

# Wisconsin Pest Survey Report

## 2015-2019 CEREAL & CORN CYST NEMATODE SURVEYS

This survey was conducted to check for exotic cereal cyst nematodes in cereal and corn producing fields of Wisconsin. Nematodes are microscopically small worm-like creatures that infest crops and affect their health and productivity. Females of cyst nematodes form pinhead-sized cysts on plant roots. They are filled with eggs containing worm-shaped juveniles ready to hatch and infest more roots. Cyst nematodes are particularly difficult to control because the cyst is a durable egg sack that can remain viable in the soil, in some cases for decades. Avoiding introduction, and early detection, are the best options in protecting Wisconsin agriculture.

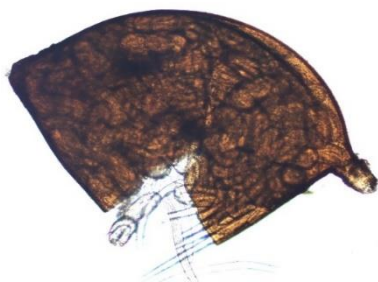
**Cereal cyst nematodes** can limit the yield of small grains (Smiley et al 2017). Heavy root predation on wheat results in stunted plants, reduced tillering, chlorotic leaves, and shallow, bushy roots. Other cyst nematode species, such as soybean cyst nematode, are economically significant and widespread in Wisconsin. Potato cyst nematodes (pale and golden cyst nematodes) that are of concern to potato growers, have so far been successfully kept out of this state.

This survey specifically targeted three exotic cereal cyst nematodes that could impact production and trade if they were accidentally introduced to Wisconsin. *Heterodera filipjevi* is present in Europe and Asia and was recently discovered in three wheat-producing Northwestern states. *Heterodera latipons*, the Mediterranean cereal cyst nematode, is not known in North America but feeds on small grains and grasses in Asia, North Africa, Europe and Russia. *Punctodera chalconensis*, the Mexican corn cyst nematode, is a serious pest of corn, found only in Mexico at this point.

The survey also included *Heterodera avenae*, a cereal cyst nematode that is widespread in North America, Asia, Europe and North Africa. It is the cereal cyst nematode most likely to be encountered in Wisconsin.

Year	Sampling time frame	Wheat	Oat	Corn
2015	4-17 to 11-2	91	9	98
2016	7-20 to 12-7	89	3	22
2017	7-26 to 11-30	15	0	80
2018	7-23 to 11-2	16	1	56
2019	10-9 to 11-5	0	0	58

The survey focused on winter wheat producing counties of the state as shown on the map on page 3. Table 1 shows the number of fields surveyed for each crop. In 2019, Wisconsin farmers planted 195,000 acres of winter wheat, 265,000 acres of oats and 3.8 million acres of corn. Wheat is the main host for *H. filipjevi* and *H. latipons*. Corn, host for *P. chalconensis*, is also grown in these counties.



Ruptured nematode cyst under 100x magnification, releasing eggs and juveniles.



Juvenile cyst nematode hatching from egg under 400X magnification.

**Soil sampling** was conducted during the growing season by collecting 15-20 soil cores from randomly chosen fields. Soil samples were taken to Plant Industry Bureau (PIB) laboratory for separation of cysts from soil by wet sieving. Cysts were sorted out under 20X to 400X magnification, and further processed with gene-based methods. Species-specific PCR's and sequence analysis allowed for identification of nematodes to species level.

**Cyst nematode species identified during surveys from 2015 to 2019.** The three targeted exotic cereal cyst nematodes *H. filipjevi*, *H. latipons* and *Punctodera chalcoensis* were not detected during five years of survey. They have never been reported in Wisconsin. There were also no finds of the cereal cyst (*Heterodera avenae*).

Table 2. Shows cyst nematode species detected during field surveys from 2015 to 2019.

