Cropping Practices and Soil Properties Associated with Plant-Parasitic Nematodes in Corn Fields in Ohio

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Abstract

Ten morphological groups of plant-parasitic nematodes (spiral, lesion, lance, dagger, stunt, pin, ring, stubby-root, cyst, and miscellaneous tylenchids) were detected in corn fields in Ohio, but the presence and population density of these groups varied among fields. Binary and ordinal logistic regression models were fitted to the data to estimate the odds of each group being present, and the lesion, lance, spiral, and pin nematode population densities being at moderate-high risk levels based on soil region, cropping sequence, tillage, and soil pH, silt content, and electrical conductivity. All covariates were associated with at least one nematode group, but soil region had the greatest and most consistent effect. Dagger and ring nematodes were more likely to be present in region 6 than in any of the other regions, whereas lance, stunt, pin, stubby-root, and spiral nematodes were more likely to be present in regions 1 to 5 than 6. Spiral, lance, and pin nematode population densities were more likely to be at

moderate-high risk levels in regions 3 and 4 than in region 6. Fields under conservation tillage were two times more likely to have moderate-high risk lance nematode population densities than fields under conventional tillage. Similarly, pin nematode population densities were two times more likely to be at moderate-high risk levels in fields under rotation than in continuous corn. For every unit increase in soil pH, the odds of the spiral nematode population density being at moderate-high risk levels increased by 43%, but the odds of the lesion and pin nematode population densities being at the same risk level decreased by 63 and 29%, respectively. The predicted probability of lesion and lance population densities being at moderate-high risk levels decreased as the silt content of the soil increased. These findings will be useful for developing future nematode sampling protocols and for assessing the risk associated with nematodes in corn fields in Ohio.

There are at least 120 species of plant-parasitic nematodes (PPN) known to be associated with corn worldwide, of which more than 60 are associated with corn in North America (Koenning et al. 1999; Norton 1983) reported that the most frequently occurring genera of PPNs on corn in the United States were *Pratylenchus*, *Hoplolaimus*, and *Meloidogyne*, with *Belonolaimus*, *Longidorus*, and *Paratrichodorus* also contributing to corn yield suppression in several states. Mueller et al. (2016) reported that PPNs were ranked among the top 10 most important diseases/pathogens of corn in the U.S. in 2012 and 2013, causing an estimated total loss of more than 294 million bushels (more than 7.4 million metric tons) from 2012 to 2015. Losses due to PPNs were estimated to be consistently higher in southern than in northern states, with nematodes being ranked among the top three most important pathogens causing yield loss in the south in each of the four years.

Prior to a study conducted by Simon et al. (2018) in 2013 and 2014, very little was known about the population structure of PPNs in corn fields in Ohio. Samples were collected from 425 fields in that study, and 10 morphological groups of PPNs, namely *Helicotylenchus* (spiral), *Pratylenchus* (lesion), *Hoplolaimus* (lance), *Xiphinema* (dagger), *Tylenchorhynchus* (stunt), *Paratylenchus* (pin), *Cricone-mella* (ring), *Paratrichodorus* (stubby-root), *Heterodera* (cyst), and “tylenchids” (several genera in the subfamily Tylenchinae grouped together) were identified. However, the incidence and abundance of these nematodes varied among soil regions, counties within soil regions, and fields within counties and soil regions (Simon et al. 2018). For instance, tylenchids, spiral, and lesion nematodes 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 15% of the fields. In addition, the abundance of all groups varied considerably among fields, with maximum individual-field population densities ranging from 26 to 1,164 nematodes/100 cm³ soil, depending on the nematode.

Results from the Ohio survey in terms of the most frequently found PPNs and field-to-field variation in nematode presence and abundance were consistent with results from other studies conducted in the United States corn belt. For instance, Tylka et al. (2011) and Niblack and Lopez-Nicora (personal communication) also reported that spiral and lesion nematodes were among the most frequently detected morphological groups in Iowa and Illinois, respectively, and that the presence and population densities of these and other nematodes varied among fields. Such field-to-field variability and even within-field variability is not uncommon for PPNs (Norton and Hoffmann 1974), and numerous attempts have been made to determine factors associated with or contributing to such high variability. Crop rotation, tillage, soil pH, and soil texture have all been identified as being associated with nematode population density and diversity (Alby et al. 1983; Edwards et al. 1988; Govaerts et al. 2006; Parmalee and Alston 1986). However, the specific effects of these factors vary among nematode genera and species 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 conventionally tilled fields when compared with no-till fields. They also showed that nematode population densities 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 significantly positive associations 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 densities of *Helicotylenchus platyurus*, *H. pseudorobustus*, and *Xiphinema americanum* occurred in soils

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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 PPNs. For instance, Schmitt and Norton (1972) showed higher population densities of *X. americanum* in silty clay-loam soils when compared with silt loam soils. *Pratylenchus crenatus* was reported to occur in soils ranging from loams and silt-loams to light sandy soils, whereas *Pratylenchus penetrans* was found more frequently on soils with high sand content (Florini et al. 1987). Other studies reported that *P. penetrans* 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 *Criconebella* species were found in sandy soils (Wallace et al. 1993); low population densities of *Tylenchorhynchus*, *Helicotylenchus*, and *Xiphinema* species in loams and sandy loams; high population densities of *Helicotylenchus* spp. in clays and silty clay soils (Norton et al. 1970); and high population 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 and nematodes, Norton and Hoffmann (1974) hypothesized that soil pH would be useful for determining where nematodes are likely to occur. However, lacking in most of the aforementioned studies on associations among cropping practices, soil properties, and nematode population densities were formal quantitative estimates of the nature, form, and strength of the associations. Such estimates would be useful for developing models to identify fields in which certain types of nematodes are most likely to be present and at high population densities in order to advance on-farm nematode research. The objective of this study was to use data from the study conducted by Simon (2015) to formally quantify and characterize associations between soil properties, cropping practices, soil regions, and PPN incidence and population density in Ohio corn fields.

Our overall null hypothesis was that there were no, or at best very weak, functional relationships between nematode population densities and soil type and region, cropping history, and soil properties. We further hypothesized that sandy soils were likely to have

nematode population densities similar to those in high silt or clay content soils, and for any given soil type (textural class), fields under continuous corn was likely to have similar population densities to fields under crop rotation away from corn. Logistic regression modeling approaches were used to formally test these hypotheses and to quantify associations among the aforementioned covariates and the population densities of different morphological groups of nematodes. Firstly, binary logistic regression models were fitted to the nematode incidence data to estimate the odds of each morphological group being present, as influenced by the covariates, then ordinal logistic regression models were fitted to the population density data to estimate the odds (and probabilities) of lance, lesion, spiral, and pin nematode populations exceeding critical damage thresholds.

