

Density-Dependent Multiplication and Survival Rates in *Heterodera glycines*

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Abstract: Seasonal multiplication and overwinter survival are density-dependent in *Heterodera glycines*. At low to moderate population densities, the nematode is capable of large population increases on susceptible soybean cultivars and high rates of oversummer or overwinter survival in the absence of a host. To improve estimates of *H. glycines* multiplication and survival rates, egg densities were monitored for 12 cropping sequences across 10 years. Log-linear regression analysis was used to describe and compare density-dependent relationships. Growing-season change in *H. glycines* egg densities was density-dependent for all crops (susceptible soybean, resistant soybean, and nonhost), with slope estimates for the density-dependent relationship greater for susceptible soybean compared with a non-host crop. Overwinter population change also was density-dependent, with similar declines in survival rates observed for all crops as population densities increased. Survival was greater following susceptible soybean compared with resistant soybean, with an intermediate rate of survival associated with non-host crops. Survival estimates greater than 100% frequently were obtained at low population densities, despite attempts to account for sampling error. Rates of growing-season multiplication and survival, when standardized for population density, declined with year of the study. Standardized overwinter survival rates were inversely related to average daily minimum temperature and monthly snow cover.

Key words: density-dependence, *Heterodera glycines*, multiplication rate, nematode, overwinter survival.

Resistant cultivars and rotation with nonhost crops remain effective methods for managing the soybean cyst nematode *Heterodera glycines* Ichinohe in soybean (*Glycine max* [L.] Merr.). Nevertheless, intrinsic factors such as high rates of reproduction on susceptible cultivars (Long and Todd, 2001) and several types of dormancy (Yen et al., 1995) combine to impede attempts to manage this pest. Survival in the absence of a host—and overwinter survival, in particular—can approach 100%, especially in the north-central United States (Riggs et al., 2001).

Density-dependent multiplication and survival rates have been observed for several nematodes, including *H. glycines* (Ferris, 1985; Francl and Dropkin, 1986). Although mediated by different factors, both relationships are described by log-linear or negative exponential models (Ferris, 1985). Comparison of density-dependent rates of population increase of *H. glycines* on soybean suggests that the relationship differs between resistant and susceptible cultivars (Wang et al., 2000), whereas density-dependent rates of population decline appear to be similar among non-host crops (Francl and Dropkin, 1986). Explicit comparisons of density-dependent effects among host and non-host crops are needed to enhance our ability to accurately predict population changes over multi-year cropping sequences and improve current advisory recommendations for *H. glycines* management. The objective of the

present study was to compare density-dependent relationships for *H. glycines* multiplication and survival across long-term (10-year) cropping sequences, incorporating the effects of fluctuating environmental factors. Specific rotation effects on the nematode-soybean interaction, including mean nematode reproduction and soybean yield loss, have been reported separately (Long and Todd, 2001).

MATERIALS AND METHODS

The study was conducted in an *H. glycines* race 3-infested soybean field near Columbus, Kansas, on a Parsons silt loam soil (34% sand, 46% silt, 20% clay, 1.5% organic matter; pH 6.9). Twelve cropping sequences were established in 1991 and continued through 2000. Rotations consisted of four general patterns: (i) continuous susceptible soybean; (ii) 2 years of non-host crops followed by susceptible soybean; (iii) a non-host crop alternated with full-season soybean, with resistant and susceptible cultivars alternated in soybean years; and (iv) a non-host crop alternated with resistant or susceptible soybean double-cropped with wheat (*Triticum aestivum* L.). Every phase of all rotation sequences was present in each year of the study. Single non-host crops (rotations 3 and 4) were corn (*Zea mays* L.) the first year and sorghum (*Sorghum bicolor* [L.] Moench) thereafter, while consecutive non-host crops (rotation 2) consisted of corn (1991 only) or sorghum followed by wheat/summer fallow. Susceptible soybean cultivars were Bay (1991–1994) and Stafford (1995–2000); resistant soybean cultivars were Pioneer 9531 (1991–1994) and Manokin (1995–2000); the corn hybrid was Pioneer 8379; the sorghum hybrid was Pioneer 8500; wheat cultivars were Karl (1991–1994) and Karl192 (1995–2000).

