A Negative Binomial Regression Model of the Observed Population Density of *Heterodera glycines* after Annual Corn Rotation in Nebraska

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Abstract

In Nebraska, rotation of soybean (*Glycine max*) fields to corn (*Zea mays*) is a major practice to manage the soybean cyst nematode (SCN; *Heterodera glycines*). However, factors associated with SCN population density decline during corn rotation are not clearly defined. This study addresses that question using a modeling approach. Seventy-nine fields were sampled in 2009, 2010, and 2011 to determine SCN population densities (eggs/100 cm³ of soil) before and after rotation. After rigorous field screening and model testing and validation, the regression model $\text{Log}(P_f) = -2.3360 + 0.8368\text{Log}P_i + 0.4333pH$ was developed, where $\text{Log } P_f$ is the natural log of SCN eggs at the end of the rotation year, P_i is the population density before rotation, and pH is the soil pH. Model goodness-of-fit was assessed through residual analysis, information criteria, and other remedial measures. Model overdispersion was 1.04. Validation in a 50 and a 75% random sample from the original data set showed

little change in model regression coefficients, standard errors, and associated significance, confirming model fit and performance. The model indicates that for one-unit increase in soil pH, SCN P_f is expected to increase by 53.7% at constant P_i , and correspondingly, a 10% change in P_i will result in about 8.3% change in P_f at constant soil pH. The model suggested that SCN population levels before corn rotation and soil pH are major determinants of observed SCN population density reduction after annual corn rotation in Nebraska. This model has potential for use in SCN risk analysis and in predicting SCN population decline after corn rotation in the state.

Keywords: cyst nematode epidemiology, negative binomial, corn rotation, nematode management, oilseeds and legumes, disease management, epidemiology, disease development and spread

Since its discovery in North Carolina in 1954 (Winstead et al. 1955), the soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe, 1952) has been considered the most economically important pathogen of soybean (*Glycine max* L.) in the United States (Doupnik 1993; Ross 1962; Wrather and Koenning 2006). Soybean yield suppression due to SCN was estimated at 35 million metric tons during 2006 (Wrather 2008), a much higher yield reduction than that caused by any other soybean disease in the country (Wrather and Koenning 2006). In Nebraska (the seventh largest soybean producing state in the United States; NASS 2018), yield losses due to SCN were estimated at \$40 million in 2012 (Wilson and Giesler 2013).

SCN is a microscopic, cyst-forming, obligate plant parasitic animal that infects soybean and other cultivated and noncultivated plants (Johnson et al. 2008; Riggs 1992). In susceptible hosts, juvenile nematodes enter the root, migrate toward the central cylinder, and establish a permanent feeding site near the vascular tissue, where they complete adulthood and reproduction after three molts (Lauritis et al. 1983; Niblack 2005). Conversely, in nonhost crops like corn, wheat, and oats, juveniles are unable to establish a permanent feeding site and reproduce, and eventually die (Chen et al. 2008). Both juveniles and other SCN developmental stages remaining in the soil are affected by soil factors, weather conditions, and management practices imposed during the nonhost rotation cycle and the off-season (Noel 2008).

In Nebraska, management of SCN relies almost entirely on rotation of soybean fields to corn (*Zea mays* L.) and use of

SCN-resistant soybean varieties (Giesler and Wilson 2011). Percent decline of SCN population densities in annually rotated corn fields in the state was recently estimated to vary between 10 and 90% (Pérez-Hernández and Giesler 2014). While it is well established that SCN population densities can be reduced significantly during the corn rotation cycle (Koenning et al. 1993; Noel 2008; Pérez-Hernández and Giesler 2014; Porter et al. 2001; Young and Hartwig 1992), the factors associated with such a decline in field conditions are not clearly defined. Soil temperature, tillage, soil texture, and pH have been associated to SCN abundance (Alston and Schmitt 1988; Pedersen et al. 2010; Ross 1964; Slack et al. 1972; Westphal et al. 2009; Workneh et al. 1999), but the inferred association has been derived from studies focused on the soybean growing season. Little is known about the relationships of those factors with the observed SCN mortality during the annual corn rotation cycle, which includes the growing season and an off-season or overwintering period. Identification of major factors associated with SCN population density reduction in corn can provide the basis for SCN risk assessment, which ultimately can be useful for better management decisions. Regression models offer a robust approach to identify and understand factors and interactions, with the advantage of elucidating quantitative variable relationships that are often difficult to identify with ordinary class variable models (Jones and Perry 1978; Kutner et al. 2004; Paul and Munkvold 2005). The objective of this research was to identify, using a modeling approach, major factors associated with the observed SCN population density reduction after annual corn rotation in Nebraska.