Materials and Methods

Sampling, extraction, and identification of plant-parasitic nematodes. During the 2013 and 2014 field seasons, 425 (184 in the first year and 241 in the second) corn fields were sampled and PPNs were extracted and identified based on morphology as described (Mai and Mullin 1996; Simon 2015). In brief, soil samples were collected from 15 to 16 fields in each of four to five counties in six naturally occurring soil regions. A 2.54-cm (1-inch) 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 a zig-zag pattern across eight rows of each selected field. Soil cores from the same field were pooled into a composite sample from which a single subsample of 100 cm³ was taken for nematode extraction by means of the sieving, decanting, and centrifugal sucrose flotation technique (Jenkins 1964). Nematodes were examined under stereoscopic and compound microscopes and categorized into morphological groups with the aid of a pictorial key. Groups were defined based on body shape and size, stylet shape and size, esophagus and intestine orientation, amphid opening, tail shape, metacarpus size, vulva position, stylet knob shape and size, body orientation when at rest, head description, and other morphological characteristics (Mai and Mullin 1996). The most frequently occurring morphological groups were then further characterized to species using morphometric and molecular techniques (Simon et al. 2018).

At the time of sampling, notes were taken on cropping practices such as tillage and crop rotation. Samples were collected from 83 fields under continuous corn (CC, four or more years of corn-after-corn), 220 under corn-soybean rotation (CS, the two crops rotated in the same field at least once in the last five years), and 122 under corn-soybean-wheat rotation (CSW, all three crops planted in the field at least once in the last five years) (Fig. 1A). In terms of tillage, samples were collected from 111 no-till fields, 99 fields under vertical or minimum tillage (defined here as conservation tillage), and 215 fields under conventional tillage (Fig. 1B). Fields from which samples were collected were almost evenly distributed across the six soil regions, which consisted of different soil series: 1) Hoytville-Nappanee-Paulding-Toledo; 2) Blount-Pewamo-Glynwood; 3) Miamian-Kokomo-Eldean; 4) Bennington-Cardington-Centerburg; 5) Clermont-Rossmoyne-Avonburg-Cincinnati; and 6) Shelocla-Brownsville-Latham-Steinsburg. Samples were collected from 61 fields each in regions 1 and 2, 76 each in regions 3, 4, and 5, and 75 in region 6. See Table 1 in Simon et al. (2018) for more details pertaining to the soil regions.

Soil properties. Particle size and texture. A subsample of the soil from each field was used to determine particle size distribution and texture based on the Pipette Method (Gee and Bauder 1986). Individual samples were air-dried and soil aggregates were crushed and passed through a sieve of 2 mm mesh size. Particle size distribution was then analyzed as described by Simon (2015). In brief, 10 grams of each sample were placed into a sedimentation bottle containing a dispersing solution (sodium hexametaphosphate and sodium carbonate) and deionized water. The bottles were sealed and placed onto a shaker overnight at 120 oscillations per minute, after which more deionized water was added. After the prescribed stirring and settling intervals, an aliquot of the soil remaining in suspension, i.e., clay, was placed into a preweighed crucible and dried in an oven at 105°C for 24 h. After cooling in a desiccator at room temperature,

Fig. 1. Number of soil samples collected from corn fields in Ohio under **A**, different crop rotation, **B**, tillage practices, and **C**, with different soil textures. CC = continuous corn, CS = corn-soybean rotation, and CSW = corn-soybean-wheat rotation. Sampled fields were either left without tillage (no-till) or subjected to conservation (vertical or minimum tillage) or conventional tillage.

the weight of the crucible + clay was recorded and percent clay calculated. The sand fraction of each sample was estimated by passing the remaining mixture through a 53 µm mesh sieve, rinsing the sand trapped in the sieve into a beaker, and then determining its dry weight. After determining the clay and sand contents, percent silt was calculated as 100 – (% clay + % sand). Textural classes were assigned to soil samples according to the USDA classification system (Schoeneberger et al. 2012). Most of soils (260) were classified as silt-loam (Fig. 1C).

Soil pH and electrical conductivity. A 1:1 mixture of soil and deionized water was placed into a plastic cup, stirred for 10 s, and allowed to stand for 30 min. Prior to running the test on the samples, the pH meter was calibrated using pH 4 and pH 7 buffer solutions. The electrode was then placed directly into the soil suspension and allowed to stabilize before reading the pH. The electrical conductivity (EC) of each sample was determined in a similar manner with an EC meter. The meter was first calibrated against a standard solution of known EC (1.41 millisiemens, mS) before being used to measure the conductance of each sample in microsiemens per centimeter (µS/cm). During both the pH and EC tests, the electrodes were rinsed with deionized water between samples to minimize cross contamination.

Data analysis. Odds and odds ratios. Odds refers to the number of times an event occurs (for instance, nematodes of a certain morphological group being present) or the probability of that event occurring relative to the number of times it does not occur (nematodes of that group not being detected) or the probability of it not occurring. In other words, the odds of finding nematodes of a certain group (lance, for example) 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 nematodes of a certain group can be estimated as:

$$o = \frac{p}{1-p} \quad (1)$$

where O is the odds, p is the probability of the response, i.e., finding the 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. For instance, one can estimate the odds of the lance 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. Suppose the nematode in question was detected in 203 fields, 112 under conservation tillage and 91 under conventional tillage, and not detected in 222 fields, 98 under conservation tillage and 124 under conventional tillage. The odds of finding the nematode in fields under conservation and conventional tillage would be 1.14 (112/98) and 0.73 (91/124), respectively; the odds of finding it in fields under conservation tillage relative to fields under conventional tillage (the odds ratio) would be 1.55 (1.14/0.73). This means that the nematode was 1.55 times (or 55%) more likely to be present in fields under conservation tillage relative to fields under conventional tillage. An odds ratio of 1 means that the nematode is equally likely to be found in the two tillage systems.

Associations among the incidence of plant-parasitic nematodes, soil characteristics, and cropping practices. The association between tillage (as well as other covariates or explanatory variables such as cropping sequence, soil region, or soil texture) and nematode presence was quantified, and odds and odds ratios were estimated through binary logistic regression with k explanatory variables and $i = 1, \dots, n$ observations (fields). For each morphological group of PPNs detected in this study, each field was assigned a code of 1 if a member of that group was detected and 0 otherwise as the dependent variable (Y_i). The model can be fitted to the logit link function of Y_i as:

$$\text{logit}(p_i) = \ln\left(\frac{p_i}{1-p_i}\right) = \alpha + \beta_1 x_{i1} + \beta_2 x_{i2} + \dots + \beta_k x_{ik} \quad (2)$$

where $\ln\left(\frac{p_i}{1-p_i}\right)$ is the log of the odds of finding a nematode of a certain morphological group in the i th field, also known as the log-odds; p_i is the probability of the morphological group in question being present in the i th field ($\Pr[Y_i = 1]$), given the

explanatory variables ($x_{i1}, x_{i2}, \dots, x_{ik}$); and α and β_1 to β_k are model parameters.

