Nutsedge Counts Predict *Meloidogyne incognita* Juvenile Counts in an Integrated Management System

Zhining Ou,[1] Leigh Murray,[2] Stephen H. Thomas,[3] Jill Schroeder,[3] James Libbin[4]

Abstract: The southern root-knot nematode (*Meloidogyne incognita*), yellow nutsedge (*Cyperus esculentus*) and purple nutsedge (*Cyperus rotundus*) are important pests in crops grown in the southern US. Management of the individual pests rather than the pest complex is often unsuccessful due to mutually beneficial pest interactions. In an integrated pest management scheme using alfalfa to suppress nutsedges and *M. incognita,* we evaluated quadratic polynomial regression models for prediction of the number of *M. incognita* J2 in soil samples as a function of yellow and purple nutsedge plant counts, squares of nutsedge counts and the cross-product between nutsedge counts . In May 2005, purple nutsedge plant count was a significant predictor of *M. incognita* count. In July and September 2005, counts of both nutsedges and the cross-product were significant predictors. In 2006, the second year of the alfalfa rotation, counts of all three species were reduced. As a likely consequence, the predictive relationship between nutsedges and *M. incognita* was not significant for May and July. In September 2006, purple nutsedge was a significant predictor of *M. incognita*. These results lead us to conclude that nutsedge plant counts in a field infested with the *M. incognita*-nutsedge pest complex can be used as a visual predictor of *M. incognita* J2 populations, unless the numbers of nutsedge plants and *M. incognita* are all very low.
Key words: alfalfa, crop rotation, *Cyperus esculentus*, *Cyperus rotundus*, interaction, *Medicago sativa*, *Meloidogyne incognita*, method, perennial weed, Poisson regression, predictive modeling, purple nutsedge, southern root-knot nematode, yellow nutsedge.

Southern root-knot nematode (*Meloidogyne incognita* (Kofoid & White) Chitwood), yellow nutsedge (*Cyperus esculentus* L., hereafter referred to as 'YNS') and purple nutsedge (*Cyperus rotundus* L., hereafter referred to as 'PNS') occur simultaneously in many crops grown throughout the southern and western regions of the US. Previous research (Bird and Hogger, 1973; Hogger and Bird, 1976; Schroeder et al., 1993) has identified relationships between *M. incognita* and common weed species, particularly YNS and PNS, that occur in sandy soils used to produce cotton and chile pepper (*Capsicum annuum* L., hereafter referred to as 'chile'). Yellow nutsedge, purple nutsedge and *M. incognita* are largely dependent on passive dissemination and are well adapted to a mutually beneficial coexistence that sustains and enhances the pest complex (Schroeder et al., 1994; Thomas et al., 1997; Schroeder et al., 2004; Thomas et al., 2004; Schroeder et al., 2005; Thomas et al., 2005). Management that targets the pests individually has not been successful or sustainable due to these beneficial interactions (Schroeder et al., 1994, 2004; Thomas et al., 2005). Successful management requires a comprehensive strategy. Crop rotation with a nondormant, *M. incognita*-resistant alfalfa, which has aggressive growth and can compete with nutsedge for light and other resources, can provide simultaneous suppression of all three pests, eliminate the need for 1,3-dichloropropene fumigation in the subsequent crop, and pro-vide an economical rotation option for New Mexico chile growers (Fiore, 2004).

Field nematode counts are frequently highly skewed and usually non-Normally distributed, so statistical techniques which assume Normality and constant variance of the response variable are not appropriate in fitting analyses of variance or regression models (i.e., linear models) (Goodell and Ferris, 1980). There are two common practices to solve the problem of non-Normality and/or non-constant variance. First, the older method is that of transformation of the data (Neter et al., 1983). For example, stability of variance and symmetry of the probability distribution can often be obtained by a transformation of the form $\log (x + c)$ on observed data x, where c is a constant (Proctor and Marks, 1975). However, sometimes the transformation that best produces Normality may be different from the transformation that best produces constant variance (McCullagh and Nelder, 1989). Moreover, statistical results obtained on the transformed scale are not necessarily applicable to the original scale of measurement and hence are not easily interpreted (Finney, 1941; Patterson, 1966). For example, the second author of this paper has seen cases where means of logged data and the corresponding means of the original data are in a different order.

The second, more modern approach is the technique of Generalized Linear Models (McCullagh and Nelder, 1989; Littell et al., 2002), which directly uses a probability distribution that is more appropriate than the Normal for a given set of data. For example, important non-Normal probability distributions for count data include the Poisson and the Negative Binomial (McCullagh and Nelder, 1989). Most nematode field data show an aggregated pattern and hence follow a Negative Binomial distribution, where the variance is larger than the mean (Ferris, 1984; McSorley, 1998). This is the case of over-dispersion, which frequently happens when population counts are high and likely

skewed (Bliss and Fisher, 1953; Onsager, 1981). A Poisson distribution is suitable for counts when the variance equals the mean. This situation frequently happens when population counts are consistently low with no high counts and hence are not over-dispersed (Bliss and Fisher, 1953; Onsager, 1981). Onsager (1981) hypothesized that in an integrated pest management system the Poisson might be a more appropriate probability distribution to model counts than the Negative Binomial because of low population counts.

The Generalized Linear Models procedure typically uses maximum likelihood estimation, applied to probability distributions (like the Poisson or Negative Binomial) to fit analysis of variance or regression models to some known function (called the "link function") of the distribution mean (McCullagh and Nelder, 1989). Unlike values generated by transforming the data and then fitting a model using Normal distribution, the predicted values from the fitted Generalized Linear Model can be correctly "inverted" or transformed back into the original scale of measurement by the "inverse link function" (McCullagh and Nelder, 1989), and results between the two scales are consistent. Note also that a good model constructed with an appropriate distribution can sometimes resolve a problem of over-dispersion due to model misspecification of the true predictive relationship.

Given the beneficial relationships between nutsedges and *M. incognita*, we hypothesized that the location of the nutsedge populations in an alfalfa field predicts occurrence of greater J2 populations and probable areas of nematode resurgence in subsequent crops. Additional management of the nutsedges in a crop following alfalfa may prolong the suppression of *M. incognita*. The objective of this work was to evaluate quadratic polynomial regression models for prediction of the number of *M. incognita* J2 in soil samples as a function of yellow and purple nutsedge counts, squares of nutsedge counts and the cross-product between nutsedge counts.

MATERIALS AND METHODS

Plot conditions: A 2-yr study was initiated in September 2004 at the Leyendecker Plant Science Research Center, New Mexico State University, near Las Cruces, NM, on an Anthony-Vinton fine sandy loam soil (coarse-loamy, mixed [calcareous], thermic Typic Torrifluvent; sandy, mixed, thermic Typic Torrifluvent) (79% sand, 12% silt, 9% clay; 0.8% organic matter; pH 8.1). Environmental conditions were monitored by a Campbell CR-10 Measurement and Control Module (Campbell Scientific Inc., Logan, UT) (Table 1).