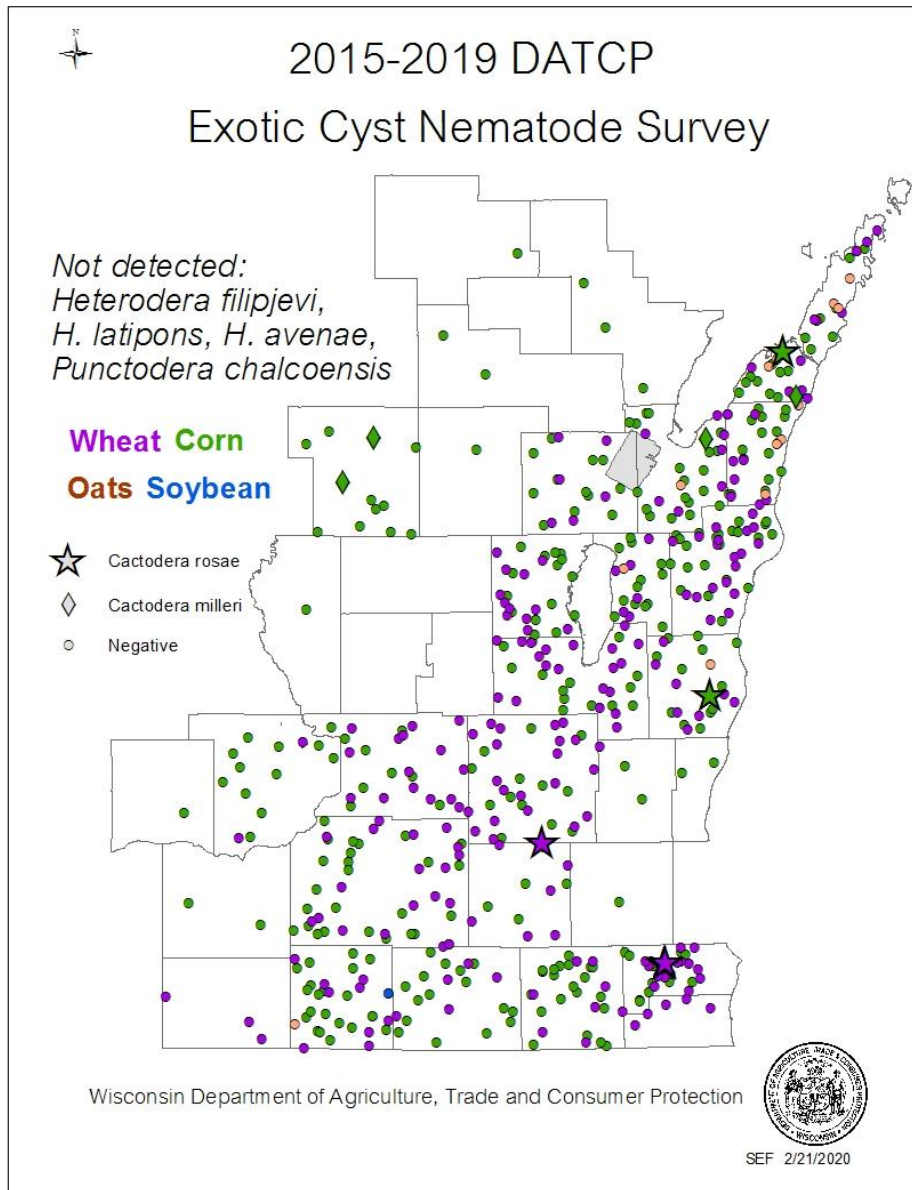
Table 2. Cyst nematode species detected during survey	2015 Fields infested	2016 Fields infested	2017 Fields infested	2018 Fields Infested	2019 Fields Infested
Cereal cyst ( <i>Heterodera avenae</i> )	0	0	0	0	0
Exotic cereal cyst ( <i>Heterodera filipjevi</i> )	0	0	0	0	0
Mediterranean cyst ( <i>Heterodera latipons</i> )	0	0	0	0	0
Mexican corn cyst ( <i>Punctodera chalcoensis</i> )	0	0	0	0	0
Cactus cyst-like ( <i>Cactodera spp.</i> )	6% (12/198)	4% (4/114)	2% (2/95)	1.4% (1/73)	10% (6/58)
Soybean cyst ( <i>Heterodera glycines</i> )	15% (29/198)	19% (22/114)	21% (20/95)	21% (15/73)	10% (6/58)
Soybean cyst-like ( <i>Heterodera spp.</i> ) including clover cyst ( <i>H. trifolii</i> )	7% (14/198)	6% (7/114)	8% (8/95)	6% (4/73)	7% (12/58)
Total number of samples containing cyst nematodes*	28% (55/198)	25% (29/114)	32% (30/95)	26% (19/73)	33% (19/58)

\*Samples may contain more than one species of cyst nematode. For instance both soybean cyst nematode and *Cactodera* cysts could be found in the same soil sample. This is why the sum of all cyst species detected may be higher than the total number of soil samples testing positive for cyst nematodes.

**Other cyst nematodes** that are commonly found in Wisconsin field soil were detected in 25% to 32% of fields tested. **Soybean cyst nematode** (*Heterodera glycines*), an economically significant pest of soybeans, is the most frequently found cyst nematode. It was confirmed in 15% (2015), 19% (2016), 21% (2017), 21% (2018) and 10% (2019) of fields. These fields were most likely planted with soybeans as a rotational crop at some point in time. Wheat can be planted in rotation with soybean to control soybean cyst. Most cysts could be assigned to genus *Heterodera* or *Cactodera*. Cyst nematodes in the genus *Heterodera* included **clover cyst** (*H. trifolii*) which were present in 7% (2015), 6% (2016), 8% (2017), 6% (2018) and 12% (2019) of fields. Clover cysts infest red and white clovers, and certain other legumes but not cereal, corn or soybean.