Materials and Methods

SCN data collection. Data of SCN population densities from 79 fields were initially used for the analysis. Field selection, sampling methodology, and determination of SCN population densities in each field are described in detail elsewhere (Pérez-Hernández and Giesler 2014). In essence, from Nebraska SCN surveys, commercial production fields (37 to 55 ha in size) with a history of SCN and in soybean-corn rotation were selected in 2009, 2010, and 2011 in the major soybean growing areas of the state. In each field, ten 3 × 3 m sampling

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grids were selected systematically in a zig-zag pattern, and the center of each grid was referenced for site-specific resampling using a hand-held global positioning system device (GeoExplorer 2008 series Trimble, Westminster, CO). From each grid, twenty 2.5-cm-diameter, 15- to 20-cm-deep soil cores were collected every 60 cm and mixed to a composite sample. The SCN population density (eggs/100 cm³ of soil) in each grid was determined from a 100 cm³ subsample using standard sieving techniques for SCN (Khan 2008). Next, an average population density from the 10 grids was determined for each field. Each field was sampled twice: in midspring, before corn planting, and in midspring of the following year, preceding soybean planting. The SCN population density determined in the first spring sampling is herein referred to as SCN initial population density (*P*_i) or population density before rotation, and the population density estimated in the second spring, after annual rotation, is referred to as SCN final population density (*P*_f).

Model construction. Construction of the model was carried out in four major steps: (i) selection of input variables, (ii) preliminary diagnostics and fit of the first model, (iii) model refinement and selection, and (iv) model validation. Each of these steps is described next.

Model input variables. The response variable was the SCN *P*_f in each field, expressed as the number of SCN eggs/100 cm³ of soil. The explanatory variables were selected based on published research on relationship of environmental factors with SCN. In addition, other variables that were considered to affect SCN during the rotation year were also included. The set of variables comprised: (i) SCN *P*_i, (ii) soil pH, (iii) soil organic matter (OM; %), (iv) a soil texture index (TI), (v) accumulated rainfall in the corn growing season (AR; mm), (vi) number of days with soil temperature below freezing in the off-season (ST; °C), (vii) tillage as a binary indicator variable (= 0 if no-till, 1 otherwise), and (viii) irrigation (= 0 if nonirrigated, 1 otherwise). Soil pH, OM, and TI for each field were determined from a composite sample representing each field and analyzed by a commercial soil testing laboratory (Wards Laboratories, Inc., Kearney, NE). The TI was derived from a principal component analysis of the sand, silt, and clay proportions in the composite soil sample (Pérez-Hernández and Giesler 2014). In such analysis, sand, silt, and clay showed high collinearity and were reduced to the first principal component, which explained 93% of the total variability in the data set. The score of that principal component represented the TI in each field.

The variables AR and ST were obtained for each field from the Nebraska Rainfall Assessment and Information Network (NeRAIN 2013), weather stations of the National Weather Service (NWS, 2013), and automated weather stations of the High Plains Regional Climate Center (HPRCC 2013). The AR was estimated for the corn growing season (1 May to 30 October), whereas ST was estimated for the off-season (1 November to 30 April). AR for each field was estimated by inverse distance weighting (IDW) interpolation using daily precipitation data from weather stations located within a 25-km radius from each target field. The distance from the weather station of interest to each target field was calculated from the latitude and longitude of both the station and the target field using the Haversine formula (Sinnott 1984) in a routine written in Python programming language (Python 3.1.5., Python Software Foundation, Delaware, U.S.A.). The procedure for the IDW is given by:

$$\hat{x} = \sum_{i=1}^n x_i w_i / \sum_{i=1}^n w_i,$$

where: $\hat{x}$ is the IDW estimate, x_i is the particular measurement at the *i*th surrounding station, and the weight function w_i is derived from the inverse of the distance between the target field and the *i*th surrounding station (Hubbard and You 2005). Data of ST, also estimated by IDW spatial interpolation, were estimated from daily average soil temperature of the closest automated weather station to a target field. Available automated stations were within a maximum radius of 35 km of the target fields, and the corresponding distance from a station to a target field was calculated as described above. Information on tillage and irrigation was obtained from direct assessments of the field during the sampling, and from agronomic questionnaires sent to the cooperating growers.