The field was uniformly cropped with oats in 2004 prior to initiating the experiment. During field preparation, fertilizer was applied at 280 kg 11–52–0 (N-P-K)/ha. The herbicide benefin at 1.34 kg a.i./ha

TABLE 1. Soil temperature and precipitation from an alfalfa field infested with *M. incognita* and yellow and purple nutsedge in Doña Ana County, NM.

Alfalfa growing season	Soil temperature (°C)		No. of d > 10°C[a]	Monthly cumulative precipitation (mm)
	Maximum	Minimum		
2004				
Sept.	37.35	7.89	30	53.09
Oct.	31.72	2.51	31	25.40
Nov.	17.14	2.47	20	38.10
Dec.	10.37	0.15	1	7.62
2005				
Jan.	13.17	2.42	10	19.05
Feb.	14.17	1.57	22	9.94
Mar.	19.36	5.64	30	10.16
Apr.	21.08	8.70	30	7.11
May	29.73	11.55	13[b]	13.97
June	38.78	16.39	23[b]	0.51
July	38.77	20.97	31	14.99
Aug.	36.22	15.75	31	28.19
Sept.	35.88	20.87	16[b]	37.08
Oct.	25.81	12.89	28	41.40
Nov.	20.28	3.32	28	0.00
Dec.	12.43	1.19	8	0.00
2006				
Jan.	10.00	2.79	0	0.76
Feb.	15.87	3.93	16	5.33
Mar.	17.24	7.46	30	0.00
Apr.	36.12	10.53	30	0.25
May	41.07	11.69	31	2.79
June	43.97	18.38	30	2.54
July	28.85	20.48	12[b]	52.58
Aug.	35.42	22.94	20[b]	91.95
Sept.	28.79	15.84	30	84.33
Oct.	24.18	0.14	31	48.51

[a] Daily maximum soil temperature >10°C.
[b] Data not available for entire month.

was applied as a preplant incorporated treatment for grass and broadleaf weed control. The *M. incognita* J2 population in fall 2004 averaged $63/100$ cm^3 soil, which is about 25 times the damage threshold for chile (Thomas et al., 1995) and is considered a heavy infestation. Yellow and purple nutsedge populations were dense and evenly distributed throughout the field.

In September 2004, the field was prepared and planted with nondormant alfalfa (*Medicago sativa*, 'Mecca 2'), which contains approx. 60% African (*M. incognita*-resistant) parentage (I. M. Ray, NMSU, pers. com.) at a seeding rate of 18 kg/ha. Once the alfalfa was established, the field was flood-irrigated from February through September on a monthly schedule. A final irrigation was applied once in the late fall each year; the fields were not irrigated again until the monthly schedule resumed in February. Alfalfa weevil (*Hypera postica*) was controlled as needed throughout the experiment using foliar applications of 0.03 kg a.i. Lambda-cyhalothrin/ha. Alfalfa was harvested six times from April through early November in 2005 and 2006. This alfalfa stand was managed for 2 yr from September 2004 through October 2006 according to production practices common in the Rio Grande valley (I. M. Ray,

NMSU, pers. com.) of southern New Mexico. Although such alfalfa fields are typically maintained for at least 3 yr, previous research suggested that suppression of the *M. incognita*-nutsedge pest complex occurred by the end of the second growing season (Fiore, 2004). Therefore, 2 yr in the alfalfa rotation were considered sufficient to address the above hypotheses.

Data collection: A 55- × 100-m section of the 1 ha alfalfa field that showed historically uniform irrigation properties and crop growth was chosen for intensive data collection. This section was partitioned into a grid with a total of 1,375 plots of size 2- × 2-m and was sampled in May, July and September in both 2005 and 2006. The goal of this sampling strategy was to determine both the density and distribution of the three pest species during the alfalfa rotation. The soil samples were paired with the nutsedge plants (if present) to determine whether the nutsedge and nematode populations were associated in the field.

At each sample date, 80 2- × 2-m plots were randomly selected out of the 1,375 plots. No plots were sampled twice in a year. Eighty was the maximum number of plots that could be sampled at any one date, given logistical constraints on personnel and time of sample processing. All samples were obtained from a 0.25- × 1-m quadrat placed in the center of a selected 2- × 2-m plot. Data at each sample date included yellow and purple nutsedge plant counts and *M. incognita* J2 counts recovered from soil. Nematode populations were estimated by compositing 10 50-cm^3 soil cores collected near nutsedge plants (if present) or at random within the 0.25- × 1-m quadrat, if no nutsedge plants were present. Nematodes were extracted from the 500 cm^3 of soil by elutriation, during which the effluent from one-fifth of the drain tubes on the sample splitter was collected (equivalent to J2 per 100 cm^3 soil) (Byrd et al., 1976) and processed using centrifugal flotation (Jenkins, 1964). Juveniles were counted from 10% of the resulting aqueous suspension (equivalent to J2 per 10 cm^3 soil) using a chambered counting slide. Instead of extrapolating to J2 per 100 cm^3 soil, the conventional means of reporting nematode data, J2 counts per 10 cm^3 soil were analyzed directly to meet the requirements of the Poisson distribution.

Statistical analysis: The objective of this work was to determine if there was a relationship between *M. incognita* J2 counts and the plant predictors, yellow nutsedge and/or purple nutsedge counts, the yellow nutsedge by purple nutsedge cross-product and/or the squares of the nutsedge counts. *Meloidogyne incognita* J2 counts are discrete non-negative integers, which often have a skewed frequency distribution and which are often modeled by the Poisson distribution (Bliss and Fisher, 1953; Littell et al., 2002) or the Negative Binomial (Ferris, 1984; McSorley, 1998). Here the Poisson distribution is briefly discussed.

The Poisson distribution has the following "probability distribution function" (hereafter referred to as 'PDF') or equivalently "likelihood function":

$$P(y) = \frac{\exp(-\mu)\mu^y}{y!}, \tag{1}$$

where y is a count measured on an experimental unit, $y = 0, 1, 2, \ldots,$

$y!$ is "y factorial," defined as $y! = y(y-1)(y-2)\ldots(2)(1)$,

$\mu > 0$ is the population mean count of y,

exp( ) is the exponential function on the base of the natural logarithm, equivalent to $e^{()}$.

In addition, the theoretical mean of the Poisson PDF μ and the theoretical variance σ^2 are equal.

