1

Anguinidae: *Anguina* and *Ditylenchus* Species

Anguina Spp.

The genus *Anguina* covers the first plant-parasitic nematode to be recorded, *Anguina tritici*, discovered by Needham in 1743 from wheat seed gall. The gall-forming nematodes, *Anguina* spp., are obligate parasites and attack aerial plants parts of cereals and forage grasses. More than 40 nominal species of *Anguina* have been described (Subbotin and Riley, 2012) and important species include:

Anguina agropyri Kirjanova, 1955
A. agropyronifloris Norton, 1965
A. agrostis (Steinbuch, 1799) Filipjev, 1936
A. amsinckiae (Steiner and Scott, 1935) Thorne, 1961
A. australis Steiner, 1940
A. balsamophila (Thorne, 1926) Filipjev, 1936
A. caricis Solovyeva and Krall, 1982
A. cecidoplastes (Goodey, 1934) Filipjev, 1936
A. funesta Price, Fisher and Kerr, 1979
A. graminis (Hardy, 1850) Filipjev, 1936
A. microlaenae (Fawcett, 1938) Steiner, 1940
A. pacificae Cid del Prado Vera and Maggenti, 1984 (Ferris, 2013).
A. tritici (Steinbuch, 1799) Filipjev, 1936

Three *Anguina* species, *A. tritici*, *A. agrostis* and *A. funesta*, are considered as invasive nematode species because of their economic importance and quarantine significance in various countries (Chizhov and Subbotin, 1990; Krall, 1991). Other species are of lower importance in a limited geographical range.

Systematic position of *Anguina* species

	Classical (after Siddiqi, 2000)	Molecular (after Decraemer and Hunt, 2013)
Phylum	Nematoda	Nematoda
Class	Secernentea	Chromadorea
Subclass	Tylenchia	Chromadoria
Order	Tylenchida	Rhabditida
Suborder	Tylenchina	Tylenchina
Infra-order	Anguinata	Tylenchomorpha
Superfamily	Anguinoidea	Sphaerularioidea
Family	Anguinidae	Anguinidae
Subfamily	Anguininae	Anguininae
Genus	*Anguina*	*Anguina*

1 *Anguina tritici*

1.1 Common name

Seed gall nematode; bunted wheat; ear cockle

1.2 Scientific name

Anguina tritici (Steinbuch, 1799) Filipjev, 1936

1.3 Synonyms

Anguillula scandens Schneider, 1866
Anguillula tritici (Steinbuch) Grube, 1849
Anguillulina scandens (Schneider) Goodey, 1932
Anguillulina tritici (Steinbuch) Gervais and Van Beneden, 1859
Rhabditis tritici (Steinbuch) Dujardin, 1845
Tylenchus tritici (Steinbuch) Bastian, 1865
Tylenchus scandens (Schneider) Cobb, 1890
Vibrio tritici Steinbuch, 1799

1.4 EPPO code

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1.5 Diagnosis

To facilitate the identification of *Anguina tritici*, morphological, biochemical and molecular diagnosis approaches are presented.

1.5.1 Morphological diagnosis

Important morphological descriptions of *A. tritici* after Southey (1972) and Krall (1991) are summarized below. For measurements, see Table 3.

Mature female: Body obese, spirally coiled ventrally. Lip region low and flattened, slightly offset, cephalic framework weak. Cuticle very finely annulated. Procorpus often swollen by gland

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Table 3. Morphometric data for *Anguina tritici*, *A. agrostis* and *A. funesta* (Adapted from ISPM-27, DP 18: *Anguina* spp.).

Morphometric characters		Range, numerous populations		
		Anguina tritici	*Anguina agrostis*	*Anguina funesta*
Mature females	Length (mm)	3.0–5.2	1.3–2.7	1.65–2.44
	Stylet (µm)	8–11	8–12	7–10
	a (nematode body length/greatest width)	13–30	13.8–25.4	16.8–20.1
	b (nematode body length/pharynx length valve)	9.8–25.0	8.0–28.7	9.3–34.0
	c (body length/tail length)	24–63	25.2–44.0	18.1–41.2
	V% (distance from the anterior end to the vulva / nematode body length)	70–95	87–92	86.9–94.0
Males	Length (mm)	1.9–2.5	1.05–1.68	0.78–1.52
	Stylet (µm)	8–11	10–12	7–10
	a	21–30	23–38	20.3–30.9
	b	6.3–13.0	6–9	6.3–9.5
	c	17–28	20.0–28.4	16.1–24.9
	Spicules (µm)	35–40	25–40	16–28
	Gubernaculum (µm)	9–10	10–14	9–14
Second-stage juveniles	Length (mm)	0.75–0.95	0.55–1.25	0.81–0.87
	Stylet (µm)	10	10	7–10
	a	47–59	44–71	48–53
	b	4.0–6.3	3.2–6.1	4.2–4.6
	c	23–28	11.7–20	12.3–15.1

secretions but constricted at junction of metacorpus. Isthmus sometimes posteriorly swollen, offset from pharyngeal glands by a deep constriction. Pharyngeal glands not overlapping, or with very slight overlap of intestine. Excretory pore near junction of pharynx and intestine or slightly more posterior. Vulval lips protruding, orifice of small glands visible on vulval lips anterior and posterior to vulva. Ovary with two or more flexures, often reaching to pharyngeal region with oogonia in multiple rows arranged around a rachis. Spermatheca pyriform, separated from oviduct by a sphincter. Postvulval uterine sac present (Fig. 1.1). Tail conoid, tapering to obtuse or rounded tip, not mucronate.

Male: Slenderer than female. Habitus upon heat relaxation curved either ventrally or dorsally. Testis with one or two flexures. Spicules stout, arcuate, with two ventral ridges running from tip to widest part (Fig. 1.1). Capitulum with distinct ventral folding at anterior. Gubernaculum simple, trough-like. Bursa leptoderan. Tail conoid, tip rounded or obtuse.

Second-stage juvenile: Body slender, not spirally coiled. Tail conoid, pointed (Fig. 1.1).

1.5.2 Biochemical and cytogenetic diagnosis

Biochemical and cytogenetic techniques are used to discover the degree of genetic similarity in a taxonomic group. Such data must be obtained for several species to compare with both in-group and out-group members. A comparative study of such data provides valuable information about characters and features that are the result of common genetic material, as well as those that are unique for a particular member of the group. The uniqueness determines the identity of the taxon (Siddiqi, 2000). Biochemical approaches such as the separation of proteins (general proteins and isozymes) by one-dimensional gel electrophoresis, isoelectric focusing, two-dimensional gel electrophoresis and sodium dodecyl sulphate–capillary gel electrophoresis are used for the biochemical diagnosis of plant-parasitic nematodes (Abrantes *et al.*, 2004). Serological techniques have also been found effective in the identification and quantification of nematodes, monoclonal antibodies being a more useful immunological tool than polyclonal antibodies (Abrantes *et al.*, 2004). Riley *et al.* (1988) characterized *A. agrostis*, *A. funesta* and *A. tritici* based on isozymes, using one-dimensional native gel electrophoresis.

1.5.3 Molecular diagnosis

Molecular diagnosis of *Anguina* spp. is based on polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) (Powers *et al.*, 2001), real-time PCR (Ma *et al.*, 2011; Li *et al.*, 2015) or sequencing of the internal transcribed spacer (ITS) region of ribosomal (r) RNA (Subbotin *et al.*, 2004). The choice of test depends on whether identification requires confirmation of both the presence and the absence of particular species, and on the availability of species standards for controls. The method described by Ma *et al.* (2011) is limited to the positive identification of *A. agrostis*, while the other methods can simultaneously distinguish multiple species within the same test. Powers *et al.* (2001) were the first to sequence the ITS1 region of *Anguina* spp. They combined PCR with analysis of RFLP and simultaneously distinguished a range of *Anguina* species from each other (Table 4). Subbotin *et al.* (2004) subsequently sequenced 58 populations of *Anguina*, *Ditylenchus*, *Heteroanguina* and *Mesoanguina* for phylogenetic analysis. At present there are 71 sequence accessions of rRNA fragments of *Anguina* spp., collected from different localities and host plants, in the United States National Center for Biotechnology Information

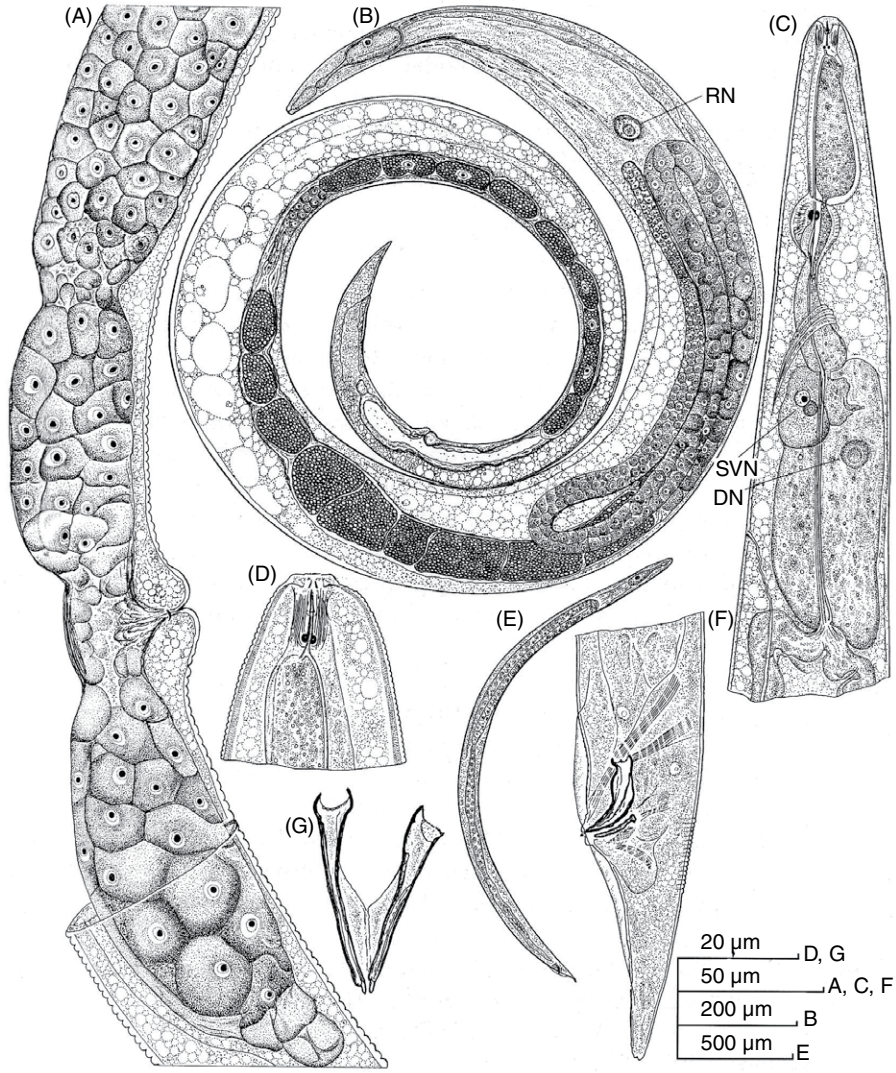

Fig. 1.1. Line drawing of *Anguina tritici from wheat grain*. Vulval region with uterus and postvulval uterine sac (A); female (B); oesophageal region of male (C); head end of female (D); male (E); tail end of male (F); spicule (G). DN, nucleus of dorsal oesophageal gland; SVN, nuclei of subventral glands; RN, nucleus of renette cell. [Reproduced from M.R. Siddiqi, 2000; courtesy CAB International]

Table 4. Restriction fragment sizes for *Anguina* species and associated restriction fragment length polymorphism (RFLP) patterns (after Powers *et al*., 2001).

Species	*AluI*	#	*BsrI*	#	*EcoRI*	#	*HaeIII*	#	*HhaI*	#	*HinfI*	#	*TaqI*	#
Anguina agrostis	548	A	295, 238, 15	B	299, 249	A	548	A	548	A	448, 100	B	355, 135, 58	A
Anguina funesta	548	A	295, 238, 15	B	299, 249	A	548	A	548	A	448, 100	B	490, 58	B
Anguina tritici	277, 274	B	550	C	550	B	550	A	462, 88	B	550	E	492, 58	B

Code for the RFLP profile for each restriction enzyme.

(NCBI) public database. Details of some molecular methods for diagnosis of *Anguina* spp. are described below.

Li *et al*. (2015) designed a TaqMan real-time PCR to identify *A. tritici*, *A. agrostis*, *A. funesta* and *A. pacificae*. This test included forward and reverse genus-specific primers combined with a fluorescent probe. These primers and probe set were designed to serve as an internal control for confirming the presence of *Anguina* spp. as well as the integrity of the PCR components and user performance. The test also included primers and probe sets specifically designed for the detection of each of the

target species mentioned above and was intended for identification of single juveniles. The sensitivity of the test was demonstrated through construction of standard curves from reactions using serially diluted nematode DNA and the test was able to detect as little as 1.25 copies of the ITS rDNA. The specificity of each primer and probe set was demonstrated in single-plex and duplex reactions (i.e. with the species-specific and genus-specific primers and probe sets) tested against all of the target species as well as several non-target nematodes, including *Meloidogyne* spp., *Pratylenchus* spp. and *Ditylenchus* spp. For the DNA extraction procedure, see Chapter 15, section 110.1. The ITS rRNA genus-and species-specific TaqMan primers and probes are:

A. tritici (ATfpr primers-probe set)

ATf (forward): 5′-GTTGCCTACGGCCGT-3′
ATr (reverse): 5′-ATGTAATCGATGTGGTACAGCCAT-3′
ATp (probe): 5′-6-FAM/ATCATGTCTTGGCTAGTG TAGACGTATCTG/BHQ-1-3′

A. agrostis (AAfpr primers-probe set)

AAf (forward): 5′-CGGTTGTTTACGGCCGT-3′
AAr (reverse): 5′-ATGTAGTCGGTGTGAAAACAGCCAT-3′
AAp (probe): 5′-6-FAM/ATCATGTCTTGGCTATTGTAGA CGTATCTG/BHQ-1-3′

A. funesta (AFfpr primers-probe set)

AFf (forward): 5′-GGTTGCTTACGGCCC-3′
AFr (reverse): 5′-GTGTAATCGATGTGATACAGCCCC-3′
AFp (probe): 5′-6-FAM/ATCATGTCTTGGCTATTAT AGACGTATCTG/BHQ-1-3′

A. pacificae (APfpr primers-probe set)

APf (forward): 5′-ACCGGTTGAATATTGGCTGT-3′
APr (reverse): 5′-ATGTAATCGATGTGAAACAGCCGT-3′
APp (probe): 5′-6-FAM/ATCATGTCTTGGAAAGTTTA GACGTATCTG/BHQ-1-3′

Anguina spp. (ASfpr primers-probe set)

ASf (forward): 5′-GTCTTATCGGTGGATCACTCGG-3′
ASr (reverse): 5′-TGCAGTTCACACCATATATCGCAG-3′
ASp (probe): 5′-TET/TCATAGATCGATGAAGAACG CAGCCA/BHQ-2-3′

The PCR amplification was performed in a real-time PCR using the following reagents and cycling parameters.

Reagent	–	Final concentration
Polymerase chain reaction (PCR)-grade water	–	final reaction volume of 25 µl
PCR buffer (including $MgCl_2$)	–	1× $MgCl_2$ 2.60 mM
dNTPs	–	0.24 mM
Species-specific primer (forward)	–	240 nM
Species-specific primer (reverse)	–	240 nM
Species-specific probe	–	120 nM
ASf internal control primer (forward)	–	160 nM
ASr internal control primer (reverse)	–	160 nM
ASp internal control probe	–	120 nM
DNA polymerase	–	1.0 U
DNA (volume)	–	1 µml
Cycling parameters		
Initial denaturation	–	95°C for 20 s
Number of cycles	–	40
Denaturation	–	95°C for 1 s
Optics	–	OFF
Annealing	–	60°C for 40 s
Optics	–	ON
Ramp	–	5°C per s
Expected amplicons size	–	74–85 base pairs

Ma *et al*. (2011) designed a species-specific TaqMan real-time PCR to identify juveniles of *A. agrostis*. It was evaluated against the target species *A. agrostis* as well as non-target species *A. tritici*, *A. wevelli* and *Ditylenchus destructor*. The ITS rRNA species-specific primers are:

PF: 5′-GTTTGCCTACCGGTTGTTTACG-3′
PR: 5′-CCACATGCAGTCGGTGTGAA-3′
TaqMan Pb: 5′-FAM-TCATGTCTTGGCTATTGTAGACG TATCTGA-TAMRA-3′

The amplification was performed in a real-time PCR using the Light Cycler according to the following cycling parameters.

Reagent	–	Final concentration
Polymerase chain reaction (PCR)-grade water	–	final reaction volume of 10 µl
PCR buffer	–	1× $MgCl_2$ 1.25 mM
dNTPs	–	0.2 mM
Primer PF (forward)	–	0.4 µM
Primer PR (reverse)	–	0.4 µM
Probe Pb	–	0.02 µM
DNA polymerase	–	0.5 U
DNA (volume)	–	1 µl
Cycling parameters		
Initial denaturation	–	94°C for 3 min
Number of cycles	–	45
Denaturation	–	94°C for 10 s
Annealing	–	60°C for 30 s
Expected amplicons size	–	88 base pairs

Detailed methodology and protocols for molecular diagnosis including DNA isolation, sequence analysis, construction of phylogenetic tree etc. are described in Chapter 15, section 110.1.

1.6 Geographical distribution

The seed gall nematode, *A. tritici* is worldwide in distribution and has been reported from the majority of wheat-growing

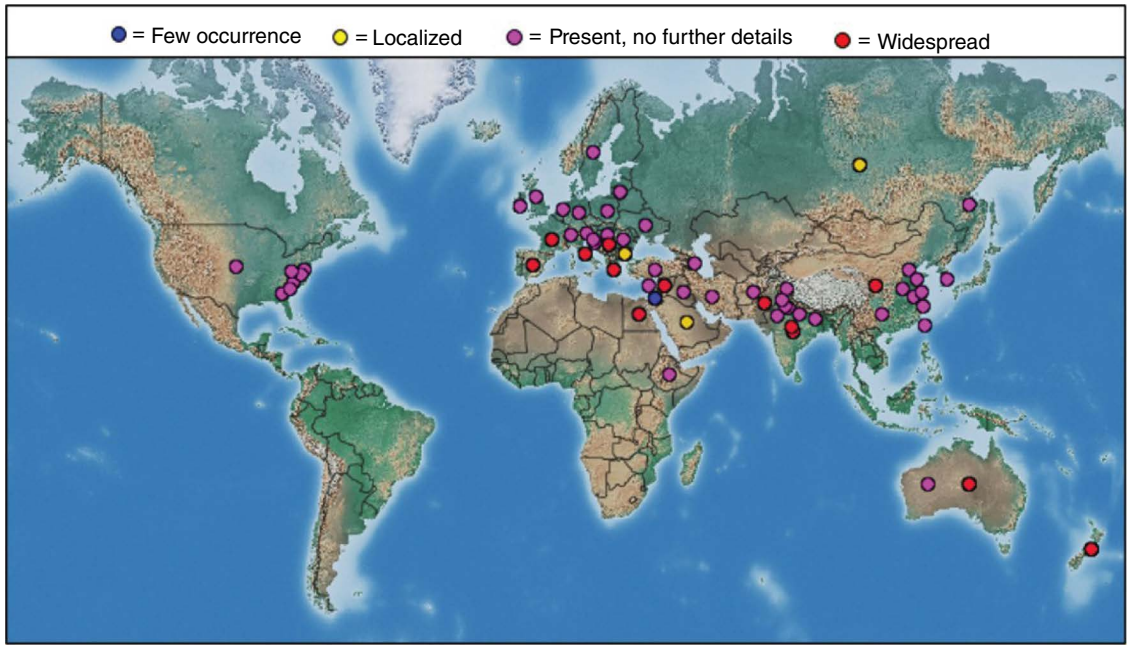

Fig. 1.2. Distribution map of *Anguina tritici*. [Adapted from CABI-ISC, 2020]

areas of the world (Fig. 1.2). At present, this nematode has almost been eradicated from all advanced European countries. *A. tritici* was present in several states of the USA, but was eliminated in 1975 and currently is not reported in the USA (Randhawa and Chitambar, 2017). However, it is still considered an important problem in several underdeveloped and developing countries like India, Pakistan, Ethiopia, Romania, Iraq, Syria and Yugoslavia (Nandal *et al*., 2010) where the farmers have been using their old stock of seed for a long time. There are indications that *A. tritici* is becoming rare or extinct in countries or regions where it had been reported previously. Therefore, past records should be treated with caution. *A. tritici* has been known to occur in the following countries (CABI-ISC, 2020).

Africa: Egypt, Ethiopia.
Asia: Afghanistan, Azerbaijan, China, India, Iran, Iraq, Israel, Pakistan, Saudi Arabia, South Korea, Syria, Taiwan, Turkey.
Europe: Austria, Bulgaria, Croatia, Cyprus, Denmark, France, Germany, Greece, Hungary, Ireland, Italy, Lithuania, Netherlands, Poland, Romania, Russia, Serbia, Serbia and Montenegro, Slovenia, Spain, Sweden, Switzerland, Ukraine, United Kingdom.
Oceania: Australia, New Zealand.

1.7 Hosts

Wheat (*Triticum aestivum*) is considered the major host of this nematode, but some other crops like triticale (× *Tritico secale*), barley (*Hordeum vulgare*), oats (*Avena sativa*), rye (*Secale orientalis*), emmer (*Triticum dicoccum*), spelt (*Triticum spelta*) and littleseed canarygrass (*Phalaris minor*) have also been reported to be attacked by this nematode (Nandal *et al*., 2010).

