Punctodera chalcoensis

Scientific Name
Punctodera chalcoensis Stone, Sosa Moss, and Mulvey, 1976

Synonyms
Heterodera punctata

Common Name
Mexican corn cyst nematode

Type of Pest
Nematode

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

Reason for Inclusion in Manual
National cyst nematode survey; Corn Commodity-Based survey.

Figure 1. *Punctodera chalcoensis* Second-stage juvenile. Top: Shape and size of stylet knob. Bottom: Long hyaline tail section. Photos courtesy of Janet A. Rowe. www.cabicompendium.org

Background Information
From Baldwin and Mundo-Ocampo (1991): The Mexican corn cyst nematode, *Punctodera chalcoensis*, was first observed by Vasquez (1976) in corn fields in Huamantla, Tlaxcala, Mexico in the late 1950's and was considered *Heterodera punctata* (=*Punctodera punctata*). In the early 1960's, a cyst nematode was observed attacking corn in the valley of Mexico at Chalco and subsequently in the states of Puebla and Tlaxcala, which was also identified as *H. punctata* (Sosa-Moss and Gonzalez, 1973; Vazquez, 1976). Sosa-Moss (1965) believed that the Chalco population of the Mexican corn cyst nematode, unlike *H. punctata*, was limited to corn as a host. In 1976, a new genus, *Punctodera*, was proposed to accommodate *P. punctata* and a new species from Canada, *P. matadorensis* (Mulvey and Stone, 1976). Meanwhile, morphological differences were noted when comparing the Mexican corn cyst nematode with *H. punctata* populations from other countries (Sosa-Moss, 1965; Villanueva, 1974) and with *P. matadorensis* (Stone et al. 1976). These observations led to a description of the Mexican corn cyst nematode as a distinct species, *P.*

chalcoensis, with the type locality in Chalco, Mexico and the corn as the type host (Stone et al., 1976).

Pest Description

Cyst nematodes that are now considered *Punctodera* have been recognized for 60 years, primarily on the basis of a cyst without a cone but with both a vulval circumfenestra and an anal fenestra. Females and cysts of *Punctodera* spp. are globose to ovoid tapering to a protruding neck (Hesling, 1978). Subsurface punctations of the female cuticle, while indicated by the genus name, also occur in other heteoderines, although their intensity and occurrence in parallel rows is striking in *Punctodera*.

From Stone et al. (1976):

Note: Measurements are from cysts collected from field soil; field specimens were smaller than females that were cultured in a glasshouse.

Eggs: Length = 114.4 ± 3.6 µm; width = 43.1 ± 1.7 µm; length/width = 2.7 ± 0.1 µm. Second stage juvenile folded four times within the egg.

Second-stage juveniles (J2s): (Fig. 1) Typical heteroderid second-stage juvenile with vermiform body adopting a slight ventral curve on heat relaxation; tail tapering to a slender point. Cuticular annulations distinct; four incisors in lateral field reducing to three anteriorly and posteriorly, aerolated intermittently throughout. Cuticle thicker for first eight annules. Head offset, apparently three-five, usually four head annules when seen by light microscopy. SEM observation shows usually three, the extra annule seen in light microscopy being part of the lips. Oral disc distinct, elongated dorso-ventrally to about 1.5 times its width and surrounded by distinct lateral lips bearing the amphid apertures; the two components of each pair of submedian lips are fused into single distinct arcs. No indication of cephalic or labial papillae. Moderately heavy hexaradiate head skeleton. Cephalids at level of second and seventh-eighth body annules. Well-developed stylet with massive basal knobs rounded posteriorly, flat, to shallowly concave anteriorly. Anterior (prorhabdial) portion of stylet less than half stylet length. Median oesophageal bulb well developed occupying full width of body cavity and often with a somewhat rectangular shape. Oesophageal gland lobes with ventral overlap, usually extending back about one third of body length but sometimes considerably shorter. No distinct oesophageal-intestinal valve observed. Hemizonid one-two annules anterior to excretory pore; hemizonion not observed. Genital primordium lying about 60% of body length from anterior end. Phasmids situated two-thirds along tail length; small refractive bodies sometimes present in matrix of hyaline terminus.

Unfixed juveniles: Length = 542 ± 26 µm; maximum body width = 21.6 ± 0.7 µm; stylet length = 24.4 ± 0.2 µm; stylet base to dorsal oesophageal gland duct junction = 5.5 ± 0.8 µm; head tip to median bulb valve = 70.4 ± 6.3 µm; head tip to excretory pore = 110.1 ± 7.9 µm; head tip to end of oesophageal glands lobe = 176.0 ± 15.1 µm; tail length = 66.2 ± 3.5 µm; hyaline terminus length = 38.6 ± 3.4 µm; hyaline terminus length/stylet length = 1.6 + 0.1.

