Investigating the Presence and Population Densities of Plant-Parasitic Nematodes and the Influence of Soil Region, Cropping Practices and Soil Properties on these Nematodes in Corn Fields in Ohio

THESIS

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ABSTRACT

Corn fields in Ohio were surveyed in 2013 and 2014 to determine the frequency and abundance of plant-parasitic nematodes. Soil samples were collected at a depth of 40 to 50 cm from 425 fields when corn was between growth stages V3 and V6. A total of 15-16 fields were sampled in each of 28 counties, across 6 soil regions, representing three cropping systems and three tillage programs. Standard laboratory techniques were used for processing and extraction, and plant-parasitic nematodes were identified to genus. Nine commonly occurring morphological groups of plant-parasitic nematodes, namely spiral (Helicotylenchus spp.), lesion (Pratylenchus spp.), lance (Hoplolaimus spp.), dagger (Xiphinema spp.), stunt (Tylenchorhynchus spp.), pin (Paratylenchus spp.), ring (Criconemella spp.), stubby-root (Paratrichodorus spp.), and cyst (Heterodera spp.), along with several genera in the subfamily Tylenchinae counted together as “tylenchids”, were identified, with maximum individual-field population densities ranging from 26 to 1,164 nematodes/100 cm³ soil, depending on the group. Generalized linear mixed models were fitted to the data to estimate county-level heterogeneity in nematode presence, while binary and ordinal logistic regression models were fitted to estimate the odds of each genus being present, and the lesion, lance, spiral, and pin nematodes at potentially damaging population densities based on soil region, cropping
sequence, tillage, and soil pH, silt content, and electrical conductivity. The spiral nematodes and tylenchids were detected in 94 and 96% of the fields, whereas the lesion, pin, lance, stunt, and dagger nematodes were recovered from 80, 57, 48, 48, and 37% of the fields, respectively. The stubby-root, cyst, and ring nematodes were the least frequent, each identified from fewer than 13% of the fields. County-level heterogeneity varied among the genera, with variance estimates ranging from 0.56 to 3.23. However, except for comparisons between the stunt and pin, stunt and dagger, and lance and dagger nematodes, the 95% confidence intervals around the variance estimates overlapped considerably, suggesting comparable county-level heterogeneity across genera. Based on odds ratios, all covariates affected nematode populations, but soil region had the greatest and most consistent effect. Dagger and ring nematodes were more likely to be present in region 6 than any of the other regions, but lance, stunt, pin, stubby-root, and spiral nematodes were more likely to be present in regions 1, 2, 3, 4, and 5 than 6. The odds of spiral, lance, and pin nematode population densities being at moderate to high levels in region 3 and 4 were greater than the odds in region 6, but region 1 was more likely than 6 to have lance nematode population densities at high risk levels. The spiral nematode was also likely to reach higher risk levels in regions 1, 2, and 5 than 6, and pin nematode was more likely to reach high risk levels in region 2 than 6. Spiral, lance, and pin nematode were more likely to be present in fields under corn-soybean or corn-soybean-wheat rotation than under continuous corn, and the odds of the pin nematode population density being at moderate to very severe risk levels were
two times greater for field under rotation than fields under continuous corn. Tillage was associated with the presence of lance, stubby-root, and stunt nematode, with the former two genera being more likely to be found in fields under conservation tillage than conventional tillage, while the opposite was true for the latter genus. The lance nematode was also more likely to reach yield-impacting population densities in conservation than conventional tillage fields. As soil pH increased, the probability of the spiral nematode population densities being in the moderate to very severe risk category increased, but the probability of the lesion and pin nematode population densities being in that same risk category decreased. For the lesion and lance nematodes, the probability of population densities being in the moderate to very severe risk category decreased as the silt content of the soil increased. Soil electrical conductivity had contrasting effects on the probability of lesion and pin nematode population densities reaching high-risk levels.
Dedicated to

My Wife, Lindani M. Abraham-Simon

My Mother, Avril Sampson

My Sisters, Natoya A. George and

Dacia C. Sampson
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CHAPTER 1: INTRODUCTION

CORN PRODUCTION IN OHIO AND NEMATODES AS PLANT PATHOGENS

Corn production in Ohio. The United States is one of the leading producers of field corn (Zea mays L.), accounting for approximately 32% (274 million metric tons) of the world’s total production in 2013, followed by China and Brazil, which produced 205 and 81 million metric tons, respectively. Leaders in corn production in the United States include the states of Iowa, Illinois, Indiana, Minnesota, Ohio and Nebraska. Ohio is ranked sixth in the nation in corn production, contributing approximately 5% of the corn produced annually. With an estimated 1.51 million hectares (3.73 million acres) of corn harvested at an average of 4.8 MT/ha (174 bushels/acre), a total production of 649 million bushels (18 million metric tons) was produced in 2013. At an average grain price of $4.41/bushel ($173.61/MT), the 2013 crop was worth an estimated $2.8 billion, according to the United States Department of Agriculture (USDA) National Agricultural Statistics Service (NASS).

Plant-parasitic nematodes. Soil dwelling nematodes are minute (0.3–5.0 mm long) roundworms which are very abundant, diverse and ubiquitous in all soils (Yeates 1979). These tiny roundworms feed on fungi, bacteria, plants and other soil flora and fauna. Nematodes that feed exclusively on plants, commonly
referred to as plant-parasitic nematodes, feed primarily on plant roots although some species parasitize aboveground plant parts. Plant-parasitic nematodes are obligate parasites that obtain their nutrients from the cytoplasm of living plant cells (Williamson and Hussey 1996). These nematodes use a stylet to puncture the plant cell wall and obtain nutrients. The major symptoms exhibited by plants affected by this group of nematodes include retarded growth, wilting, and predisposition to infection by other pathogens (Williamson and Hussey 1996).

Some plant-parasitic nematodes live mostly outside their host in the soil and may penetrate the root minimally; these are commonly referred to as ectoparasites. These nematodes can cause severe root damage and some are capable of virus transmission (Brown et al. 1995). The needle (*Longidorus*) and sting (*Belonolaimus*) nematodes are two important ectoparasitic plant-parasitic nematodes that feed on corn (McDonald and Nicol 2005). Other plant-parasitic nematodes spend most of their lives inside host plant roots as migratory or sedentary endoparasites. Migratory nematodes move throughout the roots causing extensive cellular necrosis and the sedentary forms develop a complex biological and chemical interaction with the host (Williamson and Hussey 1995). One of the most important migratory endoparasitic nematode that affects corn is the lesion nematode (*Pratylenchus*) (Tylka 2007).

According to Norton (1983), probably all corn plants grown on the millions of hectares devoted to the crop in the world are parasitized by nematodes. These minute roundworms are among a myriad of pathogens that restrict corn productivity (Windham and Edwards 1999). They may cause substantial grain
yield and quality losses, and quite often these losses go undetected or may be attributed to other causes (Norton 1983). For instance, when the lesion nematode (*Pratylenchus*) feeds on corn roots, they cause lesions on the root surface (Corbett 1976) that affect the uptake of water and nutrients and serve as ports of entry for other organisms. Other plant-parasitic nematodes, such as the needle (*Longidorus*) and dagger (*Xiphinema*) cause severe root tip damage (McDonald and Nicol 2005) and may also transmit plant pathogenic viruses. According to the Society of Nematologists, plant-parasitic nematodes cause more than $3.0$ billion worth of crop losses annually. Koenning et al. (1999) reported that the United States recorded losses as high as 20% in corn production as a result of plant-parasitic nematodes in selected states.

**NEMATODES ASSOCIATED WITH CORN**

There are at least 120 species of plant parasitic nematodes known to be associated with corn worldwide, of which more than 60 species are associated with corn in North America (Norton 1983). Koenning *et al.* (1999) reported the most frequently occurring genera of plant-parasitic nematodes on corn in the US were *Hoplolaimus*, *Meloidogyne*, and *Pratylenchus* with *Belonolaimus*, *Longidorus*, and *Paratrichodorus* also contributing to corn-yield suppression in several states. Based on data from a more recent corn nematode survey conducted in 2011, Tylka *et al.* (2011a) reported that the most common genera were the ectoparasitic *Xiphinema* (dagger), *Longidorus* (needle), *Paratylenchus* (pin), *Helicotylenchus* (spiral), *Belonolaimus* (sting), *Paratrichodorus* (stubby-
root), *Criconemella* (ring), and *Tylenchorhynchus* (stunt) and the endoparasitic *Hoplolaimus* (lance), *Meloidogyne* (root-knot), and *Pratylenchus* (lesion). However, nematodes species composition and abundance often vary considerably among fields, states and countries. For instance, Waele and Jordaan (1988) found that *Paratrichodorus minor*, *Scutellonema brachyurum*, and *Criconemella sphaerocephala* were the predominant ectoparasites in corn fields in South Africa and *Pratylenchus zeae* and *P. brachyurus* the predominant endoparasites.

Differences in the frequency, distribution, and species composition of plant-parasitic nematode assemblage from one location to another may be attributed to edaphoclimatic conditions, cropping practices and other environmental factors (Norton 1983). For instance, high numbers of plant-parasitic nematodes were found in virtually every corn field in Iowa and Illinois based on surveys conducted in 2011 (Tylka *et al.* 2011a) and 2009 (Niblack and Lopez, personal communication), respectively. The survey of 550 corn fields in Illinois showed spiral (*Helicotylenchus*) and lesion (*Pratylenchus*) nematodes were found in 99% and 84% of fields sampled at mean population densities of 150 and 26 nematodes/100 cm$^3$ soil, respectively (Niblack and Lopez, personal communication). Results from Iowa were relatively similar, showing spiral (*Helicotylenchus*) and lesion (*Pratylenchus*) nematodes most frequently occurring, being present in 77% and 51% of fields sampled, at mean population densities of 87 and 21 nematodes/100 cm$^3$ of soil, respectively (Tylka *et al.* 2011a).
The fact that nematodes were found in relatively high numbers in corn fields in Iowa and Illinois was not surprising. Current crop management practices such as conservation tillage, continuous corn and the abandonment of soil applied insecticides, which in the past provided the added benefit of suppressing nematodes, favor the buildup of nematodes. Changes in tillage, residue management and rotation practices contribute to major shifts in the numbers and composition of soil fauna, both pest and beneficial organisms (Govaerts et al. 2006). However, the effects of tillage practices on plant-parasitic nematodes are unclear and seem to vary from one genus to another and among locations. For instance, early work done by Caveness (1974) in western Nigeria found more *Pratylenchus* spp. in soil and corn root samples in conventionally-tilled plots when compared with no-till plots, but *Helicotylenchus* and *Meloidogyne* species were more abundant in no-till plots than in conventionally-tilled plots planted with corn in rotation with several crops. In contrast, *Pratylenchus scribneri* was more evenly distributed in no-till plots than in conventionally-tilled plots in Indiana (Alby et al. 1983). Additionally, Thomas (1978) reported that different tillage practices resulted in differences among nematode populations, with numbers being generally greater in no-till plots. Higher population densities of *Helicotylenchus* spp., *Pratylenchus* spp., and *Xiphinema* spp. were found in no-till plots. Results from a study conducted by Parmelee and Alston (1986) in Georgia suggested that the effects of tillage practices on nematode populations may depend on when samples are collected. They found that tillage did affect nematode trophic structure and abundance, with monthly mean population densities being
significantly greater in conventional tillage plots when compared with no-till plots. However, during the summer plant-parasitic nematodes were more abundant in no-till plot than in conventionally-tilled plots.

In continuous corn cropping systems, nematode populations may increase substantially, ultimately resulting in significant yield decline (Reversat and Germani 1985). However, the effects of crop rotation on nematode population densities seem to vary with nematode genera and tillage. Govaerts et al. (2006) reported that population densities of *Pratylenchus thornei* were higher with continuous corn when compared with corn-wheat rotation, and higher in conventional tillage when compared with no-till. They also showed that population densities of plant-parasitic nematodes increased significantly under conventional tillage with continuous corn and residue retention, but decreased under no-till with rotation and residue retention. Similarly, Edwards et al. (1988) reported that densities of stunt nematodes (*Tylenchorhynchus* spp.) in a continuous corn cropping system were significantly higher with conventional tillage than with strip tillage or no-till.

**NEMATODE DISTRIBUTION WITHIN THE SOIL PROFILE**

The vertical distribution of nematodes in the soil profile is highly variable and may be influenced by many biotic and abiotic factors (Brodie 1976). The highest population densities for most plant-parasitic nematodes usually occur in the upper 45 cm of the soil profile (Barker and Nusbaum 1971), with the vertical distribution of these pathogens being partly related to the host rooting pattern.
(MacGuidwin and Stanger 1991). According to Ferris (1981), the host plant (their food source) is the most important factor affecting nematode distribution in the soil profile. This was demonstrated by Ogiga and Estey (1973) when studying the vertical distribution of *Pratylenchus spp.* in apple orchards. They found these nematodes as deep as 80 cm, but the highest densities were observed between 20 and 40 cm. Similarly, Brodie (1976) detected the highest densities of *Pratylenchus brachyurus* between depths of 45 and 75 cm in soybean fields, and studying the vertical distribution of plant-parasitic nematode in four field crops (corn, black salsify, carrot and potato), Pudasaini et al. (2005) observed that, with the exception of corn fields where nematodes were found at 70 cm below the surface, no nematodes were recovered below 50 cm. In addition to the presence and rooting pattern of the host, Brodie (1976) showed that abiotic factors such as soil texture, temperature and moisture may greatly influence the vertical distribution of nematodes, whereas others showed that moisture and temperature only had very minor effects on vertical distribution (Pudasaini et al. 2005).

**INFLUENCE OF ABIOTIC FACTORS ON NEMATODE POPULATION**

Soil characteristics such as soil pH, soil texture, and organic matter are important relative to the occurrence and population dynamics of nematodes (Wallace 1963). Studies done by Norton and Hoffmann (1974) demonstrated significant positive correlations between nematode numbers and soil pH in Iowa. This led them to speculate that soil pH might be used as a tool to predict where nematodes are likely to occur. Additionally, the research showed that *Xiphinema*
*chambersi* was found only in soils with pH between 4.5 and 6.4, whereas high population densities of *Helicotylenchus platyurus*, *H. pseudorobustus* and *X. americanum* occurred in soils with pH above 6.0. In a separate study, a positive correlation was also observed between pH and *Tylenchorhynchus maximus* for pH ranging from 5.0 to 6.5 (Schmitt 1969).

Soil texture may also affect the distribution and population density of plant-parasitic nematodes. This is due in part to the fact that this soil property may affect the movement of nematodes. According to Wallace (1971), “the relation between nematode movement and soil texture is a function of the ratio of nematode size to pore and particle size. As the length and diameter of nematodes increase, the optimum pore and particle sizes for maximum movement also increase until pore diameter is too large to restrict lateral movement.” Brodie (1976) reported that *Belonolaimus longicaudatus* predominantly inhabited the top 30 cm of soil with 87 to 88% sand, 6 to 7% silt, and 5 to 7% clay, whereas *Pratylenchus brachyurus* was found at all depths, but population densities were greatest between 45 to 75 cm where the soil was 78 to 79% sand, 6% silt and 15 to 16% clay. However, the association between soil texture and plant-parasitic nematodes population density seems to depend on the genera and species of the nematodes. Studies by Schmitt and Norton (1972) showed higher population densities of *Xiphinema americanum* in silty clay-loam when compared with silt-loam soils in common lilac in Iowa. *Pratylenchus crenatus* was reported to occur in soils ranging from loams and silt-loams in Ohio (Brown *et al.* 1980) to light sandy soils in Europe (Loof 1978), whereas
Pratylenchus penetrans was found more frequently in soils with higher sand content (Florini et al. 1987). Other studies reported that Paratrylenchus projectus was favored by light-colored silt-loam, whereas other Paratrylenchus species population densities were positively correlated with fine silt (Wallace et al. 1993). High population densities of Criconemella species were found in sandy soils (Wallace et al. 1993); lower densities of Tylenchorhynchus, Helicotylenchus, and Xiphinema species in loams and sandy loam soils; higher densities of Helicotylenchus spp. in clays and silty clay soils (Norton et al. 1970), and high abundance of Tylenchorhynchus spp. in areas high in coarse silt or dark-colored silty clay-loam (Ferris and Bernard 1971).

