Globodera pallida

Scientific Name

Globodera pallida (Stone, 1973) Behrens, 1975

<u>Synonyms:</u> Heterodera pallida, Heterodera rostochiensis

Common Name

Pale cyst nematode, Pale potato cyst nematode, white potato cyst nematode

Type of Pest

Nematode



Figure 1. Anterior part of a second-stage juvenile of *Globodera pallida*. Photo courtesy of Christopher Hogger, Swiss Federal Research Station for Agroecology and Agriculture, <u>http://www.bugwood.org/</u>.

Taxonomic Position

Class: Secernentea, Order: Tylenchida, Family: Heteroderidae (Siddiqi, 2000).

Reason for Inclusion in Manual

PPQ Program Pest; cyst nematode

Pest Description

Globodera pallida was first described in 1973. Before then, most records referred to *Heterodera rostochiensis sensu lato*, which included both *G. pallida* and *G. rostochiensis*. Because of this, it is difficult to determine which species is referred to in work prior to 1973 (CABI/EPPO, n.d.).

Specific measurements of each of the life stages can be found in Stone (1972).

<u>Eqgs:</u> "Egg shell hyaline (colorless). "The eggs of *G. pallida* are retained within the body of the female where they develop and are not deposited in an egg sac. At its death, the female becomes a cyst filled with embryonated eggs, which contain second-stage juveniles folded four times (Friedman, 1985; taken from Stone, 1972). The surface of the eggshell is smooth; no microvilli are present. Measurements of the egg fall within the range: $108.3 \pm 2.0 \ \mu m \times 43.2 \pm 3.2 \ \mu m$ " (CABI, 2013).

<u>Second-Stage Juveniles (J2s)</u>: "J2s (Fig. 1), in soil, are motile and vermiform (wormshaped) and become sedentary and swollen once they penetrate inside the root. They have a body about 470 μ m long, a mouth with a strong stylet, which is a hollow feeding structure used for ingesting cell content after puncturing their walls, and a pointed tail" (CABI/EPPO, n.d.).

"Body vermiform...cuticular annulation prominent. Lateral field with four incisures. occasionally completely areolated. Head rounded, slightly offset with 4 to 6 annules...Cephalic framework heavily sclerotized. Stylet well developed (22.5-25.0 μ m long), basal knobs with distinct forward projection in lateral view. Median bulb elliptical with prominent valve. Esophageal glands extend ventrally for about 35 percent of body length...Hemizonid usually one annule anterior to excretory pore...Four-celled genital primordium at about 60 percent



Figure 2. *Globodera pallida* female on potato tuber. Photo courtesy of Bonsak Hammeraas, Bioforsk - Norwegian Institute for Agricultural and Environmental Research,

of body length. Tail tapers to finely rounded terminus...Line of 2 to 8 small refractive bodies sometimes visible internally near tail end. Phasmids difficult to see, slightly anterior to middle of tail" (Friedman, 1985; taken from Stone, 1972; Skantar et al., 2007).

Third (J3s) and Fourth-Stage (J4s) Juveniles: No description found. These stages are swollen, sedentary and occur within the plant root (CFIA, 2012).

<u>Females:</u> The adult females are swollen, sedentary and protruding from the root surface. "Body subspherical with projecting neck (Fig. 2) and variable diameter (400-600 μ m)..., internal color white, cuticle brown after death. Cuticle never light yellow or golden. Some populations pass through 4- to 6week internal cream stage before turning brown. Head with 1 or 2 annules, cephalic framework weakly developed. Stylet with



Figure 3. Cysts of *Globodera pallida*. Photo courtesy of Central Science Laboratory, Harpenden Archive, British Crown, <u>http://www.bugwood.org/</u>.

rounded knobs sloping backward...Median bulb large with large valve...Esophageal glands broad...frequently displaced forward by paired ovaries. Excretory pore at base of neck...Vulva not set off from body, located opposite neck in slight almost circular

depression. Anus small, at right angle to vulval slit. Cuticle reticulate, subcuticular punctations visible. Number of cuticular ridges on anal-vulval axis 12.5 ± 3.1 " (Friedman, 1985; taken from Stone, 1972; Skantar et al., 2007; Subbotin et al. 2010).