A graph of the theoretical Poisson PDF (eqn. 1), with y = counts on the horizontal axis and $nP(y)$ = expected number of experimental units on the vertical axis, shows a right-skewed unimodal shape. For example, the theoretical Poisson frequencies graphed in Figure 1 come from a PDF with population mean count of *M. incognita* J2 $\mu = 1$:

$$P(y) = \frac{\exp(-1)1^y}{y!}.$$

Therefore, the probability of an experimental unit from this specific Poisson distribution having a *M. incognita* J2 count of zero is:

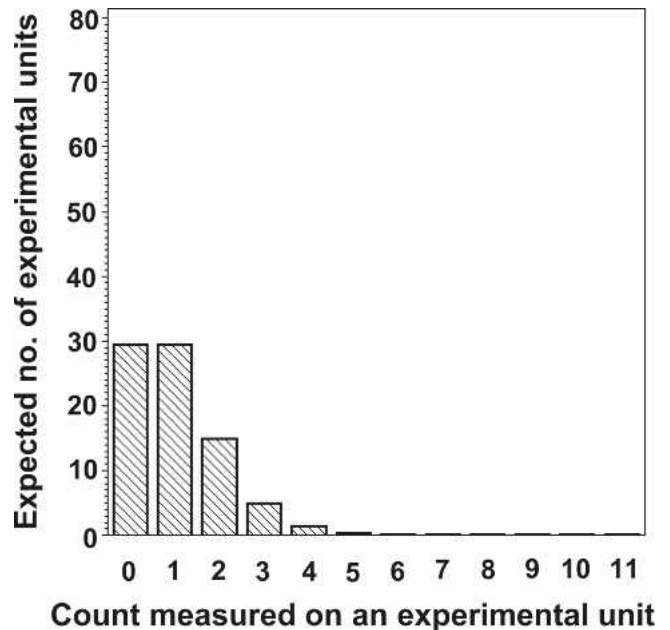$$P(y = 0) = \frac{\exp(-1)1^0}{0!} = 0.3679,$$

FIG. 1. Theoretical Poisson frequency distribution with $\mu = 1$ and $n = 80$ experimental units. Horizontal axis is y = count measured on each experimental unit. Vertical axis is $nP(y)$ = expected number of experimental units with a count of y, where $P(y)$ is the probability of y.

and therefore $nP(y = 0) = (80)(0.3679) = 29.432$ out of 80 experimental units are expected to have a zero J2 count.

Poisson models are an example of Generalized Linear Models (McCullagh and Nelder, 1989), which are commonly based on PDF from the exponential family. The exponential family has the following general PDF:

$$P(y; \theta, \phi) = \exp\left\{\frac{y(\theta) - b(\theta)}{a(\phi)} - c(y, \phi)\right\}, \qquad (2)$$

where y is an observation measured on an experimental unit,

θ is the "canonical parameter" which is some function of μ, the mean count,

$b(\theta)$ is a function of θ,

$a(\phi)$ is some function of the parameter ϕ which is called the "dispersion parameter,"

$c(y, \phi)$ is some function of y and ϕ only, not of θ or μ.

Therefore, rewriting the Poisson PDF (eqn. 1) in the form of the exponential family PDF (eqn. 2), we obtain:

$$P(y; \mu, 1) = \exp\left\{\frac{y[\ln(\mu)] - \mu}{1} - \ln(y!)\right\}, \qquad (3)$$

where $\theta = \ln(\mu)$, so that the natural log function of μ is the "canonical link,"

$$b(\theta) = \exp(\theta) = \mu,$$

$$a(\phi) = \phi = 1,$$

$$c(y, \phi) = -\ln(y!).$$

The assumption $a(\phi) = \phi = 1$ is true if the data are, in fact, generated from a theoretical Poisson distribution. If $\phi > 1$, then the count data are over-dispersed (i.e., the count data are aggregated so that the variance of y is larger than the mean of y), and the Poisson PDF is not appropriate for the data. A goodness-of-fit test discussed below provides an evaluation of this assumption.

In Generalized Linear Models, maximum likelihood is used to fit a linear (i.e., additive) model on the canonical parameter θ, which is related to the mean of the distribution through the non-linear link-function. This linear model can be either an analysis of variance model or a regression model. In this paper, model selection and diagnostics were performed on the specific polynomial Poisson regression model:

$$\begin{aligned} \theta &= \ln(\mu) \\ &= b_0 + b_1(\text{YNS}) + b_2(\text{PNS}) + b_3(\text{YNS})^2 + b_4(\text{PNS})^2 \\ &\quad + b_5(\text{YNS} \times \text{PNS}). \end{aligned} \qquad (4)$$

This model is fitted on the natural log-scale, but can be transformed to the count scale by the formula:

$$\begin{aligned} \mu &= \exp(\theta) \\ &= \exp\{b_0 + b_1(\text{YNS}) + b_2(\text{PNS}) + b_3(\text{YNS})^2 + b_4(\text{PNS})^2 \\ &\quad + b_5(\text{YNS} \times \text{PNS})\}. \end{aligned} \qquad (5)$$

Note that equation (4) is a linear model for θ, but equation (5) is a non-linear model for $\mu = \exp(\theta)$.

Approximate χ^2-tests of hypotheses and confidence limits can be calculated on θ (and equivalently on μ) using large-sample theory, either by the Likelihood Ratio method or by Wald Statistics (Littell et al., 2002; SAS Institute, Cary, NC). Confidence limits on θ are exponentiated to obtain the confidence limits on μ.

A goodness-of-fit test is recommended after fitting a particular model to evaluate the discrepancy between the data and the fitted model. This is especially important when using a one-parameter PDF like the Poisson, for which over-dispersion may be a problem. Two important measures of discrepancy are the Pearson χ^2 and the deviance. The deviance criterion, which is based on the Likelihood Ratio principle, is recommended by McCullagh and Nelder (1989) to evaluate the goodness-of-fit of a model with p predictors in comparison to the "exact fit" model where the number of predictors is equal to the number of observations (McCullagh and Nelder, 1989). The general form of deviance is:

$$2\{\ln[L_{Exact}] - \ln[L_{Fitted}]\} \sim \chi^2_{(n-p)}, \qquad (6)$$

where L_{Exact} is the likelihood function of the "exact fit" model with the number of model parameters equal to n, number of observations, and L_{Fitted} is the likelihood function of the fitted model with $p < n$ model parameters. Specifically, the Poisson deviance has the form (McCullagh and Nelder, 1989):

$$2\{\Sigma[y\ln(y/\hat{\mu}) - (y - \hat{\mu})]\} \sim \chi^2_{(n-p)}, \qquad (7)$$

where $\hat{\mu}$ is the estimated value from equation (5). A rule-of-thumb is that good model fit occurs when the deviance divided by the degrees of freedom is "close" to one (McCullagh and Nelder, 1989). This is because if a model with p parameters describes a data set of n observations very well, then the value of the deviance is expected to be close to the expected value of the χ^2 distribution, which is the degrees of freedom, $n - p$. In a formal approximate χ^2-test, a critical value for a specified significance level α and $n - p$ degrees of freedom can be used to measure the size of the deviance. The model fits the data well if the deviance is smaller than the χ^2 critical value (McCullagh and Nelder, 1989; Dobson, 1990). Otherwise, the model does not fit the data.

Assuming the Poisson is appropriate, a large deviance can occur from fitting a wrong or incomplete model for μ (e.g., important predictors are omitted or the functional form is mis-specified), or if there are influential observations or outliers. In addition, over-dispersion can occur because of standard nematode counting techniques, such as use of chambered counting slides, in which nematodes are counted in a 1 ml aliquot from the 10 ml nematode suspension recovered from a 100 cm^3 soil sample, then multiplied by 10 to extrapolate to 100 cm^3 soil, with the result that the

extrapolated counts are 0, 10, 20, ... , rather than counts of 0, 1, 2, ... , as required theoretically for the Poisson. Poisson models should, therefore, be fitted on actual counts, rather than extrapolated counts. Finally, another possible cause of a large deviance is if the variance of the data is larger than the mean of the data, i.e., the over-dispersion problem. Among the several potential reasons for over-dispersion, probably the most relevant to this study is clustering in the nematode population (McCullagh and Nelder, 1989).