Preliminary diagnostics and fit of first model. Preliminary diagnostics for the response and explanatory variables was done by examination of the marginal distribution of the response variable (*P*_f), description of *P*_f mean and variance relationship, and summary statistics for the predictors. The quantitative explanatory variables were first evaluated for their association with *P*_f using correlation analysis (PROC CORR of SAS, SAS Institute Inc., Cary, NC). Given that the response variable is a count, the standard Poisson model was fitted first. This Poisson regression model was not pursued because true, severe overdispersion was confirmed in the data (Hilbe 2011; Stroup 2013). Further, since neither too many nor no zeroes existed in the data set for consideration of other Poisson model variations, a negative binomial model with traditional parameterization (NB2; Hilbe 2011) was implemented (PROC GENMODE of SAS; *lm-nbg* function of R). The NB2 model with mean μ and variance function $\mu + \alpha\mu^2$ has density:

$$f(y_i | \mu, \alpha) = \frac{\Gamma(y_i + \alpha^{-1})}{\Gamma(y_i + 1)\Gamma(\alpha^{-1})} \left(\frac{\alpha^{-1}}{\alpha^{-1} + \mu} \right)^{\alpha^{-1}} \left(\frac{\mu}{\alpha^{-1} + \mu} \right)^{y_i}$$

where: $\alpha \geq 0$, $y = 0, 1, 2, \dots$, and $\Gamma(\cdot)$ is the gamma function (Cameron and Trivedi 2013).

Due to the relatively small data set and number of selected explanatory variables, fitting of a full second-order model was not considered and neither was the use of an automated algorithm for selection of a candidate model. Instead, a full first-order model was fitted to the data (*P*_i natural log-transformed for consistency with the negative binomial model link function), and the variables that showed significance (*P* value) of at least 0.50 were retained. To further ensure that important variables were not left out, the model was fitted to one variable at a time, and only variables with a significance ≤ 0.50 were also retained. With the retained variables up to this stage, as a final step in the selection of a candidate model, a full first-order model was fitted and the proper functional form of the predictor variables was assessed using partial regression plots (*avPlots* function in the *car* package of R: R Foundation for Statistical Computing, Vienna, Austria). Partial regression plots are scatterplots of residuals, where the residuals in the y-axis are obtained from regressing the response on all the regressors, but a regressor x_i . Therefore, these residuals are the part of the response variable that is not explained by all the regressors except x_i (Fox and Weisberg 2011). Correspondingly, the residuals in the x-axis are obtained by regressing x_i on the other regressors. These residuals represent the part of x_i that is not explained by the other regressors or that remains when the other regressors are conditioned (Fox and Weisberg 2011). Following assessment of the partial regression plots, a candidate model was constructed with the explanatory variables that showed a significant relationship (linear or quadratic) with or explanatory power to the response.

Model refinement and selection. The goodness-of-fit and refinement of the candidate model selected up to this point was done through residual analysis, determination of influential observations, and goodness-of-fit tests. Residual analysis included Pearson and Deviance residuals, with generated 95% confidence intervals as graphical envelopes for the latter according to the procedure proposed by Svetliza and Paula (Svetliza and Paula 2003). Presence of influential observations was determined through difference in fitted values (DFFITS), Cook's Distance, and DFBETAS methods, implemented in the R software. Also, the leverage of identified unusual observations was assessed using the hat-values as a leverage measure (*influenceIndexPlot* function of the *car* package of R) (Fox and Weisberg 2011). Goodness-of-fit tests included deviance, likelihood ratio tests (Hilbe 2011), as well as the Akaike's (AIC) and Bayesian information (BIC) criteria (PROC GENMOD of SAS). In addition, special attention was paid to the value of the dispersion statistics or Pearson χ^2 for assessment of the equidispersion in the model. After the refinement process was completed, goodness-of-fit tests were performed again on the model. The model developed at this stage was the final model and was fitted using robust estimation of standard errors (Hilbe 2014).

Model validation. The final model was validated in two random samples containing 50 and 75% of the observations from the original data set (25 and 35 data points, respectively). The random samples were taken from the data set using the SURVEYSELECT procedure of SAS. Model performance was validated through the relative change in the regression coefficients of each of the predictors and their associated standard errors and significance level. Predictions of single observations from the data set were also made to verify model performance. In addition, predicted versus observed P_f values for the random samples was assessed with simple linear regression.

Results

Distribution of the response, summary statistics, and correlations. The response variable (SCN P_f) showed a skewed distribution (Fig. 1) and extremely different values for the unconditional mean and variance, which had a nonlinear relationship, thus suggesting overdispersion. The suggested overdispersion was confirmed by the high value of the Pearson χ^2/df or dispersion statistics ($=18.5$) of the Poisson regression model that was fitted to the data before the construction of the negative binomial regression model. The mean and variance of P_f varied for each level of tillage, suggesting this variable as a good P_f predictor candidate. Significant positive correlations ($P \leq 0.05$) were observed between the response variable (P_f) and P_i , pH, and ST (Table 1). Positive correlations

Fig. 1. Frequency distribution of the SCN population density (eggs/100 cm³ of soil) observed after annual corn rotation (P_f) in the selected fields in Nebraska.

