



© 2011 Plant Management Network.
Accepted for publication 28 October 2011. Published 5 December 2011.

Testing for Plant-parasitic Nematodes that Feed on Corn in Iowa 2000-2010

Gregory L. Tylka, Adam J. Sisson, Laura C. Jesse, John Kennicker, and Christopher C. Marett, Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA 50011

Corresponding author: Gregory L. Tylka. gltylka@iastate.edu

Tylka, G. L., Sisson, A. J., Jesse, L. C., Kennicker, J., and Marett, C. C. 2011. Testing for plant-parasitic nematodes that feed on corn in Iowa 2000-2010. Online. Plant Health Progress doi: 10.1094/PHP-2011-1205-01-RS.

Abstract

The Iowa State University Plant and Insect Diagnostic Clinic analyzes soil and root samples for plant-parasitic nematodes. The results of samples associated with corn that were submitted from 2000 through 2010 were summarized. One or more genera of plant-parasitic nematodes were found in 92% of the samples. Spiral nematode and root-lesion nematode were most commonly found. Other nematodes recovered were dagger, lance, needle, pin, ring, and stunt nematodes. Nematodes recovered at damaging population densities were dagger, needle, ring, and spiral nematodes. An average of 15 samples were submitted per year from 2000 to 2004. Sample numbers increased nearly threefold since 2005, but overall sample numbers were low every year from 2000 through 2010. Samples were received from 53 of the 99 Iowa counties, and most samples were received in June and July, which is the recommended sampling time. Nematodes that have been associated with corn in Iowa in the past that were not recovered from the samples were sheath, sting, and stubby-root nematodes. The methods used to extract the nematodes from soil and roots and how the samples were handled during collection and processing may have affected the species and population densities recovered. Much more frequent and widespread sampling is needed in Iowa for plant-parasitic nematodes that feed on corn.

Introduction

Growers in Iowa currently produce the greatest amount of corn (*Zea mays* L.) on the largest land area of all states in the United States. In 2010, the United States Department of Agriculture (USDA) National Agricultural Statistics Service (NASS) estimated that 13.05 million acres (5.28 million hectares) of corn were harvested in Iowa with an average yield of 165 bushels per acre (10,347 kg per hectare) for a total production of 2.15 billion bushels (54.5 million metric tons) worth approximately \$11.74 billion (9).

Plant-parasitic nematodes are among a myriad of pathogens that restrict yields of corn (10), and these microscopic worms are found in virtually every field in Iowa (8). More than 60 different species of plant-parasitic nematodes have been found in association with corn in North America (7), and nearly half of those species, 28, have been found in Iowa (D. C. Norton, *personal communication*). Nematodes gained broad recognition as pathogens of corn in the United States in the late 1970s (7). But interest in nematode parasites of corn waned in the 1990s, when great attention and research funding was focused on the soybean cyst nematode, *Heterodera glycines*. Since 2009, interest in nematodes that feed on corn in Iowa has noticeably increased. Print and television advertisements for new seed treatments to protect corn roots from nematodes have raised awareness of this important group of pathogens among growers and agribusiness personnel in the state. Articles in the agricultural press about nematode parasites damaging corn often suggest that recent changes in corn production practices may have lead to more frequent instances of nematode damage (6).

Because of the increased awareness and the possible increased occurrence of yield loss, Iowa State University (ISU) personnel have received many inquiries in the past several years about how widespread and often damaging population densities of plant-parasitic nematodes occur on corn in Iowa. The ISU Plant and Insect Diagnostic Clinic tests soil and root samples for plant-parasitic nematodes, and results of analyses of samples from 2000 to 2010 were summarized and are reported herein. The results will be useful in guiding future sampling efforts for these nematode pests of corn and also can serve as a reference point if testing statistics are compiled again in the future.

Extraction of Nematodes from Soil and Root Samples

Samples were voluntarily submitted to the ISU Plant and Insect Diagnostic Clinic by growers and agribusiness personnel. Samples consisted of soil alone, soil and root masses, or just root masses (Fig. 1). If soil and roots came from the same field and were collected at the same time, both were considered to be a single sample. Research plot samples were excluded from the data that were analyzed for this paper.