<u>Cysts:</u> The cyst is a persistent tanned sac which contains the eggs and is derived from some of all components of the dead mature female body wall (Fig. 3) (CABI, 2013). It is found in the soil or attached to the roots.

"Cyst brown, subspherical with protruding neck, abullate. Vulval region forming single circular fenestra in older cysts...Anus small, may be at apex of V-shaped mark...Cyst wall pattern similar to that of female but more pronounced. Generally punctate" (Friedman, 1985; taken from Stone, 1972).

<u>Males:</u> Males are motile and present in the soil or coiled inside the cuticle of J4s. They are vermiform like the J2s and about 1200 μ m long. Copulatory apparatus close to the end of posterior body. Cuticular annulation prominent. Lateral field with 4 incisures, areolations sometimes cross outer incisures...Head rounded, offset, with 6 to 7 annuli. Cephalic framework heavily sclerotized. Stylet strong with posteriorly sloping basal knobs...Median bulb elliptical with prominent valve. Esophageal glands narrow, ventral, terminating near excretory pore...Hemizonid 2 annules long, 2 to 3 annules anterior to excretory pore...Testis single...Spicules with single pointed tips...Gubernaculum small, about 2 μ m across in side view, slightly broader in dorso-ventral aspect, without ornamentation...Phasmids not observed" (Friedman, 1985; taken from Stone, 1972).

Biology and Ecology

This species overwinters as coiled J2s within egg shells protected by a cyst (dead female). Secretions from the host roots in spring lead to the emergence of juveniles (Friedman, 1985). Hatching of potato cyst nematodes (PCN) increases rapidly after plant emergence, peaking between two to five weeks after emergence (Devine and Jones, 2003). *Globodera pallida* J2s hatch when temperatures are around 10°C (50°F) or higher. This nematode is adapted to develop at cool temperatures (10 to 18°C; 50 to 64.4°F) (CABI, 2013). The upper threshold temperature for J2 root penetration and development is 28°C; 82°F (Mugniery, 1978).

Hatched PCN J2s find host roots to penetrate, usually behind the root tip or lateral root. After root penetration, J2s move through the root tissues, feeding on the cortex, endodermis and pericycle. J2s then become sedentary and swollen establishing a permanent feeding site consisting of a large syncytium (nutrient transfer cells). In resistant potato varieties, localized necrosis and thickening of the syncytial walls may occur (reviewed in Baldwin and Mundo-Ocampo, 1991). After establishment in the syncytium, J2s molt into swollen J3s and J4s and will either develop into vermiform and motile males or swollen and sedentary females (CABI, 2013).

From hatching to adult, development takes 38 to 45 days. Sedentary adult females enlarge and burst through the root with their tail end, facilitating mating (reviewed in Baldwin and Mundo-Ocampo, 1991). The anterior body of the female remains

embedded in the root, where they feed upon the syncytyium (CSL, 2003). Females exude a sex pheromone to attract males. Mating occurs within 50 days of the root invasion by the J2s. Males develop from sedentary saccate juveniles and are mobile. They do not feed, but instead spend their time mating (with up to 10 females) over their adult lifespan of about 10 days. The females retain the eggs within their bodies (between 200 and 600). Once a female dies, she becomes a cyst, changing from white to brown (reviewed in Baldwin and Mundo-Occampo, 1991). Cysts usually may remain attached to dead root or detach from the root falling into the soil (CABI/EPPO, n.d.).

The pest infects feeder roots (USDA, 2006), taking in nutrients used for tuber production. Losses are dependent on nematode density and to a lesser extent host cultivar, soil type, moisture, planting and harvesting time, and fertilizer use (Friedman,

1985). It can take up to 20 years from the time potato cyst nematodes are introduced to an area before symptoms are observed on potato plants. Once introduced into a potato field, it can take six to seven years before nematode numbers are at a detectable level (Berg, 2006). The time from pest introduction to detection depends largely on the quantity of cysts introduced to the field, the frequency with which host crops are grown, and the field survey rate used.