Table 1. Pearson correlation coefficients between the response variable (P_f) and the quantitative explanatory variables used in model development

Variable	P_f	P_i	pH	OM	TI	AR	ST
P_f^s	1.00	0.84 a ^t	0.27 b ^t	-0.03 c ^t	-0.12 c	-0.10 c	0.29 a
P_i^u		1.00	0.05 c	-0.18 c	-0.26 b	-0.07 c	0.29 a
pH ^v			1.00	0.42 a	0.41 a	-0.11 c	-0.05 c
OM ^w				1.00	0.95 a	0.01 c	-0.25 c
TI ^x					1.00	-0.11 c	-0.22 c
AR ^y						1.00	0.17 c
ST ^z							1.00

^s SCN final population density (eggs/100 cm³ of soil) or population density after annual corn rotation.

^t a, Significant ($P \leq 0.05$). b, Marginally significant (P between 0.06 and 0.10). c, Not significant ($P > 0.10$).

^u SCN initial population density (eggs/100 cm³ of soil) or population density before corn rotation.

^v Soil pH.

^w Soil organic matter.

^x Soil texture index obtained from principal component analysis of sand, silt, and clay.

^y Accumulated rainfall in mm in the corn growing season (1 May to 30 October).

^z Number of days with soil temperature below freezing in the off-season (1 November to 30 April).

were also observed between pH and OM and pH and TI. The variable P_i was positively correlated with ST and negatively correlated with TI.

Negative binomial regression model. The full first-order negative binomial regression model fitted to the eight variables indicated significant predictor capability for P_i , pH, and tillage (Table 2). TI showed marginal significance ($P = 0.09$) and OM, AR, ST, and irrigation were not significant at the 0.05 level. Fitting of each variable at a time showed significance only for SCN P_i , soil pH, and ST (Table 3). The partial regression plots with six retained variables suggested that P_i had a linear relationship with P_f given the rest of the variables were in the model (Fig. 2A). Variable pH was also linearly related to P_f given that the other variables were present in the model (Fig. 2B). Tillage and TI showed a weak linear relationship with P_f , but neither a quadratic nor a cubic functional form was suggested by the partial regression scatter plot (Fig. 2C and D). Plots for irrigation and ST showed a random scattering of points suggesting no relationship with the response variable P_f given the other variables were in the model (Fig. 2E and F). In all the partial regression plots,

Table 2. Estimates of the regression coefficients, associated standard errors, and significance of the fixed effects (intercept and explanatory variables) from fitting of the full first-order negative binomial regression model

Effect	Estimate	Std error	95% Confidence limits	Z value	P > Z
Intercept	-2.2029	1.0124	-4.1872 -0.2186	-2.18	0.0296
Log P_i^t	0.8158	0.0579	0.7024 0.9292	14.10	<0.0001
pH ^u	0.4131	0.0683	0.2793 0.5469	6.05	0.0001
OM ^v	-0.0421	0.1468	-0.3298 0.2456	-0.29	0.7744
TI ^w	-0.0828	0.1132	-0.3047 0.1392	-0.73	0.4649
AR ^x	0.0003	0.0009	-0.0015 0.0021	0.30	0.7606
ST ^y	0.0013	0.0018	-0.0031 0.0038	0.19	0.8475
Tillage ^z	0.4266	0.1782	0.0773 0.7758	2.39	0.0167
Irrigation ^z	-0.2013	0.2062	-0.6054 0.2029	-0.98	0.3291

^t SCN initial population density (eggs 100/cm³ of soil, natural log-transformed).

^u Soil pH.

^v Soil organic matter.

^w Soil texture index obtained from principal component analysis of sand, silt, and clay.

^x Accumulated rainfall in mm in the corn growing season (1 May to 30 October).

^y Number of days with soil temperatures below freezing in the off-season (1 November to 30 April).

^z Entered in the model as binary variables.

Table 3. Estimates of the regression coefficients and their associated standard errors and significance after fitting the negative binomial model with one variable at a time

Effect	Estimate	Std error	95% Confidence limits	Z value	P > Z
Log P_i^t	0.8347	0.0592	0.7187 0.9508	14.10	<0.0001
pH ^u	0.5043	0.1522	0.2061 0.8026	3.31	0.0009
OM ^v	-0.0198	0.1603	-0.3341 0.2944	-0.12	0.9017
TI ^w	-0.0590	0.1001	-0.2553 0.1372	-0.59	0.5554
AR ^x	0.0003	0.0009	-0.0015 0.0021	0.30	0.7606
ST ^y	0.0105	0.0042	0.0023 0.0187	2.50	0.0125
Tillage ^z	0.2221	0.2863	-0.3392 0.7833	0.78	0.4380
Irrigation ^z	-0.6135	0.3464	-1.2924 0.0653	-1.77	0.0765

^t SCN initial population density (eggs 100/cm³ of soil, natural log-transformed).