Uncorrected over-dispersion will result in smaller estimates of standard errors, a higher Type I error rate, and narrower confidence intervals than should be the case theoretically. To solve the discrepancy problem caused by clumping population, models based on the Negative Binomial distribution (Young and Young, 1998) or other distributions may be necessary (Littell et al., 2002). Note that the Negative Binomial distribution converges to the Poisson as the positive exponent parameter k, the measure of aggregation, approaches positive infinity (Bliss and Fisher, 1953; Noe and Campbell, 1985).

An alternative solution to the problem of over-dispersion, called "rescaling," is to keep the Poisson distribution but to correct the over-dispersion by modifying the estimated standard errors and test statistics. This modification is done by adjusting the assumed variance by a dispersion parameter ϕ^*, so that the actual variance becomes $\phi^*\mu$ instead of μ. Estimated standard errors and test statistics are adjusted by a "scale parameter," $\sqrt{\hat{\phi}^*}$, which is estimated by the square root of the deviance divided by its degrees of freedom. In this case, the Poisson PDF or likelihood function is modified to become a "quasi-likelihood function" (Wedderburn, 1974). Similar to maximum likelihood estimation, maximizing the quasi-likelihood results in the same point estimates, but with larger standard errors, which are the original standard errors obtained from the original Poisson model but multiplied by $\sqrt{\hat{\phi}^*}$. In addition, confidence intervals are wider, and the chance of making a Type I error is reduced (McCullagh and Nelder, 1989; Littell et al., 2002). The approach using the quasi-likelihood method has high efficiency if over-dispersion is modest (Cox, 1983).

For the nutsedge and *M. incognita* J2 counts, the SAS GENMOD procedure (Littel et al., 2002; SAS Institute, Cary, NC) was used to estimate and test the regression parameters of the Poisson regression model (eqn. 5) using maximum likelihood estimation and Type 3 likelihood ratio χ^2 (Littell et al., 2002). The Poisson distribution with the log link-function was specified by DIST and LINK options, respectively, in the MODEL statement of the procedure. Nonsignificant predictors were deleted, and the models were refitted. When there was possible over-dispersion (i.e., deviance approximate χ^2-test was significant), rescaling was done using the DSCALE option in the GENMOD procedure

MODEL statement. Models fitted with and without the DSCALE option were compared for significance of regression parameters. If models fitted with and without DSCALE were equivalent, then the models fitted with the DSCALE option were reported. Otherwise, the models both with and without the DSCALE option were reported. All tests were done at a significance level of α = 0.10. After final models were obtained, bivariate graphs of fitted models were prepared using the SAS GPLOT procedure, and tri-variate graphs were prepared by the SAS GCONTOUR or G3D procedures (SAS Institute, Cary, NC). Data were analyzed separately for every sample date in each of the two alfalfa rotation years.

RESULTS

Preliminary modeling: As expected, *M. incognita* J2 counts in the sampled quadrats were not Normally distributed (e.g., Fig. 2A,B) and, indeed, visually resemble the Poisson distribution (e.g., Fig. 1). Therefore, Generalized Linear Models with the Poisson probability distribution with the log link-function were used to fit polynomial regression models on actual J2 counts (i.e., based on 10 cm^3 soil) (Table 2). Deviance approximate χ^2-test statistics were greater than the critical χ^2 values for all six fitted regression models in 2005 and 2006, indicating a modest (Cox, 1983) over-dispersion problem with the Poisson distribution. To solve the problem of over-dispersion, we chose the rescaling approach, rather than fitting a different probability distribution. Recall from earlier discussion that, in the rescaling approach, regression coefficient estimates are the same from the original Poisson model but have larger standard errors and tests of hypothesis are less powerful in the rescaled Poisson model, thus resulting in a somewhat conservative approach. Rescaled Poisson polynomial regression models were then fitted, and original and rescaled models were compared. Rescaled and original models were the same with respect to statistical significance of polynomial regression coefficients for all sample dates except July and September 2005. For these two dates, models differed only in the significance of the YNS × PNS cross-product term, which was significant in the original model but not in the rescaled model. Thus, rescaled Poisson models were not substantially different from the original models. Rescaled Poisson models for all dates plus original Poisson models for July and September 2005 are presented for comparison purposes (Table 2).

Fitted models: Results for final models are summarized in Table 2 and presented as follows in order of increasing complexity. In May and July 2006, none of the nutsedge predictors was significant, resulting in intercept-only models (Table 2). In May 2006, the mean number of *M. incognita* J2 was exp(−0.3930) = 0.68/10 cm^3 soil with 90% lower and upper confidence limits of
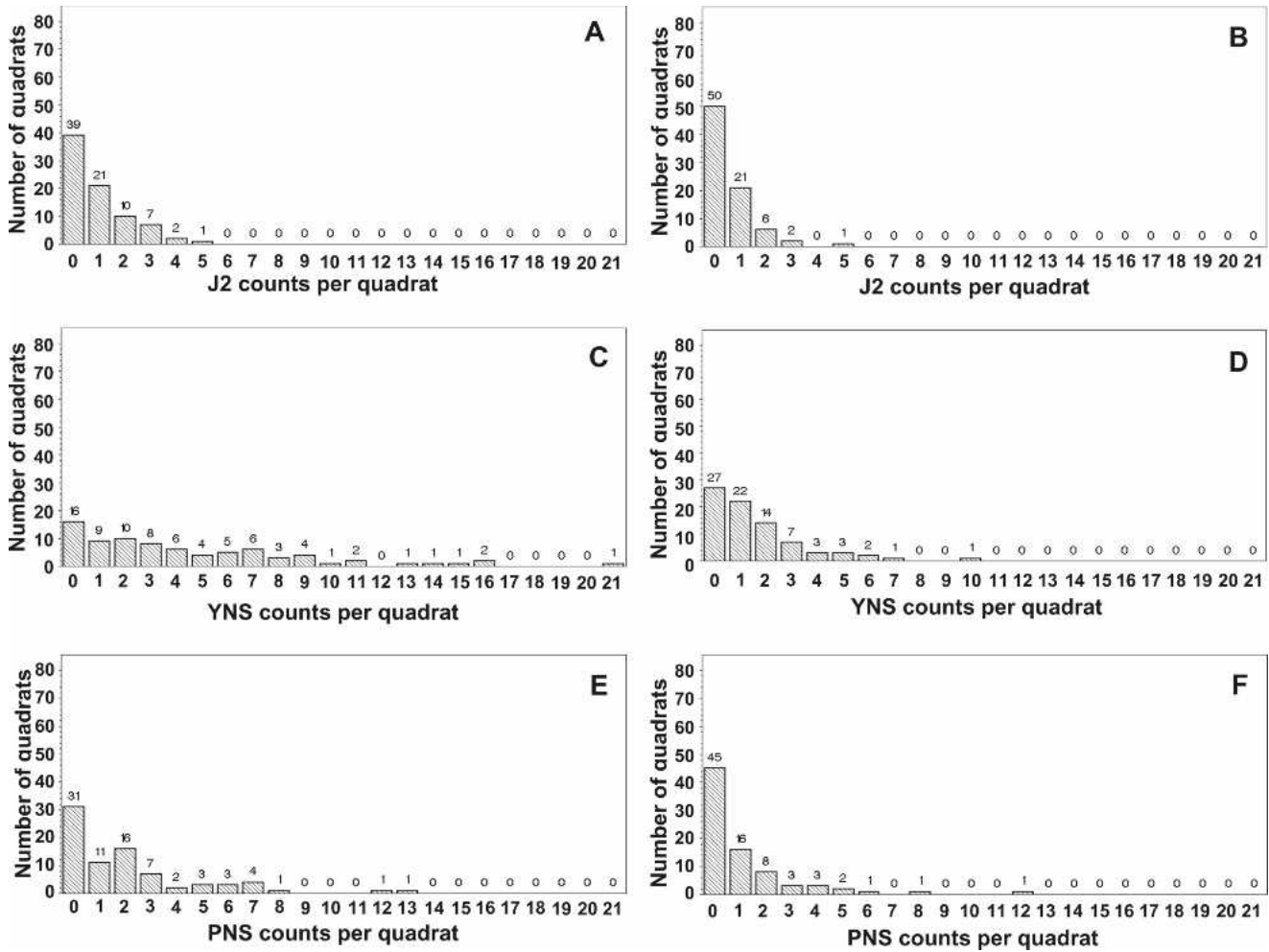
FIG. 2. Observed frequency distribution in July 2005 (A, C, E) and July 2006 (B, D, F) of *M. incognita* J2 counts, yellow nutsedge (YNS) and purple nutsedge (PNS), respectively, of $n = 80$ quadrats of size 0.25 m², from an alfalfa field infested with nutsedges in Doña Ana County, NM.