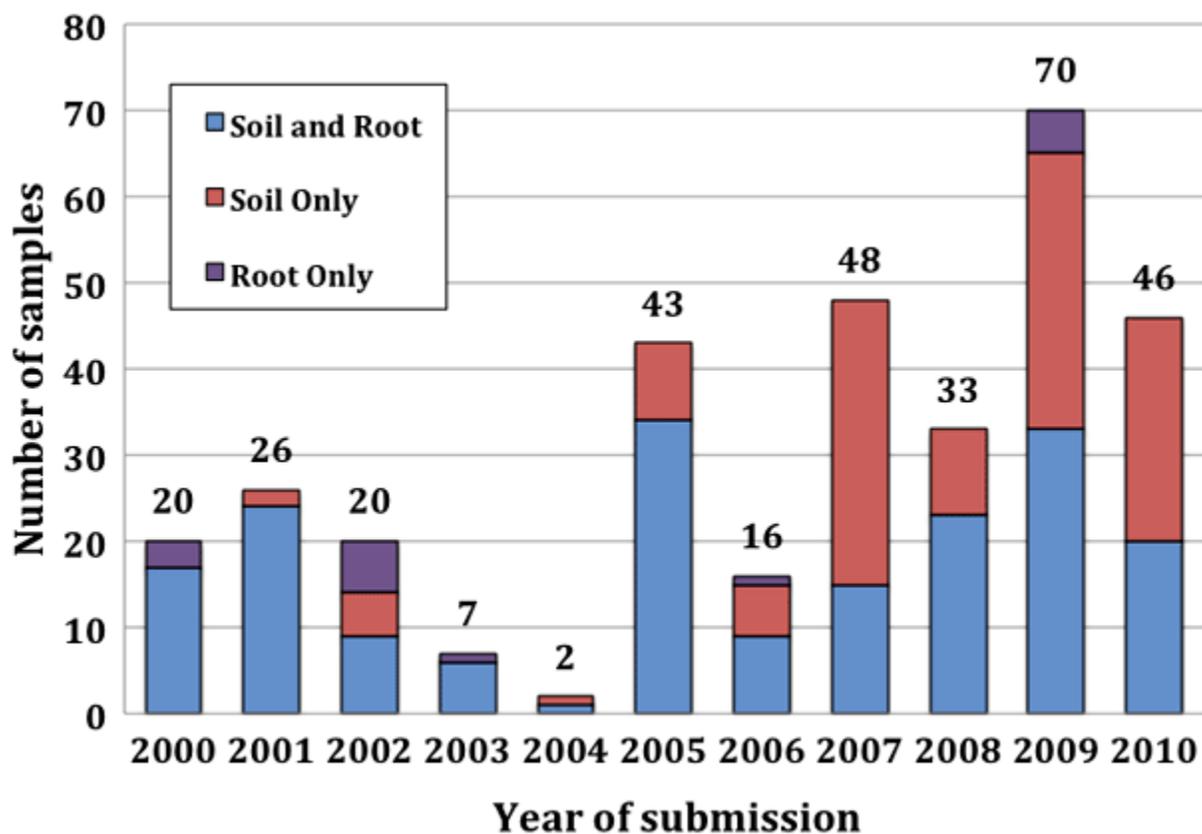


Fig. 1. Number of samples from Iowa corn fields analyzed by the ISU Plant and Insect Diagnostic Clinic for plant-parasitic nematodes from 2000 to 2010. There were 331 total samples.

To extract the nematodes from the soil, soil samples were first manually pushed through hardware cloth with ¼-inch (6.35 mm) openings to break up soil cores and aggregates and to facilitate thorough mixing of the soil. Nematodes then were extracted from an arbitrarily selected 100 cm³ subsample of mixed soil using a combination of modified wet-sieving and decanting (2) and subsequent sucrose centrifugation (4). In the wet-sieving and decanting procedure, the nematodes and sediments suspended in water were passed through an 850-µm-pore (#20) sieve nested above a 38-µm-pore (#400) sieve. The materials trapped on the 850-µm-pore sieve were observed using a dissecting microscope for the presence of very large nematodes, such as the needle nematode (*Longidorus*). The materials trapped on the 38-µm-pore sieve were centrifuged first in water, then in sucrose to separate nematodes and other

organic material from heavier sediment particles (4). Following centrifugation in sucrose, the suspension was poured through a 38- μm -pore sieve and rinsed thoroughly with tap water. The materials recovered on the 38- μm -pore sieve were observed with an inverted compound microscope, and plant-parasitic nematodes were identified to genus and counted. Nematode population densities from the soil were expressed as numbers per 100 cm^3 soil.

