Heterodera cajani

Scientific Name:
Heterodera cajani Koshy, 1967

Synonyms:
Heterodera vigni Edwards & Misra, 1968

Common Name
Pigeon-pea 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: A Cyst of *Heterodera glycines* containing eggs. This cyst is very similar in appearance to *H. cajani* cysts. Photo courtesy of Agroscope FAL Reckenholz Archive, Swiss Federal Research Station for Agroecology and Agriculture, Bugwood.org

Background
The genus *Heterodera* contains at least 80 species, some of which cause serious yield reduction in crops (Subbotin et al., 2010). The protective cyst stage of these nematodes enables them to withstand desiccation and greatly enhances their dispersal and survival (Waeyenberge et al., 2009). There are seven main morphological groups within the genus *Heterodera*: *Afenestrata*, *Avenae*, *Cyperi*, *Goettingiana*, *Humuli*, *Sacchari*, and *Schachtii*. *Heterodera cajani* is a member of the *Schachtii* group. Morphologically, its closest known relatives are *H. medicaginis* and *H. mediterranea* (Subbotin et al., 2010).

Pest Description
Heterodera cajani, the pigeon-pea cyst nematode, is the causal agent of pearly root disease on affected hosts. The nematode was originally found on a pigeon pea crop in New Delhi India by Koshy in 1967. The nematode was initially described but misidentified as *H. trifoli* by Swarup et al. (1964) (Koshy et al., 1971; Kannan and Lingaraju, 1999). Further studies showed that this nematode was indeed a new species *H. cajani* (Koshy 1967). The original description of the species has been supplemented by Koshy et al. (1971) and Sharma and Swarup (1984). *Heterodera vigni* described

from roots of *Vigna unguiculata* (cowpea) by Edward and Misra (1968) has been recognized as a junior synonym of *H. cajani* (Kalha and Edward, 1979). Redescription of the pigeon pea cyst nematode is given by Olia and Ali, 2004.