The experimental design was a randomized complete block with three replications. Plots were four rows, 9.2 m long with 75-cm row spacing, except for

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wheat, which was planted in 12 rows with 20-cm row spacing. Planting dates for full-season soybean and sorghum were late May to mid-June. Wheat was planted in October and double-cropped soybean planted mid-June through early July. Standard agronomic practices, described in detail elsewhere (Long and Todd, 2001), were followed. Conservation tillage, consisting of a single discing and field cultivation, was applied in the direction of plot rows prior to planting. Crops were harvested each year in October, except for wheat, which was harvested in late June or early July. Soybean yields are reported in Long and Todd (2001).

Soil samples for determination of nematode population densities were collected prior to planting and at harvest in each year of the study. Samples consisted of four 5-cm-diam. soil cores collected to a depth of 15 cm from the middle two rows of each plot. Cores were composited and cysts were extracted from 100-cm³ subsamples by wet-sieving over a 150- μ m-pore sieve. Cysts were mechanically ruptured to release eggs and second-stage juveniles (J2) (Niblack et al., 1993). Eggs and J2 were counted at $\times 40$ magnification.

Initial (Pi) and final (Pf) nematode counts and ratios from each plot and year were transformed to log values to reduce heterogeneity of variances and to linearize the relationship between growing-season population change (Pf/Pi) and Pi, and between overwinter survival (Pi_t/Pf_{t-1}) and Pf_{t-1}. Multiplication and survival data were subjected to a strip-plot analysis of covariance (Littell et al., 1996) across years, with Pi and Pf_{t-1} as covariates, using the MIXED procedure in SAS (SAS Institute, Cary, NC). The effect of environmental variables (daily minimum and maximum temperatures, precipitation), obtained from the Columbus, Kansas National Oceanic and Atmospheric Administration weather station (courtesy of Ted Wary), on nematode multiplication and survival was investigated by means of stepwise regression analysis.

RESULTS AND DISCUSSION

General population dynamics: Heterodera glycines egg densities fluctuated within and between years (Fig. 1). Absolute (non-transformed) nematode densities (eggs and J2/100 cm³ soil) at planting (Pi) and harvest (Pf) averaged 4,649 and 20,090, respectively, for continuous susceptible soybean, 3,109 and 7,204, respectively, for 3-year rotations, and 2,372 and 5,065, respectively, for 4-year rotations. As expected, the presence of a susceptible cultivar typically resulted in large increases in egg densities, although declines in average population densities were observed in 3 out of 10 growing seasons in plots planted to continuous susceptible soybean. Seasonal multiplication rates averaged 30 for susceptible soybean across rotations and years, while population changes in the presence of resistant soybean or non-host crops were relatively small, with Pf/Pi values aver-