The root-lesion nematode (*Pratylenchus*) and the lance nematode (*Hoplolaimus*) are two common nematodes that exist in the soil and also parasitize corn by feeding endoparasitically within the roots (8). Consequently, nematodes must be extracted from corn root tissue to assess the population densities of these nematodes. For root samples submitted to the ISU Plant and Insect Diagnostic Clinic, adhering soil was washed off of the roots, and then approximately 1-inch-long (2.5-cm-long) pieces of the roots were cut from the root masses. No attempt was made to differentiate between seminal and nodal roots. The root fragments were incubated with agitation on a platform shaker for at least 48 h at 86°F (30°C) in 125-ml-capacity Erlenmeyer flasks containing approximately 50 ml of a solution of 10 ppm mercuric chloride and 50 ppm dihydrostreptomycin sulfate (1). After incubation, the solution was poured through a 38- μm -pore sieve. The materials trapped on the sieve were rinsed thoroughly with tap water and then observed using an inverted compound microscope. The nematodes present were identified to genus and counted. The root fragments were dried to determine the mass of the roots from which the nematodes were recovered, and the numbers of nematodes obtained from the root samples were divided by dry root masses to calculate the numbers of nematodes per g dry root tissue.

The results of the sample analyses (population densities of plant-parasitic nematodes by genus) were compiled and summarized by year, associated crop, date of submission, and geographical location. If information was missing from the original sample submission form, the information was obtained by contacting the sample submitter.

Frequency and Population Densities of Nematodes Recovered from Soil and Roots

Overall, there were 495 samples analyzed for plant-parasitic nematodes by the ISU Plant and Insect Diagnostic Clinic from 2000 to 2010. Of these, 388 samples were from Iowa and 107 were from locations outside of the state. The samples primarily were associated with corn, but some samples were from other plants including soybean, turf, pumpkin, strawberry, potato, and snap bean.

The total number of samples analyzed specifically from corn was 422, with 331 samples coming from Iowa locations. Of the 331 Iowa samples, 190 were combined soil and root samples, 124 were soil only, and 17 were roots only. The number of samples that could be ascribed to a specific Iowa county was 310, and the number that could be assigned to a specific month of submission was 329.

One or more genera of plant-parasitic nematodes were found in all but 27, or 8.2%, of the soil samples associated with corn from Iowa, illustrating that these plant parasites are very common in Iowa soils.

Spiral nematodes (*Helicotylenchus*) were the most frequently found plant-parasitic nematode (Table 1), present in 77% of the soil samples. The spiral nematode also was present at the highest maximum population density (2,340 per 100 cm^3 soil) and with the greatest mean population density (87 per 100 cm^3 soil) of all nematode genera identified in the samples. The second most common plant-parasitic nematode recovered from the soil samples was the root-lesion nematode (*Pratylenchus*), which was found in 51% of the samples. The maximum population density of root-lesion nematode in a soil sample was 532 per 100 cm^3 soil and the mean population density for all samples containing this nematode was 21 per 100 cm^3 soil.

Table 1. Incidence and population densities of plant-parasitic nematodes from 314 total soil samples (190 soil + roots and 124 soil only) submitted from Iowa corn fields from 2000 to 2010 to the ISU Plant and Insect Diagnostic Clinic and relationship of population densities to damage thresholds (8).

Nematode common name (Genus)	Number of samples infested	Percent of total samples infested	Maximum (and mean) population density (per 100 cm ³ soil) when present	Damage threshold (per 100 cm ³ soil)	Percent of total samples exceeding damage threshold	Percent of infested samples exceeding damage threshold
dagger (<i>Xiphinema</i>)	131	41.7	126 (14)	30-40	5.7	13.7
lance (<i>Hoplolaimus</i>)	71	22.6	388 (19)	no threshold for soil – see Table 2 for root population density thresholds		
needle (<i>Longidorus</i>)	25	8.0	50 (9)	1	8.0	100.0
pin (<i>Paratylenchus</i> and <i>Gracilacus</i>)	2	0.6	2 (2)	no damage threshold established		
ring (<i>Mesocriconema</i>)	11	3.5	142 (25)	100	0.3	9.1
root-lesion (<i>Pratylenchus</i>)	159	50.6	532 (21)	no threshold for soil – see Table 2 for root population density thresholds		
spiral (<i>Helicotylenchus</i>)	243	77.4	2,340 (87)	500-1,000	1.3	1.6
stubby-root (<i>Trichodorus</i> and <i>Paratrichodorus</i>)	0	0.0	0 (0)	no damage threshold established		
stunt (<i>Tylenchorhynchus</i> and <i>Quinisulcius</i>)	15	4.8	24 (7)	100	0.0	0.0