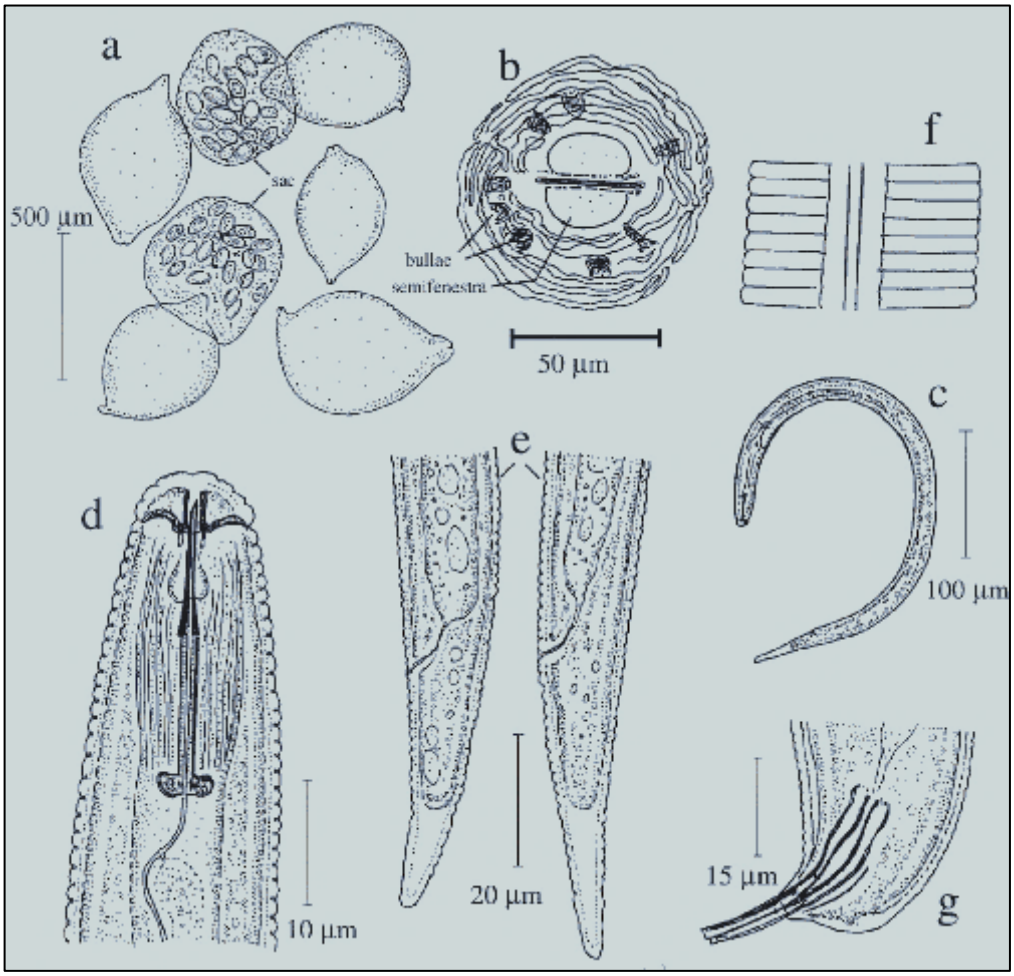

Figure 2: *Heterodera cajani* (a) Egg sacs; (b) Vulval cone of a cyst; (c) second stage juvenile (J2); (d) J2 second stage juveniles with stylet; (e) J2 T tail of second stage juvenile; (f)J2 lateral field s of juveniles; (g)Copulatory system of a male showing spicules with bidentate tip. Illustration courtesy of Catherine Githure, ECOPORT Foundation.

Eggs: Eggs are oval, 94 to 97 µm long, and 41 to 47 µm wide. The egg shell is hyaline without surface markings. Eggs are retained in the cyst or are enclosed in an egg sac produced by the female. The size of the egg sac varies between 0.5 to 2 times the size of the cyst. Other species in the *Schachtii* group have egg sacs not more than one cyst size. The color of the egg sac is usually yellow but can be purple in color occasionally. There may be a few to 200 eggs (average 54) in the egg sacs (Koshy et al., 1971).

Second-Stage Juveniles (J2s): J2's length measures 0.435 ± 0.05 mm (0.345 to 0.515 mm). Other measurements include: a= 23.9 ± 0.27 (18.3 to 28.9), b=4.1 ± 0.08 (3.2 to

5.3), b'=3.1 ± 0.04 (2.5 to 3.7), c=9.7 ± 0.13 (8.0 to 12.3), stylet= 25.6 ± 0.12 µm (23.1 to 27.0 µm), tail=45.2 ± 0.64 µm (32.0 to 52.0 µm), and hyaline tail-terminal= 23.8 ± 0.37 µm (17.0-30.0 µm) (Koshy et al., 1971).

The body is cylindrical, elongate, tapering anteriorly and posteriorly but more so posteriorly. Head is offset, bearing four annules with the first two anterior to the cephalic constriction more prominent than others. Cuticular annulation is distinct, the annules about 1.6 µm apart at mid-body. Lateral field with four incisures, covering approximately 1/4th body width. The cephalic framework is heavily sclerotized. Anterior cephalids are located one annule behind the cephalic constriction and posterior cephalids are located at 8th annule. The stylet is strong, well developed with anteriorly directed knobs, the width being more than the height (Fig. 2). Dorsal oesophageal gland opening is 4.0 to 5.0 µm behind the stylet base. The procorpus is a slender tube ending in a distinct median bulb with well-developed valve plates. The basal part of the oesophagus extends as a glandular lobe overlapping the anterior region of the intestine, mostly laterally. The hemizonid is distinct, located one annule anterior to the distinct anal excretory pore. The tail and hyaline tail-terminal vary in length, with a bluntly rounded narrow terminus; stylet/hyaline tail terminal ratio 1.0 (0.8 to 1.2) and inconspicuous phasmids (Koshy et al., 1971).

Adults:

Females: The female is 569 ± 4.01 µm (480 to 640 µm) long including the neck. The width is 378 ± 3.18 µm (285 to 480 µm). The L/B ratio is 1.52 ± 0.02 (1.25 to 1.93). The stylet measures 26± 0.19 µm (25 to 28 µm) (Koshy et al., 1971).