1.8 Symptoms

1.8.1 Ear cockle

The seed gall nematode causes typical above-ground symptoms at both the vegetative and the reproductive phases of the plant growth. Initially, the diseased plants show basal swelling of the stem (Nandal *et al*., 2010), crinkling, curling, twisting and rolling of the leaves, profuse tillering and stunted growth (Fig. 1.3A). The symptoms on leaves are more conspicuous at the early stage of plant growth and occur due to ectoparasitic feeding (Khan and Athar, 1996). The symptoms on vegetative parts normally disappear at the later stage (Lal and Lal, 2006). During the reproductive phase of plant growth, the nematode larvae enter into the developing grain and feed endoparasitically (Clark *et al*., 1991). Due to this feeding, diseased ear heads are formed (Fig. 1.3B), which are generally smaller and broader than the normal ones, with or without awns (Nandal *et al*., 2010). In such ear heads, instead of formation of grains, cockles (seed galls) are formed which are initially green and turn brown to black at maturity and hard to the touch (Khan and Athar, 1998; Fig. 1.3C). Some or all grains are transformed into galls and filled with numerous infective juvenile nematodes (Subbotin and Riley, 2012). Green immature cockles are soft in consistency and contain all the stages of the nematode, whereas black mature cockles are hard in texture and contain a

Fig. 1.3. Symptoms of *Anguina tritici* (A–F), *A. agrostis* (G–I) and *A. funesta* (J–L). Infected wheat plant (A,B); infected and healthy ear heads (C); healthy seeds and galls (D); wheat ear heads showing yellow ear rot or *tundu* disease (E); emergence of second-stage juvenile from infested seed (F); (right) healthy and (centre, left) infested bentgrass with *A. agrostis* (G); an elongated, black seed gall and sliced SEM of gall containing anhydrobiotic juveniles (H); juveniles and eggs from seed gall of the grass *Holcus mollis* (I); (left) healthy seed of *Lolium rigidum*, (centre) galled and (right) gall colonized by the bacterium *Rathayibacter toxicus* (J,K); gumming disease of ryegrass due to *Rathayibacter toxicus* (L). [Courtesy A,E: NASI, India; B: Author's own photo; C: A.L. Taylor, Nemapix picture set, Bugwood.org; D: © 2011, Ian Riley, Adelaide, Australia; F: M. McClure, University of Arizona, Bugwood.org; G: Malcolm Storey, www.bioimages.org.uk; H: J. Eisenback; I: J. Bridge/CABI BioScience; J,L: J. Allen; K: © 2006, Ian Riley, Adelaide, Australia]

large number of second-stage juveniles (J_2) of *A. tritici* in the anhydrobiotic state (dormant/quiescent, Fig. 1.3F) which may survive for as long as 38 years (Ferris, 2013). Sometimes cockles (galls) are also formed on non-floral parts like leaves, glume, lemma, palea, awns, etc.

1.8.2 Yellow ear rot (tundu disease)

Tundu disease is caused by combined infection by the nematode *A. tritici* and the bacterium *Clavibacter tritici* (= *Rathayibacter tritici*). The vector role of the nematode is essential where

A. tritici carries the bacterial cells to the shoot meristem which is well protected by the compact leaf scales, and causes yellow ear rot disease (Khan, 1993). The initial symptoms of the disease are similar to those of ear cockle disease. However, at the later stage when low temperature, high humidity and cloudy weather prevail, a bright yellow slime or gum-like substance (Fig. 1.3E) is profusely exuded by the bacterium on abortive ear heads, leaves and stems. It becomes hard and brittle on drying (Nandal *et al.*, 2010). In this disease, normally no grains are produced, due to the destruction of floral organs by the bacterium. In partially diseased ear heads, some wrinkled shaped grains of poor quality are produced (Paruthi *et al.*, 1987). Normally in such ear heads, cockles are not formed. Sahebani *et al.* (2007) reported that attachment of a large number of bacterial cells of *C. tritici* (= *R. tritici*) on the nematode body induces weakness and mortality to nematodes. Freshly harvested infected wheat cockles containing the bacterium are also toxic to cattle and sheep (Anwar *et al.*, 2001).

1.9 Biology and life cycle

The life cycle of *A. tritici* synchronizes with the development of wheat plants and one generation completes in one season on wheat (Nandal *et al.*, 2010). The cockled seeds which contain quiescent larvae in the anhydrobiotic state are the sole source of nematode inoculum (Subbotin and Riley, 2012). The nematode reaches the field whenever nematode-infested cockles are sown along with healthy wheat seeds. The cockles, when coming into contact with soil moisture, absorb water and swell. This terminates the quiescent state and facilitates the migration of the larvae. The activation of dormant larvae and their complete release into the soil may occur within 10–15 days, depending upon temperature, moisture content and soil type (Paruthi *et al.*, 1990). These larvae move here and there in the soil to make their way to the growing points of young wheat plants. A layer of moisture is essentially required for the movement of larvae on the plant surface (Khan *et al.*, 1998). The second-stage larvae, during the vegetative growth of plant phase, remain ectoparasitic under the well-protected innermost layer of compact leaf scales and bud scales at the growing point (Paruthi *et al.*, 2009). At the time of initiation of floral primordia, the larvae invade the floral tissues and feed endoparasitically. This stimulates the development of galls. During the endoparasitic feeding, the larvae become enclosed in the cavities formed by overgrowth of floral initials which eventually are turned into cockles or galls. The larvae undergo three successive moults very quickly within 3–5 days of the invasion of floral primordia and develop into adult males and females in the ratio of 1:2 (Midha and Swarup, 1972). Reproduction is amphimictic and egg-laying starts within 6–12 days, with each female laying upto 30,000 or more eggs (Subbotin and Riley, 2012). First moulting takes place inside the egg and hatching occurs quickly, giving rise to second-stage larvae (McClure, 1988). This stage coincides with the drying and maturing of the wheat plants. Under the dry conditions the larvae lose body water and enter into a quiescent state of anhydrobiosis. The nematode completes one generation from J_2 to J_2 in a growing season (Nandal *et al.*, 2010).

1.10 Movement and means of dispersal

Trading of wheat grains infested with seed galls/cockles or sowing of cockles along with healthy seeds in fields are the main means of dispersal of *A. tritici* (Nandal *et al.*, 2010). Other means of spread include infected crop straw, flooding and rainfall, natural migration (maximum 20–30 cm), and sheep, cow or bird manure. *A. tritici* is also spread by wind and by animal feet, farm machinery and implements (Subbotin and Riley, 2012).

1.10.1 Plant parts liable to carry the nematode in trade/transport

Plant parts, including roots, flowers, inflorescences, cones, calyx, leaves, seedlings, micro-propagated plants, stems (above ground), shoots, tillers and true seeds (including grain), are liable to carry all stages (eggs, juveniles and adults) of the nematode.

1.10.2 Plant parts not known to carry the nematode in trade/transport

Bark and wood.

1.11 Economic importance

Wheat is the third-largest cereal crop, cultivated throughout the world after maize and rice (FAOSTAT, 2019). Ear cockle nematodes cause quantitative and qualitative losses in wheat, through either the formation of galls (Nandal *et al.*, 2010; Subbotin and Riley, 2012) or the occurrence of yellow ear-rot disease (Ferris, 2013). Wheat produced from partially attacked ear heads or yellow ear-rot disease has less market value (Anwar *et al.*, 2001). The average incidence of the diseases and loss in yield of wheat caused by this nematode from India has been reported as 1–3% but the losses on individual farms may be up to 30–50% (Nandal *et al.*, 2010). Khan and Athar (1996) reported that yield reduction in wheat is dependent on the inoculum level of the nematode; 15,000, 20,000 and 30,000 J_2/plant caused 6–18%, 12–28% and 24–43% yield decline to wheat varieties, respectively.

1.12 Pest risk analysis and looming threat from introduction

The wheat seed gall nematode *A. tritici* is the oldest-recorded plant-parasitic nematode, reported in 1743 by Needham. In the early 20th century, this nematode was considered as one of the important problems of wheat throughout the world. Due to the adoption of seed-cleaning techniques, its occurrence has become

rarest in European countries but it is still considered an important problem in some underdeveloped and developing countries, including India. In the countries where the nematode is prevalent, it is considered as an important pest of wheat. The nematode infection in wheat inflorescence results in ear-cockle disease. The nematode in association with a bacterium, *Clavibacter tritici* (= *Rathayibacter tritici*), causes yellow ear-rot or *tundu* disease.

A. tritici is considered as a highly invasive nematode species due to its global distribution, the crop damage potential, seed-borne nature and long survival strategy. Infested grains are the principal means of international transport of the nematode, which can be very easily carried by wheat seed being moved as a food commodity. *A. tritici* has been intercepted on several occasions in consignments of wheat as a food commodity. The likelihood of introduction or spread of *A. tritici* into new areas is minimal, largely due to effective ongoing modern seed grading and other practices, but the potential economic damage by this pest should not be underestimated. As stated earlier, wheat seeds infested with low populations of the nematode may not show typical symptoms of seed galling and pose the probability of accidental introductions. Therefore, the likely risk posed to the wheat crop and the looming threat from its introduction are evaluated below.

1.12.1 Climate/host interaction

Wheat, the main host of *A. tritici*, is the third-largest cereal crop of the world after maize and rice. More of the Earth's surface is covered by wheat than by any other food crop. Wheat is a hardy crop that can grow in a wide range of climatic conditions and that permits large-scale cultivation and long-term storage. If *A. tritici* is introduced into non-native areas, it is likely to establish wherever wheat is grown.

1.12.2 Host range

Primary hosts of *A. tritici* include wheat, rye, spelt and emmer wheat. Oats, barley, durum wheat and rivet wheat are considered minor hosts.

1.12.3 Pest dispersal potential

Reproduction of *A. tritici* is amphimictic. Mating occurs and a single female produces up to 30,000 eggs over several weeks. These eggs hatch and produce second-stage juvenile (J_2s), which remain within the galls as the survival stage perpetuates as the invasive stage in the following years. Dry galls are harvested along with the healthy grains, and each gall may contain thousands of J_2 (Subbotin and Riley, 2012). *A. tritici* produces one generation per year (Ferris, 2013). It is likely to enter new areas through the transport of infected host plants or seed material, since wheat is an important commodity for import and export and its international trade is allowed in many countries, some of which are known to have *A. tritici* (USDA, 2015). Because of its high reproduction and dispersal potential, *A. tritici* receives a high rating in this category.

1.12.4 Economic impact

Seed gall nematodes can cause severe crop losses to wheat (70%) and rye (35–65%) in underdeveloped and developing countries due to poor agricultural practices, monoculture and use of seeds from the previous season or non-certified seeds (Nandal *et al*., 2010). Growth in global wheat consumption has been facilitated by imports, particularly into developing countries, including many tropical non-wheat-producing countries and those where increasing land and water constraints hinder wheat cultivation (FAOSTAT, 2019). Introduction of seed gall nematodes into developed countries may cause insignificant damage to the wheat and rye, due to the use of modern agricultural production systems in these countries. However, the introduction can severely hamper the export of wheat grains to international markets, due to the presence of the nematode in production areas. The interaction of this nematode and *C. tritici* results in *tundu* or spike blight or yellow ear-rot disease (Ferris, 2013). *Dilophospora alopecuri* in association with *A. tritici* could result in dilophosphorosis disease of wheat where the spike is covered by a black sticky mass. Since the nematode directly affects the crop produce (grain), it could lower crop yield as well as crop value (including increasing crop production costs) and could trigger the loss of markets (including quarantines). Further, the nematode can vector another pestiferous organism and can be injurious or poisonous to agriculturally important animals.

1.12.5 Environmental impact

A. tritici infestation is likely to cause ear cockle disease in all wheat-growing areas and could trigger additional official or private treatment programmes. To prevent ear cockle and the spread of this nematode species, growers would require seed cleaning and crop rotation to eliminate the pest. Hot-water treatments would be needed to eradicate *A. tritici* from seed lots or mechanical separation of nematode galls from the healthy grains using sieves or brine solution (PKB, 2016). Additionally, infestations of *A. tritici* could significantly impact irrigation, ornamental plantings and other cultural practices.

1.12.6 Global trade and nematode interceptions

The nematode has been found in shipments of wheat seed being traded as a food commodity. Shipments from infested countries are assumed to be of serious concern. Introduction of the nematode species into new areas can likely occur in seeds that pass inspection, because they may be infested with low numbers of nematode inoculum and not transformed into typical galls. The nematode has an invasive nature and alone is responsible for causing ear cockle disease, but in association with the bacterium *Clavibacter tritici* it causes yellow ear rot or *tundu* disease. Both these diseases, being seed-borne in nature, can be easily detected and controlled. The dispersal of the nematode from one country to another is through the transportation of infected seeds, straw from an infected crop, or manure of cows, sheep, sparrows, pigeons, etc. fed on the cockle grains.

1.13 Invasiveness rating

Considering the consequences of introduction and pest risk analysis of *A. tritici*, the following invasiveness rating has been assigned.

Climate/host interaction	–	High (3)
Host range	–	Medium (2)
Dispersal potential	–	High (3)
Economic impact	–	High (3)
Environmental impact	–	High (3)
Post-entry distribution	–	Medium (–2)
Final rating (score)	–	High (12)

Based on the above rating, *A. tritici* receives a score of 12 out of 15 and is rated as HIGHLY INVASIVE NEMATODE.

1.14 Region-wise status of invasiveness

A. tritici is in the 'Harmful Organisms' list for Argentina, Brazil, Chile, Colombia, Ecuador, Egypt, Guatemala, Indonesia, Israel, Madagascar, Namibia, Nepal, New Zealand, Paraguay, Peru, South Africa, Taiwan, Thailand, Timor-Leste, and Uruguay (USDA-PCIT, 2017). Based on the worldwide geographical distribution of *A. tritici* and likely introduction in the major wheat-producing countries where it has so far been absent, the region-wise status of invasiveness is evaluated (for Regional Plant Protection Organizations, see Chapter 16, section 112). Region-wise top wheat-producing countries where *A. tritici* attains invasive status are:

COSAVE: Argentina, Brazil
EPPO: Belarus, Belgium, Czech Republic, Kazakhstan, Morocco, Turkmenistan, Uzbekistan
IAPSC: Algeria
NAPPO: Canada, Mexico, USA.

1.15 Management measures

1.15.1 Cultural methods

Since both the pathogens (*A. tritici* and *C. tritici*) are seed-borne in nature, use of cockle-free seeds is the best approach to control the disease (Nandal *et al.*, 2010). Seeds can be cleaned and cockles can be separated from healthy seeds using mechanical and physical methods, or healthy seeds can be procured from seed agencies. Sukul (1970) reported decreased incidence of disease caused by *A. tritici* by growing wheat along with *Polygonium hydropiper*. Inter-culturing of mustard (*Brassica campestris*) and rapeseed (*B. juncea*) with wheat also showed a remarkable decrease in the diseases caused by *A. tritici* and improved wheat yield (Verma, 1991). Singh *et al.* (2009) studied the effect of sowing dates on the incidence of ear cockle disease of wheat and reported that delay in sowing by 35 days from November onwards increased the incidence of disease in most of the cultivars of wheat.

1.15.2 Mechanical methods of separation of cockles

A method of separation that is common in certain parts of India and China is to use a winnowing basket in an open area or in front of a fan. The contaminated seeds (cockles), being lighter in weight, fall away from the heap of healthy grains and can be separated. The efficiency of removal of galls by this method depends upon the velocity of wind at the time of winnowing. An alternative method is screening infested grains by putting them on a sieve (3 mm size) and rotating sidewise for some time. The galls, being smaller than the healthy seeds, fall down while the healthy seeds are retained on the sieve. Again, complete removal of galls is not achieved in this method, because some large-sized cockles are retained on the sieve along with the healthy grains (Paruthi *et al.*, 2009).

1.15.3 Physical methods of separation of cockles

WATER FLOTATION This method is based on putting the nematode-infested wheat seeds into a drum containing water and stirring it well with a wooden stick or by hand for some time. The galls being lighter in weight than the healthy grains start floating on the surface of the water and can then be removed by ordinary sieves. The cockles should be removed as early as possible, because they start sinking along with the seed after absorbing water.

SALT SEDIMENTATION Another method of removing the cockles from the grains is putting the seed in different concentrations of salt solution. Addition of salt increases the range and duration of floating by the cockles, hence they are efficiently removed from healthy seeds. Complete removal of cockles from the grains can be achieved at or above 10% concentration of salt. This method is more efficient than the water flotation method but is less often adopted because the treated seeds need to be washed in water several times before sowing, to avoid the injurious effect of salt on germination of seeds (Paruthi and Bhatti, 1992).

HOT-WATER TREATMENT The hot-water method is based on the principle that animal tissue is slightly more sensitive to temperature (45–55°C) than the plant tissue. Hence immersing the nematode-infested wheat seeds in hot water at 54–56°C for 10–12 min or 50°C for 30 min or 30°C for 4 h effectively killed *A. tritici* larvae inside the galls (Nandal *et al.*, 2010). Although this method has been found very effective, it could not be used in farmers' fields due to the involvement of several technical difficulties in maintaining the temperature of the water, and duration of exposure.

MICROWAVE TREATMENT A few researchers have explored the feasibility of using the energy of microwaves to kill J_2 of *A. tritici* inside the gall. Khan and Hayat (1999) exposed water-soaked and unsoaked wheat seeds and galls to microwaves at 2450 MHz/sec for 4–30 s. The exposure for 14 s onwards induced 40–76% and 20–48% mortality in the juveniles from soaked and unsoaked seeds, respectively.

RESISTANT VARIETIES The use of resistant varieties is one of the most economical and feasible methods of controlling the disease. A large number of varieties and lines have been screened

by various workers without much success (Khan and Athar, 1998). However, some lines and varieties, such as Saberbeg (Saleh and Fattah, 1990) and AUS 15854 (Baheti and Verma, 2001), have been reported to be resistant against this nematode.

1.15.4 Chemical control

Limited information is available on chemical control of *A. tritici*. Spraying of ethyl parathion at 0.05% provided complete control of *A. tritici* on wheat (Singh and Prasad, 1972). Foliar application of monocrotophos on 1-month-old wheat plants at 1 kg/ha led to the recovery of plants from nematode attack and improved the yield of commonly grown varieties of wheat by 46% (Khan *et al.*, 1998). Soil application of carbofuran at 1 kg a.i./ha after 50 days of sowing resulted in 18.3% disease incidence caused by *A. tritici* in comparison with 46.7% in an untreated check (Paruthi *et al.*, 2009).

1.15.5 Phytosanitary measures

Ear cockles are the only source for the perpetuation of the disease and their removal from contaminated seed lots can completely eradicate the disease (Luc *et al.*, 1990). *A. tritici* has been eliminated, or reduced to a minimal number of infestations, in Europe and the USA by seed cleaning, crop rotation and fallow (Brown, 1987). Selective quarantine should be imposed in regions of the world where the disease is not under control. For further details on International Sanitary and Phytosanitary Measures (ISPMs), see Chapter 16 (International Regulations), section 115.

2 *Anguina agrostis*

2.1 Common name

Bentgrass nematode

2.2 Scientific name

Anguina agrostis (Steinbuch, 1799) Filipjev, 1936

2.3 Synonyms

Anguillula agrostidis (Steinbuch, 1799) Warming, 1877
Anguillula agrostis (Steinbuch, 1799) Ehrenberg, 1838
Anguillula phalaridis (Steinbuch, 1799) Ehrenberg, 1838
Anguillulina agrostis (Steinbuch, 1799) Goodey, 1932
Anguillulina phalaridis (Steinbuch, 1799) Goodey, 1932
Anguina phalaridis (Steinbuch, 1779) Chizhov, 1980
Anguina poophila Kiryanova, 1952
Anguina lolii Price, 1973
Tylenchus agrostidis Bastian, 1865
Tylenchus agrostis (Steinbuch, 1799) Goodey, 1930
Tylenchus phalaridis (Steinbuch, 1799) Orley, 1880
Tylenchus phlei Horn, 1888
Vibrio phalaridis Steinbuch, 1799
Vibrio agrostis Steinbuch, 1799

2.4 EPPO code

ANGUAG

2.5 Diagnosis

2.5.1 Morphological diagnosis

Important morphological descriptions of *Anguina agrostis* after Southey (1973) and Krall (1991) are summarized below. For measurements, see Table 3.

Mature female: Based on specimens from the type host. Body obese, C-shaped to spirally coiled ventrally. Lip region low and flattened, offset by a fine constriction. Cuticle marked by fine annulations. Lateral fields not discernible on fully developed adult females, six incisures visible on immature specimens. Neither procorpus nor isthmus exhibiting marked swellings, the former slightly contracted at junction with metacorpus. Isthmus occasionally folded in mature specimens by forward pressure of gonad. Pharyngeal glands not overlapping, or with very slight overlap of intestine. Vulval lips prominent. Ovary usually with two flexures, often reaching to pharyngeal region with oogonia in multiple rows arranged around a rachis. Spermatheca pyriform, separated from oviduct by a constriction. Postvulval uterine sac present, 36–63% of vulva–anus distance. Tail conoid with acute terminus (Fig. 1.4A).
Male: Smaller and slenderer than female. Habitus upon heat relaxation curved ventral to almost straight. Lateral field difficult to discern on mature specimens, reported to have six incisures. Testis usually reflexed once, spermatocytes arranged about a rachis. Spicules slenderer in build than those of *A. tritici*, capitulum showing little or no ventral folding at anterior. Two ventral ridges running from tip of each spicule to widest part, before converging and joining capitulum. Gubernaculum simple, bursa leptoderan, ending just short of acute or finely mucronate tail tip (Fig. 1.4B).
Second-stage juvenile: Body slender, not spirally coiled; tail conoid, pointed.

2.5.2 Biochemical and molecular diagnosis

Morphologically *A. agrostis* can be easily differentiated from *A. tritici* but not from *A. funesta*. To distinguish *A. agrostis* from *A. funesta*, biochemical diagnosis using isozymes (Riley *et al.*, 1988) and molecular analyses (Powers *et al.*, 2001; Li *et al.*, 2015) are more reliable. For details, see section 1.5.3.

2.6 Geographical distribution

A. agrostis is worldwide in distribution and has been reported from different countries on bentgrass, especially in the Pacific

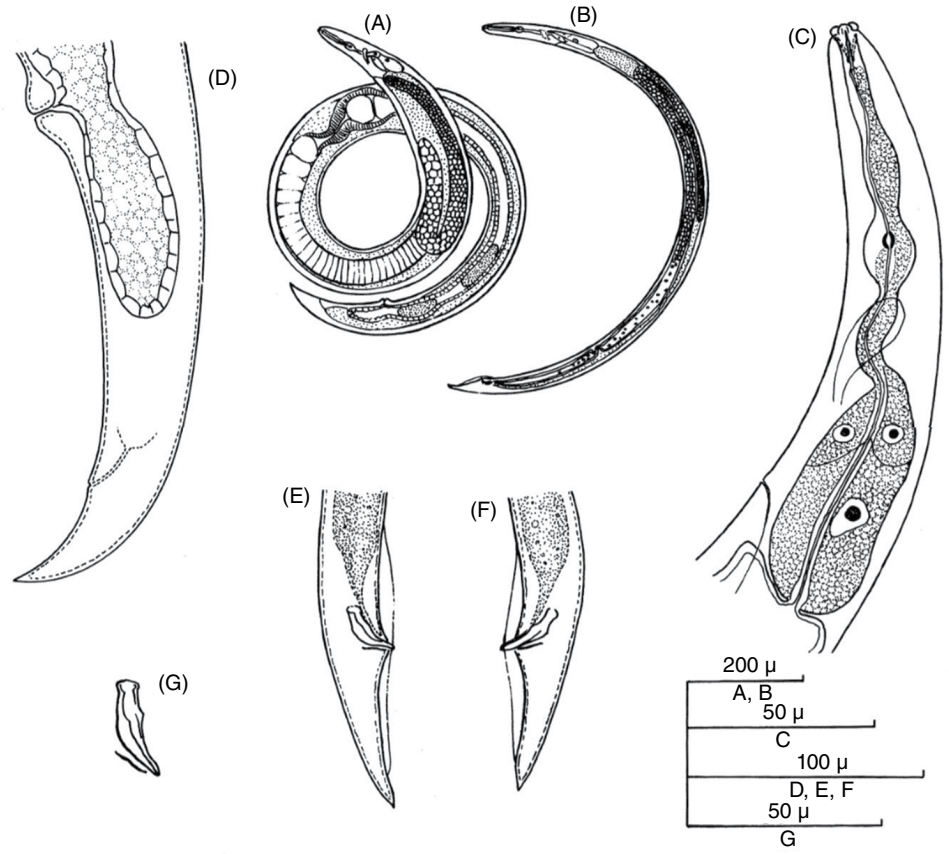

Fig. 1.4. Line drawing of *Anguina agrostis*. Adult female (A); adult male (B); head and oesophageal region of female (C); tail of female (D); bursa (E,F); spicule (G). [Reproduced from Southey, 1973, CIH Descriptions of Plant-parasitic Nematodes, Set 2, No. 20; courtesy CAB International]

Northwest USA and New Zealand. The nematode is known to occur in the following countries (CABI-CPC, 2010).