^u Soil pH.

^v Soil organic matter.

^w Soil texture index obtained from principal component analysis of sand, silt, and clay.

^x Accumulated rainfall in mm in the corn growing season (1 May to 30 October).

^y Number of days with soil temperatures below freezing in the off-season (1 November to 30 April).

^z Entered in the model as binary variables.

observations 32 and 37 stood out as potential outliers. This issue was dealt with more carefully in the model refinement step.

Based on all the aforementioned assessments, a candidate model with the variables P_i , pH, tillage, and TI was selected (Table 4). The model is written as:

$$\widehat{\text{Log}}(Pf) = -\hat{\beta}_0 + \hat{\beta}_1 \text{Log}P_i + \hat{\beta}_2 pH + \hat{\beta}_3 \text{Tillage} - \hat{\beta}_4 TI.$$

The Pearson χ^2/df corresponding to the dispersion statistics for this model was 1.02.

Model refinement and selection. *Residual plots.* Pearson and deviance residual plots showed no systematic features or patterns, indicating overall a good fit of the candidate model (Figs. 3 and 4). Only the residuals for texture index show a slight pattern for low and high values. The 95% confidence interval for the deviance residuals comprised all observations, except a couple of observations that appeared slightly out of the interval boundaries (Fig. 4). The lack-of-fit test computed for the numerical predictors in this model indicated no lack-of-fit (t values of 0.036, 0.025, and 0.013 for P_i , pH, and TI, respectively, with associated P values of 0.85, 0.87, and 0.91). However, the Pearson residual value of observations 32 and 37 consistently appeared isolated from the rest of the cloud, suggesting these observations could be extreme and, likely, influential in the model fit (Fig. 3A–D). These observations also stood out as extreme in the partial regression plots (Fig. 2A–F).

Influential observations. The difference in fitted values (DFFITS) method suggested observation 32 as influential on single fitted values according to the DFFITS guidelines for influential observations for small to medium sized data sets (Fig. 5). Cook's Distance (D) method identified observations 22, 32, and 37 as having the greatest Cook's Distance (Fig. 6), yet none of these observations were influential on all predicted values based on the Cook's Distance guidelines for

influential observations: if $D > F(0.50, p, n-p)$, where p is the number of predictors and n is the number of observations in the data set, then observation is influential. Value of D for observations 22, 32, and 37 was 0.1208, 0.1251, and 0.1252, respectively. The DFBETAS method, which measures the influence of the i th observation on each estimated regression coefficient, identified observation 32 as influential only on the regression coefficient for texture index (Fig. 7).

Follow up assessment of observations 32 and 37 confirmed they had a high leverage in the estimators of the regression coefficients and their associated significance level in the candidate model (Table 5; Fig. 6). In other words, the coefficient estimates and their corresponding P -values in the candidate model changed significantly with and without these observations (Table 5). In particular, the sign of the regression coefficient for tillage changed from positive to negative, and the regression coefficient for TI changed by 55% (Table 5) when the model was fitted to the data set without observations 32 and

Table 4. Model information criteria and deviance of the starting full first order model and the selected candidate negative binomial regression model

Model	Information criteria		
	AIC ^v	BIC ^w	DVC ^x
Starting model ^y	671.97	682.81	2.22
Candidate selected model ^z	527.64	541.64	1.32

^v Akaike's information criterion.

^w Bayesian information criterion.

^x Deviance.

^y Full first-order negative binomial regression model with the predictors SCN initial population density (P_i , natural log-transformed), soil pH, texture index (TI), tillage and irrigation.

^z Negative binomial regression model: $\widehat{\text{Log}}(Pf) = -\hat{\beta}_0 + \hat{\beta}_1 \text{Log}P_i + \hat{\beta}_2 pH + \hat{\beta}_3 \text{Tillage} - \hat{\beta}_4 TI$ selected after fitting the full first order model and application of remedial measures.

Fig. 2. Partial regression plots of the SCN population density after annual corn rotation (P_f) on A, SCN initial population density (natural log-transformed, $\text{Log}P_i$), B, Soil pH, C, Tillage, D, Texture index (TI), E, Irrigation, and F, Number of days with soil temperature below freezing (ST). Regression model was a negative binomial.

