Heterodera ciceri

Scientific Name:
Heterodera ciceri Vovlas, Greco, and Di Vito, 1985

Common Name
Chickpea cyst nematode

Type of Pest
Plant parasitic nematode

Taxonomic Position (Siddiqi, 2000)
Class: Secernentea, **Order:** Tylenchida, **Family:** Heteroderidae

Reason for Inclusion in Manual
Cyst Nematode Survey

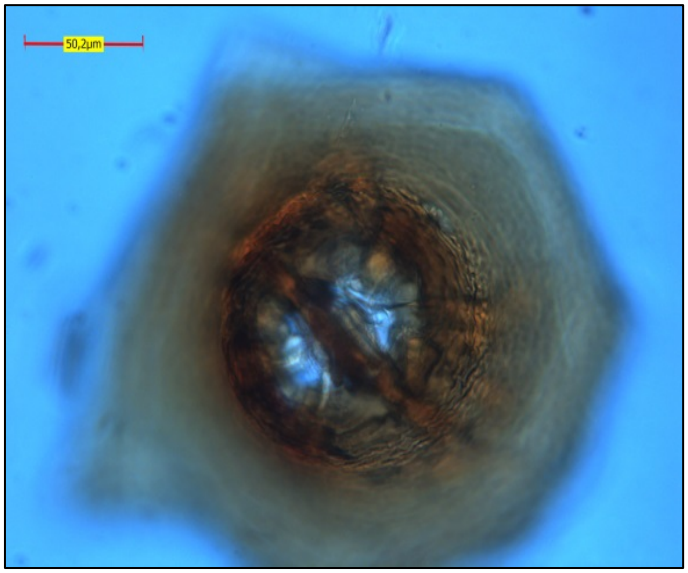

Figure 1: Perineal patterns of a cyst of *Heterodera ciceri* showing semifenestrae and bullae around them. Bar=50µM. Courtesy of Nicola Greco, CNR - Institute of Plant Protection, Bari, Italy.

Pest Description
Heterodera ciceri, the chickpea cyst nematode, is a severe pest of chickpea and a few other host plants. This nematode was initially reported as an unidentified *Heterodera* species by Bellar and Kebabeh (1983) during a survey of a chickpea crop in Syria.

As described in Vovlas et al. (1985):
Eggs: Eggs are oval, 123-143 µm long, and 48-53 µm wide. The egg shell is hyaline without surface markings. Second stage juveniles are folded four times within the egg shell.

Second-Stage Juveniles (J2s): J2's measure 440-585 µm in length. Other measurements include: Stylet = 27-30 µm, maximum width at mid-body = 21 µm, tail length = 53-72 µm, and hyaline tail-terminal = 31-42 µm.

The head is hemispherical and slightly offset, 4-5 µm in length and 8-9 µm in width with three post labial annules. An oral disc plate is dorso-ventrally elongated and two rounded lateral sectors bear large semilunar amphidial apertures. The stylet is robust with knobs 2-2.5 µm long, 4-5µm wide with concave anterior surfaces. Oesophageal glands are well developed and are 30-42 µm away from the head. The hemizonid is distinct and two annules long, with 1-2 annules anterior to the excretory pore. The oval genital primordium is 13-15 µm long, 10-13 µm wide, and rests 210-232 µm anterior to the tail tip. Cuticular annulations are distinct. Lateral field has four incisures, and 20-

25% of the body has aerolated outer bands. The tail is irregularly annulated with uniform tapering to end abruptly to a rounded terminus. The terminal hyaline portion is 48-58% of the tail length (Fig. 2).

