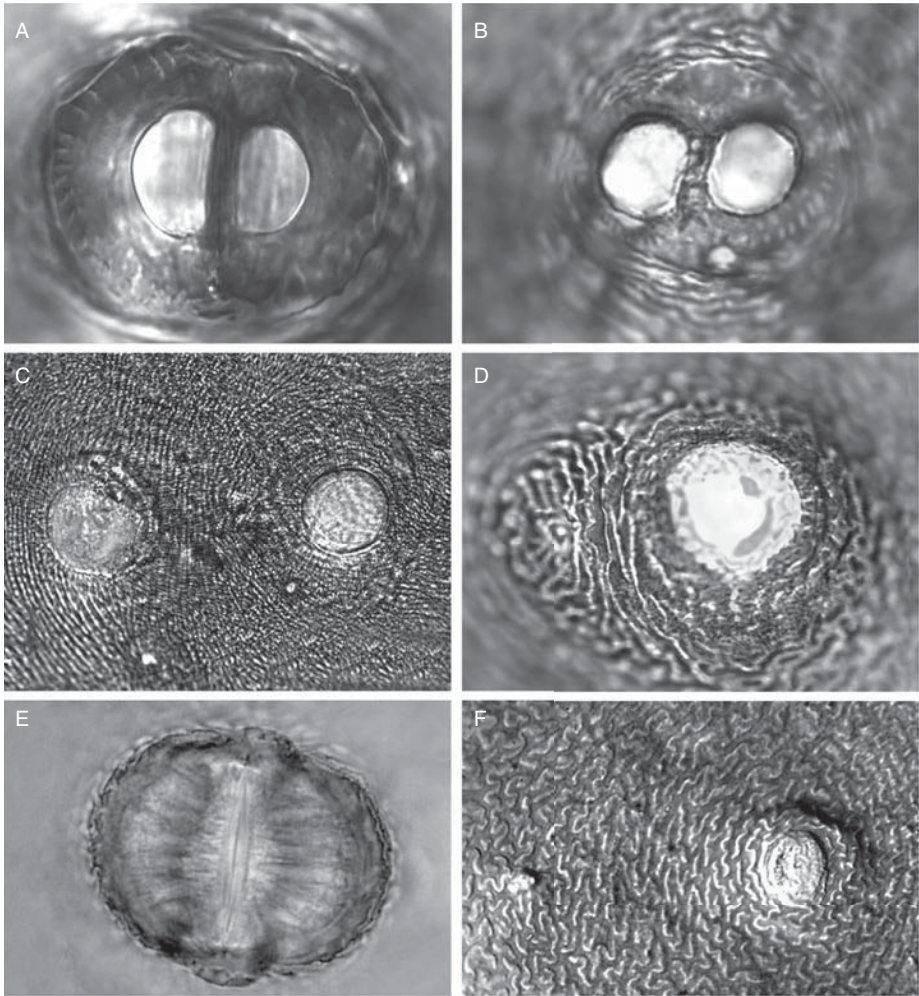


**Fig. 4.2.** Cyst and second-stage juvenile (J2) characteristics A: Cysts of *Globodera*. B: Cysts of *Heterodera*. C: Anterior regions of J2 of *G. rostochiensis*, *H. schachtii*, *Punctodera punctata*. D: Posterior region of J2 of *G. rostochiensis*, *H. schachtii*, *Punctodera punctata*.



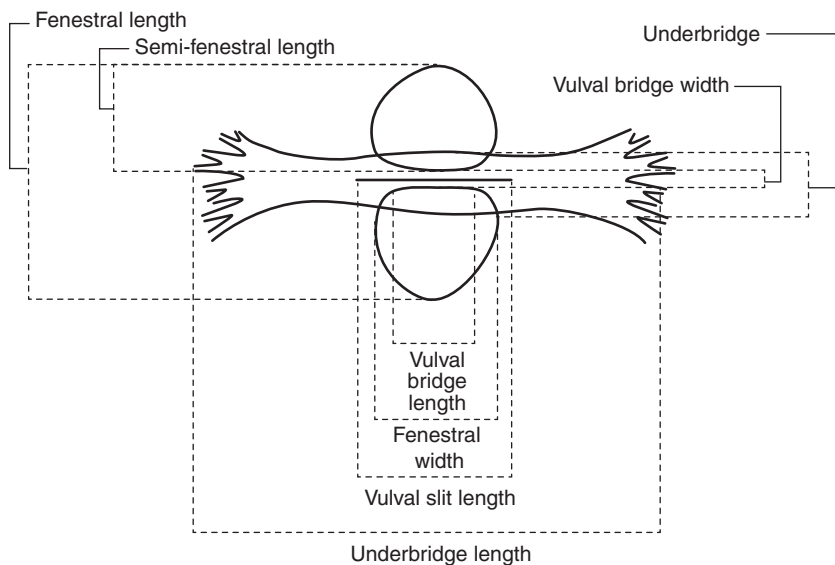
**Fig. 4.3.** Drawings depicting basic structures of terminal regions of cyst nematodes used for diagnosis. A: Overview of female. Box indicates terminal region as enlarged in B and C. B: Ventrolateral 3D view with cutaway showing internal structures. C: Right lateral view of *Heterodera schachtii*. D: Transverse view through vulva (v) at level of vaginal sphincter (vs) and vaginal dilators (vd) of *H. schachtii*. E: Dorsal view of mature cyst. Main features for diagnosis are fenestrae type and measurements, underbridge and vulval features. (After Subbotin *et al.*, 2010a.)

**Second-stage juveniles.** J2 are vermiform, with an offset, dome-shaped head and conical tail tapering to a point. On death, the body assumes a gentle curve with the ventral surface concave so that the nematode lies on its side. The cuticle is regularly annulated with the lateral fields running from near the head to the tail; the number of incisures may be reduced anteriorly and posteriorly. The head skeleton, stylet and pharynx are well developed, the latter occupying approximately one-third of the body length (Fig. 4.2C). The median bulb is rounded in shape with a prominent valve. The pharyngeal glands overlap the intestine ventrally and subventrally; the single dorsal pharyngeal gland nucleus is more prominent than, and anterior to, the two



**Fig. 4.4.** Fenestration of cyst nematodes. The word fenestra (meaning window) refers to the thin-walled area on the vulval cone or perineal area of mature cysts. In young cysts the fenestral area is membranous but later decays, leaving a hole in the cyst wall. There are three main types of fenestration: circumfenestrate, bifenestrate and ambifenestrate. The fenestration shown by a particular cyst is an important feature in identification. A: *Heterodera glycines* (ambifenestrate). B: *H. avenae* (bifenestrate). C: *Punctodera punctata* (circumfenestrate). D: *Cactodera cacti* (circumfenestrate). E: *H. orientalis* (no fenestration). F: *Globodera rostochiensis* (circumfenestrate). (After Subbotin *et al.*, 2010a, with modifications and courtesy of V.N. Chizhov.)

subventral gland nuclei. The excretory pore is clearly visible on the ventral surface opposite the pharyngeal glands with hemizonid anterior to it. The anus may be marked by a small notch or step in the cuticle and the tail has a clear tip, the hyaline portion (Figs 4.2D and 4.6). Phasmids are visible in some species as small refractive points lying laterally on the tail surface, usually within the lateral field. Measurements from the J2 are used in diagnosis of genera and species (Fig. 4.6).



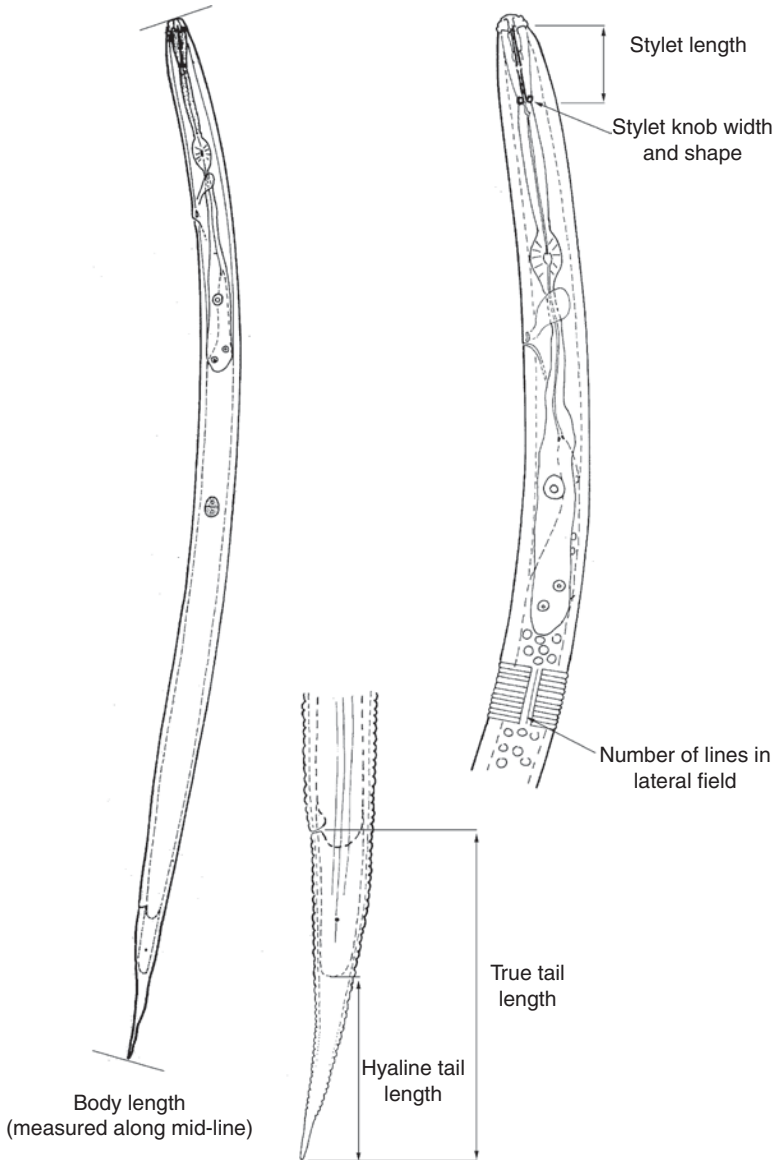
**Fig. 4.5.** Measurements of the fenestral area (fenestrae and underbridge) of a cyst, important for species and genus diagnostics.