When potatoes are harvested, cysts can become detached from the roots, falling into the soil where they serve as a



Figure 4. Symptoms of *Globodera pallida* infestation. Photo courtesy of Florida Division of Plant Industry Archive, Florida Department of Agriculture and Consumer Services, <u>http://www.bugwood.org/</u>.

source of infestation for future crops (CSL, 2003). Repeated potato cultivation on infested land can lead to crop loss of up to 80% due to buildup of the nematode population (CABI/EPPO, n.d.).

Depending on the duration of the crop cycle, this species may have one or more generations per year and remains in the soil as a dormant stage (cyst) for years (reviewed in Baldwin and Mundo-Ocampo, 1991). The cysts can stay dormant for up to 30 years while the eggs remain viable, making them extremely persistent in the soil (USDA, 2006).

There are many different pathotypes of *G. pallida*, and although a potato variety may be resistant to one pathotype, it may be susceptible to another (CIP, n.d.). This species has three recognized pathotypes, which are characterized by their ability to reproduce

on certain *Solanum* clones and hybrids (reviewed in CABI/EPPO, n.d.: Finlay and de Scurrah, 1998; Fleming and Powers, 1998). Hybrids between *G. pallida* and *G.*

rostochiensis can exist in nature (reviewed in Baldwin and Mundo-Ocampo, 1991).

Symptoms and Signs

Infested plant material can suffer from poor growth. Areas may be small and then enlarge and increase in number with succeeding years. Symptoms are similar to that of water or nutrient deficiencies (Friedman, 1985). Tuber size can be significantly reduced, even when only minor symptoms are observed. Infested plants are smaller than normal with yellow leaves (Fig. 4) (USDA, 2006). Plants may wilt during the warmest part of the day



Figure 5. Signs of *Globodera pallida* infestation. Photo courtesy of Florida Division of Plant Industry Archive, Florida Department of Agriculture and Consumer Services, <u>http://www.bugwood.org/</u>.

(Friedman, 1985). Infested potato plants have a reduced root system. Because of decreased water and nutrient uptake and concomitant infection of other pathogens, plant death may occur (EPPO, 2004; Turner and Evans, 1998).

Symptoms are not diagnostic for potato cyst nematodes and may resemble symptoms caused by other pathogenic soil-borne nematodes as well as certain soil or environmental factors, including compaction, flooding, drought, herbicide injury, or nutritional deficiency (Sabaratnam, 2012).

In general, potato cyst nematodes can reduce yields by around 30% (Trudgill et al., 1998). However, growers may overlook infestations due to their small size and lack of specific symptoms aboveground (CIP, n.d.). Mature females and cysts can be seen with the visible eye as minute globular structures (200 to 500 μ m) (Fig. 5). When looking at plant roots, females and cysts can be readily dislodged from host material (NAPPO, 2007).

Pest Importance

Potato cyst nematodes (*G. pallida* and *G. rostochiensis*) are considered major pests of potato in cool, temperate areas and also in warmer climates in Mediterranean and Chilean localities where potatoes are grown during the winter-spring season (Greco et al., 1982; Greco and Moreno, 1992). The amount of damage (especially tuber weight) is closely related to the amount of nematode eggs per soil unit (CABI/EPPO, n.d.; Turner and Evans, 1998). *Globodera pallida* can reduce yields by up to 80% if left unchecked (APHIS, 2006b; USDA, 2006). In the United Kingdom, loss estimates due to

PCN are around £50 million (about \$82 million) per year (CABI, 2013) or about 9% yield loss of annual national production. Annual costs within the European Union are estimated at €300 million (\$410 million) (Moxnes and Hausken, 2007).

According to the United States Potato Board, total U.S. potato and potato product exports reached record levels in fiscal year 2013. The value of these exports is estimated to be \$1.6 billion and consists of 17% of U.S. potato production (USPB, 2013). *Globodera pallida* serves as a serious threat to both domestic and international trade in potatoes and nursery stock (USDA, 2006). This species is regulated as a harmful organism in 81 countries worldwide (USDA-PCIT, 2013).

Potato trade between the United States and Canada was temporarily halted with the find of *G. pallida* in Idaho and a putative find of *G. pallida* in Alberta followed by an additional find of *G. rostochiensis* in Quebec. In order to resume potato trade between the United States and Canada, the Canada and United States Guidelines on Surveillance and Phytosanitary Actions for Potato Cyst Nematodes was developed and implemented to address current and future detections of potato cyst nematodes in the two countries (APHIS, 2006a; CFIA and USDA, 2009; Zink, 2014, personal communication).