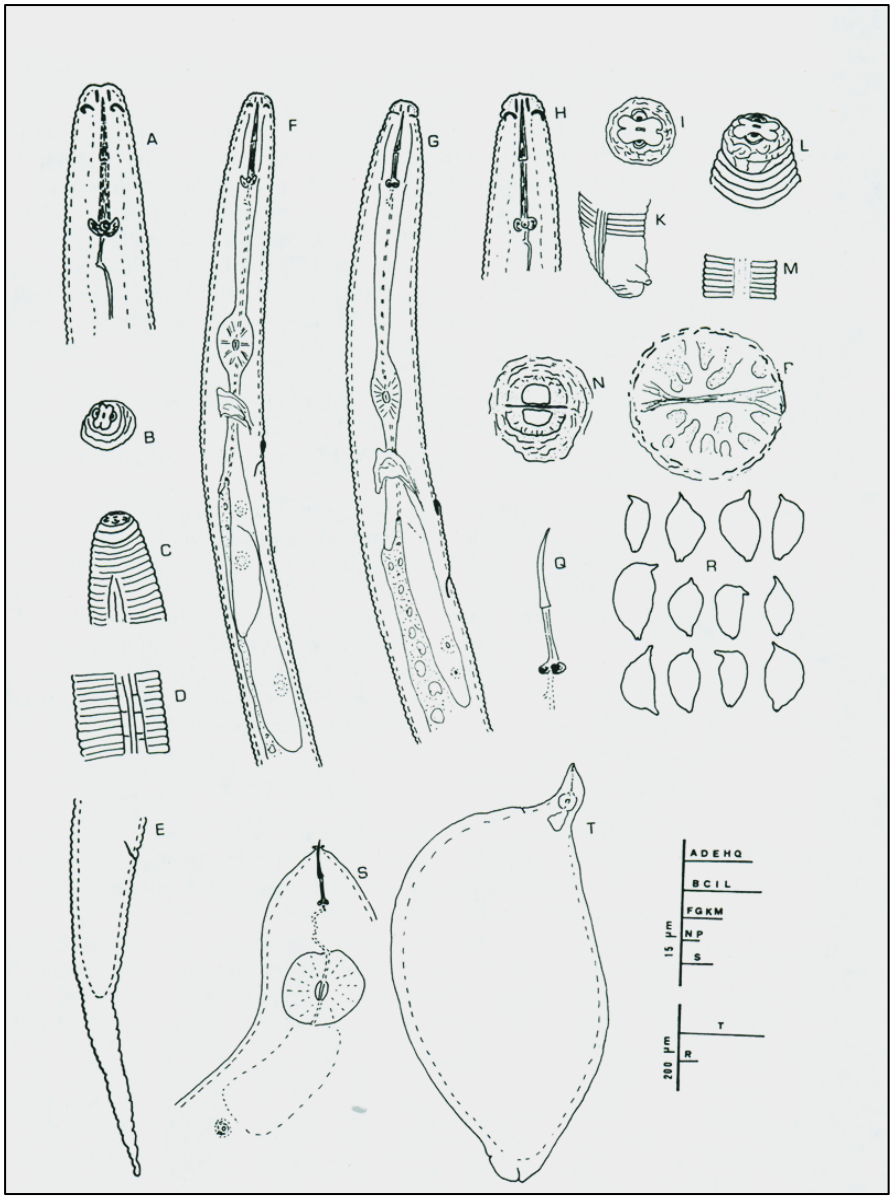

Figure 2. *Heterodera ciceri.* A) Anterior end of second stage juvenile; B), C) second stage juvenile lip pattern and profile; D) second stage juvenile mid-body lateral field; E) second stage juvenile tail; F, G) Oesophageal region of second stage juvenile and male respectively; H) Anterior end of male; I, L) Lip pattern and profile of male; K) Male tail; M) Male mid-body lateral field; N) Fenestrae and vulval slit; P) Bullae and underbridge; Q) Female stylet; R) Outline drawing of cysts; S) Anterior portion of female; T) Outline drawing of a gravid female body. Illustration from Vovlas et al.,1985.

Adults:
Females: The female is 773 µm (550-950 µm) long excluding the neck. The width is 451 µm (300-520 µm). The L/W ratio is 1.7 (1.5-2.0). The stylet measures 30 µm (29-31 µm) (Fig. 2). The DGO = 56 µm, and the excretory pore is 158 µm (154-169 µm) from the end of the body.

The female body is lemon-shaped with a well-defined neck and prominent terminal cone. The adult females are opaque in color, turning yellow to yellow-brown during tanning. There could be a thin, sub-crystalline layer covering the entire female body. A gelatinous matrix without eggs is present after the female becomes gravid. The vulva appears as a terminally positioned transverse slit. The anus is subterminal, located in a depression 7-10 µm in diameter. The cuticle has zig-zag ridges at mid-body, but the cuticle around the neck and cone regions consist of a series of unbroken lines which appear as concentric circles in SEM.

Males: The males are 1.308 mm (1.235-1.488 mm) long. Other measurements include: Maximum body width = 29-30 µm, stylet length = 29 µm (28-30 µm), spicule length along axis = 36 µm (34-38 µm), gubernaculum = 9-10 µm, head tip to excretory pore = 170 µm (153-180 µm).