A variety of *Cactodera* species, most of which do not have common names, were found in 6% (2015), 4% (2016), 2% (2017), 1.4% (2018) and 10% (2019) of fields surveyed. *Cactodera*

species such as *C. estonica* (Norgren 1986), *C. cacti* (cactus cyst), *C. weissii* and *C. milleri* (Schroeder et. al 2008) have previously been documented in this state. The 2019 survey detected *Cactodera milleri* in Brown, Door and Portage Counties. *Cactodera* are usually found on non-crop hosts and are probably feeding on weeds in the surveyed cereal and corn fields.



The 2015 cereal cyst survey found a **Cactodera cyst nematode new to Wisconsin**, called ***Cactodera rosae***. Nematode cysts found in a soil sample from a corn field in Racine Co. were determined to be *C. rosae*. This species was first described in 2008 by Cid del Prado on barley roots and soil in Mexico. DNA-based testing at PIB lab showed 100% homology of recovered cysts to *C. rosae*, based on comparison of partial 28S rRNA gene sequences. The identification was confirmed by the USDA Nematologist. This is a first detection of *Cactodera rosae* in Wisconsin and possibly the US. Our cereal cyst survey

detected six fields with *Cactodera rosae* cysts as shown on the map. In corn, the cysts were found in one field in Door (2016), two in Racine (2015, 2016) and one in Sheboygan (2015) Counties. One wheat field each in Dodge (2015) and Racine Counties (2016) were also infected with *C. rosae*. In each case very few cysts were recovered. These findings suggest that low levels of *C. rosae* may be more widespread than previously recognized. Our knowledge of this species is very limited at this point, including whether there is any effect on corn, wheat or any other cereal crop in Wisconsin. Our pest survey will continue to watch for this cyst nematode to further assess its prevalence.

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## Appendix

### Method

Cyst extraction from soils followed CAPS Cyst Nematode Approved Methods, specifically the wet sieving method. All extracted cysts were examined for morphological characteristics of cysts and second stage juveniles following keys by Mulvey & Golden (1983), Handoo (2002), and the CAPS Cyst Nematode Survey Reference datasheets for the three target species. Examination under 20X and 400X magnification showed that *Punctodera chalcoensis* was not present. Cysts were assigned to genus *Heterodera* or *Cactodera* based on vulval and juvenile morphology. Highly degraded single cyst material recovered from soil samples could not be identified reliably.

DNA was extracted from cysts, eggs and juveniles using the nematode lysis buffer and methodology described by Yan & Smiley (2010). Samples were tested using ITS species-specific primers for *Heterodera filipjevi*, *H. avenae* (Yan et al., 2013) and actin gene based primers for *H. latipons* (Toumi et al., 2013). A real time PCR (Ye 2012) was used to screen for *H. glycines*. Positive controls of *H. filipjevi* and *H. avenae* were kindly provided by Dr. Richard W. Smiley, Oregon State. Suspect cysts were verified by PCR, sequencing and BLAST analysis of partial 28S rRNA gene (Baldwin 1997) and cytochrome oxidase subunit 1 (COI) genes (Derycke 2005). Thanks to Dr. Zafar Handoo at USDA Nematology laboratory for confirming *Cactodera rosae*.

### Literature:

- Derycke, S., Remerie, T., Vierstraete, A., Backeljau, T., & Vanfleteren, J. 2005. Mitochondrial DNA variation and cryptic speciation within the free-living marine nematode *Pellioditis marina*. Marine Ecology Progress Series, 300, 91–103.
- Handoo, Z. 2002. A key and compendium to species of the *Heterodera avenae* group (Nematoda: Heteroderidae). Journal of Nematology 34(3): 250-262.
- Mulvey and Golden (1983) Journal of Nematology 15 (1). 1-59.
- Norgren, R. L. 1986. First report of *Cactodera estonica* in the United States. Plant Disease 70:1159.
- Schroeder, N. E., Gerhardt, D. J., Phibbs, A., & MacGuidwin, A. E. (2008). First report of *Cactodera milleri* in Wisconsin. *Plant Disease*, 92(4), 656.
- Smiley, R. W., Dababat, A. A., Iqbal, S., Jones, M. G. K., Maafi, Z. T., Peng, D., Subbotin S., A., Waeyenberge, L. 2017. Cereal Cyst Nematodes: A Complex and Destructive Group of *Heterodera* Species. *Plant Disease* 101(10):1692-1720.
- 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.
- 2018 State Agriculture Overview Wisconsin USDA National Agricultural Statistical Service.
- Yan, G.P., Smiley, R.W., Okubara, P.A., and Skantar, A.M. 2013. Species-specific PCR assays for differentiating *H. filipjevi* and *H. avenae*. *Plant Disease* 97(12): 1611-1619.
- Ye, Weimin. 2012. Development of Prime Time-Real-Time PCR for Species Identification of Soybean Cyst Nematode (*Heterodera glycines* Ichinohe, 1952) in North Carolina. *Journal of Nematology* 44(3):284–290.

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