DAMAGE CAUSED BY PLANT-PARASITIC NEMATODES

Plant-parasitic nematodes possess a hollow stylet that they use to puncture cortical cell walls, inject secretions and ingest nutrients from the plant root cells (Wondafrash et al. 2013). In one study by Norton et al. (1978) corn yield was shown to be inversely related to the combined population densities of lesion (Pratylenchus) and lance (Hoplolaimus) nematodes. Many nematodes may cause yield reduction at relatively low population densities, particularly when host plants are stressed, and losses may be compounded by secondary infection of roots by other organisms and the transmission of plant viruses. However, nematodes vary in their ability to cause damage and yield loss in corn. Some species are capable of causing damage to corn at very low population densities and other species are not harmful until population densities reach many...
hundreds or more per 100 cm$^3$ soil (Tylka 2007). For instance, it was reported that the needle nematode (*Longidorus*), which is highly localized and restricted largely to highly sandy soils, is probably the most devastating nematode to corn in Iowa when present at only one or two nematodes/100 cm$^3$ soil (Norton and Hoffmann 1975). Conversely, the moderately pathogenic spiral nematode (*Helicotylenchus*) found more predominantly in heavier soils than in sandy soils needs to be present at 1,000 or more nematodes/100 cm$^3$ to cause yield loss (Tylka 2007). McSorley and Dickson (1989) demonstrated that *Belonolaimus longicaudatus* caused particularly severe damage to seedlings in corn plots, with many seedlings exhibiting characteristic *B. longicaudatus* root injury. Severe root tip damage on sandy soils and yield decline were caused by *Longidorus* and *Xiphinema*, especially under moisture stress, and *B. longicaudatus* caused major losses to sweet corn on sandy soils in Florida (Rhoades 1977). Nevertheless, the literature on the specific level of damage caused by plant-parasitic nematodes in corn under field conditions is limited. For instance, *Pratylenchus penetrans*, a notoriously damaging species of lesion nematode on crops such as potato (Rowe and Powelson 2002) has been shown to cause severe damage to corn in greenhouse studies, but the level of damage caused by this species in corn fields is unknown.

**THE LESION NEMATODE (Pratylenchus)**

**Description.** Members of this genus are usually stout and cylindroid, with bluntly rounded tails and broad heads. Adults are less than 1.0 mm long and
possess a strong stylet with large basal knobs (Thorne 1961). They are commonly referred to as lesion nematodes because they cause necrotic lesions within root tissue while feeding and/or migrating. When this plant-parasitic nematode is viewed under a microscope, the overlapping esophagus, the flat and broad head and the sclerotized cephalic framework are useful diagnostic features (Mai and Mullin 1996). When at rest, lesion nematodes tend to lie in a straight line (Mai and Mullin 1996).

**Feeding habits.** The endoparasitic lesion nematode, *Pratylenchus* spp., is among the most economically damaging plant-parasitic nematodes and is found in a wide variety of crops worldwide. Apparently, crops differ in their suitability as hosts to a given species and, sometimes a single crop species may be susceptible to more than one species of *Pratylenchus* (Mai and Mullin 1996). For instance, *P. scribneri* is a common pest of corn (*Zea mays* L.) in irrigated sandy soils in the north-central region of the United States (Todd 1991); however, *P. neglectus* is less often associated with corn (Norton et al. 1984). According to Zirakparvar (1980), *P. hexincisus* is probably the most important *Pratylenchus* species in the Midwest. In a greenhouse study, Zirakparvar (1980) demonstrated that within 60 days of inoculation with *P. hexincisus*, dark brown discrete lesions developed in coarse and fibrous corn roots and pruning of the fibrous roots occurred, as well as proliferation of lateral roots after 90 days. These nematodes were observed only in the cortical parenchyma, and sloughing of the cortical tissue occurred in fibrous roots. According to Olowe (1977), *P. zeae* causes mechanical breakdown of cells and necrosis of stelar and cortical tissues,
resulting in cavity formation. Patel et al. (2002) reported reduced root and shoot weights, plant height and chlorophyll content and an increase in *P. zaeae* population in corn grown in pots.

Estimates of yield loss in corn due to *Pratylenchus* species are limited by the confounding effects of other factors (Dickson and McSorley 1990; Todd and Oakley 1996). However, in Nigeria, one *Pratylenchus* species alone, *P. brachyurus*, was reported as being responsible for over 25% yield reduction (Egunjobi 1974). Smolik and Evenson (1987) demonstrated that yield loss caused by *P. scribneri* and *P. hexincisus* in irrigated and dryland corn, respectively, in South Dakota was relatively minor. The estimated rate of yield loss was approximately 1% per 1,000 nematodes/g root. Relatively similar results were reported by Todd and Oakley (1996) in south-central Kansas, and by Norton and Hinz (1976), working with *P. hexincisus* in sandy soils in Iowa. Indirect evidence of the effect of lesion nematode on grain yield was obtained by Riekert (1996) from a nematicide trial in which yields were higher in treated nematode-infested plots than in the untreated check.

**THE LANCE NEMATODE (Hoplolaimus)**

**Description.** These nematodes are relatively large, approximately 0.95-1.80 mm, with a massive stylet which possesses a characteristically tulip-shaped knob. When members of this genus are viewed under a microscope, the esophagus overlapping the intestine, the round tail, heavy cephalic framework
and the massive stylet with tulip-shaped knobs are some useful morphological features for identification (Sher 1963).

**Feeding Habits.** Members of the genus *Hoplolaimus* usually feed some distance behind the root tip and may or may not enter the roots (Mai and Mullin 1996). During feeding, cortical cells of the root are destroyed and necrotic lesions may be extensive (Mai and Mullin 1996). Species of this migratory endoparasitic nematode have a wide host range that includes cereals, soybeans, wheat, corn, cotton, sugarcane, grasses, and trees (Dropkin 1989).

**THE SPIRAL NEMATODE (Helicotylenchus)**

**Description.** Adult nematodes of this genus are 0.6 to 0.8 mm long and the stylet length is approximately 0.03 mm. Members of this genus possess the characteristic overlapping of the intestine by the esophagus and the vulva is located approximately 60% of the body from the head (Fortuner et al. 1984). Adult females have two amphidelphic ovaries and the tail is curved dorsally (Sher 1966). When at rest or dead these nematodes tend to curl their bodies into a spiral.

**Feeding Habit.** Nematodes of this genus are frequently found in soil samples collected in the rhizosphere. They are usually ectoparasites, feeding with not only the stylet inserted into the root but also the anterior part of the body (Thorne 1961). According to Mai and Mullin (1996), feeding by adults and juveniles of the spiral nematode produces small discolored lesions in the root cortex and in other underground parts. Preferably, these nematodes would enter
behind root tips and at the junction of lateral and main roots. The spiral nematode can severely damage roots when population densities are high. For instance, Norton et al. (1978) reported significant negative correlation between *H. pseudorobustus* and corn yields, and Taylor (1961) found that this nematode disrupted cortical cells; however, other evidence suggested that this species and *H. dihysteria* are only moderately or slightly pathogenic to corn (Sledge 1956).

**THE PIN NEMATODE (Paratylenchus)**

**Description.** These nematodes are cylindrically elongated and are less than 0.5 mm long. Pin nematodes are among the smallest plant-parasitic nematodes that parasitize plants. When at rest or dead they assume a J- or C-shape. Males have reduced or no stylet (Siddiqi and Goodey 1963) and younger juveniles of most species possess poorly developed stylets (Raski 1962).

**Feeding Habit.** Large population densities of species of *Paratylenchus* are found in the root zone in different soil types worldwide. Many species are reported as migratory ectoparasites. Host plants for this plant-parasitic nematode include carrot, celery, parsley, cabbage, grapes, clover, grasses and fruit trees (Dropkin 1989). In general, infection by high population densities results in reduced crop growth without clear symptoms on the roots. Pin nematodes insert their stylets into epidermal cells at the base of root hairs (Dropkin 1989) and may feed on the same cell for several days without killing it. Feeding on roots by *Paratylenchus* species results in brown necrotic areas in some hosts (Mai and Mullin 1996).
NEMATODE IDENTIFICATION

Generally plant-parasitic nematodes can be identified to the genus level by using morphological traits such as stylet shape and size, overall body shape, size, and several other gross body morphological measurements (Tylka et al. 2011b). Common morphological traits used for identification include buccal and pharyngeal structure, and other anatomical features such as the intestine, lip region, cuticle, reproductive system, sense organs and tail are also used, as well as life history traits such as host plant (Anderson 1992). For instance, when viewing the lesion nematode (*Pratylenchus*) under a dissecting microscope, some useful diagnostic features are the flat head, the overlapping esophagus, and the relatively graceful and slow movement (Mai and Mullen 1996). Another diagnostic feature that is considered when identifying nematodes is their shape when at rest. For instance, the pin nematode (*Paratylenchus*) assumes a J- or C-shape when at rest (Mai and Mullen 1996). Dichotomous keys developed by Mai and Mullen (1996) can be used to identify many common genera of plant-parasitic nematodes based on morphological traits using a stereoscopic and compound microscopes; which is a very helpful resource for nematode identification. Differentiating species of plant-parasitic nematodes varies by genus and depends on criteria and methods that include minute microscopic observation of different adult and juvenile life stages to measure subtle morphological details (Tylka et al. 2011b). Identifying nematodes to species using morphological traits only can be very tedious and would require an
experienced nematologist. Therefore, DNA-based techniques were developed to discriminate among species of plant-parasitic nematodes. For instance, Uehara et al. (1998) were able to distinguish between Pratylenchus loosi and P. coffeae using DNA amplification with species-specific primer sets. Additionally, Al-Banna et al. (2004) demonstrated the use of a PCR-based assay to discriminate among six Pratylenchus species (P. brachyurus, P. neglectus, P. scribneri, P. penetrans, P. thornei and P. vulnus) using species-specific primers. Recent work by Floyd et al. (2002) demonstrated the development of a molecular operational taxonomic unit (MOTU) scheme for soil nematodes. This MOTU technique allows for a rapid assessment of nematode taxon diversity in soils and essentially works by using a molecular barcode, derived from a single-specimen polymerase chain reaction (PCR) and sequencing of the 5’ segment of the small subunit ribosomal RNA (SSU) gene.

**SAMPLING AND EXTRACTING NEMATODES**

Nematodes recovered from a field not only reflect the population levels at the time and depth of sampling, but also reflect the effects of sampling and extraction procedures (Yeates and Bongers 1999). Nematode feeding habits and life cycles must also be considered in relation to the time of sampling (Ferris et al. 1981). Plant-parasitic nematode population densities and species diversity may differ greatly over short distances. Sampling the nematode population in a field makes it possible to determine the abundance and types of nematodes present, in order to determine susceptibility of a certain crop (Ferris et al. 1981).
Samples should be collected midseason when nematode numbers are likely greatest for most plant-parasitic nematodes that feed on corn; however, for the needle nematode, samples should be collected in the spring or fall (Tylka 2007). According to Tylka et al. (2011b) a 2.54 cm (1 inch) diameter soil probe can be used to collect at least 20 soil cores from the section identified for sampling. The soil probe should be angled in the seed row so as to collect the soil from within the rhizosphere of the growing corn plant. Nematodes that feed on corn may be found deep in the soil profile and some, such as the needle nematode, may migrate further down as the growing season progresses. Therefore these nematodes can go undetected if soil cores are taken within the upper 6-8 inches. However, soil cores should be taken at least in the upper 12 inches (30 cm) of the soil profile and if possible 18 inches (45 cm) deep when sampling for corn nematodes. Other guidelines were given for proper handling and submission of samples to the laboratory for processing (Tylka et al. 2011b).

Ferris et al. (1981) suggested stratification as one approach for improving efficiency in estimating nematode population densities, where fields can be divided into regions of probable difference of nematode population densities. Ferris et al. (1981) also mentioned these differences may result from variations in cropping histories, soil type or climatic conditions. Moreover, each stratum can be represented by at least one sample. Consequently, each sample usually represents as large an area of the field as possible. A sampling pattern for an established crop is necessary to minimize variability within a stratum in a field and one such pattern is to sample from the region of the root zone of the crop.
For instance, in row crops, cores are taken from the root zone of the crop being sampled. Ferris *et al.* (1981) recommended that one sample of about 12 to 20 soil cores should be used to represent no more than 5 acres of uniform soil texture.

Extracting vermiform nematodes from the soil usually requires decanting plus sieving, centrifugation, active migration and elutriation (Yeates and Bongers 1999). Moreover, differences in the nematode size, shape and activity can affect the actual genera extracted. Whitehead and Hemming (1965) suggested that 75% of the nematodes were extracted when 200 ml soil suspension was settled in rising water current in a simple container, which utilized centrifugal flotation. This technique relies solely on the specific gravity of nematodes. However, when mineral and heavy organic particles were separated from the concentrated suspension about 95% of the nematodes were extracted. Yeates and Bongers 1999 also reported extraction efficiencies as high as 90%; however, typical recoveries are between 30-60%. Earlier work done by Caveness and Jensen (1955) also demonstrated the extraction of nematodes and their eggs from the soil mineral content using centrifugal flotation. An overview of the active migration technique which utilizes the Baermann funnel is given by Brown and Boag (1988).
MANAGEMENT OF PLANT-PARASITIC NEMATODES

Cultural. It is widely accepted that the presence of the host plant has a major influence in plant-parasitic nematode community dynamics (Norton 1989). Therefore, host range studies of various crop species showing the effect of various cropping regimes on populations of plant-parasitic nematodes may be a useful cultural management practice to develop strategic crop rotation systems. For instance, greenhouse studies by Todd (1991) supported sorghum as being more suitable for Belonolaimus spp. than corn, soybeans, or wheat; however, field studies revealed that corn and sorghum were both suitable hosts for Belonolaimus spp., but corn was the better host for Pratylenchus scribneri in sandy soil. Additionally, alfalfa was found not to be a suitable host for either Belonolaimus spp. or Pratylenchus scribneri; however, soybean was intermediate in host efficiency for both nematode species under field conditions. Zirakparvar (1980) indicated that P. hexincisus has an extensive host range and certain crops such as soybean, garden pea, and white Dutch clover could be used in rotation with corn to control this plant-parasitic nematode.

Altering practices such as planting date, crop rotation, tillage, weed control, application of organic amendments and sanitation have been tested, and were demonstrated to be effective in reducing nematode populations in many cases (Becerra and Sosa-Moss 1977; Idowu 1981; Cabanillas et al. 1999). For instance, the use of radish and French and African marigold proved very effective against Pratylenchus species populations in a corn-based rotation Knuth (2002). According to Youssef (1998), population densities of Heterodera and
Tylenchorhynchus species were reduced by weeding corn plots in Egypt. Some of the major weeds associated with the corn field were Echinochloa colunum, Digitaria sanguinalis, Portulaca oleracea and Xanthium strumarium. Research in Georgia showed that conservation tillage and poultry litter can greatly influence nematode population densities (Sumner et al. 2002). According to Sumner et al. (2002) Meloidogyne incognita population densities increased with conventional tillage after a three-year period when compared with conservation tillage, whereas population densities of Paratrichodorus christiei were reduced by poultry litter treatments and Helicotylenchus dihystera numbers were reduced by non-composted broiler litter when compared with no litter. Sosa-Moss (1987) reported that earlier sowing dates and an adequate fertilizer regime reduced damage caused to corn by Punctodera chalcoensis in Mexico. Similarly, Ivezic et al. (1996) demonstrated up to 60% reduction in nematode numbers, particularly Pratylenchus thornei, in corn fields following high levels of potassium application. On the other hand, fertilizer amendment had no effect on damage caused by Heterodera zeae to corn (Krusberg et al. 1997).

Chemical. The use of nematicides, especially broad-spectrum soil fumigants has been effective in controlling nematodes worldwide. However, nematicides are highly toxic and have contributed to human health risks and environmental pollution (Abawi and Widmer 2000). These undesirable features resulted in a total ban or restriction on the use of most nematicides. For instance, methyl bromide, widely used for control of nematodes, weeds and other soil borne pathogens for decades, is currently being phased out in the United States
(Abawi and Widmer 2000). However, a recent study showed the efficacy of 1,3-dichloropropene and chloropicrin as alternative soil fumigants for control of soilborne pests under good agricultural practices (Chellemi et al. 2013). Moreover, a separate study by Qiao et al. (2014) demonstrated that reduced rates of 1,3-dichloropropene and abamectin (a microcyclic lactone derived from the soil bacterium, *Streptomyces avermitilis*) can be used as an effective alternative for methyl bromide for root-knot nematode (*Meloidogyne incognita*) control in tomato production in China. Nevertheless, the current trend indicates that chemical nematicides may not be available in the near future and therefore, alternative control strategies and tactics need to be developed to replace or complement current chemical nematicide options for management of plant-parasitic nematodes (Barker and Koenning 1998).