Due to the importance of PCN to potato production and trade, development of resistant varieties and other control options have been explored, including crop rotation, nematicides, trap cropping, and soil sterilization (Kerry et al., n.d.; Whitehead, 1995; Schouten and Beniers, 1997; Whitehead and Turner, 1998; Minnis et al., 2004; Castelli et al., 2005a; 2005b; EPPO, 2006; Moxnes and Hausken, 2007). A computer program called SAMPLE has been created to develop sampling methods for the detection of infestation foci of potato cyst nematodes to be used by seed and ware potato growers and governments. The purpose is to reduce high, ineffective, and many times unnecessary applications of soil fumigants (Been and Schomaker, 2000).

Known Hosts

According to a review by Sullivan et al. (2007), PCN (*G. pallida* and *G. rostochiensis*) have about 150 host species in the genus Solanum. A few species of Datura, *Hyoscyamus, Lycopersicon, Physalis, Physoclaina, Salpiglossis, and Saracha* also can allow reproduction of PCN in geographical areas where these nematodes occur. Many of these species are wild species found in South America, including *S. tuberosum* subsp. *andigena* (yellow potato), *S. vernei* (purple potato), and *S. brevicaule* (=*S. sucrense*). The authors did not differentiate records from *G. pallida* and *G. rostochiensis* due to many of these reports occurring prior to 1973 before the two species were separated and recognized as distinct species.

Weed hosts in Europe include *S. sarrachoides* (hairy nightshade), *S. dulcamara* (bitter nightshade), and *Datura stramonium* (jimsonweed), which may exacerbate nematode persistence in certain agricultural areas (reviewed in Baldwin and Mundo-Ocampo, 1991). The most important agricultural host is *Solanum tuberosum* (potato) (Baldwin

and Mundo-Ocampo, 1991). *Globodera pallida* has several pathotypes which differ in their ability to attack different potato cultivars (CSL, 2003). **Major hosts**

Solanum tuberosum (potato) (EPPO, 2007).

Minor hosts

Solanum dulcamara (bittersweet nightshade), S. lycopersicum (tomato), S. melongena (eggplant) (EPPO, 2007).

Experimental Hosts

Solanum alatum (hairy nightshade), S. carolinense (Carolina horsenettle), S. durum (no common name), S. gigantum (red bitter berry), S. gilo (scarlett eggplant), S. nigrum (black nightshade), S. physalifolium (hairy nightshade), S. rostratum (spiny nightshade), S. torvum (turkey berry), S. triflorum (cutleaf nightshade), and S. villosum (wooly nightshade) (Stelter, 1987; Boydston et al., 2013).

Pathogen or Associated Organisms Vectored

This species is not known to vector any organisms or other associated organisms. However, nematode feeding can lead to infection by *Rhizoctonia solani*, *Verticillium* spp., and other fungal diseases (CSL, 2003; Wale et al., 2008).

A synergistic relationship between Verticillium wilt (*Verticillium dahliae*) and *G. pallida* has been observed with some potato cultivars.

Known Distribution

Globodera pallida is found in cooler areas; while the similar *G. rostochiensis* can tolerate slightly warmer climates (Baldwin and Mundo-Ocampo, 1991). However, both species can suppress potato yield in warm climates in Mediterranean and Chilean localities where potatoes are grown during the winter-spring season (Greco et al., 1982; Greco and Moreno, 1992).

Africa: Algeria and Tunisia; Asia: India, Iran, Japan, and Pakistan; Europe: Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Faroe Islands, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal (including Madeira), Romania, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, and the United Kingdom; Central America: Costa Rica and Panama; North America: Canada, United States (Idaho); Oceania: New Zealand; South America: Argentina, Bolivia, Chile, Colombia, Ecuador, Falkland Islands, Peru, and Venezuela (Zasada and Gatt, 2000; Zouhar et al., 2003; Andrés et al., 2006; EPPO, 2007; Širca, 2012; CABI, 2013; Nježić et al., 2013; Narubu et al., 2016).