Fixed juveniles: Length = 533 ± 29 µm; maximum body width = 20.1 ± 0.6 µm; stylet length = 24.7 ± 0.6 µm; stylet base to dorsal oesophageal gland duct junction = 5.1 ± 0.7 µm; head tip to median bulb valve = 73.7 ± 2.7 µm; head tip to excretory pore = 107.8 ± 4.9 µm; tail length= 63.2 ± 3.3 µm; hyaline terminus length = 38.2 ± 3.1 µm; hyaline terminus length/stylet length = 1.5 + 0.1.

Female: Mature females white in color (Fig. 2), spherical or subspherical with projecting neck containing an oesophagus; length excluding neck/width ratio close to one. Head with one or two prominent annules. Stylet slender with rounded basal knobs. Oesophagus strongly developed with massive circular median bulb with prominent valve; oesophageal glands lobe with distinct dorsal and subventral gland cells. Excretory pore at base of neck. Two large ovaries filling enlarged body cavity, which in mature females is occupied by eggs. Cuticle greatly thickened except in head region, covered with a rugose or lace-like pattern of shallow ridges, with a sub-surface pattern of rows of fine refractive spots (the "punctations").

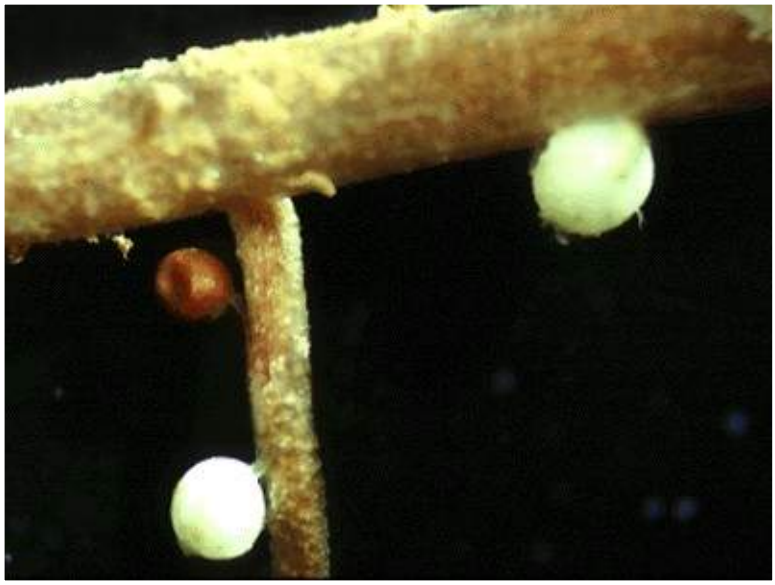

Figure 2. *P. chalcoensis* females (white) and cyst (brown) on corn roots. Photo courtesy of Janet A. Rowe. www.cabicompendium.org

Vulva a short transverse slit situated at the opposite pole of the body to the neck, lying centrally in a circular zone lacking ridges and punctations, with the cuticle of reduced thickness (the vulval fenestra). Vulval slit on slight circular prominence. The vulval fenestra appears more transparent than the surrounding body wall and is itself surrounded by a narrow zone of less thick cuticle. The anus is a transverse slit smaller than the vulva and lying 'dorsal' to the vulva within a thin-walled circular zone (the anal fenestra). The anal fenestra is similar in size and appearance to the vulval fenestra but lacks the surrounding clear zone; the anus does not lie centrally in the fenestra but towards the ventral side. Clusters of elongate bodies are associated with the anal and vulval apertures. The size of the vulval aperature precludes extrusion of eggs but a very small gelatinous matrix (egg sac) was observed on some specimens and may have been lost from other specimens in extraction. A thick white sub-crystalline layer is typically present consisting of polygonal plates and resembling that described from *P. punctata*.

Length excluding neck = 473 ± 105 µm; width = 429 ± 120 µm, neck length = 142 ± 24 µm; length excluding neck/width ratio =1.14 ± 0.18; stylet length = 25.8 ± 0.9 µm, stylet

base to dorsal oesophageal gland duct junction = 5.7 ± 0.9 µm; length of median oesophageal bulb = 25.7 ± 1.9 µm; width of median oesophageal bulb = 24.9 ± 2.4 µm; head tip to median oesophageal bulb valve = 77.4 ± 8.4 µm; head tip to excretory pore = 131.0 ± 0.6 µm.