Africa: South Africa
Asia: China, Georgia, Kyrgyzstan
Europe: Czech Republic, Estonia, Finland, Germany, Ireland, Netherlands, Norway, Poland, Russian Federation, Slovakia, Sweden, Ukraine, United Kingdom
North America: Canada, USA
Oceania: Australia, New Zealand

2.7 Host plants

A. agrostis is considered to be a species complex with pathotypes differing in host range (Krall, 1991; Brzeski, 1998). Subbotin *et al*. (2004) supported the concept of narrow specialization of seed gall nematodes, concluding that *A. agrostis* occurs in only one host, *Agrostis capillaris*, and that other *Agrostis* species are hosts for two further undescribed species of *Anguina*. The type host of *A. agrostis* is *Agrostis tenuis*. In addition to various bentgrass species like *Agrostis canina* (velvet bentgrass), *Agrostis capillaris* (brown bentgrass), *Agrostis exarata* and *Agrostis stolonifera* var. *palustris* (bentgrass). *Anguina agrostis sensu lato* has been reported from other grass genera, including *Bromus erectus* (upright brome grass), *Dactylis glomerata* (orchard grass), *Festuca nigrescens* (Chewings fescue), *Festuca ovina* (sheep's fescue), *Lolium rigidum* (rigid ryegrass), *Phleum boehmeri*, *Phleum phleoides*, *Phleum pratense* (timothy), *Poa alpina*, *Poa annua* (annual meadowgrass), *Poa pratensis* (smooth-stalked meadowgrass) and *Trisetum flavescens* (yellow oatgrass).

2.8 Symptoms

A. agrostis causes no obvious symptoms on grass shoots except that of the inflorescence, which is so distorted that the parasitized plants were once described as a new plant species, *Agrostis sylvaticus*. In grasses, seed galls are difficult to detect as they are covered by lemmas and paleae (Fig. 1.3G). A small scarifier can be used to remove lemmas and paleae without damage to seeds or galls. This allows visual identification of galls (Alderman *et al*., 2003). Galled flowers have glumes of two or three times the normal length, lemmas five to eight times the normal length, projecting beyond the glumes as a

sharp point, and paleae developing to about four times the normal length. Lodicules, stamens and sometimes other flower parts are suppressed in such parasitized flowers (Goodey, 1932). Galls are at first greenish and later become dark purple-brown. Seed galls containing the nematodes are dark brown (Fig. 1.3H). They may look similar to normal seeds but are less heavy and hence can be separated mechanically from them. Adult nematodes (one to three females, one to three males) are found within these galls. Later, the females lay eggs and die, the eggs hatch and the second-stage juveniles fill the gall, which then turns into a seed gall.

2.9 Biology and life cycle

Reproduction is amphimictic and females and males mate within the gall. The female lays as many as 1000 eggs. Shortly after oviposition, the females and males die and disintegrate. The eggs are embryonated and the first-stage juveniles moult within the egg. The second-stage juveniles hatch and aggregate and undergo quiescence as the gall dries off (Bird and Stynes, 1981). The optimum temperature for egg development, from laying to hatching, is 18–20°C. The minimum time recorded for embryogenesis to hatching is 9–10 days. Embryogenesis is inhibited at temperatures of 27°C and above and hatching at temperatures greater than 23°C. Second-stage juveniles hatch and invade young grasses early in May (Europe) or at the end of August (Australia). The juveniles migrate to the inflorescence and incite seed galls. Only one generation completes in a year (Bird and Stynes, 1981).

In dry galls, the second-stage juveniles remain viable for up to 10 years. During rains, galls are softened and the juveniles escape (Southey, 1974). When the galls are soaked in water, the mucoprotein in which the juveniles are embedded swells, causing the walls of the gall to rupture, leading to expulsion of the juvenile mass. The second-stage juveniles feed ectoparasitically on the growing points of the shoots of their host grasses until the plant begins to form its inflorescence. The larvae enter young flower tissues, invade developing ovules and quickly moult three times to become adults. The ovary of each flower is thus replaced by a gall which contains one to three adult females and about as many males, although sometimes only a single individual is found in a gall. In the Pacific Northwest USA, the life cycle is completed in 3–4 weeks (Bird and Stynes, 1981).

2.10 Interactions with other pathogens

The nematodes sometimes introduce the bacterium *Corynebacterium rathayi* into the galls induced by *A. agrostis* in annual ryegrass (*Lolium rigidum*), which subsequently become toxic to grazing animals (Bird and Riddle, 1984). The bacterium *C. rathayi* is carried on the cuticle surface of the nematodes. Dauer juveniles of *A. agrostis* are not attracted to *C. rathayi* (from bacterial galls), but on contact, the bacteria attach to the nematode cuticle surface (Bird and Riddle, 1984). Shaw and Muth (1949) reported that in Oregon, USA, cattle, horses and sheep developed symptoms of poisoning such as nervous disorders, muscle trembling and lack of coordination, sometimes resulting in death, when they had fed on *Festuca nigrescens* infested with *A. agrostis* galls.

2.11 Economic impact

Bentgrass represents the most widely used grass for golf-course putting greens, gardens, etc. *A. agrostis* is considered to be a serious and potentially important nematode pest of bentgrass, especially in the Pacific Northwest USA and New Zealand (Bird, 1985). Samples from Oregon, USA, during 1996–2000 recovered 35–50% of orchard grass seeds containing *Anguina* galls and it caused 75% reduction in grass seed production. Fresh galls of *A. agrostis* from Chewing fescue (*Festuca rubra*) are poisonous to cattle and sheep (Apt *et al*., 1960).

Galls induced by this nematode in annual ryegrass *Lolium rigidum* in Western Australia replace the seeds and the nematode sometimes introduces *Rathayibacter rathayi* into the galls (Riley and McKay, 1990). These subsequently become toxic to grazing animals. Seed galls containing the nematodes are dark brown and those colonized by *R. rathayi* are yellow. Walls of the former are twice as thick as those of the latter. Both types of gall are originally initiated by the nematode (Bird *et al*., 1980).

2.12 Movement and means of dispersal

The primary means of dispersal is by seeds infested with seed galls/cockles. Other means of spread include the following.

2.12.1 Plant parts liable to carry the nematode in trade/transport

Plant parts including roots, flowers, inflorescences, cones, calyx, leaves, seedlings, micro-propagated plants, stems (above ground), shoots, tillers and true seeds (including grain) are liable to carry all stages (eggs, juveniles and adult) of the nematode.

2.12.2 Plant parts not known to carry the nematode in trade/transport

Bark and wood.

2.13 Pest risk analysis and looming threat from introduction

A. agrostis is considered as a serious and potentially important nematode pest of bentgrass (Southey, 1973). Due to its global distribution, wide host range and crop damage potential, the likelihood of introduction or spread of *A. agrostis* into new areas puts golf-course greens and gardens at risk. The chance of introduction is minimal, largely due to dispersal

potential, but the economic damage potential of this pest should not be underestimated.

2.14 Invasiveness rating

Considering the consequences of introduction and pest risk analysis of *A. agrostis*, the following invasiveness rating has been assigned.

Climate/host interaction	–	Medium (2)
Hosts range	–	High (3)
Dispersal potential	–	Low (1)
Economic impact	–	High (3)
Environmental impact	–	Medium (2)
Post-entry distribution	–	Low (–1)
Final rating (score)	–	Medium (10)

Based on the above invasiveness rating, *A. agrostis* receives a score of 10 out of 15 and is rated as MEDIUM INVASIVE NEMATODE.

2.15 Region-wise status of invasiveness

APPPC: Afghanistan, Armenia, Azerbaijan, India, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Pakistan, Saudi Arabia, Syria.
COSAVE: Argentina, Chile.
EPPO: Albania, Bulgaria, France, Greece, Italy, Morocco, Portugal, Spain, Switzerland, Turkmenistan, Uzbekistan, Yugoslavia (former).
IAPSC: Algeria, Egypt, Libya, Tunisia.

2.16 Management measures

2.16.1 Mechanical control

In infested areas, all harvesting machinery, including cutting and threshing machines, tractors, trucks and other equipment, including seed bags, clothing and boots of farm workers, should be cleaned to remove all trash and chaff before moving to clean areas. Mechanical separation to remove lighter infested galls removes most but not all galls from grass seed. Seed harvesting procedures can also minimize the disease, as indicated by the reduction of seed head galls in a *Trisetum flavescens* field from 8.2% to 0.11% by cutting for fodder in one year and taking the first seed harvest in the following year (Wagner and Domling, 1974).

2.16.2 Hot-water treatment

Hot-water treatment of infested seeds at 52°C for 15 min, after a 2 h pre-soak in tepid water, controls nematodes and reduces germination by only 5%. Hot-water treatment is effective only when the galls are 'naked', not covered by the inner paleae of the inflorescence (Christie, 1959).

2.16.3 Chemical control

Satisfactory control without serious effect on seed germination was obtained by fumigation of *Agrostis tenuis* seeds with methyl bromide (Hague, 1963).

2.16.4 Phytosanitary measures

See Chapter 16, section 115.

3 *Anguina funesta*

3.1 Common name

Seed gall nematode

3.2 Scientific name

Anguina funesta Price, Fisher and Kerr, 1979

3.3 Synonym

Anguina lolii Bird and Stynes, 1977

3.4 EPPO code

ANGUFU

3.5 Diagnosis

3.5.1 Morphological diagnosis

Important morphological descriptions of *Anguina funesta* after Price *et al.* (1979) are summarized below. For morphometric measurements, see Table 3.

FEMALE: Young females are fully motile, but older females, in which gross expansion of the ovary has occurred, are strongly ventrally curved and capable of only weak movements of head and tail. Habitus following heat relaxation ventrally curved, forming a complete circle, with head and tail overlapping (Fig 1.5B). Lips slightly offset and rounded in front, cephalic framework lightly cuticularized. Stylet length 7–10 µm with conus and shaft of roughly equal length and with well-developed knobs. Pharynx 64–178 µm long with a wide procorpus opening to a muscular metacorpus, ovate to spheroid, 17–25 µm long. Pharyngeal–intestinal junction obscured by large dorsal gland, 45–72 µm in length, ovate to spatulate with a prominent nucleus. Pharyngeal glands slightly overlapping the intestine. Hemizonid between base of metacorpus and anterior end of dorsal pharyngeal gland, at 80–100 µm from anterior. Excretory pore located more posteriorly, 105–155 µm from anterior. Lateral field difficult to discern. Vulva with prominent lips. Anterior ovary with

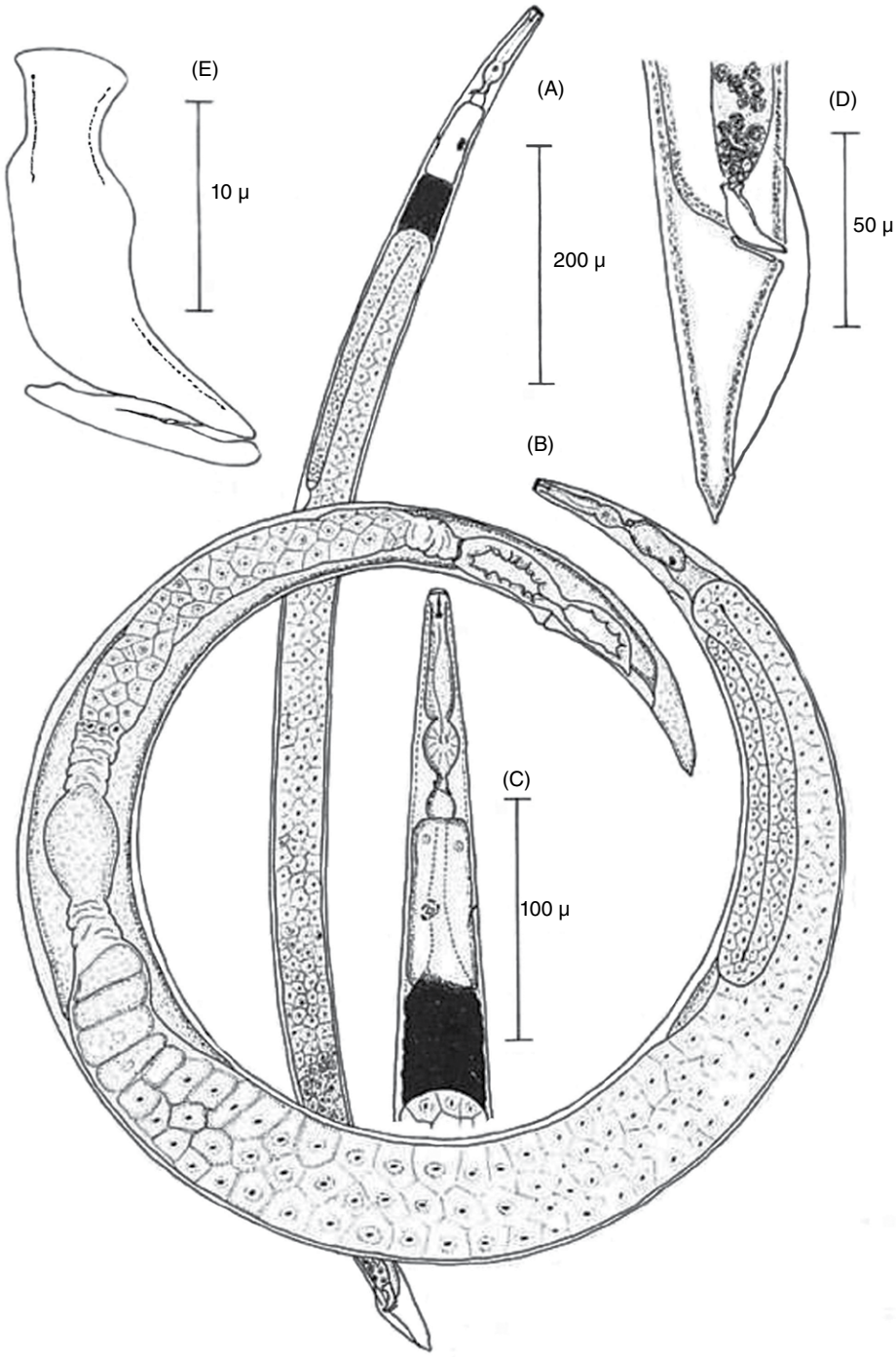

Fig. 1.5. Line drawing of *Anguina funesta*. Adult male (A); adult female (B); male anterior (C); male tail (D); and spicule and gubernaculum (E). [Price *et al.,* 1979; courtesy Nematologica]

one or two flexures. Rarely, gonad not reflexed but rather extended anteriorly to base of procorpus. Oocytes arranged in three or four rows about a rachis, except near base of ovary where these increase to five or six rows. Spermatheca ovate or elongated, long and 25–40 µm wide. Crustaformeria long and slender, made up of more than four columns of cells and up to 350 µm long, separated from spermatheca and uterus by short constrictions, 10–15 µm long. Crustaformeria often containing sperm cells and up to eight eggs. Uterus thick-walled, 70–100 µm long. Postvulval uterine sac approximately same length as uterus, 62–112 µm. Tail 48–112 µm long, vulva–anus distance 86–73 µm. Body width at anus approximately half that at vulva. Tail occasionally bluntly rounded, more usually conically pointed, sometimes with mucronate tip (Fig. 1.5B).

Male: Shorter and thinner than female (Fig. 1.5A). Habitus upon heat relaxation straight or slightly curved. Lip and pharyngeal regions of male similar to that of female, although dorsal pharyngeal gland larger, almost rectangular, 50–78 µm long (Fig. 1.5C). Hemizonid and excretory pore 71–90 µm and 102–147 µm from anterior, respectively. Lateral field with five or six incisures, broken at intervals and occupying one-quarter to one-third of body width. Testis nearly always reflexed once. Testis with spermatocytes in multiple rows about a rachis. Spicules paired, non-fused and arcuate, each 16–28 µm long with characteristic bulges on manubrium and where manubrium and shaft join. Gubernaculum slim and trough-like (Fig. 1.5D). Bursa leptoderan, extending almost to tail tip, 44–114 µm in length. Tail 43–72 µm long with terminus conically pointed (Fig. 1.5C). Body width at cloaca 17–43 µm.

3.5.2 Biochemical and molecular diagnosis

Morphologically *A. agrostis* and *A. funesta* are similar and *A. funesta* was considered a junior synonym of *A. agrostis*. However, despite their morphological affinities, these two species can be differentiated by biochemical diagnosis using isozymes (Riley *et al.*, 1988) and molecular analyses (Powers *et al.*, 2001; Li *et al.*, 2015). For details, see section 1.5.3.

3.6 Geographical distribution

A. funesta was reported from Australia (Price *et al.*, 1979) and has been detected in Oregon, USA (Meng *et al.*, 2012).

3.7 Host plant

The principal host of *A. funesta* is annual ryegrass *Lolium rigidum*.

3.8 Symptoms

The nematodes congregate near the apical meristems until ovary initiation, then stimulate ovary primordia to develop into galls. Occasionally, galls are produced in stamen primordia or, in very heavily infested plants, on glumes or rachis (McCay and Ophel, 1993). *A. funesta* is recorded as a vector of *Rathayibacter toxicus*, which causes the disease annual ryegrass toxicity when consumed by livestock. Rangeland infested by the nematode and bacterium is unusable for grazing. Symptoms of infestation are shown in Fig. 1.3J-L.

3.9 Biology and life cycle

The life cycle of *A. funesta* is similar to that of *A. agrostis*. The second-stage juveniles (J_2) emerge from the seed galls in the soil and crawl on the newly germinated ryegrass seedlings. The J_2 become located between young leaves and penetrate flower buds at the time of flower bud initiation, where they stimulate gall formation in place of normal grains. Juvenile development is completed in the galls. Newly formed females deposit eggs, which hatch, producing J_2, which remain, encased in the galls, and perpetuate the plant infection in the following years (Price *et al.*, 1979). Dried galls are harvested with seeds. During dry summers, *A. funesta* survives in the soil within these seed galls as anhydrobiotic second-stage juveniles. During winter, the nematodes are released from decaying galls and, via water droplets in moist soil, they invade new host seedlings, where they feed upon the young leaves. The nematodes congregate near the apical meristems until ovary initiation, then invade and stimulate ovary primordia to develop into galls. Occasionally, galls are produced in stamen primordia or, in very heavily infested plants, on glumes or rachis (McCay and Ophel, 1993).

3.10 Interactions with other pathogens

A. funesta is associated with a yellow slime bacterium, *Rathayibacter toxicus* (formerly *Clavibacter toxicus* and *Corynebacterium toxicus*). The J_2 can acquire bacterial cells from the soil or from infected plants. The bacterium is introduced in the plant tissue during seed gall formation. Such galls are fatally toxic to grazing livestock.

3.11 Economic impact

A. funesta is recorded as a vector of *R. toxicus*, which causes the disease annual ryegrass toxicity when consumed by livestock. Rangeland infested by the nematode and bacterium is unusable for grazing. Annual ryegrass toxicity is responsible for severe losses in the livestock industry in Australia (Price *et al.*, 1979).

3.12 Movement and means of dispersal

See section 2.12.

3.13 Pest risk analysis and looming threat from introduction

The nematode is a serious damaging pest for the livestock industry, pastures, rangeland and land for recreational uses. It is also of major regulatory significance. A complete pest risk assessment should be prepared for this nematode. The accidental introduction of this nematode would have devastating repercussions on grain exports to domestic and international markets because of the quarantines imposed against this pest by many countries.

3.14 Invasiveness rating

Considering the consequences of introduction and pest risk analysis of *A. funesta*, the following invasiveness rating has been assigned.

Climate/host interaction	–	Medium (2)
Hosts range	–	Low (1)
Dispersal potential	–	Low (1)
Economic impact	–	High (3)
Environmental impact	–	Medium (2)
Post-entry distribution	–	Not known (0)
Final rating (score)	–	Medium (9)

Based on the above invasiveness rating, *A. funesta* receives a score of 9 out of 15 and is rated as LOW INVASIVE NEMATODE.

3.15 Region-wise status of invasiveness

APPPC: Afghanistan, Armenia, Azerbaijan, Georgia, India, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Pakistan, Saudi Arabia, Syria.
COSAVE: Argentina, Chile.
EPPO: Albania, Bulgaria, France, Greece, Italy, Morocco, Portugal, Russian Federation, Spain, Switzerland, Turkey, Turkmenistan, Ukraine, Uzbekistan, Yugoslavia (former).
IAPSC: Algeria, Egypt, Libya, South Africa, Tunisia.
NAPPO: Mexico.

3.16 Management measures

Management measures, including phytosanitary methods, for *A. funesta* are similar to those for *A. agrostis*. For details, see section 2.16.

Ditylenchus Spp.

The genus *Ditylenchus* (Filipjev, 1936) contains a large number of species which are mostly myceliophagous. A few species are phytophagous, but are of great importance as they attack more than 1200 species of cultivated and wild plants (Sturhan and Brzeski, 1991). Over 60 species of *Ditylenchus* have been described (Siddiqi, 2000; Jeszke *et al.,* 2013) and important plant-parasitic species are:

Ditylenchus africanus Wendt, Swart, Vrain and Webster, 1995
D. angustus (Butler, 1913) Filipjev, 1936
D. destructor Thorne, 1945
D. dipsaci (Kuhn, 1857) Filipjev, 1936
D. humli Skarbilovich, 1972
D. gigas Vovlas *et al*., 2011
D. medicaginis Wasilewska, 1965
D. melongena, Bhatnagar and Kadyan, 1969
D. procerus (Bally and Reydon, 1930) Filipjev, 1936
D. sorghi, Verma, 1966
D. weischeri Chizhov *et al*., 2010

Among these, *D. africanus*, *D. angustus*, *D. destructor*, *D. gigas* and *D. dipsaci* are considered as highly invasive nematode species because of their economic importance and quarantine significance in various countries (Sturhan and Brzeski, 1991; Lal and Lal, 2006; Bora and Rahman, 2010; EPPO, 2013). These *Ditylenchus* species attack more than 450 higher plant species (Siddiqi, 2000; Chizhov *et al*., 2010; Vovlas *et al*., 2011). Other species are of lesser importance, having limited geographical distribution.