37 (Table 5). This confirmed that those observations were influential in the model fit. Regression coefficients for Pi and soil pH, in their turn, underwent little change.

Based on all the above remedial measures and model refinement, the final selected model is the one having SCN initial population density and soil pH as predictors:

$$\widehat{\text{Log}(Pf)} = -\hat{\beta}_0 + \hat{\beta}_1 \text{Log}Pi + \hat{\beta}_2 pH.$$

Fig. 3. Pearson residual plots for the candidate negative binomial regression model: $\widehat{\text{Log}(Pf)} = -\hat{\beta}_0 + \hat{\beta}_1 \text{Log}Pi + \hat{\beta}_2 pH + \hat{\beta}_3 \text{Tillage} - \hat{\beta}_4 TI$. A, SCN initial population density (natural log-transformed, $\text{Log}Pi$), B, Soil pH, C, Tillage, and D, Texture index (TI).

Fig. 4. Plot of deviance residuals (with generated 95% confidence envelopes) against the percentile of a normal distribution for the candidate negative binomial regression model: $\widehat{\text{Log}(Pf)} = -\hat{\beta}_0 + \hat{\beta}_1 \text{Log}Pi + \hat{\beta}_2 pH + \hat{\beta}_3 \text{Tillage} - \hat{\beta}_4 TI$. where $\text{Log}Pi$ is the SCN initial population density (natural log-transformed), pH is the soil pH, and TI is the soil texture index.

With the actual values of the regression coefficients, the model is written as:

$$\widehat{\text{Log}(Pf)} = -2.3360 + 0.8368 \text{Log}Pi + 0.4333 pH,$$

or equivalently: $Pf = \exp^{-2.3360 + 0.8368(\text{Log}Pi) + 0.4333 pH}$. The Pearson χ^2 statistics for this model was 1.04.

Goodness-of-fit tests. The goodness-of-fit tests (log-likelihood ratio and deviance) indicated good fit of the model: Log-likelihood ratio was 61.87 ($P < 0.0001$), and the χ^2 test for the deviance was 34.89 ($P = 0.2878$). The Akaike's and Bayesian information criteria of the

Fig. 5. Difference in fitted values (DFFITS) of the observations after fit of the candidate negative binomial regression model: $\widehat{\text{Log}(Pf)} = -\hat{\beta}_0 + \hat{\beta}_1 \text{Log}Pi + \hat{\beta}_2 pH + \hat{\beta}_3 \text{Tillage} - \hat{\beta}_4 TI$. Influential observations on single fitted values are shown outside the border lines with absolute value >1 .

Fig. 6. Bubble plot of hat-values, studentized residuals, and Cook's distances of the observations after fit of the candidate negative binomial regression model: $\widehat{\text{Log}(Pf)} = -\hat{\beta}_0 + \hat{\beta}_1 \text{Log}Pi + \hat{\beta}_2 pH + \hat{\beta}_3 \text{Tillage} - \hat{\beta}_4 TI$. Size of the circle is proportional to Cook's Distance. Observations with large hat values are observations with high leverage.

Fig. 7. DFBETAS values of the observations after fit of the candidate negative binomial regression model: $\text{Log}(Pf) = -\beta_0 + \beta_1 \text{Log}Pi + \beta_2 pH + \beta_3 \text{Tillage} - \beta_4 TI$, where **A**, SCN initial population density, **B**, Soil pH, **C**, Tillage, and **D**, Texture index. Influential observations on the estimated coefficients are shown outside the border lines with absolute value >1 .

final model were 510.82 and 516.93, respectively, whereas for the intercept-only model, these information criteria were 568.7 and 571.8. The Pearson χ^2/df or dispersion statistics was 1.04 for the final model and 1.16 for intercept-only model.

Model validation. Little change in the regression coefficients and their associated significance level was observed in the model fitted to the 50 and 75% random samples from the data set (Table 6). In the 50% random sample, the relative change in the regression coefficients was 11.3% for the intercept, 1.2% for β_1 , and 10.1% for β_2 , whereas in the 75% sample the relative change was 3.7, 1.6, and 0.07%, for the intercept, β_1 , and β_2 , respectively (Table 6). The sign and level of significance for the coefficients did not change in either sample. The R^2 value from the regression between the observed and estimated SCN final population density values in the 50% sample data set was 0.79, and the slope of the regression line was not significantly different from 1 ($P = 0.99$, Fig. 8). Likewise, in the 75% sample data set, R^2 was 0.84 and the slope was not significantly different from 1 ($P = 0.97$, Fig. 9). The model showed a good performance in predicting single observations (Table 7).