The body is swollen and pearl-white in color. Females are basically lemon-shaped with protruding neck and vulva. The cuticle is thick and exhibits a zig-zag pattern externally. The head is distinctly set off from the neck, bearing two prominent annules. The second annule is larger than the first and generally disc shaped. Cephalic sclerotization is weak. There is slight dorsal curvature to the rather delicate stylet with knobs that slope posteriorly. The median bulb is large, occupying ½ of the body width across the valve. Oesophageal glands apparently contained in a single lobe and variable in size and shape. Excretory pore is distinct and located at a level posterior to the end of the oesophageal glands, usually about 127 µm from the anterior end. The two ovaries of the female are convoluted and are almost indistinguishable due to presence of eggs. The vulva is conspicuous and protrudes posteriorly. An egg sac is present. The anus is distinct and located approximately 81 to 84 µm anterior to the vulva. The female turns into a brown-colored cyst upon death (Koshy et al., 1971).

Males: The males are 1.09 ±0.01 mm (0.78 to 1.28 mm) long. Other measurements include: a=41.1 ± 0.50 (31.6 to 51.4), b=7.6 ±0.16 (5.8 to 11.8), b'=6.2 ± 0.11 (5.0 to 9.0), c=115.9 ± 1.7 (84.0 to 137.0), stylet=28.0 ± 0.20 µm (27.0 to 30.0 µm), T=57.5 ± 1.53 mm (18.0 to 76.5 mm), spicules=34.0 ± 0.97 µm (31.0 to 41.0 µm), and gubernaculum=10.6 ± 0.7 µm (8.0 to 12.0 µm) (Koshy et al., 1971).

The body is cylindrical, elongate, and tapering gradually at the anterior ends. The head is offset from the body with 4 to 5 distinct annules on the lip region. Cephalic sclerotization is heavy. Anterior cephalids usually on the second annule from the head constriction; posterior cephalid on the 8th to 10th annule. Cuticular annulation of body is distinct; approximately 2 µm apart. The lateral field is marked by four incisures without prominent areolation, occupying about ¼ to 1/5 of body width. Stylet is strong with slightly anteriorly directed knobs. The dorsal oesophageal gland opening is 4 to 6 µm behind the stylet base. The procorpus is a slender tube ending in a distinct median bulb with well-developed valve plates. A nerve ring encircles the isthmus, and the basal part of the oesophagus extends as a glandular lobe overlapping the anterior regions of the intestine, mostly laterally.

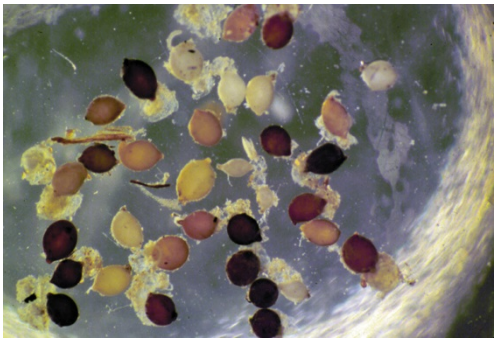

Figure 3: *Heterodera schachtii* cysts. *H. cajani* cysts have similar characteristics. Courtesy of Howard Ferris, UC Davis.

The excretory pore is generally opposite to middle of posterior oesophageal gland. Hemizonid one annulle anterior to the excretory pore. There is a single testis that outstretches anteriorly. There are paired bidentate spicules between 31 and 41 µm in length. The males have a simple rod shaped gubernaculum, 8 to 12 µm in length. The tail is very short, bluntly rounded with a slight dorsal curvature. The phasmids are not clear. There may also be abnormally short males (0.48 to 0.65 mm) present in the population that still have the capacity to reproduce (Koshy et al., 1971).

Cysts: The cysts of *H. cajani* protect the embryonated eggs containing the second stage juveniles from adverse conditions (Fig. 1, 2). The cysts are 0.546 ± 0.06 mm (0.39-0.69 mm) long and 0.354 ± 0.05 mm (0.175 to 0.55 mm) wide with a L/B ratio=1.58 ± 0.02 (1.00 to 2.50). Cysts are light to dark brown and similarly shaped to live females (lemon-shaped with a protruding neck and vulva (Fig.3). In some cases, the vulval cone tapers gradually into the cyst; while in others it is set off more abruptly and is prominent. Subcrystalline layer is present on young cysts. The cyst wall markings on the main body are “short, broken, angularly arranged lines, showing the typical zig-zag pattern”. The vulval cone has numerous “concentric lines surrounding the vulval slit and fenestrae”. Punctations are irregular and scanty, visible only under high magnification at a lower depth than the zig-zag pattern. Fenestra on vulval cone-top are of ambifenestrate type with two semifenestrae separated by the vulval bridge; length of fenestra 39.4 ± 0.65 µm (31 to 51 µm); width of fenestrae 31.8 ± 0.49 µm (25 to 40 µm); length of vulval slit 45.9 ± 0.38 µm (39 to 51 µm). The anus is indistinct. Prominent dark brown bullae present in the vulval cone region below the underbridge (Koshy et al., 1971).