Systematic position of *Ditylenchus* species

	Classical (after Siddiqi, 2000)	Molecular (after Decraemer and Hunt, 2013)
Phylum	Nematoda	Nematoda
Class	Secernentea	Chromadorea
Subclass	Tylenchia	Chromadoria
Order	Tylenchida	Rhabditida
Suborder	Tylenchina	Tylenchina
Infra-order	Anguinata	Tylenchomorpha
Superfamily	Anguinoidea	Sphaerularioidea
Family	Anguinidae	Anguinidae
Subfamily	Anguininae	Anguininae
Genus	*Ditylenchus*	*Ditylenchus*

4 *Ditylenchus africanus*

4.1 Common name

Peanut or groundnut pod nematode

4.2 Scientific name

Ditylenchus africanus Wendt, Swart, Vrain and Webster, 1995

4.3 EPPO code

DITYAF

4.4 Diagnosis

The confounding species of *Ditylenchus* cannot be distinguished categorically from the target species based only on morphological characters. When indispensable, the distinction should be supported by molecular and biochemical identification of the species. The morphological, biochemical and molecular characters of *Ditylenchus africanus* are presented below.

4.4.1 Morphological diagnosis

Detailed morphological descriptions of *D. africanus* were given by De Waele *et al*. (1989) and Wendt *et al*. (1995) and are summarized below. For morphometric measurements, see Table 5.

Table 5. Morphometric data for *Ditylenchus africanus*, *D. angustus*, *D. destructor*, *D. dipsaci* and *D. gigas* (adapted from ISPM 27, DP 8).

Morphometric characters		Range, numerous populations				
		D. africanus (after Wendt *et al.*, 1995)	*D. angustus* (after Seshadri and Dasgupta, 1975)	*D. destructor* (after Goodey, 1952; Hooper, 1973a)	*D. dipsaci* (after Hooper, 1972)	*D. gigas* (after Vovlas *et al.*, 2011)
Females	Length (mm)	0.7–1.1	0.8–1.20	0.8–1.9	1.0–1.7	1.6–2.2
	Stylet (µm)	8–10	10–11	10–14	10–12	10.5–13.0
	a	–	50–62	32 (18–49)	62 ± 5.6	
	b	–	6–9	7 (4–12)	15 ± 1.4	
	c	8.8–16.9	18–24	17 (9–30)	11–20	15.7–27.6
	V%		78–80	80 (73–90)	80 ± 1.5	
	Post uterine sac/vulva–anus length (%)	37–85		53–90	40–70	About 50
	Lateral lines	6–15	4	6	4	4
	Tail terminus	Rounded	Conoid	Rounded	Pointed	Pointed to finely rounded
	Posterior bulb	Short, dorsally overlapping	Slightly overlapping	Short, dorsally overlapping	Not overlapping	Slightly overlapping
Males	Length (mm)		0.7–1.18	0.96 (0.76–1.35)	1.3 mm ± 0.017	
	Stylet (µm)		10	10–12		
	a		40–55	35 (24–50)	63 ± 11.3	
	b		6–8	7 (4–11)	15 ± 1.7	
	c		19–26	14 (11–21)	14 ± 2.1	
	Tail		60–73	65 (40–84)	72	
	Bursa length (as % of tail length)	48–66	–	50–70	40–70	72–76
	Spicules (µm)	17–21	16–21	24–27	23–28	23.5–28
Host	Host preference	Groundnuts and fungi	Rice	Higher plants and mycelia of fungi	Higher plants and fungi	Higher plants

Female: Flattened head, about 1.3 µm high and 6.4–7.3 µm wide, narrower than rest of body, but not offset. Labial area with pore-like stomal opening encircled by six outer labial papillae and two large medial lips, each with single pair of cephalic papillae. Outline of labial region with hexagonal head. Amphidial aperture oval, directed towards stoma opening. First annule of head discontinuous, instigated by position of amphidial apertures. Four lip annuli present in lip region, apart from labial disc. Stylet delicate with distinct knobs, detached, sloping backwards. Shaft long, around 60% of stylet length. Median bulb with crescentic valves and overlapping the intestine (Fig. 1.6A). Postvulval uterine sac long (50–143 µm), comprising about 8% of total body length or 37–85% of vulva–anus distance and equal to 1.5–3.7 times vulval body diameter. Tail conoid elongate, tapering in posterior one-third to finely rounded terminus (Fig. 1.6C).

Male: Male similar to female (Fig. 1.6B). Bursa long (33–60 µm), leptoderan, cover 48–66% of tail length. Spicule ventrally arcuate, cephalated slightly (Fig. 1.6D).

4.4.2 Biochemical diagnosis

One-dimensional gel electrophoresis was the first biochemical method to be applied in nematology, to distinguish *Ditylenchus* from *Panagrellus* (Benton and Myers, 1966) and four *Meloidogyne* species (Dickson *et al.*, 1970). Immunological approaches using polyclonal antibodies (pAbs) which identify multiple antigenic determinants in nematodes have been applied to the nematode diagnosis, including *Ditylenchus* spp. (Webster and Hooper, 1968; Abrantes *et al.*, 2004).

4.4.3 Molecular diagnosis

Molecular diagnosis using DNA-based approaches, such as microsatellites or single sequence repeats (SSRs), satellite DNA (satDNA), sequence characterized amplified regions (SCARs), PCR–single-strand conformational polymorphism (PCR–SSC) and DNA microarrays have been used to characterize different *Ditylenchus* spp. (Zouhar *et al.*, 2002; Abrantes *et al.*, 2004; Kerkoud *et al.*, 2007; Subbotin *et al.*, 2011b). Wendt *et al.* (1993 and 1995) used restriction fragment length polymorphisms (RFLPs) of ITS regions to separate *D. africanus*, *D. destructor*, *D. dipsaci*, *D. gigas* and *D. myceliophagus* by four restriction enzymes. Numerous adults or juveniles were used for DNA extraction using the method described by Webster *et al.* (1990; see Chapter 15, section 110.2.1). The ITS rRNA universal primers (Vrain *et al.*, 1992) used in this test are:

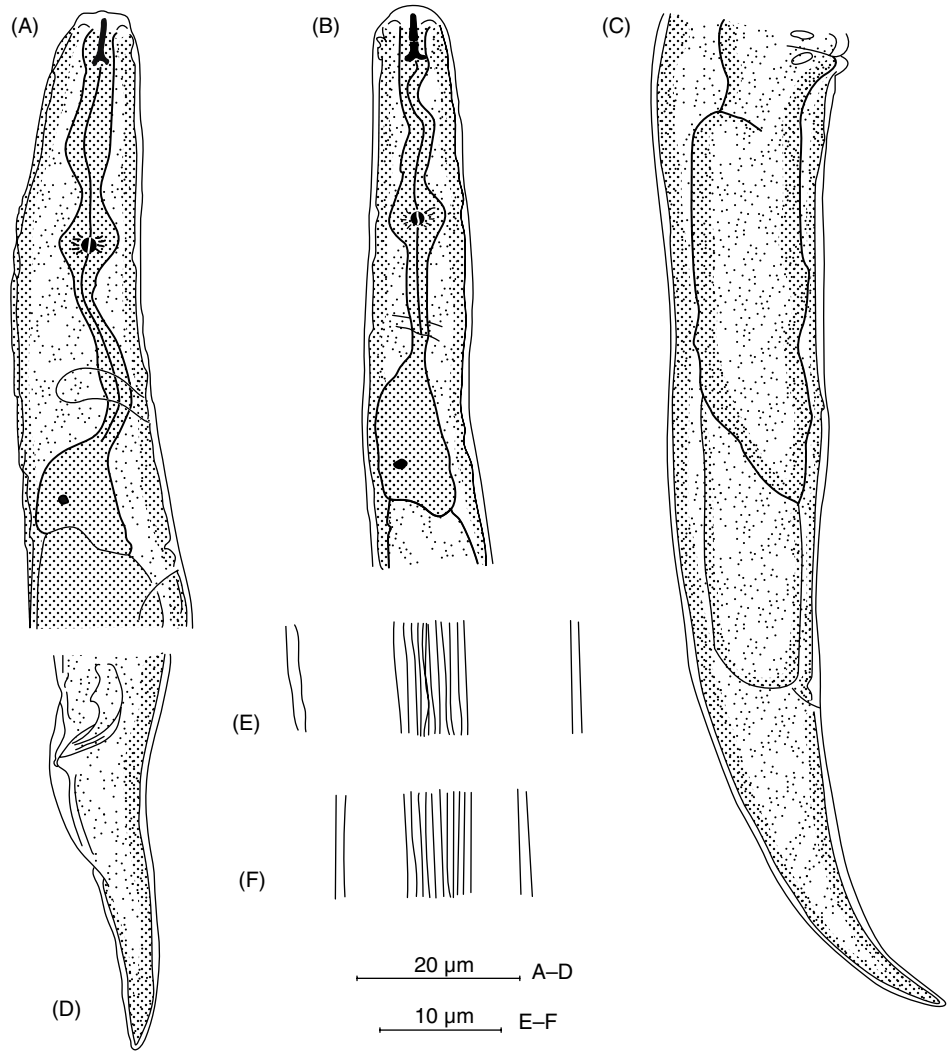

Fig. 1.6. Line drawing of *Ditylenchus africanus*. Anterior part of female body (A); anterior part of male body (B); posterior part of female body (C); male tail (D); female lateral field (E); male lateral field (F). [De Waele *et al.*, 1989; courtesy *Journal of Nematology*]

18S: 5′-TTG ATT ACG TCC CTG CCC TTT-3′
26S: 5′-TTT CAC TCG CCG TTA CTA AGG-3′

The PCR cycling parameters comprise the first cycle of 1.5 min at 96°C, 30 s at 50°C and at 72°C for 4 min; 40 cycles at 96°C for 45 s, at 50°C for 30 s and at 72°C for 4 min; and a final cycle at 96°C for 45 s, at 50°C for 30 s and at 72°C for 10 min. The 2–5 µl amplified DNA is analysed by 0.1% agarose gel electrophoresis. The amplicon size is 1000 base pairs (bp) for *D. africanus*, 900 bp for *D. dipsaci*, *D. gigas* and *D. myceliophagus* and 1200 bp for *D. destructor*. The approximate lengths (bp) of RFLP fragments of the ITS-rRNA generated by four restriction enzymes are presented in Table 6.

4.5 Geographical distribution

The peanut or groundnut pod nematode has limited distribution and has only been reported from South Africa (Wendt *et al.*, 1995) and Mozambique (CABI-ISC, 2020). In China, the reports of *D. destructor* from American ginseng (Zhang *et al.*, 2009) and *D. arachis* from groundnut (Zhang *et al.*, 2014) are possibly junior synonyms of *D. africanus* and both nematodes need to be further studied and validated.

4.6 Hosts

Groundnut (*Arachis hypogea*) is the principal host (Dickson and De Waele, 2005). Other agronomic crops and weeds are listed in Table 7.

4.7 Symptoms

On the below-ground parts of groundnuts, *D. africanus* does not cause any visible lesions. However, on roots, the infestation

Table 6. Approximate length (base pair) of RFLP fragments of the ITS-rRNA for *Ditylenchus* species generated by four restriction enzymes (adapted from ISPM-27, DP 8).

Species	Unrestricted PCR product	*Hae*III	*Hpa*II	*Hin*fI	*Rsa*I
D. africanus	1000	650, 540	950	450, 340, 150, 130, 100	690, 450
D. destructor	1200	450, 170	1000	780, 180	600, 250, 170
D. dipsaci	900	900	320, 200, 180	440, 350, 150	450, 250, 140
D. gigas	900	800, 200	600, 200	350, 150	490, 450
D. myceliophagus	900	450, 200	900	630, 310	900

Source: Wendt *et al*. (1993, 1995).

Table 7. Hosts of *Ditylenchus africanus* (adapted from CABI-ISC, 2020).

Main hosts
Peanut/groundnut (*Arachis hypogea*)
Other hosts
Glycine max (soybean)
Gossypium hirsutum (Bourbon cotton)
Helianthus annuus (sunflower)
Lupinus albus (white lupin)
Medicago sativa (lucerne)
Nicotiana tabacum (tobacco)
Phaseolus vulgaris (common bean)
Pisum sativum (pea)
Solanum tuberosum (potato)
Sorghum bicolor (sorghum)
Triticum aestivum (wheat)
Vigna unguiculata (cowpea)
Zea mays (maize)
Wild hosts
Chenopodium album (fat hen)
Datura stramonium (jimson weed)
Eleusine indica (goosegrass)
Tagetes minuta (stinking Roger)
Xanthium strumarium (common cocklebur)

affects appearance and sometimes reduces weight. The germination of the seeds is also reduced (Dickson and De Waele, 2005). Infected hulls exhibit greyish-black to brown necrotic tissue mainly at the point of connection with the peg, and broad bands are formed along the longitudinal veins that extend in the exocarp just beneath the pod surface (Dickson and De Waele, 2005). Infected seeds are usually shrivelled with dark brown to black micropyles and yellow to dark flaccid testae with dark vascular strands (Fig. 1.7A-C). The embryos may also become darkly discoloured (Jones and De Waele, 1990). Around 40–60% of the pods and seeds are destroyed in a heavily infested field (Dickson and De Waele, 2005).

4.8 Biology and life cycle

The biology of *D. africanus* coincides with the growth of the groundnut plant (Dickson and De Waele, 2005). The nematode usually survives in very low numbers in the soil on fungi, groundnut roots, alternative hosts and weeds, until the groundnut pegs appear in the soil (about 60 days after sowing). The nematode penetrates the young pod at its connection point with the peg, entering the exocarp and moving either longitudinally in the cell layer towards the pod beak-end and producing the pod discoloration, or through the mesocarp into the endocarp of the hull-shell (Venter *et al*., 1995). It then moves to the seed micropyle, from where it invades the seed testa and embryo and produces symptoms on the seeds. The nematode has not been recorded within the cotyledons of the seed (Jones and De Waele, 1990). The nematode multiplies within the pod and seed, and the life cycle from egg to adult is completed in 8 days at 25°C (De Waele and Wilken, 1990). At 28–30°C, egg hatching is initiated at around 3 days and by the 6th day 90% of eggs hatch (Dickson and De Waele, 2005). The nematode can enter into a state of anhydrobiosis, with around one-third of anhydrobiotic nematodes becoming active after rehydration to invade hulls and seeds of the newly planted crop (Dickson and De Waele, 2005). From 17 to 21 weeks after sowing (varying among cultivars), as the plant matures the relative number of eggs and anhydrobiotes in the pod and seed tissues increases (Venter *et al*., 1995).

The infective stage is unknown and the nematode can survive for at least 28–32 weeks in planting seeds, which may be symptomless, and in the field soil in the absence of the host plant, or in groundnut hulls buried in the soil (Basson *et al*., 1993). This period is long enough to survive the dry winter season in South Africa. With the first spring rains, the eggs hatch and anhydrobiotes rehydrate. Basson *et al*. (1993) showed that rehydrated soil populations of the nematode can invade the next groundnut crop and cause damage. Although comparatively few *D. africanus* survive in whole stored seed at 10°C, the surviving nematode population is also able to reproduce to levels capable of causing damage to the next crop (Basson *et al*., 1993).

4.9 Economic importance

D. africanus was first reported from south-western Transvaal province in South Africa in 1987 (De Waele *et al*., 1989). In South Africa, it was found in all the major groundnut production areas on about 75% of all fields (De Waele *et al*., 1989). Nematode infection causes serious qualitative damage and results in 65% of under-graded groundnut seeds in South Africa. In the greenhouse, the fresh weight of harvested seed was suppressed by 20–50% (Dickson and De Waele, 2005). A 1987 survey showed that 73% of the samples from 877 farms of the major groundnut production areas were infected with *D. africanus* (De Waele *et al*.,

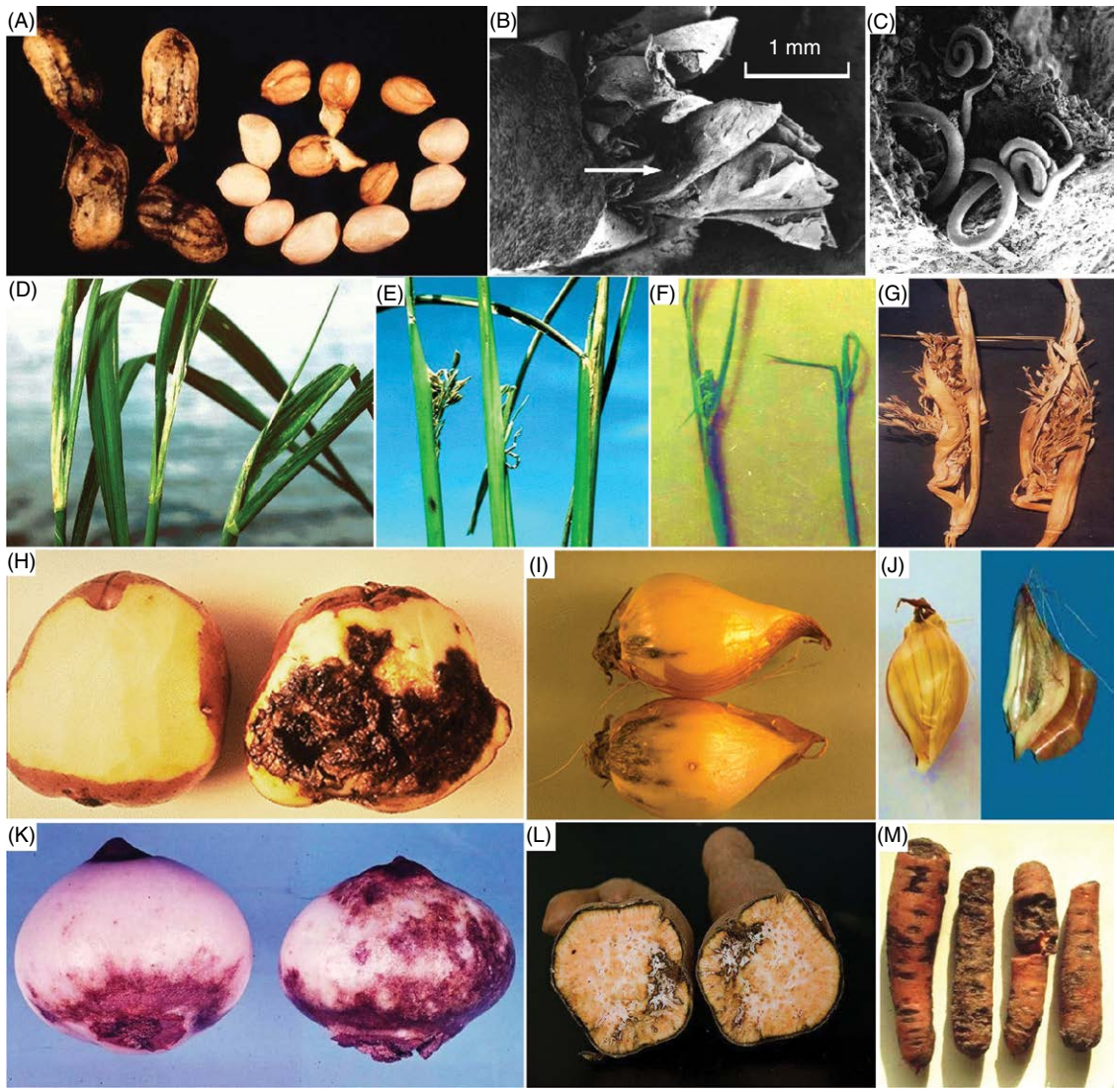

Fig. 1.7. Symptoms of *Ditylenchus africanus* on groundnut (A–C), *D. angustus* on rice (D–G) and *D. destructor* on different hosts (H–M). Nematodes on pods and seed of groundnut (A); SEM overview of *D. africanus* on the embryo of groundnut seed (B); close-up SEM (C); initial symptoms of *ufra* disease – white patches on the leaf bases (D); *ufra* symptom – partial emergence and distorted panicles due to nematode infection (E); twisting and loop formation (F); swollen *ufra* (G); infected potato tuber (H); infected iris rhizome (I); healthy and infected iris rhizome (J); healthy and infected tulip bulbs (K); infected sweet potato (L); infected carrots (M). [Photo courtesy A-C: ARC-Grain Crops Institute, South Africa; D,E: J. Bridge, CABI BioScience; F,G: NASI, India; H,M: Ephytia, INRAe; I: M.V. McKenry and P.A. Roberts, University of California; J: USDA; K: PPO Wageningen UR; L: : © 2007, Ian Riley, Adelaide, Australia]

1989). The average number of nematodes per seed was 160, with up to 3338 per seed in the south-western Transvaal area. Further, infestations of up to 97,000 nematodes per pod are not uncommon for groundnut grown in the field (Dickson and De Waele, 2005). The nematode can multiply to heavy infections (100,000 nematodes per pod), causing 100% losses to groundnut crops in some fields (Venter, 1994). In heavy infestations, the final nematode density exceeds 700 nematodes per seed and the seed mass may be reduced by up to 50%. Lower germination of seeds with 25% decrease in the number of harvestable seeds has been recorded (Venter *et al*., 1991).

4.10 Similarities to other species/conditions

Morphologically *D. africanus* is very similar to *D. myceliophagous*, *D. destructor*, *D. dipsaci and D. gigas* (see Table 5). Earlier, *D. africanus* was reported as *D. destructor* on groundnut/peanut in South Africa, later separated as *D. africanus* (Wendt *et al*., 1995). The histopathology of *D. africanus* resembles that of *Aphelenchoides arachidis* on groundnut. In South Africa, groundnut producers often confuse *D. africanus* symptoms with those of the fungus *Chalara elegans*. However, symptoms can be differentiated on the pattern of development of pod discoloration. The *D. africanus*-infected seeds of pods

have yellow to brown flaccid testae, frequently with darkened veins, and discoloured embryos, while *C. elegans*-infected seeds are not discoloured (Dickson and De Waele, 2005).

4.11 Movement and means of dispersal

The main means of dispersal is by seeds containing nematodes in trade and by the sowing of infected seeds in fields. Other means of spread include infected crop straw, flooding and rainfall, natural migration (maximum 20–30 cm), and sheep, cattle or bird manure.

4.11.1 Plant parts liable to carry the nematode in trade/transport

Plant parts including roots, flowers, inflorescences, cones, calyx, leaves, seedlings, micro-propagated plants, stems (above ground), shoots, tillers and true seeds, and fruits (including pods) are liable to carry all stages (eggs, juveniles and adults) of the nematode.

4.11.2 Plant parts not known to carry the nematode in trade/transport

Bark and wood.

4.12 Pest risk analysis and looming threat from introduction

D. africanus is a damaging pest in South Africa but is not present in any quarantine regions within the continent of Africa. This nematode is also reported from Mozambique. However, *D. africanus* can become a major economic pest if introduced in other major groundnut-producing countries, especially China, India, the USA and Sudan. Due to its limited distribution, the chances of introduction into other groundnut-producing countries are very limited. However, a complete pest risk assessment should be prepared for this nematode. Its accidental introduction would have devastating repercussions on groundnut exports to domestic and international markets; hence, strict quarantine regulation and extreme cautions should be taken in importing groundnut pods or seeds from Africa.

4.13 Invasiveness rating

Considering the consequences of introduction and pest risk analysis, the following invasiveness rating has been assigned to *D. africanus*.

Climate/host interaction	–	Medium (2)
Hosts range	–	High (3)
Dispersal potential	–	High (3)
Economic impact	–	High (3)
Environmental impact	–	Medium (2)
Post-entry distribution	–	Low (–1)
Final rating (score)	–	High (12)

Based on the above invasiveness rating, *D. africanus* receives a score of 12 out of 15 and is rated as HIGHLY INVASIVE NEMATODE.