Discussion

A modeling approach was used in this study in an attempt to identify major factors associated with the SCN population density reduction observed after annual corn rotation in Nebraska. A final regression model with SCN spring population density (Pi) and soil pH as predictors was developed. The final model, based on the

Table 5. Estimates of the regression coefficients, significance level, and relative percent change in the coefficient estimates after fit of the candidate negative binomial regression model before and after removal of observations 32 and 37

Coefficient	Whole data set			Without observation 32			Without observation 37			Without 32 and 37
	Estimate	Std error	$P > Z$	Estimate	$P > Z$	Relative change (%)	Estimate	$P > Z$	Relative change (%)	Relative change (%)
Intercept	-2.5384	0.6009	<0.0001	-2.4292	<0.0001	4.3	-2.6171	<0.0001	3.1	1.2
$\text{Log}Pi^v$	0.8342	0.0511	<0.0001	0.8492	<0.0001	-1.8	0.8325	<0.0001	0.2	-1.6
pH^w	0.4395	0.0690	<0.0001	0.4136	<0.0001	5.9	0.4446	<0.0001	1.16	4.6
Tillage ^x	0.3340	0.1488	0.0248	-0.2688	0.0423	180.5	-0.3796	0.0030	213.7	5.4
TI^y	-0.0830	0.0550	0.1312	-0.0578	0.2200	30.4	-0.0680	0.2327	18.1	46.9
		1.02 ^z			1.15 ^z			0.98 ^z		1.11 ^z

^v SCN initial population density natural-log transformed.

^w Soil pH.

^x As a binary variable: till versus no till.

^y Texture index calculated from a principal component analysis of sand, silt, and clay.

^z Model dispersion statistics or Pearson χ^2/df .

Table 6. Relative change in the estimates of the regression coefficients of the final negative binomial regression model fitted to two random samples compared with fit to the entire data set

Coefficient	Whole data set			50% random sample			75% random sample		
	Estimate	Std error	$P > Z$	Estimate	$P > Z$	Relative change (%)	Estimate	$P > Z$	Relative change (%)
Intercept	-2.3360	0.6009	<0.0001	-2.0731	0.0035	11.3	-2.2457	0.0038	3.9
$\text{Log}Pi^x$	0.8368	0.0511	<0.0001	0.8266	<0.0001	1.2	0.8234	<0.0001	1.6
pH^y	0.4333	0.0690	<0.0001	0.3870	<0.0001	10.1	0.4336	<0.0001	0.07
		1.04 ^z			1.01 ^z			0.95 ^z	

^x SCN initial population density (eggs 100 cm^{-3} of soil, natural log-transformed).

^y Soil pH.

^z Model dispersion statistics or scaled Pearson χ^2/df .

Table 7. Analysis of fixed effects (intercept and explanatory variables) from fitting of the final negative binomial regression model using robust estimation of standard errors

Effect	Estimate	Std error	95% Confidence limits		Z value	$P > Z$
Intercept	-2.3360	0.7171	-3.7414	-0.9306	-4.40	<0.0001
Pi^x	0.8368	0.0627	0.7138	0.9597	18.49	<0.0001
pH^y	0.4333	0.0849	0.2670	0.5997	8.01	<0.0001
	1.04 ^z					

^x SCN initial population density (eggs $100/\text{cm}^3$ of soil, natural log-transformed).

^y Soil pH.

^z Model dispersion statistics or Pearson χ^2/df .

negative binomial distribution, was developed through a careful cycle of model specification, estimation, testing, and validation. The type of parameterization of the negative binomial used in this study, referred to as traditional negative binomial model or NB2, is the most common implementation of the negative binomial (Cameron and Trivedi 2013). In this type of parameterization, the conditional variance is modeled as a quadratic function of the mean: $\mu + \alpha\mu^2$ and model parameters are estimated with standard maximum likelihood (Hilbe 2014). Equidispersion, a condition assumed by the standard Poisson distribution, stipulates equality of the conditional mean and variance and is a crucial requirement in count data models for efficient estimators and valid inference (Hilbe 2014). In the present study, a rigorous assessment of overdispersion was made during the model development process. The overdispersion statistics in the final model developed in the study indicated 4% of unexplained correlation in the data. Given the relatively small data set on which the model was built and the parsimony of the model itself, this unexplained correlation was regarded as acceptable in selection of the final model.