Subbotin et al. (2010) contains detailed morphometrics of cysts, females, and J2s of *Heterodera cajani*.

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 plant cells and withdraw nutrients. The lifecycle of the pigeon-pea cyst nematode, *H. cajani*, 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 of plant roots. Females do not move after establishing a feeding site in root tissue. After death, the cuticle of the female nematode tans to form a brown cyst that serves to protect the retained eggs, although numerous eggs are also laid in an external gelatinous matrix by the females before dying. Cysts are the survival stage and 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) emerges from the egg. The J2, the infective stage, seeks a host root and penetrates the cortex (usually within 48 hours), which leads ultimately to growth reduction and yield loss (Koshy and Swarup, 1979; Kannan and Lingaraju, 1999). The vermiform (worm-like in shape) nematode then becomes sedentary and feeds via specialized trophic cells (syncytia) formed in the stele of the roots by the host in response to secretions from the nematode. Developing nematodes become increasingly obese and molt to the J3 usually around the 6th day. Ultimately, the J4 stage is reached. If the J4 molts to the female, it remains in position within the root cortex. If the J4 molts to the vermiform male, it escapes from the J4 cuticle and the root and searches for females to mate with. Though males are encountered in large numbers, the nematode can reproduce even in the absence of males (Koshy and Swarup, 1971a; Sharma and Swarup, 1984).

Koshy and Swarup (1971e) found that *H. cajani* is capable of causing infections on cotyledons and stems of pigeon pea seedlings. Mature adult females, however, did not develop but adult males were plentiful. The authors hypothesized that the juveniles did not develop to adult females and instead mostly turned into adult males due to stress conditions such as lack of nutrition in the aerial plant parts.

The life cycle of *H. cajani* is completed within 16 days at 29°C (84°F), and there can be many generations (up to nine possible) in a single growing season (Koshy and Swarup, 1971a, b; Koshy and Swarup, 1979). At 25.5^{o}C, 28-29 days are necessary for this nematode to complete a lifecycle (Senthamizh et al., 2005). Under cool conditions (10-25°C; 50-77°F), the time required for *H. cajani* to complete one generation, however, was extended to 45 to 80 days (Koshy and Swarup, 1971a).

H. cajani prefer soils called vertisols and are most damaging in this soil type (Sharma et al., 1992a; Elyas and Sharma, 1997). This type of soil has little organic material, has high clay content, and can shrink and or expand depending on the season and moisture content. Rao et al. (2011) indicate that the nematode is particularly widespread in sandy loams in northern India and vertisols in southern India. Other soil textures are thought to influence nematode mobility, gas exchange, water retention, and microbes

antagonistic to *H. cajani*. The highest populations of juveniles and eggs appear during crop maturity and crop harvest. Rates of decline after harvest are dependent on depth of soil. Populations of emerged juveniles deeper than 15 cm (5.9 in.) were not affected by summer fallow (Sharma and Nene, 1992).

Juvenile emergence from cysts takes place between 20-35°C (68-95°F) with an optimum of 28°C (82.4°F) (Sharma and Swarup, 1984). Koshy and Swarup (1971d), however, observed emergence of juveniles at a slightly different range (from 15 to 37°C (59-98.6°F) with an optimum of 29°C (84.2°F). Emergence from egg sacs is not as closely related to temperature (Sharma et al., 1992b). The J2s also emerge at a wide pH range of from 3.5 to 11.5 with 10.5 being optimal (Koshy and Swarup, 1971d). Root leachates stimulate emergence of J2s from cysts (Koshy and Swarup, 1971d). Juvenile emergence is more efficient from egg masses than from cysts. Koshy and Swarup (1971d) observed that over a 20-day period, 100% of larvae emerged from egg masses as compared to 89% and 16% from white and brown cysts, respectively.