## 4.4. Genera and Principal Species

### 4.4.1. Genus *Heterodera* Schmidt, 1871

Females and cysts are usually lemon-shaped, the neck protruding from the anterior end and at the opposite pole the posterior usually ending in a cone (Fig. 4.7). The cone carries the fenestra that internally is associated with the reproductive organs and, from the exterior, will provide diagnostic features used for identification. Some members of this genus, such as *H. schachtii*, have a very high conspicuous cone and others, such as *H. cruciferae*, have much lower cones. The mature female containing eggs dies, causing her cuticle to tan and dry out, thus protecting the eggs until the invasive J2 hatch. The cyst can range in colour from light brown to dark brown or almost black. The cuticle surface displays folds and ridges often in specific patterns, e.g. zigzag or parallel, which are helpful in the diagnosis of species. *Heterodera* cysts may or may not have an underbridge. Bullae may also be present and both features are very diagnostic of the groups contained within this genus. To fully investigate the posterior part of the cyst (perineae), it must first be mounted on a slide, ideally containing 5–10 additional perineal patterns for further detailed analysis. Molecular and morphological data support division of most *Heterodera* species into several groups: *Afenestrata*, *Avenae*, *Cyperi*, *Goettingiana*, *Humuli*, *Sacchari* and *Schachtii*. Key features for study include the formation of the fenestra. These are classified as without fenestration (*Afenestrata* group), ambifenestrata (two openings divided by a narrow vulval bridge) or bifenestrata (two openings separated by a much wider vulval bridge). The length of the vulval slit varies. In the *Avenae* group it is very short at 8–10  $\mu\text{m}$ , whereas members of the *Schachtii* group have a much longer slit averaging 65  $\mu\text{m}$  in length.

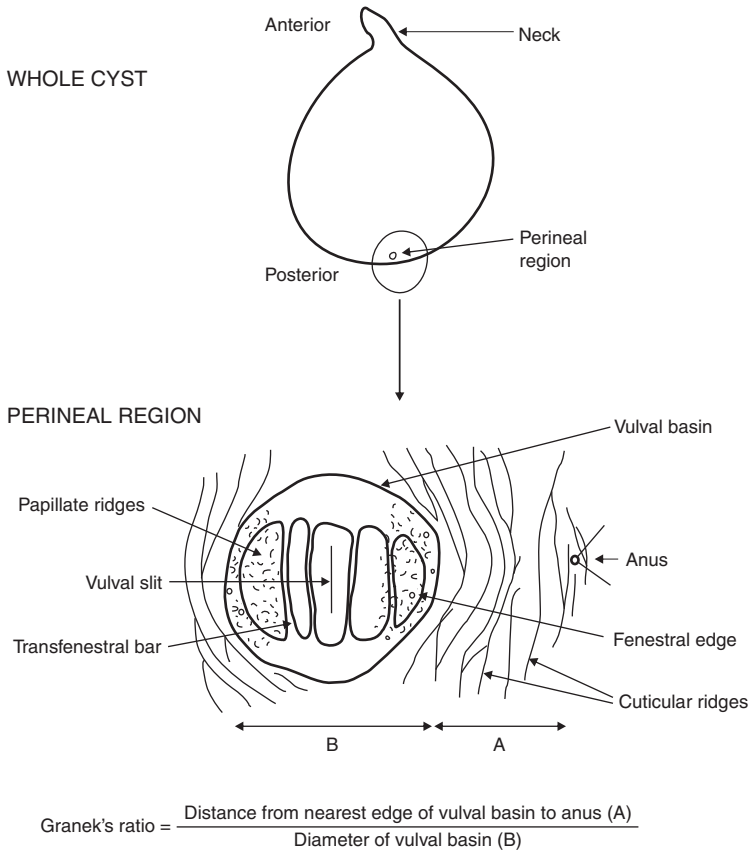




**Fig. 4.6.** Measurements of second-stage juvenile, important for species and genus diagnostics.

The J2 are also used for diagnosis together with the cyst features. The stylet length and the position and shape of the basal knobs are important features. The number of lateral fields is usually three or four. The number of head annules present, the width of the body at the excretory pore and the anus, and the length of both the true tail, i.e. from the anus to the tail tip, and the hyaline tail length are diagnostic (Fig. 4.6).

The type species of the genus is *H. schachtii*.



**Fig. 4.7.** The whole cyst shape and vulval area details for calculation of Granek's ratio.

#### 4.4.1.1. Cereal cyst nematode, *Heterodera avenae* Wollenweber, 1924

The cereal cyst nematode was first recorded by Kühn (1874) as a parasite of cereals in Germany and was later found in other countries. *Heterodera avenae* is now found in most wheat-growing regions of the world. The cereal cyst nematode is an important pest of cereals and is the principal nematode species on temperate cereals. In Europe, more than 50% of the fields in major cereal-growing areas are infected by this nematode (Rivoal and Cook, 1993), with annual yield losses reaching £3 million (Nicol and Rivoal, 2008). At least \$US3.4 million is estimated to be lost annually in wheat production in the states of Idaho, Oregon, and Washington because of cereal cyst nematodes. The yield losses it causes on wheat range from 15–20% in Pakistan, and 40–92% on wheat and 17–77% on barley in Saudi Arabia. In China yield losses of wheat crops induced by this nematode can reach 70%. Hosts of *H. avenae* include species of cereals and grasses from the following genera: *Agropyron*, *Agrostis*, *Alopecurus*, *Anisantha*, *Arrhenatherum*, *Avena*, *Brachypodium*, *Bromus*, *Dactylis*, *Echinochloa*, *Festuca*, *Hordeum*, *Koeleria*, *Lolium*, *Phalaris*, *Phleum*, *Poa*, *Polypogon*, *Secale*, *Setaria*, *Sorghum*, *Trisetum*, *Triticum*, *Vulpia*, *Zerna* and *Zea* (Williams and Siddiqi, 1972). *Heterodera avenae* has only one

generation per year, with J2 hatch from the eggs determined largely by temperature (Rivoal and Cook, 1993).

#### DESCRIPTION

Cysts: L = 518–801  $\mu\text{m}$ ; W = 432–744  $\mu\text{m}$ ; L/W ratio = 0.8–1.8; fenestral length = 32–55  $\mu\text{m}$ ; vulval slit = 7–12  $\mu\text{m}$ .

Male: L = 1020–1590  $\mu\text{m}$ ; stylet = 27–33  $\mu\text{m}$ ; spicules = 33–38  $\mu\text{m}$ ; gubernaculum = 10–13  $\mu\text{m}$ .

J2: L = 505–598  $\mu\text{m}$ ; stylet = 24–27.5  $\mu\text{m}$ ; hyaline region = 34–50  $\mu\text{m}$ ; tail = 52–79  $\mu\text{m}$ . Cyst lemon-shaped, with prominent neck and vulval cone. Subcrystalline layer conspicuous, sloughing off with formation of dark brown cyst. Bifenestrate, bullae prominent, crowded beneath vulval cone. J2 vermiform, with a sharply pointed tail. Stylet well developed, with large, anteriorly flattened to concave basal knobs.

*Heterodera avenae* belongs to the *Avenae* group and to the *H. avenae* complex (also called Cereal Cyst Nematodes (CCN)). It differs from species it closely resembles (*H. australis*, *H. aucklandica*, *H. riparia*, *H. pratensis* and *H. arenaria*) by morphometrical characters of J2 and cysts and by PCR–ITS–RFLP and IEF of proteins. Based on the ITS rRNA gene sequences and PCR–ITS–RFLP all world populations of *H. avenae* can be divided into several types: (i) *H. avenae*, European and North American populations, type A; (ii) *H. avenae*, Asian and African populations, type B; (iii) *H. avenae*, several French populations; and (iv) *H. 'avenae'* from China, type C.