4.14 Region-wise status of invasiveness

Region-wise, groundnut-producing countries where *D. africanus* is absent and attains invasive status are:

APPPC: Bangladesh, Cambodia, China, India, Indonesia, Iraq, Israel, Japan, Jordan, Kazakhstan, Kyrgyzstan, Lebanon, Malaysia, Myanmar, Pakistan, Philippines, Saudi Arabia, South Korea, Sri Lanka, Syria, Tajikistan, Thailand, Vietnam
CAHFSA: Barbados, Belize, Cuba, Dominican Republic, Haiti, Jamaica, Saint Kitts & Nevis
CAN: Bolivia, Colombia, Ecuador, Peru
COSAVE: Argentina, Brazil, Guyana, Paraguay, Suriname, Uruguay
EPPO: Bulgaria, Cyprus, Greece, Hungary, Italy, Morocco, Portugal, Spain, Turkey, Uzbekistan
IAPSC: Algeria, Angola, Benin, Botswana, Burkina Faso, Burundi, Cameroon, Cape Verde, Central African Republic, Chad, Comoros, Congo, Côte d'Ivoire, Egypt, Eritrea, Ethiopia, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Kenya, Liberia, Madagascar, Malawi, Mali, Mauritania, Mauritius, Namibia, Niger, Nigeria, Réunion, Rwanda, Senegal, Sierra Leone, Somalia, Sudan, Swaziland, Togo, Uganda, Zambia, Zimbabwe
NAPPO: Mexico, USA
OIRSA: Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua
PPPO: Australia, Fiji, Papua New Guinea, Tonga, Vanuatu

4.15 Management measures

4.15.1 Prevention and control

The spread of the pest can be stopped through the elimination of infection sources by burning off the infested groundnut (Dickson and De Waele, 2005). Timely harvesting also helps in the drying of the hulls and seeds before eggs are laid, and such hull stubble and seed for planting contain lower numbers of anhydrobiotic nematodes (Venter, 1994). Recently, the groundnut breeding line PC254K1 has been identified as resistant to *D. africanus* and has been included in the groundnut breeding programme (Steenkamp *et al.*, 2016).

4.15.2 Phytosanitary measures

See Chapter 16, section 115.

5 *Ditylenchus angustus*

5.1 Common name

Rice stem nematode; *ufra* disease

5.2 Scientific name

Ditylenchus angustus (Butler, 1913) Filipjev, 1936

5.3 Synonyms

Anguillulina angusta (Butler, 1913) Goodey, 1932
Tylenchus angustus Butler, 1913

5.4 EPPO code

DITYAN

5.5 Diagnosis

Morphological, biochemical and molecular diagnosis approaches of *Ditylenchus angustus* are presented below.

5.5.1 Morphological diagnosis

Detailed morphological descriptions of *D. angustus* were given by Seshadri and Dasgupta (1975). Additional morphological detail was provided by Mian and Latif (1995). For measurements, see Table 5.

Female: Slender body, nearly straight to ventrally arcuate when heat relaxed. Cuticle with fine annules, transverse striations about 1 μm wide at mid-body (Fig. 1.8G). Lip region low, flattened, not distinctly offset, unstriated, wider than high at lip base. Cephalic framework hexaradiate, (Fig. 1.8D) slightly sclerotized, *en face* view shows six lips of roughly equal size (Fig. 1.8C). Lateral fields with four incisures, outer incisures different than inner ones, one-fourth of body width or marginally less, extending almost to tail tip. Deirids present, nearly posterior to the level of excretory pore. Stylet moderately developed, conus reduced, around 45% of total stylet length, knobs small about 2 μm across but distinct, frequently with posteriorly sloping anterior surfaces, rather amalgamated with one another. Procorpus cylindrical, narrowing at median oesophageal bulb junction, 3–3.6 times as long as body width in that region. Median oesophageal bulb elliptical, with distinct valvular plate anterior to centre. Isthmus cylindrical, narrow, 1.5–1.9 times as long as procorpus; posterior oesophageal bulb usually clavate, ventrally overlapping the intestine, with three distinct gland nuclei. Cardia absent. Nerve ring conspicuous, behind median oesophageal bulb. Excretory pore slightly anterior to starting of posterior oesophageal bulb. Hemizonid present, anterior to excretory pore. Vulva with a transverse slit, vaginal tube slightly oblique, attaining more than halfway across body. Spermatheca extremely elongated, filled with large rounded sperms. Genital tract single anteriorly outstretched ovary, oocytes in single row, rarely in double rows. Post-uterine sac present, collapsed, without sperm, 2.5 times as long as vulval body width, extending about half to two-thirds distance to anus. Tail conoid, around five times anal body width in length, narrowing to sharply pointed terminus looking like a mucro (Fig. 1.8F). Phasmids pore-like, difficult to see, close behind mid-part of tail.
Male: Morphologically similar to females. Body approximately straight to slightly curved ventrally when heat fixed. Bursa present, but narrow in some specimens, starting opposite the proximal end of spicules and extending nearly to tail tip. Spicules simple, ventrally curved. Gubernaculum simple and short (Fig. 1.8I).
Juvenile: Morphologically similar to adults. Oesophagus proportionally shorter than adult's.

5.5.2 Biochemical diagnosis

Ibrahim *et al*. (1994b) used esterase and protein patterns to differentiate *D. angustus* from two new species of *Aphelenchoides* on rice. Each nematode species exhibited distinct protein bands and a characteristic esterase pattern. *D. angustus* showed two moderately strong protein bands at Rm = 0.63 and 0.65.

5.5.3 Molecular diagnosis

PCR–RFLP involving the amplification of the ITS region of the ribosomal DNA and restriction enzyme digestion has been used to differentiate *D. angustus* from an *Aphelenchoides* species and an undescribed *Aphelenchoides* population (Ibrahim *et al*., 1994a). For details of molecular characterization of *Ditylenchus* species, see section 6.5.3 below.

5.6 Geographical distribution

The rice stem nematode (RSN) has been reported to occur in South-East Asia (Bora and Rahman, 2010). Since the report by Butler (1913a) on occurrence of the nematode in Noakhali and Tippera of East Bengal (now Bangladesh), this nematode has been recorded in many other countries, including Bangladesh, India, Indonesia, Malaysia, Myanmar, Philippines and Thailand (CABI-ISC, 2020).

5.7 Hosts

Almost all the cultivated deep-water rice cultivars, including some of the transplanted rice varieties, are good hosts of *D. angustus* (Bora and Rahman, 2010). Thirteen species of *Oryza,* namely, *O. perennis*, *O. sativa* var. *fatua*, *O glaberima*, *O. cubensis*, *O. officinalis*, *O. meyaiana*, *O. latifolia*, *O. eichingeri*, *O. alta*, *O. minuta*, *O. nivera*, *O. rufipogon* and *O. spontanese* (Bridge *et al*., 2005a; Peng *et al*., 2018) are reported as host crops of *D. angustus*. Apart from *Oryza* spp., duckweed *Hygroryza aristata*, swamp rice grass *Leersia hexandra* (Miah and Bakr, 1977), *Paspalum scorbiculatum* (Pathak, 1992), *Sacciolepsis interrupta* and *Echinochola colona* (Cuc,1982) are recorded as alternative hosts of *D. angustus*. Four different fungi, namely *Curvularia oryzae*, *Epicoccum purpurascens*, *Pestalotia oryzae* and *Rhizoctonia solani*, can also harbour this nematode.

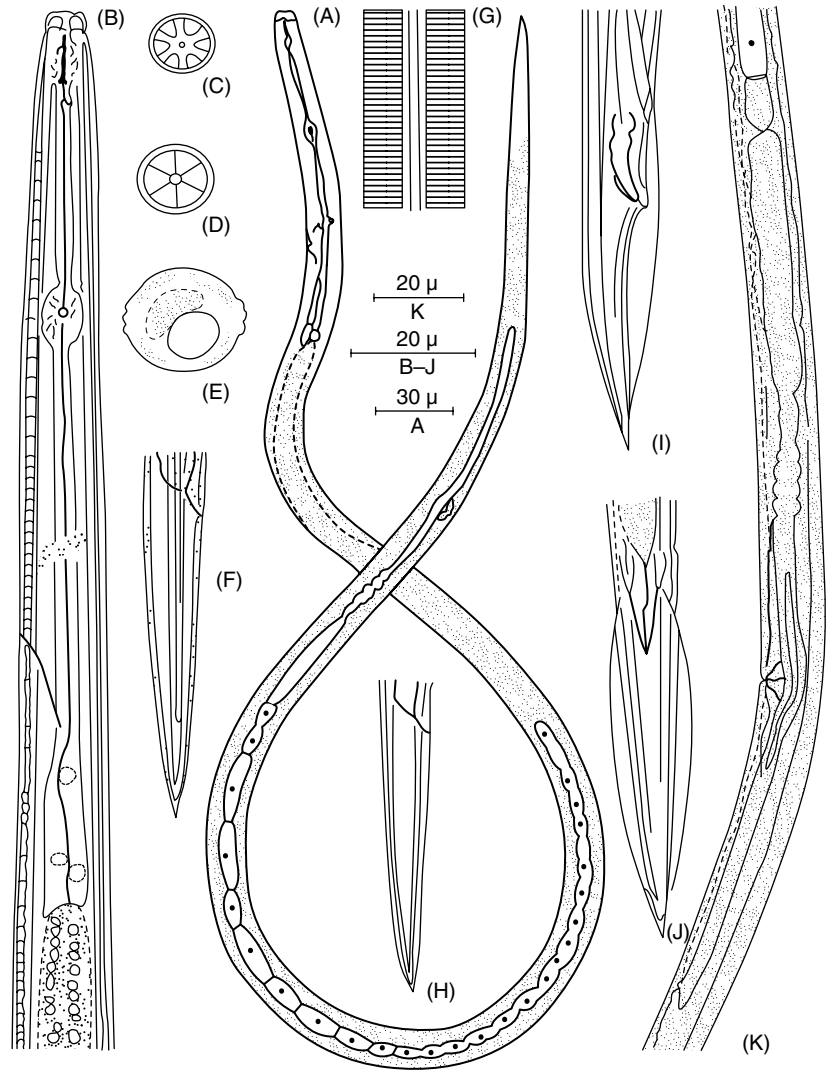

Fig. 1.8. Line drawing of *Ditylenchus angustus*. Whole female body (A); oesophageal region of a female (B); *en face* view (C); cephalic framework (D); cross-section of a female at mid-body (E); female tail (F); lateral field at mid-body (G); juvenile tail (H); lateral view of a male tail (I); ventral view of a male tail (J); vulva region of an adult female (K). [Source: Nemaplex, http://nemaplex.ucdavis.edu/, H. Ferris]

5.8 Symptoms

RSNs cause characteristic symptoms of *ufra* which vary considerably with the degree and time of infestation. The infection starts from the early seedling stage but it is difficult to recognize the nematode attack at this stage. In seedling stages, the first visible symptom appears as mosaic-like discoloration arranged in a splash pattern, or chlorosis or white streaks on young leaves and sheaths; becoming more evident with time. The entire leaf may become twisted or severely malformed. In some cases, the basal portion of the young leaf becomes wrinkled, following whitish-green discoloration (Bora and Rahman, 2010). Gradually the chlorotic portion becomes brown to dark brown with the advancement of the disease. In severe cases, the leaf margins may crinkle and the leaf tip is twisted. Often, the central leaf may emerge from the side of the stem by forming a loop or the central leaf may be curly, and later it becomes dried (Ou, 1985). Sometimes several branches are produced from the infested node which make the plant bushy. Sometimes the symptoms are masked and the plant looks healthy. Before the heading stage, plants remain symptomless. Most typical and conspicuous symptoms, which again vary considerably, are seen at the heading stage of the crop.

Butler (1913b) classified the disease into two types: *thor ufra* or swollen *ufra*; and *pucca ufra* or ripe *ufra*. Swollen *ufra* develops when a large number of nematodes are present which damage the panicle at an early stage; the panicle coils and remains enclosed within the leaf sheath (Fig. 1.7D-F). Ripe *ufra* represents the secondary or tertiary infestation where the nematode population is comparatively less, which damages the panicle at

a later stage; the panicle emerges but is distorted, bears sterile or empty grains at the base and produces normal grains only near the tip (Fig. 1.7G). Cox (1980) reclassified the symptoms into three types: *ufra* I, where the panicle remains completely enclosed within the leaf sheath (equivalent to swollen *ufra*); *ufra* II, where the panicle partially emerges and is weak, with unfilled grains (Fig. 1.7E); and *ufra* III, where the panicle emerges normally with partly filled grains near the tip (equivalent to ripe *ufra*). *Ufra* symptoms generally appear in patches in the field and expand with the advancement of crop age. Severely infected plants with *ufra* I symptoms are more often noticed at the centre of the patch than at the edges. In fields with severe *ufra* I and *ufra* II symptoms, yield may reduce to zero; whereas with *ufra* III, fields may produce some yield.

5.9 Biology and life cycle

The rice stem nematode *D. angustus* is an obligate ectoparasite and stubble-borne in nature. Infested plant residues are believed to be the primary source of inoculum. After harvesting of the infested crop, the nematode survives on wild rice, ratoons, weed grass and left-over stubbles (Miah and Bakr, 1977). In the infested rice stubbles, the fourth-stage larvae remain dormant, forming a coil, particularly inside peduncles, under upper leaf sheaths and within the glumes of the lower grains of the panicle. The number of nematodes in single infested stubbles varies from 1 to 30,000 (Rathaiah, 1988) and in seeds varies from 5.3 to 2400 (Ibrahim and Perry, 1993). Occurrence of this nematode in soil and seed has been reported (Cuc and Giang, 1982), but this nematode is not soil-borne (Pathak, 1992). In seed, the larvae are located at the overlapping region of the rice husk. The infestation starts at seedling stages of a few days old. Under favourable environmental conditions, with temperatures at 28–30°C and humidity above 85% (McGeachie and Rahman, 1983), the active nematodes climb up on the surface of newly germinated rice seedlings and feed ectoparasitically on the epidermal cells of the tender growing tissues and developing inflorescence (Ou, 1985). Because of the compact nature of the innermost leaf that ensheaths the shoot meristem, the nematode is not able to invade the apical meristem. It is probably for this reason that the infested plants are not killed, but grow and produce flower and seeds. The nematode does not produce any toxic substances while feeding but continues to suck sap through its small stylet.

The fourth-stage larvae, usually under the compact leaves, moult to become adults. The reproduction of the nematode is amphimictic and takes place on the host plant during the months of May–June and November, but the number of generations in a single season is not certain and quantitative estimation of eggs laid per female is difficult to ascertain (Perry, 1995). Three generations in a cropping season and 50–100 eggs per female was reported by Butler (1913a), and this was confirmed by the observation of three population peaks in a cropping season by Cox and Rahman (1979). In artificial conditions, the developmental cycle of *D. angustus* takes 15 days from second-stage juvenile (J_2) to become adult, 21 days from J_2 to egg and 24 days to complete its life cycle (Cuc, 1989). However, Plowright and Gill (1994) mentioned that the period of the life cycle of *D. angustus* is 10–20 days at 30°C. When crops proceed towards maturity, nematodes are found in large numbers at the top of the stem, in the panicle and in the seeds. At near maturity of the crop, predominantly fourth-stage larvae become inactive and undergo quiescence, each being tightly coiled circularly with the head at the centre. This state is commonly known as eelwool. When placed in water, larvae in the eelwool state uncoil and become active with vigorous wrinkling. The larvae become active more quickly at 31°C. The larvae can move on a solid surface at an atmospheric humidity of 85% or more.

D. angustus can survive in the quiescent stage in grains (under glumes or husk), in dry paddy stubbles, in the infested field, or on the surface of the soil until the next cropping season (Bora and Rahman, 2010). It also survives in the active stage in ratoons, weeds and wild rice after the harvest of paddy (Pathak, 1992). In dry paddy stubbles, the nematode can survive desiccation for at least 15 months (Catling *et al*., 1979). However, after harvest, the population in stubbles reduces drastically, leaving no live nematodes 4–5 months after harvest (Kinh, 1981). There are no specific 'survival stages' of *D. angustus* and in dry diseased stubbles three stages of *D. angustus* are found: J_3, J_4 and adult, with J_4 being predominant (Bora and Rahman, 2010).

5.10 Economic importance

Although this disease is most devastating to deep-water rice, it can also attack transplanted winter rice and summer rice (Chakraborti *et al*., 1985). The infestation is greater if the nematode inoculums are present in the field at the time of sowing (Rahman and Evans, 1987). Field experiments have revealed that a level of 10% infested seedlings at the time of transplanting is sufficient to cause significant yield loss (Mondal *et al*., 1989). Loss in grain yield depends on the percentage of *ufra* infestation. If there are more than 40% *ufra* II symptoms in a field, the yield loss may reach 100% (Cox and Rahman, 1980). In India, the yield reduction may be 20–90% (Rao *et al*., 1986a). Sometimes the disease is so severe that farmers cut the crop for cattle feed (Bora and Rahman, 2010). Miah and Bakr (1977) recorded that *ufra* disease affected only 2% of deep-water rice in Bangladesh, resulting in an average yield loss of 50% and even 100% in individual farms. Similarly, Mondal and Miah (1987b) reported 60–70% yield reduction in rice in low-lying areas in Bangladesh and 10–100% in transplanted rice (Rahman *et al*., 1994).

5.11 Similarities to other species/conditions

D. angustus is very similar to other *Ditylenchus* species, but is the only pest of rice. Morphologically it appears similar to *Aphelenchoides besseyi* under low microscopic resolution, but can be distinguished by head shape and type of oesophageal bulb. Males are more simply differentiated based on tail shape, spicule shape and presence or abundance of bursa.

5.12 Movement and means of dispersal

Dissemination of *D. angustus* from one field to another or from one plant to another takes place through plant residues, water (flood or irrigation water), rain splash and stem and leaf contact under conditions of high humidity (Rahman and Evans, 1987). Chances of transmission of this nematode through seed are minimal (Seshadri and Dasgupta, 1975). However, Prasad and Varaprasad (2001) showed that *D. angustus* is seed-borne and there is a possibility of dispersal of this nematode through seeds from infested field to other rice belts, located away from the RSN endemic zone. Considerable numbers of *D. angustus* have also been reported from filled grains containing more than 12% moisture (Ibrahim and Perry, 1993). Mondal and Miah (1987a) reported that tidal water plays an important role in the spread of this nematode.

5.12.1 Plant parts liable to carry the nematode in trade/transport

Plant parts including roots, flowers, inflorescences, cones, calyx, panicle (including ear heads), leaves, seedlings, micropropagated plants, stems (above ground), shoots, tillers and true seeds (including grain) are liable to carry all stages (eggs, juveniles and adults) of the nematode.

5.12.2 Plant parts not known to carry the nematode in trade/transport

Bark and wood.

5.13 Pest risk analysis and looming threat from introduction

D. angustus is solely a pest of rice, mainly in different types of flooded rice from deep-water to lowland irrigated. The nematode is confined to rice cultivation in India, Bangladesh, Myanmar and Thailand, which are major rice-growing countries of the world. There are also discrete reports of its occurrence in Egypt, Indonesia, Iran, Madagascar, Malaysia, Pakistan, Philippines and Sudan but there has not been any evidence about the economic damage by *D. angustus* in these countries. At a global level, *D. angustus* draws significant attention as a quarantine pest of rice in the countries where it is absent or has limited distribution or interception. Samplings during quarantine inspection have shown large numbers of seed lots to be infected with *D. angustus* traded from the nematode's endemic zone. The accidental introduction of *D. angustus* would have devastating repercussions on rice export to domestic and international markets.

5.14 Invasiveness rating

Considering the consequences of introduction and pest risk analysis, the following invasiveness rating for *D. angustus* has been assigned.

Climate/host interaction	–	High (3)
Hosts range	–	High (3)
Dispersal potential	–	High (3)
Economic impact	–	High (3)
Environmental impact	–	Medium (2)
Post-entry distribution	–	Low (–1)
Final rating (score)	–	High (13)

Based on the above invasiveness rating, *D. angustus* receives a score of 13 out of 15 and is rated as HIGHLY INVASIVE NEMATODE.

5.15 Region-wise status of invasiveness

Region-wise, top rice-producing countries where *D. angustus* attains invasive status are:

APPPC: Afghanistan, Cambodia, China, Iran, Japan, Nepal, North Korea, Pakistan, South Korea, Sri Lanka
CAHFSA: Lao People's Democratic Republic
CAN: Colombia, Ecuador, Peru, Venezuela
COSAVE: Argentina, Brazil, Uruguay
EPPO: Italy, Russian Federation, Spain, Turkey
IAPSC: Côte d'Ivoire, Egypt, Guinea, Mali, Madagascar, Nigeria, Sierra Leone, Senegal, Tanzania
NAPPO: USA
PPPO: Australia, Melanesia, Micronesia, New Zealand

5.16 Management measures

5.16.1 Seed treatment

Seed treatment is quite an effective and economically feasible management practice for *D. angustus*. Seed treatments include soaking of rice seeds in thiabendazole (0.1%) or ethylthiocyanoacetate (0.13%) or phosphamidon (0.1%) for 24 h (Vuong and Rodriguez, 1972). Dipping of seedling roots in carbofuran and applications of carbofuran during transplanting and every 20 days thereafter will effectively control the nematode infestation in rice (Chakraborti, 2000).

5.16.2 Cultural methods

Burning of diseased stubbles, followed by ploughing, reduces the incidence of *ufra* disease and is suggested as an effective control measure (Ou, 1985), but burning may create a problem of air pollution which has been observed in some states of India in recent years. Rotation of deep-water rice with a non-host crop such as jute (*Corchorus olitorius*) and mustard (*Brassica* sp.) reduces the *ufra* disease in the next crop (Butler, 1919). In areas with good irrigation facilities, cultivation of autumn rice (*boro* rice) followed by summer rice (*ahu* rice) will control the *ufra*, as the nematode will be deprived of the susceptible host during its active period. However, this practice may not be

feasible in most of the deep-water rice-growing areas, where floodwater rises to a high level that does not allow the cultivation of *ahu* rice.

5.16.3 Host resistance

A good number of rice cultivars have expressed tolerance/resistance against *D. angustus*, including Bazail-65, Jalamagna, Basudeu, AR-9(C), IR 13437-20-4E-PI, IR 17643-4, Lakhi, Karkati, BR 308-3-3-2, Rayada 16-011, Rayda 16-013, Rayada 16-05, Rayada 16-06, Rayada 16-07, Ba Tuc, etc. (Bora and Rahman, 2010).

5.16.4 Chemical control

Many chemical pesticides have been known to be useful for controlling *ufra* disease. Soil application of carbofuran at the time of transplanting, at 0.67 kg a.i./ha, followed by benomyl spray at 2.5 kg a.i./ha effectively suppressed *ufra* infestation (Rahman, 1993). Root-dip treatment of nursery stock with either carbofuran 3%, Miran 3% at 7.5–10% or Tecto 40FL at 2.5–10% for 12 h resulted in a healthy and *ufra*-free crop (Mondal and Miah, 1987b). Seed dressing with carbosulfan 25 STD plus soil application of carbofuran 3G at 3 kg a.i./ha plus foliar spray with carbosulfan 40 EC at 40, 120 days after sowing the seeds and before panicle initiation stage satisfactorily controlled *ufra* disease in deep-water rice (Das, 2004).