The hatching response of cysts is also dependent on the age of the host plant. As the plant senesces due either to age or to pathogenic damage, females lay eggs that are more dependent on the stimulus of root leachate to hatch (Gaur et al., 1992; 1996). This is hypothesized to act as a survival measure during periods with the host is not present (fallow or intercropping periods). Older plants produce less root leachate, so hatching dependence on this chemical ensures that the next generation does not hatch until the host plant conditions are optimal (Gaur et al., 1992). Second stage juveniles within the cyst from later generations also have greater lipid reserves than those in the egg sacs, which will also aid in survival (Gaur et al., 1992). The areas of the world where *H. cajani* is present are characterized by lack of moisture and high temperature. Guar et al. (1996) also demonstrated experimentally that the *H. cajani* cysts are able to retain viability through extreme periods of dessication. Koshy and Swarup (1971d) showed that cysts stored in air-dried soil under laboratory conditions remained viable for a period of at least two years.

Singh and Sharma (1995) measured infectivity, development, and reproduction of *Heterodera cajani* on pigeon pea at a range of soil moistures (16, 24, 32, and 40%) at 20-25°C (68-77°F). The greatest percentage of juveniles that penetrated roots (34.3%) and greatest percentage of females per root system (79.6%) at four weeks after soil infestation occurred at 24% soil moisture when averaged across temperature. Optimal reproduction for *H. cajani* was observed at 24% soil moisture and 25°C (77°F) (Singh and Sharma, 1995).

Intraspecific variation and the occurrence of three races based on host differential reaction have been reported with this nematode (Walia and Bajaj, 1986, 1988; Siddiqui and Mahmood, 1993; Subbotin et al., 2010). The pigeon pea (*Cajanus cajan*) race (race A) develops on all plants, whereas the other races do not reproduce either on cluster bean (*Cyamopsis tetragonolobus*) (race B) or cluster bean and sunn hemp (*Crotalaria juncea*) (race C). Walia and Bajaj (2000) found that these races could be distinguished by vulval cone structure and male morphology (Subbotin et al., 2010).

Another study discriminated three races among 14 populations from seven districts of Uttar Pradesh, India (Siddiqui and Mahmood, 1993). Use of host differentials of cowpea, mung bean, soybean, and pigeon pea accessions confirmed the presence of races of *H. cajani* (Mehta and Bajaj, 2005). Rao et al. (2011) used an Amplified Fragment Length Polymorphism (AFLP) and morphometric procedures to compare interspecific variation in *H. cajani* populations in India.

Symptoms and Signs

The main symptoms of an *H. cajani* infection are stunted growth and reduced yield due to damage to the host plant's root system. A reduction in height and vigor of the infected plants can be discerned by careful comparison with healthy plants. Stunting, reduced leaf lamina size, and yellowing on cotyledonary leaves may or may not be readily visible. Flowers and pods are reduced in size and number, and the root system may also be poorly developed. Yellowing (chlorosis) is also common after an extended infection.

In sesame, reduction in shoot length and weight, root length and weight, number of capsules and in chlorophyll content are specifically observed in soils infested with *H. cajani* in India. Poor seed setting in the locule is also seen with plants devoid of productive branching. In most cases, plants were found with a small, single mummified capsule at the plant tip. These capsules were found to be filled with only six seeds per locule whereas 112 seeds were observed in plants in uninfested soil (Balasubramanian and Vadivelu, 2003). In cowpea, *H. cajani* can cause delays in leaf emergence, delayed differentiation of flowers and pods, and a reduced number of flowers and pods per plant (Aboul-Eid and Ghorab, 1974).

The first symptoms of nematode parasitism are evident after 30 to 45 days, around that time the pearly white lemon-shaped females start appearing on the roots of infected plants (Sharma et al., 1992b). Brown cysts can also be observed in the soil (Fig. 3). Plant height can be reduced by as much as 34%.

Damage due to *H. cajani* infection is caused by the J2s as they feed intracellularly on the elongation region of the growing root. The females also damage the roots as the formation of syncytial cells around their cephalic region interrupt xylem and phloem elements, thus hampering root efficiency (Aboul-Eid and Ghorab, 1974; Sharma et al., 1992b).

Pest Importance

Legume crops are important staple crops worldwide. Chickpea was harvested on 131,000 acres, kidney beans on 47,000 acres, and the common pea on over 159,000 acres in 2011 in the United States (NASS, 2012). *H. cajani* could have detrimental effects to production and yield if introduced to these fields. In India, it was revealed that little resistance is currently available within pigeon pea germplasm. Although tests are being conducted in wild pigeon pea and other hosts for resistance genes to *H. cajani*, most of the tested 7,000 accessions of pigeon pea germplasm were determined to be susceptible or highly susceptible to *H. cajani* (Elyas and Sharma, 1997).