In most instances the use of nematicides is limited for economic and political reasons, as well as inconsistent results with their application (McDonald et al. 1987; Riekert 1996). Nevertheless, the responsible use of chemical to control nematodes in corn could always be an effective production management tool, particularly when used in integrated nematode management systems (Johnson and Leonard 1995). Currently, products available to manage plant-parasitic nematodes on corn in Iowa include the soil-applied insecticide/nematicide Counter® and two relatively new protectant seed treatments, Avicta® and Votivo® (Tylka et al. 2012). Counter® is a contact and systematic nematicide with active ingredient terbufos, Avicta® is a contact nematicide with active ingredient abamectin (Syngenta), and Votivo® is a special
strain of *Bacillus firmus*, a natural soil borne bacterium that grows on the root. *Bacillus firmus* reportedly creates a barrier as a thin film on the root which prevents nematodes from penetrating (Bayer Crop Science). Tylka *et al.* (2012) concluded that the aforementioned nematode management products had no effect on corn yields in an experiment where spiral (*Helicotylenchus*) and lesion (*Pratylenchus*) nematodes were predominant and dagger (*Xiphinema*) and lance (*Hoplolaimus*) were present at low population levels. However, Tylka *et al.* (2012) mentioned that the effectiveness of these seed treatments and soil applied nematicides may be more meaningful in fields with greater plant-parasitic nematodes population densities or in fields with more damaging nematode species such as needle (*Longidorus*) and sting (*Belonolaimus*) nematodes. Therefore, more research is needed to ascertain the effects of these seed treatments and soil-applied nematicides on specific plant-parasitic nematode populations and corn yield.

**Resistance.** Breeding without selecting for nematode resistance can be very costly in any crop production system if the crops are highly susceptible or intolerant (McDonald and Nicol 2005). However, nematologists should endeavor to work with plant breeders to ensure that the end-product is acceptable by producers, consumers and processors (McDonald and Nicol 2005). Many corn hybrids were reported to be resistant to *Meloidogyne, Helicotylenchus*, and *Paratrichodorus* nematode (Johnson 1975). However, Jordaan and De Waele (1987) stated that it could be more difficult to identify resistance to migratory than sedentary endoparasites, but cited several reports of resistance in corn to other
nematode species. For instance, Davis and Timper (2000) concluded that corn is generally more resistant to *Meloidogyne* species *M. incognita* and *M. arenaria*, and resistance to *M. javanica* was found in many hybrids (Lordello et al. 1989). Additionally, immunity was claimed as a dominant trait in one hybrid, (Lordello and Lordello 1992). Lordello *et al.* (1985) also identified several corn genotypes resistant to *Pratylenchus zeae* and *P. brachyurus*. Two wild corn species, *Zea diploperennis* and *Z. mexicana*, demonstrated resistance against *Pratylenchus scribneri* and *Helicotylenchus pseudorobustus* (Norton *et al.* 1985), and Singh and Patel (1999) reported a corn variety resistant to *Tylenchorhynchus vulgaris*. Wicks *et al.* (1990) reported a yellow corn line with resistance to *Pratylenchus hexincisus* and *P. scribneri*. Importantly, this corn line also demonstrated resistance to ear rot and other important fungal diseases to corn. Additionally, Hashmi *et al.* (1993) demonstrated resistance to *Heterodera zeae* in a greenhouse screening of inbred corn lines.

Although the use of nematode resistance alone or in combination with other nematode management tools is highly recommended by many, the introduction of resistance should always focus on the production of a genotype with acceptable agronomic traits and durable resistance (McDonald and Nicol 2005).
RESEARCH GOALS AND OBJECTIVES

Presently, there is no information on the statewide distribution or effects of nematodes associated with corn in Ohio. Therefore, a comprehensive survey of corn fields in Ohio was conducted to determine which nematode genera are present and at what population densities. The ultimate goals of this research are to i) determine nematode population density and factors affecting population density, ii) establish quantitative relationships between these factors and nematode population densities, iii) model the relationships between nematode populations and yield in order to develop damage thresholds and iv) to evaluate nematicide seed treatments and develop nematode management recommendations. However, the specific objectives of this study were to i) to determine the frequency and population densities of different morphological groups (genera) of plant parasitic nematodes in corn fields in Ohio, ii) to estimate variability in the presence and absence of plant-parasitic nematodes in Ohio, and iii) to quantify associations among soil region, cropping practices and soil properties on the presence and abundance of plant-parasitic nematodes in corn fields in Ohio.
REFERENCES


www.bayercropscience.us/products/seegrowth/poncho-votivo


CHAPTER 2: Frequency, Abundance, and Heterogeneity of Plant-Parasitic Nematodes in Corn Fields in Ohio

INTRODUCTION

Over the last seven years or so, interest in plant-parasitic nematodes in field corn, and consequently, the use of nematicide seed treatments to manage these pathogens, have increased in the U.S. Corn Belt. This is consistent with a recent increase in the overall use of other pesticides in agriculture, even in the absence of known disease or insect threat. Although some plant-pathogenic nematodes are notorious for their ability to cause losses in field crops, including corn (Norton 1983), the threat posed by these round worms to corn production is Ohio is largely unknown. Recent research in Iowa showed that when baseline nematode populations are below established damage thresholds, nematicide seed treatments may not provide a yield benefit, even if they are effective at reducing nematode population density (Batista da Silva 2013). This has led to questions as to whether nematodes are really a concern and whether nematicide seed treatments are warranted.

There are more than 60 different species of plant-parasitic nematodes known to feed on corn in North America (Norton 1983), of which the most common genera are the ectoparasites *Longidorus, Paratylenchus,*
Mesocriconema, Hemicyclophora, Helicotylenchus, Belonolaimus, Paratrichodorus, and Tylenchorhynchus and the endoparasites Hoplolaimus, Xiphinema, Meloidogyne, and Pratylenchus (Tylka et al 2011a). From a survey of 550 fields in Illinois, plant-parasitic nematodes were found in every field, at population densities ranging from 100 to 4000+ nematodes/100 cm$^3$ soil (Niblack and Lopez, personal communication). The spiral nematode (Helicotylenchus) was the most frequent, being found in 99% of the samples, in most cases above 150 nematodes/100 cm$^3$ soil. The lesion nematodes (Pratylenchus) were the second most prevalent in the Illinois survey, being present in 84% of the fields. These were also found at populations considered above moderate risk levels (26 nematodes/100 cm$^3$ soil) in more than 50% of the fields in which they were present. Results from Iowa were very similar to those from Illinois, with plant-parasitic nematodes being found in 92% of the 331 samples analyzed. Spiral and lesion nematodes were again the most frequently occurring genera, being present in 77% and 51% of the samples, respectively, at mean population densities of 87/100 cm$^3$ soil for the former and 21/100 cm$^3$ soil for the latter (Tylka et al 2011a).

The fact that nematodes were found at relatively high population densities in corn fields in Iowa and Illinois is not surprising. Current crop management practices such as conservation tillage and continuous corn may be contributing to the buildup of plant-parasitic nematodes, because the frequency and distribution of these pathogens may be influenced by soil type, edaphoclimatic conditions, cropping practices, and complex interactions among these factors. For instance,
Caveness (1974) found more *Pratylenchus* spp. in soil and corn root samples collected from conventionally-tilled plots when compared with no-till plots, but *Helicotylenchus* and *Meloidogyne* species were more abundant in no-till plots than in conventionally-tilled plots planted with corn in rotation with several other crops. In contrast, *Pratylenchus scribneri* was more evenly distributed in no-till plots than in conventionally-tilled plots in Indiana (Alby *et al.* 1983), and Thomas (1978) reported that higher population densities of *Helicotylenchus* spp., *Pratylenchus* spp., and *Xiphinema* spp. were found in no-till plots. Results from a study conducted by Parmelee and Alston (1986) in Georgia suggested that the effects of tillage practices on nematode populations may depend on when samples are collected. They found that tillage did affect nematode species assemblage and population density, with monthly abundance being significantly greater in conventional tillage plots than in no-till plots; however, during the summer plant-parasitic nematodes were more abundant in no-till plot than in conventionally-tilled plots.

In spite of the similarities among crop production practices in Illinois, Iowa, and Ohio, data on nematode frequency and population density from the former two states cannot be extrapolated to the latter, because other factors such as soil pH, organic matter, and texture, known to vary among the three states, may also affect nematode population structure (Florini *et al.* 1987, Norton and Hoffmann 1974, Schmitt 1969, Wallace *et al.* 1993). Moreover, because the damage caused by any given species of nematode may vary with hybrid, crop management practices, weather conditions, and interactions among these factors
(Norton 1983, Tylka et al 2011a), no single population density will be adequate for estimating losses under all conditions (Tylka et al 2011a). Therefore this study was conducted specifically to 1) determine which genera of plant-parasitic nematodes were most frequently found in corn fields in Ohio and at what population densities, and 2) to estimate county-level variability in the presence and abundance of these organisms. Fifteen to 16 corn fields were sampled in 28 counties during the 2013 and 2014 growing seasons, and plant-parasitic nematodes were identified to genus and enumerated. Linear and generalized linear mixed models were then fitted to the data to estimate population variability among counties.

MATERIALS AND METHODS

Sampling. A systematic survey of plant-parasitic nematodes was conducted in corn fields in the state of Ohio. Samples were collected during the 2013 and 2014 growing seasons between growth stages V3 to V6 according to a stratified sampling technique. The vegetative stage 3 refers to corn plants with three leaves below the collar leaf and V4, V5 and V6 represented corn plants with four, five and six leaves below the collar leaf respectively (Abendroth et al 2011). The state was divided into strata based on six naturally occurring soil regions located in the western half of the state where 95% of the corn is cultivated (Fig. 2.1). A total of 28 counties were randomly selected, and from each county, 15 or 16 fields were sampled (Fig 2.2 and Table 2.1). Soil samples were ultimately taken from fields with different cropping histories, including fields
with continuous corn (five or more years of corn-after-corn), corn-soybean rotation (no more than two consecutive years in the last five years with any one of the two crops), and corn-soybean-wheat rotation (all three crops planted in the field at least once in the last five years). Crop husbandry and management of the selected fields were consistent with field corn production practices in the state (Barker et al. 2005). All fields were grown in monoculture and planted during the months of April and May. Land preparation across selected fields included conventional, vertical, minimum, or zero tillage. According to the Conservation Technological Information Center webpage these tillage practices are precisely defined crop residue management. Conventional tillage had less than 15 percent residue cover after planting which includes disturbing the entire soil surface prior to or during planting by full width tillage. On the other hand, no till or zero till represents undisturbed soil from harvesting to planting with the exception of disturbed strips by planting or drilling equipment. This form of tillage entails more than 30 percent crop residue. Conservation tillage which includes minimum and vertical tillage covers more than 30 percent or more of the soil surface with crop residue after planting to mitigate soil erosion.

Samples were collected in an N-shaped pattern across each of the selected fields as described by (Tylka et al. 2011b). A 2.5-cm diameter soil probe (MODEL LS, Oakfield Apparatus and Fond du Lac) was used to collect up to 20 soil cores at a depth of 30 to 50 cm from every sampled field and pooled into one composite sample. The soil probe was angled at approximately 45° under the row of corn plants to collect sample within the rhizosphere. Undisturbed soil
cores along with root fragments were placed into labeled plastic bags. Each sample was then carefully placed into a cooler with Styrofoam and disposable ice packs, transported back to the laboratory, and stored at 4°C until processed.

**Nematode extraction.** Nematodes were extracted from 100 cm³ soil subsamples with sieving, decanting and centrifugal sucrose flotation (Jenkins 1964). After carefully breaking up large pieces of soil cores and passing the sample through a 1.27-cm mesh, each sample was thoroughly mixed, and a 100 cm³ subsample was taken. Batches of six samples were prepared for processing and extraction. Into 500 mL tap water in a 1,000 mL beaker, soil was added until the water level reached the 600 mL mark, ensuring that a 100 cm³ soil sample was used. This mixture was stirred gently until a soil/water suspension was formed. The suspension was poured through a combination of 850 µm/250 µm sieves over a 4.73 L pail. Water was repeatedly added to the remaining soil in the beaker, and the suspension was poured through the 850 µm/250 µm sieve combination until the water looked relatively clear.

The 4.73 L pail with the soil suspension was set aside for subsequent processing, while the material captured in the 850 µm/250 µm sieves was transferred to a Baermann funnel mounted on a universal ring stand (Staniland 1954). The funnel was placed on a shelf and left for 48 hr during which time the water level was replenished as needed to maintain the level. After 48 hours, samples were collected in a 38 µm sieve through the opening at the bottom of the funnel. At the same time, the material on the mesh in the funnel was thoroughly rinsed and collected in the sieve. Nematodes in the sieve were then
rinsed into a capped tube, and the tube was stored at 4°C until processed for nematode identification and counting.

Nematodes were also extracted from the soil suspension in the 4.73 L pail. After resuspension, the mixture was gradually poured through a 150µm/38µm sieve set. The sieves were rinsed thoroughly until the water passing through them looked clear, and the captured material from each sieve was transferred to a 50-ml centrifuge tube. Tubes were then placed into a centrifuge for five minutes at 2,800 rpm. After centrifugation, the supernatant was slowly poured from the tubes, and 30 to 35 mL 45% sucrose solution was added to the pellet and stirred. Tubes were immediately placed into a centrifuge for two minutes at 1,500 rpm (Jenkins 1964). The resulting clear supernatant was poured over an angled 38 µm sieve and rinsed with water to remove sucrose from captured nematodes. The nematodes in the sieve were then poured into a 50-ml capped tube and the tube was stored at 4°C until used for nematode identification and counting.

**Nematode identification and enumeration.** Plant-parasitic nematodes and free-living nematodes were identified to genus using overall body shape and size, stylet shape and size, esophagus and intestine orientation, amphid opening, tail shape, metacorpus size, vulva position, stylet knob shape and size, shape when at rest, head description and several other measures of gross body morphology (Mai and Mullin 1996). This was done according to the key provided in Mai and Mullin (1996). For instance the spiral nematode was characterized by well-developed stylets, overlapping esophagus which is typically ventrally and lip
region not having longitudinal striations. When at rest or dead this nematode tend to form a spiral shape. Other plant-parasitic nematodes such as the dagger nematode was characterized by long stylets with sclerotized basal flanges with the guiding rind located near the base of the stylet and the stubby-root nematode was characterized by thick and short body with short curved stylets. Lesion nematodes were also characterized by well-developed stylets, ventrally overlapping esophagus, relatively broad heads and bluntly rounded tails (Mai and Mullin 1996).

Nematode population densities, were determined by counting the number of nematodes of each genus in a counting dish under a stereoscopic microscope. Abundance was then expressed as the number of nematodes per 100 cm$^3$ soil. Mean abundance was calculated as the sum of nematodes per 100 cm$^3$ soil divided by the total number of samples in which the genus in question was detected. For example, to estimate the mean abundance of lesion nematodes in Adams county, the abundance for each field sampled in that county was added and that total was divided by the number of fields that were positive for lesion nematode. A similar approach was used to estimate mean abundance for tillage and crop rotation practices, and soil regions. For each genus, percent frequency was calculated as the total number of samples in which that genus was detected, divided by the total number of samples collected and multiplied by 100.

**County-level heterogeneity of 10 genera of plant-parasitic nematodes in corn fields in Ohio.** For each of the 10 genera of plant-parasitic nematode detected, each field was assigned a code of 1 if the nematode genus in question
was detected and 0 if it was not. Because nematodes were extracted from a single composite sample per field, each field was considered a sampling unit. The expected probability of a nematode of a certain genus being present in a given county was estimated as \( p = Y/n \), where \( Y \) is the number of fields testing positive for the nematode in question and \( n \) is the total number of fields sampled per county (15 or 16). Since \( p \) was assumed to have a conditional binomial distribution, PROC GLIMMIX of SAS (Littell et al 2006) was used to fit a generalized linear mixed model to the data to estimate the random effect of county (\( C \)) on the expected value of \( p \). Models were fitted to the logit link function of \( p (\eta) \), given as \( \eta = \ln \left[ \frac{p}{1 - p} \right] \), as previously described (Gbur et al 2012, Kriss et al 2012 and Stroup 2013).