5.16.5 Phytosanitary measures

See Chapter 16, section 115.

6 *Ditylenchus destructor*

6.1 Common names

Potato tuber nematode; potato rot nematode

6.2 Scientific name

Ditylenchus destructor Thorne, 1945

6.3 EPPO code

DITYDE

6.4 Diagnosis

Morphological, biochemical and molecular diagnosis approaches of *Ditylenchus destructor* are presented below.

6.4.1 Morphological diagnosis

Morphological descriptions of *D. destructor* after Sturhan and Brzeski (1991) and Brzeski (1998) are summarized below. Substantial morphometric variation occurs in adults according to their host and age. For morphometric measurements, see Table 5.

Female: Minute, around 800–1400 µm long, 23–47 µm wide and ventrally arcuate slightly. Lateral field with six incisures (Fig. 1.9F), narrowed to two on neck and tail areas. Cuticle with fine annulation including on head. About four head annules distinguished by scanning electron microscopy (Wendt *et al*., 1995). Head often narrower than adjoining body. Stylet long, 10–12 µm in length, sometimes as long as 14 µm. Stylet cone comprising 45–50% of stylet length, distinct knobs, rounded and sloping backwards. Muscular median bulb, with thickenings of lumen valve and around 3 µm long. Posterior oesophageal bulb overlaps intestine dorsally for a short distance (Fig. 1.9D), though specimens with offset glandular bulb are also rarely seen. Excretory pore behind oesophageal glands. Postvulval sac present spreading about three-quarters of vulva–anus distance (Fig. 1.9D). Eggs twice as long as wide (Andrássy, 2007). Lips of vulva elevated and thick (Fig. 1.9E). Genital tract single with ovary outstretched anteriorly, sometimes touching oesophageal region. Postvulval uterine sac 40–98% of vulva–anus distance, but not functioning as a spermatheca (Fig. 1.9D).
Male: Similar to female in general morphological appearance (Fig. 1.9A). Bursa covers 50–90% of the tail length. Spicules long, 24–27 µm (Fig. 1.9C). Testis outstretched reaching base of oesophagus. Tail conical like female, usually ventrally curved, three to five anal body widths long with rounded terminus.
Juvenile: Four juvenile stages present, first stage occurring within the egg, almost twice as long as wide. Similar to female in general morphology but genital structures absent.

6.4.2 Biochemical diagnosis

One-dimensional SDS gel electrophoresis of proteins has been used to distinguish species and races of *Ditylenchus*, including *D. destructor* (Evans, 1971), *D. dipsaci* (Tenente and Evans, 1997) and *D. myceliophagus* (Ibrahim *et al*., 1994b).

6.4.3 Molecular diagnosis

Molecular diagnosis of *D. destructor* based on PCR–RFLP or sequencing of the ITS region of the rRNA gene has been given by different workers. Wendt *et al*. (1993, 1995) used PCR–RFLPs of ITS regions to separate *D. destructor*, *D. africanus*, *D. dipsaci* and *D. myceliophagus* by four restriction enzymes; see section 4.4.3, above, and Table 6. Several populations of *D. destructor* from sweet potato have been characterized by RFLP profiles and showed differences in their RFLP profiles (Ji *et al*., 2006; Subbotin *et al*., 2011b). More than 50 ITS1 region sequence accessions of rRNA fragments acquired from *D. destructor* collected from different geographical regions and host plants are currently available in the GenBank database (Powers *et al*., 2001).

6.5 Geographical distribution

The potato rot nematode (PRN) is a near-cosmopolitan species and most common in temperate regions (EPPO, 2013). It has

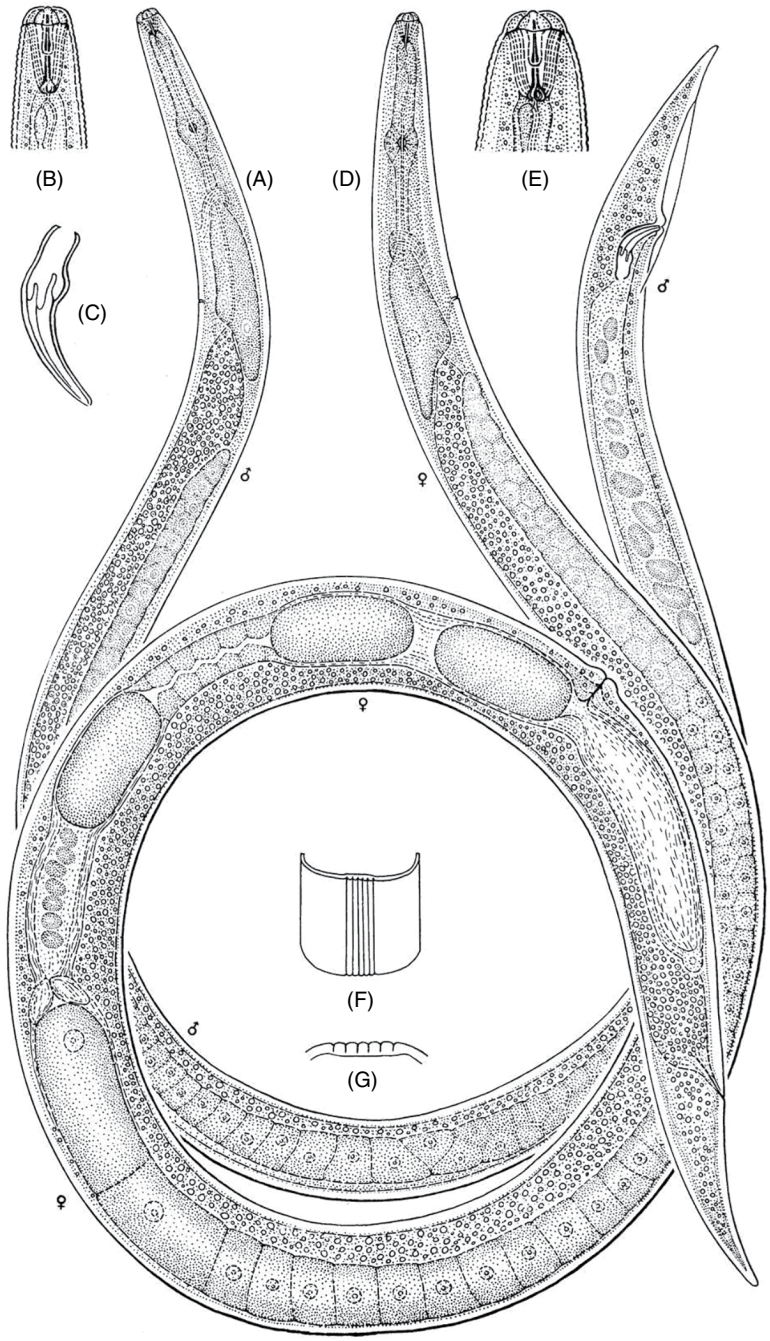

Fig. 1.9. Line drawing of *Ditylenchus destructor*. Male (A); head of male (B); spicule (C); female (D); head of adult female (E); section of cuticle at mid-body showing 6 incisures of lateral field (F); cross-section of lateral field (G). [Reproduced from CIH description, Set 2, No. 21 (after Thorne, 1945); courtesy Helminthology Society of Washington].

been reported to occur worldwide (Fig. 1.10). *D. destructor* is known to occur in the following countries (CABI-ISC, 2020).

Africa: South Africa
Asia: Azerbaijan, China, Iran, Japan, Kazakhstan, Kyrgyzstan, Pakistan, Saudi Arabia, South Korea, Tajikistan, Turkey, Uzbekistan.
Europe: Albania, Austria, Belarus, Belgium, Bulgaria, Czech Republic, Estonia, France, Germany, Greece, Hungary, Ireland, Italy, Jersey, Latvia, Lithuania, Luxembourg, Moldova, Netherlands, Norway, Poland, Romania, Russia, Slovakia, Slovenia, Sweden, Switzerland, Ukraine, United Kingdom.
North America: Canada, Mexico, USA.
Oceania: New Zealand.
South America: Ecuador, Peru.

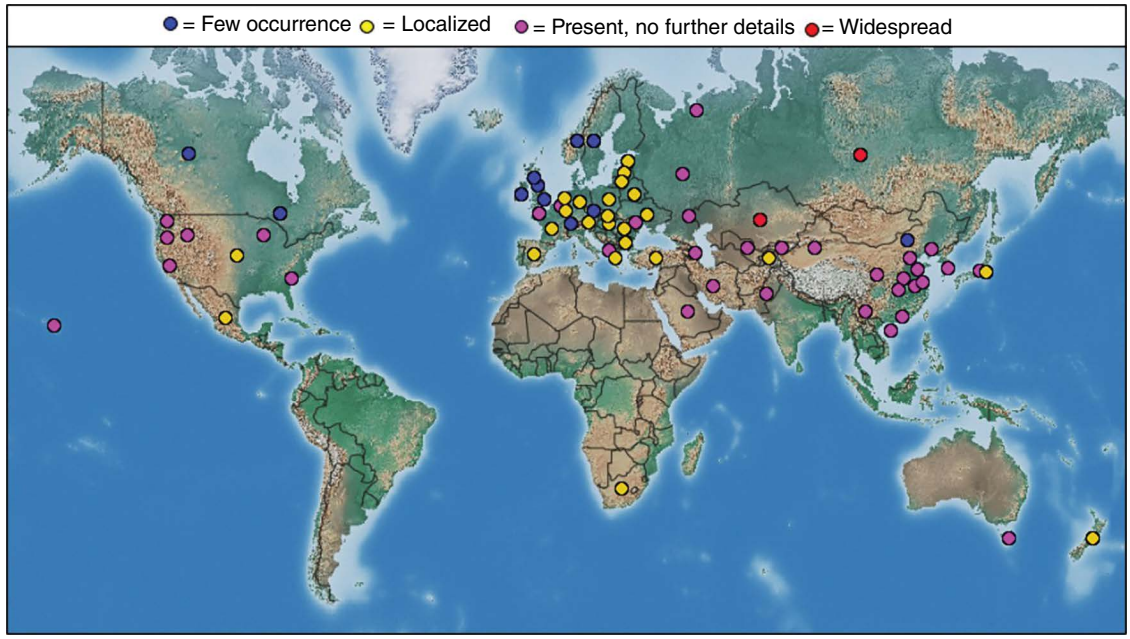

Fig. 1.10. Distribution map of *Ditylenchus destructor*. [Adapted from CABI-ISC, 2020]

6.6 Hosts

PRN attacks exclusively the below-ground parts of plants (tubers, rhizomes and stem-like subterranean parts). Potato is the principal host, but the nematode has an extensive host range, comprising more than 90 plant species, which include crop plants, ornamental plants and weeds (Sturhan and Brzeski, 1991). Main, and wild hosts of *D. destructor* are listed in Table 8.

6.7 Symptoms

PRN attacks mainly below-ground plant parts (tubers and stolons of potato, rhizomes of mint, and roots of hop and lilac), instigating discoloration and rotting of plant tissue. The above-ground parts are sometimes also infected, showing dwarfing, thickening and branching of the stem and curling, dwarfing and discoloration of the leaves (Sturhan and Brzeski, 1991). Symptoms on different crops are described below.

6.7.1 Potato

No noticeable symptoms appear during the growth period. The nematodes enter potato tubers typically through the stolons. The maximum numbers of nematodes are located at the edge of the undamaged and browning parts; here, the mass of small nematodes is visible even with a simple magnifying glass. The initial symptoms of *D. destructor* infection are small, white, chalky or light-coloured spots that can be seen just below the skin of the tuber (Brodie, 1998). These spots later become larger and slowly darker (through grey to dark brown and black) and develop a spongy texture (Fig. 1.7H). This is mainly a result of the secondary attack by fungi, bacteria and other saprophytic nematodes (Brodie, 1998). On severely affected tubers there are typically vaguely sunken areas with wrinkled, cracked, papery skin. The skin is not damaged but becomes thin and cracked as core infected tissues become dry and shrink (Brodie, 1998). Finally, the whole tuber may be mummified. Such damaged tubers drift in water (Fig. 1.7H). In contrast, the skin of potato infested with *D. dipsaci* is generally not cracked. The *D. destructor* nematodes continue to multiply inside the tubers even after harvest and may build up to huge numbers. Symptoms are more visible after storage, and secondary infections by fungi, bacteria and free-living nematodes occur.

6.7.2 Ornamental plants (iris, tulip, dahlia, etc.)

Infestation on iris results in greyish linear marks that expand upwards from the basal plate on the outer fleshy scales (Haglund, 1983). As nematode infestation intensifies, the destruction spreads over and reaches across the tissue of the bulb (Fig. 1.7I-K). This leads to a secondary dry, fibrous rotting which results in the breakdown of the bulb. Ring-like brown spots are noticeable in the cross-section of infested iris and tulip bulbs (Southey, 1993). Infested dahlia tubers develop symptoms similar to those in potato (Hooper, 1973a). Yellowing and dieback of the leaves are secondary symptoms caused by injury to the bulb and subsequent cessation of root functioning. *D. destructor* infestation of ornamental *Liatris spicata* corms ('Gayflower', 'Blazing Star' or 'Button Snakeroot') in cold storage in South Africa caused a blackish rot with living nematodes at different stages in the tissue adjoining the decaying areas (Van der Vegte and Daiber, 1983).

Table 8. List of main and wild host plants of *Ditylenchus destructor* (adapted from CABI-ISC, 2020).

Main hosts
Allium cepa (onion)
Allium sativum (garlic)
Arachis hypogaea (groundnut)
Beta vulgaris (beetroot)
Beta vulgaris var. *saccharifera* (sugar beet)
Camellia sinensis (tea)
Capsicum annuum (bell pepper)
Chrysanthemum morifolium
Citrus sinensis (navel orange)
Cucumis sativus (cucumber)
Cucurbita moschata (pumpkin)
Dahlia hybrids
Daucus carota (carrot)
Fragaria ananassa (strawberry)
Gladiolus hybrids (sword lily)
Glycine max (soybean)
Humulus lupulus (hop)
Ipomoea batatas (sweet potato)
Iris (irises)
Mentha (mints)
Panax ginseng (Asiatic ginseng)
Panax quinquefolius (American ginseng)
Solanum lycopersicum (tomato)
Solanum melongena (aubergine)
Solanum tuberosum (potato)
Trifolium (clovers)
Triticum aestivum (wheat)
Tulipa (tulip)
Zea mays (maize)
Wild hosts
Chenopodium album (fat hen)
Cyperus rotundus (purple nutsedge)
Datura stramonium (jimsonweed)
Eleusine indica (goosegrass)
Elymus repens (quackgrass)
Fumaria officinalis (common fumitory)
Solanum (nightshade)
Solanum nigrum (black nightshade)
Sonchus arvensis (perennial sowthistle)
Tagetes minuta (stinking Roger)
Taraxacum officinale complex (dandelion)
Xanthium strumarium (cocklebur)

6.7.3 Carrot

Infestation results in diagonal cracks in the skin of the carrot with white stripes in the cortical tissue (Fig. 1.7M). Secondary infections by fungi and bacteria in these areas may also occur, resulting in decay of the tissue. The damage is simply seen in a cross-section of the carrot. The nematode continues its damaging activity during storage and the carrots become unacceptable for consumption (Stoeen, 1977).

6.7.4 Beetroot and sweet potato

In sugar beet, infestation causes dark, necrotic lesions on rhizomes and roots. The symptoms are similar to crown canker (Dallimore and Thorne, 1951). On sweet potato, symptoms develop similar to potato. The damage is conspicuous in a cross-section of the tuber (Fig. 1.7L). In addition to yield loss, sugar content decreases.

6.8 Biology and life cycle

D. destructor is a migratory endoparasite of roots and belowground modified plant parts. The nematode seldom attacks the aerial parts of plants. It enters potato tubers through the lenticels, and then starts to multiply quickly and colonize the whole tuber. The life cycle is completed inside potato tubers, where nematodes feed on starch grains. They live inside the living tissue, where they aggregate rapidly and the fecund females produce up to 250 eggs individually. Two days after being laid, eggs hatch at 28°C, with an average interval of 4.4 days between egg-laying and hatch, and development from egg to adult completes in 6–7 days. The nematodes can survive and develop within harvested tubers as well as in stored tubers during the winter. Soil plays only a secondary role in the transfer of this nematode (Hooper, 1973a). The PRN attacks carrots at the base of the lateral roots, and tissue breaks down the cortex. The damaged tissue becomes discoloured; subsequently, external lesions appear which serve as infection sites for other secondary pathogens (Stoeen, 1977). It was also found that PRNs invade stems, buds and leaves of *Cimicifuga racemosa* (Planer, 1972) and roots of ginseng in Korea (Young and Seung, 1995). Stem infestations are unusual but have also been reported on potato haulm (Goodey, 1951) and *Vicia sativa* (Duggan and Moore, 1962).

D. destructor can survive on other host plants and about 70 crops and weeds have been recorded as hosts. Unlike the closely related *D. dipsaci*, it does not form 'eelworm wool'. *D. destructor* is incapable of withstanding excessive desiccation, and for this reason is generally important only in cool and moist soils. Without a resistant resting stage, this species hibernates in the soil as adults or larvae and may even reproduce by feeding on alternative weed hosts like *Mentha arvensis*, *Sonchus arvensis* and fungal mycelia. It may also possibly overwinter as eggs and hatch in the spring and larvae are instantly able to parasitize hosts.

6.9 Economic importance

D. destructor is accountable for severe losses in potato and hops production (EPPO, 2013). The intensity of infestation of potato tubers by *D. destructor* on Estonian farms ranged from 2 to 9% and up to 80–90% of tubers from various fields became infected during storage (Kikas, 1969). *D. destructor* was frequently recorded on seed potatoes from Central Asia and it is prevalent on potatoes in Kazakhstan (German, 1972) and Azerbaijan (Ismailov, 1976) and causes substantial yield losses. In Uzbekistan, PRN

represented 84.7% of the total nematodes found on potato tubers (Adylova and Vasilevskii, 1983). Severe infestations of potatoes have been recorded in the Samarkand, Tashkent and Fergana regions of Uzbekistan (Usmanova, 1972). Sturhan and Brzeski (1991) reported that *D. destructor* is also able to destroy the hyphae of cultivated mushroom (*Agaricus hortensis*).

6.10 Interaction with other pathogens

Rojankovski and Ciurea (1986) found 55 species of fungi and bacteria associated with *D. destructor* in potato tubers, with *Fusarium* spp. being the most common. *Rhizoctonia solani* infections of potato tubers were maximum in pots to which the largest number of *D. destructor* was added (Janowicz and Mazurkiewicz, 1982). The damage to potato tubers was greater when both the dry rot fungus (*Fusarium solani* var. *coeruleum*, *F. culmorum* and *F. oxysporum*) and *D. destructor* were present concomitantly (Janowicz, 1984).

6.11 Similarities to other species

D. destructor is similar to *D. dipsaci* but is distinguished by the lateral field demonstrating six incisures (Fig. 1.9F), the longer postvulval sac and the finely rounded tail terminus (Fig. 1.9D). Morphologically *D. destructor* differs from *D. africanus* mainly in its stylet length, which may overlap slightly, and the spicule length, which implies that males must be present in the population. The spiculum shape of *D. dipsaci* differs from *D. destructor* in having a ventral tumulus in the calomus area (Karssen and Willemsen, 2010). When observed in the lateral view, the spicule is less arched in *D. dipsaci* than in *D. destructor* (Fig. 1.9C).

6.12 Movement and means of dispersal

The nematode can be easily disseminated through the seed, tuber, bulbs and other plant parts.

6.12.1 Plant parts liable to carry the nematode in trade/transport

Roots, bulbs, tubers, corms, rhizomes, fruits (including pods), flowers, inflorescences, cones, calyx, leaves, stems (above ground), shoots, true seeds, seedlings/micro-propagated plants.

6.12.2 Plant parts not known to carry the nematode in trade/transport

Bark, wood and trunks.

6.13 Pest risk analysis and looming threat from introduction

D. destructor has a very wide host range and attacks several important food crops and ornamental plants in most of the temperate areas of the world (CABI-ISC, 2020). *D. destructor* is of quarantine significance for many countries, particularly in APPPC and COSAVE regions, and at present it is targeted in regulatory programmes worldwide. However, its dissemination varies between regions, countries and localities. Seed potatoes and bulbous ornamentals transmit the infection and disperse the disease, and hence must be subject to proper phytosanitary regulations.

6.14 Invasiveness rating

Considering the consequences of introduction and pest risk analysis, the following invasiveness rating has been assigned to *D. destructor*.

Climate/host interaction	–	High (3)
Hosts range	–	High (3)
Dispersal potential	–	High (3)
Economic impact	–	High (3)
Environmental impact	–	Medium (2)
Post-entry distribution	–	Medium (–2)
Final rating (score)	–	High (12)

Based on the above invasiveness rating, *D. destructor* receives a score of 12 out of 15 and is rated as HIGHLY INVASIVE NEMATODE.

6.15 Region-wise status of invasiveness

At the international level, *D. destructor* draws significant quarantine attention and has attained invasive status in the following countries, where it is absent or has very limited distribution.

APPPC: Bangladesh, Cambodia, India, Indonesia, Lao, Malaysia, Myanmar, Nepal, Philippines, Sri Lanka, Thailand, Tonga, Vietnam

CAHFSA: Anguilla, Bahamas, Barbados, Bermuda, Cayman Islands, Colombia, Cuba, Dominica, Dominican Republic, French Guiana, Grenada, Guadeloupe, Guyana, Haiti, Honduras, Jamaica, Martinique, Netherlands Antilles, Nicaragua, Panama, Puerto Rico, Saint Lucia, Saint Kitts & Nevis, Suriname, Trinidad and Tobago, Turks and Caicos Islands, Virgin Islands (British), Virgin Islands (US).

CAN: Bolivia, Venezuela

COSAVE: Argentina, Chile, Paraguay, Uruguay

EPPO: Croatia, Cyprus, Finland, Georgia, Guernsey, Jordan, Macedonia, Serbia, Tunisia

IAPSC: Angola, Benin, Botswana, Burkina Faso, Burundi, Cameroon, Cape Verde, Central African Republic, Chad, Comoros, Congo (Republic of), Congo (Democratic Republic of), Côte d'Ivoire, Djibouti, Egypt, Equatorial Guinea, Eritrea, Ethiopia, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Kenya, Lesotho, Liberia, Madagascar, Malawi, Mali, Mauritania, Mauritius, Mozambique, Niger, Nigeria, Rwanda, São Tomé & Principe, Senegal, Seychelles,

Sierra Leone, Somalia, Sudan, Swaziland, Tanzania, Togo, Uganda, Zaire, Zambia, Zimbabwe
NEPPO: Algeria, Jordan, Libya, Malta, Morocco, Syria
OIRSA: Costa Rica, El Salvador, Guatemala, Honduras
PPPO: American Samoa, Australia, Cook Islands, Fiji, French Polynesia, Guam, Kiribati, Marshall Islands, Micronesia, Nauru, New Caledonia, Niue, Northern Mariana Islands, Palau, Pitcairn, Samoa, Solomon Islands, Tokelau, Tonga, Tuvalu, Vanuatu, Wallis and Futuna Islands

6.16 Management measures

6.16.1 Prevention and control

The spread of *D. destructor* can be prevented through the removal of infection sources by fumigation and the application of strict quarantine restricting the movement of infected tubers. Other phytosanitary methods for controlling *D. destructor* include the following.