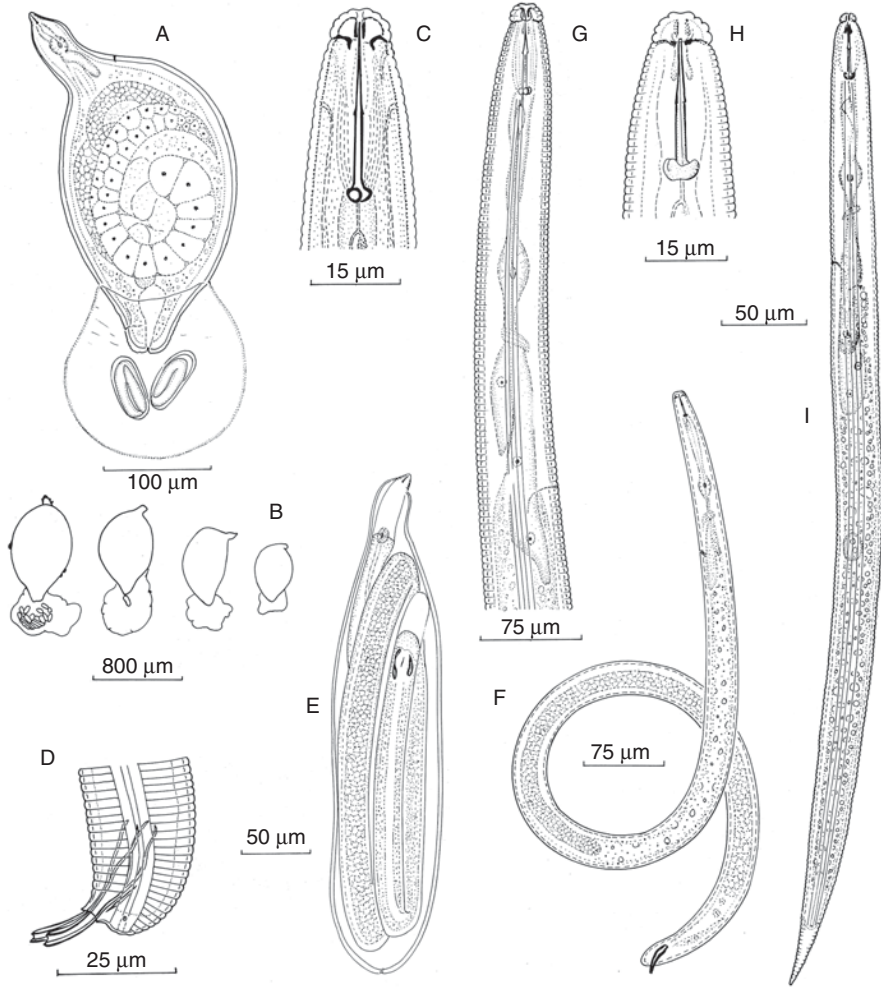
#### 4.4.1.2. Sugar beet cyst nematode, *Heterodera schachtii* Schmidt, 1871

The sugar beet cyst nematode (Fig. 4.8) has been recognized as a plant pathogen since 1859 when it was associated with stunted and declining sugar beet in Germany. In the following years it became recognized as a pest of great importance in beet-growing areas of several European countries. *Heterodera schachtii* is found in all major sugar beet production areas of the world, favouring temperate regions but apparently tolerating a broad range of climates. It is widespread in Europe, the USA and Canada (Baldwin and Mundo-Ocampo, 1991). Annual yield loss in EU countries based upon world market sugar prices was estimated in 1999 at up to €90 million (Müller, 1999). The optimum temperature for development is around 25°C. In some climates, three to five generations may complete development on sugar beet in one season (Franklin, 1972). *Heterodera schachtii* was described from the host *Beta vulgaris* and it parasitizes mainly plants of the families Amaranthaceae (Caryophyllales) (many species of *Beta* and *Chenopodium*) and Brassicaceae (Brassicales) (*Brassica oleracea*, *B. napus*, *B. rapa*, *Rhaphanus sativus* and many others including a diversity of common weeds) (Franklin, 1972). Some plants from Polygonaceae, Scrophulariaceae, Caryophyllaceae and Solanaceae are susceptible to nematode infection.

#### DESCRIPTION

Cysts: L = 480–960  $\mu\text{m}$ ; W = 396–696  $\mu\text{m}$ ; L/W ratio = 0.9–2.0; fenestral length = 28–48  $\mu\text{m}$ ; vulval slit = 33–54  $\mu\text{m}$ .





**Fig. 4.8.** *Heterodera schachtii*. A: Adult female with egg sac. B: Cysts and egg sacs. C: Anterior region of male. D: Male tail. E: Fourth-stage male moulting. F: Adult male. G: Male pharyngeal region. H: Anterior region of J2. I: J2. (After Franklin, 1972.)

Male: L = 1038–1638 µm; stylet = 27–30 µm; spicules = 27–39 µm; gubernaculum = 10–11 µm.

J2: L = 400–512 µm; stylet = 23–28 µm; hyaline region = 17–33 µm; tail = 40–56 µm (Fig. 4.8).

Cyst colour light to dark brown. Ambifenestrate, within cone, remnants of vagina attached to side walls by underbridge and a number of irregularly arranged, dark brown molar-shaped bullae situated a short distance beneath the vulval bridge. J2 labial region offset, hemispherical, with four indistinct annuli. Stylet moderately heavy with

prominent, forwardly-directed knobs. Tail acutely conical with rounded tip, distinct hyaline terminal section 1–1.25 stylet lengths long.

*Heterodera schachtii* belongs to the *Schachtii* group and is distinguished from closely related species (*H. trifolii*, *H. glycines*, *H. betae* and others) by a combination of morphological and morphometric characteristics. PCR-ITS-RFLP profile generated by *Mva*I is unique for *H. schachtii*. Diagnostics of this species using PCR with species-specific primers has also been developed.

#### 4.4.1.3. Soybean cyst nematode, *Heterodera glycines* Ichinohe, 1952

A cyst nematode parasitizing soybean plants, *Glycine max* and causing ‘yellow dwarf’ symptoms was recorded from Shirakawa, Fukushima Prefecture, Japan, in 1915. Ichinohe (1952) was the first to make careful morphological comparisons with other *Heterodera* species and to give a specific name and brief description of this nematode. In Japan, yield losses have been estimated at 10–70% (Ichinohe, 1988). Presently, *H. glycines* occurs in most countries of the world where soybean is produced. In a study of losses predicted in ten soybean-producing countries together accounting for 97% of the world crop, *H. glycines* appeared to be the most important constraint (losses of 8,969,400 t) on yield and estimated at \$US1960 million (Wrather *et al.*, 2001). In these countries, total yield losses attributed to *H. glycines* were greater than those for any other pest of the crop. *Heterodera glycines* is widely distributed throughout the north-central USA where different maturity groups with the same source of resistance to *H. glycines* are grown. State surveys in the region report from 14 to 63% of fields are infested with *H. glycines*.

*Heterodera glycines* has a broad host range, especially Fabaceae, but also on other families. More than 66 weed species of nine families are suitable hosts. Riggs (1992) provided a list of non-fabaceous hosts comprising 63 species in 50 genera from 22 families (e.g. Boraginaceae, Capparaceae, Caryophyllaceae, Chenopodiaceae, Brassicaceae, Lamiaceae, Fabaceae, Scrophulariaceae, Solanaceae). In field conditions, *H. glycines* was also found in several other plants, including henbit (*Lamium amplexicaule*), purple deadnettle (*Lamium purpureum*), mouse-ear chickweed (*Cerastium holosteoides*) and common chickweed (*Stellaria media*) (Riggs, 1992).

Three to five generations develop during the cropping season. Optimum temperature is 23–28°C; development stops below 14°C and above 34°C. In the absence of a host, J2 and eggs in cysts may remain viable in soil for 6–8 years.

*Heterodera glycines* disturbs root growth, interferes with nodulation and causes early yellowing of soybean plants. The above-ground symptoms of damage on individual plants and appearance of infested fields are usually not sufficiently specific to allow direct identification. Infected plants are predisposed to *Fusarium* wilt. Sudden death syndrome is a soil-borne disease of soybean caused by the fungus *Fusarium solani* in association with *H. glycines*.

#### DESCRIPTION

Cysts: L = 340–920 µm; W = 200–688 µm; L/W ratio = 1.0–2.4; fenestral length = 35–72 µm; vulval slit = 36–60 µm.

Male: L = 911–1400 µm; stylet = 24–27 µm; spicules = 28–45 µm; gubernaculum = 8–13 µm.

J2: L = 345–504 µm; stylet = 21–25 µm; hyaline region = 18–36 µm; tail = 35–59 µm.

Cyst mainly lemon-shaped, sometimes round with a protruding neck and cone. Ambifenestrata, bullae prominent, located at or anterior to underbridge, extending into vulval cone from interior of body wall cuticle. Shape varying from round to finger-like, round bullae differently sized, finger-like bullae of variable length and thickness. Underbridge well developed. J2 body vermiform with regularly annulated cuticle. Stylet robust with anteriorly protruding knobs. Tail tapering uniformly to a finely rounded terminus.

*Heterodera glycines* belongs to the *Schachtii* group and is distinguished from similar species (*H. medicaginis*, *H. schachtii*, *H. trifolii*, *H. daverti* and others) by a combination of morphological and morphometric characteristics. It differs from *H. schachtii* by the shape of the stylet knobs of J2 (slightly convex vs moderately or strongly concave), shorter average J2 stylet and longer average fenestral length.

#### **4.4.1.4. Pea cyst nematode, *Heterodera goettingiana* Liebscher, 1892**

In 1890, G. Liebscher reported infection and yield loss of pea (*Pisum sativum*) and vetch (*Vicia sativa*) by nematodes identified as *H. schachtii* at fields of the Agricultural Institute at Göttingen, Germany. Two years later he described this species as *H. goettingiana*. Several researchers have reported diseases of peas, primarily in European countries, caused by this nematode; however, little was known about biology and pathogenicity of *H. goettingiana* until the mid-1900s (Franklin, 1951). Infected pea fields show sharply delineated patches with dwarfed, poorly branched and yellowing plants that die prematurely. Infected plants either fail to flower or flower too early. The root system is poorly developed. Development takes 3–15 weeks depending on soil temperature and moisture as well as host species. One or two generations occur during the growing season in the UK, and three generations may develop in southern Italy. Cysts with eggs can remain viable in the absence of a host for 12 years.

##### DESCRIPTION

Cysts: L = 400–780 µm; W = 310–540 µm; L/W ratio = 1.3–2.2; fenestral length = 43–71 µm; vulval slit = 43–61 µm.

Male: L = 1270 µm; stylet = 27 µm; spicules = 27 µm; gubernaculum = 12 µm. (No ranges available.)

J2: L = 408–519 µm; stylet = 23–26 µm; hyaline region = 27–38 µm; tail = 54–74 µm.