The dagger nematode (*Xiphinema*), lance nematode (*Hoplolaimus*), and needle nematode (*Longidorus*) were present in 42%, 23%, and 8% of the samples, respectively (Table 1). The pin nematode (*Paratylenchus*) was found in only two samples. And the sheath (*Hemicycliophora*) and stubby-root (*Paratrichodorus*) nematodes were not found in any of the samples, although these nematodes were reported to be associated with corn in Iowa in the past [(8), D. C. Norton, *personal communication*].

Most genera of plant-parasitic nematodes must reach a certain population density, a damage threshold, before they result in reduction of corn growth and yield (7,8). The damage thresholds established by ISU plant nematologist D. C. Norton for nematodes that feed on corn in Iowa are included in Table 1. The specific damage potential of most nematode populations can be affected by host genotype and various physical and chemical conditions of the soil (7,8), so it is difficult to designate a single population density of a nematode species that will result in yield loss under all conditions. Damage thresholds are generally useful for plant-parasitic nematodes that feed on corn because low population densities of many genera are not believed to cause yield reduction, however population densities of one worm per 100 cm³ soil are thought to cause damage for other nematode genera.

Of the Iowa soil samples in which dagger, ring (*Mesocriconema*), and spiral nematodes were present, population densities exceeded the damage threshold in only 14%, 9%, and less than 2% of the samples, respectively. The damage threshold for needle nematode is one nematode per 100 cm³ soil (Table 1), which is the minimum population density that can be detected in any sample. So 100% of the 25 samples in which needle nematode was detected had population densities that exceeded the damage threshold.

A total of 207 corn root samples were tested for the presence of endoparasitic nematodes from 2000 through 2010. The root-lesion nematode was found in 42% of the samples and lance nematode was recovered from approximately 7% of the samples (Table 2). None of the samples had population densities exceeding the damage thresholds established for Iowa.

Table 2. Incidence and population densities of plant-parasitic nematodes from 207 total root samples (190 roots + soil and 17 roots only) submitted from Iowa corn fields from 2000 to 2010 to the ISU Plant and Insect Diagnostic Clinic and relationship of population densities to damage thresholds (8).

Nematode common name (Genus)	Number of samples infested	Percent of total samples infested	Maximum (and mean) population density (per g dry root) when present	Damage threshold (per g dry root)	Percent of total samples exceeding damage threshold	Percent of infested samples exceeding damage threshold
lance (<i>Hoplolaimus</i>)	14	6.8	80 (22)	300-400	0	0
root-lesion (<i>Pratylenchus</i>)	87	42.0	915 (76)	1,000	0	0

From 2000 through 2004, there was an average of 15 samples submitted for testing per year from Iowa and never more than 26 samples in any individual year (Fig. 1). From 2005 through 2010, there was nearly a three-fold increase in the mean number of samples (42) submitted annually. The greatest number of samples ever received in a year was 70, in 2009. Although the number of samples received in 2009 represented a large increase in testing for plant-parasitic nematodes on corn in Iowa, it is still extremely low (less than one sample per county) in relation to the very large area of the crop grown in the state.

A great majority of the samples submitted for testing were received between June and September (Fig. 2). Nearly 64% of the samples (210 samples) were received in June and July and 20% (65 samples) were received in August and September (Fig. 2). This trend in month of submission of samples was not unexpected; mid-season sampling for nematodes that feed on corn is generally recommended (8).