Tillage was assigned codes of 1 (conservation tillage = no-till, vertical tillage, or minimum tillage) and 2 (conventional tillage i.e., tillage operations other than those in category 1); cropping sequence was coded as 1, 2, and 3 for CC, CS, and CSW, respectively, and soil regions 1-6 were assigned corresponding codes of 1, 2, 3, 4, 5, and 6. Soil texture (silt content), EC, and pH were used as continuous explanatory variables. Separate models were fitted to the data for each morphological group using PROC LOGISTIC of SAS (SAS 9.4, SAS Inc. Cary, NC), with maximum likelihood as the estimation method (Allison 2012; Derr 2013; Paul and Munkvold 2004). Conventional tillage (tillage factor 2), CSW (cropping sequence factor 3), and soil region 6 were used as the baseline or reference factor levels for tillage, cropping sequence, and soil region, respectively. Other levels of these factors could have been used as references, but in keeping with our hypotheses, the selected levels allowed us to more directly evaluate PPN presence and population densities relative to cropping practices commonly recommended for nematode management (tillage and a three-crop rotation) and to a soil region with properties (Simon et al. 2018) believed to be less favorable for these pathogens. Odds ratios were estimated for each factor relative to their respective reference. The initial model was fitted with all explanatory variables, and then the backward stepwise variable selection option was used in the model statement to eliminate nonsignificant variables ($P > 0.10$).

Associations among soil characteristics and cropping practices and the abundance of lesion, spiral, lance, and pin nematodes. The logistic regression model was refitted to the data to estimate the probability (and consequently the odds) of four of the most frequently encountered morphological groups (lance, lesion, spiral, and pin) exceeding important damage thresholds. For this analysis, a modified version of a 6-category risk scale, developed by nematologists through The Society of Nematologists (Niblack 2009), was used to assign the four groups to risk classes based on population density (Table 1). However, since the proportions of nematodes in the moderate, severe, and very severe risk categories on the original scale were low, and such low proportions could be problematic for model fitting, the scale was compressed to a 3-class scale by combining the last four categories in Table 1 into a single risk class called moderate-high risk. The population density of nematodes (nematodes per 100 cm³ soil) in each group in each field was then assigned either to risk class 0 (no risk), 1 (insignificant risk), or 2 (moderate-high risk), representing the potential damage each is capable of causing based on population density (Table 1).

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_i , nematode risk class in this case) with $j = 3$ levels (0, 1, or 2), k explanatory variables, and $i = 1, \dots, n$ observations (fields), the model can be written as:

Table 1. Four morphological groups of plant-parasitic nematodes and risk categories for the potential damage they are capable of causing in corn at different population densities

Risk category	Population density ^a				Risk class ^b
	Spiral	Lesion	Lance	Pin	
Not detected	0	0	0	0	0
Not significant	1 to 75	1 to 10	1 to 10	1 to 50	1
Minor risk	75 to 150	11 to 25	11 to 40	51 to 100	2
Moderate risk	151 to 300	26 to 50	41 to 75	101 to 500	2
Severe risk	301 to 500	51 to 100	76 to 100	501 to 1000	2
Very severe risk	>500	>100	>100	>1000	2

^a Nematodes per 100 cm³ of soil.

^b Risk categories or combination of categories reclassified as 0 = no risk, 1 = insignificant risk, and 2 = moderate-high risk.

$$\text{logit}(\theta_{i0}) = \ln\left(\frac{p_{i0}}{1-p_{i0}}\right) = \alpha_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \dots + \beta_k x_{ik} \quad (3a)$$

$$\text{logit}(\theta_{i1}) = \ln\left(\frac{p_{i0} + p_{i1}}{1 - (p_{i0} + p_{i1})}\right) = \alpha_1 + \beta_1 x_{i1} + \beta_2 x_{i2} + \dots + \beta_k x_{ik} \quad (3b)$$

where $\text{logit}(\theta_{ij})$ is the cumulative logit link function of Y_i , θ_{ij} is the cumulative probability of Y_i being $\geq j$ ($\Pr[Y_i \geq j]$) given the

explanatory variables ($x_{i1}, x_{i2}, \dots, x_{ik}$), p_{ij} is the probability of Y_i being $= j$ in the i th field ($\Pr[Y_i = j]$), and α_0, α_1 , and β_1 to β_k are model parameters. For any given j , the model is an ordinal logistic regression model for $Y_i \geq j$ and is interpreted as the probability of the population density of the morphological group in question reaching or exceeding a certain risk level (j) in the i th field, given the set of explanatory variables. Separate models were fitted to the data for each morphological group in PROC LOGISTIC of SAS (Derr 2013; Paul and Munkvold 2004). Soil silt content, pH, and EC were used as continuous explanatory variables, whereas tillage practice (conventional and conservation), cropping sequence (CC, CS, and CSW) and soil region (regions 1-6) were used as categorical explanatory variables. 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 explanatory variables, and then the backward stepwise variable selection option was used to eliminate nonsignificant ($P > 0.10$) explanatory variables.

Results

Presence and population density of plant-parasitic nematodes.

Four of the most frequently encountered morphological groups of plant-parasitic nematodes (PPNs) were spiral, lesion, pin, and lance, occurring in 94, 80, 57, and 48% of the fields, respectively. Other PPNs detected were the tylenchids (several genera morphologically similar to members of the subfamily Tylenchinae [NCBI Taxonomy] including *Cephenchus*, *Filenchus*, *Malenchus*, and *Tylenchus*), stunt, and dagger, in 96, 48, and 37% of the fields, respectively, and the stubby-root, cyst, and ring in less than 15% of the samples. 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 cm³ soil, respectively. Six of the most frequently occurring morphological groups were characterized into three species of lesion (*P. thornei*, *P. scribneri*, and *P. crenatus*) and one each of spiral (*Helicotylenchus pseudorobustus*), lance (*Hoplolaimus magnistylus*), dagger (*Xiphinema rivesi*), stunt (*Tylenchorhynchus claytoni*), and pin (*Paratylenchus neoamblycephalus*) nematodes (Simon et al. 2018).

The population density (also referred to here as abundance) of the spiral nematode ranged from 0 to 990 nematodes/100 cm³ soil (Fig. 2A), and from 0 to 300, 0 to 106, and 0 to 1,164 nematodes/100 cm³ of soil for lesion, lance, and pin nematodes, respectively (Fig. 2B, C, and D). Averaged across fields, the spiral nematode was the most abundant, while the lesion and lance nematodes were the least abundant in all soil regions, crop rotational programs, and tillage systems (Fig. 2). For the spiral nematode, the mean population density was higher in continuous corn than in CS or CSW rotations (Fig. 2A). Contrastingly, mean population density of the pin nematode was higher in fields under rotation than under continuous corn (Fig. 2D). Among the tillage systems, the spiral nematode was more abundant in conservation (vertical and minimum) than in no-till or conventional tillage systems (Fig. 2A), whereas mean population density was similar across tillage systems for the other three morphological groups (Fig. 2B, C, and D). The spiral nematode was more abundant in soil regions 1, 2, 3, and 4 than in 5 and 6, and pin was more abundant in regions 2 and 4 than in 1, 3, 5, and 6, whereas lance and lesion nematodes had similar mean population densities in the six soil regions (Fig. 2).