The models fitted to the data can be written as \( \eta_i = \mu + C_i \), where \( \mu \) is the intercept or the overall mean on the logit scale and \( C_i \) is the random effect of the \( i \)th county. \( C_i \) was assumed to have a normal distribution with mean 0 and variance \( \sigma^2 \). Separate models were fitted to the data for each nematode genus with maximum likelihood as the parameter estimation method, and estimates of \( \mu \) and \( \sigma^2 \) and their standard errors for each genus were obtained. The significance of \( \sigma^2 \) was determined with a likelihood ratio test, and the profile confidence interval around \( \sigma^2 \) was calculated as explained by Kriss et al (2012). After fitting the models, the inverse link function was used to obtain estimated probability of each nematode genus being detected in each count, conditioned on the random effect, as \( \hat{p} = e^{\hat{\mu}} \left( 1 + e^{\hat{\mu}} \right) \).
RESULTS

Plant-parasitic nematode complex in corn fields in Ohio. Plant-parasitic nematodes associated with corn in Ohio accounted for over 50% of the total nematodes recovered in this survey. Bacterial-feeding nematodes were found at varying levels, while fungivores, omnivores and predators were detected in 3.8, 3.3 and 1.1% of the fields, respectively; the remaining 39.2% consisted of bacterial feeders Fig 2.3. Ten common morphological groups of plant-parasitic nematodes, namely Helicotylenchus (spiral), Pratylenchus (lesion), Hoplolaimus (lance), Xiphinema (dagger), Tylenchorhynchus (stunt), Paratylenchus (pin), Criconemella (ring), Paratrichodorus (stubby-root), and Heterodera (cyst) were identified, several genera in the subfamily Tylenchinae were counted together as “tylenchids”. These nematodes were identified by delicate stylets, non-overlapping esophagus, and filiform tails. All 10 common groups were detected in Clark, Jackson, Pickaway, and Shelby counties; 9 in Adams, Fairfield, Fayette, Hocking, Huron, Logan, Morrow, Paulding, and Vinton counties; 8 in Brown, Clermont, Clinton, Fulton, Greene, Henry, Mercer, Pike, Richland, Scioto, and Warren counties, 7 in Highland, Union, and Wood counties, and 6 in Licking county. Spiral, lesion, dagger, pin, and “tylenchids” nematodes were detected in all 28 counties; lance in all but Fulton and Henry counties; stunt in all but Licking, Morrow, and Vinton counties; stubby-root nematode was not detected in Highland, Licking, Mercer, Pike, Richland, Scioto, Union, and Wood; ring was only detected; and cyst and ring nematodes were only detected in 14 and 11 of the 28 counties sampled, respectively.
“Tylenchids” were observed in 96% of the samples. The most frequently occurring plant-parasitic nematodes were spiral, and lesion detected in 94 and 80% of the fields, respectively (Table 2.2). Pin, lance, stunt, and dagger were identified in 57, 48, 48, and 37%, respectively. Stubby root, cyst, and ring nematodes all occurred with frequencies less than 13%. Spiral was also the most abundant plant-parasitic nematode detected, with mean population density of 90 nematodes/100 cm$^3$ soil. The second most abundant plant-parasitic nematode was the pin nematode with 61 nematodes/100 cm$^3$ soil. Stunt, lance, and lesion were detected at mean population densities of 23, 20 and 16 nematodes/100 cm$^3$ soil respectively. Other plant-parasitic nematode genera detected below mean population density of 10 nematodes/100 cm$^3$ of soil included ring, dagger, stubby root, and cyst nematodes.

Spiral, lesion, lance, and pin nematodes were detected in nearly all 28 counties included in the Ohio survey. The abundance of spiral nematodes ranged from 16 to 208 nematodes/100 cm$^3$ soil among counties, with Union county recording the highest and Pike the lowest mean population density. For lesion and lance nematodes, Fulton and Scioto (37 nematodes/100 cm$^3$ soil) and Mercer (31 nematodes/100 cm$^3$ soil) counties recorded the highest abundance, respectively. On the other hand, lance nematode was not detected in either Henry or Fulton counties, and lesion nematode was recorded at the lowest abundance in Clinton county (4 nematodes/100 cm$^3$ soil). Pin nematode was detected in all counties, with Union county recording the highest (245
nematodes/100 cm$^3$ soil) and Wood the lowest (6 nematodes/100 cm$^3$ soil) mean population density (Table 2.1).

**Plant-parasitic nematode abundance by cropping practices and soil region.** No clear pattern was observed for mean population densities of plant-parasitic nematodes across the three cropping systems accounted for in this survey. Spiral was the most abundant plant-parasitic nematode for all three cropping systems. Pin nematode was the second most abundant for corn after soybean and wheat (C-S-W-R) and corn after soybean (C-S-R), whereas lower abundance was recorded for corn after corn (C-C-C) when compared with other plant-parasitic nematode genera. Additionally, lesion, lance, dagger, and stunt showed relatively consistent mean population densities across all three cropping systems (Fig. 2.4 and Table 2.3).

No clear pattern was observed for mean population densities of plant-parasitic nematodes across the three tillage systems (conventional, no-till and conservation) accounted for in this survey. Mean population densities for spiral nematode were highest for conventional and conservation tillage compared with other plant-parasitic nematodes. In the no tillage system, pin was the most abundant plant parasitic nematode detected and the second most abundant for the other two tillage systems. Comparing the data from the three tillage systems demonstrated that the mean population densities of lesion and stunt nematodes were highest in the conventional tillage system. Abundance was highest for lance in the conservation tillage system, whereas dagger showed consistent mean population densities across the three tillage systems. No tillage had the highest
abundance or ring nematode when compared to the other tillage systems (Fig. 2.5).

Plant-parasitic nematodes were detected in all six soil regions, with the exception of ring and cyst in soil region 1 and 5, respectively. The most predominant plant-parasitic nematode across all soil regions was spiral; however, pin was the most abundant nematode detected in soil region 2. Lesion, dagger, and stunt were most abundant in soil region 1, whereas soil region 5 had the highest abundance of lance nematodes (Fig. 2.6).

**County-level heterogeneity of 10 commonly morphological groups of plant-parasitic nematodes in corn fields in Ohio.** The estimated proportion of samples testing positive for the nine genera of plant-parasitic nematodes, plus several genera in the subfamily Tylenchinae grouped together as “tylenchids” (called frequency here) on the logit (\( \hat{\mu} \)) and original (\( \hat{p} \)) scales are presented in Table 2.4, along with county-level variance estimate (\( \hat{\sigma}^2 \)) for each genus. Based on both the \( \hat{\mu} \) and the corresponding \( \hat{p} \) values, the estimated mean proportion of nematode-positive fields, averaged across all samples, varied considerably among the genera, ranging from -3.73 (for the ring nematode) to 4.12 (tylenchids) and 0.02 and 0.98, respectively. Based on the \( \hat{\sigma}^2 \) values, county-level heterogeneity also varied among the genera, ranging from 0.56 to 3.23 (Table 2.4 and Fig. 2.7). However, the confidence intervals around \( \hat{\sigma}^2 \) overlapped considerably, suggesting comparable county-level heterogeneity across genera. The only exceptions were for comparisons between the stunt and pin, stunt and dagger, and lance and dagger nematodes for which the confidence intervals
overlapped slightly or not at all. Given that the sample size, which affects the width of the 95% confidence interval, was the same for all genera, non-overlapping intervals could be interpreted to mean differences in county-level heterogeneity. Therefore, based on the confidence intervals, the stunt nematode was more variable at the county level than the dagger and pin nematodes, and the lance nematode was more variable than the dagger nematode at this same spatial scale.

**DISCUSSION**

Prior to 2013, a few small scale studies were conducted to ascertain the presence of plant-parasitic nematodes in corn fields in Ohio. However, there was minimal scientific evidence to demonstrate whether plant-parasitic nematodes posed a real threat to corn production in Ohio, particularly when compared with other diseases of corn such as Stewart’s Wilt (caused by the bacterium *Erwinia stewartii*) and Gibberella ear rot (caused by the fungal pathogen *Fusarium graminearum*). The ability of plant-parasitic nematodes to parasitize corn plants and cause yield loss is well established (Norton 1983). Corn is generally most susceptible to plant-parasitic nematode damage when these obligate parasites are present in high numbers, but some species may also cause substantial damage at relative low numbers (Tylka *et al* 2011a). Given the paucity of information pertaining to the presence and diversity of nematodes in corn fields in Ohio, research was embarked upon to answer questions raised by stakeholders.
and researchers alike pertaining to the potential impact of nematodes on corn production in Ohio.

Based on the survey, plant-parasitic nematodes made up 50% of the total nematode population extracted, and at least one plant-parasitic nematode genus was found in all of the 425 fields surveyed in Ohio during 2013 and 2014. Based on these summary statistics, one can safely assume that plant-parasitic nematodes are very common in corn fields in Ohio. Ten commonly occurring morphological groups were identified, including spiral, lesion, lance, stunt, dagger, pin, stubby-root, ring, and cyst, along with several genera in the subfamily Tylenchinae counted together as “tylenchids”. In similar surveys conducted in Iowa and Illinois, eight and eleven plant-parasitic nematode genera were found in corn fields, respectively (Tylka et al 2011a; Niblack and Lopez, personal communication). Norton (1983) reported that more than 60 species of plant-parasitic nematodes are associated with corn in North America. Twenty-eight of those species were reported in Iowa (Tylka et al 2011a), however, plant-parasitic nematodes were only identified to genus in the current survey.

The plant-parasitic nematode genus detected in almost all fields surveyed in Ohio was *Helicotylenchus*, the spiral nematode. This genus was found in 94% of the fields sampled, at a mean population density of 90 nematodes/100cm² soil. These findings are comparable to those reported by Tylka et al. (2011a) and Niblack and Lopez (personal communication) from similar surveys conducted in Iowa and Illinois, respectively. However, despite its high frequency, the spiral nematode was generally not found at damaging population densities in most
fields. Population densities were generally below the 500-1000 nematodes/100 cm³ soil severe damage threshold reported by Tylka et al. (2011a). High frequency and relatively low population density seems to be fairly common for the spiral nematode in the Mid west (Niblack and Lopez, personal communication), Tylka et al. (2011a). In contrast, the spiral nematode was found in <10% of Colorado wheat fields (Todd et al. 2014), but at relatively high population densities in soybean fields in Missouri (Niblack 1992). This suggests that there may be an association between this plant-parasitic nematode and corn and soybean when compared with wheat. When compared with other migratory ectoparasitic nematodes such as the needle (Longidorus) and sting nematode (Belonolaimus), which are considered major threats to corn at relatively low population densities (1-2 nematodes/100 cm³ soil, McDonald and Nicol 2005), the spiral nematode was considered to be only moderately to slightly pathogenic to corn (Sledge 1956).

It seems reasonable to conclude that the spiral nematode is currently not a major threat to corn production in most Ohio corn fields. However, further research will be needed to determine how damaging this plant-parasitic nematode genus really is under conditions in Ohio, and what population levels. In the absence of Ohio-specific data, the damage threshold of 500-1000 nematodes/100 cm³ soil proposed (Tylka et al. 2011a) can be used as a guide to understand what the spiral nematode numbers from the current survey mean in terms of threat to corn production in Ohio. At least one field in Union, Fulton and Fairfield counties had spiral nematode populations above the suggested damage
threshold level. Additionally, Fayette, Logan and Clermont counties had at least one field approaching the damage threshold. This suggests that these counties could potentially have problems with this plant-parasitic nematode, and should be immediate targets for future and more detail research. For instance, further investigations should be conducted to ascertain which species of *Helicotylenchus* are present in these counties, since the damage potential may vary among species. *Helicotylenchus pseudorobustus* is one of those species known to negatively affect corn yields (Norton *et al.* 1978).

Fortunately, needle and sting nematodes were not confirmed in Ohio. Comparing the nematode genera found in the Ohio survey to a survey conducted in Illinois (Niblack and Lopez, personal communication), all of the plant-parasitic nematode genera detected in Illinois were found in Ohio, with the exception of *Longidorus* and *Meloidogyne*. Similarly, when compared with the Iowa survey, a similar set of plant-parasitic nematode genera were detected in the two states, with the exception of *Longidorus* found in Iowa but not Ohio. *Longidorus* is often highly localized and restricted largely to very sandy soils (at least 49% sand) (Norton and Hoffman 1975). Only 7% of the soil samples collected in Ohio had at least 49% sand. However, for *Longidorus* and other nematodes genera not found in Ohio, one cannot conclude that they are not present in the state, since only 28 counties were surveyed, and only 15-16 fields were sampled in each county. Moreover, Tylka *et al.* (2011b) suggested that when sampling for plant-parasitic nematodes and the needle nematode is suspected to be present, samples should be collected in the spring or fall. All samples in the current survey were
collected in June and early July. This suggests that the needle nematode may have been missed or the soil type found in Ohio may not have favored its presence.

Pratylenchus was another important plant-parasitic nematode genus found in this survey. The lesion nematode was the second most frequently occurring, present in 80% of fields sampled. Similar frequencies, 84 and 51%, respectively, were reported in Illinois and Iowa (Tylka et al. 2011a; Niblack and Lopez personal communication). The lesion nematode is one of the most economically damaging plant-parasitic nematodes, found in a wide variety of crops worldwide. Lesion nematodes were recovered from 78% of soil samples taken from wheat fields in Colorado (Todd et al. 2014) and was identified in soybean fields in Missouri (Niblack 1992). It’s possible that corn, wheat and soybean may be suitable hosts for this plant-parasitic nematode. This in part provides some explanation for the relatively high frequency of this genus in Ohio where all three crops are widely cultivated. The mean population density for lesion nematode in the three cropping sequences evaluated for this survey was very similar (16 nematodes/100 cm³ soil). Therefore rotation with corn, soybean and wheat may not be a suitable management tactic against lesion nematode. The fact that this nematode is present at such high frequencies in corn fields in these three states, especially Ohio and Illinois, suggest more research should be done to establish damage threshold levels and management strategies to combat this pathogen.

Based on the current survey, mean population density for the lesion nematode in Ohio, averaged across samples, was 16 nematodes/100 cm³ soil,
compared to 26 and 21 nematodes/100 cm³ soil in Illinois and Iowa, respectively (Tylka et al. 2011a; Niblack and Lopez personal communication). The suggested severe damage population threshold for this nematode in Illinois is 51 to 100 nematodes/100 cm³ soil, and 26 to 50 nematodes/100 cm³ soil is considered moderate risk (Niblack personal communication). When considering the presence of lesion nematode in corn fields surveyed in Ohio, Mercer, Fulton, Hocking, Licking, Wood, Richland, Scioto, Greene, Adams and Clermont counties had at least one field at potentially damaging population densities. Additionally, another 14 counties had at least one field with the lesion nematode at moderate risk population levels. These 24 counties may be at risk for damage by the lesion nematode, but further research would be needed to ascertain which species are present, since the threat may vary among species. Several species of lesion nematode were identified as being capable of causing damage to corn under different circumstances. *Pratylenchus scribneri*, a common pest of corn in irrigated sandy soils (Todd 1991) and *Pratylenchus hexincisus*, probably the most important *Pratylenchus* species in the Midwest (Zirakparvar 1980), are two such species. Another *Pratylenchus* species, *Pratylenchus brachyurus* was responsible for over 25% yield reduction in Nigeria (Egunjobi 1974).

*Hoplolaimus, Paratylenchus, and Tylenchorhynchus*, the lance, pin and stunt nematodes were found in 48, 57, and 48%, respectively of the fields sampled in Ohio, at mean population densities of 20, 61, and 23 nematodes/100 cm³ soil, respectively. The suggested severe and moderate risk population thresholds for the lance nematodes, are 76 to 150 and 41 to 75 nematodes/100
cm$^3$ soil, respectively, (Niblack personal communication). Fayette, Clark, Mercer, Licking, Greene, Adams, Brown and Clermont counties all had at least one field with population density above the severe threshold level, whereas Hocking, Fairfield, Richland, Scioto, and Clinton counties had fields with populations at the moderate risk threshold levels.

Compared with Ohio, relatively lower frequencies of the pin nematode were recovered in Illinois and Iowa, 24.3 and 0.6%, respectively (Tylka et al. 2011a; Niblack and Lopez, personal communication). The pin nematode was recovered from 12% of Colorado wheat fields surveyed (Todd et al. 2014), and was detected at relatively high densities in Missouri soybean fields (Niblack 1992). This nematode is considered to be of severe risk at population densities of 501 to 1000 nematodes/100 cm$^3$ soil and at moderate risk at 101 to 500 nematodes/100 cm$^3$ soil. Using these threshold levels as a guideline, Pickaway, Union, Paulding, Morrow and Richland counties all had at least one field at potentially damaging populations. Given these numbers, one can deduce that fields within these counties may be at a high risk for damage by the pin nematode. Additionally, there were thirteen other counties with fields at moderate risk pin nematode population levels. The stunt nematode was also found at relatively low frequency in Iowa (4.8%), but at higher frequencies in Ohio (48%) and Illinois (36%) (Tylka et al. 2011a; Niblack and Lopez, personal communication). The mean population density was 23 nematodes/100 cm$^3$ soil in Ohio. Henry, Fulton and Scioto counties had at least one field with populations
above the 100 nematodes/100 cm$^3$ soil severe damage threshold level proposed by Tylka et al. (2011a).

Although the needle nematode was not found in this survey, another genus closely related to this nematode was found. The dagger nematode, which is capable of causing damage at population levels as low as 30 to 40 nematodes/100 cm$^3$ soil (Tylka et al. 2011a), was found at a frequency of 37% and maximum population density of 26 nematodes/100 cm$^3$ soil. This same nematode was found in Iowa and Illinois at frequencies of 42 and 6% and maximum population densities of 126 and 18 nematodes/100 cm$^3$ soil, respectively. Based on the 30 to 40 nematodes/100 cm$^3$ soil damage threshold, none of the fields sampled in Ohio are at risk for damage and loss caused by this nematode. Nevertheless, there were a few individual fields approaching the suggested damage threshold for the dagger nematode in Henry, Fulton, Hocking and Morrow counties.

