Heterodera latipons

Scientific Name

Heterodera latipons (Franklin, 1969)

Synonyms:

Bidera latipons (Franklin, 1969) Krall and Krall, 1978; *Ephippiodera latipons* (Franklin, 1969) Shagalina and Krall, 1981

Common Name

Mediterranean cereal cyst nematode

Type of Pest Nematode

Taxonomic Position (Siddiqi, 2000)

Class: Secementea, Order: Tylenchida, Family: Heteroderidae

Reason for Inclusion in Manual

CAPS Target: AHP Prioritized Pest List - 2006 through 2011; Cyst Nematode Survey; Small Grains Survey.

Pest Description

<u>Note:</u> There has been considerable disagreement with respect to the taxonomic classification of *Heterodera latipons* and closely associated species referred to as the *"H. avenae* group". *H. avenae*, together with other bifenestrate cyst nematodes having a short vulval slit, were placed in the genus *Bidera* by Krall and Krall in 1978, but Mulvey and Golden (1983) synomized *Bidera* with *Heterodera* (Handoo, 2002). This synonomy was not universally accepted (Baldwin and Mundo-Ocampo, 1991; Davis and Venette, 2004) until 2010 (Subbotin et al., 2010).

Measurements (From Franklin (1969)):

Eggs: length 100-124 μm; width 44-56 μm.

<u>Second-stage juveniles (J2s)</u>: Body length 401-478 μ m; body width 19-22 μ m; tail length 42-54 μ m; length of the hyaline tail tip 20-31 μ m; stylet length 23-25 μ m; lateral field with four incisures.

<u>Cysts:</u> Cysts are typically ovoid to lemon-shaped as those of *H. avenae* with short vulva slits (< 16 μ m) (Fig. 1). Fenestral length 58-76 μ m; fenestral width 15-27 μ m; semi-fenestral length 13- 19 μ m; vulval slit length 6-9 μ m; vulval bridge length

18-39 μ m; underbridge length 80-125 μ m; underbridge width 7-14 μ m; sub-crystalline layer present.

<u>Females:</u> Body length (excluding neck) 348-645 μ m; body width 277-510 μ m; neck length 58-103 μ m; stylet length 21-28 μ m.

<u>Males:</u> Body length 960-1406 μ m; body width 25-32.5 μ m; stylet length 22-29 μ m; spicule length 32-36 μ m; lateral field with four longitudinal incisures.

Biology and Ecology

The life cycle of *H. latipons* is essentially the same as that for other species of Heterodera. Second-stage juveniles (J2s) emerge from the cyst at planting time, penetrate host roots just behind the root tip and establish a specialized feeding site (a syncytium) in the stele. Then, they develop into swollen females, which retain the eggs. Females rupture the root cortex and protrude from the root surface leaving the head and neck embedded. Males that have become worm-like move through the soil to inseminate females. Before the female dies, the cuticle turns brown, and the body becomes a cyst filled with embryonated eggs which develop into the second juvenile stage. Cysts persist in soil for many years and serve as the survival stage of the nematode. The cysts break off from the roots and become free in the soil



Figure 1. Micrographs and photographs showing the anatomical characteristics of *H. latipons* cysts. A) Scanning electron micrograph of the vulval area of a newly formed cyst. Note: the short vulval slit (v) and the area in which fenestration (S) will occur at a late stage of cyst formation. B) Light microscopy photograph of the perineal pattern of a nematode cyst showing wellseparated and circular semi-fenestra. C) Perineal patterns observed with a light microscope showing a well-developed and rather long underbridge, bifurcated at both ends and with a sclerotized enlargement in the middle. Photos courtesy of Greco et al. (2002). (USDA, 1985). Like other cyst forming nematodes, it has sedentary endoparasitic habits.