Soil characteristics and cropping practices associated with the presence of plant-parasitic nematodes. The observed presence of PPNs in the 425 fields varied among the morphological groups (Simon et al. 2018). Based on results from binary logistic regression analyses (Table 2), the effects of cropping sequence, tillage, soil region, silt content, pH, and EC on the log-odds of nematodes being present varied among morphological groups. Cropping sequence had a significant association with the presence of spiral and pin nematodes ($P < 0.05$), a marginally significant association with the lance nematode ($P = 0.083$), but was not associated with the presence of the other seven morphological groups. Tillage was significantly associated with the presence of three of the 10 groups (lance, stunt, and stubby), whereas soil region had a significant association with six

Fig. 2. Distribution of population densities (nematodes per 100 cm³ of soil) of **A**, the spiral (*Helicotylenchus* spp.), **B**, lesion (*Pratylenchus* spp.), **C**, lance (*Hoplolaimus* spp.), and **D**, pin (*Paratylenchus* spp.) nematodes in Ohio corn fields sampled between the V3 and V6 growth stages during the 2013 and 2014 growing seasons. Three species of lesion (*Pratylenchus thornei*, *P. scribneri*, and *P. crenatus*) and one each of spiral (*Helicotylenchus pseudorobustus*), lance (*Hoplolaimus magnistylus*), and pin (*Paratylenchus neoamblycephalus*) nematodes were identified. Samples were collected from fields under continuous corn (CC), corn-soybean rotation (CS), or corn-soybean-wheat rotation (CSW), left without tillage (no-till) or subjected to conservation (vertical or minimum tillage) or conventional tillage in six soil regions as defined by the Ohio Department of Natural Resources (1 = Hoytville-Nappanee-Paulding-Toledo, 2 = Blount-Pewamo-Glynnwood, 3 = Miamian-Kokomo-Eldean, 4 = Bennington-Cardington-Centerburg, 5 = Clermont-Rossmyrne-Avonburg-Cincinnati, and 6 = Shelocla-Brownsville-Latham-Steinsburg). Note that the Y axes are not on the same scale.

groups, namely lesion, dagger, stunt, pin, ring, and stubby-root ($P < 0.05$; Table 2). The continuous covariates silt content, pH, and EC, also had statistically significant associations ($P < 0.06$) with at least two of the morphological groups. 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. The presence of the other morphological groups was associated with two factors (Table 2).

Odds ratios (OR) are depicted in Figure 3A, B, and C for associations between cropping sequence and spiral, lance, and pin nematodes, respectively, using CSW as the reference crop-rotation system for comparison. An OR of 1 (the reference line in Fig. 3) signifies equal odds of finding the nematode in question in fields under the two cropping sequences 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 spiral, lance, and pin nematodes being present in a field under CC were respectively 0.233, 0.515, and 0.509 times the odds of them being present in a field under the three-crop rotation (CC versus CSW; Fig. 3). This means that a field under the three-crop rotation was 4.29 (1/0.233), 1.94 (1/0.515), and 1.96 times more likely to have spiral, lance, and pin nematodes, respectively, than a field under CC (Fig. 3A, B, and C). Similarly, a field under CS rotation was 2.58 (1/0.387) and 1.92 (1/0.521) times more likely to have spiral and pin nematodes, respectively, than a field under CC (Fig. 3A and C), but the two rotational schemes were equally likely to have lance nematodes (Fig. 3B; the OR of 0.791 was not significantly significant). Fields under CS were just as likely to have spiral, lesion, and pin nematodes as fields under CSW, i.e., ORs for comparisons between CS and CSW were not statistically significant (Fig. 3A, B, and C; the 95% confidence intervals included 1).

Figure 3D 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 fields under conservation tillage (coded as 1) were almost twice (ORs of 1.79 and 1.89, respectively) the odds of finding them in fields under conventional tillage (the reference, coded as 2 in Fig. 3D). In other words, the odds of lance and stubby-root nematodes being present in fields under conservation tillage were 79 $[(1.79-1) \times 100]$ and 89% $[(1.89-1) \times 100]$ greater, respectively, than the odds of them being present in conventional tillage fields. However, the opposite was true for stunt nematodes, which were 1.63 (1/0.614) times or 63% more likely to be present in fields under conventional tillage than under conservation tillage.

Associations between soil region and the presence of PPNs depended on the nematode. With the exception of the dagger and ring

nematodes, the odds of PPNs being present in one or more of soil regions 1, 2, 3, 4, or 5 were greater than the odds of them being present in soil region 6 (the reference soil region). Lance, stunt, pin and stubby-root nematodes were more than twice as likely to be present in soil region 3 as in region 6 (Fig. 4A, C, D, and F). The odds of stunt or spiral nematodes being present in region 5 were more than 7 times the odds of them being present in region 6 (Fig. 4C and G). Soil region 4 was 2.9 times more likely to have lance nematodes (Fig. 4A), 3.9 times more likely to have pin nematodes (Fig. 4D), and 6.5 times more likely to have spiral nematodes (Fig. 4G) than region 6. Contrastingly, dagger and ring nematodes were respectively 2 to 3 and 5 to 15 times more likely to be present in soil region 6 than in any of the other soil regions (Fig. 4B and E). Lance nematodes were also more (5.5 times) likely to be present in soil region 6 than in region 1 (Fig. 4A), and stunt nematodes were more than twice as likely to be present in soil region 6 as in regions 2 or 4 (Fig. 4C).

There were significant positive relationships between log-odds and soil pH for spiral and cyst nematodes ($P < 0.05$). For both morphological groups, there was a 0.74 unit increase in the log-odds of cyst or spiral nematodes being present (all other factors in the model being held constant) per unit increase in pH. In other words, for every unit increase in pH, the odds of spiral or cyst nematodes being present were more than twice ($OR = 2.10 = e^{0.74}$) 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, meaning that for every unit increase in pH, there was a 0.46 unit reduction in the log-odds of this nematode being present. In other words, lesion nematode was about 1.6 times more likely to be absent than present as pH increased. For every percent increase in the silt content of the soil, log-odds for the presence of tylenchids increased (slope = 0.04), whereas log-odds for the presence of stunt nematodes decreased (slope = -0.02). Soil EC had significant positive relationships with log-odds ($P < 0.10$) for the presence of tylenchids and lesion nematodes (slopes of 0.02 and 0.005, respectively).

