Globodera rostochiensis

Scientific Name
Globodera rostochiensis (Wollenweber, 1923) Skarbilovich, 1959.

Synonyms:
Heterodera rostochiensis Wollenweber, *Heterodera schachtii rostochiensis* Wollenweber, *Globodera schachtii solani* Zimmermann

Common Name
Golden nematode, golden potato cyst nematode, yellow potato cyst nematode

Type of Pest
Nematode

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

Reason for Inclusion in Manual
PPQ Program Pest; cyst nematode

Pest Description
The similar species *Globodera pallida* was 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 earlier work (CABI/EPPO, n.d.).

Eggs: The eggs of *G. rostochiensis* are always retained within the body of the female where they develop and are not deposited in an egg sac. At its death, the

Figure 1. Second stage juveniles and eggs of *Globodera rostochiensis*. Photo courtesy of Ulrich Zunke, University of Hamburg. http://www.bugwood.org/.
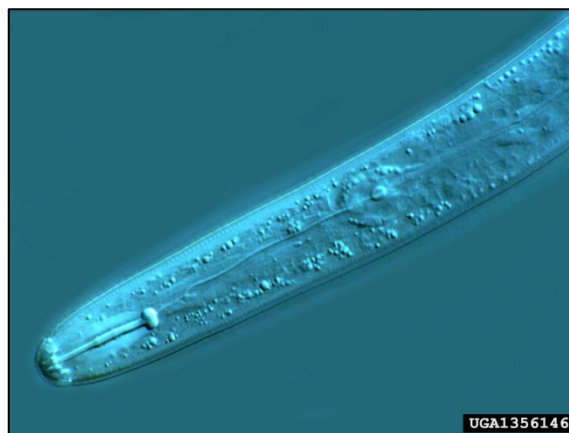

Figure 2. Close-up of the anterior region, showing the stylet, metacorpus, and the anterior end of the esophageal dorsal gland of *Globodera rostochiensis.* Photo courtesy of Ulrich Zunke, University of Hamburg. http://www.bugwood.org/.

female becomes a cyst filled with embryonated eggs, which contain second-stage juveniles (J2s) folded four times. The egg shell is hyaline with a smooth surface. No microvilli are present. Length=101-104 μm; width=46-48 μm; L/W ratio=2.1-2.5 (Friedman, 1985, taken from Golden and Ellington, 1972; CABI, 2013).

Second-Stage Juveniles (J2s): "J2s, in soil, are motile and vermiform and become sedentary and swollen once they penetrate inside the root. They have a body length of about 430 (366-470) *µ*m, a mouth with a strong stylet, which is a hollow feeding structure used for ingesting cell content after puncturing their walls (Fig. 1), and a pointed tail" (CABI/EPPO, n.d.) (Fig. 2).

"Body vermiform, cuticular annulation prominent. Lateral field with 4 incisures. Head slightly offset, rounded, bearing 4 – 6 annules. Cephalic framework heavily sclerotized. Stylet well developed, 22 (21-23) *µ*m long, knobs rounded, slightly backward sloping. Median bulb ellipsoidal with prominent valve. Esophageal glands extend ventrally for about 35 percent of body length. Hemizonid 1 annule anterior to excretory pore. Four-celled genital primordium about 60 percent of body length. Tail tapered to finely rounded terminus. Phasmids difficult to see, about half way on tail" (Friedman, 1985, taken from Golden and Ellington, 1972).

Figure 3. Adult female of *Globodera rostochiensis.* Photo courtesy of Pest and Diseases Image Library, http://www.bugwood.org.

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

Females: The adult females are swollen, sedentary, and protruding from the root surface. "Body subspherical, 540 (250-810) *µ*m in diameter, with projecting neck, white then passing through golden yellow phase as cyst is formed (Fig. 3). Cuticle reticulate, punctate just below surface. Head with one or two annules. Cephalic framework weakly developed.