Cyst lemon-shaped with light to dark brown cyst wall. Subcrystalline layer not visible. Vulval cone ambifenestrata. In some old cysts, vulval bridge ruptured, fenestrae joining to form a large oval fenestrum. Bullae absent, although bullae-like structures and vulval denticles present. Underbridge weak. J2 body vermiform, curved ventrally after fixation. Labial region hemispherical, with 2–5 annuli, slightly offset from body. Lateral field with four incisures, not areolated. Stylet knobs rounded, slightly projecting anteriorly. Tail tapering uniformly to a finely rounded terminus.

*Heterodera goettingiana* belongs to the *Goettingiana* group. It differs from several other representatives of the *Goettingiana* group (*H. cruciferae*, *H. carotae*, *H. circaeae*, *H. scutellarariae* and *H. persica*) by longer average J2 body, longer tail and longer hyaline region.

#### 4.4.1.5. Mediterranean cereal cyst nematode *Heterodera latipons* Franklin, 1969

In the early 1960s, a cyst nematode similar to *H. avenae* was detected in Israel and Libya on the roots of stunted wheat and barley plants. It was morphologically studied and described by Franklin (1969) as a new species under the name *H. latipons*, based on characteristics of the Israel population. This nematode was later recorded in many countries, mainly from the Mediterranean and the Orient. *Heterodera latipons* often occurs in mixed populations with *H. avenae* in cereal cropping systems. Hosts include barley (*Hordeum vulgare*), oat (*Avena sativa*), rye (*Secale cereale*), *Phalaris minor*, *P. paradoxa* and *Elytrigia repens*. *Triticum durum* was considered to be a poor host of this nematode. Yield losses as high as 50% were reported on barley in Cyprus. In Syria, the nematode causes average yield losses of 20 and 30% in barley and durum wheat, respectively, and the nematode was more damaging under water stress conditions. Moreover, damage is more severe in fields infested concomitantly with *H. latipons* and the fungus *Bipolaris sorokiniana*, the causal agent of the common root rot and seedling blight of barley, i.e. the presence of the nematode increases the aggressiveness of the fungus (Scholz, 2001). In all areas studied, *H. latipons* completed only one life cycle during the growing season (Mor *et al.*, 1992).

##### DESCRIPTION

Cysts: L = 300–700  $\mu\text{m}$ ; W = 320–560  $\mu\text{m}$ ; L/W ratio = 0.6–1.7; fenestral length = 52–76  $\mu\text{m}$ ; vulval slit = 6–11  $\mu\text{m}$ .

Male: L = 960–1406  $\mu\text{m}$ ; stylet = 22–29  $\mu\text{m}$ ; spicules = 32–36  $\mu\text{m}$ ; gubernaculum = 8  $\mu\text{m}$ .

J2: L = 401–559  $\mu\text{m}$ ; stylet = 22–25  $\mu\text{m}$ ; hyaline region = 20–36  $\mu\text{m}$ ; tail = 42–68  $\mu\text{m}$ .

Cysts are dark to mid-brown covered with white subcrystalline layer. Bifenestrate, semifenestrae separated by a distance greater than fenestral width, vulval slit short. Strong underbridge with pronounced thickening in middle and with ends splayed. Bullae usually absent, sometimes present at underbridge level. J2 body slightly curved dorsoventrally when killed by heat. Stylet with well-developed, anteriorly concave knobs.

*Heterodera latipons* belongs to the *Avenae* group and closely resembles *H. hordecalis* and *H. turcomanica*. These nematodes share similar circular semifenestrae separated by a distance longer than the semifenestra diameter and a rather typical underbridge but with a pronounced enlargement underlying the vulval slit. The most important differentiating character between *H. latipons* and *H. hordecalis* is the vulval slit, which in *H. latipons* is much shorter.

#### 4.4.2. Genus *Globodera* Skarbilovich, 1959

Mature females and cysts are spheroid, lacking terminal cone. Vulval area is circumfenestrate. Vulva located in a cavity beneath outline of body, vulval slit <15  $\mu\text{m}$ . There is no anal fenestra. Vaginal remnants, underbridge and bullae rarely present

(Fig. 4.7). Cuticle with distinct D layer (Cordero and Baldwin, 1990). All eggs retained in body, egg sac absent. Colouration is helpful in making a diagnosis of species, especially separation of the PCN species *G. rostochiensis* and *G. pallida*. Egg surface is smooth. Male lateral field with four lines, spicules >30 µm, distally pointed. J2 has four incisures in lateral field. Tail conical, pointed, phasmids punctiform. *En face* pattern typically with six separate lips, sometimes with fusion of adjacent sub-medial lips. The type species of the genus is *G. rostochiensis*.

PCN include two species, *G. rostochiensis* and *G. pallida*, which have been reported from many countries and are considered to be one of the most economically important pests of potato. Molecular analysis, however, indicates presence of several still undescribed species of PCN. It is now generally accepted that PCN are native to South America (Grenier *et al.*, 2010), where it is the principal pest of Andean potato crops. *Globodera rostochiensis* and *G. pallida* are differently distributed in the Andes. Factors that may be responsible include day length, temperature, altitude, rainfall or the interaction of any of them with the host potato. Human activities over centuries may also have influenced distribution. In South America, these species are mainly found between 2000 m and 4000 m above sea level, with the heaviest infestations between 2900 m and 3800 m above sea level. The two species occupy different zones in the Andes. The demarcation line between the two species is near 15.6°S. With few exceptions, populations north of this line are mainly *G. pallida*. Those from areas around Lake Titicaca and further south are predominantly *G. rostochiensis* with few *G. pallida* or mixtures of both species. The most southerly population, from the east side of the Andes in Bolivia, are mixtures of *G. rostochiensis* and *G. pallida*. These two species have been introduced in many parts of the world, particularly to Europe, and also to the USA, Canada, New Zealand and numerous other countries where potatoes are grown.

#### **4.4.2.1. Golden potato cyst nematode *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959**

The golden cyst nematode associated with potato plants, *Solanum tuberosum*, from Rostock, Germany was first reported in 1881 and was considered to be *H. schachtii*, this being the only known species of cyst nematode at that time. During the early 1900s, the PCN became more widely known throughout Europe and was described in 1923. In temperate regions, *G. rostochiensis* usually completes only one generation, although a second generation may be initiated but not completed; J2 hatched from first-generation eggs, but they were unable to reach the adult stage. In subtropical regions two generations might occur. Development of one generation requires 6–10 weeks. The J2 can go into diapause and remain viable for many years, hatching continuing for 25 or more years. Heavily infected plants become yellow and stunted. Infected plants have reduced root systems, which are abnormally branched and brownish in colour. Symptoms in the field first appear in small patches. At low nematode densities tuber sizes are reduced, whereas at higher densities both number and size of tubers can be reduced. At eight and 64 eggs g<sup>-1</sup> of soil, yield losses of about 20 and 70%, respectively, can be expected. Seinhorst (1982) and Elston *et al.* (1991) proposed models that described the relationships of PCN population densities before planting with potato yield and post-harvest nematode

populations (see Chapter 10). The damaging effect of PCN is not only determined by nematode density, but also by such factors as cultivar, crop husbandry and environmental conditions. PCN are responsible for annual potato tuber losses of up to 9% in Europe. Information on economic importance in some South American countries is scarce or unavailable although yield losses in Bolivia and Peru have been estimated to be around US\$13,000,000 and US\$128,000,000, respectively (Franco and Gonzalez, 2010).

Hosts include potato, *Solanum tuberosum* (Solanaceae, Solanales), tomato, *S. lycopersicum* and eggplant, *S. melongena*. Other hosts include many *Solanum* spp., *Datura* spp., *Hyoscyamus niger*, *Nicotiana acuminata*, *Physalis* spp., *Physochlaina orientalis*, *Salpiglossis* spp., *Capsicum annuum* and *Saracha jaltomata*.

#### DESCRIPTION

Cysts: L = 450–990  $\mu\text{m}$ ; W = 250–810  $\mu\text{m}$ ; L/W ratio = 0.9–1.8; fenestral diameter = 14–21  $\mu\text{m}$ ; number of ridges between anus and fenestra = 16–22; Granek's ratio = 2.3–7.0.

Male: L = 960–1406  $\mu\text{m}$ ; stylet = 22–29  $\mu\text{m}$ ; spicules = 32–36  $\mu\text{m}$ ; gubernaculum = 8  $\mu\text{m}$ .

J2: L = 366–502  $\mu\text{m}$ ; stylet = 19–23  $\mu\text{m}$ ; DGO = 2.4–6.7  $\mu\text{m}$ ; hyaline region = 18–30  $\mu\text{m}$ ; tail = 37–57  $\mu\text{m}$ .

Female colour changing from white to yellow to light golden as female matures to cyst stage. Cyst brown, ovate to spherical in shape with protruding neck, circumfenestrate (Fig. 4.3F), abullate. Fenestra circular, anus conspicuous at apex of a V-shaped subsurface cuticular mark. J2 body tapering at both extremities but more at posterior end. Stylet well developed, with prominent rounded knobs as viewed laterally. Lateral fields with four lines extending for most of body length. Tail tapering to small, rounded terminus.

*Globodera rostochiensis* is morphologically similar to *G. pallida* and *G. tabacum*. It differs from *G. pallida* by yellow or gold vs cream coloured maturing females, higher number of ridges between the vulva and anus, larger mean for Granek's ratio, stylet knob shape, shorter average stylet length and rounded vs more pointed J2 tail terminus (Box 4.2).