Soil characteristics and cropping practices associated lesion, spiral, lance, and pin nematode population densities. The number of fields with nematode population densities in the no, insignificant, and moderate-high risk categories varied among the four morphological groups. For the spiral, lesion, lance, and pin nematodes, 236 (56%), 191 (45%), 91 (21%), and 183 (43%) fields, respectively, had population densities in the insignificant risk category (category 1), whereas 162 (38%), 149 (35%), 112 (26%), and 58 (14%), respectively, had population densities in the moderate-high risk category (category 2; Table 1). The corresponding percentages for the no risk category were 6, 20, 52, and 43% for spiral, lesion, lance, and pin

Table 2. Probability values (levels of significance) for Wald χ^2 test from binary logistic regression analyses of associations between soil characteristics, cropping practices, and the presence of 10 plant-parasitic nematode morphological groups from a survey of corn fields in Ohio in 2013 and 2014

Explanatory variables ^a	Plant-parasitic nematodes ^b									
	Spiral	Tylenchids	Cyst	Lesion	Lance	Dagger	Stunt	Pin	Ring	Stubby
Cropping sequence	0.036	ns	ns	ns	0.083	ns	ns	0.041	ns	ns
Tillage	ns	ns	ns	ns	0.009	ns	0.047	ns	ns	0.047
Soil region	0.039	ns	ns	ns	<0.001	0.022	<0.001	<0.001	0.002	0.037
Silt content	ns	0.013	ns	ns	ns	ns	0.057	ns	ns	ns
Soil pH	<0.001	ns	<0.001	<0.001	ns	ns	ns	ns	ns	ns
Soil EC	ns	0.019	ns	0.059	ns	ns	ns	ns	ns	ns
Likelihood ratio test ^c	<0.001	0.002	<0.001	<0.001	<0.001	0.020	<0.001	<0.001	<0.001	0.003

^a Cropping sequence refers to fields under continuous corn, corn-soybean rotation, or corn-soybean-wheat rotation. Tillage refers to fields left without tillage (no-till) or subjected to vertical, minimum tillage, or conventional tillage. For the purpose of data analysis, no-till, vertical tillage, and minimum tillage were collectively considered conservation tillage. Soil region refers to six naturally occurring soil regions as defined by the Ohio Department of Natural Resources (see the Materials and Methods for details). Silt content = percent silt and EC = electrical conductivity ($\mu\text{S}/\text{cm}$).

^b Morphological groups = Spiral (*Helicotylenchus*), cyst (*Heterodera*), lesion (*Pratylenchus*), lance (*Hoplolaimus*), dagger (*Xiphinema*), stunt (*Tylenchorhynchus*), pin (*Paratylenchus*), ring (*Criconeimella*), and stubby-root (*Paratrichodorus*). Three species of lesion (*Pratylenchus thornei*, *P. scribneri*, and *P. crenatus*) and one each of spiral (*Helicotylenchus pseudorobustus*), lance (*Hoplolaimus magnistylus*), dagger (*Xiphinema rivesi*), stunt (*Tylenchorhynchus claytoni*), and pin (*Paratylenchus neoamblycephalus*) nematodes were identified. The group tylenchids represents several genera morphologically similar to members of the subfamily Tylenchinae (NCBI Taxonomy) including *Cephelelenchus*, *Filenchus*, *Malenchus*, and *Tylenchus*.

^c Likelihood ratio χ^2 test of the null hypothesis that all explanatory variables have coefficients of zero. In all cases the null hypothesis was rejected, meaning that in each case at least one of the coefficients is different from zero. The fitted models are acceptable.

nematodes, respectively. Of the fields in the moderate-high risk category, 16 had more than 300 spiral nematodes/100 cm³ soil, 19 had more than 50 lesion nematodes/100 cm³ soil, eight had more than 75 lance nematodes/100 cm³ soil, and six had more than 500 pin nematodes/100 cm³ soil.

The statistical significance of specific soil characteristics and cropping practices on the odds of these nematode population densities being assigned to the moderate-high risk category (category 2) depended on the nematode (Table 3). For instance, the odds of spiral nematode density being assigned to category 2 (over 0 and 1) was influenced by soil region and soil pH ($P < 0.05$; Table 3), whereas the odds of the lesion nematode density being assigned to that same category was influenced by the silt content, pH, and EC of the soil. For the lance and pin nematodes, tillage, soil region, and silt content, and cropping sequence, soil region, soil pH, and EC, respectively, were significantly associated with the odds of the population densities of these nematodes reaching their respective moderate-high risk thresholds (Tables 1 and 3).

The odds ratios and coefficients in Table 4 can be interpreted in terms of odds of the population densities of the nematodes in question being in risk category 2 (moderate-high risk, used here as the reference category) versus categories 1 or 0 or in terms of the odds of it being in categories 2 or 1 versus category 0. Hereafter, all references to the odds of being in the moderate-high risk category will be made

relative to the odds of being in the combined insignificant- and no-risk categories. For instance, for the spiral nematode, soil region 1 had 3.9 times higher odds of having a population density in the moderate-higher risk category (category 2 versus 1 and 0) than soil region 6. This means that soil region 1 was almost 4 times more likely to have spiral nematode population densities at the moderate-high 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-high risk levels in those soil regions being 2.6 to 4.5 times greater than the odds of that occurring in soil region 6 (Table 4). Similarly, soil regions 2, 3, and 4 were more likely to have pin nematode population densities at the moderate-high 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 observed for the lance nematode, with the primary exception being for soil region 1, which was less likely than soil region 6 to have lance nematode population densities in the moderate-high risk category.

Table 4 also shows odds ratios for the association between tillage and lance nematode population density and between cropping sequence and the population density of pin nematodes. Tillage system 1 (conservation tillage = the combination of no-till, minimum till, and vertical tillage) was two times more likely to have lance nematode population densities in the moderate-high risk category than tillage system 2 (conventional) (OR = 2.03). For the pin nematode,

Fig. 3. Adjusted 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* spp.), and stubby-root (*Paratrichodorus* spp.) nematodes. The species identified were *Helicotylenchus pseudorobustus* (spiral), *Hoplolaimus magnistylus* (lance), *Paratylenchus neoamblycephalus* (pin), and *Tylenchorhynchus claytoni* (stunt). Cropping sequences CC, CS, and CSW 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), respectively. Conventional tillage (tillage factor 2) and corn-soybean-wheat (cropping sequence CSW) were the baseline or reference factor levels for tillage and cropping sequence, respectively. Odds ratios were estimated for each factor relative to their respective reference.