In untreated potted *Sesamum* (sesame) plants growing in soil infested with *H. cajani*, shoot length, shoot weight, root length, root weight, number of capsules, and chlorophyll content were suppressed by 65.5, 80.6, 55.9, 86.5, 84.4, and 49.7%, respectively, when compared with treated pots (Balasubramian and Vadivelu, 2003). In cowpea in Egypt, infestation by *H. cajani* resulted in 34% reduction in plant height, 52% reduction in flowering buds, and a 70% reduction in the total number of dry pods (Aboul-Eid and Ghorab, 1974). The largest reported losses in yield induced by the nematode are about 80% (Kannan and Lingaraju, 1999).

Heterodera cajani is listed as a harmful organism in Brazil and Ecuador (USDA-PCIT, 2014). There may be trade implications with these countries if this nematode becomes established in the United States.

Known Hosts

Major hosts:

Cajanus cajan (pigeon pea), *Cajanus crassus*, *Cajanus platycarpus* (wild pigeon pea), *Cicer arietinum* (chickpea), *Cyamopsis tetragonoloba** (cluster bean, guar), *Desmodium triflorum* (threeflower ticktrefoil), *Flemingia strobilifera* (wild hops), *Glycine max** (soybean), *Lablab purpureus* (hyacinth, lablab bean), *Macrotyloma uniflorum* (horsegram), *Macroptilium atropurpureum* (purple bush bean), *Macroptilium lathyroides* (wild bush bean), *Phaseolus acutifolus* (tepary bean), *Phaseolus lunatus* (sieve bean), *Phaseolus vulgaris* (kidney bean), *Phyllanthus maderaspatensis* (Madera leaf flower), *Pisum sativum** (common pea), *Sesamum indicum* (Indian sesame), *Sesamum orientale* (sesame), *Sesbania aculeata* (dhaincha, dunchi fiber), *Vicia narbonensis* (purple broad vetch), *Vicia sativa* (garden vetch), *Vigna aconitifolia* (moth bean), *Vigna mungo* (black gram), *Vigna radiata* (green gram, mungbean), *Vigna trilobata* (African gram), *Vigna umbellata** (rice bean), and *Vigna unguiculata* (cowpea) (Koshy, 1967; Bhatti and Gupta, 1972; Janarthanan, 1972; Koshy and Swarup, 1972; Verma and Yadav, 1975; Guar and Sing, 1977; Dalal and Bhatti, 1983; Sharma and Nene, 1985; Walia et al., 1985; Zaki and Bhatti, 1986; Sharma et al., 1992b; Jain et al., 1994; Luc, 1996; Balasubramanian and Vadivelu, 2003; SON, 2003; Subbotin et al., 2010).

Minor hosts

Crotalaria juncea (sunn hemp), *Rhyncosia* spp., and *Sesbania cannabina* (corkwood tree) (Sharma and Nene, 1985; Walia et al., 1985; SON, 2003).

*****Koshy and Swarup (1972) determined that some cultivars of *Glycine max* (soybean), *Cyamopsis tetragonoloba* (cluster bean, guar), *Vigna umbellata* (rice bean), *Pisum sativum* (common pea), and several *Vicia* species were non-hosts or poor hosts of the pigeon-pea nematode. Others authors, however, have recorded these cultivars/species as plant hosts and as non-hosts (Jain et al., 1994). Some of the discrepancies may be a result of the presence of physiological races of *H. cajani*.

Known Vectors or Associated Organisms

Heterodera cajani is commonly found in association with the fungus *Fusarium udum* in fields where pigeon pea has been grown continuously for a number of years (Sharma et al., 1985). *F*. *udum* is the causal agent of *Fusarium* wilt on pigeon pea and is one of the crop's most threatening soil-borne diseases (Sharma and Nene, 1989). Concurrent infection of *H cajani* and *F. udum* on pigeon pea can result in a more damaging wilt disease complex, however, this reaction appears to vary with genotype/cultivar (*i.e*., observed with wilt susceptible pigeon pea but not observed in wilt-tolerant or wilt-resistant genotypes) (Hasan, 1984; Sharma and Nene, 1989; Siddiqui and Mahmood, 1995).