TABLE 2. Poisson regression models with log link-function of *M. incognita* J2 counts from an alfalfa field infested with yellow and purple nutsedge in Doña Ana County, NM.

Sample dates[c]	Intercept	Estimated predictor coefficients $(\hat{b}_i)$[a,b]					Deviance/*df*	Mean estimates and 90% confidence limits[e]		
		YNS[d]	PNS[d]	$(YNS)^2$	$(PNS)^2$	YNS × PNS		Lower	Mean	Upper
2005										
May, rescaled	0.5893*	—	0.5099*	—	—	—	2.1410			
July, original	−0.6309*	0.0890*	0.1266*	—	—	−0.0148†	1.4760			
July, rescaled	−0.6309*	0.0890*	0.1266†	—	—	−0.0148[NS]	1.4760			
Sept, original	−0.7295*	0.3879*	0.1604*	—	—	−0.1184*	1.6666			
Sept, rescaled	−0.7295*	0.3879*	0.1604†	—	—	−0.1184[NS]	1.6666			
2006										
May, rescaled	−0.3930*	—	—	—	—	—	1.5708	−0.6873	−0.3930	−0.1250
July, rescaled	−0.5978*	—	—	—	—	—	1.2471	−0.8881	−0.5978	−0.3331
Sept, rescaled	−1.2639*	—	0.2617*	—	—	—	1.2155			

[a] Reported as natural log-scale fitted model: $\ln(\hat{\mu}) = \hat{b}_0 + \hat{b}_1(YNS) + \hat{b}_2(PNS) + \hat{b}_3(YNS)^2 + \hat{b}_4(PNS)^2 + \hat{b}_5(YNS \times PNS)$. *Meloidogyne incognita* J2 counts are calculated per 10 cm³ soil sample.

[b] Regression coefficient observed significance level was $P \leq 0.05 = (*)$, $P \leq 0.10 = (†)$ or $P > 0.10 = (NS)$.

[c] Original models were fitted using the original Poisson distribution; rescaled models were fitted using the Poisson distribution with rescaling to correct for over-dispersion problems.

[d] YNS = yellow nutsedge (*Cyperus esculentus*) counts. PNS = purple nutsedge (*Cyperus rotundus*) counts.

[e] If none of the predictors were significant, the predictive model was an intercept-only model: $\ln(\hat{\mu}) = \hat{b}_0$. The 90% lower and upper confidence limits of the predicted mean are reported.

exp(−0.6873) = 0.50 and exp(−0.1250) = 0.88 J2/10 cm³ soil, respectively. Similarly, in July 2006, an intercept-only model indicated that the mean number of J2 was exp(−0.5978) = 0.55/10 cm³ soil with 90% lower and upper confidence limits of exp(−0.8881) = 0.41 and exp(−0.3331) = 0.72 J2/10 cm³ soil. Yellow and purple nutsedge counts were especially low in May 2006 (data not presented), with only four out of 80 quadrats containing YNS plants, one quadrat containing PNS plants, and no quadrat containing both. We note however that three of the five quadrats (60%) with either YNS or PNS also contained *M. incognita* J2, in comparison with *M. incognita* J2 occurring in only 28 out of 75 quadrats (37.3%) with no nutsedge plants. As a comparison, in July 2006, nutsedge populations had increased over May 2006 (Fig. 2D,F), with 27 quadrats out of 80 containing YNS plants, nine containing PNS plants, and 26 containing both. In this case, however, of the 62 quadrats containing one or both nutsedge species, 22 (35%) also contained *M. incognita* J2, while of the 18 quadrats with no nutsedges, 10 (44%) contained no *M. incognita* J2.

In May 2005 and September 2006, which were the beginning and the end of the alfalfa rotation, respectively, the only significant predictor of *M. incognita* J2 counts was PNS count per 0.25 m² quadrat on both sample dates. Both predictive relationships were positive (Table 2; Fig. 3A,B). Recall that the fitted model on the natural log scale is a straight line (eqn. 4), while on the count scale, the predictive relationship is an exponential curve (eqn. 5; Fig. 3A,B). The width of confidence limits increases with increasing predicted J2 mean because of the nature of the Poisson distribution where mean equals variance. The May 2005 predictive curve is higher than the curve of September 2006 with respect to the same number of PNS counts, with no overlap in the area bounded by the lower and upper

90% confidence limits. Thus, nematode counts were higher overall and also increased more rapidly with increasing PNS at the beginning of the first growing season than at the end of the second growing season. In May 2005 (data not presented), only five out of 80 quadrats contained YNS plants, seven contained PNS plants and two contained both (data not presented). However, eight of the nine quadrats (89%) with plants of the significant predictor PNS also contained *M. incognita* J2, as compared to only 51 out of the 71 quadrats without PNS (71.8%) but containing *M. incognita* J2. In September 2006 (data not presented), 22 out of 80 quadrats contained YNS plants, with one quadrat containing the maximum of four YNS plants, 11 out of 80 quadrats contained PNS plants, with two quadrats containing the maximum of three PNS plants, and 26 quadrats containing both YNS and PNS, with one quadrat containing the maximum of three YNS and seven PNS plants. Of the 37 quadrats containing plants of the significant predictor PNS, 10 (27%) also contained *M. incognita* J2, while of the 43 quadrats without PNS, only eight (18.6%) contained *M. incognita* J2.