Reports of this species being reported in Finland, Japan, Libya, Malaysia, Mexico, Russia, Serbia & Montenegro, and South Africa are considered invalid or unreliable records (CABI, 2013).

This species was thought to have been eradicated from Denmark (EPPO, 2007). However, in 2012 it was detected there for the first time in 25 years (IPPC, 2013) and eradication efforts are underway at this time.

Pathway

The J2s of PCN (*G. pallida* and *G. rostochiensis*) do not move long distances by themselves in the soil. They can only move a maximum of 1 m (approx. 3 ¼ ft.) to locate a suitable host (EPPO, 2004). This species is distributed to new infestation sites by passive transport via wind, rain, flood water, and many other means such as contaminated propagative material, agricultural tools, animals, machinery, and workers (Turner and Evans, 1998; CABI, 2013).

The primary means of spread is through movement of cysts in soil which are the most environmentally resistant and easily transportable stage (CABI, 2013). This can occur through movement of infested farming equipment, seed potatoes, and tare dirt (USDA, 2006). While the import of soil and potatoes is highly regulated, there is a risk of moving the nematode through soil and potato smuggling as well as different plant and non-plant material that may have minute amounts of infested soil present, for example second-hand vehicles (Turner and Evans, 1998; NAPPO, 2007).

This species has been intercepted at least 31 times at U.S. ports of entry since 1984. Interceptions occurred on *Solanum tuberosum* (20), soil (4), other plant material (4), and tractors (2). Most of these interceptions originated from South America (24): Ecuador (13), Peru (8), Chile (2), and Colombia (1) with the remaining originating from Europe (7): Germany (3), United Kingdom (3), and Russia (1). Interceptions occurred in both stores (28) and general cargo (3) (AQAS, 2013).

Processed potatoes are not considered a viable pathway as nematodes will not survive the cooking process (steaming and drying) (APHIS, 2006b).

It is thought that PCN (*G. pallida* and *G. rostochiensis*) were introduced into Europe along with the potato from South America around the 1600s. From Europe, PCN were further spread through infested seed pieces (Turner and Evans, 1998; Baldwin and Mundo-Ocampo, 1991).

PCN are known to spread by similar methods as potato wart (*Synchytrium endobioticum*). Widespread provisions to prevent the spread of potato wart disease have helped limit the spread of potato cyst nematodes (Friedman, 1985).

Potential Distribution within the United States

Globodera pallida was detected in Idaho in April 2006 (USDA, 2006; Hafez et al, 2007). As of 2013, there are 21 known infested fields within a five mile radius. Infested fields are found in both Bingham and Bonneville counties. As of January 10, 2014, 8,478 acres of farmland in these two counties are regulated, of which 2,300 acres are infested fields (Gresham, 2014, personal communication).



Figure 6. Left: States with the most planted acres of fall potatoes in 2013. Right: States with the most production of fall potatoes in 2013. Statistics taken from USDA-NASS (2013).

Potato producing states are most likely to be impacted by the presence of *Globodera pallida*. According to a recent report by USDA-NASS (2013), the states with the most fall potato acreage planted and the largest quantity of fall potatoes produced are as follows: Colorado, Idaho, Maine, Michigan, Minnesota, Nebraska, New York, North Dakota, Oregon, Washington, and Wisconsin (Fig. 6). However, there are known hosts of *G. pallida* throughout the United States, and potatoes are cultivated to some level in virtually every state, so this nematode could possibly be found in any state. State surveyor coordinators should determine the suitability of a survey for *G. pallida* in their local areas.

Information on the Idaho quarantine can be found in the most recent version of the CFR under regulation 7 CFR 301.86, which can be accessed here: <u>www.ecfr.gov</u>. Regulated items include:

- potatoes,
- nursery stock,
- soil, compost, humus, muck, peat, and decomposed manure,
- grass sod,
- small grains and soybeans,
- hay, straw fodder, and plant litter,
- unshucked ear corn,
- used farm equipment,
- and any other material that may convey PCN.

Survey CAPS-Approved Method*:

There are two approved methods for survey including soil sampling and collection of host roots. Laboratory methods that are acceptable for cyst extraction include sugar centrifugation, USDA cyst extractor, Fenwick can sieving, wet sieving, and elutriation.