Fig. 4. Adjusted 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 (*Criconeimella* spp.), **F**, stubby-root (*Paratrichodorus* spp.), and **G**, spiral (*Helicotylenchus* spp.). The species identified were *Helicotylenchus pseudorobustus* (spiral), *Hoplolaimus magnistylus* (lance), *Xiphinema rivesi* (dagger), *Tylenchorhynchus claytoni* (stunt), and *Paratylenchus neoamblycephalus* (pin). Soil regions 1, 2, 3, 4, 5, and 6 represent Hoytville-Nappanee-Paulding-Toledo, Blount-Pewamo-Glywood, Miamian-Kokomo-Eldean, Bennington-Cardington-Centerburg, Clermont-Rossmoyne-Avonburg-Cincinnati, and Shelocta-Brownsville-Latham-Steinsburg soil regions, respectively, as defined by Ohio Department of Natural Resources. Odds ratios were estimated for soil region 1, 2, 3, 4, and 5 relative to soil region 6, the baseline or reference soil region. Note that the Y axes are not on the same scale.

population densities in the moderate-high risk category were more likely to occur in fields under CS and CSW rotation (Table 4) than in CC fields (OR < 1 for the CC versus CS and CC versus CSW comparison, $P < 0.05$). However, the odds of this same nematode being in the moderate-high risk category in fields under CS and CSW rotations were equally likely (OR = 0.97 for CS versus CSW, $P > 0.10$ in Table 4).

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 of spiral nematode, a 0.49 unit decrease in the log-odds of lesion nematode, and a 0.25 unit decrease in the log-odds of pin nematode population densities being in risk category 2 (Table 4). In other words, for every unit increase in pH from 4 to 9.6 (with all other factors being held constant), the odds of the spiral nematode population density being in the moderate-high risk category (category 2 over categories 1 or 0) increased by 43%, whereas the odds of the lesion and pin nematode densities being in that same risk category (over categories 1 or 0) decreased by 63 and 29%, respectively (Fig. 5).

Relationships between predicted probability of nematode population densities being in risk category 2 and soil pH varied with soil region for spiral nematodes (Fig. 5A), with the silt content of the soil for lesion nematodes (Fig. 5B), and with soil region and cropping sequence for pin nematodes (Fig. 5C and D). At all recorded pH values, soil region 6 had lower predicted probabilities than regions 1–5 for spiral nematodes (Fig. 5A), whereas soil region 1, 5, and 6 had lower probabilities than regions 2, 3, and 4 for the pin nematode (Fig. 5C and D, respectively). However, the magnitude of the differences in predicted probabilities among soil regions varied with pH. Differences were smaller at lower pH values for spiral nematodes (Fig. 5A), but larger at similar pH values for pin nematodes (Fig. 5C and D). At any given pH, in any given soil region, the predicted probabilities of pin nematode population density being in the moderate-

high risk category were higher in fields under CSW rotation than under CC (Fig. 5C and D).

The effects of silt content and EC of the soil on the probability of nematode population densities reaching moderate-higher risk levels also varied among nematodes. Slopes for relationships between the silt content of the soil and log-odds were negative for both the lesion and lance nematodes, whereas the slope for the relationship between EC and log-odds was positive for the lesion nematode but negative for the pin nematode (Table 4). For both the lesion and lance nematodes, the predicted probability of population densities being in the moderate-high risk category decreased as the silt content of the soil increased, at fixed levels of other covariates (Fig. 6A, B, and C). The predicted probability of population densities of the lesion nematode being in the moderate-high risk category increased as the EC of the soil increased (Fig. 6F), but the probability of pin nematode population densities being in that same risk category decreased with increasing EC (Fig. 6D and E).

As was the case with soil pH, relationships between predicted probability and silt content and predicted probability and EC varied

Table 3. Probability values (level of significance) for Wald χ^2 test from proportional odds ordinal logistic regression analyses of associations between soil characteristics, cropping practices, and the abundance of four of the most frequently occurring morphological groups of plant-parasitic nematode based on a survey of corn fields in Ohio in 2013 and 2014

Explanatory variables ^a	Plant-parasitic nematodes ^b			
	Spiral	Lesion	Lance	Pin
Cropping sequence	ns	ns	ns	0.014
Tillage	ns	ns	<0.001	ns
Soil region	<0.001	ns	<0.001	<0.001
Silt content	ns	0.051	0.063	ns
Soil pH	0.002	<0.001	ns	0.027
Soil EC	ns	0.032	ns	0.024
Likelihood ratio test ^c	<0.001	<0.001	<0.001	<0.001
Score test ^d	0.120	0.551	0.113	0.535

^a Cropping sequence refers to fields under continuous corn, corn-soybean rotation, and corn-soybean-wheat rotation. Tillage refers to fields left without tillage (no-till) or subjected to vertical, minimum tillage, or conventional tillage. For the purpose of data analysis, no-till, vertical tillage, and minimum tillage were collectively considered conservation tillage. Soil region refers to six naturally occurring soil regions as defined by the Ohio Department of Natural Resources (see Materials and Methods for details). Silt content = percent silt and EC = electrical conductivity ($\mu\text{S}/\text{cm}$).

^b Three species of lesion (*Pratylenchus thornei*, *P. scribneri*, and *P. crenatus*) and one each of spiral (*Helicotylenchus pseudorobustus*), lance (*Hoplolaimus magnistylus*), and pin (*Paratylenchus neoamblycephalus*) nematodes were identified.

^c Probability value for the likelihood ratio χ^2 test of the null hypothesis that all explanatory variables have coefficients of zero. In all cases the null hypothesis was rejected, meaning in each case at least one of the coefficients is different from zero.

^d Probability value for the score test for the proportional odds or parallel slopes assumption. In other words, the assumption that coefficients for relationships between pairs of risk groups are the same. Failing to reject the null hypothesis ($P > 0.10$) means that the assumption was met in all cases.

Table 4. Adjusted odds ratios for categorical variables, regression slopes for continuous covariates, and corresponding statistics (based on Wald χ^2 test) from proportional odds ordinal logistic regression analyses of associations among soil characteristics, cropping practices, and the abundance of four of the most frequently occurring morphological groups of plant-parasitic nematode based on a survey of corn fields in Ohio in 2013 and 2014

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

^a Three species of lesion (*Pratylenchus thornei*, *P. scribneri*, and *P. crenatus*) and one each of spiral (*Helicotylenchus pseudorobustus*), lance (*Hoplolaimus magnistylus*), and pin (*Paratylenchus neoamblycephalus*) nematodes were identified.

^b Region = naturally occurring soil regions as defined by the Ohio Department of Natural Resources (1 = Hoytville-Nappanee-Paulding-Toledo, 2 = Blount-Pewamo-Glynnwood, 3 = Miami-Kokomo-Eldean, 4 = Bennington-Cardington-Centerburg, 5 = Clermont-Rossmyrne-Avonburg-Cincinnati, and 6 = Shelocta-Brownsville-Latham-Steinsburg); TILL = tillage practice (1 = conservation tillage [the combination of no-till, and minimum and vertical tillage] and 2 = conventional tillage; cropping sequence CC = continuous corn, CS = corn-soybean rotation, and CSW = corn-soybean-wheat rotation; and soil pH and electrical conductivity (EC)).