There is also a noted synergistic relationship between *Heterodera cajani* and *Macrophomina phaseolina*, the causal agent of root rot of black gram (*Vigna mungo*). When the cyst nematode was inoculated one week prior to the root rot pathogen, the onset of root rot disease was earlier by 15 days and with disease incidence of 95% and maximum reduction in pod yield when compared to either of them being inoculated separately or simultaneously (Latha and Narasimhan, 2000).

Known Distribution

Africa: Egypt. **Asia:** India and Pakistan (Koshy, 1967; Koshy and Swarup, 1971c; Aboul-Eid and Ghorab, 1974; SON, 2003; Subbotin et al., 2010).

Pathway

The wide host range of *H. cajani* among leguminous crops coupled with the ability of this pest to become established in tropical and subtropical areas makes this nematode a dangerous species. This nematode can be introduced with legumes, plant debris, and soil contaminated with cysts (SON, 2003). India has begun to employ extensive intercropping for *H. cajani* management with sorghum, maize (corn), pearl millet, and guar (Dwivedi and Upadhyay, 2001). Transport of *H. cajani* could occur with soil adhering to all types of planting material not just host crops (Rao et al., 2011).

There have been shipments of the following host propagative material from countries with records of presence of *Heterodera cajani* since 2004: *Cajanus* spp. (24), *Cicer arietinum* (7), *Cyamopsis tetragonoloba* (11), *Desmodium* spp. (1), *Glycine max* (15), *Phaseolus* spp. (10), *Phyllanthus* spp. (2), *Sesamum* spp. (2), *Sesbania* spp. (1), *Vicia* spp. (1), and *Vigna* spp. (5) (AQAS, 2014).

In addition to the shipments of host material, there have also been interceptions of the following host material from known host countries since 2004: *Cajanus* spp. (2), *Cicer arietinum* (416), *Cyamopsis tetragonoloba* (5), *Glycine max* (562), *Lablab purpureus* (40), *Phaseolus* spp. (457), *Phyllanthus* spp. (12), *Sesamum* spp. (21), *Sesbania* spp. (1), *Vicia* spp. (3), and *Vigna* spp. (115) (AQAS, 2014).

Potential Distribution within the United States

The presence of *H. cajani* in Egypt shows that it could become established in tropical, subtropical, and even Mediterranean countries (Luc, 1996). A recent risk analysis by APHIS-PPQ-CPHST based on the presence of susceptible host plants shows that Idaho, Michigan, Minnesota, Montana, Nebraska, North Dakota, Pennsylvania, Washington, and Wisconsin are at the highest risk from this nematode.

The top ten dry-bean producing states in 2006-2008 were: North Dakota (38%), Michigan(14%), Nebraska (11%), Minnesota (10%), Idaho (7%), California (4%), Washington (3%), and Colorado (3%) (USDA-ERS, 2014).

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 Sampling. As for other cyst-forming nematodes, in the absence of a host crop, soil samples must be collected and processed to extract cysts. Cyst nematodes are often extracted from soil using some form of elutriation, flotation, or sieving. The Fenwick flotation can is frequently used for this purpose. Characteristics of cysts and second stage juveniles must then be prepared, observed under a microscope, and compared with those of the original description (Koshy, 1967).

Sharma and Nene (1992) collected 200 cm^3 (200 g) soil samples with a 75 cm (29.5 in.) long 2.5 cm (1 in.) wide tube agar when sampling for *Heterodera cajani*. Samples were taken at 0 to 15 and 15 to 30 cm (0 to 5.9 and 5.9 to 11.8 in.) depths from at least 15 randomly selected places in each field in question. Vermiform nematodes were extracted from the sample using the decanting and sieving technique described by Cobb (Cobb, 1918). Sieves were used to extract cysts (180 µm) and vermiform nematodes (38 µm) (Sharma and Nene, 1992). Dwivedi and Upadhyay (2001) also collected 200 cm^3 of soil and extracted cysts by Cobb's decanting and sieving technique. Although the Cobb methods usually utilizes 20 and 60 mesh sieves, Sharma and Nene (1986) found that small cysts pass through the 60 mesh sieve and that a 80

mesh sieve should be used as an alternative to the 60 mesh sieve for extraction and counting of *H. cajani* cysts.