The body is vermiform, typically in an open C-shape after heat relaxation, with a short bluntly rounded tail (Fig. 2). The tail is about 1/3 of the body width long, and there is a long (10-14 µm) cloacal tube ending in a circular opening. The head region is hemispherical, 5-6 µm long and 10 µm wide. It is offset from the body. The prestoma is rectangular and situated in the center of the slightly raised oral disc plate. The labial disc plate is partially fused with the rounded subdorsal and subventral pairs of lip sectors. Large amphidial apertures lie between the labial disc and the ellipsoidal lateral sectors. Posterior to the lip sectors, the head annules are irregular and incomplete, except for the basal annule, which is larger and less subdivided by longitudinal striae. The cephalic framework and the stylet are robust, the stylet having rounded basal knobs 5-6 µm wide. The DGO opens 5-6 µm behind the stylet knobs.

Cysts: The cysts are 796 µm (570-930 µm) long excluding the neck and 452 µm (350-550 µm) wide with a L/W ratio = 1.77 (1.56-2.37).

Cysts are typically lemon-shaped with a distinct neck and prominent cone. The neck could be off set and curved posteriorly. The cysts are well-ornamented with irregular zig-zag ridges, forming a fine network over the body. The excretory pore is 10-14 µm across within a depression encircled by 3-4 continuous cuticular ridges. Cysts are completely or partially covered with a thin, white subcrystalline layer.

Biology and Ecology
Nematodes are unsegmented roundworms. Most plant parasitic types are very small and feed on roots by means of a stylet, a hollow, needle-like structure used to pierce

Figure 3: A field of chickpea in Syria severely damaged by *H. ciceri.*
Photo courtesy of Nicola Greco, CNR - Institute of Plant Protection, Bari, Italy.

plant cells and withdraw nutrients. The lifecycle of chickpea cyst nematode, *H. ciceri*, is similar to other cyst nematodes. Six stages are involved in the life cycle of this nematode: an egg stage, four juvenile stages designated J1 to J4, and the adult stage. Adult females are lemon-shaped and are semi-endoparasites, parasitic organisms that have the posterior region of the body outside the host and the anterior region inserted inside the host on which they feed. After death, the cuticle of the female nematode tans to form a brown cyst that serves to protect the retained eggs. Cysts can remain viable in the soil for years, creating a problem for complete eradication from the field.

The first stage juvenile (J1) undergoes the first molt while still inside the egg and the second stage juvenile (J2) emerge from the egg. The J2, the infective stage, migrates through the soil and penetrates the host roots. Once inside, the J2s become sedentary, swell, and feed on specialized stellar cells in the roots while they complete development (Greco et al., 1992). At 20°C, Kaloshian et al. (1986) observed J2 in chickpea roots five days after sowing pre-germinated chickpea seeds in soil infested with 20 eggs/cm^3 of *H. ciceri*. J3 were observed nine days after sowing. Adult vermiform males were observed 19 days after sowing. J2, J3, and J4 males developed inside the root tissue, but the J4 females ruptured the tissue and were visible externally. Twenty days after sowing, well developed eggs were observed in the females. By thirty seven days after sowing, females had an average of 256 eggs, and began to change color. Forty-eight days after sowing, half of the female population within the roots was brown cysts (Kaloshian et al., 1986). At 68 °F (20 °C), *H. ciceri* development from J2 to appearance of cysts is complete by 36 days (Greco et al., 1992). The life cycle of *H. ciceri* requires a minimum

temperature of 50 °F (10 °C) to be completed, but root invasion by J2s can occur at as low as 46 °F (8 °C). Early spring would lead to massive root invasion and development, but low soil moisture in late spring would decrease egg hatching, therefore likely limiting *H. ciceri* to one generation per growing season (Kaloshian et al., 1986).

Symptoms and Signs

The chickpea cyst nematode is a particularly aggressive legume pest (Fig. 3) (Greco et al., 1992; Gaur et al., 2011). Symptoms of infection are most visible at the flowering stage of the host. Host roots infected with *H. ciceri* are poorly developed, have small necrotic spots, and lacks *Rhizobium* nodules (Fig. 4) (Greco et al., 1992; Gaur et al., 2011). Above-ground symptoms are not specific, but instead are typical of any plant experiencing root damage, including stunting, lower yield, yellowing, and early senescence. Adverse effects are observable in soils with *H. ciceri* infestations exceeding 1 egg/cm^3 soil. Higher population densities lead to higher yield losses, up to 100% in soil infested with 32 eggs/cm^3 (Fig. 5). In addition to overall seed yield, chickpea nematode infections suppress the protein content of host seeds (Greco et al., 1986).