^c Estimate represents odds ratios (OR) for Region, TILL, and cropping sequence, and regression slopes (rate of change in predicted probability per unit increase in continuous variables) for pH and EC; se, CI_L, and CI_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.

with soil region, tillage practice, cropping sequence, and soil pH, depending on the nematode (Fig. 6). At a fixed soil silt content (within the range of values observed in this study), predicted probabilities of lance nematode population density being in the moderate-high risk category were lower for soil region 1 than for regions 2–6, and for any given soil region, higher in fields under conservation tillage than under conventional tillage (Fig. 6A and B). Soils with low and high pH had the highest and lowest predicted probabilities, respectively, of the lesion nematode population density being in the moderate-high risk category across all soil silt contents and EC levels observed in this study (Fig. 6C and F). For the pin nematode, soil regions 1, 5, and 6, and in any given soil region, CC fields had the lowest predicted probabilities at any given level of soil EC (within the range observed in this study).

Discussion

Based on results from a survey of 425 Ohio corn fields (184 in 2013 and 241 in 2014), Simon et al. (2018) observed considerable variability in the presence and population density of PPNs among soil region, counties within soil region, and particularly among fields within counties and soil regions. Such variability is quite common for nematodes (Gavassoni et al. 2001, 2007; Norton and Hoffmann 1974) and could be attributed to several factors, including soil properties, cropping practices, and edaphoclimatic conditions, among

others. Here we expanded on the work done by Simon et al. (2018) by using logistic regression models (Allison 2012) to more formally quantify association among these factors and nematode presence and population density.

Cropping sequence was associated with the presence of the spiral, lance, and pin nematodes, but not the other morphological groups, in the corn fields sampled. The odds of finding the spiral, lance, or pin nematodes in a CS or a CSW rotation were greater than the odds of finding them in a field under CC. Furthermore, pin nematodes were more likely to be found at moderate-high risk population densities in fields under rotation than in a continuous-corn field. In contrast, cropping system did not affect the population density of the lance or spiral nematode, suggesting that although these nematodes may be more prevalent in fields under rotation than under CC, when present in the latter cropping system, the population density is likely to be comparable to that of the former system. These results are somewhat surprising given that the general thinking was that CC was going to harbor more nematodes and favor higher population densities. The observed associations (or lack thereof) between nematodes and cropping sequence could be indicative of host preference. For those PPNs with moderate-high incidence across the state such as the dagger and stunt nematodes (Simon et al. 2018), the nonsignificant cropping system effect could be interpreted to mean that they likely thrive on all three crops.

Fig. 5. Relationship between soil pH and predicted probabilities of population densities being in the moderate-high risk category (see Table 1) for **A**, spiral nematodes in different soil regions, **B**, lesion nematodes in soils with different baseline silt content, and **C** and **D**, pin nematodes in different soil regions under continuous corn (**C**) and corn-soybean-wheat rotation (**D**). Three species of lesion (*Pratylenchus thornei*, *P. scribneri*, and *P. crenatus*) and one each of spiral (*Helicotylenchus pseudorobustus*) and pin (*Paratylenchus neoamblycephalus*) nematodes were identified. Soil regions (lines in **A**, **C**, and **D**) 1, 2, 3, 4, 5, and 6 represent Hoytville-Nappanee-Paulding-Toledo, Blount-Pewamo-Glywood, Miami-Kokomo-Eldean, Bennington-Cardington-Centerburg, Clermont-Rossmyrne-Avonburg-Cincinnati, and Shelocla-Brownsville-Latham-Steinsburg soil regions, respectively, as defined by Ohio Department of Natural Resources. The silt content (lines in **B**) represent minimum, mean, and maximum % silt across all samples. Note that the lines for soil regions 1, 5, and 6 overlap considerably in **C** and **D**.

Indeed, Hodda et al. (2012) reported multiple species of *Xiphinema* and *Tylenchorhynchus* associated with corn, soybean, and wheat, including *T. claytoni*, the species identified in this study. On the other hand, the lance, spiral, and pin nematodes, groups with higher population densities under rotation than under CC, likely have a greater preference for soybean than corn. Results from a study in Ohio (Simon, unpublished) provide some support for this hypothesis for the spiral nematode. In one particular field in that study, the population density of the spiral nematode was substantially higher in the second year (2017) under soybean than in the first year (2016) under corn. Samples were collected from the same georeferenced zones in both years. For other nematodes (cyst, ring, stubby-root), the nonsignificant cropping system effect could be due to their low overall incidence, which in turn could mean that either they are not prevalent in Ohio's corn fields, the sample size was too small for them to be detected, or in the case of the cyst nematode (confirmed morphologically to be soybean cyst), corn is simply not a host. However, further research would be needed to formally evaluate the aforementioned explanations and hypotheses for the observed associations between cropping sequence and the presence and population density of PPNs.

Others have reported on the influence of cropping system on the population density of PPNs, with results somewhat contrary to those observed in the current investigation. For instance, Govaerts et al. (2006) reported that the population densities of PPNs were higher with CC when compared with corn-wheat rotation, but that the statistical significance of the cropping sequence effects varied with nematode genera and tillage. In agreement with Govaerts et al. (2006), Edwards et al. (1988) found evidence of an interaction effect of crop rotation and tillage on the population density of the stunt nematode, a morphological group influenced by tillage but not cropping sequence in our study. They observed higher population densities of the stunt nematode in continuous corn with conventional tillage than in continuous corn with no-till or strip tillage. Results from our analyses

showed that the odds of finding the stunt nematode in fields with conventional tillage were approximately twice the odds of finding it in no-till, vertical- or minimum-till fields (collectively considered as conservation tillage in this study), regardless of the cropping sequence.

Contrary to the stunt nematode, the lance and stubby-root nematodes were almost twice as likely to be found in fields under conservation tillage when compared with fields under conventional tillage. In addition, the odds of lance nematode reaching moderate-high risk population levels in a conservation tillage system were twice the odds of it reaching that same damage threshold in a conventional tillage system. This is consistent with the general thinking that no-till or minimum-tillage (collectively defined here as conservation tillage) is more favorable for nematodes, but is certainly not the case for all the nematodes detected in this study. 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 nematodes reaching moderate-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 (*Pratylenchus*) were observed in conventionally tilled plots than in no-till plots, and conversely, higher densities of the spiral nematode were observed in no-till plots when compared with conventionally tilled plots. Thomas (1978), however, reported higher population densities of the spiral and lesion nematodes (as well as the dagger nematode) in no-till plots. These differences among studies and among nematodes in terms of the tillage effect suggest that, as was the case with cropping system, the effect of tillage on PPN populations may vary with nematode genera and interactions among cropping practices and other factors (Edwards et al. 1988; Govaerts et al. 2006).