#### 4.4.2.2. Pale potato cyst nematode, *Globodera pallida* Stone, 1973

The pale PCN, *G. pallida*, is considered to be a major pest of potato crops in cool temperate climates. It is reported from several counties in Europe, Asia, Africa and South America. In Central and North America *G. pallida* has been reported in Panama, the USA and Canada, but in the last two countries *Globodera* species on potato have a rather restricted distribution with small infested areas, because of rigorous phytosanitary regulations and seed potato certification programmes, compared to the widespread infestations found in European countries. Recently, mtDNA analysis has been used to study genetic relationships among Peruvian populations of *G. pallida*, thus identifying the origin of Western European populations of this species (Picard *et al.*, 2007; Plantard *et al.*, 2008). Using the mtDNA gene, cytochrome *b* (*cytb*) sequences and microsatellite loci, Plantard *et al.* (2008) showed



**Box 4.2. Morphometric comparison between *Globodera rostochiensis* and *G. pallida* (measurements in  $\mu\text{m}$ ; values for second-stage juveniles are ranges compiled from various sources).**

**Second-stage juvenile characters**

	Body length	Stylet length	Stylet knob shape	Tail length	Hyaline region tail length
<i>G. rostochiensis</i>	366–502	19–23	Rounded	37–57	18–30
<i>G. pallida</i>	380–533	23–25	Robust; square hooked	40–57	20–31

**Cyst characters**

	Distance from anus to vulval basin; mean (range)	Fenestral diameter	Number of ridges between anus and vulval basin	Granek's ratio
<i>G. rostochiensis</i>	66.0 (50.0–77.0)	Usually <19.0	16–22	2.3–7.0
<i>G. pallida</i>	45.0 (35.0–55.0)	Usually >17.0	7–17	1.2–3.6

that the *G. pallida* presently distributed in Europe derived from a single restricted area in the extreme south of Peru, located between the north shore of Lake Titicaca and Cusco. *Globodera pallida* develops one generation for a vegetation season. This species is adapted to cool temperatures and is able to hatch earlier in the year and develop at 2°C cooler than *G. rostochiensis* (Langeslag *et al.*, 1982). The symptoms of attack by *G. pallida* are similar to those for *G. rostochiensis* and the damage threshold is 1–2 eggs g<sup>-1</sup> soil. Hosts includes potato (*S. tuberosum*), eggplant (*S. melongena*), tomato (*S. lycopersicum*), many other species of *Solanum*, and black henbane (*Hyoscyamus niger*).

**DESCRIPTION**

Cysts: L = 420–748  $\mu\text{m}$ ; W = 400–685  $\mu\text{m}$ ; fenestral diameter = 17.5–25  $\mu\text{m}$ ; number of ridges between anus and fenestra = 7–17; Granek's ratio = 1.2–3.6.

Male: L = 1198  $\mu\text{m}$ ; stylet = 27  $\mu\text{m}$ ; spicules = 36  $\mu\text{m}$ ; gubernaculum = 11  $\mu\text{m}$ .

J2: L = 380–533  $\mu\text{m}$ ; stylet = 22.5–25  $\mu\text{m}$ ; DGO = 2.7–5  $\mu\text{m}$ ; hyaline region = 20–31  $\mu\text{m}$ ; tail = 40–57  $\mu\text{m}$ .

Female is white in colour, some populations passing, after 4–6 weeks, through a cream stage, turning glossy brown when dead. Cyst vulval region intact or fenestrated



with single circumfenestrate (Fig. 4.3D) opening occupying all or part of vulval basin, abullate. J2 lateral field with four incisures but with three anteriorly and posteriorly, occasionally completely areolated. Stylet well developed, basal knobs with distinct anterior projection as viewed laterally. Tail tapering uniformly with a finely rounded point, hyaline region forming about half of tail region.

*Globodera pallida* is most closely related to *G. rostochiensis* and *G. tabacum*. It differs from *G. rostochiensis* by cream-coloured females vs yellow or gold, smaller number of ridges between the vulva and anus, smaller mean for Granek's ratio, stylet knob shape, longer stylet length, tail terminus and presence of refractive bodies on hyaline part of tail (usually 4–7 refractive bodies vs absence) in J2.

#### **4.4.2.3. Tobacco cyst nematode *Globodera tabacum* (Lownsbery & Lownsbery, 1954) Skarbilovich, 1959**

*Globodera tabacum* is considered as a serious and important pest of shade and broad-leaf tobacco. It is recorded from several countries in Europe, Asia, Africa, South and North America. *Globodera tabacum* is a polytypic species containing the following subspecies: *G. tabacum tabacum* (Lownsbery & Lownsbery, 1954), *G. tabacum virginiae* (Miller and Gray, 1968) and *G. tabacum solanacearum* (Miller & Gray, 1972). All three subspecies develop on tobacco and horse nettle (*Solanum carolinense*), but otherwise differ in host preference. *Globodera tabacum* parasitizes *Nicotiana tabacum*, *S. carolinense*, tomato and other species of the genera *Nicotiana* and *Solanum*, as well as *Atropa belladonna*, *Hyoscyamus niger*, *Nicandra physalodes* and *Capsicum annuum*. Two or more generations usually occur. Infected tobacco plants have small root systems and above-ground symptoms are similar to those associated with severe root-knot and lesion nematode infestations. Nematode infection is often associated with increased damage from bacterial wilt, black shank. Farmers in Virginia, USA, have recorded complete crop failures, but losses generally average 15%. A high density of nematode populations early in the growing season can reduce flue-cured tobacco yield by 25–50%, although tobacco may escape significant losses from moderate populations, especially under favourable growing conditions.

#### DESCRIPTION

Cysts: L = 337–937 µm; W = 232–812 µm; L/W ratio = 0.9–1.5; fenestral diameter = 13–36 µm; number of ridges between anus and fenestra = 5–15; Granek's ratio = 1.4–4.2.

Male: L = 710–1450 µm; stylet = 24–29 µm; spicules = 26–35 µm; gubernaculum = 9–12 µm.

J2: L = 458–621 µm; stylet = 20–27 µm; DGO = 4.3–9 µm; hyaline region = 17–35 µm; tail = 34–64 µm.

Female body ovate to spherical with elongate neck, white, becoming yellow. Cyst light shiny brown, circumfenestrate, abullate. J2 with well-developed rounded basal knobs. Terminus of tail finely rounded.

*Globodera tabacum* differs from *G. rostochiensis* by J2 with longer mean values of body length, mean stylet and by cysts with smaller mean number of cuticular ridges.

It differs from *G. mexicana* by J2 with longer mean body length and from *G. pallida* by cysts with a smaller mean number of cuticular ridges and by J2 with longer mean body length.

#### 4.4.3. Genus *Punctodera* Mulvey & Stone, 1976

Mature females and cysts are spherical, pear-shaped or ovoid, with short projecting neck and heavy subcrystalline layer. Cuticle reticulate, subcuticle with punctations. D-layer present. Terminal region not cone-shaped; cyst light to dark brown. Vulval slit extremely short ( $<5\ \mu\text{m}$ ), anus at a short distance from vulval fenestra. Circumfenestrate, fenestra surrounding vulva  $16\text{--}40\ \mu\text{m}$  (approx.  $20\ \mu\text{m}$  in type species) in diameter, anus offset toward ventral margin of fenestra, an anal fenestra of similar shape and size to vulval fenestra present. Underbridge and perineal papilla-like tubercles absent. Bullae present or absent. Egg retained in body, no egg sac. Males vermiform, less than 1.5 mm long. DGO  $2.6\text{--}4.6\ \mu\text{m}$ . Spicules  $31\text{--}33\ \mu\text{m}$  long, distally pointed. Tail less than 0.5 anal body diameter long, cloacal lips not forming a tube. J2 body length is  $0.35\text{--}0.49\ \text{mm}$ , stylet  $24\text{--}26\ \mu\text{m}$  and tail conical,  $63\text{--}78\ \mu\text{m}$  long, hyaline region in type species  $38\text{--}41\ \mu\text{m}$  long. Lateral field with four incisures. Phasmid openings punctiform, without a lens-like structure. Parasites of monocotyledonous plants. The type species of the genus is *Punctodera punctata*.

##### 4.4.3.1. Grass cyst nematode, *Punctodera punctata* (Thorne, 1928) Mulvey & Stone, 1976

This species was described by Thorne based on specimens from heavily infected wheat roots from a field in the Humboldt area, Saskatchewan, Canada. Several further attempts to collect topotype specimens failed. Subsequently, *P. punctata* was also reported as a common species infecting grasses from Europe, the USA and Canada. However, all attempts to infect wheat or other cereals by these nematodes failed to give any positive results. Several authors suggested that *P. punctata* might represent a complex of several closely related species. Many grasses are good hosts of this nematode. Only a single generation occurs each year.

##### DESCRIPTION

Female and cysts: L =  $330\text{--}901\ \mu\text{m}$ ; W =  $170\text{--}720\ \mu\text{m}$ ; L/W ratio = 1.2–3.0; vulval fenestral diameter =  $16\text{--}33\ \mu\text{m}$ ; anal fenestral diameter =  $33\ (25.2\text{--}42.0)\ \mu\text{m}$ .

Male: L =  $910\text{--}1270\ \mu\text{m}$ ; stylet =  $26\text{--}33\ \mu\text{m}$ ; spicules =  $28\text{--}36\ \mu\text{m}$ ; gubernaculum =  $8\text{--}10\ \mu\text{m}$ .

J2: L =  $520\text{--}643\ \mu\text{m}$ ; stylet =  $23\text{--}32\ \mu\text{m}$ ; DGO =  $3.5\text{--}6.5\ \mu\text{m}$ ; hyaline region =  $38\text{--}64\ \mu\text{m}$ ; tail =  $63\text{--}93\ \mu\text{m}$ .