Perineal portions of females: Vulval fenestra length = 30.7 ± 7.0 µm; width 32.1 ± 7.0 µm; length of vulval slit = 4.0 ± 0.5 µm; anal fenestra length = 29.5 ± 5.7 µm; width = 31.1 ± 5.3 µm; length of anal slit = 3.0 ± 1.0 µm; distance between fenestrae = 67.7 ± 14.5 µm.

Cysts: Cyst shape spherical as that of female, color pale to dark brown (Fig. 2), darkening with age. New cysts often retain the subcrystalline layer. In old cysts, the thin walls of the vulval and anal fenestrae are lost; younger cysts show incomplete fenestration. Some specimens have small scattered bullae in the perineal region or closely sited in an area just below the vulval fenestra but they are lacking from many cysts. New cysts typically contain from 200-400 embryonated eggs.

Cysts entire, length excluding neck = 510 µm; maximum width = 470 µm; neck length = 88 µm; length excluding neck/width ratio =1.1. Dry cyst length excluding neck = 441 ± 69 µm; width = 416 ± 61 µm; neck length = 95 ± 26 µm; length excluding neck/width ratio= 1.06 ±0.10.

Perineal portions of cysts: Vulval fenestra length = 18.1 ± 2.7 µm; width 19.8 ± 2.9 µm; diameter of clear zone surrounding vulval fenestra ~ 30 µm; length of vulval slit = 4.2 ± 0.4 µm; anal fenestra length = 21.1 ± 3.4 µm; width = 22.4 ± 3.1 µm; length of anal slit = 2.8 ± 0.4 µm; distance between fenestre = 142.3 ± 8.8 µm.

Males: Typical heteroderid male morphology. Body vermiform, heat relaxed specimens with strong ventral curvature and tail frequently twisted through 180°. Head offset with 5-7 annules as seen by light microscopy. Tail bluntly rounded less than one quarter of body width long. Cuticle with regular annulations, four lateral incisures, areolated, lateral field terminating on tail. Heavy hexaradiate cephalic skeleton. Cephalids at level of second and eighth body annules. Stylet well developed with shallow basal knobs, flat to slightly concave anteriorly. Median oesophageal bulb ellipsoidal, not filling body cavity; oesophageal glands lobe overlapping intestine ventrally. Hemizonid extending over two annules, one annule anterior to excretory pore, hemizonion not observed. Single gonad extending for about half body length, less in small specimens. Paired spicules flask-shaped proximally and tapering distally, curved ventrally with single points, single un-ornamented gubernaculums. Phasmids not observed.

Length = 985 ± 69 µm; body width = 25.8 ± 1.7 µm; stylet length = 26.8 ± 0.8 µm; stylet base to dorsal oesophageal gland duct junction = 4.0 ± 0.6 µm; head tip to oesophageal median bulb valve = 82.7 ± 3.7 µm; head tip to excretory pore = 131.3 ± 7.7 µm; tail length = 2.8 ± 1.4 µm; spicule length (across chord) = 31.9 ± 1.8 µm; gubernaculum length 7.3 ± 0.8 µm; overall gonad length 476 ± 107 µm; G=48.

Additional morphological and molecular characters of *P. chalcoensis* are provided by Subbotin et al. (2010).

Biology and Ecology

This cyst forming nematode has sedentary endoparasitic habits. Cysts are persistent (>10 years) tanned sacs derived from the female body and contain eggs. Cysts of this species are subspherical or ovoid, lack posterior protuberance, have large vulval and anal fenestrae and small scattered or absent bullae (Sosa Moss, 1987). Nematodes overwinter in the soil as cysts (containing embryonated juveniles). Diapause is broken by rainfall, and J2s hatch out into the soil and are attracted to roots, where they feed. Mature males leave the roots and mate with females whose bodies then form cysts. The fertilized female retains the eggs in her sack-like body until they are embryonated, then only a limited number of eggs hatch out each year, until the cyst is empty or the eggs are no longer viable (CABI, 2012).

Figure 3. *P. chalcoensis* damage to corn. Photo courtesy of Laurence I. Miller, Virginia Tech. University. www.forestryimages.org

Several authors concluded that the life cycle of *P. chalcoensis* spans the growing season of corn, therefore only one generation occurs per year and that the cysts must remain in the soil during the winter to initiate the next cycle (Baldwin and Mundo-Ocampo, 1991). Others have reported a typical heteroderine life cycle of about 30 days (Baldwin and Mundo-Ocampo, 1991). Fresh eggs may hatch readily, but only a low percentage of eggs hatch after diapause is established within cysts. Villanueva (1974) reported that root exudates of most plants induce egg hatching of the Mexican population, but that exudates from corn roots resulted in a significantly greater degree of hatching.