*Criconemella, Paratrichodorus, and Heterodera* were other plant-parasitic nematodes recovered from soil samples. These were infrequently or never found above the damage threshold level. *Longidorus, Meloidogyne,* and *Hemicycliophora* were not found in any of the samples collected in this survey. However, these plant-parasitic nematode genera were associated with corn fields in other related studies (Norton, 2011; Niblack and Lopez personal communication; Tylka et al. 2011a). The reasons for the absence of these plant-parasitic nematodes in Ohio may range from differences in soil type, farming practices, sampling date and extraction techniques. Some nematologists also
suggest that plant-parasitic nematodes may be ruptured by aggressive handling of soil samples and these ruptured nematodes cannot be recovered for identification (Tylka et al. 2011b). Perhaps, due to the geographical location and consequently the soil type in Ohio, these nematodes may genuinely not be present in the corn fields sampled. Nevertheless, given the economic importance of these plant-parasitic nematode not detected in the Ohio survey, especially *Longidorus*, more research is needed to further ascertain their presence and abundance.

In our survey, most of the corn fields sampled were below the suggested damage threshold for all plant-parasitic nematode genera detected. Frequencies and mean population densities varied among nematode genera detected, and for a given genus, among soil regions, counties within soil regions and fields within counties and soil region. Considerable variation was also observed among cropping and tillage practices. However, there were individual corn fields in several counties with mean population densities above the suggested damage threshold level for *Pratylenchus, Hoplolaimus, Helicotylenchus, Paratylenchus* and *Tylenchorhynchus*. The prevalence of *Pratylenchus* in this study, which is considered as one of the most economically damaging plant-parasitic nematodes in a wide variety of crops, warrants greater recognition as a potential limiting biological factor for corn production in Ohio. Corn growers should be apprised of the commonness of plant-parasitic nematodes in their fields, and the potential threat posed by these pathogens should be communicated to the relevant stakeholders; however, further research is necessary to quantify relationships
between plant-parasitic nematode populations and yield loss in order to establish Ohio-specific damage thresholds, and to identify location-specific risk factors contributing to the high frequency and abundancy observed in some fields.
REFERENCES


Batista da Silva, M. 2013. Studies on extraction and control of plant-parasitic nematodes on corn. M.S. thesis, Department of Plant Pathology and Microbiology, Iowa State University, Ames.


www.ctic.purdue.edu/resourcedisplay/322/

Table 2.1  Frequency and abundance of *Helicotylenchus* spp., *Pratylenchus* spp., *Hoplolaimus* spp., and *Paratylenchus* spp. in 28 Ohio counties sampled between the V3 and V6 growth stages during 2013 and 2014 growing seasons.

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<th>S.D</th>
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<th>Frequency</th>
<th>Abundance</th>
<th>S.D</th>
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</table>

Continued
Table 2.1: Continued

| Percentage of N samples that were positive for nematodes of a certain genus (total number of samples in which the nematode genus was detected divided by the total number of samples collected [N = 15 or 16], multiplied by 100). |
| Mean abundance = total number of nematodes of a certain genus per 100 cm³ soil divided by the total number of samples in which that genus was detected. |
| S.D = standard deviation of mean abundance. |
| Sample with the highest abundance (maximum number of nematodes of a certain genus detected per 100 cm³ of soil). |
Table 2.2 Frequency and abundance of nine genera of plant-parasitic nematodes, along with several genera in the subfamily Tylenchinae grouped together as “tylenchids” detected in corn fields sampled in Ohio between the V3 and V6 growth stages during the 2013 and 2014 growing seasons.

<table>
<thead>
<tr>
<th>Nematode common name</th>
<th>Frequency(^a)</th>
<th>Abundance(^b)</th>
<th>S.D(^c)</th>
<th>Maximum(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiral</td>
<td>94</td>
<td>90</td>
<td>109</td>
<td>990</td>
</tr>
<tr>
<td>Tylenchids</td>
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<td>25</td>
<td>26</td>
<td>218</td>
</tr>
<tr>
<td>Cyst</td>
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<td>12</td>
<td>58</td>
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<tr>
<td>Lance</td>
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<td>21</td>
<td>106</td>
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<tr>
<td>Dagger</td>
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<td>5</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td>Stunt</td>
<td>48</td>
<td>23</td>
<td>37</td>
<td>240</td>
</tr>
<tr>
<td>Pin</td>
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<td>61</td>
<td>137</td>
<td>1164</td>
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<tr>
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<td>10</td>
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<tr>
<td>Stubby</td>
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<td>56</td>
</tr>
</tbody>
</table>

\(^a\)Percentage of 425 samples that were positive for nematodes of a certain genus (total number of samples in which the nematode genus was detected divided by 425, multiplied by 100).

\(^b\)Mean abundance = total number of nematodes of a certain genus per 100 cm\(^3\) soil divided by the total number of samples in which that genus was detected.

\(^c\)S.D = standard deviation of mean abundance.

\(^d\)Sample with the highest abundance (maximum number of nematodes of a certain genus detected per 100 cm\(^3\) of soil).
Table 2.3 Frequency and abundance of nine genera of plant-parasitic nematodes, along with several genera in the subfamily Tylenchinae grouped together as “Tylenchids” detected in soil samples collected between the V3 and V6 growth stages during 2013 and 2014 growing seasons from corn field under continuous corn (C-C-C), corn-soybean-wheat rotation (C-S-W-R), and corn-soybean rotation (C-S-R)

<table>
<thead>
<tr>
<th>Nematode common name</th>
<th>C-C-C N=83</th>
<th>C-S-W-R N=122</th>
<th>C-S-R N=220</th>
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</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>Abundance</td>
<td>Maximum</td>
<td>Frequency</td>
</tr>
<tr>
<td>Spiral</td>
<td>88</td>
<td>117</td>
<td>990</td>
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<tr>
<td>Tylenchids</td>
<td>98</td>
<td>28</td>
<td>180</td>
</tr>
<tr>
<td>Cyst</td>
<td>15</td>
<td>12</td>
<td>58</td>
</tr>
<tr>
<td>Lesion</td>
<td>80</td>
<td>16</td>
<td>62</td>
</tr>
<tr>
<td>Lance</td>
<td>37</td>
<td>29</td>
<td>106</td>
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<tr>
<td>Dagger</td>
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<td>22</td>
</tr>
<tr>
<td>Stunt</td>
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<tr>
<td>Pin</td>
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</tr>
<tr>
<td>Stubby</td>
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<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

aPercentage of N samples that were positive for nematodes of a certain genus (total number of samples in which the nematode genus was detected divided by the total number of samples collected, multiplied by 100).
bMean abundance = total number of nematodes of a certain genus per 100 cm³ soil divided by the total number of samples in which that genus was detected.
cS.D = standard deviation of mean abundance.
dSample with the highest abundance (maximum number of nematodes of a certain genus detected per 100 cm³ of soil).
Table 2.4 Estimated mean proportion of 425 corn fields testing positive for each plant-parasitic nematode genus on the logit ($\hat{\mu}$) and original scale ($\hat{\mu}$), estimated county-level variance ($\hat{\sigma}^2$), and corresponding standard errors

<table>
<thead>
<tr>
<th>Nematode common name</th>
<th>$\hat{\mu}$</th>
<th>SE($\hat{\mu}$)</th>
<th>$\hat{\mu}^a$</th>
<th>SE($\hat{\mu}$)</th>
<th>$\hat{\sigma}^2$</th>
<th>SE($\sigma$)</th>
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</thead>
<tbody>
<tr>
<td>Spiral</td>
<td>2.93</td>
<td>0.314</td>
<td>0.95</td>
<td>0.015</td>
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<td>Tylenchids</td>
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<td>0.98</td>
<td>0.010</td>
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<td>Cyst</td>
<td>-2.97</td>
<td>0.474</td>
<td>0.05</td>
<td>0.022</td>
<td>2.553</td>
<td>1.386</td>
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<tr>
<td>Lesion</td>
<td>1.66</td>
<td>0.250</td>
<td>0.84</td>
<td>0.034</td>
<td>1.033</td>
<td>0.468</td>
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<td>Lance</td>
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<td>0.292</td>
<td>0.48</td>
<td>0.073</td>
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<td>0.747</td>
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<tr>
<td>Dagger</td>
<td>-0.58</td>
<td>0.175</td>
<td>0.36</td>
<td>0.040</td>
<td>0.521</td>
<td>0.247</td>
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<tr>
<td>Stunt</td>
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<td>0.368</td>
<td>0.45</td>
<td>0.091</td>
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<td>0.33</td>
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<td>Ring</td>
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<td>0.02</td>
<td>0.012</td>
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<td>1.129</td>
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<tr>
<td>Stubby</td>
<td>-2.22</td>
<td>0.268</td>
<td>0.10</td>
<td>0.024</td>
<td>0.907</td>
<td>0.476</td>
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</table>

$^a$Estimated from $\hat{\mu}$ by applying the inverse link function.

$^b$Statistically for genera based on likelihood ratio tests.
Fig 2.1. A map of the state of Ohio showing the six soil regions in which corn fields were surveyed and sampled for plant-parasitic nematodes. (Ohio Department of Natural Resources Map)
Fig 2.2. A map of the state of Ohio showing counties in which corn fields were sampled for plant-parasitic nematodes.
Fig. 2.3. Percentage of nematodes in different trophic groups detected in soil samples collected in corn field between the V3 and V6 growth stages during the 2013 and 2014 growing seasons.
Fig. 2.4. Mean abundance (nematodes per 100 cm$^3$ soil) of nine genera of plant-parasitic nematodes *Helicotylenchus* (spiral), *Pratylenchus* (lesion), *Hoplolaimus* (lance), *Xiphinema* (dagger), *Tylenchorhynchus* (stunt), *Paratylenchus* (pin), *Criconemella* (ring), *Paratrichodorus* (stubby-root), and *Heterodera* (cyst), along with several genera in the subfamily Tylenchinae grouped together as “tylenchids” in Ohio corn fields sampled between the V3 and V6 growth stages during the 2013 and 2014 growing seasons. Samples were collected from fields under continuous corn (C-C-C), corn-soybean rotation (C-S-R) or corn-soybean-wheat rotation (C-S-W-R).
Fig. 2.5. Mean abundance (nematodes per 100 cm³ soil) of nine genera of plant-parasitic nematodes *Helicotylenchus* (spiral), *Pratylenchus* (lesion), *Hoplolaimus* (lance), *Xiphinema* (dagger), *Tylenchorhynchus* (stunt), *Paratylenchus* (pin), *Criconemella* (ring), *Paratrichodorus* (stubby-root), and *Heterodera* (cyst), along with several genera in the subfamily Tylenchinae grouped together as “tylenchids” in Ohio corn fields sampled between the V3 and V6 growth stages during the 2013 and 2014 growing seasons. Samples were collected from fields left without tillage (no-till) or subjected to conservation (vertical or minimum tillage) or conventional tillage (includes all standard tillage operations).
Fig. 2.6. Mean abundance (nematodes per 100 cm³ soil) of nine genera of plant-parasitic nematodes *Helicotylenchus* (spiral), *Pratylenchus* (lesion), *Hoplolaimus* (lance), *Xiphinema* (dagger), *Tylenchorhynchus* (stunt), *Paratylenchus* (pin), *Criconemella* (ring), *Paratrichodorus* (stubby-root), and *Heterodera* (cyst), along with several genera in the subfamily Tylenchinae grouped together as “tylenchid” in Ohio corn fields sampled between the V3 and V6 growth stages during the 2013 and 2014 growing seasons. Samples were collected from fields in six different soil regions (1 - Hoytville, 2 - Blount, 3 - Miamian, 4 - Bennington, 5 – Clermont, and 6 - Eden).
Fig. 2.7. Estimated variance parameters (dots) and 95% confidence intervals (error bars) at the county level for frequency of nine genera of plant-parasitic nematodes (*Helicotylenchus* (spiral), *Pratylenchus* (lesion), *Hoplolaimus* (lance), *Xiphinema* (dagger), *Tylenchorhynchus* (stunt), *Paratylenchus* (pin), *Criconemella* (ring), *Paratrichodorus* (stubby-root), and *Heterodera* (cyst), along with several genera in the subfamily Tylenchinae grouped together as “tylenchid” in Ohio corn fields sampled between growth stages V3 and V6 during the 2013 and 2014 growing seasons.
CHAPTER 3: Influence of Soil Region, Cropping Practices, and Soil Properties on the Presence and Abundance of Plant-Parasitic Nematodes in Corn Fields in Ohio

INTRODUCTION

Prior to a survey conducted by Simon et al. (Chapter 1) in 2013 and 2014, very little was known about the population structure of plant-parasitic nematodes in corn fields in Ohio. Samples were collected from 425 fields in that study, and ten commonly occurring morphological groups of plant-parasitic nematodes, namely spiral (*Helicotylenchus* spp.), lesion (*Pratylenchus* spp.), lance (*Hoplolaimus* spp.), dagger (*Xiphinema* spp.), stunt (*Tylenchorhynchus* spp.), pin (*Paratylenchus* spp.), ring (*Criconemella* spp.), stubby-root (*Paratrichodorus* spp.), and cyst (*Heterodera* spp.), along with several genera in the subfamily Tylenchinae grouped together as “tylenchids”, were identified. However, the frequency and abundance of these nematodes varied among soil regions, counties within soil regions, and fields within counties. For instance spiral and lesion were found in almost every field sampled; lance, stunt, and pin in approximately 50% of the fields; and ring, stubby-root, and cyst in less than 13% of the fields. In addition, the abundance of all genera varied considerably among
fields, with maximum individual-field population densities ranged from 26 to 1,164 nematodes/100 cm$^3$ soil, depending on the genus.

Results from the Ohio survey in terms of the most frequently found plant-parasitic nematodes and field-to-field variation in nematode presence and abundance were consistent with results from other studies conducted in the US Corn Belt. For instance, Tylka et al. (2011a) and Niblack and Lopez (personal communication) also reported that spiral and lesion nematodes were among the most frequently found genera in Iowa and Illinois, respectively, and the presence and population densities of these and other genera varied among fields. Such field-to-field variability and even within-field variability is not uncommon for plant-parasitic nematodes (Norton and Hoffman 1974), and numerous attempts were made to determine factors responsible for or contributing to such high variability. Crop rotation, tillage, soil pH, and soil texture have all been identified as factors associated with nematode population density and diversity (Alby et al. 1983, Edwards et al. 1988, Govaerts et al. 2006, Parmelee and Alston 1986). However, the specific effects of these factors vary among nematode genera and are influenced by interactions with other factors.

Govaerts et al. (2006) reported that population densities of *Pratylenchus thornei* were higher in fields under continuous corn than under corn-wheat rotation, and higher in conventional tillage fields when compared to no-till fields. They also showed that nematode populations increased significantly under conventional tillage with continuous corn, but decreased under no-till with rotation. Similarly, Edwards et al. (1988) reported that population densities of
stunt nematodes in a continuous corn cropping system were significantly higher with conventional tillage than with strip tillage or no-till. Norton and Hoffmann (1974) demonstrated significant positive correlations between nematode population density and soil pH in Iowa. *Xiphinema chambersi* was found only in soils with pH between 4.5 and 6.4, while high populations of *Helicotylenchus platyurus*, *H. pseudorobustus* and *Xiphinema americanum* occurred in soils with pH above 6.0. In a separate study, a positive correlation was also observed between pH and *Tylenchorhynchus maximus* for pH ranging from 5.0 to 6.5 (Schmitt 1969).

Soil texture has also been shown to affect the distribution and population density of plant-parasitic nematodes. However, the association between soil texture and plant-parasitic nematodes population density seems to depend on the genera and species of the nematodes. For instance, Schmitt and Norton (1972) showed higher population densities of *Xiphinema americanum* in silty clay loam when compared to silt loam soil; *Pratylenchus crenatus* was reported to occur in soils ranging from loams and silt-loams in Ohio to light sandy soils in Europe, whereas *Pratylenchus penetrans* was found more frequently on soils with higher sand content (Florini *et al.* 1987). Other studies reported that *Paratylenchus projectus* was favored by light colored silt loam, whereas the population densities of other *Pratylenchus* species were positively correlated with fine silt (Wallace *et al.* 1993). High population densities of *Criconemella* species were found in sandy soils (Wallace *et al.* 1993); low densities of *Tylenchorhynchus, Helicotylenchus* and *Xiphinema* species in loams and sandy
loam soils; higher densities of *Helicotylenchus* spp. in clays and silty clay soils (Norton *et al.* 1970), and high densities of *Tylenchorhynchus* species in coarse silt or dark colored silty clay loam (Ferris and Bernard 1971).