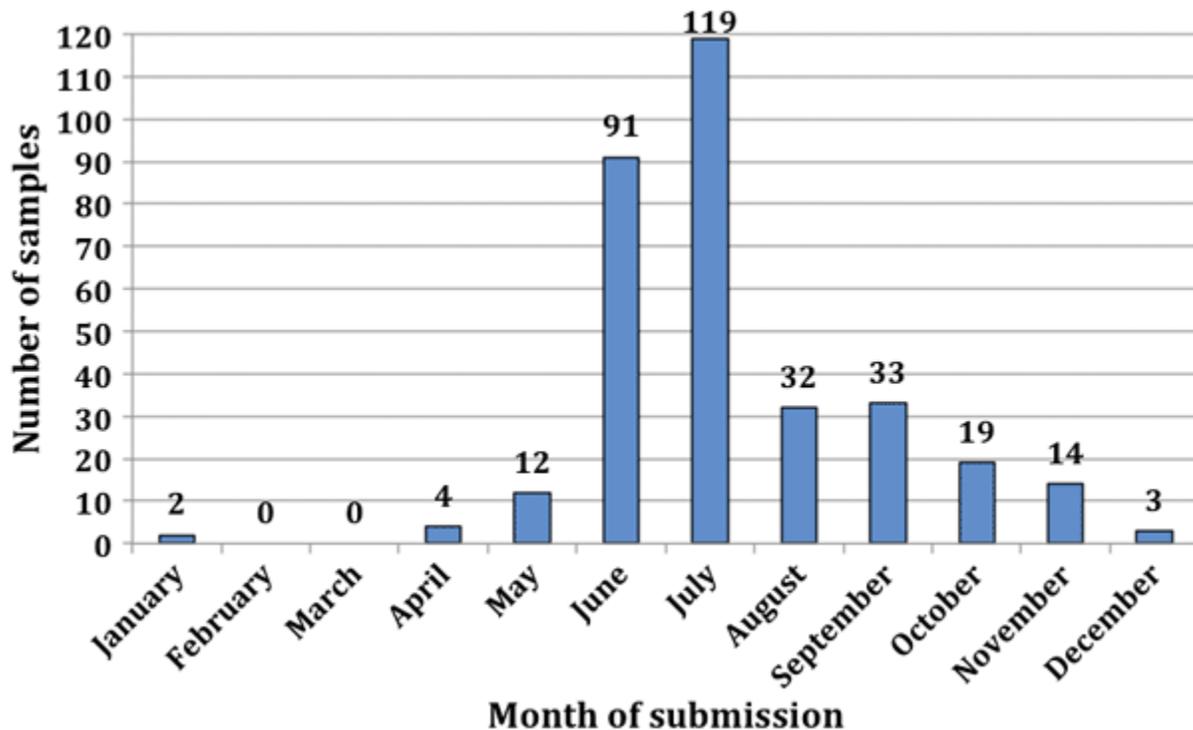


Fig. 2. Monthly distribution of the number of samples submitted to the ISU Plant and Insect Diagnostic Clinic from Iowa corn fields to be tested for plant-parasitic nematodes from 2000 to 2010. There were 329 total samples with a corresponding month of submission.

The geographical distribution of the locations in Iowa from which samples were submitted for testing was not uniform or widespread across the state; most of the samples were received from counties in the eastern third of the state (Fig. 3). Samples were received from only 53 of the 99 Iowa counties, and the number of samples submitted per county ranged from 1 to 55 in total for the years 2000 to 2010. Samples with nematode population densities exceeding the damage threshold were received from counties throughout the state (Fig. 4). The high number (20) of damaging populations of needle nematode in Muscatine Co. in east central Iowa is likely because of the high prevalence of sandy soils in that area of Iowa; needle nematode occurs only in soils with at least 49% sand (5).