The environmental requirements of *P. chalcoensis* are unknown, although the distribution at high elevations (above 2000 meters) in Mexico, and its absence in corn fields in warmer, subtropical regions suggests that it does not tolerate continuous, warm, humid conditions. The nematode is most abundant in sandy soils (Stone et al., 1976).

Symptoms/Signs

P. chalcoensis is highly damaging to corn in Mexico, causing severe yellowing, stunting (Fig. 3), and even death of young seedlings. Pale stripes on the leaves can also be observed. Plants without supplementary fertilization showed more damage and less tolerance to the nematode. Heavily attacked plants have stunted root systems and many short laterals, giving a bottle brush effect, and the aerial parts of the plants appear unthrifty.

Later sown corn suffers greater damage than early sown corn because the peak emergence of infective juveniles of *P. chalcoensis* occur at the same time as seed germination (Sosa-Moss, 1987). Early sown corn has already developed a good root system before the rains provide sufficient moisture to stimulate hatching of juveniles.

White females can be observed on roots of corn (Fig. 2).

Pest Importance

Punctodera chalcoensis is a serious pest of corn in Mexico. When associated with fungi, *P. chalcoensis* can cause yield suppression of 90% (Sosa-Moss, 1987). The plants that remain are so stunted and yellow that no straw can be harvested. In the most severe cases of nematode damage, as many as 500 cysts per 100 g of soil have been recorded (Vazquez, 1971).

Corn is the most widely cultivated feed grain in the United States, and commercial production takes place in every state in the continental United States (USDA-NASS, 2016). In 2015, corn was cultivated on over 88 million acres of land in the United States (USDA-NASS, 2016). The total 2015 U.S. corn harvest was approximately 13.6 billion bushels, with an estimated value of over $60 billion. The United States is the number one corn producer in the world, and 10-20% of U.S. grown corn is exported (USDA-ERS, 2013).

Punctodera chalcoensis is listed as a harmful organism in the following countries: Brazil, Ecuador, and South Korea (USDA-PCIT, 2013). There may be trade implications with these countries if this nematode becomes established in the United States.

Known Hosts

Zea mays (corn), and *Zea mexicana* (teosinte, wild maize) (Stone et al., 1976; EPPO, 2012).

Graminaceae tested and proved to be non-hosts were: *Avena sativa* (oats), *A. fatua* (wild oat), *Triticum aestivum* (wheat), *Secale cereale* (rye), triticale, *Sorghum vulgare* (sorghum), *Agropyron* spp. (crested wheatgrass), *Bromus* spp. (brome), *Dactylis* spp. (orchard grass), *Elymus* spp. (wild rye), *Festuca* spp. (fescue), *Lolium* spp. (ryegrass), and *Phleium* spp. (timothy grass) (Stone et al., 1976).