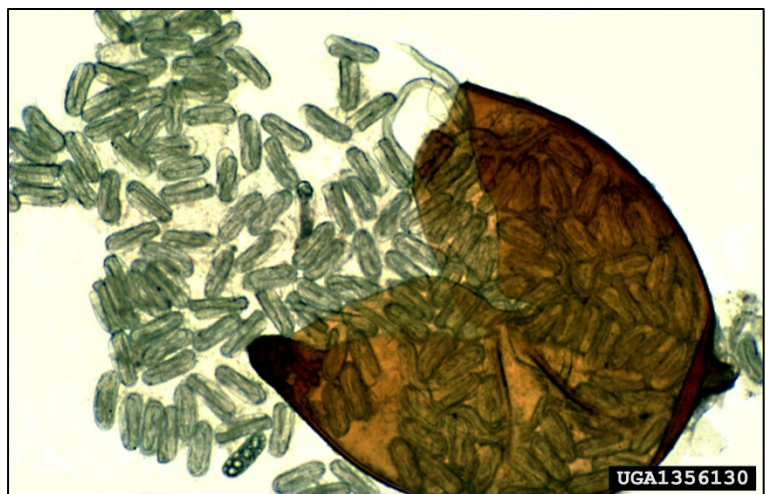**Figure 4.** Crushed cyst of *Globodera rostochiensis* with numerous eggs. Photo courtesy of Ulrich Zunke, University of Hamburg. http://www.bugwood.org/.

Stylet knobs rounded, sloped posteriorly. Median bulb large, nearly spherical, with well-

developed valve. Esophageal glands in broad lobe, often obscure, frequently displaced forward by developing paired ovaries. Excretory pore near base of neck. Vulva not set off from body, opposite neck, in slight almost circular depression. Anus smaller than vulva, at right angle to axis of vulval slit. Number of cuticular ridges on anal-vulval axis 17-24" (Friedman, 1985, taken from Golden and Ellington, 1972; Subbotin et al., 2010).

Cysts: Cysts are similar in shape to the females, but have a tanned skin and degenerated organs. They are like a persistent tanned sac which contains the eggs and is derived from some or all components of the dead mature female body wall (CABI, 2013). This life stage is found in the soil or attached to the roots (CABI/EPPO, n.d.) (Fig. 4). "Cyst brown, subspherical to spherical, with projecting neck. Vulval region forming single circular fenestra in older cysts, abullate. Anus small, may be at apex of V-shaped structure. Cyst wall pattern similar to that of female but often more prominent. Usually punctate" (Friedman, 1985, taken from Golden and Ellington, 1972).

Cyst length without neck=445 ± 50 μm; width=382 ± 60 μm; neck length=104 ± 19 μm; mean fenestral diameter=19.0 ± 2.0 μm; anus to fenestra=66.5 ± 10.3 μm; Granek's ratio=3.6 ± 0.8 (CABI, 2013).

Males: Males are motile and present in the soil or coiled inside the cuticle of J4s. They are vermiform like the J2d and about 1200±100 μm long. The copulatory apparatus is close to the end of posterior body (CABI/EPPO, n.d.). "Body vermiform, cuticular annulation prominent. Lateral field with 4 incisures. Head slightly offset, hemispherical, 6-7 annules. Cephalic framework heavily sclerotized. Stylet strong with prominent knobs slightly backward sloping. Median bulb ellipsoidal with prominent valve. Esophageal glands narrow, ventral, terminating near excretory pore. Hemizonid 2 annules long, 2-3 annules anterior to excretory pore. Testis single, spicules with tips rounded, unnotched. Tail short, length and shape variable. Phasmids not reported" (Friedman, 1985, taken from Golden and Ellington, 1972).

Detailed descriptions of the life stages can be found in Golden and Ellington (1972), while Golden (1986) gives life stage descriptions of cyst nematodes in general.

Biology and Ecology
This species overwinters as J2s within eggs inside the cyst. Each cyst may have 200 to 500 embryonated eggs (Friedman, 1985) (Fig. 4). Hatching of potato cyst nematode (PCN) J2s increases rapidly after plant emergence, peaking between two to five weeks after emergence (Devine and Jones, 2003). Host root secretions help stimulate J2s to hatch (Friedman, 1985) as well as soil temperatures above 10°C (50°F) (Ferris, 2005). Optimal soil temperature for hatching and development is 15 to 20°C (59 to 68°F) (Eyres et al., 2005). The upper threshold temperature for J2 root penetration and development is 28°C (82°F) (Mugniery, 1978), indicating that no nematode parasitization occurs at this and above soil temperatures.