Females and cysts are ovoid, pear- or flask-shaped without vulval cone, white. Vulva slit approx.  $4\ \mu\text{m}$  long, bordered by thickened ridges, set in a subcircular translucent area of cuticle. Anal slit less than  $4\ \mu\text{m}$  long, positioned towards ventral side of a similar subcircular area. Newly formed cysts with conspicuous subcrystalline layer.

J2 with well-developed projecting anteriorly basal knobs. Conspicuous hyaline region at least twice as long as stylet, distal third of tail tapering, ending in a rounded point.

*Punctodera punctata* differs from other *Punctodera* species by the pear-shaped cysts and the absence of bullae.

#### 4.4.4. Genus *Cactodera* Krall & Krall, 1978

Mature females and cysts are lemon-shaped to spherical, with posterior protuberance. Vulva terminal, vulval slit <30 µm, fenestra circumfenestrate. Anus without fenestration. Bullae and underbridge absent, vulval denticles usually present. Cuticle with D-layer. The eggs are usually retained within the cyst body. The eggshell surface may be covered by tiny punctations. The surface structure of eggs is important diagnostically within *Cactodera* as some species have smooth surfaced eggs, e.g. *Cactodera weissii* and *C. amaranthi*, whilst others, such as *C. milleri*, *C. eremica* and *C. thornei*, are punctuated. J2 have a lateral field with four incisures, phasmid openings punctiform. The type species of the genus is *C. cacti*.

##### 4.4.4.1 *Cactus cyst nematode, Cactodera cacti* (Filipjev & Schuurmans Stekhoven, 1941) Krall & Krall, 1978

A cyst nematode infecting cacti, *Discocactus akkermannii* and *Cereus speciosus*, both of which were expressing declining symptoms, was first recorded and described from Maartensdijk, near Utrecht, The Netherlands. The cactus cyst nematode is distributed worldwide, mainly on plants of the family Cactaceae grown in glasshouses as ornamentals. The dispersal of *C. cacti* from native regions in the Americas is beyond doubt associated with the international trade of infested ornamental cactus plants around the world. The cactus cyst nematode has been associated with or found to infect plants belonging to three families: Cactaceae (Caryophyllales): *Cereus*, *Cleistocactus*, *Coryphantha*, *Discocactus*, *Echinocactus*, *Echinopsis*, *Echinocereus*, *Epiphyllum*, *Gymnocalycium*, *Hattiora*, *Heliocereus*, *Hylocereus*, *Leuchtenbergia*, *Mammillaria*, *Melocactus*, *Notocactus*, *Nopalea*, *Notocactus*, *Opuntia*, *Oreocereus*, *Rebutia*, *Rhipsalis*, *Schlumbergera*, *Selenicereus*, *Thelocactus*; Umbelliferae (order Apiales): *Apium*; and Euphorbiaceae (order Malpighiales): *Euphorbia*. Infected plants may exhibit various symptoms including branched roots and increased numbers of rootlets. Plants become reddish-brown to yellow in colour, wilted and stunted, with reduced flower production and shortening of the flowering period. With high infection the host may die.

#### DESCRIPTION

Cysts: L = 328–780 µm; W = 240–598 µm; L/W ratio = 1.1–2.0; fenestral diameter = 16–48 µm.

Male: L = 910–1113 µm; stylet = 22–29 µm; spicules = 30–37 µm; gubernaculum = 10–15 µm.

J2: L = 344–584 µm; stylet = 21–26 µm; hyaline region = 12–21 µm; tail = 34–60 µm.

Female body lemon-shaped to almost spherical, pearly white, yellow or golden, maturing to light brown. Cyst usually lemon-shaped, but may be rounded with protruding neck and vulva, light or medium brown, sometimes reddish-brown. Vulval denticles generally present, visible beneath fenestral surface. Cone tops abullate, circumfenestrate. J2 vermiform, body tapering anteriorly and posteriorly. Tail tapering, with hyaline region often shorter than stylet. Eggshells heavily punctuate as seen under optical microscope with oil immersion.

*Cactodera cacti* resembles *C. weissi*, *C. acnidae*, *C. milleri* and *C. galinsogae*. It differs from *C. weissi* and *C. acnidae* in having eggshells heavily punctate vs shells without visible markings, and J2 with larger stylet. It differs from J2 of *C. galinsogae* by a longer tail and from *C. milleri* by cysts with a larger fenestral diameter.

#### 4.4.5. Genus *Dolichodera* Mulvey & Ebsary, 1980

Females and cysts: body elongate to oval, without terminal protuberance, white, swollen part 400–500 µm long, 140–270 µm wide, 2–2.8 times as long as wide, neck moderately long. Cuticle not annulated but with fine irregular striae. Vulval area terminal or just subterminal, circumfenestrate, fenestra approx. 20 µm in diameter, bullae present, perineal tubercles absent. Anus pore-like, lacking a fenestra, located 10–13 µm dorsal to vulval fenestral margin. Cyst with several large bullae. Perineal tubercles absent. Vulva circumfenestrate, underbridge absent. Anus lacking fenestra. Male not found. J2 with long tail (95–120 µm). Lateral field with three incisures, inner one faint. Labial region hemispherical, offset, with two annuli. Tail tip narrowly rounded. Phasmid openings lacking a lens-like ampulla, located about one anal body diameter posterior to anus. The type species of the genus is *Dolichodera fluvialis* Mulvey & Ebsary, 1980, parasitizing *Spartina pectinata*.

#### 4.4.6. Genus *Betulodera* Sturhan, 2002

Cysts are lemon-shaped, pear-shaped or spheroid with insignificant, obtuse vulval cone. Cyst wall thick, with irregular network-like pattern, D-layer absent (no punctations in inner, deeper layers of cyst wall), subcrystalline layer heavily developed. Vulva terminal, surrounded by circumfenestration, vulval slit short (<10 µm), underbridge absent, denticles occasionally present, anus without fenestration. Male body twisted, no cloacal tube, spicules with bifid distal tips, phasmid openings punctiform. J2 has lateral field with three incisures, phasmid openings punctiform, without lens-like structure, labial region with three or four labial annuli and labial disc fused with submedial lips. The type and only species: *Betulodera betulae* (Hirschmann & Riggs, 1969) Sturhan, 2002.

#### 4.4.7. Genus *Paradolichodera* Sturhan, Wouts & Subbotin, 2007

Mature female and cyst are elongate to ovoid, with rounded posterior end. Cuticle transparent, with faint transverse striations on anterior part of body and posteriorly

mostly with faint irregular ridges superimposed on distinct punctations. Cuticle turning yellowish to light brown on death, covered by a subcrystalline-like film. Eggs retained in body, egg sac not observed. Labial disc squarish. Stylet well developed. Vulva terminal or subterminal, vulval slit short, circumfenestrate. Anus lacking fenestration. Male body not twisted, lateral field with four incisures. Cloacal tube present, spicules rounded at tip. Phasmids lacking. J2 long, extremely slender for family, lateral fields indistinct. Stylet short ( $<20\ \mu\text{m}$ ). Dorsal gland orifice located more than half stylet length posterior to stylet base, pharyngeal glands long, filling body cavity. Tail long, slender, phasmid openings punctiform. Type and only species: *Paradolichodera tenuissima* Sturhan, Wouts & Subbotin, 2007.

#### 4.4.8. Genus *Vittatidera* Bernard, Handoo, Powers, Donald & Heinz, 2010

Cysts are orange-brown to brown, lemon-shaped with short necks and vulval cone. Vulval aperture circular to rhomboid, circumfenestrate, with irregular denticle-like protuberances around the periphery of orifice. Bullae, vulval bridge and vulval underbridge absent. It resembles representatives of the genera *Cactodera* and *Betulodera* in having lemon-shaped cysts and circumfenestrate vulval area. Male variable length, stylet knobs rounded. J2 having conoid tail with narrowly rounded tip, phasmid apertures pore-like. Stylet length  $<18\ \mu\text{m}$ . Stylet knobs rounded. Lateral field with four incisures. Eggshell smooth. The type and only species is *Vittatidera zeaphila* Bernard, Handoo, Powers, Donald & Heinz, 2010, parasitizing maize in north-western Tennessee, USA.

### 4.5. Pathotypes and Races

As resistant varieties were increasingly developed as a means of controlling cyst nematodes in several major crops (see Chapter 14), it became apparent that genetic variation existed within populations able to overcome such resistance (Cook and Rivoal, 1998). This led to the growing realization that within cyst nematode species that are morphologically identical, distinct virulent strains occur. Various pathotype schemes for the major cyst nematodes were proposed, with ‘pathotype’ being regarded as a group of individual nematodes with common gene(s) for (a)virulence and differing from gene or gene combinations found in other groups. Three cyst nematode groups have been extensively studied and pathotype schemes proposed, which are all based on the ability (or inability) of populations within each species to reproduce on a range of ‘differential’ host plants; these three groups are PCN (*G. rostochiensis* and *G. pallida*), cereal cyst nematode (*H. avenae*) and the soybean cyst nematode (*H. glycines*).

Two pathotype schemes for *G. rostochiensis* and *G. pallida* were proposed in 1977 that described the virulence of populations from Europe and South America (Table 4.4). In the pathotype/differential clone interactions, susceptible (+) indicated a multiplication rate ( $P_f/P_i$ )  $>1.0$ , and resistant (-) indicated a  $P_f/P_i$   $<1.0$ , where  $P_i$  and  $P_f$  are the initial and final population sizes, respectively. This standardized disparate national schemes, especially those used within European countries, but it soon became clear that environmental influences and the extensive heterogeneity of

**Table 4.4.** Pathotype groups of potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*. (Adapted from Cook and Noel, 2002.)