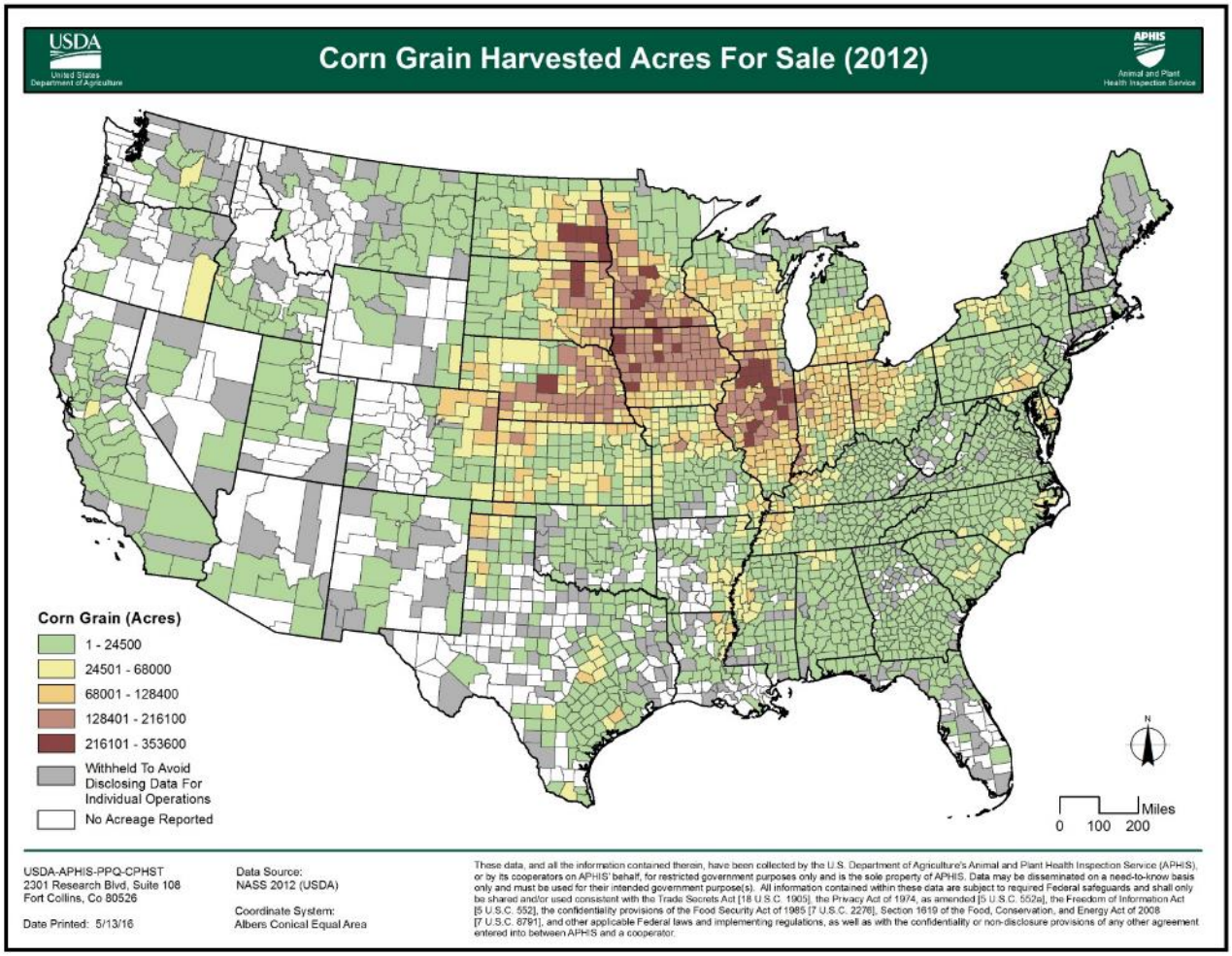

Figure 4. Corn commodity acreage. Map courtesy of USDA-APHIS-PPQ-CPHST.

Known Vectors (or associated organisms)

P. chalcoensis is not a known vector, nor is it known to be vectored. However, when this pest attacks the roots of host plants, the hosts become more prone to secondary infections by other pathogens, particularly pathogenic fungi (Sosa-Moss, 1987; EPPO, 2012).

Known Distribution

North America: Mexico (Jalisco, México, Michoacán, Puebla, Querétaro, Tlaxcala, Veracruz) (EPPO, 2012).

Pathway

Infested soil and growing media, plants for planting, bulbs, and tubers from areas where *P. chalcoensis* occurs are the most probable pathways to introduce this pest into new areas. Soil attached to machinery, tools, footwear, or plant products is another possible pathway (EPPO, 2012). The presence of this pest in Mexico is cause for concern due

to the shared land border with the United States and the high volume of traffic between the two countries. In 1984, there was an interception of a live *P. chalcoensis* cyst at the U.S. border station in Brownsville, Texas. The cyst was found in soil of a *Rosa* spp. plant carried by a pedestrian who was crossing the border from Mexico (AQAS, 2013).

Since 2003, there were 8 shipments of *Zea* spp. propagative material from Mexico, with the largest shipment consisting of 1850 kg of seed. There were also 111 interceptions of *Zea* spp. plant material from Mexico since 2003 (AQAS, 2013).

Potential Distribution within the United States

Biological information for this pest is not currently available to determine the potential distribution of this nematode within the United States. Once a cyst nematode is introduced into a country, however, it is very difficult to minimize spread without extensive quarantine measures and expensive eradication strategies.

A recent commodity acreage map developed by USDA-APHIS-PPQ-CPHST (Fig. 4) shows that states in the upper Midwest are most vulnerable to *P. chalcoensis* based on host availability. Corn is grown commercially in every state in the continental United States (USDA-NASS, 2016). Corn cultivars grown in the United States are susceptible to *P. chalcoensis* (Mundo et al., 1987).

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: The soil around corn plants with cysts should be sampled. Cyst nematodes are then extracted from soil, identified, and counted. A composite soil sample is collected from a sampling unit such as an entire field, a specific area of a field, or an experimental plot. Multiple (15 to 20) cores of soil are collected from the upper 8 to 12 inches (20-30 cm) of soil and combined for each composite sample. A subsample is removed from the larger sample and air-dried.