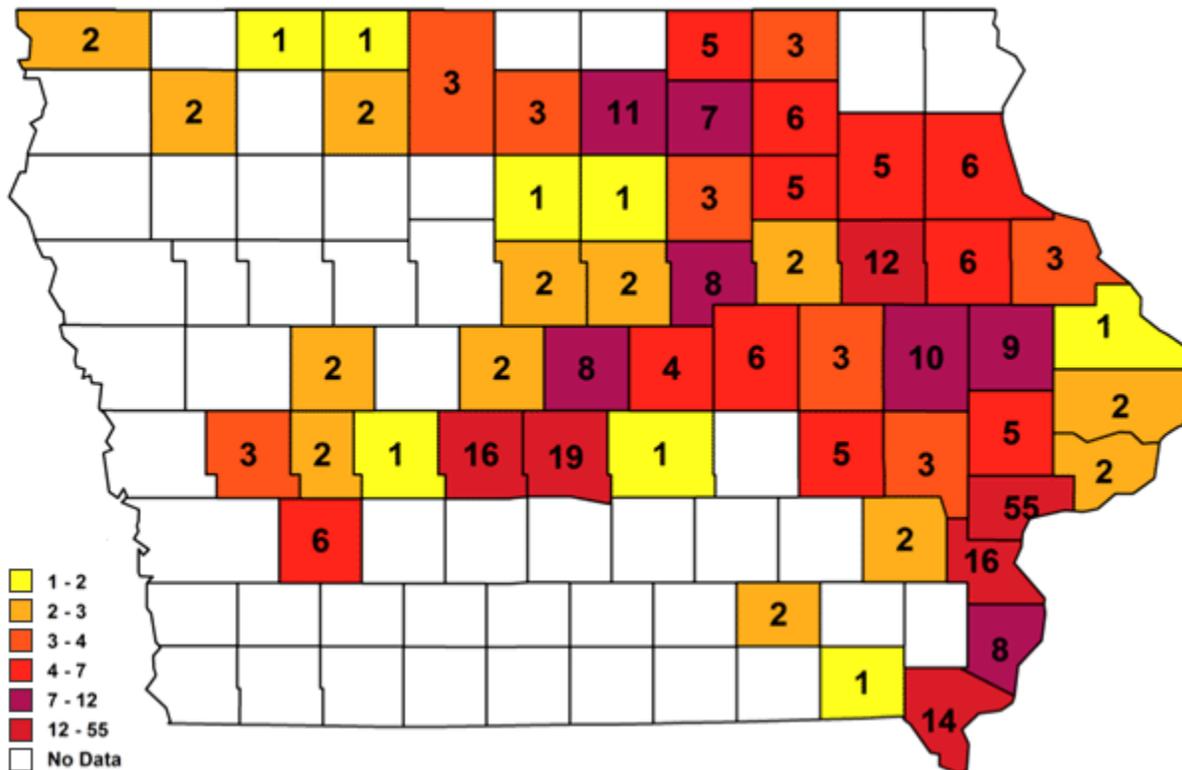


Fig. 3. Geographic distribution of the number of samples submitted to the ISU Plant and Insect Diagnostic Clinic from corn fields to be tested for plant-parasitic nematodes from 2000 to 2010. There were 310 total samples with a corresponding county of submission. An additional 75 samples were submitted from out of state corn fields (not shown on map).