In both July and September 2005, both YNS and PNS counts were significant positive predictors of *M. incognita* J2 counts with $P \leq 0.10$, with the negative YNS × PNS cross-product term being significant in the original Poisson models but not significant in the more conservative rescaled Poisson models (Table 2). Both original and rescaled Poisson models are reported for these two dates. In particular, in July 2005 (Fig. 2C,E), of quadrats with one or both nutsedge species, 36 contained no *M. incognita* J2, while 40 contained *M. incognita* J2. In comparison, of the four quadrats containing neither nutsedge species, three also had no *M. incognita* J2. In September 2005 (data not presented), of quadrats containing one or both nutsedge species, 37 contained no *M. incognita* J2, and 29 contained *M. incognita* J2. Of the 14
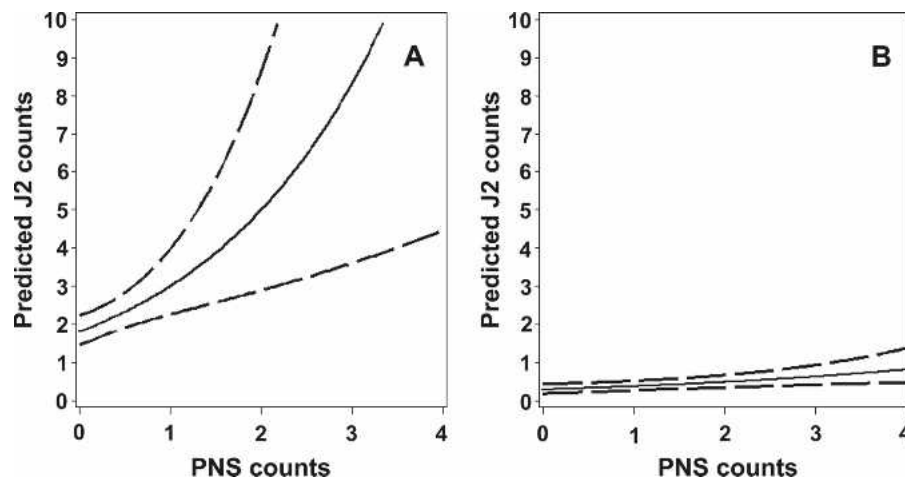

FIG. 3. Predicted *M. incognita* J2 counts (solid line) and 90% confidence upper and lower limits (dashed line) for May 2005 (A) with predicted model $\hat{\mu}$ = exp{0.5893 + 0.5099(PNS)} and September 2006 (B) with predicted model $\hat{\mu}$ = exp{−1.2639 + 0.2617(PNS)}, from an alfalfa field infested with nutsedges in Doña Ana County, NM. Horizontal axis is purple nutsedge (PNS) counts per 0.25 m². Vertical axis is the predicted number of *M. incognita* J2 counts per 10 cm³ soil.
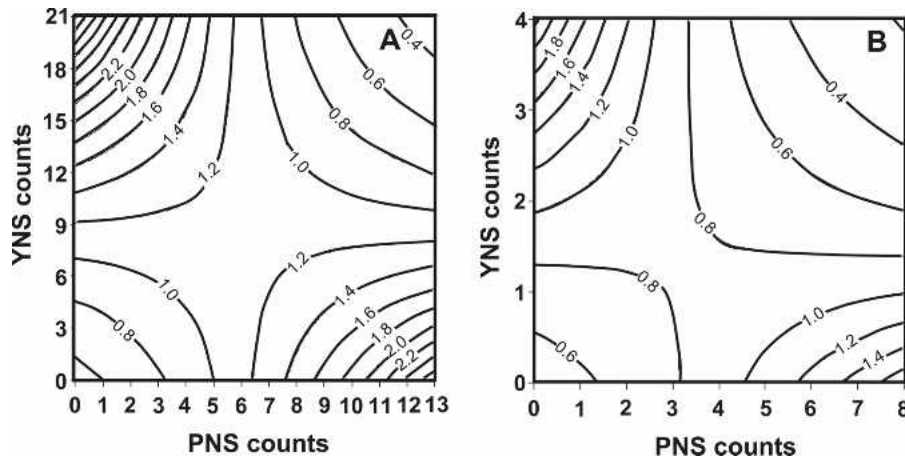
FIG. 4. Contour plots of *M. incognita* J2 counts (labeled curves) vs. yellow nutsedge (YNS) and purple nutsedge (PNS) counts for July 2005 (A) with predicted model $\hat{\mu} = \exp[-0.6309 + 0.0890(\text{YNS}) + 0.1266(\text{PNS}) - 0.0148(\text{YNS} \times \text{PNS})]$ and September 2005 (B) with predicted model $\hat{\mu} = \exp[-0.7295 + 0.3879(\text{YNS}) + 0.1604(\text{PNS}) - 0.1184(\text{YNS} \times \text{PNS})]$, from an alfalfa field infested with nutsedges in Doña Ana County, NM.

quadrats without nutsedge, nine also contained no *M. incognita* J2.

For the fitted Poisson regression models (Table 2), recall that point estimates of regression coefficients were preserved from original Poisson models to re-scaled models, with the only differences being larger standard errors of regression coefficients and wider confidence intervals for rescaled models, and thus larger *P*-values compare to the original model. The decision to keep the nonsignificant cross-product in the model will be discussed later. From a geometric stand-point, the cross-product in the predictive model pro-duces a surface with a twist instead of a flat plane in a three-dimensional space (Fig. 4A,B). The predicted model showed a lower *M. incognita* J2 count when both YNS and PNS counts were small and when both were large. On the other hand, a high *M. incognita* popula-tion was related to increased counts of either YNS or PNS, while counts of the other nutsedge species re-mained low. The predictive relationship was consistent from July to September in 2005.