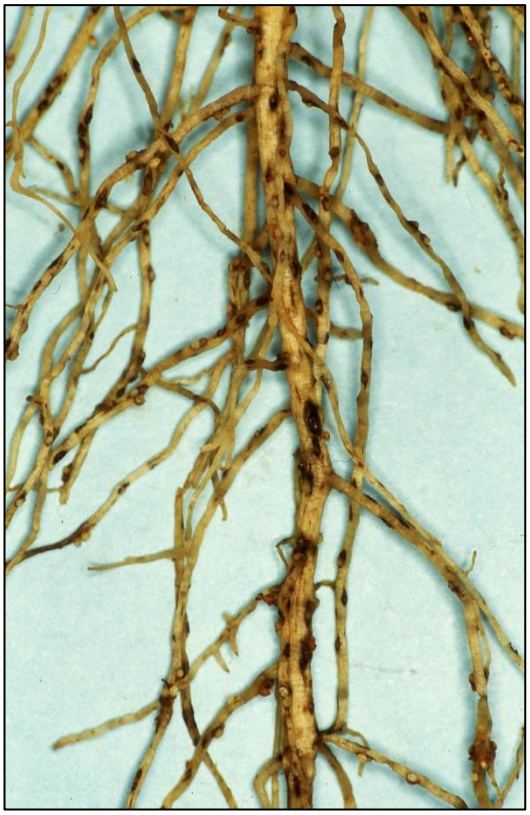

Figure 4: *Heterodera ciceri* females on a root of chickpea. Courtesy of Nicola Greco, CNR - Institute of Plant Protection, Bari, Italy.

Mature females and cysts are just visible to the naked eye and can be seen as minute, lemon-shaped bodies on the root surface (Fig. 4).

Pest Importance

Legume crops are important staple crops in the United States and worldwide. In 2011, chickpea harvests topped 131,000 acres, lentil harvests topped 460,000 acres, and common pea harvests topped 159,000 acres in the United States alone (NASS, 2012). Introduction of *H. ciceri* to these fields could cause detrimental effects to production and yield. None of the 6,000 lines of chickpea tested so far have demonstrated any resistance to the chickpea nematode. Surveys, conducted in Syria to determine the initial spread of *H. ciceri* in 1984 and 1988, found this nematode on 30% of chickpea and 39% of lentil fields. In the most heavily infected areas, nearly all fields were infested (Greco et al., 1992). In greenhouse experiments, some wild *Cicer* species lines (*C. bijugum, C. pinnatifidum, C. reticulatum*) allow very low reproduction of the chickpea nematode, and were deemed resistant (Di Vito and Vovlas, 1990; Di Vito et al., 1996). Back crossing investigations continue to develop resistant lines, but so far no resistant chickpea cultivar is available (Di Vito et al., 1996).

Figure 5: Microplots containing non-infested soil (left) and soil infested with 64 eggs of *H. ciceri* per gram of soil (right) and sown with chickpea. Photo courtesy of Nicola Greco, CNR - Institute of Plant Protection, Bari, Italy.

Heterodera ciceri is listed as a harmful organism in Brazil (USDA-PCIT, 2013). There may be trade implications with this large trading partner if this nematode becomes established in the United States.

Known Hosts

Major hosts: *Cicer arietinum* (chickpea), *Cicer* spp., and *Lens culinaris* (lentil).

Minor hosts: *Lathyrus sativa* (grasspea) and *Pisum sativum* (pea).

Experimental hosts: *Dianthus caryophyllus** (carnation), *Lupinus albus** (lupin), *Medicago rigidula** (annual medics), *Medicago sativa** (alfalfa), *Phaseolus vulgaris** (bean), *Trifolium incarnatum** (crimson clover), *Trifolium pretense** (red clover), *Vicia faba** (faba bean), and *Vicia sativa** (vetch) (Greco et al., 1986; Greco et al., 1992; Di Vito et al., 1996; CABI, 2010).

*marked species are considered very poor hosts for *H. ciceri.* Reproduction rates of the nematode on these hosts are generally less than one.

Known Vectors or Associated Organisms
Heterodera ciceri is not known to be vectored by any other organism, nor is it known to vector any other pathogens.

Known Distribution
Asia: The nematode has been reported only in Middle Eastern countries, including Jordan, Lebanon, Syria, and Turkey (Greco et al., 1992, SON, 2003; CABI, 2010).

Europe: A report by CABI (2010) of occurrence of this nematode in Italy and Spain was disproved by Greco and Inserra (2007). This nematode pest does not occur in Europe.