Laboratory experiments (Scholz and Sikora, 2004) demonstrated that eggs hatch in greater numbers from older (4 to 5 months) than from younger (1 to 2 months) cysts after exposure to temperatures of 5°C (41°F) or 10°C (50°F). Temperatures in the range of 5 to 15°C (41 to 59°F) appear to be the most suitable for egg hatch, while at 20 to 25°C (68 to 77°F) egg hatch seems to be suppressed. Field studies under Mediterranean conditions indicate that nematode J2s occur in the soil from November to February when soil temperatures do not exceed 18°C (64.4°F). Usually, J2s in soil peak at plant emergence. Juveniles invade roots behind the root apex (at root apex in *H. avenae*) at the beginning of plant emergence in November-December.

Females and males both develop by the end of January, and females lay eggs by February. Embryonated eggs can be observed by early March, when well-developed white females can be easily observed on the roots. From April onwards, white females turn into brown cysts coated by a sub-crystalline layer. In cooler areas, such as inland Syria, the development of the nematode can be delayed. Accumulated day degrees, above 7°C (44.6°F), for the development of white females and cysts with coiled embryos were 215 and 386, respectively. Only one generation per growing season is completed (Mor et al., 1992; Scholz and Sikora, 2004).



Figure 2. Symptoms of infection by *Heterodera latipons* in: A) A durum wheat field in Syria, and B) A barley field in Cyprus. Photos courtesy of Greco et al. (2002).

Symptoms/Signs

Slight to severe yellowing of cereal stands can be observed at an early stage of nematode infestation. Later, infested fields show patchy plant growth associated with poor tillering and shorter spikes. Symptoms occur in patches, and the patches enlarge as the nematode population spreads from the initial focus (Fig. 2). These symptoms are similar to those caused by other biotic or and abiotic stresses. Before plant flowering,

white lemon-shaped females can be observed on the roots by the naked eye or under a dissecting microscope after gently shaking or washing the roots to remove adhering soil (Greco et al., 2002). Plants also tend to wilt during warmer portions of the day (USDA, 1985).

Pest Importance

Hajihasani et al., (2010) did a recent study on the effects of *H. latipons* infestation on the yield and growth parameters of *Triticum aestivum* cv. Sardari (Bread Wheat). They concluded that an *H. latipons* population density of 20 eggs/J2s per gram of soil was shown to reduce grain yield by up to 55%, root dry weight by up to 70%, aerial shoot dry weight by up to 48%, spike height by up to 36%, and plant height by up to 32%.

In addition to yield loss in wheat, Philis (1988) reported up to 50% yield loss of barley in infested fields in Cyprus. A 24% yield loss was observed in barley infested with 28 eggs and juveniles/g of soil located in an area with 279 mm annual rainfall, but no significant yield reduction was observed in soil infested with up to 10 eggs/g soil in areas with 411 mm annual rainfall (Scholz, 2001). These findings corroborate field observations that the nematode is more damaging under water stress conditions.

Each of the main hosts of *Heterodera latipons* (wheat, rye, oat, and barley) are widely grown commodities in the United States. Planted acreage of these four commodities in 2013 totaled over 63 million (USDA-ERS, 2013). Wheat was by far the most widely cultivated, encompassing 56 million of those acres. The total 2013 U.S. wheat harvest consisted of 2.13 billion bushels and had an estimated value of \$14.9 billion (USDA-ERS, 2013). Approximately 51% of the wheat produced in the United States in 2013 was destined for export, comprising a total of 19.5% of the global export market (USDA-ERS, 2013). *H. latipons* has the potential to become a serious threat to both production and trade of these commodities if it becomes established in the United States.

At the genus level, *Heterodera* is listed as a harmful organism in the following countries: Australia, Chile, Madagascar, Namibia, Nauru, South Africa, and Syria (USDA-PCIT, 2013). There may be trade implications with these countries if *H. latipons* becomes established in the United States.

Known Hosts

Main Hosts:

Avena sativa (oats), Hordeum spp. (barley), Secale cereale (rye), and Triticum spp. (wheat) (Subbotin et al., 2010).

Note: *Triticum aestivum* is reported to be the main wheat host (EPPO, 2013), and *T. durhum* is reported as a poor host (Scholz, 2001).