Based on observed associations between soil physical properties, Norton and Hoffmann (1974) hypothesized that soil pH would be useful for predicting where nematodes are likely to occur. However, lacking form most of the aforementioned studies on associations among cropping practices, soil proprieties, and nematode were formal quantitative estimates of the nature, form and strength of the associations. Such estimates are important for predicating or assessing the risk of nematodes being present or exceeding critical population levels. Using data from the survey described in chapter 1, logistic regression modeling approaches were used to formally quantify and characterized associations between soil properties, cropping practices, soil regions and plant-parasitic nematode presence and abundance in Ohio corn fields. First, binary logistic regression models were fitted to the nematode frequency data to estimate the odds of each genus (or group of genera in the case of tylenchid) being present, as influenced by the covariates, then ordinal logistic regression models were fitted to the abundance data to estimate the odds (and probabilities) of lance, lesion, spiral and pin nematode populations exceeding critical damage thresholds. Finding from this study would be useful for developing future nematode sampling protocols and for assessing the risk associated with nematodes in corn fields in Ohio.
The null hypothesis for the current study states that nematode population and density will not vary with soil type and will not be influenced by cropping history. Sandy soils will likely have similar nematode population when compared to high silt or clay content soils, and for any given soil type, fields under continuous corn will likely have similar populations with fields under crop rotation away from corn.

**MATERIALS AND METHODS**

**Sampling, extraction and identification of plant-parasitic nematodes.**

During the 2013 and 2014 growing seasons, 425 corn fields were sampled and plant-parasitic nematodes were extracted and identified as described in Chapter 1. In brief, the state was divided into six strata defined by naturally-occurring soil regions located in the western half of the state, and soil samples were collected from 15 to 16 randomly selected fields in each of 4 to 5 counties within each soil region. A 2.5 cm diameter soil probe (MODEL LS, Oakfield Apparatus and Fond du Lac) was used to collect up to 20 soil cores at a depth of 30 to 50 cm along an N-shaped transect across each of the selected fields. Samples from the same field were pooled into a composite sample from which subsampled were taken for nematode extraction using the sieving, decanting and centrifugal sucrose floatation technique (Jenkins 1964). Plant-parasitic nematodes were enumerated and identified to genus with the aid of a stereoscopic and compound microscope and a pictorial key based on body shape and size, stylet shape and size, esophagus and intestine orientation, amphid opening, tail shape, metacorpus
size, vulva position, stylet knob shape and size, shape when at rest, head
description and several other measures of gross body morphology (Mai and
Mullin 1996).

At the time of sampling, notes were taken of cropping practices such as
tillage and crop rotation. Samples were collected from 111 no-till fields, 99 from
fields under vertical or minimum tillage, and 215 fields under conventional tillage.
In terms of cropping sequence, samples were collected from 83 fields under
continuous corn (five or more years of corn-after-corn), 220 under corn-soybean
rotation (no more than two consecutive years in the last five years with any one
of the two crops), and 122 under corn-soybean-wheat rotation (all three crops
planted in the field at least once in the last five years). Sampled fields were
almost evenly distributed across soil region 1 (Hoytville), 2 (Blount), 3 (Miamian),
4 (Bennington), 5 (Clermont), and 6 (Eden), with 122 fields in region 1 and 2, 228
in regions 3-5 and 75 in region 6.

**Soil physical and chemical properties.** *Particle size determination.* A
subsample of the soil from each field was collected for soil analysis. Soil texture
was determined using the pipette method. Individual soil samples were air dried,
passed through an electrical grinding unit, and then through a 2-mm-mesh sieve.
Particle size analysis was done in batches of 24, consisting of 22 soil samples, 1
standard sample and 1 solution blank. Ten grams of each soil sample was
placed into a 450-mL square sedimentation bottle and 10 mL of dispersing
solution (sodium hexametaphosphate and sodium carbonate) was added. Bottles
were then filled to the halfway mark with deionized water, sealed with rubber
stoppers, and placed on a horizontal reciprocating shaker overnight at 120 oscillations per minute. After shaking, the rubber stoppers were removed and soil adhering to the stopper or bottle neck was washed back into bottles. Each bottle was then placed on a torsion balance and deionized water was added until the suspension weighed 410 g. An electric mixer was then used to stir the content of each bottle for approximately 30 seconds and a timer was used to determine settling time for each bottle. The bottles were removed from the mixer at 1-minute intervals and a timer was started after bottle no.1 was removed. After 211.54 minutes, an automatic pipette was placed at a 5-cm depth in bottle no. 1 and a portion of the solution was pipetted and placed into a crucible. This was done for the remaining bottles at 1 minute intervals. The crucibles were then placed on a crucible rack and dried at 105°C for 24 hr in an oven. Once crucibles were dried, the rack was placed into a desiccator until cooled to room temperature before recording the crucible + clay weight.

Sand was separated from the remaining sample suspension by pouring the entire suspension through a 300 mm-mesh sieve. The sand was carefully rinsed into a beaker and a portion of the supernatant was carefully decanted after the sand within the beaker settled. Beakers were then dried in an oven at 105°C for 24 hr. Beaker + sand weights were then determined after removing beakers from drying oven and allowed to cool to room temperature. After calculations for determining percent clay, sand and silt; a soil textural triangle was used to determine soil type.
Soil pH and Electrical Conductivity. A 1:1 soil: deionized water solution was placed into a plastic cup and allowed to stand for 30 minutes after stirring for 10 seconds. The pH meter was calibrated using pH 4 and pH 7 buffer solutions. The pH meter electrode was then inserted directly into the soil suspension and the meter was allowed to stabilize before reading pH. The electrode was rinsed with deionized water after each sample. The EC meter was calibrated by adjusting the temperature correction on the conductance meter to match the standard solution EC value. After taking the pH reading, a similar volume of deionized water was added to the soil suspension. The supernatant was then poured into a 15 mL falcon tube and the EC meter electrode was inserted directly into the solution. The meter was allowed to stabilize before reading conductance in micro Siemens per centimeter (µS/cm). The electrode was rinsed with deionized water between each sample.

Data Analysis. Odds and Odds Ratio nematodes being present or exceeding critical thresholds. Odds refers to the number of times an event occurs (for instance, nematodes of a certain genus being present) relative to the number of times it does not (nematodes of that genus not being detected). In other words, the odds of finding nematodes of a certain genus (lance for instance) can be estimated as the ratio of the number of samples testing positive to the number of samples testing negative. For instance, expressed in terms of probabilities, the odds of finding the lance nematode can be estimated as:

\[ O = \frac{p}{1 - p} \] (1)
where \( O \) = odds, \( p \) is the probability of finding the lance nematode and \( 1 - p \) is the probability of not finding it.

The odds ratio, as the name suggests, is computed as the ratio of two odds. Again, using the lance nematode as an example, one can estimate the odds of this nematode being present in fields under conservation tillage (defined here as no-till, minimum tillage or vertical tillage) relative to the odds of it being present in fields under conventional tillage. Based on the 2 x 2 contingency table (Table 3.1), the lance nematode was present in 203 of the 425 fields sampled, 112 under conservation tillage and 91 under conventional tillage. Conversely, this genus was not detected (absent) in 98 fields under conservation tillage and 124 under conventional tillage. Therefore, the odds of finding the lance nematode in fields under conservation and conventional tillage would be 1.14 (112/98) and 0.73 (91/124), respectively, and the odds of finding this genus in fields under conservation tillage relative to fields under conventional tillage would be 1.55 (1.14/0.73). This means that lance nematode was 1.55 times (or 55%) more likely to be present in fields under conservation tillage relative to fields under conventional tillage.

Modeling associations among the presence of plant-parasitic nematodes, soil characteristics and cropping practices. The association between tillage (or other factors such as cropping sequence, soil region, soil texture etc) and nematode presence (or abundance) can be formally modeled and the odds and odds ratios estimate through binary logistic regression with \( k \) predictor or explanatory variables as:
\[
\ln \frac{p}{1-p} = \alpha + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k
\]  

where \( \ln \frac{p}{1-p} \) is the log of the odds of finding the nematode, also known as the log-odds, logit, or the link function, \( p \) is the probability of the nematode genus in question being present, \( \alpha \) and \( \beta_1 \) to \( \beta_k \) are model parameters, and \( x_1 \) to \( x_k \) are explanatory variables such as tillage, cropping sequence, soil region and soil pH.

For each of the 10 genera of plant-parasitic nematode detected, each field was assigned a code of 1 if the nematode genus in question was present and 0 if it was absent. Tillage was assigned codes of 1 (conservation tillage = no-till, vertical tillage, or minimum tillage) and 2 (conventional tillage); cropping sequence was coded as 1, 2, and 3 for continuous corn, corn-soybean rotation, and corn-soybean-wheat rotation, respectively, and soil regions 1-6 were assigned corresponding codes of 1, 2, 3, 4, 5 and 6. Soil texture (silt content), electrical conductivity, and pH were used as continuous explanatory variables. Separate models were fitted for each nematode genus using PROC LOGISTIC of SAS (Allison 1999, Derr 2013, Paul and Munkvold 2004), with conventional tillage (tillage factor 2), corn-soybean-wheat (cropping sequence factor 3), and soil region 6 as the baseline or reference factor levels for tillage, cropping sequence, and soil region, respectively. Therefore odds ratios were estimated for each factor relative to their respective reference. The initial model was fitted with all predictor variables, and then the backward stepwise variable selection option was used in the model statement to eliminate non-significant variables (\( P > 0.10 \)).

*Modeling the effects of soil characteristics and cropping practices on the odds of lesion, spiral, lance, and pin nematodes exceeding critical thresholds.*
The logistic regression model was refitted to the data to estimate the probability (and consequently the odds) of four of the most frequently encountered nematodes, lance, lesion, spiral and pin, exceeding critical thresholds. For each of the four nematode genera, the abundance (nematodes per 100 cm³ of soil) in each field was assigned to one of three classes, 0, 1 and 2, representing the risk of the damage each is capable of causing based on population density. Level 0 represents no risk, 1 low risk, and 2 moderate-to-high risk (Table 3.2).

The proportional odds (PO) logistic regression model, based on cumulative probabilities, was used to model the relationship between soil characteristics, cropping practices and population density classes of lesion, spiral, lance, and pin nematodes. For a dependent variable (Y, nematode risk class in this case) with k levels, the model can be stated as:

$$Pr[Y \geq j / X] = \frac{1}{1 + e^{-(\alpha_j + \beta X)}}$$ (3)

where \(j\) is the cutoff level of \(Y\), \(X\) is the predictor or explanatory variable, \(\alpha_j\) and \(\beta\) are model parameters. For each cutoff level of \(Y\), the model is an ordinal logistic model for \(Y \geq j\) and is interpreted as the probability of a given response being greater than or equal to the cutoff, given the set of predictor variables. Separate models were fitted to the data for each nematode in PROC LOGISTIC of SAS, using soil silt content, pH, and electrical conductivity as continuous predictor variables, and tillage practice (conventional and conservation), cropping sequence (continuous corn, corn-soybean rotation, and cone-soybean-wheat rotation) and soil region (Regions 1-6) as categorical predictors. Categorical variables were coded and references were selected as described above. As was
the case with the binary logistic regression models, the PO models were initially fitted with all predictors, and then the backward stepwise variable selection option was used to eliminate non-significant \( P > 0.10 \) predictors.

**RESULTS**

**Presence and population density of plant-parasitic nematodes.** Soil samples were collected from 425 fields in 28 counties covering six soil regions. Most of the samples were from silt-loam soil (260), and fields under corn-soybean rotation (220) and conventional tillage (215) (Fig. 3.1). The most frequently encountered plant-parasitic nematodes were spiral (*Helicotylenchus* spp.), lesion (*Pratylenchus* spp.), pin (*Paratylenchus* spp.), and lance (*Hoplolaimus* spp.), occurring in 94, 80, 57, and 48% of the fields, respectively. Other plant-parasitic nematodes detected were those grouped as “tylenchids”, stunt (*Tylenchorhynchus* spp.) and dagger (*Xiphinema* spp.), in 96, 48, and 37% of the fields, respectively, and stubby-root (*Paratrichodorus* spp.), cyst (*Heterodera* spp.) and ring (*Criconemella* spp.) at frequencies below 15%. The latter six groups occurred at population densities ranging from 0 to 218, 0 to 240, 0 to 26, 0 to 56, 0 to 58, and 0 to 82 nematodes/100 cm\(^3\) of soil.

Of the most frequently occurring genera, the population density (referred to here as abundance) of the spiral nematode ranged from 0 to 990 nematodes/100 cm\(^3\) of soil, and from 0 to 300, 0 to 106, and 0 to 1,164 nematodes/100 cm\(^3\) of soil for lesion, lance, and pin nematodes, respectively. Averaged across fields, the spiral nematode was the most abundant, while the
lesion and lance nematodes were the least abundant in all soil regions, cropping rotational programs, and tillage systems (Fig. 3.2). For the spiral nematode, the mean abundance was higher in continuous corn than in corn-soybean or corn-soybean-wheat rotations. Contrastingly, for the pin nematode, the population density was higher in corn rotated with soybean or soybean and wheat than in continuous corn (Fig. 3.2A). Among the tillage systems, the spiral nematode was more abundant in conservation (vertical and minimum) than in no-till or conventional tillage systems, while the abundance of the other three genera was consistent across tillage systems (Fig. 3.2B). The spiral nematode was more abundant in soil Regions 1, 2, 3 and 4 than in 5 and 6; pin was more abundant in Regions 2 and 4 than in 1, 3, 5 and 6, whereas lance and lesion nematodes had similar population densities in the six soil regions (Fig. 3.2C).

With the exception of the lesion nematode, which was not detected in any of the 16 fields sampled in Greene County, all four genera of plant-parasitic nematodes were found in at least one field in the 28 counties surveyed (Fig. 3.3). However, the population density of each genus varied considerably among fields and among counties. In terms of their potential to cause damage to corn, populations of the four nematode genera ranged from not-detected to very severe risk (Table 3.2), however, more than 80% of the fields had populations considered to be below moderate risk threshold levels. Averaged across fields, the spiral nematode was detected at non-significant risk levels in 12 counties, minor risk levels in 13 counties, and at moderate risk levels in 3 counties (Fig. 3.3A); the lesion nematode was detected at non-significant risk levels in 16
counties, minor risk levels in 9 counties, and moderate risk levels in 3 counties (Fig. 3.3B); the lance nematode population density was below 1/100 cm$^3$ of soil in 2 counties (consisted as non-detected), at non-significant risk levels in 14 counties, and at low risk levels in 12 counties (Fig. 3.3C); and the pin nematode was detected at non-significant, low and moderate risk levels in 23, 3, and 2 counties, respectively (Fig. 3.3D).

**Associations among the presence of plant-parasitic nematodes, soil characteristics and cropping practices.** The observed presence to absence ratio of plant-parasitic nematode in the 425 fields varied among the genera (Fig. 3.4). Negative fields represented 6, 4, and 20% of the total for spiral, tylenchid, and lesions nematodes, respectively (Fig. 3.4A, B, and D), compared to 89, 95, and 87% for cyst, ring, and stubby-root nematodes, respectively (Fig. 3.4C, I, and J). The presence to absence ratio was close to 50:50 for the lance, dagger, stunt and pin nematodes (Fig. 3.4E, F, G, and H). Based on results from the binary logistic regression analyses (Table 3.2), the effects of cropping sequence, tillage, soil region, silt content, pH and electrical conductivity (EC) on the log-odds of the nematodes being present (heretofore referred to simple as the presence) varied among nematode genera. Cropping sequence had a significant effect on the presence of spiral, lance and pin nematodes, but not of the other seven genera (at $P = 0.10$). Tillage was associated with the presence of three of the 10 genera (lance, stunt and stubby), whereas soil region had a significant effect on six genera, namely lesion, dagger, stunt, pin, ring, and stubby-root. The continuous covariates, silt content, pH, and EC, each significantly affected the presence of
tylenchid and stunt, cyst and lesion, and tylenchid and lesion, respectively. The presence of spiral, lance, and stunt nematodes was associated with three of the factors evaluated, whereas the presence of cyst, dagger, and ring nematodes was associated with one of the covariates evaluated (Table 3.3). The presence of all other genera was associated with two factors.