Species and accession	Species Ploidy, resistance gene	<i>G. rostochiensis</i>					<i>G. pallida</i>						
		Ro1		Ro3		Ro5	Pa1		-	-	Pa2/3		
		Ro1	Ro4	Ro2	Ro3	Ro5	Pa1		-	-	Pa2	Pa3	-
		R1A	R1B	R2A	R3A	-	P1A	P1B	P2A	P3A	P4A	P5A	P6A
<i>Solanum tuberosum</i> ssp. <i>tuberosum</i>	4x, (minor)	+/-	+	+	+	+	+	+	+	+	+	+	+
<i>S. tuberosum</i> ssp. <i>andigena</i> CPC 1673	4x, H1 on chromosome 5	-	-	+	+	+	+	"	"	"	+	+	"
<i>S. kurtzianum</i> KTT 60.21.19	2x, K1 K2 A and B	-	(+)	-	(+)	(+)	+	+	+	+	+	+	+
<i>S. vernei</i> GLKS 58.1642.4	2x, quantitative	-	+	-	-	+	+	+	+	-	+	+	+
<i>S. vernei</i> Vt 62.33.3	2x, quantitative	-	-	-	-	+	-	+	-	-	-	+	+
ex. <i>S. multidissectum</i> hybrid P55/7	2x, 1 + polygenes H2	+	+	+	+	+	-	-/+	-	+	+	+	+
<i>S. t.</i> ssp. <i>andigena</i> CIP 280090.10	H3 + polygenes Quantitative	+	"	"	"	"	(-)	"	"	"	(-)	(-)	"
<i>S. vernei</i> hybrid 69.1377/94	2x, polygenes	-	-	-	-	-	-	"	"	"	-	-	"
<i>S. vernei</i> hybrid 63.346/19	2x, polygenes	-	-	-	-	-	+	"	"	"	+	+	"
<i>S. spegazzinii</i>	2x, Fa = H1	-	-	+	+	+	"	"	"	"	"	"	"
<i>S. spegazzinii</i>	2x, Fb + 2 minor Glo1 on chromosome 7	+	-	+	-	-	"	"	"	"	"	"	"
		(-)	+	+	+	+	"	"	"	"	"	"	"

<sup>a</sup>Trudgill (1985); <sup>b</sup>Kort *et al.* (1977); <sup>c</sup>Canto-Saenz and de Scurrah (1977).

Note: + = compatible interaction: nematode multiplication, potato susceptible; - = incompatible interaction: nematode no multiplication, potato resistant; () = partial or uncertain interaction; " = no information.

some populations, especially those of *G. pallida*, caused problems. Populations in the centres of origin of the two species in South America are more heterogeneous in virulence characteristics than those introduced and dispersed in the rest of the world. Some populations are relatively homozygous for virulence, e.g. Ro1 (R1A) and Pa1 (P1A). Others, such as most *G. pallida* populations, are heterogeneous and give varying results; thus, these populations cannot reliably be described as pathotypes and are increasingly referred to as virulence groupings within pathotypes.

The pathotype scheme for cereal cyst nematodes (*H. avenae*) is based on their multiplication on host differentials of barley, oats and wheat crops, with the major division into three pathotype groups based upon reactions of the barley cultivars with known resistance genes (*Rha1*, *Rha2*, *Rha3*). Each pathotype group is further subdivided by their reactions on other differentials. Resistance is defined as fewer than 5% new females on susceptible controls (Table 4.5). As with the PCN pathotype schemes, because the genetics of field populations are largely unknown and variability exists within them, the term ‘virulence phenotype’ has been proposed. Evidence suggests that the different species of cereal cyst nematodes (*H. avenae*, *H. filipjevi* and *H. australis*) have populations with different virulence phenotypes. There is limited evidence for loss of effectiveness of resistance genes used in widely grown cultivars and part of the reason for this may be that endemic biological control develops when cereals are intensively cultivated in moist temperate soils and, therefore, selection pressures for virulent strains are reduced.

Differences in virulence between soybean cyst nematode (*H. glycines*) populations are referred to as races rather than pathotypes. Such differences were recognized during breeding programmes in the USA for resistant soybean varieties. Using four soybean differentials (Pickett, Peking, PI 88788 and PI 90763), 16 races were characterized (Table 4.6). A resistant response (avirulence) is defined as a Female Index of <10% of that obtained on the susceptible cultivar Lee. Although the soybean cyst nematode race scheme predicts whether a cultivar will control the nematode population within a field in a particular season, as with other schemes, it cannot predict the consequences of selection pressure with a heterogeneous population.

Despite the limitations of the various pathotype/race schemes for cyst nematodes, providing their limitations are recognized, they continue to give a useful indication of the virulence characteristics of particular nematode gene pools. As such, they can provide critical information necessary for effective management and the emergence of new virulent strains.

## 4.6. Biochemical and Molecular Diagnosis

Biochemical approaches such as gel electrophoresis for separating protein and enzyme profiles have shown great potential for helping to identify cyst nematodes. Isoelectric focusing (IEF) is currently used in nematology laboratories for routine diagnostics of *G. pallida* and *G. rostochiensis*. By comparison with the biochemical approaches, analysis of DNA for diagnostics has several advantages. The main DNA region targeted for diagnostics of cyst nematodes is the internal transcribed spacer 1 (ITS1) and internal transcribed spacer 2 (ITS2), which are situated between 18S and 5.8S and 5.8S and 28S rRNA genes, respectively. Genes of mitochondrial DNA (mtDNA), with their relatively higher rate of mutations relative to rRNA genes, have



**Table 4.5.** Pathotype groups of cereal cyst nematodes, *Heterodera avenae*, *H. filipjevi* and *H. australis*. (Adapted from Cook and Rivoal, 1998.)

Species	<i>H. avenae</i>							<i>H. australis</i>	<i>H. filipjevi</i>		
	Ha1 group							Ha2 group	Ha3 group		
	Ha11	Ha21	Ha31	Ha41	Ha51	Ha61	Ha71	Ha12	Ha13	Ha23	Ha33
Pathotype											
Different species and cultivar											
<i>Barley</i>											
Varde	+	"	"	+	"	+	+	+	+	+	+
Emir	+	+	"	+	"	-	+	+	+	+	+
Ortolan/Drost	-	-	-	-	-	-	-	+	+	+	+
Morocco	-	-	-	-	-	-	-	-	-	-	-
Siri	-	-	-	+	+	+	-	-	+	+	+
KVL 191	-	-	-	"	+	+	+	-	"	"	"
Bajo Aragon	-	"	"	-	"	-	-	-	+	+	+
Herta	+	+	-	"	-	"	-	+	+	"	"
Martin 403	-	"	"	-	"	-	-	-	-	+	+
Dalmatische	(-)	"	"	+	"	-	(+)	+	+	(-)	+
La Estanzuela	"	"	"	"	"	"	+	"	"	(-)	"
Hartian 43	-	"	"	"	"	"	-	-	"	-	+
<i>Oat</i>											
Nidar	+	"	"	(+)	"	+	-	+	+	+	+
Sol II	+	-	-	-	-	+	-	+	+	+	+
Pura Hybrid BS1	-	-	"	-	-	-	-	-	+	-	+
<i>Avena sterilis</i> 1376	-	-	"	-	-	-	-	-	-	-	-
Silva	(-)	"	"	-	"	(-)	-	(-)	(-)	(-)	+
IGV.H. 72-646	-	"	"	-	"	-	-	-	+	+	+
<i>Wheat</i>											
Capa	+	+	"	+	"	+	+	+	+	+	+
AUS10894	-	"	"	-	"	-	+	-	(-)	+	+
Loros	-	-	"	-	"	(-)	-	-	(-)	+	+
Psathias	"	"	"	+	"	"	"	+	+	+	-
Iskamish K-2-light	+	"	"	-	"	(-)	"	+	+	+	+

Note: + = susceptible; - = resistant (<5% new females compared to numbers on susceptible control); () = intermediate; " = no information

**Table 4.6.** Races of soybean cyst nematode, *Heterodera glycines*. (Adapted from Cook and Rivoal, 1998.)

Differential cultivar	Race	3	6	13	9	1	5	11	2	8	10	12	14	7	15	1	4
	Virulence phenotype	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Pickett		-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Peking		-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
PI 88788		-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
PI 90763		-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+

Note: - resistant (female index <10% cultivar Lee); + susceptible (female index >10% that of susceptible control cultivar Lee).

great potential for diagnostics of races and populations of cyst nematodes. PCR-RFLP and PCR with species-specific primer(s) presently are used for diagnostics of many cyst nematode species (Subbotin *et al.*, 2010a,b; see Chapter 2).

## 4.7. Interactions with Other Plant Pathogens

As with all plant-parasitic nematodes, the mechanism of feeding on plant tissue results in wounding and provision of entry sites for other pathogens (Barker and McGawley, 1998). However, more specific associations, which can result in either synergistic or antagonistic responses by the host plant, demonstrate that more complex interactions have evolved. Most investigations of the interrelationships of cyst nematodes and other plant parasites have focused on those with fungi, especially those causing wilt and root rot (Table 4.7). A number of cyst nematode species interact with *Fusarium* wilt species, causing the wilt disease to be more severe than in the absence of the nematode, e.g. *G. tabacum* on tobacco, *H. cajani* on pigeon pea and *H. glycines* on soybean. Generally, these interactions involve synergism with regard to disease development but often result in restricted nematode reproduction because of the associated root damage. Only limited examples of interactions between cyst nematodes and root-rot fungi (*Rhizoctonia* spp.) have been documented, but those that have generally show an enhancement of the disease in the presence of the nematode, for example *G. rostochiensis* on potato, *H. avenae* on wheat, *H. glycines* on soybean and *H. schachtii* on sugar beet. Although such associations usually have an adverse effect on the host plant, the interaction of *H. cajani* with *Rhizoctonia bataticola* suppresses the associated damage caused by the fungus.