Cysts can be extracted from the subsample using a Fenwick can method (20 mesh screen over a 60 mesh screen), a modified Baermann funnel technique, or a modified Fenwick can elutriation method (Fig. 5a) with further separation of cysts from the plant debris by flotation in an ethanol and glycerin solution (Fig. 5b) (Caswell et al., 1985; Ingham, 1994). Cysts are then picked from the remaining debris and identified. Cysts can be crushed to determine the number of eggs plus juveniles, which can then be adjusted to reflect the nematode density per pound of oven-dry soil.

Survey for *P. chalcoensis* should crossover easily and inexpensively if a state already has equipment to survey for potato cyst nematodes. The Fenwick can method used to survey for *Globodera pallida and G. rostochiensis* should also work for the extraction of *P. chalcoensis* cysts.

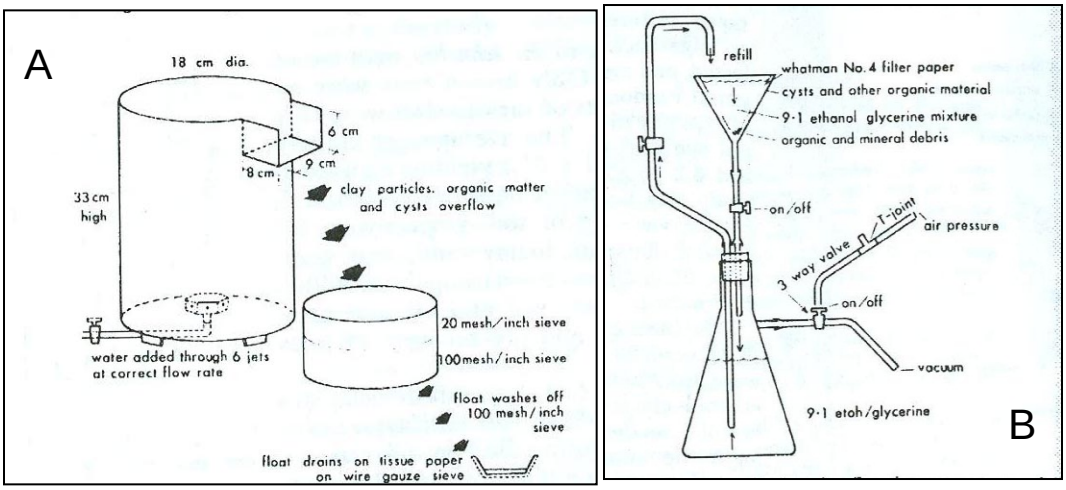

Figure 5. A) Modified Fenwick can used to separate nematode cysts and organic debris from soil sample. B) Ethanol-glycerine flotation apparatus for separation of nematode cysts from sample organic matter. Reproduced from Caswell et al., 1985.

There are a variety of additional methods available for cyst extraction from soil. Soil can also be processed using Cobb's decanting and sieving technique to determine the presence of males, white females, and cysts. Subbotin et al. (2003) used sieving-decanting and centrifugation-flotation methods to isolate cysts from soil. Holgado et al. (2004) air dried soil samples, passed the samples through a 5 mm sieve, and extracted cysts using a fluidizing column. Abidou et al. (2005) processed soil samples through a Kort elutriator.

Motile nematodes (*e.g.* juveniles) can be extracted using the Whitehead tray method (Smiley et al., 2007).

Key Diagnostics

CAPS-Approved Method*: Confirmation of *P. chalcoensis* is by morphological identification. Characteristics of second-stage juveniles, females, and cysts can be used to differentiate from other *Punctodera* species.

Literature-Based Methods: Most diagnoses are made via morphological characteristics. The Society of Nematologists provide an illustrated key to selected genera and species of cyst forming Heteroderidae in the Western Hemisphere (based on cysts and second-stage larvae) at http://nematode.unl.edu/cystkey.htm.

Stone et al. (1976) provide a morphological key to the three *Punctodera* spp.:

1. Cyst pear-shaped, second-stage juvenile 350-470 µm ……*Punctodera punctata*

- Cyst spherical or sub-spherical shaped, second stage juvenile >500 µm ……2

2. Massive bullae between vulval and anal fenestrae, second stage juvenile with oesophageal gland lobe extending to about 50% of body length and stylet knob strongly concave anteriorly, host grasses ……*Punctodera matadorensis*

- Bullae small, scattered or absent, second-stage juvenile with oesophageal gland lobe extending to about 30% of body length and stylet knobs flattish to slightly concave anteriorly, host maize ……*Punctodera chalcoenisis*

Sabo et al (2002), sequenced the internal transcribed spacer (ITS) 1, 5.8S ribosomal RNA gene, and ITS2 of *P. chalcoensis*, which may provide for molecular identification.

Gibson et al. (2011) sequenced the partial mitochondrial genome sequence of *P. chalcoensis*, opening the possibility of future diagnostic methods based on this sequence.