Odds ratios (OR) are depicted in Figure 3.5A, B, and C for the association between cropping sequence and spiral, lance and pin nematodes, respectively, with corn-soybean-wheat rotation as the reference crop-rotation system for comparison. An OR of 1 (the reference line in Fig. 3.5) signifies equal odds of finding the nematode in question in fields under the two rotational systems being compared. Odds ratios > 1 mean that the odds of finding the nematode is lower in the reference rotational system (the denominator of the ratio), whereas odds ratios < 1 mean the opposite. For instance, the odds of finding spiral, lance, and pin nematodes in a field under corn-soybean rotation (coded as 2) were respectively 0.602, 0.651, and 0.976 times the odds of finding them in a field under corn-soybean-wheat rotation (coded as 3) (Fig. 3.5). This means that a field under the three-crop rotation was 1.66 (1/0.602) and 1.54 (1/0.651) times more likely to have spiral and lance nematodes, respectively, that a field under corn-soybean rotation (Fig. 3.5A and B), but the two rotational systems were equally likely to have the pin nematode (OR = 0.976, not significantly different from 1). Similarly, the odds of finding spiral, lance, pin nematodes in fields under corn-soybean-wheat rotation were greater than the odds of finding them in no-till fields (OR < 1 for the 1 vs 3 comparisons in Fig. 3.5A, B and C), and the odds of
finding spiral and pin nematodes in corn-soybean rotation fields were greater than the odds of finding them in no-till fields (1 vs 2 in Fig. 3.5A and C, with 2 as the reference).

Figure 3.5D shows odds ratios for the effect of tillage on lance, stunt, and stubby-root nematodes. The odds of finding lance and stubby-root nematodes in a field under conservation tillage (coded as 1 in Fig. 3.5D) were respectively 1.79 and 1.89 times the odds of finding them in a field under conventional tillage (the reference, coded as 2 in Fig. 3.5D). In other words, lance and stubby-roots were almost twice as likely to be found in conservation tillage fields then in conventional tillage fields. However, the opposite was true for stunt nematodes, which was 1.6 times more likely to be present in fields under conventional tillage than under conservation tillage (OR < 1 [0.614] in Fig. 3.5D).

The association between soil region and plant-parasitic nematodes depended on the genus of the nematode and the specific soil region. With the exception of the dagger and ring nematodes (Fig. 3.6B and E), the odds of plant-parasitic nematodes being present in soil regions 1-5 tended to be greater than or equal to the odds of them being present in soil region 6 (the reference; Fig. 3.6). Dagger and ring nematodes were generally more likely to be present in soil region 6 than any of the other soil region (OR < 1 in Fig. 3.6D and E). However, lance, stunt, pin, stubby-root, and spiral nematodes were more or equally likely to be present in soil regions 1-5 as in soil region 6 (OR ≥ 1 in Fig. 3.6A, C, D, F and G). The exceptions were for the lance nematode in soil region 1 (Fig. 3.6A) and stunt nematode in soil regions 2 and 4 (Fig. 3.6C).
There were significant positive relationships between log-odds and soil pH for spiral and cyst nematodes. For both genera, for every unit increase in pH, there was a 0.73 unit increase in the log-odds of cyst and spiral nematodes being present (all other factors in the model being held constant). In other words, for every unit increase in pH, the odds of spiral and cyst nematodes being present were almost twice (OR = 2.10 = e^{0.73}) the odds on them being absent (data not shown). However, an inverse relationship was observed between soil pH and log-odds for the lesion nematode. The slope of the log-odds/pH relationship was -0.46 for lesion nematode, meeting that for every unit increase in pH there was a 0.46 reduction in the log-odds of this nematode being present. Lesion nematode was about 1.6 times more likely to be absent than present as pH increased. Similar trends were observed for relationships between log-odds for the presence of tylenchid and stunt nematodes and silt content of the soil, with the log-odds for the former group increasing as the silt content increased (slope = 0.04), whereas the log-odds for the latter decreased (slope = -0.02). Soil electrical conductivity had significant positive relationships with log-odds (P < 0.10) for tylenchid and lesion nematodes (slopes of 0.02 and 0.005, respectively).

**Effects of soil characteristics and cropping practices on the odds of lesion, spiral, lance, and pin nematodes populations exceeding critical thresholds.** The number of fields with nematodes in the none, low, and moderate-high risk categories varied among the four genera (Fig. 3.7). For all four genera, most of the fields had populations densities considered to be of negligible or low risk. For the spiral, lesion, lance, and pin nematodes, 62, 65, 74,
and 86% of the fields, respectively, had population below the moderate risk threshold (Fig. 3.7 and Table 3.2). The statistical significant of specific soil characteristics and cropping practices on the odds of these nematodes being assigned to the moderate-very severe risk category (2, the reference risk category) varied among genera (Table 3.4). For instance, the odds of spiral nematode being assigned to category 2 was influenced by soil region and soil pH, while the odds of the lesion nematode being assigned to this same category was influences by the silt content, pH, and electrical conductivity of the soil. For the lance and pin nematodes, tillage, soil region and silt content, and cropping sequence, soil region, soil pH and electrical conductivity, respectively, significantly affected the odds of these genera exceeding their respective moderate-very severe risk thresholds (Tables 3.2 and 3.4).

The odds ratios in Table 3.5 can be interpreted in terms of odds of being in risk category 2 (moderate to very severe risk) versus the odds of being in categories 1 or 0 and in terms of the odds of being in categories 2 or 1 versus category 0. For instance, for the spiral nematode, soil region 1 had 3.9 times the odds of having a population density in the high risk category than soil region 6, both for risk category 2 versus 1 or 0 and category 2 or 1 versus 0. This means that the soil region 1 was more likely to have spiral nematode populations at the moderate-very severe risk level than soil region 6. The same was true for soil regions 2, 3, 4, and 5, with the odds of this nematode reaching moderate-very severe levels in those soil regions being 2.6 to 4.5 times greater than the odds in soil region 6 (Table 3.5). Similarly, for the pin nematode, soil regions 2, 3, and 4
were more likely to have populations at the moderate-very severe risk levels than soil region 6, but soil regions 1 and 5 were just as likely as soil region 6 to have pin nematode population density in risk category 2. Very similar trends were also observed for the lance nematode, with the only exception being for soil region 1 which was less likely to have lance nematode population in moderate-very severe risk class than soil region 6.

Table 3.5 also shows odds ratios for the effects of tillage on lance nematode population density and cropping sequence on the population density of the pin nematode. Tillage system 1 was two times more likely to have lance nematode populations in the moderate-very severe risk category than tillage system 2 (OR = 2.03). For the pin nematode, abundant in the moderate-very severe risk category was more likely in fields under corn-soybean and corn-soybean-wheat rotation (coded as 2 and 3, respectively, in Table 3.5) than in continuous corn fields, the reference cropping sequence (OR < 1 for the 1 vs. 2 and 1 vs. 3 comparison, $P < 0.05$). However, the odds of pin nematode abundance being in the moderate-very severe risk category in fields under corn-soybean and corn-soybean-wheat rotation were equality likely (OR = 0.97 for 2 vs. 3, $P > 0.10$ in Table 3.5).

Soil pH had a significant positive relationship with log-odds for the spiral nematode, but a negative relationship with log-odds for the lesion and pin nematodes. For every unit increase in pH, there was a 0.36 unit increase in the log-odds for spiral nematode, a 0.49 unit decrease in the log-odds for lesion nematode and a 0.25 unit decrease in the log-odds for the pin nematode (Table
3.5). In other works, as pH increased from 4.0 to 9.6 (with all other factors being held constant) the predicted probability of the spiral nematode populations being in the moderate-very severe risk category (2) increased, but the probability of the lesion and pin nematode populations being in that same risk category decreased (Fig. 3.8).

The effects of silt content and electrical conductivity of the soil on the odds of nematode populations reaching high-risk levels also varied among genera. Slopes for relationships between slit content and log-odds were negative for both the lesion and lance nematodes; whereas the slope for the relationship between electrical conductivity and log-odds was positive for the lesion nematode and negative for the pin nematode (Table 3.5). For both the lesion and lance nematodes, the predicted probability of populations being in the moderate-very severe risk category decreased as the silt content of the soil increased at fixed levels of other covariates (Fig. 3.9 A and B). The predicted probability of populations of the lesion nematode being in the moderate-very severe risk category increased as the electrical conductivity (EC) of the soil increased (Fig. 3.9 C), but the probability of pin nematode populations being in that same risk class decreased with increasing EC (Fig. 3.9 D).

DISCUSSION

In a survey of 425 Ohio corn fields, Simon et al. (Chapter 2) observed considerable variation in the presence and abundance of plant-parasitic nematodes among soil region, counties within soil region, and fields within
counties and soil regions. Such variability is quite common for nematodes (as well as other soil inhabiting or soil-borne organisms) and could be attributed to several factors, including soil properties, cropping practices, and edaphoclimatic conditions, among others. Logistic regression models were fitted to the survey data to more formally quantify association among these factors and nematode presence and abundance. Binary logistic regression (Allison 1999), an approach appropriate for modeling yes-no type responses (more formally referred to event-not event), was used to quantify the effects of soil region, crop rotation and tillage practices, and soil physical and chemical properties on the presence of the plant-parasitic nematode genera found in the survey. In addition, ordinal logistic regression, an expansion of the binary approach to model responses with three or more ordered categories, was used to quantify associations among the aforementioned factors and ordered categories of nematode population density. In the latter case, the ordered categories were defined based on damage thresholds proposed by Tylka et al (2011a) and Niblack (personal communication).

The survey results showed that cropping sequence was associated with the presence of spiral, lance and pin nematodes in Ohio corn fields. However, the other plant-parasitic nematode detected in this study were not affected by cropping sequence. The odds of finding the spiral, lance or pin nematodes in a corn-soybean or a corn-soybean-wheat rotation were greater than finding them in a field with continuous corn. Furthermore, the pin nematode was more likely to be found at moderate-very severe risk population levels in fields under corn-
soybean and corn-soybean-wheat rotation than in a continuous corn field. This could be interpreted to mean that the pin nematode is not only likely to be more present in fields with crop rotation than in continuous corn fields, it is also more likely to exceed damage thresholds in the former than the latter cropping system. In contrast, cropping system did not affect the population density of the lance or spiral nematode, suggesting that these two genera are more likely to be found in fields in rotation than in continuous corn, but when present in the latter system, the population density is likely to be comparable to that of the former cropping system. Others have reported on the influence of cropping system on the population density of plant-parasitic nematodes, with results somewhat contrary to those observed in the current investigation. For instance, in contrast to what we observed, Govaerts et al. (2006) reported that population densities of one species of the lesion nematode, *Pratylenchus thornei*, were higher with continuous corn when compared to corn-wheat rotation, but that the effects of crop rotation on nematode populations may vary with nematode genera and tillage. In agreement with Govaerts et al. (2006), Edwards et al. (1988) found evident of an interaction effect of crop rotation and tillage on the population density of *Tylenchorhynchus*, a genus influenced by tillage, but not cropping sequence in our study. They observed higher population densities in continuous corn with conventional tillage, when compared to continuous corn with no-till or strip tillage.

Results from our analyses suggested that the stunt nematode was more likely be present in fields under conventional tillage than under conservation
tillage. The odds of finding this genus in fields with conventional tillage were approximately twice the odds of finding in no-till, vertical- or minimum-till fields (collectively considered as conservation tillage in this study). However, the lance and stubby-root nematodes were about twice as likely to be found in fields under conservation tillage when compared to fields under conventional tillage. In addition to being more present in conservation tillage systems than in conventional tillage systems, the lance nematode was also likely to be more abundant in the former system than the latter. The odds of this genus reaching moderate-high risk population levels in a conservation tillage system were twice the odds of it reach that same damage threshold in a conventional tillage system. In contrast, population densities of the spiral, pin, and lesion nematodes were consistent across tillage systems, since tillage did not have a significant effect on the odds of these genera reaching high-risk population levels. This is at odds with results from studies conducted by Caveness (1974) and Thomas (1978). In the former study, higher population densities of the lesion nematode were observed in conventionally-tilled plots then in no-till plots, and conversely, higher densities of the spiral nematode (and Meloidogyne, the root-knot nematode) were observed in no-till plots when compared to conventionally-tilled plots. Thomas (1978) however reported higher population densities of both the spiral and lesion nematodes (as well as the dagger nematode) in no-till plots. These differences among studies suggest that as was the case with cropping system, the effect of tillage on plant-parasitic nematode populations may vary with nematode genera and interaction among cropping practices (Govaerts et al.}
Therefore practicing conventional tillage or crop rotation to manage nematodes may not be the ultimate answer. Nevertheless, more specific research can be done to evaluate the presence and abundance of various plant-parasitic nematode genera in different tillage and crop rotation systems.

Soil region had a significant effect on the presence of seven of the nine plant-parasitic nematode genera recovered in the current survey. The association between soil region and nematode presence was significant for the lance, dagger, stunt, pin, ring, stubby-root, and spiral nematodes but not the lesion or cyst nematodes or the tylenchid group of nematodes. The odds of finding the spiral, lance, pin, stunt or stubby-root nematodes in soil regions 1 through soil region 5 were more than or equally likely to the odds of finding them in soil region 6. However, the lance nematode was more likely to be found in soil region 6 than soil region 1. Not only did soil region affect the presence of most of the nematodes investigated in this study, this factor also affected nematode abundance and the odds of populations exceeding established damage thresholds. For instance, the odd of finding spiral nematode populations at the moderate-severe risk level was greater in soil region 1 (Hoytville), 2 (Blount), 3 (Miamian), 4 (Bennington), and 5 (Clermont) than in soil region 6 (Eden). Similarly, pin nematode population in a moderate-severe-risk category in soil region 2 (Blount), 3 (Miamian) and 4 (Bennington) was more likely than in soil region 6 (Eden). However, it was less likely to have populations of the lance
nematode in a moderate-severe risk category in soil region 1 when compared to soil region 6.

All of the soil regions evaluated had soil series that were typically silt loams, with the exception of Hoytville and Eden, which were typically clay loam or silty clay loam soils, respectively. Because, the odds of finding the spiral or pin nematodes in the Hoytville, Blount, Miamian, Bennington, and Clermont soil series were greater than in the Eden soil series. It may be reasonable to suggest that it is more likely to find these nematodes in a silt loam soil, instead of a silty clay loam soil. Moreover, the odds of finding the dagger and ring nematodes in the Eden soil series were greater than finding them in the other soil series, suggesting that the dagger and ring nematodes may be favored by silty clay loam soils in Ohio. Similarly, higher population densities of *Xiphinema americanum* were found in silty clay loam when compared to silt loam soils in common lilac in Iowa (Schmitt and Norton 1972). Moreover, other studies showed that lower population densities of *Xiphinema* were found in loams and sandy loam soils, higher population densities of *Helicotylenchus* were found in clays and silty clay soils (Norton *et al.* 1970), and high densities of *Criconemella* were found in sandy soils (Wallace *et al.* 1996). This may be indicative of the fact that the spiral and dagger nematodes may be favored by heavier fine textured soils when compared to coarser texture soils. Wallace (1971) explained the effects of soil texture of nematodes based on ratio of nematode size to soil pore and particular size, stating that “the relation between nematode movement and soil texture is a function of the ratio of nematode size to pore and particle size. As the length and
When the effect of soil texture on the abundance of lesion, lance, spiral, and pin nematodes was more directly investigated using silt content as a continuous covariate, silt content only had a significant effect on populations of the lesion and lance nematodes, but not the pin or spiral nematodes. Both plant-parasitic nematode genera showed a negative relationship between soil silt content and the probability of population density in a moderate-severe risk level category. This could be interpreted to mean that as soil silt content increased, high-risk populations of lesion and lance nematodes decreased. These effects (or lack of effects) of silt content on nematode abundance were somewhat contrary to what was discussed above based on soil region. This apparent disparity could be due to the fact that other soil-region-specific factors likely had greater effect on nematode abundance than the silt content. Brodie (1976) also reported high population densities of *Pratylenchus brachyurus* in soil with relatively low silt content (6% silt), which seems to suggest that the lesion nematode may be less abundant in soils with relatively high silt content. However, as was the case with most of the covariates discussed thus far, the effect of soil texture on plant-parasitic nematode presence and abundance may vary among genera and species with genera, since one particular species of *Pratylenchus, Pratylenchus crenatus*, was detected in soils ranging from loams to silt loams in Ohio (Brown *et al.* 1980) to light sandy soils in Europe (Loof 1978), and another, *Pratylenchus*
*penetrans*, was found more frequently on soils with higher sand content (Florini *et al.* 1987).

Studies demonstrated that soil physical characteristics such as soil pH and soil texture can affect the occurrence and population dynamics of nematodes (Wallace 1963). In our study, soil pH was associated with the presence of the spiral, lesion and cysts nematodes. However, there was a significant negative relationship between lesion nematode population at a moderate-severe risk level and soil pH, and conversely, a significant positive relationship between soil pH and spiral nematode population at a moderate-severe risk level. This indicates that the predicted probability of lesion nematode populations at the moderate-severe risk level would decrease as soil pH increases from 4.0 to 9.6 and the opposite would be true for the spiral nematode. Studies in Iowa also showed significant positive correlations between spiral nematode populations and soil pH (Norton and Hoffman 1974), and a positive correlation was also observed between *Tylenchorhynchus* and soil pH (Schmitt 1969).