Other hosts:

Elytrigia repens (couch grass), *Phalaris minor* (canarygrass), and *P. paradoxa* (Hood canarygrass) (Subbotin et al., 2010).

Barley and wheat are the most seriously damaged cereals, but the nematode also infects oats and rye. Mackintosh (1970) reported *Ammophila arenaria* (marram grass) as a host of *H. latipons*. However, this was probably a misidentification of *H. hordecalis* (Davis and Venette, 2004).

There is a record of an interception of *Heterodera latipons* at a port of entry in the United Kingdom (UK) on a shipment of *Solanum tuberosum* (ware potatoes) from Israel (Defra, 2007). However, there is no record of *S. tuberosum* as a host for this nematode.

Known Vectors (or associated organisms)

Damage is more severe in fields infested concomitantly by *H. latipons* and the fungus *Bipolaris sorokiniana* (Sacc,) Shoemaker, the causal agent of common root rot and seedling blight of barley, as the nematode increases the aggressiveness of the fungus (Scholz, 2001).

Known Distribution

Asia: Armenia, Iran, Israel, Japan, Jordan, Syria, Tajikistan, Turkey, and Turkmenistan. **Africa:** Libya, Morocco, and Tunisia. **Europe:** Bulgaria, Cyprus, Czech Republic, Greece, Italy, Poland, Spain, and Ukraine. (Greco et al., 2002; CABI, 2004; Davis and Venette, 2004; Subbotin et al., 2010; Mokrini et al., 2012, EPPO, 2013).

There are unconfirmed reports of *Heterodera latipons* in Canada (Prince Edward Island) and the United Kingdom (Scotland) (EPPO, 2013). These reports, however, are likely misidentifications of *H. hordecalis* (USDA, 1985).

Pathway

There are currently no restrictions on the import of *Avena sativa*, *Hordeum vulgare*, *Phalaris paradoxa*, or *Secale cereale* seed material other than their required sampling as an FSA-A seed (USDA, 2013). Cyst nematodes can be transported on infested soil adhering to seed material (Greco et al., 1992). Since 2003, there have been shipments of *Avena* spp. (30), *Hordeum* spp. (18), *Phalaris* spp. (2), and *Secale* spp. (8) from known host countries. There were also interceptions of *Avena* spp. (23), *Hordeum* spp. (20), *Phalaris* spp. (2), and *Secale* spp. (2) from known host countries. In addition, there were 211 interceptions of *Triticum* spp. from known host countries (AQAS, 2013).

In addition to transport on host material, *Heterodera latipons* can be transferred to new areas via contaminated soil debris or plant material, primarily root tissue. Greco et al. (1992) suggest that increased traffic of machinery and personnel between the United States and the Middle East could increase the likelihood of accidental introduction of cyst nematodes which are present in this region. This suggestion was put forward referring to the 1991 Persian Gulf conflict, but it is just as relevant now in light of the United States presence in the Middle East.

The aforementioned interception record of *H. latipons* on a *Solanum tuberosum* shipment (Defra, 2007) is cause for concern because *S. tuberosum* is not a known host of this nematode. This interception suggests that contaminated soil or plant material

containing *H. latipons* can be transported on just about anything originating from host countries.

Potential Distribution within the United States

Considering the large variety of climatic conditions under which *H. latipons* has been reported and its temperature requirements for development, there is no doubt that this cyst-forming nematode would become a very noxious pest for winter cereals, especially in temperate states in the United States (Greco et al., 2002).

Survey

CAPS-Approved Method*:

Use soil sampling, collection of host roots, or of a combination of both methods.

<u>Soil sample:</u> 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.

<u>Collect host roots:</u> 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 <u>http://caps.ceris.purdue.edu/</u>.