The reliability of the predicted values for the July and September 2005 models can be assessed by examining the width of their confidence intervals for the mean *M. incognita* J2 count: the narrower the width, the better the prediction that can be obtained. Figure 5A and B shows plots of the widths of confidence intervals (upper limit minus lower limit) at each observed data point for July and September 2005, respectively. A higher vertical bar represents a wider range between upper and lower confidence limits on the *M. incognita* J2 at given values of YNS and PNS counts. Note that, in both graphs, confidence interval widths were increasing as the esti-mated mean *M. incognita* J2 was increasing, that is, as the YNS and PNS counts increased. This occurs natu-rally because of the nature of the Poisson distribution (i.e., the variance increases as the mean increases) but may also occur because of the low number of quadrats with very high (i.e., > 10) YNS and/or PNS counts. For example, Figure 2C shows that, in July 2005, 16 out of 80 quadrats had YNS plant counts of zero, but 56 quad-rats had counts between one and 10, while only eight
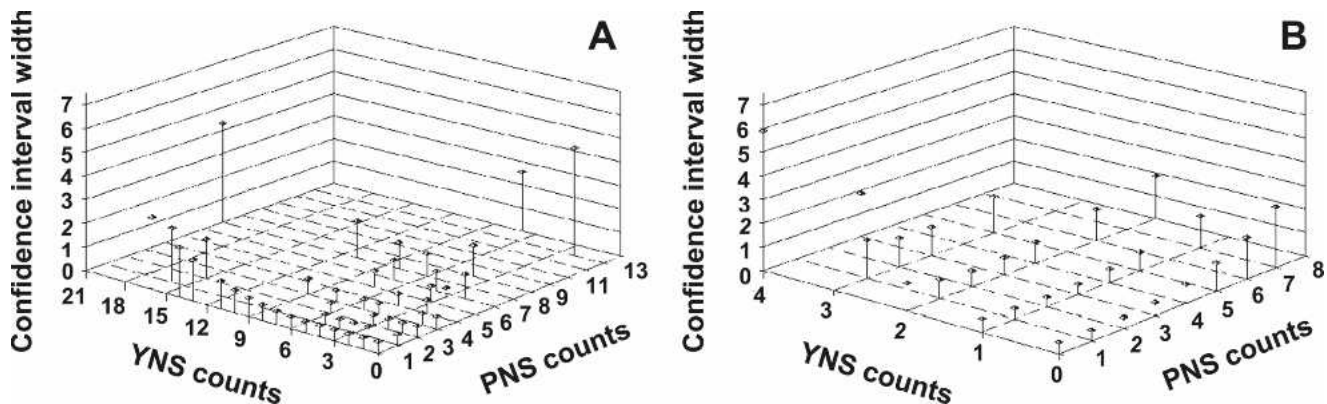

FIG. 5. Three-dimensional graphs showing 90% confidence interval widths (upper minus lower) on predicted *M. incognita* J2 counts vs. yellow nutsedge (YNS) and purple nutsedge (PNS) counts for July 2005 (A) with predicted model $\hat{\mu} = \exp\{-0.6309 + 0.0890(\text{YNS}) + 0.1266(\text{PNS}) - 0.0148(\text{YNS} \times \text{PNS})\}$ and September 2005 (B) with predicted model $\hat{\mu} = \exp[-0.7295 + 0.3879(\text{YNS}) + 0.1604(\text{PNS}) - 0.1184(\text{YNS} \times \text{PNS})\}$, from an alfalfa field infested with nutsedges in Doña Ana County, NM.

quadrats had counts over 10. Similarly, Figure 2E shows that 31 quadrats had no PNS, but 47 quadrats had counts between one and 10, while only two had PNS counts over 10. Given both the width of the confidence interval (Fig. 5A) and the numbers of quadrats with counts of zero to 10 (Fig. 2C, E), the July 2005 model appears reliable in that range of nutsedge values where the majority of this data occurred. September 2005 had 48 quadrats with no YNS, with remaining quadrats having YNS counts of four or fewer, and also had 20 quadrats with PNS counts of zero, with remaining quadrats having counts of eight or fewer. Therefore, the model for September 2005 may be reliable (Fig. 5B) over a smaller range of nutsedge accounts than the July 2005 model, where YNS and PNS counts are both four or fewer.

In summary, nutsedge as a stand-alone predictor of *M. incognita* J2 counts was significant with a positive regression coefficient in all of the predictive models. On the other hand, the YNS × PNS cross-product was significant with a negative regression coefficient in the original July and September 2005 models. A positive predictor implies a monotone increasing relationship between a predictor and the predicted variable. In this study, a one-unit count increase in the nutsedge predictor implies a change equal to the slope coefficient in the natural log function of the predicted number of *M. incognita* J2 for the Poisson Generalized Linear Model (eqn. 4). Based on the natural log link-function, the predicted *M. incognita* J2 count was calculated by exponentiating the predicted value obtained from the log-scale model. For example, for the regression model of May 2005 where PNS was the only significant predictor, if no PNS is observed, then the natural log of predicted *M. incognita* J2 count (from eqn. 4) is $\ln(\hat{\mu}) = 0.5893 + 0.5099(0) = 0.5893$. If a single PNS plant is observed, then the link-function value is $\ln(\hat{\mu}) = 0.5893 + 0.5099(1) = 1.0992$. On the log-scale, *M. incognita* J2 counts increase linearly by the regression coefficient of PNS in the model. Equivalently, on the count-scale, at a PNS count of zero, the predicted J2 count (from eqn. 5) will be $\hat{\mu} = \exp\{0.5893 + 0.5099(0)\} = 1.8027$. For a PNS count of one, the predicted J2 count is $\hat{\mu} = \exp\{0.5893 + 0.5099(1)\} = 3.0018$. Thus, the increment of the predicted J2 on the count scale is not constant; instead, it is a result of exponentiating a value obtained from the log-scale model. Hence, the fitted Poisson regression models indicate J2 counts increase exponentially with increasing nutsedges counts.

Discussion

Previous research evaluating the influence of alfalfa on the *M. incognita*-nutsedge pest complex showed that YNS population density was greatest in June, while PNS density was highest from July through September (Fiore, 2004). Overall, both *M. incognita* and nutsedge populations declined from 2005 to 2006 as a result of competition from the *M. incognita*-resistant alfalfa, consistent with previous research (Fiore, 2004).

At the beginning of the growing season in the first year of the alfalfa rotation (May 2005), when the field was heavily infested with all three pests but the seasonal populations were just developing, PNS count was a more important predictor for *M. incognita* J2 count than was YNS count. Previous greenhouse studies have also shown that PNS is a more consistent host than YNS for *M. incognita* J2 (Schroeder et al., 1999). However, YNS did become important as the growing season progressed.

In July and September 2005, when the nutsedges were at their growing peak in the first year of the rotation (Fiore, 2004), the two predictive models were consistent. Both YNS and PNS plant counts were positive significant predictors of *M. incognita* J2 count. The YNS × PNS cross-product term was a significant negative predictor in the original Poisson models, but was not significant in the more conservative rescaled models. From a statistical standpoint, maintaining the cross-product term in the rescaled model is not reasonable, because it is not significant. On the other hand, the original models with the cross-product term suggested a biologically competitive interaction between YNS and PNS, and this interaction may have an effect on predicting *M. incognita* J2 counts. Therefore, rescaled models with the YNS × PNS cross-product were reported. Further research with a larger sample size is desired. By increasing sample size, a higher number of quadrats which contain a large number of nutsedges (i.e., >10) is expected, thus filling the area where there were not many observations in the current study and perhaps confirming the significance of each potential predictor. This study suggests that the predictability of *M. incognita* J2 counts by nutsedge counts is more reliable when both YNS and PNS counts are relatively small, as was evident in the region where the confidence limit width was fairly narrow. Such regions occurred where YNS ≤ 12 plants/0.25 m² quadrat and PNS ≤ 7 plants/0.25 m² quadrat in July 2005 and where YNS ≤ 3 plants/0.25 m² quadrat and PNS ≤ 4 plants/0.25 m² quadrat in September 2005.