Soil EC significantly affected the presence of *Pratylenchus* and *Paratylenchus*. However, there was a significant positive relationship between soil EC and lesion nematode population at the moderate-severe risk level, conversely, a negative relationship was observed between soil EC and populations of pin nematodes at the same risk category. This suggests that the lesion nematode may be better able to thrive in soils with higher salinity levels than the pin nematode.
In this study, we were able to quantify relationships among cropping practices, soil regions, and plant-parasitic nematode presences and abundance. This knowledge will help us to better understand and manage factors effecting nematode presence and build-up in corn fields in Ohio. However, given the observed different in nematode responses to the different factors evaluated, no single manage strategy will be equally effective against all genera. An approach that may be effective at reducing one genus may increase another. Moreover, as alluded to earlier, difference species of the same genus may respond differently to any given factor or set of factors. Therefore further research would be needed before one can truly determine how the knowledge generated here could be used to manage plant-parasitic nematodes. In fact, information from this study on nematode presence, abundance, and variability in different soil regions, county within soil regions, and field within counties and soil region, and factors such as tillage, cropping sequence, and soil properties affecting presence and abundance will be invaluable to the establishment of future studies, particularly on-farm research. For instance, this information could be used to develop sampling protocols and to identify on-farm trial locations.
REFERENCES


Table 3.1 Contingence table of the association between the presence/absence of lance nematode and tillage practice

<table>
<thead>
<tr>
<th></th>
<th>Present</th>
<th>Absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conservationa</td>
<td>112</td>
<td>98</td>
<td>210</td>
</tr>
<tr>
<td>Conventional</td>
<td>91</td>
<td>124</td>
<td>215</td>
</tr>
<tr>
<td>Total</td>
<td>203</td>
<td>222</td>
<td>425</td>
</tr>
</tbody>
</table>

*a Conservation tillage represents the combination of no-till, and minimum and vertical tillage.*
Table 3.2 Four genera of plant-parasitic nematodes and risk categories for the potential damage they are capable of causing in corn at different population densities

<table>
<thead>
<tr>
<th>Risk Category</th>
<th>Population density&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Risk&lt;sup&gt;b&lt;/sup&gt; Class</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spiral</td>
<td>Lesion</td>
</tr>
<tr>
<td>Not detected</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Not significant</td>
<td>1 to 75</td>
<td>1 to 10</td>
</tr>
<tr>
<td>Minor risk</td>
<td>75 to 150</td>
<td>11 to 25</td>
</tr>
<tr>
<td>Moderate risk</td>
<td>151 to 33</td>
<td>26 to 50</td>
</tr>
<tr>
<td>Severe risk</td>
<td>301 to 500</td>
<td>51 to 100</td>
</tr>
<tr>
<td>Very severe risk</td>
<td>&gt; 500</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

<sup>a</sup>Nematodes per 100 cm<sup>3</sup> of soil.

<sup>b</sup>Risk categories or combination of categories reclassified as 1 = no risk, 2 = low risk and 3 moderate-to-high risk.
Table 3.3 Probability values (level of significant) from binary logistic regression analyses of the effects of different soil characteristics and cropping practices on the presence of ten plant-parasitic nematode genera (or group of genera in the case of tylenchid) based on a survey of corn fields in Ohio in 2013 and 2014.

<table>
<thead>
<tr>
<th>Explanatory Variables&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Spiral</th>
<th>tylenchid</th>
<th>Cyst</th>
<th>Lesion</th>
<th>Lance</th>
<th>Dagger</th>
<th>Stunt</th>
<th>Pin</th>
<th>Ring</th>
<th>Stubby</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cropping sequence</td>
<td>0.036</td>
<td>ns</td>
<td>Ns</td>
<td>0.083</td>
<td>Ns</td>
<td>Ns</td>
<td>0.041</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Tillage</td>
<td>ns</td>
<td>ns</td>
<td>Ns</td>
<td>Ns</td>
<td>0.009</td>
<td>Ns</td>
<td>0.047</td>
<td>ns</td>
<td>ns</td>
<td>0.047</td>
</tr>
<tr>
<td>Soil region</td>
<td>0.039</td>
<td>ns</td>
<td>Ns</td>
<td>Ns</td>
<td>&lt;0.001</td>
<td>0.022</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.037</td>
</tr>
<tr>
<td>Silt content</td>
<td>ns</td>
<td>0.075</td>
<td>Ns</td>
<td>Ns</td>
<td>ns</td>
<td>Ns</td>
<td>0.057</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Soil pH</td>
<td>&lt;0.001</td>
<td>ns</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>ns</td>
<td>Ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Soil EC</td>
<td>ns</td>
<td>0.032</td>
<td>Ns</td>
<td>0.059</td>
<td>ns</td>
<td>Ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

<sup>a</sup>Cropping sequence includes samples collected from fields under continuous corn (C-C-C), corn-soybean rotation (C-S-R) and corn-soybean-wheat rotation (C-S-W-R). Tillage includes samples collected from fields left without tillage (no-till) or subjected to vertical, minimum tillage, or conventional tillage (includes all standard tillage operations). For the purpose of data analysis, no-till, vertical tillage, and minimum tillage were collectively considered conservation tillage. Soil region includes samples collected from fields in six soil regions (1 = Hoytville, 2 = Blount, 3 = Miamian, 4 = Bennington, 5 = Clermont and 6 = Eden). Silt content = percent silt and EC = electrical conductivity.
Table 3.4 Probability values (level of significant) from proportional odds ordinal logistic regression analyses of the effects of different soil characteristics and cropping practices on the abundance of four of the most frequently occurring genera of plant-parasitic nematode based on a survey of corn fields in Ohio in 2013 and 2014.

<table>
<thead>
<tr>
<th>Explanatory Variables</th>
<th>Plant-parasitic nematodes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spiral</td>
<td>Lesion</td>
<td>Lance</td>
</tr>
<tr>
<td>Cropping sequence</td>
<td>Ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Tillage</td>
<td>Ns</td>
<td>ns</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Soil region</td>
<td>&lt;0.001</td>
<td>ns</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Silt content</td>
<td>Ns</td>
<td>0.051</td>
<td>0.063</td>
</tr>
<tr>
<td>Soil pH</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>ns</td>
</tr>
<tr>
<td>Soil EC</td>
<td>Ns</td>
<td>0.032</td>
<td>ns</td>
</tr>
</tbody>
</table>

*Cropping sequence includes samples collected from fields under continuous corn (C-C-C), corn-soybean rotation (C-S-R) and corn-soybean-wheat rotation (C-S-W-R). Tillage includes samples collected from fields left without tillage (No-till) or subjected to vertical, minimum tillage, or conventional tillage (includes all standard tillage operations). For the purpose of data analysis, no-till, vertical tillage, and minimum tillage are collectively considered conservation tillage. Soil regions includes samples collected from fields in six different soil regions (1 = Hoytville, 2 = Blount, 3 = Miamian, 4 = Bennington, 5 = Clermont and 6 = Eden). Silt content = percent silt and EC = electrical conductivity.
Table 3.5 Odds ratios for categorical variables, regression slopes for continuous covariates, and corresponding statistics from proportional odds ordinal logistic regression analyses of associations among soil characteristics, cropping practices and the abundance of four of the most frequently occurring genera of plant-parasitic nematode based on a survey of corn fields in Ohio in 2013 and 2014

<table>
<thead>
<tr>
<th>Genus</th>
<th>Effect</th>
<th>Estimate</th>
<th>se</th>
<th>CL$_L$</th>
<th>CL$_U$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiral</td>
<td>REGION 1 vs 6</td>
<td>3.90</td>
<td>1.480</td>
<td>1.86</td>
<td>8.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>REGION 2 vs 6</td>
<td>2.55</td>
<td>0.998</td>
<td>1.18</td>
<td>5.49</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>REGION 3 vs 6</td>
<td>3.70</td>
<td>1.291</td>
<td>1.87</td>
<td>7.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>REGION 4 vs 6</td>
<td>4.48</td>
<td>1.561</td>
<td>2.27</td>
<td>8.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>REGION 5 vs 6</td>
<td>3.27</td>
<td>1.141</td>
<td>1.65</td>
<td>6.48</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>0.36</td>
<td>0.112</td>
<td>...</td>
<td>...</td>
<td>0.002</td>
</tr>
<tr>
<td>Lesion</td>
<td>pH</td>
<td>-0.490</td>
<td>0.101</td>
<td>...</td>
<td>...</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>SILT</td>
<td>-0.014</td>
<td>0.007</td>
<td>...</td>
<td>...</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>0.003</td>
<td>0.002</td>
<td>...</td>
<td>...</td>
<td>0.032</td>
</tr>
<tr>
<td>Lance</td>
<td>TILL 1 vs 2</td>
<td>2.03</td>
<td>0.419</td>
<td>1.36</td>
<td>3.04</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>REGION 1 vs 6</td>
<td>0.10</td>
<td>0.054</td>
<td>0.04</td>
<td>0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>REGION 2 vs 6</td>
<td>0.72</td>
<td>0.250</td>
<td>0.36</td>
<td>1.42</td>
<td>0.340</td>
</tr>
<tr>
<td></td>
<td>REGION 3 vs 6</td>
<td>2.80</td>
<td>0.886</td>
<td>1.51</td>
<td>5.21</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>REGION 4 vs 6</td>
<td>2.51</td>
<td>0.797</td>
<td>1.35</td>
<td>4.68</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>REGION 5 vs 6</td>
<td>1.10</td>
<td>0.362</td>
<td>0.58</td>
<td>2.10</td>
<td>0.769</td>
</tr>
<tr>
<td></td>
<td>SILT</td>
<td>-0.021</td>
<td>0.011</td>
<td>...</td>
<td>...</td>
<td>0.063</td>
</tr>
<tr>
<td>Pin</td>
<td>CS 1 vs 2</td>
<td>0.48</td>
<td>0.128</td>
<td>0.28</td>
<td>0.81</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>CS 1 vs 3</td>
<td>0.47</td>
<td>0.135</td>
<td>0.26</td>
<td>0.82</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>CS 2 vs 3</td>
<td>0.97</td>
<td>0.215</td>
<td>0.63</td>
<td>1.50</td>
<td>0.905</td>
</tr>
<tr>
<td></td>
<td>REGION 1 vs 6</td>
<td>0.98</td>
<td>0.362</td>
<td>0.47</td>
<td>2.02</td>
<td>0.952</td>
</tr>
<tr>
<td></td>
<td>REGION 2 vs 6</td>
<td>3.16</td>
<td>1.182</td>
<td>1.52</td>
<td>6.58</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>REGION 3 vs 6</td>
<td>2.64</td>
<td>0.859</td>
<td>1.40</td>
<td>5.00</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>REGION 4 vs 6</td>
<td>5.39</td>
<td>1.790</td>
<td>2.81</td>
<td>10.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>REGION 5 vs 6</td>
<td>0.99</td>
<td>0.323</td>
<td>0.52</td>
<td>1.88</td>
<td>0.976</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>-0.254</td>
<td>0.115</td>
<td>...</td>
<td>...</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>-0.003</td>
<td>0.001</td>
<td>...</td>
<td>...</td>
<td>0.024</td>
</tr>
</tbody>
</table>

$^a$Plant-parasitic nematodes genera Helicotylenchus spp. (spiral), Pratylenchus spp. (lesion), Hoplolaimus spp. (lance) and Paratylenchus spp. (pin).

$^b$Effect = Six soil regions (1 = Hoytville, 2 = Blount, 3 = Miamian, 4 = Bennington, 5 = Clermont and 6 = Eden); TILL = tillage practice (1 = conservation tillage [the combination of no-till, and minimum and vertical tillage] and 2 = conventional tillage (includes all standard tillage operations); CS = cropping sequence (1 = continuous corn, 2 = corn-soybean rotation and 3 = corn-soybean-wheat rotation); and soil pH and electrical conductivity (EC).

$^c$Estimate represents odds-ratios (OR) for class variables REGION, TILL and CS, and regression slope for continuous variables pH and EC; se, CL$_L$, and CL$_U$ are standard error and lower and upper limits for the 95% confidence interval around ORs and slopes, and $P$ is the level of significance.
Fig 3.1. Number of soil samples collected from corn fields in Ohio with A, different soil textures, and B, under different crop rotation, and C, tillage practices. C-C-C = continuous corn, C-S-R = corn-soybean rotation and C-S-W = corn-soybean-wheat rotation. Sampled fields were either left without tillage (No-till) or subjected to conservation (vertical or minimum tillage) or conventional tillage.
Fig. 3.2. Mean abundance (nematodes per 100 cm$^3$ of soil) of the four most frequently encountered genera of plant-parasitic nematodes *Helicotylenchus* [spiral], *Pratylenchus* [lesion], *Hoplolaimus* [lance], and *Paratylenchus* [pin] in Ohio corn fields sampled between the V3 and V6 growth stages during the 2013 and 2014 growing seasons. Samples were collected from fields under A, different crop rotation and B, tillage practices in C, different soil regions. C-C-C = continuous corn, C-S-R = corn-soybean rotation and C-S-W = corn-soybean-wheat rotation. Sampled fields were either left without tillage (No-till) or subjected to conservation (vertical or minimum tillage) or conventional tillage (includes all standard tillage operations) in six soil regions (1 = Hoytville, 2 = Blount, 3 = Miamian, 4 = Bennington, 5 = Clermont, and 6 = Eden).
Fig 3.3. Maps of the state of Ohio showing counties in which A, spiral (*Helicotylenchus* spp.), B, lesion (*Pratylenchus* spp.), C, lance (*Hoplolaimus* spp.) and D, pin (*Paratylenchus* spp.) nematodes were detected and the abundance of each genera in terms of nematodes per 100 cm³ soil. Color-coded abundance classes were defined based on the potential of each nematode to cause damage (reduce yield) at different population levels.
Fig. 3.5. Odds ratios (dots) and corresponding 95% confidence intervals (error bar) for the associations between cropping sequence and the presence of A, spiral (Helicotylenchus spp.), B, lance (Hoplolaimus spp.), and C, pin (Paratylenchus spp.) plant-parasitic nematodes, and D, between tillage practice and the presence of lance, stunt (Tylenchorhynchus sp.), and stubby-root (Paratrichodorus sp.) nematodes. Cropping sequence 1, 2 and 3 represent continuous corn, corn-soybean rotation, and corn-soybean-wheat rotation, respectively; and tillage 1 and 2 represent conservation tillage (the combination of no-till, and minimum and vertical tillage) and conventional tillage (includes all standard tillage operations).
Fig. 3.6. Odds ratios (dots) and corresponding 95% confidence intervals (error bar) for associations among soil regions and the presence of plant-parasitic nematodes A, lance (Hoplolaimus spp.), B, dagger (Xiphinema spp.), C, stunt (Tylenchorhynchus spp.), D, pin (Paratylenchus spp.), E, ring (Criconemella spp.), F, stubby-root (Paratriechodorus spp.) and, G, spiral (Helicotylenchus spp.). Soil regions 1, 2, 3, 4, 5, and 6 represent Hoytville, Blount, Miamian, Bennington, Clermont, Eden soil regions, respectively.
Fig. 3.7. Number of fields (samples) with nematode abundance in each of three risk classes based on populations densities of A, spiral (*Helicotylenchus* spp.), B, lesion (*Pratylenchus* spp.), C, lance (*Hoplolaimus* spp.) and D, pin (*Paratylenchus* spp.) nematodes (Table 3.1).
Fig. 3.8. Relationship between soil pH and predicted probabilities of A, spiral, B, lesion, and C, pin nematodes populations being in three risk categories at fixed levels of other covariates used in the fitted models. Risk categories 0, 1, and 2 represent no risk, low risk, and moderate-very severe risk based on population densities in Table 2. REGION = 4 represents soil region 4 (Bennington), EC = soil electrical conductivity, and SC = 2 represents corn-soybean rotation.
Fig. 3.9. Relationship between soil A and B soil silt content (%) and C and D electrical conductivity and predicted probabilities of lesion (A and C), lance (B), and pin (D) nematodes populations being in three risk categories at fixed levels of other covariates used in the fitted models. Risk categories 0, 1, and 2 represent no risk, low risk, and moderate-very severe risk based on population densities in Table 2. REGION = 4 represents soil region 4 (Bennington), EC = soil electrical conductivity, SILT = soil silt content, pH = soil pH, and TILL = 1 represents continuous corn, SC = 2 represents corn-soybean rotation.
BIBLIOGRAPHY


Batista da Silva, M. 2013. Studies on extraction and control of plant-parasitic nematodes on corn. M.S. thesis, Department of Plant Pathology and Microbiology, Iowa State University, Ames.


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www.bayercropscience.us/products/seegrowth/poncho-votivo

www.ctic.purdue.edu/resourcedislay/322/