Literature-Based Methods:

<u>Soil Sampling:</u> As for other cyst-forming nematodes, in the absence of a host crop, soil samples must be collected and processed to extract cysts. Cyst nematodes are often extracted from soil using some form of elutriation or flotation. The Fenwick flotation can is frequently used for this purpose (Davis and Venette, 2004). Perineal patterns of cysts must then be prepared, observed under a microscope, and compared with those of the original description (Franklin, 1969) or processed using molecular analyses. In the presence of a host crop (winter cereals), close examination of roots will reveal the presence of white lemon-shaped females. However, females become easily detached from the roots at a later stage of development, making the detection of the nematode very difficult even in cases of heavy infestation, especially in non-sandy soils. Roots of cereals infested by *H. latipons* do not show the typical branching of those infested by *H. avenae* they tend to be grouped (Mor et al., 1992).

In the field, the most reliable method for detection is the collection of soil samples in a grid pattern and processing by a wet screening method (USDA, 1985). For identification, a minimum of 10 cysts with juveniles is desirable. Males and females will help in identifying field infestations (USDA, 1985). Davis and Venette (2004) give considerable detail about soil sampling (e.g., number of samples to take, subsampling, and factors that influence efficiency of this method).

Key Diagnostics

<u>CAPS-Approved Method</u>*: Confirmation of *H. latipons* is by morphological identification. Keys are available for identification by morphological characteristics of the cyst, second stage juvenile, male, and female.

Mulvey (1972) provides a key to 39 species of *Heterodera*, including *H. latipons*, based on characteristics of cysts. Handoo (2002) provides a key to the species within the *H. avenae* group as well as a thorough review of morphological studies to date.

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

Literature-Based Methods:

Most diagnoses are made via morphological characteristics. *Heterodera latipons* cysts are typically ovoid to lemon-shaped as those of *H. avenae*. They belong to the *H. avenae* group because they have short vulva slits. The fenestration of *H. latipons* cysts shows two distinct semi-fenestrae, which are more than a semifenestral width apart. The underbridge is strong and shows a pronounced thickening in the middle from which the name of the species is derived. The extremities of the underbridge are bi-trifurcate. Bullae are few to absent (Greco et al., 2002).

Cyst, J2, male, and female morphology provide diagnostic information for the identification of this nematode species (Handoo, 2002). Handoo (2002) provides a key to the species within the *H. avenae* group as well as a thorough review of morphological studies to date. Greco et al. (2002) give detailed information on the morphological differentiation of four *Heterodera* species and include scanning electron micrographs of key anatomical characteristics.

Restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and internal transcribed spacer ribosomal DNA (ITS-rDNA) have all been used to differentiate morphologically similar species (Bekal et al., 1997; Subbotin et al., 1999; Subbotin et al., 2001; Nicol, 2002; Rivoal et al., 2003; Tahna Maafi et al., 2003; Madani et al., 2004; Abidou et al., 2005).

Toumi et al. (2013) developed a species specific PCR based on actin protein sequences that can detect *H. latipons*.

Heterodera latipons, H. avenae, H. filipjevi, and *H. mani*, were also differentiated by electrophoresis on cellulose acetate plates using the enzymes esterase and malate dehydrogenase and aliquots of 25 females (Mokably et al., 2001).

Easily Confused Species

Heterodera latipons may occur by itself or in mixed populations that include closely related *H. avenae* or *H. trifolii*. *H. latipons* has been confused with several other cyst nematode species that parasitize cereals, including (but not limited to) *H. avenae*, *H.*

bifenestra, H. filipjevi, H. hordecalis, H. mani, H. pakistanensis, H. turcomanica, H. zeae, and a more taxonomically distant species, *Punctodera punctata* (Kort, 1972; Nicol, 2002). Franklin (1969), who first described *H. latipons*, compared morphological characters of *H. latipons, H. avenae*, and *H. turcomanica*. Due to technological advances in molecular diagnostics, differentiating among morphologically similar cyst nematodes can be completed most reliably by restriction fragment length polymorphism (PCR-RFLP) of ribosomal DNA (Bekal et al., 1997; Subbotin et al., 1999; Subbotin et al., 2001; Handoo, 2002; Nicol, 2002; Maafi et al., 2003; Rivoal et al., 2003; Madani et al., 2004; Subbotin et al., 2010).