TREATMENT OF POTATO TUBERS Soaking potatoes in nematicides before planting is effective against *D. destructor*. In Azerbaijan, sorting out of 'clean' seed potatoes, or treatment with dimethoate and carbathion (metam), were both effective against *D. destructor*. Dipping potato tubers in thionazin controlled PRN and enhanced the yield (Wilski, 1972).

TREATMENT OF BULBS Application of diazinon as bulb treatment decreased *D. destructor* populations by 37–48% (Rasinya, 1972). Immersion of iris bulbs in hot water containing 0.5% formaldehyde at 43.5°C for 3 h can control PRN infestations. The bulbs may be soaked for 2.5 h in a solution of thionazin plus formaldehyde. In another study, hot-water treatment of iris bulbs (cv. 'Wedgewood') for 3 h at 43.6°C after warm storage for 7 days at 30°C effectively controlled *D. destructor* (ISEHS, 1974). In Japan, infestation in iris bulbs can be controlled by immersion in water containing formaldehyde at 43.5°C for 2–3 h, but some varieties may be damaged during this treatment. In garlic bulbs, *D. destructor* was managed by drying at 34–36°C for 12–17 days (Fujimura *et al*., 1989).

6.16.2 Phytosanitary measures

See Chapter 16, section 115.

7 *Ditylenchus dipsaci*

7.1 Common name

Stem and bulb nematode

7.2 Scientific name

Ditylenchus dipsaci (Kuhn, 1857) Filiopjev, 1936

7.3 Synonyms

Anguillula devastatrix Kühn, 1869
Anguillula dipsaci Kühn, 1857
Anguillula secalis Nitschke, 1868
Anguillulina dipsaci (Kühn, 1857) Gervais and Van, 1859
Anguillulina dipsaci var. *communis* Steiner and Scott, 1935
Ditylenchus allocotus (Steiner, 1934) Filip'ev and Sch. Stek., 1
Ditylenchus amsinckiae (Steiner & Scott, 1935) Filipev and Sch.
Ditylenchus dipsaci var. *tobaensis Schneider, 1937*
Ditylenchus fragariae Kir'yanova, 1951
Ditylenchus sonchophila Kir'yanova, 1958
Ditylenchus trifolii Skarbilivich, 1958
Tylenchus allii Beijerinck, 1883
Tylenchus devastator
Tylenchus devastatrix (Kühn) Oerley
Tylenchus dipsaci (Kühn, 1857) Bastian, 1865
Tylenchus havensteinii Kühn, 1881
Tylenchus hyacinthi Prillieux, 1881
Tylenchus putrefaciens Kühn, 1879

7.4 EPPO code

DITYDI

7.5 Diagnosis

Morphological, biochemical, karyological and molecular studies of different populations and races of *Ditylenchus dipsaci sensu lato* (*s.l.*) revealed it as a complex species composed of a large number of biological races mainly differing in host preference (Jeszke *et al*., 2013). Subsequently, a total of 13 nominal species have been synonymized with *D. dipsaci* and up to 30 biological races have been distinguished, mainly differentiated by host range and usually named after their primary host plant. Further, Jeszke *et al*. (2013) grouped this complex into two, the first comprising diploid (n =12) populations categorized by their 'normal' size and named *D. dipsaci sensu stricto* (*s.s.*). This group contains most of the populations documented so far. The second group is polyploidal (n = 18–28) and currently includes: the 'giant race' of *D. dipsaci* (*Ditylenchus gigas* Vovlas *et al*., 2011) parasitizing broad bean (*Vicia faba*); *D. weischeri* Chizhov *et al*., 2010, parasitizing creeping thistle (*Cirsium arvense*); and three undescribed *Ditylenchus* spp. called D, E and F. D is associated with plant species of the Fabaceae, E is associated with plant species of the Asteraceae and F is associated with the plant species of Plantaginaceae (Jeszke *et al*., 2013). Of all these *Ditylenchus* species, only *D. dipsaci s.s.* and its morphologically bigger variant *D. gigas* are plant parasites of higher plants and of economic importance. The following diagnosis protocols facilitate the precise identification of *D. dipsaci s.s* using morphological, biochemical, karyological and molecular diagnosis approaches.

7.5.1 Morphological diagnosis

Detailed morphological descriptions of *D. dipsaci* are given after Hooper (1972), Sturhan and Brzeski (1991), Wendt *et al.* (1995) and Brzeski (1998). For morphometric measurements, see Table 5.

Female: Body almost straight after heat relaxed. Lateral field with four incisures (Fig. 1.11F). Head continuous with adjoining body (Fig. 1.11B). Stylet long, 10–12 µm. Stylet cone around half of stylet length, knobs well developed and rounded. Phasmid-like structures located dorsal to the lateral fields. Lip region unstriated, low, somewhat flattened, hardly offset from body. Median bulb muscular, 4–5 µm long with thickenings of lumen walls (Fig. 1.11A). Basal bulb overlapping intestine for a few micrometres or occasionally offset. Excretory pore behind posterior part of glandular bulb or isthmus. Postvulval uterine sac covering about half to marginally more of vulva–anus distance. Tail conical with a pointed tip (Fig. 1.11E).
Male: Anterior region similar to female (Fig. 1.11G). Stylet 10–12 µm long. Bursa envelops tail about three-quarters (Fig. 1.11D, H). Spicules 23–28 µm long. Gubernaculum short and simple.

7.5.2 Biochemical and karyological diagnosis

Different workers used one-dimensional SDS gel electrophoresis of isozymes and proteins to distinguish species and races of *D. dipsaci* (Hussey and Krusberg, 1971). *D. dipsaci* was also categorized based on enzymes, using isoelectric focusing (Ibrahim, 1991). Two-dimensional gel electrophoresis (2-DGE) is a significantly more informative demonstration of proteins in a nematode extract (Abrantes *et al.*, 2004) and has been used to

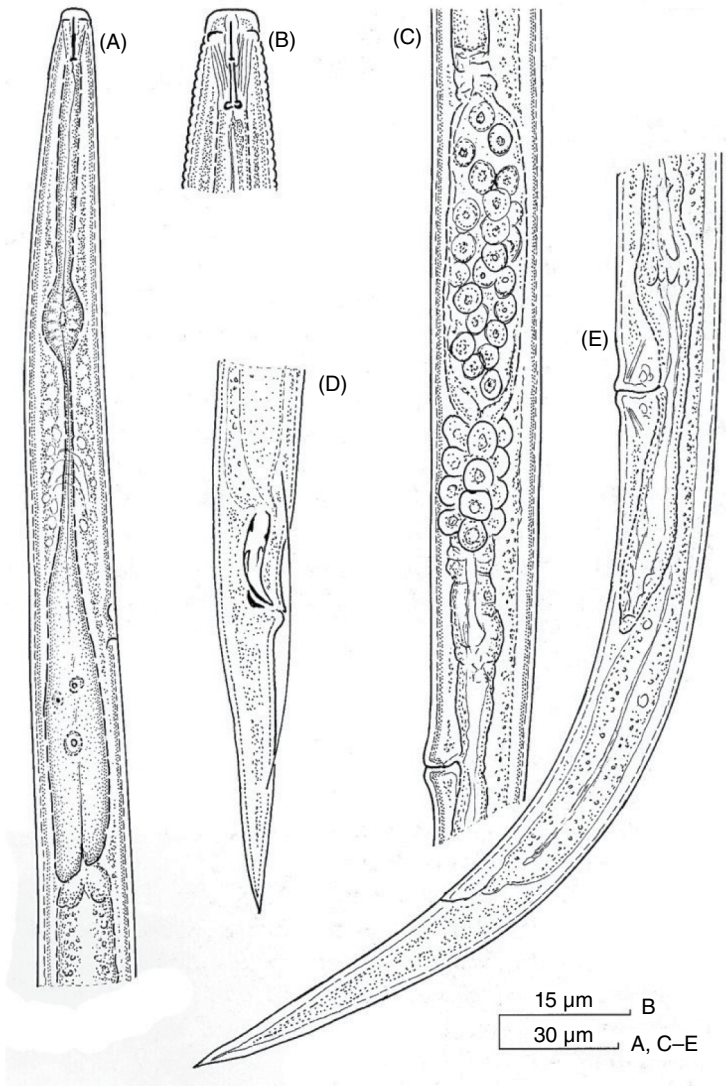

Fig. 1.11. Line drawing of *Ditylenchus dipsaci*. Female with oesophageal region (A); head of female (B); female reproductive system (C); tail end of male (D); posterior region of female (E). [Reproduced from M.R. Siddiqi, 2000; courtesy CAB International].

examine inter- and intraspecific variations in proteins of *D. dipsaci* (Bossis *et al*., 1998). Southern hybridization (Wendt *et al*., 1993) and electrophoresis (Palazova and Baicheva, 2002) have also been used to examine the races concept within *D. dipsaci* and genetic diversity amongst *Ditylenchus* species.

Barabashova (1978) distinguished the *D. dipsaci* complex in two groups based on karyological analyses. The first forms a fairly homologous group that includes the parasites of cultivated plants, with chromosome number $n = 12$. The majority of the races in the first group cross-breed successfully. The second forms a heterogeneous group that includes parasites of wild plants and these differ in both karyotype ($n = 18$ to 28) and morphology. The 'giant race', parasitic in *Vicia faba* var. *equina*, where $n = 27$, is also considered in the second group. At least partial incompatibility is perceived among races in the second group. Genetically, the two groups are incompatible.

7.5.3 Molecular diagnosis

The literature reveals that numerous molecular methods have been developed and used for *D. dipsaci* diagnosis. Most of the molecular approaches are PCR or PCR–RFLP based, and for identification and detection of population difference by sequence analysis (Leal-Bertioli *et al*., 2000; Esquibet *et al*., 2003; Subbotin *et al*., 2005a; Zouhar *et al*., 2007). Madani *et al*. (2015) designed species-specific primer sets based on the nucleotide sequence of the heat shock protein (hsp90) gene for *D. dipsaci*. Some of these techniques are described below.

18S AND ITS1-SPECIFIC PCR TEST Subbotin *et al*. (2005a) developed a species-specific identification for *D. dipsaci s.s*. (normal race only; the giant race, later called *D. gigas*, not included). The test was evaluated against *D. destructor* (one population), *D. dipsaci* (18 populations from various hosts and locations) and *Ditylenchus* sp. (12 populations from different hosts and locations). The *D. dipsaci*-specific primers are:

rDNA2: 5′-TTT CAC TCG CCG TTA CTA AGG-3′ (Vrain *et al*., 1992)
DitNF1: 5′-TTA TGA CAA ATT CAT GGC GG-3′

The amplification is performed using the following cycling parameters.

Reagent	–	Final concentration
PCR mixture	–	25 µl (1× from 10× PCR buffer)
$MgCl_2$	–	15 mM
dNTPs	–	0.5 mM
Primer rDNA2	–	60 nM
Primer DitNF1	–	60 nM
Taq DNA polymerase	–	1 U
Cycling parameters		
Initial denaturation	–	94°C for 4 min
Number of cycles	–	35
Denaturation	–	94°C for 15s
Annealing	–	57°C for 30s
Extension	–	72°C for 30s
Final elongation	–	72°C for 10 min

The PCR products are analysed by agarose gel electrophoresis. The amplicon size is almost 263 bp for *D. dipsaci s.s*. (normal race). No amplification is observed with non-target species.

5.8S RDNA-SPECIFIC PCR TEST Marek *et al*. (2005) developed a species-specific test for *D. dipsaci*. It was evaluated against three European populations of *D. dipsaci* obtained from different hosts and some non-target genus populations such as *Globodera pallida*, *Bursaphelenchus xylophilus* and *Rhabditis* spp. Two specific primer sets were designed for *D. dipsaci* identification, but the most sensitive (10 pg of target DNA detected) primers are:

PF1: 5′-AAC GGC TCT GTT GGC TTC TAT-3′
PR1: 5′-ATT TAC GAC CCT GAG CCA GAT-3′

The PCR amplification is performed using the following reagents and cycling parameters.

Reagent	–	Final concentration
PCR mixture	–	25 µl (1× Taq buffer)
$MgCl_2$	–	1.5 mM
dNTPs	–	200 µM
Primer PF1	–	10 pmol
Primer PR1	–	10 pmol
Taq DNA polymerase	–	1.5 U
Cycling parameters		
Initial denaturation	–	94°C for 3 min
Number of cycles	–	30
Denaturation	–	94°C for 2 min
Annealing	–	62°C for 30 s
Extension	–	72°C for 2 min
Final elongation	–	72°C for 10 min

The PCR products are analysed by agarose gel electrophoresis. The amplicon size with this primer set is around 327 bp for *D. dipsaci*.

5.8S RDNA AND ITS-SPECIFIC PCR TEST FOR *D. DIPSACI* Kerkoud *et al*. (2007) developed 5.8S rDNA and ITS-specific PCR test for *D. dipsaci* and it was evaluated against *D. dipsaci*, *D. africanus*, *D. destructor*, *D. myceliophagus*, *Ditylenchus* sp. (now described as *D. gigas*) and *Aphelenchoides ritzemabosi*. Two sets of specific primers are used, one for *D. dipsaci* alone and one for *D. gigas* and *D. dipsaci* combined. The primer sets allow separation of *D. dipsaci* from *D. gigas*.

The first primer set is:

DdpS1: 5′-TGG CTG CGT TGA AGA GAA CT-3′
rDNA2: 5′-TTT CAC TCG CCG TTA CTA AGG-3′ (Vrain *et al*., 1992)

The second primer set is:

DdpS2: 5′-CGA TCA ACC AAA ACA CTA GGA ATT-3′
rDNA2: 5′-TTT CAC TCG CCG TTA CTA AGG-3′ (Vrain *et al*., 1992)

The amplification is performed using the following reagents and cycling parameters.

Reagent	–	Final concentration
PCR mixture	–	20 µl
PCR buffer	–	1.5 mM
$MgCl_2$	–	5 mM
dNTPs	–	200 µM
First primer DdpS1	–	0.5 µM
First primer rDNA2	–	0.5 µM
Second primer DdpS1	–	1 µM
Second primer rDNA2	–	1 µM
Taq DNA polymerase	–	1 U
Cycling parameters		
Initial denaturation	–	94°C for 1 min
Number of cycles	–	40
Denaturation	–	94°C for 30 s
Annealing	–	60°C for 45 s
Extension	–	72°C for 45 s

The PCR products are analysed by agarose gel electrophoresis. The amplicon size for *D. dipsaci* alone is approximately 517 bp. No amplification is recorded with non-target species, including *D. gigas*. When both primer sets are used, the amplicon size for *D. dipsaci* and *D. gigas* is approximately 707 bp.

SCAR PCR TEST Esquibet *et al.* (2003) developed a species-specific test for *D. dipsaci* using sequence characterized amplified region (SCAR) PCR test and it was designed to differentiate normal and giant races of *D. dipsaci*. The test was evaluated against *D. dipsaci* normal race (11 populations from different hosts and locations), *D. dipsaci* giant race (*D. gigas*, 11 populations isolated from *V. faba* from different locations) and *D. myceliophagus* (one population). The *D. dipsaci*-specific primers used are as follows.

Normal race:

H05: 5′-TCA AGG TAA TCT TTT TCC CCA CT-3′
H06: 5′-CAACTG CTA ATG CGT GCT CT-3′

Giant race:

D09: 5′-CAA AGT GTT TGA TCG ACT GGA-3′
D10: 5′-CAT CCC AAA ACA AAG AAA GG-3′

The amplification is performed using the following reagents and cycling parameters.

Reagent	–	Final concentration
PCR mixture	–	10 µl
$MgCl_2$	–	1.5 mM
dNTPs	–	250 µM
Primer H05-H06 or D09-D10	–	690 nM (for duplex PCR)
Primer H05-H06 or D09-D10	–	500 nM (for multiplex PCR)
Taq DNA polymerase	–	0.5 U
Cycling parameters		
Initial denaturation	–	94°C for 3 min
Number of cycles	–	30
Denaturation	–	94°C for 1 min
Annealing	–	59°C for 1 min
Extension	–	72°C for 1 min

The PCR products are analysed by agarose gel electrophoresis. The amplicon size is approximately 242 bp for *D. dipsaci* (normal race) and 198 bp for *D. dipsaci* (giant race). When both primer sets are used, no amplification is recorded with non-target race and non-target species (Esquibet *et al.*, 2003).

Another SCAR PCR test was designed by Zouhar *et al.* (2007) using a species-specific primer for *D. dipsaci*. It was evaluated against ten European populations of *D. dipsaci* from different hosts. The two following specific primer sets have been developed.

First primer:

DIT_2 forward: 5′-GCA ATG CAC AGG TGG ATA AAG-3′
DIT_2 reverse: 5′-CTG TCT GTG ATT TCA CGG TAG AC-3′

Second primer:

DIT_5 forward: 5′-GAA AAC CAA AGA GGC CGT AAC-3′
DIT_5 reverse: 5′-ACC TGA TTC TGT ACG GTG CAA-3′

The PCR is performed with the following reagents and cycling parameters.

Reagent	–	Final concentration
PCR mixture	–	25 µl
$MgCl_2$	–	1.5 mM
dNTPs	–	200 µN
Forward primer DIT_2 or DIT_5	–	10 pmol
Reverse primer DIT_2 or DIT_5	–	10 pmol
Taq DNA polymerase	–	1.5 U
DNA template	–	50 ng
Cycling parameters		
Initial denaturation	–	94°C for 3 min
Number of cycles	–	30
Denaturation	–	94°C for 1 min
Annealing	–	60°C for 1 min
Extension	–	72°C for 1 min
Final elongation	–	72°C for 10 min

The PCR products are analysed by agarose gel electrophoresis. The amplicon with the first primer set is approximately 325 bp for *D. dipsaci*. The amplicon with the second primer set is approximately 245 bp for *D. dipsaci*.

7.6 Geographical distribution

The stem and bulb nematode (SBN) has been reported to occur all over the world, especially in temperate regions (Fig. 1.12). There are several reports about the presence of this nematode in India (Sturhan and Brzeski, 1991; Chakraborti, 2001), although other workers noted its absence (EPPO, 2009b; CABI-ISC, 2020). *D. dipsaci* is known to occur in the following countries (EPPO, 2013; CABI-ISC, 2020).

Africa: Algeria, Kenya, Morocco, Réunion, South Africa, Tunisia.

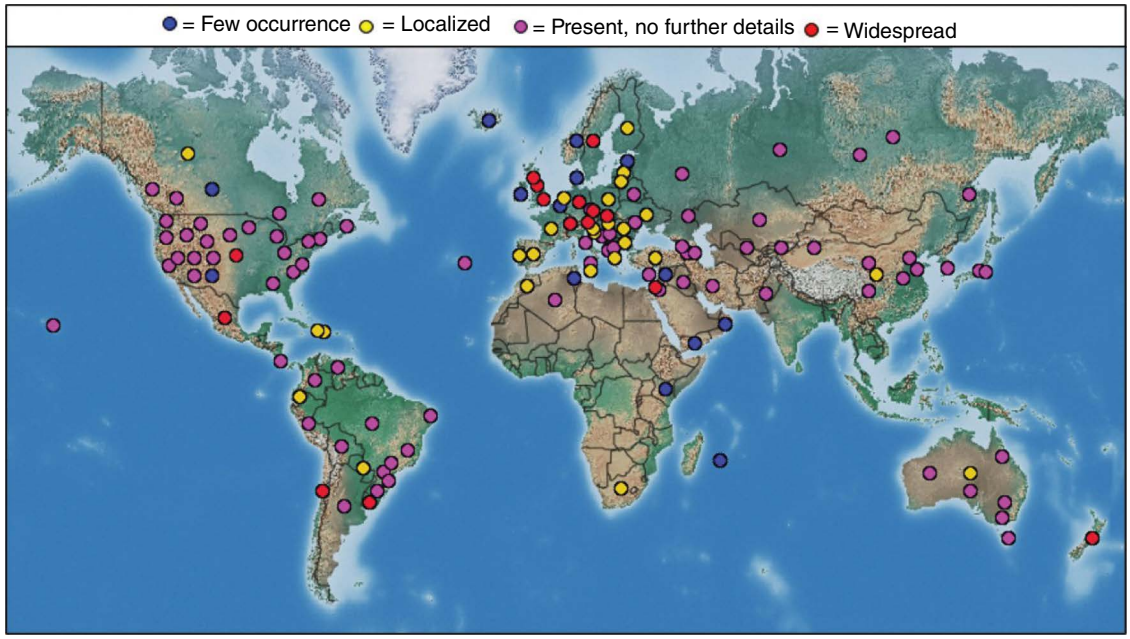

Fig. 1.12. Distribution map of *Ditylenchus dipsaci*. [Adapted from CABI-ISC, 2020]

Asia: Armenia, Azerbaijan, China, Georgia, Iran, Iraq, Israel, Japan, Jordan, Kazakhstan, Kyrgyzstan, Oman, Pakistan, South Korea, Syria, Taiwan, Turkey, Uzbekistan, Yemen.
Europe: Albania, Austria, Belarus, Belgium, Bosnia–Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Macedonia, Malta, Moldova, Netherlands, Norway, Poland, Portugal, Romania, Russian, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine, United Kingdom.
North America: Canada, Costa Rica, Dominican Republic, Haiti, Mexico, USA.
Oceania: Australia, New Zealand.
South America: Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Paraguay, Peru, Uruguay, Venezuela.

7.7 Hosts

SBNs parasitizes more than 1200 species of cultivated and wild plants (Jeszke *et al.*, 2013). Among these over 40 angiosperm families are known to be hosts of *D. dipsaci*. According to Sturhan and Brzeski (1991), the primary hosts of *D. dipsaci* from different families are:

Amaryllidaceae: *Narcissus* spp., *Allium cepa*, *A. sativum*, *Tulipa* spp.
Brassicasae (=Cruciferae): *Brassica campestris*
Poaceae (=Gramineae): *Avena sativa*, *Secale cereale*, *Zea mays*, *Triticum aestivum*
Fabaceae (=Leguminosae): *Medigo sativa*, *Vicia* spp., *Pisum sativum*, *Trifolium* spp.
Solanaceae: *Solanum tuberosum*, *Nicotiana* spp.

Several weeds and grasses are also recorded as hosts for *D. dipsaci* and may play an important role in its survival in the absence of cultivated plants. Many of the biological races of *D. dipsaci* have limited host ranges (EPPO, 2013). Some important host plants of *D. dipsaci* are presented in Table 9.

7.8 Symptoms

D. dipsaci is one of the most devastating nematode pests on a wide range of plants. Specific symptoms in the principal hosts are as follows.