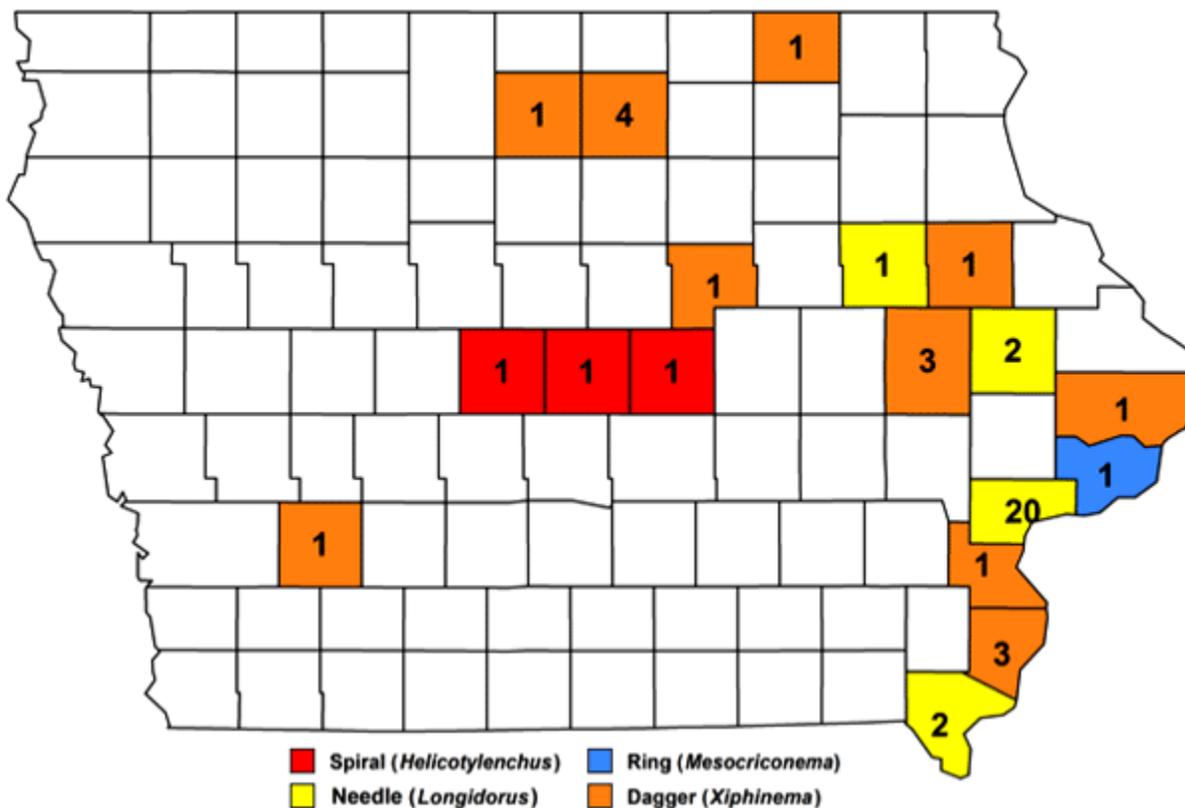


Fig. 4. Geographic distribution of damaging population densities of plant-parasitic nematodes from corn from samples submitted to the ISU Plant and Insect Diagnostic Clinic from 2000 to 2010.

Plant-parasitic Nematodes as Pathogens of Corn in Iowa

The information presented herein must be interpreted carefully. Samples were not collected randomly or systematically, and samples from all 99 Iowa counties were not tested. No inferences can be drawn about the prevalence or population densities of plant-parasitic nematodes in corn fields in counties from which no samples were received. And limited conclusions can be drawn for the counties from which samples were submitted because of the low sample numbers and the arbitrary and sporadic nature of sample submission. In order to make broad, reliable inferences, samples should be collected from randomly selected fields, such as was done by Workneh et al. in surveying the north central United States for the soybean cyst nematode (11). Also, many more samples from the entire state would need to be tested each year than the number that was submitted to the ISU Plant and Insect Diagnostic Clinic from 2000 to 2010. Nonetheless, some summary statements can be made about the results.

The spiral nematode was the most common plant-parasitic nematode associated with corn in Iowa. Despite being commonly found, the spiral nematode was infrequently present at damaging population densities. Consequently, the spiral nematode generally should not be of great concern for corn producers in Iowa.

The root-lesion nematode was commonly reported at damaging population densities in Iowa in the 1980s (8). Although it was recovered frequently in the samples tested from 2000 through 2010, the nematode was not found at damaging population densities in any of the samples. The lack of recovery of root-lesion nematode at damaging population densities may have been due, at least in part, to the methodology used to extract the nematodes from the root samples. Preliminary results of recent experiments (*data not shown*) indicate that significantly more root-lesion nematodes are recovered from corn roots when roots are incubated in tap water than when incubated in the mercuric chloride/streptomycin sulfate solution (1) that was used for these samples. Also, *Pratylenchus* species adults are less than 38 μm in diameter (3) and *Pratylenchus* juveniles are smaller than adults. Many root-lesion nematodes could have passed through the 38- μm -pore (#400) sieve used to extract nematodes from soil and root samples and gone uncounted, partially explaining why the root-lesion nematode was never recovered at high population densities. Norton primarily used the same mercuric chloride/streptomycin sulfate solution used by the ISU Plant and Insect Diagnostic Clinic for extraction of endoparasitic nematodes from corn root tissue in Iowa, but it is not known what porosity sieve he used to recover the endoparasites extracted from roots when testing for root-lesion nematode.

The incidence of recovery of the root-lesion and lance nematodes from soil and from root samples was incongruous. These endoparasitic nematodes were recovered 9 to 16% more frequently in soil samples than root samples even though both nematode genera reportedly feed within corn roots throughout the growing season (8) and most samples were received during the growing season. It is possible that our use of the mercuric chloride/streptomycin sulfate solution resulted in ineffective recovery of the root-lesion and lance nematodes from the root samples. But it is not likely that the nematodes would have been missed completely in these root samples, rather they would have been detected at lower population densities than previously reported (8).

Overall, 15% of the soil and root samples submitted to the ISU Plant and Insect Diagnostic Clinic from 2000 to 2010 contained population densities of plant-parasitic nematodes that exceeded damage thresholds; no sample had damaging population densities of more than one nematode genus. The plant-parasitic nematodes found most often at damaging population densities in the samples were the needle nematode and the dagger nematode. As stated above, the needle nematode is considered damaging at very low population densities, so any population density (even one nematode per 100 cm^3 soil) is considered damaging. Needle nematode was found in 8% of the samples received from 2000 to 2010 overall. The needle nematode has only ever been found in the north central United States in soils with at least 49% sand (5), and 20 of the 25 samples in which needle nematode was found in Iowa were from Muscatine Co., where sandy soils are common. The dagger nematode was found at damaging

population densities in 14% of the samples in which it was present and in nearly 6% of the samples overall.