In May and July 2006, the predictive relationship was not significant. Neither YNS nor PNS was a significant predictor of *M. incognita* J2 counts, and therefore fitted models were intercept-only models, in which the mean J2 count was predicted by $\exp(\hat{b}_0)$. One reason for the lack of a predictive relationship might be that the number of nutsedge per quadrat was greatly reduced after one year of the alfalfa rotation and that these data, especially in May, were at the beginning of the growing season. Another reason may be due to the small sample size: the logistically affordable size of 80 quadrats at each sample date in the current study may have prevented any predictive relationship from being detected.

Finally, in September 2006, at the end of the second growing season of the weed-suppressing alfalfa, both YNS and PNS started to reemerge. The more consistent *M. incognita* host, PNS (Schroeder et al., 1999), again became a significant positive predictor of J2 counts, as in the beginning of the alfalfa rotation in May 2005. From the practical standpoint of the efficacy of the two-year alfalfa rotation in controlling *M. incognita* J2, the majority of predicted *M. incognita* J2 counts in September 2006 were low but still above $0.24/10$ cm^3 soil, the economic threshold for pre-plant populations that are damaging to chile (Thomas et al., 1995). At the lowest point of the curve, where PNS count was zero, the predicted *M. incognita* J2 mean was $0.28/10$ cm^3 soil, and the 90% confidence interval was between 0.18 and 0.43 J2/10 cm^3 soil, a range which contains the economic threshold. However, destruction of the alfalfa stand, tillage operations and winter mortality would be expected to reduce J2 levels further. As PNS counts increased, the predicted number of *M. incognita* J2 also increased. This indicates that surviving PNS tubers which also protect *M. incognita* from 1,3-dichloropropene (Thomas et al., 2004) may be the primary source of *M. incognita* resurgence the following year. Hence, these results suggest that the three-year alfalfa rotation suggested by Fiore (2004) is necessary to reduce *M. incognita* J2 below the economic threshold.

In conclusion, the results of the Poisson regression models suggest that nutsedge counts in a field infested with the *M. incognita*-nutsedge pest complex can be used as a visual predictor of *M. incognita* J2 populations, unless the number of nutsedge plants ≤ 1 plant/0.25 m^2 quadrat over the study area.

Literature Cited

Bird, G. W., and Hogger, C. H. 1973. Nutsedges as hosts of plant-parasitic nematodes in Georgia cotton fields. Plant Disease Reporter 57:402.

Bliss, C. I., and Fisher, R. A. 1953. Fitting the negative binomial distribution to biological data and note on the efficient fitting of the negative binomial. Biometrics 9:176–200.

Byrd, D.W., Barker, K. R., Ferris, H., Nusbaum, C. J., Griffin, W. E., Small, R. H., and Stone, C. A. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. Journal of Nematology 8:206–212.

Cox, D. R. 1983. Some remarks on overdispersion. Biometrika 70:269–274.

Dobson, A. J. 1990. An introduction to generalized linear models. London: Chapman and Hall.

Ferris, H. 1984. Probability range in damage predictions as related to sampling decisions. Journal of Nematology 16:246–251.

Finney, D. J. 1941. On the distribution of a variate whose logarithm is Normally distributed. Journal of the Royal Statistical Society 7:155–161.

Fiore, C. 2004. A sustainable approach to nematode and nutsedge management in chile using nematode-resistant alfalfa as a rotation crop. M.S. thesis, New Mexico State University, Las Cruces, NM.

Goodell, P. B., and Ferris, H. 1980. Plant-parasitic nematode distributions in an alfalfa field. Journal of Nematology 12:136–141.

Hogger, C. H., and Bird, G. W. 1976. Weed and indicator hosts of plant-parasitic nematodes in Georgia cotton and soybean fields. Plant Disease Reporter 60:223–226.

Jenkins, W.R. 1964. A rapid centrifugal flotation technique for separating nematodes from soil. Plant Disease Report 48:692.

Littell, R. C., Stroup, W. W., and Freund, R. J. 2002. SAS for linear models. Cary, NC: SAS Institute.

McCullagh, P., and Nelder, J. A. 1989. Generalized linear models. New York: Chapman and Hall.

McSorley, R. 1998. Population dynamics. Pp. 109–133 in K. R. Barker, G. A. Pederson, and G. L. Windham, eds. Plant and Nematode Interactions. Madison, WI: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America.

Neter, J., Wasserman, W., and Kutner, M. H. 1983. Applied linear regression models. Homewood, IL: Richard D. Irwin.

Noe, J. P., and Campbell, C. L. 1985. Spatial pattern analysis of plant-parasitic nematodes. Journal of Nematology 17:86–93.

Onsager, J. A. 1981. A note on the poisson distribution in integrated pest management. Bulletin of the Entomological Society of America. 27:119–120.

Patterson, R. L. 1966. Difficulties involved in the estimation of a population mean using transformed sample data. Technometrics 8:535–537.

Proctor, J. R., and Marks, C. F. 1975. The determination of normalizing transformations for nematode count data from soil samples and of efficient schemes. Nematologica 20:395–406.

Schroeder, J., Kenney, M. J., Thomas, S. H., and Murray, L. W. 1994. Yellow nutsedge response to southern root-knot nematode, chile pepper, and metolachlor. Weed Science 42:534–540.

Schroeder, J., Thomas, S. H., and Murray, L. W. 1993. Yellow and purple nutsedge and chile peppers host southern root-knot nematode. Weed Science 41:150–156.

Schroeder, J., Thomas, S. H., and Murray, L. W. 1999. Yellow and purple nutsedge are not injured by root-knot nematodes inoculated at increasing population densities. Weed Science 47:201–207.

Schroeder, J., Thomas, S. H., and Murray, L. W. 2004. Root-knot nematodes affect annual and perennial weed interactions with chile pepper. Weed Science 52:28–46.

Schroeder, J., Thomas, S. H., and Murray, L. W. 2005. Impacts of crop pests on weeds and weed-crop interactions. Weed Science 53:918–922.

Thomas, S. H., Murray, L. W., and Cardenas, M. 1995. Relationship of preplant population densities of *Meloidogyne incognita* to damage in three chile pepper cultivars. Plant Disease 79:557–559.

Thomas, S. H., Schroeder, J., Kenney, M. J., and Murray, L. W. 1997. *Meloidogyne incognita* inoculum source affects host suitability and growth of yellow nutsedge and chile pepper. Journal of Nematology 29:404–410.

Thomas, S. H., Schroeder, J., and Murray, L. W. 2004. *Cyperus* tubers protect *Meloidogyne incognita* from 1,3-dichloropropene. Journal of Nematology 36:131–136.

Thomas, S. H., Schroeder, J., and Murray, L. W. 2005. The role of weeds in nematode management. Weed Science 53:923–928.

Wedderburn, W. M. 1974. Quasi-Likelihood Functions, Generalized Linear Models, and the Gauss-Newton Method. Biometrika 61:439–447.

Young, L. J., and Young, G. H. 1998. Statistical Ecology: A population perspective. Boston: Kluwer Academic.