<u>Soil sampling</u>: Send samples to the nematology diagnostic lab where nematodes will be extracted and identified (preferred method).

<u>Collecting host roots:</u> Send samples to the nematology diagnostic lab where nematodes will be extracted and identified.

<u>Signs:</u> Mature females and cysts are just visible to the naked eye and can be seen as minute white globes on the root surface. At the appropriate stage of development, females are prolonged white (slightly cream colored but no yellow phase as compared to *G. rostochiensis*).

<u>Symptoms:</u> Patches of poor growth, chlorosis, and wilting with poor top growth may be seen. Affected plants suffer yield loss and tubers are smaller.

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

Note: This datasheet should not be used for any area where the pest is known to occur in the United States. Individuals should contact the Program Manager Jonathan Jones (Jonathan.M.Jones@aphis.usda.gov) for more information.

The Pale Potato Cyst Nematode National Survey and Diagnostic Sample Forwarding Protocols (2009) can be found here:

http://www.aphis.usda.gov/plant_health/plant_pest_info/potato/downloads/pcndocs/PCN_ __NatlSurvey.pdf.

Survey Site Selection

The most important host of this species is *Solanum tuberosum* (potato), so surveys should be focused on potato crops. The Pale Potato Cyst Nematode National Survey and Diagnostic Sample Forwarding Protocols (2009) states that surveying should occur on certified seed potato acreage, commercial potato acreage, and all other land use for seed potato research including universities, government, and other research organizations.

Time of year to survey

Cysts can be found throughout the year, but surveying usually occurs right after harvest when nematode populations are high.

Literature-Based Methods:

Visual survey/soil sampling:

The CFIA and USDA (2009, updated in 2014) Guidelines list three different survey protocols for field soil sampling which depend on where the sample is taken (http://www.aphis.usda.gov/plant_health/plant_pest_info/nematode/downloads/potato_g_uidelines.pdf). The survey protocol for PCN regulated areas requires 6,000 cc of soil per acre be collected with a soil probe. The protocol for detection surveys for non-regulated areas requires collection of 2,000 cc of soil per acre. Finally, the third protocol for PCN infested fields in the eradication program requires 18,000 cc of soil per acre be collected (CFIA and USDA, 2009; Gresham, 2014, personal communication). The third protocol is probably not relevant to general detection surveys.

The CFIA and USDA (2009) Guidelines also describe how to perform a PCN viability assay protocol. This helps to determine the viability status of the eggs within the PCN cysts collected from infested fields. If this test comes out negative, a soil bioassay should be completed.

During certain times, cysts and young females on the roots can be seen with the naked eye as small white, yellow, or brown globes on the root surface when plants are lifted from the ground. Detection by this method is only possible during a narrow time period. It is also time-consuming (EPPO, 2004).

Key Diagnostics/Identification

CAPS-Approved Method*:

<u>Morphological</u>: Characters of diagnostic values include the white color, number of cuticular ridges (12.5 \pm 3.1) in the vulval-anus axis of adult females and the stylet length (22-25 μ m) of J2. Other characteristics of cysts, females, and J2 can be found in Baldwin and Mundo-Ocampo (1991).

<u>Molecular:</u> Immunological, protein, and DNA-based techniques are available. RAPD and RFLP analyses are also available. There are PPQ-CPHST-validated conventional PCR and real-time PCR methods available to detect *G. pallida*, *G. rostochiensis*, and *G. tabacum* (Nakhla et al., 2008). A real-time PCR has been developed by Nakhla et al. (2010) that shortens the time required for diagnosis. A validated work instruction is available.

<u>Notes:</u> Globodera pallida, G. rostochiensis, and G. tabacum all occur in the United States. Globodera tabacum parasitizes tobacco (*Nicotiana tabacum*), but not potato. The molecular analysis allows the identification of this species and its separation from *G. pallida* and *G. rostochiensis* in fields suspected to be infested with mixed populations of both PCN and *G. tabacum*. Globodera pallida is present in Idaho.

Work instruction for molecular testing of PCN are available. Contact Ashlee Barth (301-504-7100 x 9227, <u>ashlee.k.barth@aphis.usda.gov</u>) for the most up-to-date work instructions.