Balasubramanian and Vadivelu (2004) collected six composite soil samples per hectare, each with 20 cores weighing 200g each at a soil depth of 15 to 30 cm (5.9 to 11.8 in.), when sampling for *H. cajani* in sesame in India. The soil sample was thoroughly mixed, and cysts were recovered from dry soil using a Fenwick can. Recovered cysts were crushed in a water suspension and eggs and larvae counted with a stereoscope. The free second stage larvae present in the soil were also extracted using Cobb's sieving and decanting method followed by a modified Baermann funnel technique. Olia and Ali Alaeddini (2004) also used a Fenwick can method to extract *H. cajani* cysts.

Koshy and Swarup (1971c) collected samples from fields that showed uneven patchy growth for *Heterodera avenae*, *H. zeae*, and *H. cajani* cyst nematodes in India. Each sample consisted of approximately 1000 g of soil composed of several subsamples taken from a depth of 7 to 30 cm (2.8 to 11.8 in.). Cobb's sieving and sifting method followed by a modified Baermann's funnel method was used for analysis of the soil samples. The residue on 60 µm mesh screen was examined under a stereomicroscope for cysts, which were identified and counted. Cysts attached to root pieces were separately collected and counted.

Host root samples: In the presence of a host crop (legumes), close examination of roots will reveal the presence of pearly white, lemon-shaped females. However, at a later stage of development, females become easily detached from the roots making the detection of the nematode very difficult even in cases of heavy infestation, especially in non-sandy soils.

Balasubramanian and Vadivelu (2004) collected 2 g of roots from the plant rhizosphere.

Key Diagnostics/Identification

CAPS-Approved Method*: Confirmation of *Heterodera cajani* is by morphological identification. Characteristics of J2s, males, females, and cysts can be used for discrimination from other species (Koshy, 1967; 1971; Subbotin et al., 2010).

*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: Complete morphological and molecular data on *H. cajani* life stages are provided by Subbotin et al. (2010). Olia and Ali Alaeddini (2004) provide a redescription of the J2s, cysts, and eggs of *Heterodera cajani*. Abdollahi et al. (2006, 2007) provide detailed information on the morphometric variation of *H. cajani* J2s, eggs, and cysts in India. Rao et al. (2011) also provide information on the morphometric variation of *H. cajani* cyst vulval cones and second stage juveniles in India. Walia and Bajaj (2000) examined the morphological variation between the pigeon pea race and the cluster bean race of *H. cajani* in vulval cone structure and male morphology.

Mulvey (1972) also provides a key to 39 species of *Heterodera*, including *H. cajani*, based on characteristics of cysts.

Sharma and Swarup (1983) provide morphological keys, based on cyst, cone top structures, and second-stage larvae, to cyst nematode species occurring in India, including *H. cajani*.

Biochemical: Singh et al. (1998) used the isozyme patterns of esterases and malate dehydrogenase to separate two races of *H. cajani* and three races of *H. zeae*.

Molecular: *Heterodera* species can be identified by sequencing the ITS–rRNA genes and by PCR-RFLP profiles; these tools remain the best available for identifying cyst-forming nematodes (Waeyenberge et al., 2009; Subbotin et al., 2010).

Easily Confused Species

Heterodera cajani was originally described incorrectly under the name *H. trifoli*. It differs morphologically from this species by the number of incisures in the lateral field (J2), the size of the cysts, and the dimensions of the fenestrae and vulval slit. The J2 of *H. cajani* are shorter, and also have a different distance between the oesphageal gland opening and the stylet base (Koshy et al., 1971).

Heterodera cajani resembles species in the *Schachtii* group, including *H. trifolii*, *H. glycines*, *H. sacchari*, *H. schachtii*, *H. mothi*, *H. galeopsidis*, and *H. lespedezae*.

Characteristics of the J2s, cyst size, and vulval cone characteristics are useful to separate *H. cajani* from *H. trifolii*, *H. glycines*, *H. sacchari*, *H. schachtii*, and *H. mothi* (Mulvey, 1972; Subbotin et al., 2010). The presence of males separates *H. cajani* from *H. galeopsidis*, and a different host range separates *H. cajani* from *H. lespedezae*. Taya and Bajaj (1986) provide details distinguishing *H. cajani* larvae from *H. avenae* and *H. mothi*.

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Updates

July, 2014: Updated the Biology/Ecology section to include reviewer comments and an additional reference.

July, 2016: Deleted figure 4.