Easily Confused Species

Punctodera chalcoensis can be confused with the two other *Punctodera* spp. (*P. punctata* and *P. matadorensis*), although neither of those species occur on corn. *P. punctata* is distributed worldwide on turf, wheat, and weed grasses, and *P. matadorensis* is apparently limited to weed grasses at the type location in Canada. *P. matadorensis* was, however, recently detected in North Dakota in February, 2010. *P. chalcoensis* and *P. matadorensis* can be separated from *P. punctata* by the spherical to subspherical shape of the females and cysts in the former versus more elongate, pear-shaped females and cysts in the latter (Baldwin and Mundo-Ocampo, 1991). Cysts of *P. chalcoensis* differ from those of *P. matadorensis* by small, scattered bullae or no bullae versus massive and consistently present bullae in *P. matadorensis*. *P. chalcoensis* also differs slightly by the shape of the stylet knobs of the second-stage juvenile, which are flat to slightly concave anteriorly in *P. chalcoensis* versus strongly concave and anchor-shaped in *P. matadorensis* and rounded in *P. punctata*. The esophagus of the second-stage juveniles of *P. chalcoensis* and *P. punctata* occupies about 30% of the body length, whereas in *P. matadorensis* it includes about 50% of the body length (Baldwin and Mundo-Ocampo, 1991).

In Mexico, corn and potatoes are sometimes grown in the same areas. *Globodera* cysts sometime occur in the same soil sample. *Globodera* cysts are similar in size, shape, and color. The cyst nematode *Heterodera zeae* is present in India and North America on corn. *Heterodera* spp. have lemon-shaped cysts, however, so confusion should not arise between the two genera (CABI, 2012).

The Japanese cyst nematode *Heterodera elachista* also feeds on corn, but it is not known to be present in the western hemisphere. This nematode can be distinguished from *P. chalcoensis* due to variations in ITS1-rDNA sequences (De Luca et al., 2013).

References

Abidou, H., Valette, S., Gautheire, J.P., Rivoal, R., El-Ahmed, A., and Yahyaoui, A. 2005. Molecular polymorphism and morphometrics of species of *Heterodera avenae* group in Syria and Turkey. Journal of Nematology 37(2): 146-154.

AQAS. 2013. Agricultural Quarantine Activity Systems. Queried December 27, 2013 from, https://aqas.aphis.usda.gov/aqas/.

Baldwin, J.G. and Mundo-Ocampo, M. 1991. Heteroderinae, cyst- and non-cyst forming nematodes. *In:* Manual of Agricultural Nematology. W.R. Nickle (ed). Pp. 275-362. New York: Marcel Dekker.

CABI. 2012. Crop Protection Compendium [CD]. Wallingford, UK: CAB International. http://www.cabi.org/cpc/.

Caswell, E.P., Thomason, I.J., and McKinney, H.E. 1985. Extraction of cysts and eggs of *Heterodera schachtii* from soil with an assessment of extraction efficiency. Journal of Nematology 17: 337-340.

De Luca, F., Vovlas, N., Lucarelli, G., Troccoli, A., Radicci, V., Fanelli, E., Cantalapiedra-Navarrete, C., Palomares-Rius, J.E., and Castillo, P. 2013. *Heterodera elachista* the Japanese cyst nematode parasitizing corn in Northern Italy: Integrative diagnosis and bionomics. European Journal of Plant Pathology 136 (4): 857-872.

EPPO. 2012. *Punctodera chalcoensis*. European and Mediterranean Plant Protection Organization. Retrieved December, 2013, from, http://www.eppo.int/QUARANTINE/Alert_List/nematodes/Punctodera_chalcoensis.htm.

Gibson, T., Farrugia, D., Barrett, J., Chitwood, D.J., Rowe, J., Subbotin, S., and Dowton, M. 2011. The mitochondrial genome of the soybean cyst nematode, *Heterodera glycines*. Genome 54 (7): 565-574.

Hesling, J.J. 1978. Cyst nematodes: Morphology and identification of *Heterodera*, *Globodera*, and *Punctodera*. Tech. Bull. G B Minist. Agric. Fish Food 7: 125-155.

Holgado, R., Rowe, J.A., and Magnusson, C. 2004. Morphology of cysts and second stage juveniles of *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984 from Norway. Journal of nematode morphology and systematics 7(1): 77-84.

Ingham, R.E. 1994. Nematodes. Pages 459-490. *In* R.W. Weaver et al. (ed). Methods of soil analysis, Part 2. American Society of Agronomy. Madison, WI.