Hatched PCN J2s move in the soil searching for host roots. Usually, they penetrate root behind the tip. After root penetration, J2s move through the root, feeding on the cortex,

endodermis, and pericycle.   J2s then establish a permanent feeding site consisting of a syncytium in the root stele and become sedentary and swollen (Baldwin and Mundo-Ocampo, 1991). The syncytium provides nutrients to the nematode (EPPO, 2004). In resistant potato varieties, localized necrosis and thickening of the syncytial walls may occur (reviewed in Baldwin and Mundo-Ocampo, 1991). The syncytium provides nutrients to the nematode (EPPO, 2004). In resistant potato varieties, localized necrosis and thickening of the syncytial walls may occur (reviewed in Baldwin and Mundo-Ocampo, 1991).

J2s undergo three molts before they reach the adult stage (Friedman, 1985). From hatching to adult, development takes 38 to 45 days. The ratio of sexes is possibly determined by food supply (Ferris, 2005). Sedentary adult females enlarge and burst through the root with their posterior body portion, facilitating mating (reviewed in Baldwin and Mundo-Ocampo, 1991). The head of the female remains embedded in the root (CSL, 2003). Females exude a sex pheromone to attract mates. Males are vermiform and motile leaving the roots to mate (Friedman, 1985). Mating occurs within 50 days of J2 root invasion.

Figure 5. Infected plant on right compared to healthy plant on left. Photo courtesy of Ulrich Zunke, University of Hamburg. http://www.bugwood.org/.

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 and continue to feed. Once a female dies, she becomes a cyst (toughened cuticle), changing from white to yellow or gold and then finally to brown (reviewed in Baldwin and Mundo-Ocampo, 1991). Cysts can remain attached to the roots or they detach from the root and fall into the soil (CABI/EPPO, n.d.).

Depending on the duration of the crop cycle, this species may have one or more generations per year and persists in the soil as a dormant stage (cyst) for years (reviewed in Baldwin and Mundo-Ocampo, 1991). Friedman (1985) states that this species usually produces one generation per year. According to USDA (2012), this species produces one generation per year in the New York temperature zone.

Hybrids between *G. pallida* and *G. rostochiensis* can exist in nature (reviewed in Baldwin and Mundo-Ocampo, 1991). This species has five recognized pathotypes which are characterized by their ability to multiply on certain *Solanum* clones and hybrids (reviewed in CABI/EPPO, n.d.; Dale and de Scurrah, 1998; Fleming and Powers, 1998).

Symptoms and Signs

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.). Size of tubers can be reduced even when visible symptoms are minor (CABI/EPPO, n.d.). Infested potato plants have a reduced root system. Because of decreased water and nutrient uptake, plant death could potentially occur, especially in presence of concomitant infections of other pathogens (Turner and Evans, 1998; EPPO, 2004). Light infestations can lead to reduced tuber size while heavy infestations can lead to reduction in both number and size of tubers (Berg, 2006) (Fig. 5 and 6).

Figure 6. Healthy potato variety Désirée on right compared to infected potato on left. Photo courtesy of Christopher Hogger, Swiss Federal Research Station for Agroecology and Agriculture, http://www.bugwood.org/.

Figure 7. Potato plants infected with *Globodera rostochiensis*. Photo courtesy of Bonsak Hammeraas, Bioforsk - Norwegian Institute for Agricultural and Environmental Research, http://www.bugwood.org/.

Symptoms initially appear as localized areas of poor growth. Infested plants are usually smaller, may have yellow leaves (Fig. 7), and may also wilt, especially around midday. If plants are removed carefully around flowering time, females may be observed on the

roots, partially exposed. As the population increases, patchy areas can expand and more will pop up (Friedman, 1985).

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).

Symptoms in tomato are similar to those seen in potato. Poor growth and wilting may occur. Tomato leaves of heavily infested plants are purplish instead of yellow, and lower leaves may die. During the early stages of attack, roots may slightly swell. This could be confused with galling caused by root-knot nematodes (Spears, 1968).

Pest Importance

Potato cyst nematodes 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.). Yield losses are also dependent on potato cultivar, environmental conditions, and agronomic practices. Yields may be reduced even when no apparent symptoms are seen aboveground. Depending on potato cultivar, yield losses can range from 19 to 90% (Friedman, 1985). 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.). In the UK, 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).