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

Literature-Based Methods:

Skantar et al., (2007) describe a species specific PCR for identification of *G. pallida*, *G rostochiensis*, and *G. tabacum*. Quader et al. (2008) provide a detailed description of morphological and molecular identification of *Globodera pallida* and *G. rostochiensis*. Several conventional PCR assays for the molecular identification of *Globodera pallida* and *G. rostochiensis* cysts and juveniles have been developed (Yu et al., 2011; Douda et al., 2014; Van de Vossenberg et al., 2014). Papayiannis et al. (2013) developed a real-time PCR which enables the simultaneous detection of *G. pallida* and *G. rostochiensis*.

Some DNA-based approaches have been reported for other *Globodera* spp., which indicates that a molecular method for identification may be available in the future. Alenda et al. (2013) analyzed sequence polymorphism of effector genes in subspecies *G. tabacum* for the development of PCR tools for identification of this species complex.

Easily Confused Pests

This species can be confused with other *Globodera* species, particularly *Globodera rostochiensis* and *G. tabacum*. In addition, a new species of *Globodera*, *G. ellingtonae* (Handoo et al., 2012), was recently discovered in potato fields in Oregon. The impact of this nematode is currently under investigation, but preliminary research suggests that potato is a poor host for *G. ellingtonae* (Bullock, 2014, personal communication). *G. ellingtonae* can be confused with the three *Globodera* mentioned above. Chronis et al. (2014) developed a PCR assay that provides reliable identification of *G. ellingtonae*.

The potato cyst nematodes can be differentiated from each other if the female is at the appropriate stage. *G. rostochiensis* females turn from white to yellow and then brown while *G. pallida* changes from white to brown (Fig. 7) (EPPO, 2004). Generally "the J2s of *G. pallida* are longer, have a more robust-looking stylet with forward pointing basal knobs, and have longer true tail lengths than other species in the genus" (CABI, 2013).

Golden (1986) provides a key to 6 genera and 59 species of cyst forming nematodes. Mulvey and Golden (1983) provides a key to the 6 genera and 34 species of cyst forming nematodes known to occur in the Western Hemisphere at that time. Both keys include *G. pallida*, *G. rostochiensis*, and *G. tabacum*. Morphological characters for the separation of these *Globodera* from *G. ellingtonae* are provided by Handoo et al. (2012).

that can rapidly detect and quantify

ellingtonae are provided by Handoo et al. (2012).
A multiplex PCR has been developed to distinguish *G. pallida* and *G. rostochiensis* (Pylypenko et al., 2005). Madani et al. (2005) developed a real-time PCR assay
Figure 7. Compar rostochiensis and *G. pallida* turn dire gold before they t Ulrich Zunke, Unit http://www.bugwo



Figure 7. Comparison of *Globodera rostochiensis* and *Globodera pallida*: females of *G. pallida* turn directly to brown cysts whereas *G. rostochiensis* females change from yellow to gold before they turn brown. Photo courtesy of Ulrich Zunke, University of Hamburg, <u>http://www.bugwood.org/</u>.

G. pallida and *Heterodera schachtii* (beet cyst nematode). Madani et al. (2008) have developed a multiplex real-time PCR that can distinguish *G. pallida*, *G. rostochiensis*, and *G. tabacum*. A real-time PCR has been developed by Nakhla et al. (2010) that shortens the time required for diagnosis. A validated work instruction is available.

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Reviewed by: David Chitwood (USDA-ARS Nematology Laboratory, Beltsville, MD), Tina Gresham (Program Director, Pale Cyst Nematode Program USDA-APHIS-PPQ, Idaho Falls, ID), Zafar Handoo (USDA-ARS Nematology Laboratory, Beltsville, MD), Renato Inserra (Division of Plant Industry, Florida Department of Agriculture and Consumer Services), Jonathan Jones (USDA-APHIS pest management team, Riverdale, MD), Mark Nakhla (USDA-APHIS-PPQ-CPHST laboratory, Beltsville, MD), and Andrea Skantar (USDA-ARS Nematology Laboratory, Beltsville, MD).

Update History:

July, 2014: Added additional references that were recommended by Andrea Skantar. Updated the Key Diagnostic and Easily Confused Species sections to reflect these new references.

November, 2016: Added Japan to distribution.