H. avenae, *H. filipjevi*, *H. latipons* and *H. mani*, were also differentiated by electrophoresis on cellulose acetate plates using the enzymes esterase and malate dehydrogenase and aliquots of 25 females (Mokabli et al., 2001).

References

AQAS. 2013. Agricultural Quarantine Activity Systems. Queried December 13, 2013 from, <u>https://aqas.aphis.usda.gov/aqas/</u>.

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

Bekal, S., Gauthier, J.P., and Rivoal, R. 1997. Genetic diversity among a complex of cereal cyst nematodes inferred by RFLP analysis of the ribosomal internal transcribed spacer region. Genome 40: 479-486.

CABI. 2012. Crop Protection Compendium Wallingford, UK: CAB International. <u>http://www.cabi.org/compendia/cpc/</u>.

Davis, E.E., and Venette, R.C. 2004. Mini Risk Assessment: Mediterranean cereal cyst nematode, *Heterodera latipons* Franklin [Nematoda: Heterodidae]. http://www.aphis.usda.gov/plant_health/plant_pest_info/pest_detection/downloads/pra/hlatiponspra.pdf.

Defra. 2007. Plant Health Interception & Outbreak Chart. Department for Environment, Food and Rural Affairs. Retrieved December 5, 2007, from <u>http://www.defra.gov.uk/planth/interc/intercold.htm</u>.

EPPO. 2013. PQR - EPPO database on quarantine pests (available online). http://www.eppo.int

Franklin, M.T. 1969. *Heterodera latipons* n.sp., a cereal cyst nematode from the Mediterranean region. Nematologica15:535-542.

Greco, N., Di Vito, M., and Saxena, M. C. 1992. Plant parasitic nematodes of cool season food legumes in Syria. Nematologica Mediterranea 20: 37-46.

Greco, N., Vovlas, N., Troccoli, A., and Inserra, R.N. 2002. The Mediterranean cereal cyst nematode, *Heterodera latipons*: a menace to cool season cereals of the United States. Nematology Circular No. 221, Florida Dept. Agriculture and Conservation Services, Division of Plant Industry. http://www.doacs.state.fl.us/pi/enpp/nema/nemacirc/nemcirc221.pdf.

Hajihasani, A., Tanha Maafi, Z., Nicol, J. M., and Seraji, A. 2010. Relationships between population densities of the cereal cyst nematode, *Heterodera latipons* and yield losses of winter wheat in microplots. Australasian Plant Pathology 39(6): 530-535.

Handoo, **Z.A.** 2002. A key and compendium to species of the *Heterodera avenae* group (Nematoda: Heteroderidae). Journal of Nematology 34: 250-262.

Kort, J. 1972. Nematode Diseases of Cereals of Temperate Climates, pp. 97-126. *In* J.M. Webster [ed.], Economic Nematology. Academic Press, London.

Maafi, Z.T., Subbotin, S.A., and Moens, M. 2003. Molecular identification of cyst forming nematodes (Heteroderidae) from Iran and a phylogeny based on ITSrDNA sequences. Nematology 5: 99-111.

Mackintosh, G.M. 1970. Nematodes associated with marram grass (*Ammophila arenaria* (L.) Link) in Scotland. In: International Symposium on Nematology (1960-1986), pp. 112. European Society of Nematologists.

Madani, M., Vovlas, N., Castillo, P., Subbotin, S.A., and Moens, M. 2004. Molecular characterization of cyst nematode species (*Heterodera* spp.) from the Mediterranean basin using RFLPs and Sequences of ITS-rDNA. Journal of Phytopathology 152: 229-234.

Mokabli, A., Valette, S., and Rivoal, R. 2001. Différenciation de quelques espèces de nématodes à kystes des céréales ed des graminées par èlectrophorèse sur gel d'acétate de cellulose. Nematologia Mediterranea 29: 103-108.

Mor, M., Cohn, E., and Spiegel, Y. 1992. Phenology, pathogenicity and pathotypes of cereal cyst nematodes, *Heterodera avenae* and *H. latipons* (Nematoda: Heteroderidae) in Israel. Nematologica: 494-501.