Figure 8. Yellow females of *Globodera rostochiensis* on root. Photo courtesy of Bonsak Hammeraas, Bioforsk - Norwegian Institute for Agricultural and Environmental Research, http://www.bugwood.org/.

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 at $1.6 billion and consists of 17% of U.S. potato production (USPB, 2013). *Globodera rostochiensis* serves as a serious threat to both domestic and international trade in potatoes and nursery stock. This species is regulated as a harmful organism in 60 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).

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 source of infection for future crops (CSL, 2003).

Due to the importance of potato cyst nematodes to potato production and trade, the development of resistant varieties (Brodie, 1996; Castelli et al., 2005; EPPO, 2006a; Gerbhardt et al., 2006) and other control options have been explored, including crop rotation, nematicides, trap cropping, and soil sterilization (Kerry et al., n.d.; Minnis et al., 2004; Turner et al., 2006; Moxnes and Hausken, 2007). Commercial cultivars have been developed that can lead to 80 to 95% population reductions each year (reviewed in Friedman, 1985). Work has previously been done to identify potential biocontrol agents as well (Andreoglou et al., 2003). 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 often 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), *S. nigrum* (black nightshade), and *Datura stramonium* (jimsonweed). Weed hosts may exacerbate nematode persistence in certain agricultural areas (reviewed in Baldwin and Mundo-Ocampo, 1991). The most important agricultural host of *Globodera rostochiensis* is *Solanum tuberosum* (potato) (Baldwin and Mundo-Ocampo, 1991). *G. rostochiensis* has several pathotypes which differ in their ability to attack different potato cultivars (CSL, 2003). This nematode has been found, as a contaminant, on many different plant species that are not necessarily hosts (Friedman, 1985).

Major hosts
Solanum tuberosum (potato)

Minor hosts
Solanum donianum (=*S. blodgettii*) (mullein donianum), *Solanum elaeagnifolium* (silverleaf nightshade), *Solanum lycopersicum* (tomato), *Solanum dulcamara* (bitter nightshade), *Solanum aethiopicum* (=*S. integrifolium*) (tomato eggplant), *Solanum mauritianum* (tree tobacco), *Solanum melongena* (eggplant), *Solanum rostratum* (buffalo bur), *Solanum triflorum* (cutleaf nightshade), and *Solanum xanti* (purple nightshade) (Spears, 1968; CABI, 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).

Known Distribution
Africa: Algeria, Egypt, Libya, Sierra Leone, South Africa, and Tunisia; **Asia:** India, Indonesia, Japan, Philippines, Sri Lanka, Tajikistan, and Turkey; **Central America:** Panama; **Europe:** Albania, Austria, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Faroe Islands, Finland, France, Germany, Greece (including Crete), Hungary, Iceland, Ireland, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal (including Madeira), Romania, Russia, Serbia, Spain (including Balearics Islands and Canary Islands), Slovakia, Sweden, Switzerland, Ukraine, and United Kingdom (England, Channel Islands); **Middle East:** Armenia; Lebanon, Iran, Oman, Pakistan; **North America:** United States (New York), Canada, and Mexico; **Oceania:** Australia, New Zealand, and Norfolk Island; **South America:** Bolivia, Chile, Colombia, Ecuador, Peru, and Venezuela (CABI/EPPO, n.d.; Ibrahim et al., 2000; Indarti et al., 2004; Andrés et al., 2006; Gitty and Tanha Maafi, 2009; Ostojić et al., 2011; Bačić, 2012).

This species has been eradicated from Israel (CABI/EPPO, n.d.). This species is listed as previously occurring in Argentina and Costa Rica. Records of this species listing its occurrence in Brazil are considered invalid (EPPO, 2006b).

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).

This species has been intercepted at least 31 times at U.S. ports of entry since 1984. Interceptions occurred on *Solanum tuberosum* (14), other plant material (11), and tractors/machinery (3). Most of these interceptions originated from Europe (21): Poland (7), Netherlands (4), United Kingdom (4), Germany (2), Spain (2), Italy (1), and Soviet Union (1) with the remaining occurring from Central/South America (9): Venezuela (3), Chile (2), Peru (2), Azores (1), and Dominican Republic (1), and Asia: South Korea (1). Most interceptions occurred in stores (18), general cargo (6), and baggage (4) (AQAS, 2013).