7.8.1 Poaceae (=Gramineae)

OATS, RYE AND WHEAT Infested leaves are distorted, stems swollen with abnormal number of tillers. Overall, the plant is stunted, bushy and short. In rye (*Secale cereal*), *D. dipsaci* occurs mostly in light soils with poor humus and naturally in areas where rye is regularly cultivated. The first symptom can be detected in late autumn, but they are most noticeable in spring. In oats (*Avena sativa*), several patches of plants with stunted growth in the field indicate damage by the nematode. As infestation intensifies, plant growth ceases and the damage is visible as chlorosis of the crop (McDonald and Nicol, 2005). Wheat plants attacked by *D. dipsaci* show symptoms similar to those in rye and oats (Rivoal and Cook, 1993).

MAIZE Maize is a poor host of *D. dipsaci* but young plants may be killed. Invasion by *D. dipsaci* on young stem tissues produces necrosis, mainly in young plants, and the plants eventually die (Rivoal and Cook, 1993). Infected plants show crispy and twisted leaves resembling a corkscrew. The internodes are also shortened, and the bottom of the stem becomes hollow, causing the maize plant to fall over before harvest.

Table 9. List of host plants of *Ditylenchus dipsaci* (adapted from CABI-ISC, 2020).

Main host
Allium cepa (onion)
Allium porrum (leek)
Allium sativum (garlic)
Avena sativa (oats)
Begonia
Beta vulgaris (sugar beet)
Cannabis sativa (hemp)
Fragaria ananassa (strawberry)
Gladiolus hybrids (sword lily)
Hyacinthus orientalis (hyacinth)
Medicago sativa (lucerne)
Narcissus (daffodil)
Narcissus pseudonarcissus (wild lent lily)
Nicotiana tabacum (tobacco)
Phaseolus (beans)
Phlox drummondii (annual phlox)
Phlox paniculata (perennial phlox)
Pisum sativum (pea)
Polyphagus (polyphagous)
Secale cereale (rye)
Solanum tuberosum (potato)
Trifolium pratense (purple clover)
Trifolium repens (white clover)
Tulipa (tulip)
Vicia faba (broad bean)
Zea mays (maize)
Other hosts
Apium graveolens (celery)
Allium cepa var. *aggregatum*
Brassica napus var. *napus* (rape)
Carduus acanthoides
Crocus sativus (saffron)
Cucurbitaceae (cucurbits)
Dianthus caryophyllus
Helianthus annuus (sunflower)
Hydrangea (hydrangeas)
Ipomoea batatas (sweet potato)
Lens culinaris subsp. *culinaris* (lentil)
Onobrychis viciifolia (sainfoin)
Phaseolus coccineus (runner bean)
Pimpinella anisum (aniseed)
Petroselinum crispum (parsley)
Triticum (wheat)
Wild host
Astrantia
Avena sterilis (winter wild oat)
Bergenia
Brassica rapa subsp. *rapa* (turnip)
Chenopodium murale
Cirsium arvense (creeping thistle)
Convolvulus arvensis (bindweed)
Hieracium pilosella
Lamium album
Lamium amplexicaule (henbit deadnettle)
Lamium purpureum (purple deadnettle)
Myriophyllum verticillatum
Nerine sarniensis (Guernsey lily)
Stellaria media (common chickweed)
Raphanus raphanistrum (wild radish)
Ranunculus arvensis (corn buttercup)
Taraxacum officinale complex

7.8.2 Amaryllidaceae

NARCISSUS SPP. On above-ground parts, symptoms are the presence of chlorotic leaves with blister-like swellings (spickels). Swellings are conspicuous before flowering when leaves are actively growing. In low infestations, the swellings can be more easily felt between finger and thumb than seen. On bulbs, concentric brown rings can be seen when they are cut transversely (Fig. 1.13A–C). The necrosis can also be seen when bulbs are cut longitudinally. The necrosis starts at the neck and spreads downwards. Nematode infection can be noticed in dry bulbs with nominal bulb damage by cutting just below the neck. In the early stages of infestation, careful inspection reveals shining, spongy areas where cells have been detached. This is promptly followed by brown necrosis (Southey, 1993). Other Amarylliaceae plants usually show similar symptoms as observed in *Narcissus* spp.; for example, *Galanthus* spp. and *Nerine* spp. also express swellings on their foliage and concentric, brown necrotic rings in the bulbs.

ALLIUM SPP. (ONION, GARLIC, LEEK AND SHALLOT) In most *Allium* spp. the characteristic symptoms of SBN infestation appear on the leaves and bulbs, which become deformed (Fig. 1.13D,E,J). The base of young plants becomes swollen and leaves become twisted. Older infected bulbs exhibit bloat (swelling) of scales with open cracks frequently occurring at the root disc of the bulbs (Potter and Olthof, 1993). In onion (*A. cepa*), nematode feeding in leaves causes maceration and dissolution of cells, resulting in blisters and frosted appearance of the plants (Ferris and Ferris, 1998). In storage, the infested bulbs are predisposed to rot readily (Duncan and Moens, 2013). The inner scales of the bulb are generally more severely attacked by the nematode than the outer scales. As the season progresses the bulbs become soft and, when cut open, show browning of the scales in concentric circles. Above-ground symptoms are stunting and yellowing, while symptoms in the bulbs are necrosis, under-development and distortion. In garlic, in contrast to onion, *D. dipsaci* does not prompt deformation of leaves or swelling but does cause leaf yellowing and death of the plants (Netscher and Sikora, 1990). No visible symptoms of nematode infestation are observed on infested seeds of *Allium* spp.

7.8.3 Liliaceae

TULIP On tulips, the symptoms of *D. dipsaci* attack appear on both growing plants and bulbs, and they are quite different from those in *Narcissus* spp. In the field, infestation is best noticed at flowering. The first sign of nematode infestation is a pale or purplish lesion on one side of the stem immediately below the flower, which bends in the direction of the lesion (Southey, 1993). The lesion size increases and the epidermis

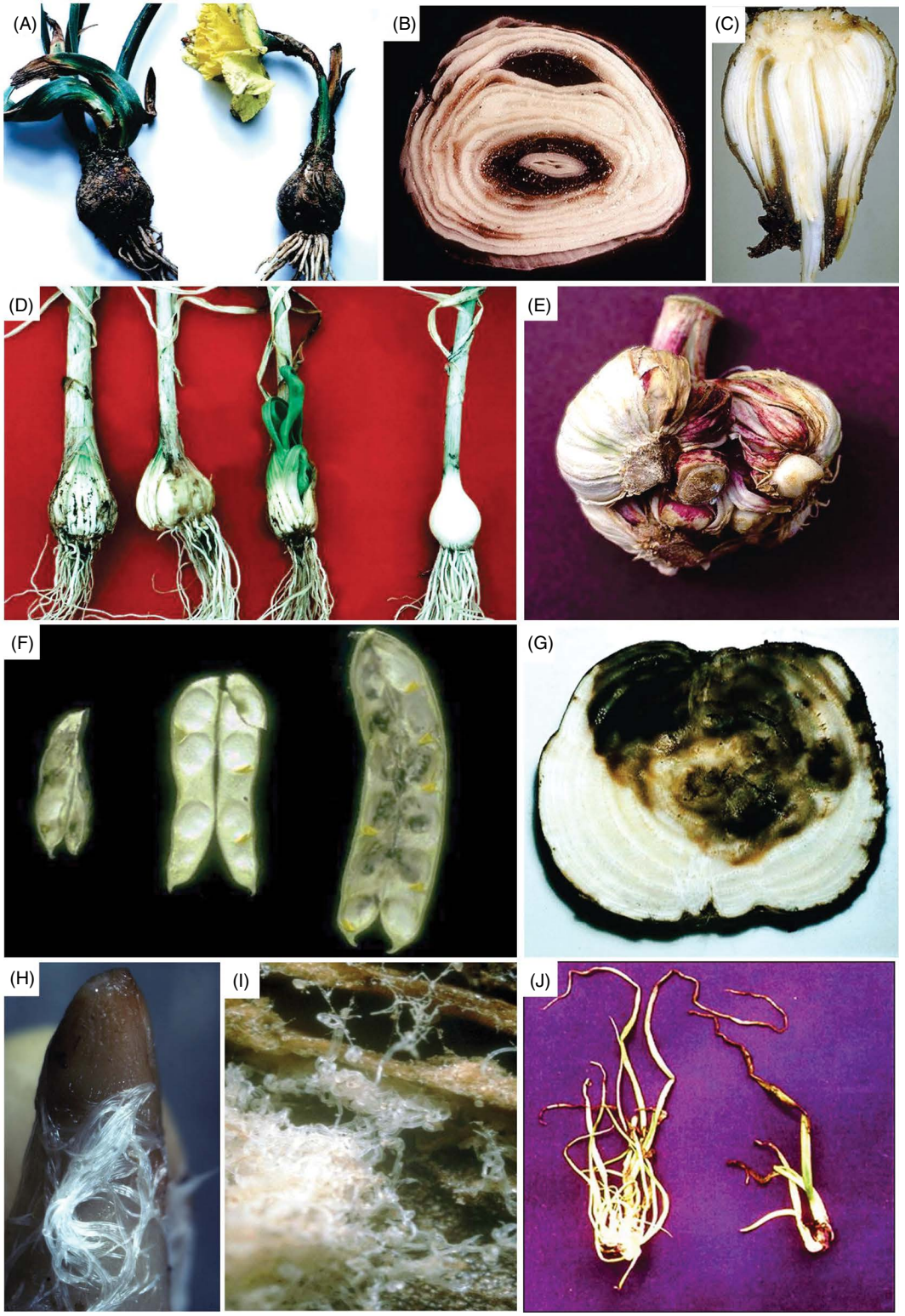

Fig. 1.13. Symptoms of *Ditylenchus dipsaci* on different plants. On narcissus bulbs (A); cross-section of narcissus (B,C); on garlic bulb (D,E); bean (F); cross-section of sugar beet (G); nematode wool (H); nematode in narcissus bulb tissue (I); young onion plants (J). [Courtesy A,D,E,H: G. Caubel, Nemapix, (1999); B: C.W. Laughlin, Nemapix, (2002); C,I: Central Science Laboratory, Harpenden, British Crown, Bugwood.org; F: Dr Augustin/Landesanstalt fur Pflanzenbau und Pflanzenschutz, Mainz, Bugwood.org; G: C. Hogger, Nemapix, (1999); J: E. Hennig, State Plant Health and Seed Inspection Service, Torun, Poland]

splits, revealing typical loose tissue beneath. The damage spreads downwards and often upwards on to the petals. In a severe infestation, the lesions extend down stems from leaf axils and growth may become retarded. On below-ground parts, the infestations start at the base of new bulbs, which arise as lateral offset buds from the base of the previous stems. The infection can be seen and detected on the removal of the outer brown scales as grey or brown soft patches on the outer fleshy scales. Infected tulip bulbs do not show brown rings as in narcissus and hyacinth (Southey, 1993).

7.8.4 Fabaceae (=Leguminosae)

FABA BEANS, VETCH, CHICKPEA, PEA AND LENTIL On leguminous crops, *D. dipsaci* induces swelling and distortion of stem tissue or lesions which turn reddish-brown and then black, depending on environmental factors and cultivar. Newly developed pods take on a dark brown appearance (Fig.1.13F). The lesions wrap the stem and increase in length, often progressing to the edge of an internode. Leaf and petiole necrosis are also frequent under heavy infestations but can be confused with symptoms induced by fungal pathogens. Infected seeds are darker, distorted, smaller in size and may have speckle-like spots on the surface. Heavy infestations regularly kill the main shoots, stimulating secondary tiller formation. The symptoms with greater severity are generally caused by the 'giant race' (Sikora and Greco, 1990). On faba bean (*Vicia faba*), *D. dipsaci* (now *D. gigas*) causes swelling or necrosis of the tissue. Infested stems of lentil and vetch (*Vicia* spp.) are also swollen and display shortened internodes. *D. dipsaci* induces confined necrosis on pea and complete necrosis of the stem on vetch (Caubel *et al*., 1998).

LUCERNE *D. dipsaci* is one of the most important nematode pests of lucerne (*Medicago sativa*). The infestation is more common in heavier soils and during high rainfall or in sprinkler-based irrigated areas. 'White flagging' related to loss of leaf chlorophyll is often a feature of infested crops under conditions of low moisture (Griffin, 1985). Infested fields exhibit irregular areas of sparse growth. Typical symptoms of nematode attack include basal swelling, dwarfing and twisting of stalks and leaves, shortening of internodes, and the formation of many axillary buds, producing an abnormal number of tillers that give the plant a bushy appearance (McDonald and Nicol, 2005). Infested plants occasionally do not grow high enough for hay (Ferris and Ferris, 1998) and they often fail to produce flower spikes (McDonald and Nicol, 2005). No symptoms of nematode infestation are observed in *Medicago* seeds.

CLOVER In general, the symptoms on clover are very similar to those expressed by *M. sativa*, except in red and white clovers. The nematode invades red clover in cool, rainy climates. Large, patchy areas of diseased plants appear in the field, with more diseased plants towards the inner side of the area. Central plants often show wilting (Cook and Yeates, 1993). The infested plants have swollen bases like bulbs and shrivelled papery leaves with conspicuously thick veins. Flower initiations are swollen like galls, and a single flower gall may comprise 5000 nematodes (Courtney, 1962). On white clover, the infected stems are swollen and short, buds are tufty, and the diseased parts become brown in summer or autumn. Leaves are slimmer than usual, and their petioles are shorter and thicker. Infested flower buds are swollen at their bases (Andrássy and Farkas, 1988).

7.8.5 Solanaceae

POTATO On potato, *D. dipsaci* produces a funnel-shaped rot, which expands further into the tuber, hence differing from the superficial rot produced by *D. destructor*. Leaves and stems are also invaded by *D. dipsaci* and this results in the characteristic stunting of the plant, supplemented by severe alteration of stems and petioles (Evans *et al*., 1993).

TOBACCO On tobacco, *D. dipsaci* enters the leaves and stems of seedlings during rainy weather and produces small, yellow galls (swellings) that may reach 40 cm or more above the soil. As disease progresses, the number of galls increases and plant tissue starts to die prematurely. The lower leaves may fall off and upper leaves may turn chlorotic. Galls ultimately rot and further growth is stopped in infected plants. In heavy soils, during cool and damp weather, the infested stems break down and the plants dislodge (Johnson, 1998).

7.8.6 Brassicaceae (=Cruciferae)

In mature *B. campestris*, severe crown rot may develop after infestation with *D. dipsaci*.

7.8.7 Other hosts

HYACINTHS The symptoms produced on hyacinth bulbs are similar to those on *Narcissus* spp., but prominent swellings are not typically seen on the plant leaves. The leaves may produce yellow streaks, distortion and frequently slight swelling (Southey, 1993).

STRAWBERRY *D. dipsaci* is the primary species of *Ditylenchus* that is regarded as an important pest of strawberry (Brown *et al*., 1993). The symptoms appear as small, twisted leaves with short, thick and distorted petioles.

BEETROOT Infestation by *D. dipsaci* results in the death of the growing point of seedlings and leads to the formation of multiple crowns. The cotyledons and leaves may become distorted, swollen and twisted. Galls may also develop on the leaves or petioles, usually on older plants. In severe conditions, feeding by the nematode on the crown may result in crown canker, crown rot or collar rot. The first noticeable symptom appears as raised, greyish pustules, generally among the leaf scars, followed by rotting outwards and downwards, expanding across the shoulder of the plant. Rotten crowns are easily detached when pulled (Cooke, 1993). The cross-section cutting of sugar beet root shows necrotic infection (Fig. 1.13G).

CARROT On carrot, the main infestation occurs on the root and stem of the plant around 1 inch (2.5 cm) below and above the ground level (Cooke, 1993). The symptoms may appear

as overlapped leaves and discoloration of the main root head below-ground. Serious damage causes the death of leaves and crown rot, particularly in autumn.

PHLOX AND OTHER ORNAMENTAL PLANTS On phlox, *D. dipsaci* causes typical thickening of shoots, brittleness of stems and shortening of internodes, which tend to split. Crinkling and reduction of the laminae of upper leaves and reducing of the uppermost leaf to attenuated filaments are characteristic and unique symptoms on phlox (Southey, 1993).

Other ornamental plants, such as *Anemone*, *Calceolaria*, *Cheiranthus*, *Gypsophila*, *Helenium*, *Heuchera*, *Lychnis*, *Lysimachia*, *Penstemon* and *Primula* spp., have been recorded as hosts of *D. dipsaci*, with symptoms including abnormal growth, swelling, rotting and failure to produce flowers (Roberts *et al*., 1981). Woody plants are tougher to attack than succulent plants, but *Hydrangea* may be invaded by SBN, causing alteration of non-woody shoots and swelling of petioles and main veins. Pronounced crinkling of leaf laminae also occurs. Another woody plant, *Yucca smaliana*, shows distortion and blister-like swellings on leaves (ISPM 27, DP-8, 2015).

7.9 Biology and life cycle

The life cycle is similar to that of other *Ditylenchus* species. Reproduction is by amphimixis and numerous generations can occur in a single growing season. Population growth can be very rapid, as the females have very high fecundity rate and may lay 200–500 eggs, from which the J_2 hatch within 2 days and develop into females within 4–5 days. Females live more than 10 weeks and the life cycle requires 19–23 days at 15°C (Duncan and Moens, 2013). Within onion and garlic tissue, the life cycle completes from egg to egg in approximately 21 days at 15°C. The best temperature range for egg development is 15–22°C (Becker and Westerdahl, 2018). The pre-adults or fourth-stage larvae can survive in freezing or extremely dry conditions in an anhydrous state as 'nematode wool' (Fig. 1.13H) for quite long periods, sometimes 20 years or more in plant tissue, leaves, stems, bulbs, seeds or in soil (Agrios, 2005). The rapid population growth of this nematode can result in severe crop damage even when the initial population density is low (Duncan and Moens, 2013).

7.10 Economic importance

D. dipsaci is one of the highly devastating plant-parasitic nematodes, mainly in temperate regions. The nematode can cause complete failure of host crops such as garlic, onions, cereals, legumes, strawberries and ornamental plants and affect the production of flower and bulbs. Sturhan and Brzeski (1991) observed that crop losses of 60–80% are not uncommon in severe infestations. In onion, up to 60% of seedlings died before attaining the planting stage in Italy. Losses of 50% were recorded on garlic in Italy and more than 90% in France and Poland.

7.11 Similarities to other species/conditions

In the first instance, *D. dipsaci* looks similar to other *Ditylenchus* species (*D. destructor*, *D. gigas*, *D. myceliophagus*, etc.). However, morphologically the number of lateral incisures (four), relatively long stylet, length of the postvulval sac and the pointed tail terminus are the differentiating characters of this species (Andrássy, 2007). *D. dipsaci* can be differentiated from *D. gigas* by the shorter body of females and the longer vulva–anus distance (Vovlas *et al*., 2011). In the lateral view, the spicule of *D. dipsaci* is more arched than in *D. destructor*. Several plants, such as lucerne, beets and clover, are infested by *D. dipsaci* and *D. destructor*, but the two species seldom occur together in the same plant (Andrássy and Farkas, 1988).

7.12 Movement and means of dispersal

Dispersal of SBN is primarily through infested plant material such as leaves, stems, bulbs and seeds, and infested soil. Contaminated agricultural tools and equipment, irrigation and splash water also help in dispersal and spread of *D. dipsaci* (Chitambar, 2018). Dissemination from one field to another or from one plant to another takes place through plant residues, water (flood or irrigation water), rain splash and stem and leaf contact. *D. dipsaci* lives as an endoparasite in stems, leaves, flowers, bulbs, tubers and rhizomes. The seed-borne nature of this nematode has also been reported by Sousa *et al*. (2003) and Sikora *et al*. (2005) in *V. faba*, *Medicago sativa* (alfalfa), *Allium cepa* (onion), *Trifolium* spp. (clovers), *Dipsacus* spp. (teasel) and *Cucumis melo* (melon). There is a possibility of dispersal of this nematode to new host areas through seeds from infested fields.

7.12.1 Plant parts liable to carry the nematode in trade/transport

Plant parts including roots, flowers, inflorescences, rhizomes, tubers, bulbs, cones, calyx, panicle (including ear heads), leaves, seedlings, micro-propagated plants, stems (above ground), shoots, tillers and true seeds are liable to carry all stages (eggs, juveniles and adult) of the nematode.

7.12.2 Plant parts not known to carry the nematode in trade/transport

Bark and wood.

7.13 Pest risk analysis and looming threat from introduction

At present, *D. dipsaci* is distributed throughout the world, but the distribution of different races is sporadic. *D. dipsaci* is categorized as a major quarantine nematode in many countries and in some countries official control measures have been implemented to limit the spread of this nematode. However, due to its wide host range, economic significance and seed-borne nature, imports of planting materials, stems, leaves, cut

flowers, bulbs, tubers, rhizomes and seeds of host plants from countries where this nematode occurs should be restricted.

7.14 Invasiveness rating

Considering the consequences of introduction and pest risk analysis, the following invasiveness rating has been assigned to *D. dipsaci*.

Climate/host interaction	–	High (3)
Hosts range	–	High (3)
Dispersal potential	–	High (3)
Economic impact	–	High (3)
Environmental impact	–	Medium (2)
Post-entry distribution	–	Low (–1)
Final rating (score)	–	High (13)

Based on the above invasiveness rating, *D. dipsaci* receives a score of 13 out of 15 and is rated as HIGHLY INVASIVE NEMATODE.

7.15 Region-wise status of invasiveness

Currently, *D. dipsaci* is on the 'Harmful Organisms' lists for many countries (USDA-PCIT, 2018). Region-wise countries where *D. dipsaci* is absent and attain invasive status are;

APPPC: Bangladesh, India, Indonesia, Sri Lanka, Taiwan, Thailand, Timor-Leste, United Arab Emirates, Vietnam
CAHFSA: Antigua and Barbuda, French Polynesia, Georgia, Grenada, Guatemala, Honduras, Nicaragua, San Marino
CAN: Cuba, El Salvador, Panama
COSAVE: Costa Rica
EPPO: Lebanon, Holy See (Vatican City State)
IAPSC: Egypt, Madagascar, Monaco, Namibia, Tanzania United Republic, South Africa, Uganda
PPPO: Cook Islands, New Caledonia

7.16 Management measures

7.16.1 Prevention and control

Preventing the nematode spread is an essential measure to save the crop from damage by *D. dipsaci*. Growers are strongly recommended to use certified nematode-free seeds and planting material. Hot-water treatments with different temperature–time regimes, depending on type and state of infected planting material and seed, are practically efficient for controlling *D. dipsaci* (Gratwick and Southey, 1972). Hot-water treatments of narcissus bulbs that may effectively control the nematode comprise either storage for 1–2 weeks at 25–30°C, followed by dipping in water for 24 h and hot-water treatment at 45°C for 4 h, or storage for 1–2 weeks at 25–30°C, followed by hot-water treatment at 47°C for 4 h (Hooper, 1991). Commercial cleaning of the seed along with associated plant debris has proved to be useful in removing nematode contamination.

7.16.2 Phytosanitary measures

See Chapter 16, section 115.