Mulvey, R.H., and Stone, A.R. 1976. Description of *Punctodera matadorensis* n. gen., n. sp. (Nematoda: Hetoroderidae) from Saskatechewan with lists of species and generic diagnosis of *Globodera* (n. rank), *Heterodera*, and *Sarisodera*. Can. J. Zool. 54: 772-785.

Mundo, M., Baldwin, J.G., and Jeromino, R.J. 1987. Distribution, morphological variation, and host range of *Punctodera chalcoensis*. J. Nematology 19: 545.

Sabo, A., Reis, L.G.L., Krall, E., Mundo-Ocampo, M., and Ferris, V.R. 2002. Phylogenetic relationships of a distinct species of *Globodera* from Portugal and two *Punctodera* species. Journal of Nematology 34(3): 263-266.

Siddiqui, M. R. 2000. Tylenchida Parasites of Plants and Insects. 2nd Edition. CAB International, Wallingford, UK.833 pp.

Smiley, R., Sheedy, J., Pinkerton, J., Easley, S., Thompson, A., and Yan, G. 2007. Cereal cyst nematode: distribution, yield reduction, and crop management strategies. Oregon State University. Pages 15-29. http://extension.oregonstate.edu/catalog/html/sr/sr1074-e/05.pdf.

Society of Nematologists. n.d. *Punctodera chalcoensis*. Exotic Nematode Plant Pests of Agricultural and Environmental Significance to the United States. Assessed 2/11/10. http://nematode.unl.edu/pest29.htm.

Sosa Moss, C. 1965. Recheches sur *Heterodera punctata* Thorne, parasite du maiss au Mexique Comptes rendus du huiteme. Symposium International de Nematologie, Antibes, E.J. Brill, p. 54.

Sosa-Moss, C. 1987. Cyst nematodes of Mexico, Central America, and South America. Nematologica Mediterranea 15: 1-12.

Sosa Moss, C. and Gonzales, C.P. 1973. Respuesta de maiz chalqueno ferilizado y no fertilizado a 4 differentes niveles de *Heterodera punctata* raza Mexicana (Nematoda: Heterodidae). Nematropica 3: 13-14.

Stone, A.R., Sosa Moss, C., and Mulvey, R.H. 1976. *Punctodera chalcoensis* n. sp. (Nematoda: Heteroderidae) a cyst nematode from Mexico parasitizing *Zea mays*. Nematologica 22: 381-389.

Subbotin, S.A., Mundo Ocampo, M., and Baldwin, J.G. 2010. Systematics of Cyst Nematodes (Nematoda: Heteroderinae). Nematology Monograph and Perspectives Volume 8A. Brill, Leiden-Boston, 351 pp.

Subbotin, S.A., Sturhan, D., Rumpenhorst, H.J., and Moens, M. 2003. Molecular and morphological characterization of the *Heterodera avenae* species complex (Tylenchida: Heteroderidae). Nematology 5(4): 515-538.

USDA-ERS. 2013. Economic Research Service. Retrieved December 13, 2013 from, http://www.ers.usda.gov/data-products/.

USDA-NASS. 2016. Crop Production, 2015 Summary. United States Department of Agriculture, National Agricultural Statistics Service, www.nass.usda.gov/

USDA-PCIT. 2013. Phytosanitary Certificate Issuance & Tracking System. *Punctodera chalcoensis.* Queried December 13, 2013 from, https://pcit.aphis.usda.gov/pcit/.

Vazquez J.T. 1971. Principales problemas nematológicos en México. Nematropica 1(1): 30.

Vasquez, J.T. 1976. Infesaciones de nematodos fitoparasitos como factor limitante en la produccion de maiz en el altiplano mexicano. Produccion del Departamento Mexicano. CODAGEM, pp. 79.

Villnueva, R.M. de J. 1974. Comparacion morfometrica entre uno poblacion inglesa y una Mexicana de *Heterodera punctata* Thorne (nematode Heteroderodae), influencia de exudados radiculares en la poblacion Mexicana. Tesis professional, I.P.N. Mexico.

This datasheet was developed by USDA-APHIS-PPQ-CPHST staff. Cite this document as:

Sullivan, M. and D. Mackesy. 2012. CPHST Pest Datasheet for *Punctodera chalcoensis.* USDA-APHIS-PPQ-CPHST. Revised June, 2014.

Reviewed by: Zafar Handoo (USDA-ARS Nematology Laboratory, Beltsville, MD), **Renato Inserra** (Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL), and **Andrea Skantar** (USDA-ARS Nematology Laboratory, Beltsville, MD).

Updates

July, 2014: Added additional references to the Key Diagnostics and Easily Confused Species sections.

July, 2016: Updated mapping and potential distribution information.