The other genera of plant-parasitic nematodes recovered from the samples were rarely or never found at damaging population densities. The sheath and stubby-root nematodes were not found in any of the samples that were submitted for testing. The absence of these two nematode genera from the results was somewhat unexpected because these genera have been associated with corn in Iowa in the past [(8), D. C. Norton, *personal communication*]. There are no known or reported methodological difficulties in extracting sheath and stubby-root nematodes from soil, as was described for the root-lesion nematode. Nematologists have observed that rough handling of soil samples may rupture some plant-parasitic nematodes that feed on corn (G. L. Tylka, *data not shown*, T. L. Niblack, *personal communication*), and the ruptured nematodes would not be recovered for identification and counting. Perhaps the sheath and stubby-root nematodes were not found in any of the samples because the nematodes were destroyed during the collection and transportation process and/or when the soil cores were broken up and the soil mixed in the laboratory prior to extraction.

There are several private soil-testing laboratories in Iowa that analyze soil samples for soybean cyst nematode (cysts and/or eggs, specifically), but the ISU Plant and Insect Diagnostic Clinic is the only facility in the state that extracts, identifies, and counts vermiform plant-parasitic nematodes that parasitize corn. Although a small number of samples from Iowa fields might have been sent to university or private soil-testing laboratories in other states, we feel that the data presented herein are an accurate depiction of the extent of nematode testing on corn that occurred in Iowa from 2000 to 2010.

Few Iowa corn fields were tested for the presence of plant-parasitic nematodes from 2000 to 2010, and the sample number per year during that time was too low to discern if there were any changes or trends occurring over time relating to damaging population densities of these pests. Much more frequent sampling of fields is needed in the future to be able to draw more convincing conclusions about the status of plant-parasitic nematodes as yield-reducing pathogens of corn in Iowa.

Acknowledgments

The authors wish to thank Winston Beck for assistance in data compilation and the Iowa State University Integrated Pest Management Program and the National Plant Diagnostic Network for financial support for this work.

Literature Cited

1. Bird, G. W. 1971. Influence of incubation solution on the rate of recovery of *Pratylenchus brachyurus* from cotton roots. *J. Nematol.* 3:378-385.
2. Gerdemann, J. W. 1955. Relation of a large soil-borne spore to phycomycetous mycorrhizal infections. *Mycologia* 47:619-632.
3. Handoo, Z. A., and Golden, A. M. 1989. A key and diagnostic compendium to the species of the genus *Pratylenchus* Filipjev, 1936 (lesion nematodes). *J. Nematol.* 21:202-218.
4. Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Dis. Rep.* 48:692.
5. Malek, R. B., Norton, D. C., Jacobsen, B. J., and Acosta, N. 1980. A new corn disease caused by *Longidorus breviannulatus* in the Midwest. *Plant Dis.* 64:1110-1113.
6. Morrison, L. 2008. The other nematode. Online. January 2008, *Corn and Soybean Digest*, Minneapolis, MN.
7. Norton, D. C. 1983. Maize nematode problems. *Plant Dis.* 67:253-256.
8. Norton, D. C., and Nyvall, R. F. 2011. Nematodes that attack corn in Iowa. Online. Coop. ext. publ. PM 1027. Iowa State Univ., Ames, IA.
9. USDA-NASS. 2010. Corn: Area planted for all purposes, harvested for grain, yield and production, Iowa by county, 2009-2010. USDA-National Agricultural Statistics Service (NASS), Washington, DC.

10. Windham, G. L., and Edwards, D. I. 1999. Nematode diseases. Pages 56-62 in: Compendium of Corn Diseases, 3rd Edn. D. G. White, ed. American Phytopathological Society, St. Paul, MN.
11. Workneh, F., Tylka, G. L., Yang, X. B., Faghihi, J., and Ferris, J. M. 1999. Regional assessment of soybean brown stem rot, *Phytophthora sojae*, and *Heterodera glycines* using area-frame sampling: Prevalence and effects of tillage. *Phytopathology* 89:204-211.