Cysts of this species can travel as a contaminant on many different non-host plants and materials, including: "garlic, horseradish, and onion cargoes for consumption, hay packing around dishes, packing for old toys, new and used autos and trucks, used bagging, old cordage, ballast, boots and shoes, military tanks and equipment, crates…, and farm tractors" (Friedman, 1985). 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 were introduced into Europe, along with the potato from South America around the 1600s. From Europe, PCN were further spread through infested seed pieces (Baldwin and Mundo-Ocampo, 1991).

PCN spread by similar methods as potato wart (*Synchytrium endobioticum*) and widespread provisions to prevent the spread of potato wart disease has helped limit the spread of potato cyst nematodes (Friedman, 1985).

Potential Distribution within the United States

Globodera rostochiensis is currently only found in certain counties of New York, specifically Cayuga, Livingston, Nassau, Orleans, Seneca, Steuben, Suffolk, and Wayne. Genesee County was once on the list of regulated counties, but it was removed from the list in 2010 because *G. rostochiensis* was never found there. It is not considered widely present nor distributed throughout the United States (USDA, 2012).

Information on the New York quarantine can be found in the most recent version of the Code of Federal Regulations (CFR) under regulation 7 CFR 301.85, which can be

accessed here: www.ecfr.gov. "Quarantine items include: soil, compost, peat, manure, live plants, grass sod, plant crowns, roots for propagation, bulbs, corms, rhizomes, root crops, small grain and soybeans (unless in approved containers), hay and straw (unless in approved containers), plant litter, ear corn (except shucked ear corn), used farm product containers, burlap bags, used farm tools (unless free of soil), used cultivating or harvesting equipment (unless free of soil), and seed potatoes" (Ferris, 2005). Additional quarantine information can be found on the USDA-APHIS Golden Nematode Program website, http://www.aphis.usda.gov/plant_health/plant_pest_info/nematode/index.shtml
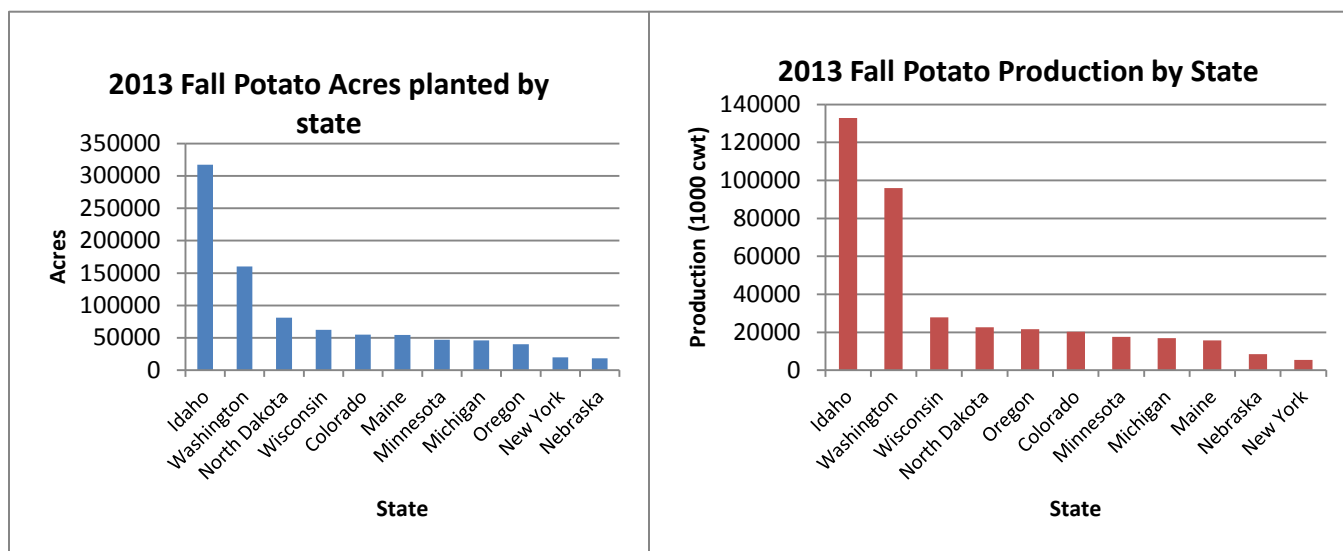
2013 Fall Potato Acres planted by state

2013 Fall Potato Production by State

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).