On model construction. Given the size of the data set on which the model was built, little emphasis was placed on fitting models with main effects, quadratic forms, and interactions. Instead, focus was on fitting a few models with explanatory variables for which an association with the observed population density after annual corn rotation (P_f) was consistently suggested by the diagnostics, goodness-of-fit tests, and remedial measures. Since P_f is a count type response variable and because its unconditional distribution was highly skewed, a Poisson model appeared to be the first natural model choice to pursue. However, true overdispersion was confirmed, thus suggesting use of an alternative model to Poisson.

Model interpretation. The final model $P_f = \exp^{(-2.3360 + 0.8368(\text{Log}P_i) + 0.4333\text{pH})}$ developed in this study suggested that the SCN population density reduction after annual corn rotation depended greatly on the SCN P_i and soil pH. In the context of the population of inference for this study and applying the model equation to different scenarios of P_i and pH, the model indicates that within the SCN initial population density range 363 to 16,954 eggs/100 cm³ of soil, a

10% change in P_i will result in an 8.3% change in P_f , holding pH constant. Likewise, within the pH range 5.5 to 8.1, for every unit increase in soil pH, SCN P_f is expected to increase by 53.7% holding P_i constant. In terms of SCN population density reduction, the general interpretation of the model is that in fields with high pH soils, the annual SCN population density reduction is expected to be lower at constant P_i . Likewise, the higher the SCN spring population densities (before rotation), the lower the SCN population density reduction at constant soil pH. The pH range values on which this model was built (5.5 to 8.1) is representative of the soil pH values found in Nebraska fields where soybean is grown. Our model establishes a quantitative relationship of field SCN mortality with pH for the first time. The range values of the response variable varied from 590 to about 16,000 eggs/100 cm³ of soil, which encompasses what in the literature is considered as low and high ranges of SCN population densities.

Relationship between variables. The relationship between soil pH and the SCN final population densities revealed from our model is in agreement with the association between soil pH and SCN reported in other studies (Francel 1993; Pedersen et al. 2010; Rogovska et al. 2009). The general observation is that higher SCN population densities occur in higher pH soils. The specific mechanisms by which soil pH affects SCN are not well understood, although it is reported that pH can affect hatching and survival of nematode species other than SCN (Rogovska et al. 2009). We speculate that high pH could prevent SCN cyst hatching during annual corn rotation, that is, in the absence of soybean root stimulus.

While in this data set only SCN P_i and pH showed the strongest explanatory power of the SCN population density reduction after annual corn rotation, the extra 4% correlation (Pearson dispersion statistics = 1.04) not explained by the model suggests that other variables and interactions could contribute to the observed SCN population density decline. In our analysis, other variables suggested as potential predictors of SCN P_f were tillage, texture index, and soil temperature. These variables were relatively near the marginal level of significance in the model construction process both in the first-order and the candidate model, and they have been associated to

Fig. 8. Relationship between the log of the observed SCN population density after annual corn rotation in Nebraska (P_f) and the log of the SCN P_f predicted with the final negative binomial regression model built on a 50% random sample. Model included SCN initial population density and soil pH as predictors. Solid line is the regression line and dotted line is the line with slope 1 passing through the origin. The regression equation and the R^2 , rounded to nearest two decimals, are shown in the graphic.

Fig. 9. Regression of the log of the observed SCN population density after annual corn rotation in Nebraska (P_f) versus the log of the SCN P_f predicted with the final negative binomial regression model in a 75% random sample from the original data set. Model included SCN initial population density and soil pH as predictors. Solid line is the regression line and dotted line is the line with slope 1 passing through the origin. The regression equation and the R^2 , rounded to nearest two decimals, are shown in the graphic.

SCN population densities (Workneh et al. 1999). Previous studies on the relationship of soil texture with SCN indicate that higher SCN population densities are more commonly found in sandy than in fine-textured soils (Pérez-Hernández and Giesler 2014). However, how texture affects SCN mortality remains unclear. In the present study, it is suggested that in the latitudinal range where this study was carried out, texture may be a weak driving factor of the SCN population fluctuations in a normal year (Pérez-Hernández and Giesler 2014). With respect to tillage, previous research suggests that in no-till fields, SCN population aggregation is increased, whereas in till fields aggregation remains low due to SCN dissemination, especially in the direction of tillage (Workneh et al. 1999). If future enhancements are to be done on the model developed in this study, soil texture, tillage, and soil temperature are suggested as explanatory variables to consider with a larger data set than the one used in this study.

In summary, the model developed in this study suggests that SCN spring population densities and soil pH are major determinants of the observed SCN population density reduction after annual corn rotation in Nebraska. This piece of information can be helpful in making decisions regarding SCN management, for example, in deciding on the number of cycles to keep a field under corn cultivation to further abate SCN population densities. The model has potential for use in SCN risk analysis and in predicting SCN population density decline after corn rotation in the state.

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