Pathway
Heterodera ciceri can be transferred to new areas via contaminated soil debris or plant material, primarily roots (SON, 2003). Greco et al. (1992) suggest that increased traffic of machinery and personnel between the United States and the Middle East could increase the likelihood of accidental introduction of the pest. This suggestion was put forward referring to the 1991 Persian Gulf conflict, but it is just as relevant now in light of the United States presence in the Middle East.

There have been 11 shipments of *Cicer* spp. propagules since 2003 from host countries (AQAS, 2013). All of these shipments appear to be seed, however, and *H. ciceri* is only known to infect root material. There have also been 12 shipments of *Lens* spp. propagules from host countries since 2003 (AQAS, 2013). Two of those shipments contained plant material, and the rest was seed. There were also shipments of *Lathyrus* spp. (10) and *Pisum* spp. (8) propagative material from host countries since 2003 (AQAS, 2013), but they all appear to be seed.

Potential Distribution within the United States
A recent risk map developed by APHIS-PPQ-CPHST, based on the presence of susceptible host plants, shows that areas of California, Colorado, Idaho, Iowa, Kentucky, Michigan, Minnesota, Montana, New York, North Dakota, Ohio, Oklahoma, South Dakota, Utah, and Washington are at the highest risk from this nematode pest. Despite the lack of host plants, Greco et al. (1992) stress that the soils of Florida combined with local outdoor climate could favor nematode development and reproduction, suggesting that populations could become established on the few host plants that are grown in the region.

Survey
CAPS-Approved Method*:
Use soil sampling, collection of host roots, or of a combination of both methods.

Soil sample: send sample to a nematology diagnostic lab where nematodes will be extracted from the soil and identified (preferred method). Laboratory methods that are acceptable for cyst extraction include sugar centrifugation, USDA cyst extractor,

Fenwick can sieving, wet sieving, and elutriation. Cobb's sieving technique has been used regularly to extract second stage juveniles from the soil.

Collect host roots: Send sample to nematology diagnostic lab where nematodes will be extracted and identified.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:
Soil sample: Collect soil and extract nematode samples via the Cobb decanting and sieving method or the Fenwick can method as described in Methods and Techniques for Nematology (van Bezooijen, 2006). Vovlas et al. (1985) and Kaloshian et al. (1986) used these methods to extract the nematode from the soil in Syria.

Key Diagnostics/Identification

CAPS-Approved Method*:
Morphological: Characteristics of second-stage juveniles, males, females, and cysts (Vovlas et al., 1985).

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:
Morphological: In addition to the original description of *H. ciceri* from Vovlas et al. (1985), Abiodou et al. (2005) compares the morphometric variation of cysts and second stage juveniles between multiple populations of *Heterodera* species in the *H. avenae* group.

Molecular: Clapp et al. (2000) developed a technique for rapid identification of *Heterodera* spp. based on ITS2 sequence variation detected by PCR single-strand conformational polymorphism (PCR-SSCP). Restriction fragment length polymorphism analysis has been developed for distinguishing between nematodes in the genus *Heterodera* (Subbotin et al., 2000; Sabo et al., 2001; Madani et al., 2004). The analysis is conducted on ribosomal DNA in the internal transcribed spacer region with multiple restriction enzymes. *Heterodera ciceri* has specific RFLP profiles for numerous restrictions enzymes that are described and compared to other species in the *Heterodera* genus (Subbotin et al., 2010). Imren et al. (2012) characterized 46 populations of *H. ciceri* using ITS sequencing.

Easily Confused Species

Heterodera ciceri is part of the large *H. schachtii* group of cyst nematodes. This group is similar in their lemon-shaped ambifenestrate cysts, long vulval slits and well developed bullae and underbridge. Among these similarities are specific differences that distinguish *H. ciceri* as a separate species. *H. ciceri* differs from *H. trifolii* in the presence of males, the host range, and in the fenestral measurements. *H. ciceri* differs

from *H. rosii* mainly in fenestral measurements, the number of annules, and the stylet length of second stage juveniles. Also, a main host of *H. rosii*, *Rumex crispus*, is not a host of *H. ciceri*, and the leguminosae hosts that are very conducive to *H. ciceri* growth are very poor for *H rosii*.

H. ciceri differs from *H. daverti* by the longer body length and longer stylet in J2, and more anterior stylet knobs. *H. ciceri* also has a longer underbridge and has a different host range that *H. daverti*.

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Updates

July, 2014: Updated the Key Diagnostics section and added more references to the literature-based methods section.

July, 2016: Deleted figure 6.