Large scale potato producing states are most likely to be impacted by *G. rostochiensis*. 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. 9). However, there are known hosts of *G. rostochiensis* throughout the United States, and potatoes are cultivated in every state, so this nematode could possibly be found in any state. State surveyors should determine the suitability of a survey for *G. rostochiensis* in their local areas.

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.

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

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

Signs: Mature females and cysts are just visible to the naked eye and can be seen as minute yellow globes on the root surface. At the appropriate stage of development, females change from white to yellow in *G. rostochiensis* (compare to *G. pallida*) (Fig. 8, 10).

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

Detailed information on survey procedures can be found in the Golden Nematode Program Manual found here:
http://www.aphis.usda.gov/import_export/plants/manuals/domestic/downloads/gnpm.pdf
(USDA, 2012).

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

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.

Survey Site Selection:
Surveys may occur in any area where host plants are grown, including certified seed potato acreage, commercial potato acreage, and all other land use for seed potato research including universities, government, and other research organizations (USDA, 2012).

Time of year to survey:
Surveys can occur after crop harvest. Surveying should occur immediately after harvest "to avoid interfering with normal post-harvest farming operations. The principle sampling methods for post-crop survey are systematic manual soil sampling or mechanical (wheel) soil sampling" (USDA, 2012).

If requested by the owner and the land will not have the host-crop grown that year, surveys can be scheduled for the spring of that year (USDA, 2012).

Literature-Based Methods:
Visual survey/soil sampling:
Agricultural Handbook No. 353, The Golden Nematode Handbook has historical information on survey and laboratory procedures (Spears, 1968).

The Golden Nematode Program Manual (USDA, 2012) states that soil sampling can be done either manually or mechanically. Manual soil sampling includes the following methods:

- 8 x 8 Block Sampling Method: Fields are divided into units of approximately ½ acre. This method has 56 sample points. Soil sample bags should contain 56 scoops of soil and weigh between 4 and 6 lbs.
- Simplified 8 x 8 Block Sampling Method: Collection bags are distributed along the edge of the field at intervals in multiples of 8 paces.
- Modified 8 x 8 (4 x 8) Block Sampling Method: This modified method increases the sensitivity by doubling the number of samples points to 112 total. Samples are collected every 4 paces instead of every 8 paces.

Mechanical sampling is when soil samples are taken by a tractor mounted machine with either two or three rotating wheals with probes that penetrate the group by about 4 inches (USDA, 2012).

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*:
Morphological: Characters of diagnostic values include the golden color, number of cuticular ridges (17-24) in the vulval-anus axis of adult females and the stylet length (20-23) μm of J2s. Other characteristics of J2s, females, and cysts can be found in Baldwin and Mundo-Ocampo (1991) and Subbotin et al. (2010).

Molecular: 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 needed. A validated work instruction is available.

Notes: *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 rostochiensis* is present in New York. Protocols for processing soil samples for PCN are available.

The Golden Nematode Program Manual is available here:
http://www.aphis.usda.gov/import_export/plants/manuals/domestic/downloads/gnpm.pdf

Work instruction for molecular testing of PCN are available. Contact Ashlee Barth

(301-504-7100 x 9227, ashlee.k.barth@aphis.usda.gov) 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 https://caps.ceris.purdue.edu/approved-methods.

Literature-Based Methods:
Skantar et al., (2007) describe a species specific PCR for identification of *G. pallida*, *G rosthochiensis*, 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. rosthochiensis*.

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 pallida* 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*.

Figure 10. 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, http://www.bugwood.org/.

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. 10) (EPPO, 2004). Generally "the second-stage

juveniles 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).

A key to differentiate between different round cyst species can be found in Golden and Ellington (1972) and Subbotin et al. (2010). 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 at that time in the Western Hemisphere. 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).

A key to mature cysts of the more common species of *Heterodera* (*G. rostochiensis* is listed as *H. rostochiensis*) can be found in Spears (1968).

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 that can rapidly detect and quantify *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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