The economic effect of these interactions varies but their effect can be important with major high-value crops such as soybean. A major disease of soybean is 'sudden death syndrome' (SDS) caused by *Fusarium solani*, which is sometimes associated with the presence of *H. glycines*. Although experimental data indicate that the nematode is not necessary for the development of SDS, field observations have shown that soybean cultivars resistant to *H. glycines* were less affected by SDS than susceptible ones. The decreased *H. glycines* population levels correspond to the restriction in root and shoot growth attributable to the additive root damage by the fungus and nematode.

**Table 4.7.** Summary of interrelationships of selected cyst nematodes of the genera *Globodera* and *Heterodera* and plant-pathogenic fungi. (Adapted from Barker and McGawley, 1998.)

Nematode	Associated fungi	Host	Comments
<i>G. rostochiensis</i>	<i>Rhizoctonia solani</i>	Potato	Yield loss, but only small interaction effect
	<i>Pyrenochaeta lycopersici</i>	Tomato	Fungus probably prevents syncytium formation by the nematode
<i>G. rostochiensis</i> and <i>G. pallida</i>	<i>Verticillium dahliae</i>	Potato	Results in 'early dying disease'
<i>G. tabacum</i>	<i>Fusarium oxysporum</i>	Tobacco	Wilt disease enhanced
	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> , <i>Verticillium albo-atrum</i>	Tomato	More <i>Verticillium</i> but less <i>Fusarium</i> wilt in the presence than absence of <i>G. tabacum</i>
<i>H. avenae</i>	<i>Rhizoctonia solani</i>	Wheat	In combination, greater reduction in tillering, height, weight, root number and length than with either alone
	<i>Gaeumannomyces graminis</i>	Wheat	Antagonism
<i>H. cajani</i>	<i>Fusarium udum</i>	Pigeon pea	Wilt enhanced
	<i>Rhizoctonia bataticola</i>	Cowpea	Damage suppressed
<i>H. glycines</i>	<i>Rhizoctonia solani</i>	Soybean	Limited nematode reproduction
	<i>Calonectria crotalariae</i>		Enhanced activity of both parasites
	<i>Phytophthora megasperma</i> var. <i>sojae</i>		Increased seedling disease (additive)
	<i>Fusarium oxysporum</i>		Increased wilt
	<i>Fusarium solani</i>		Variable; increased foliar symptoms of fungus; suppressed nematode reproduction
	<i>Macrophomina phaseolina</i>		Synergism
<i>H. oryzaicola</i>	<i>Sclerotium rolfsii</i>	Rice	Variable, but usually synergistic
<i>H. schachtii</i>	<i>Fusarium oxysporum</i>	Sugar beet	Damage was less when fungus present; fungus inhibited nematode invasion
	<i>Pythium ultimum</i>		Synergistic or damping off when in combination
	<i>Pythium aphanidermatum</i>		Additive
	<i>Pythium solani</i>		Synergism
	<i>Rhizoctonia solani</i>		Synergism, especially at high inoculum levels

The relationship of cyst nematodes with mycorrhizal fungi has focused on the vesicular-arbuscular mycorrhizal fungi, *Glomus* spp. This group is important as it may be useful in enhancing crop yields on nematode-infested soil by rendering nematode-susceptible plants more tolerant to these pathogens or through the suppression of nematode infections and reproduction. Although most research has focused on root-knot nematodes, observations of interactions of *H. glycines*,

*H. cajani* and *G. rostochiensis* with various *Glomus* species have been shown. For example, *Glomus fasciculatum* suppresses *H. glycines* on soybean and *H. cajani* on cowpea where the fungus may actually parasitize the nematode eggs.

Nematode communities usually contain many species that potentially may interact with each other. In most instances, where cyst nematodes are present, they are antagonistic to other plant-parasitic species. Examples of this include *G. tabacum* and *Pratylenchus penetrans* on tobacco, *H. avenae* and *P. neglectus* on wheat, *H. cajani* and *Helicotylenchus retusus* in pigeon pea, and *H. glycines* and *Meloidogyne incognita* on soybean. Very few examples of neutral or stimulatory responses between cyst and other nematode species have been documented. The potential interactions between cyst nematodes and bacteria, insects and other pests have received only limited study. One notable exception is that of *G. pallida* and the bacteria *Ralstonia solanacearum* on potato, in which the nematode enhances damage caused by the associated wilt.

Thus, cyst nematodes show a wide range of important interrelationships with associated organisms in a wide range of habitats. As such, full evaluations of host–parasite relationships should be undertaken in the presence of other pathogens likely to be present in their natural habitat. Such evaluations are needed for effective management strategies.

## 4.8. Management

The cyst nematodes that cause major damage to cultivated crops are mainly those species within the genera *Globodera* and *Heterodera* and these present a unique problem in their management (Riggs and Schuster, 1998). Many or all of the eggs are produced inside the female body that, upon death, becomes a cyst with a hardened protective wall. This structure is resistant to invasion by potential parasites and protects the eggs inside from rapid desiccation, enhancing their ability to remain dormant for many years. In many cases (e.g. *Globodera* spp.) substantial hatch will only occur in the presence of a hatching factor produced by a potential host (see Section 4.3 and Chapter 7), so that any management strategy must be effective over a period of years or usable year after year. However, unlike root-knot nematodes (Chapter 3), cyst nematodes have a relatively narrow host range, making appropriate crop rotation a viable option in certain situations.

### 4.8.1. Prevention

Fundamental to the prevention of cyst nematodes spreading into non-infested regions is the use of certified planting material, and strict legislation for those commodities being traded both internationally and locally. This tactic has been the mainstay for controlling several major pests such as *G. rostochiensis*, *G. pallida* and *H. schachtii* (Chapter 12). Efficient management and containment of an infestation may be compromised by the ease with which cysts can be dispersed by, for example, wind, in small aggregates of soil, on small roots attached to other parts of plants, by flood water run-off, or by adhering to machinery or animals passing through infested land. General hygiene practices should be adopted in higher risk situations when the pest is known to be present in the locality. Such measures would include cleaning machinery both before and after use, restricting movement of soil outside the field boundary and construction of natural wind breaks.

### 4.8.2. Crop rotation

Where host range is limited, crop rotation has proved an important component in managing cyst nematode levels. Alternative non-host crops can safely be cultivated, during which time a combination of spontaneous hatch and natural mortality will reduce the field population to below threshold levels (Chapter 11). Cyst nematodes that have only one to three cultivated host plants include *G. rostochiensis*, *G. pallida* (potato, eggplant and tomato), *H. avenae* (oat, barley and wheat), *H. zaeae* (cultivated and wild maize) and *H. carotae* (cultivated and wild carrot). Even those cyst nematodes with broader host ranges, such as *H. schachtii* and *H. glycines*, have relatively few cultivated hosts, facilitating the potential for control by use of rotations.

### 4.8.3. Resistance

Cultivar resistance remains the most economical practice for managing cyst nematodes (Chapter 14), although these are not always available. Resistance of major crop hosts to *G. rostochiensis*, *G. pallida*, *G. tabacum tabacum*, *G. tabacum solanacearum*, *H. avenae*, *H. glycines*, *H. schachtii* and *H. cajani* have been found and attempts made to incorporate it into commercial cultivars. Only low level, or no, resistance is known in the major crop hosts of *H. cruciferae*, *H. oryzae*, *H. sacchari* and *H. oryzicola*. However, in many cases resistance is found only in wild species, with the accompanying inherent difficulties of transferring into commercial cultivars (Riggs and Schuster, 1998). The inappropriate continuous planting of resistant cultivars is now known to increase selection pressure for virulent populations (e.g. potatoes and *G. rostochiensis* and *G. pallida*), limiting the durability of resistance in some cultivars, or resulting in the increase of other nematode problems (e.g. cereal cyst nematode with the associated build-up of *Pratylenchus neglectus*).

### 4.8.4. Biological control

Cyst nematodes would appear to be the perfect target for the use of biological agents in their management. Eggs of cyst nematodes are contained either inside the female's body/cyst or in a gelatinous sac, so they should be very susceptible to parasitism by fungi or bacteria in the rhizosphere. Numerous studies using nematophagous fungi and bacteria against economically important cyst nematodes have been undertaken with varying degrees of success and failure. However, the best example of suppressiveness of soils toward cyst nematodes is in the control of *H. avenae* by the fungi *Nematophthora gynophila* and *Pochonia chlamydosporium* (Chapter 14).

### 4.8.5. Chemical control

Nematicides have been very effective in controlling cyst nematodes (Whitehead, 1998; see Chapter 16) but several of the most effective have now been withdrawn because of health and safety concerns. The effectiveness of nematicides is reduced by their biological degradation by soil organisms, which may be increased by the multiple use

of a nematicide. Examples of cyst nematodes that produce only one or two generations a year, and for which nematicides have been extensively used as a management strategy, are *G. rostochiensis* and *G. pallida* on potatoes, *H. goettingiana* on peas and *H. avenae* on cereals. However, those species that produce several generations in a year, such as *H. glycines*, appear to be more difficult to control.

#### **4.8.6. Integrated management**

The repeated use of a single control measure is likely to fail, sooner or later, from selection of virulent biotypes, accelerated microbial degradation of nematicide, or the selection of populations better able to overcome such diverse management programmes; in general, selection of individuals unaffected by any control measure that may be applied. The potential for managing cyst nematodes by combining two or more control strategies in an integrated programme has been widely demonstrated. Usually some level of crop rotation is practised, alongside additional measures. The advantages of this approach include the use of partially effective strategies and protection of highly effective ones that are vulnerable to nematode adaptation or environmental risk; examples include integrated control of *G. rostochiensis* and *G. pallida* in Europe, and *H. glycines* in the USA (Roberts, 1993).