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, cyst, or tylenchid nematodes. The odds of finding the spiral, lance, pin, stunt, or stubby-root nematodes

Fig. 6. Relationship between **A, B,** and **C,** soil silt content (%), **D, E,** and **F,** electrical conductivity and predicted probabilities of population densities being in the moderate-high risk category (see Table 1) for lance nematodes in different soil region for fields under conservation (**A**) and conventional tillage (**B**), lesion nematodes in soils with different baseline pH levels (**C** and **F**), and pin nematodes in different soil regions under continuous corn (**D**) and corn-soybean-wheat rotation (**E**). Three species of lesion (*Pratylenchus thornei*, *P. scribneri*, and *P. crenatus*) and one each of spiral (*Helicotylenchus pseudorobustus*) and pin (*Paratylenchus neoamblycephalus*) nematodes were identified. Soil regions (lines in **A, B, D,** and **E**) 1, 2, 3, 4, 5, and 6 represent Hoytville-Nappanee-Paulding-Toledo, Blount-Pewamo-Glynwood, Miami-Kokomo-Eldean, Bennington-Cardington-Centerburg, Clermont-Rossmoyne-Avonburg-Cincinnati, and Shelocta-Brownsville-Latham-Steinsburg soil regions, respectively, as defined by the Ohio Department of Natural Resources. Soil pH levels (lines in **C** and **F**) represent minimum, mean, and maximum pH across all samples.

in soil regions 1, 2, 3, 4, or 5 tended to be greater, with a few exceptions, than the odds of finding them in soil region 6. However, the dagger and ring nematodes were more likely to be present in region 6 than regions 1–5. Not only was soil region associated with the presence of most of the nematodes investigated in this study, it was also associated with the population density and the odds of populations exceeding suggested damage thresholds for the spiral, lance, and pin nematodes, with similar trends in terms of the differences between region 6 and the other regions. These associations may be related to inherent differences among the soil regions in terms of the physical and chemical properties and genesis of the soil, and cropping practices (Broders et al. 2009; Simon et al. 2018). For instance, according to the Ohio Department of Natural Resources, the most common soils in regions 1–5 were formed from glacial deposits and have relatively high organic matter and clay content and a shallow seasonal water table (Broders et al. 2009; Simon et al. 2018). On the other hand, the most common soils in region 6 were formed from weathered sedimentary rocks (sandstone, siltstone, and shale) and have low organic matter and clay content. It therefore seems reasonable to conclude that, except for the dagger and ring nematodes, several of the morphological groups found in this study are likely to be more prevalent, and in the case of the spiral, lance, and pin nematodes, reach moderate-high population densities, in a soil region with silty clay loam soils with relatively higher organic matter than in the low-organic-matter silt loam soils of region 6. Results from a study by Norton et al. (1970) provide some support for this conclusion, showing contrasting associations among soil clay and organic matter contents and spiral (*H. pseudorobustus*) and dagger (*X. americanum*) nematodes.

When the effect of soil texture on the population density of lesion, lance, spiral, and pin nematodes was more directly investigated through the use of silt content as a continuous covariate, silt content had a significant association with population densities of the lesion and lance nematodes, but not the pin or spiral nematodes. For the former two nematodes, there was a significant negative relationship between soil silt content and the probability of population density in the moderate-high risk category, meaning that as soil silt content increased, the odds of lesion and lance nematode populations reaching the proposed moderate-high risk levels decreased. Brodie (1976) also reported high population densities of *Pratylenchus brachyurus* in soil with relatively low silt content (6% silt), suggesting that the lesion nematode may be less abundant in soils with relatively high silt content. However, these results do not support what was discussed above regarding possible effects of soil region on population density of lesion, spiral, and pin nematodes. This apparent disagreement could be due to the fact that other soil-region-specific factors likely had greater effects on nematode population than silt content or those factors interacted with silt content to affect population density. Indeed, our results showed that although the probability of the lesion and lance nematode populations reaching moderate-high risk levels decreased as silt content increased, the relationship between predicted probability and silt content (slopes and heights of the regression lines) varied with soil region, and was influenced by tillage for the former and soil pH for the latter nematode.

Like silt content, soil pH and EC were also associated with the presence and population densities of PPNs, but the nature of these relationships again varied among nematodes and was influenced by other factors. There were significant negative relationships between predicted probability for the lesion and pin nematode population densities at the moderate-high risk level and soil pH, and conversely, a significant positive relationship between soil pH and predicted probability for the spiral nematode population density at the moderate-high risk level. Studies in Iowa also showed significant positive correlations between spiral nematode population densities and soil pH (Norton and Hoffmann 1974; Norton et al. 1970), with high densities of *Helicotylenchus platyurus* and *H. pseudorobustus* occurring in soils with pH above 6.0. Although some nematodes are able to tolerate a fairly wide range of pH, others may be suppressed or killed by pH extremes (Oka 2010). For instance, McElderry et al. (2005) showed that artificial acidification of a sand-soil mix to pH below 4.5 significantly increased the mortality of stunt nematodes

(*Tylenchorhynchus* spp.) relative to the control, whereas alkalization to a pH of 11 killed root-knot nematode juveniles in another study (Oka 2010). Such pH extremes may affect nematodes directly or indirectly by changing the nutrient status and root structure of the host, altering the chemical and physical structure and microbial activity of the soil, enhancing the nematicidal activity of naturally occurring compounds in the soil, negatively affecting the nematodes' ability to regulate their osmotic pressure, or simply by killing the nematode directly (Korthals et al. 1996; Oka 2010). Like pH, the observed associations between EC and nematode population densities were likely indirect due to the fact that EC is often corrected with several other factors such as soil texture, salinity, pH, water content, cation exchange capacity, and organic matter (Friedman 2005; Valente et al. 2012), all of which may affect nematode population densities (Oka 2010; Widmer et al. 2002). There was a significant positive relationship between soil EC and lesion nematode population densities at the moderate-high risk level, but a negative relationship was observed between soil EC and populations of pin nematodes.

Our results show that soil region, cropping practices, and soil pH and EC may all be good indicators of the presence and population density of one or more important groups of PPNs. We successfully quantified association between these factors and nematode populations, generating data that will help us to better understand and manage factors affecting nematode presence and potential build-up in corn fields in Ohio. However, as stated by Niblack (1992), "The association of a plant-parasitic nematode species with a particular host through field sampling does not demonstrate a host-parasite relationship." The same can be said for associations among PPN presence, population density, production practices, and soil properties. Therefore, further research would be needed to establish causation for the observed associations, to quantify the effects of interactions among covariates on PPNs, and to develop manage strategies. For instance, since soil properties and production practices are known to vary within and among fields, studies would be needed to evaluate and develop site-specific PPN management programs, and since different genera and species of the same genus may respond differently to any given factor or set of factors, management will have to be tailored to nematode taxa. Information from this study will be invaluable for the establishment of future studies, particularly on-farm research, to develop sampling protocols, and to identify on-farm trial locations to target specific nematodes. In particular, our results will be very useful for future effort to revisit and update the information presented in Table 1 with damage thresholds based on more quantitative estimates of associations between PPN populations and crop loss.

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