Mokrini, F. Waeyenberge, L., Viaene, N., and Moens, N. 2012. First report of the cereal cyst nematode *Heterodera latipons* on wheat in Morocco. Plant Disease 96 (5): 774.

Mulvey, R.H., and Golden, A.M. 1983. An illustrated key to the cyst-forming genera and species of Heteroderidae in western hemisphere with species morphometrics and distribution. Journal of Nematology 15: 1-59.

Nicol, J.M. 2002. Important nematode pests, pp. 345-366. *In* Anon. [ed.], *Bread Wheat*: improvement and production. FAO, Rome, Italy.

Philis, I. 1988. Occurrence of *Heterodera latipons* on barley in Cyprus. Nematologia Mediterranea 16:223.

Rivoal, R., Valette, S., Bekal, S., Gauthier, J.P., and Yahyaoui, A. 2003. Genetic and phenotypic diversity in the graminaceous cyst nematode complex, inferred from PCR-RFLP of ribosomal DNA and morphometric analysis. European Journal of Plant Pathology 109: 227-241.

Scholz, U. 2001. Biology, pathogenicity and control of the cereal cyst nematode *Heterodera latipons* Franklin on wheat and barley under semiarid conditions, and interactions with common root rot *Bipolaris sorokiniana* (Sacc.) Shoemaker [teleomorph: *Cochliobolus sativus* (Ito et Kurib.) Drechs. Ex Dastur.]. PH.D. Thesis Dissertation, University of Bonn, Germany, 161 pp.

Scholz, U. and Sikora, R.A. 2004. Hatching behaviour and life cycle of *Heterodera latipons* Franklin under Syrian agro-ecological conditions. Nematology 6: 245-256.

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

Subbotin, S.A., Mundo Ocampo, M, and Baldwin J.G. 2010. Systematics of cyst nematodes (Nematoda: Heteroderinae). Nematology Monographs and Perspectives Volume 8B (Hunt, D.J., and Perry, R.N., series editors). Brill, Leiden, The Netherlands, 512 pp.

Subbotin, S.A., Vierstraete, A., DeLey, P., Rowe, J., Waeyenberge, L., Moens, M., and Vanfleteren. J.R. 2001. Phylogenic relationships within the cyst-forming nematodes (Nematoda, Heteroderidae) based on analysis of sequences from the ITS regions of ribosomal DNA. Molecular Phylogenics and Evolution 21: 1-16.

Subbotin, S.A., Waeyenberge, L., Molokanova, I.A., and Moens, M. 1999. Identification of *Heterodera* avenae group species by morphometrics and rDNA RFLPs. Nematology 1: 195-207.

Tacconi, R. 1976. The presence of *Heterodera carotae* Jones, 1950 and *H. latipons* Franklin, 1969 in Veneto. Redia (Italy) 59: 305-310.

Toumi, F., Waeyenberge, L., Viaene, N., Dababat, A., Nicol, J.M., Ogbonnaya, F., and Moens, M. 2013. Development of a species-specific PCR to detect the cereal cyst nematode, *Heterodera latipons*. Nematology 15 (6): 709-717.

USDA. 1985. Pests not known to occur in the United States or of limited distribution, No. 71: A cereal cyst nematode.

USDA. 2013. Plants for Planting Manual. Updated July, 2013. Retrieved from, http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/plant s_for_planting.pdf

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

USDA-PCIT. 2013. Phytosanitary Certificate Issuance & Tracking System. *Heterodera latipons.* Queried December 13, 2013 from, <u>https://pcit.aphis.usda.gov/pcit/</u>.

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

Sullivan, M. and D. Mackesy. 2010. CPHST Pest Datasheet for *Heterodera latipons*. USDA-APHIS-PPQ-CPHST. Revised June, 2014.

Reviewed by: Nicola Greco (Plant Nematology Institute of CNR, Bari, Italy), **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 a reference to the Key Diagnostics section.

July, 2016: Updated the 'Potential Distribution in the United States' section, deleted old map.