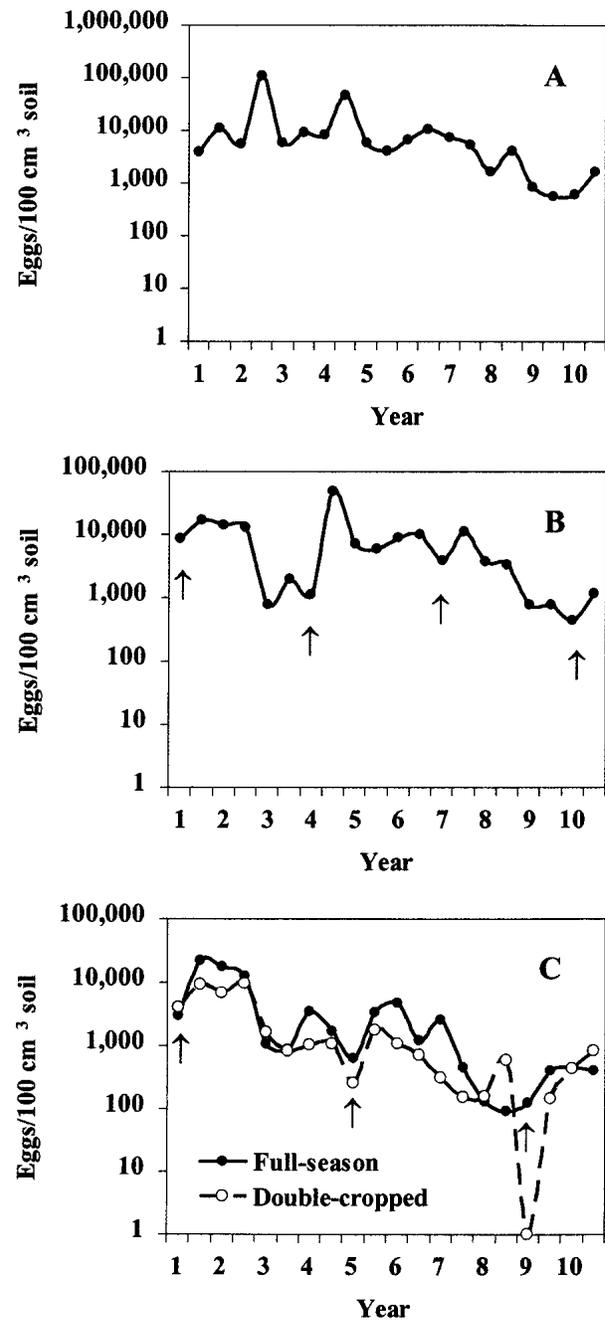


FIG. 1. Temporal changes in *Heterodera glycines* egg densities for continuous susceptible soybean (A); representative sequences of: 2 years of non-host crops followed by susceptible soybean (B); and non-host-rotation, with resistant and susceptible cultivars grown in alternate soybean years (C). Arrows indicate a susceptible soybean crop in rotation sequences. ●, full season; ○, double-cropped.

aging 0.7 and 2.5, respectively. Multiplication rates on susceptible soybean were extraordinarily high, but similar Pf/Pi values have been documented for this maturity zone (Wang et al., 2000). Log-adjusted (i.e., back-transformed means of log-transformed data) Pf/Pi values averaged 6.9, 0.5, and 1.0 for susceptible soybean, resistant soybean, and a non-host crop, respectively. Overwinter survival averaged 1.0 or greater following all

crops, while log-adjusted averages of overwinter survival were less than 1.0 (0.38 to 0.57; $P \leq 0.05$) for all crops. As reported by Francl and Dropkin (1986), survival was unaffected by differences in non-host crop (wheat vs. sorghum, in this case). A general trend of declining egg densities was observed for all rotation sequences.

Density-dependent multiplication and survival rates: Both growing-season and overwinter changes in *H. glycines* densities were strongly density-dependent, with initial egg densities the most significant factor affecting population change across rotations and years (Table 1; Fig. 2). The relationship between Pf/Pi and Pi varied with the current crop for intercept (Crop, $P = 0.03$) and slope (Pi \times crop, $P = 0.07$) parameters. Greater slope and intercept values were indicated for susceptible soybean compared with a non-host crop, reflecting stronger density-dependent effects for the host crop and resulting in predicted differences in Pf/Pi at low, but not high, Pi (Table 2; Fig. 2A). In contrast, the estimate for intercept, but not for slope, was greater for susceptible compared to resistant soybean, suggesting that relative multiplication rates of resistant and susceptible cultivars were stable across Pi levels. Density-dependent multiplication of *H. glycines* commonly occurs on resistant, as well as susceptible, soybean cultivars (Chen et al., 2001b; Todd et al., 1995), but rates of density-dependence rarely have been compared between the two. In one recent comparison of linear regression models for Pf/Pi vs. Pi across 40 environments in the north-central United States, intercept estimates for resistant and susceptible soybean cultivars were consistently different, while slope estimates were similar or different depending on environment (Wang et al., 2000). An expectation of stable relative multiplication rates across Pi levels could be argued, based on observations of similar infection rates for *H. glycines* J2 on resistant and susceptible soybean cultivars (Endo, 1964; Ross, 1958).

Declines in overwinter survival rates of *H. glycines* with increasing egg densities were similar for all crops but

TABLE 1. Analysis of covariance for density-dependent multiplication and survival rates in *Heterodera glycines* egg densities.

Source of variation	F-value	
	Growing season ^a	Overwinter ^b
Year	0.77 ($P = 0.65$)	1.50 ($P = 0.22$)
Crop	8.85 ($P = 0.034$)	0.76 ($P = 0.53$)
Yr \times crop	0.54 ($P = 0.92$)	1.63 ($P = 0.12$)
$P_x(\ln)^c$	42.04 ($P < 0.0001$)	26.71 ($P < 0.0001$)
$P_x(\ln) \times$ year	0.42 ($P = 0.92$)	2.07 ($P = 0.034$)
$P_x(\ln) \times$ crop	2.78 ($P = 0.065$)	0.73 ($P = 0.48$)
$P_x(\ln) \times$ year \times crop	0.41 ($P = 0.98$)	1.66 ($P = 0.053$)

^a Growing-season multiplication or survival (Pf/Pi) = egg densities at harvest/egg densities at planting.

^b Overwinter survival = egg densities at planting/egg densities at previous harvest.

^c $P_x = P_i$ for the growing season and $P_{f,-1}$ for the overwinter period.

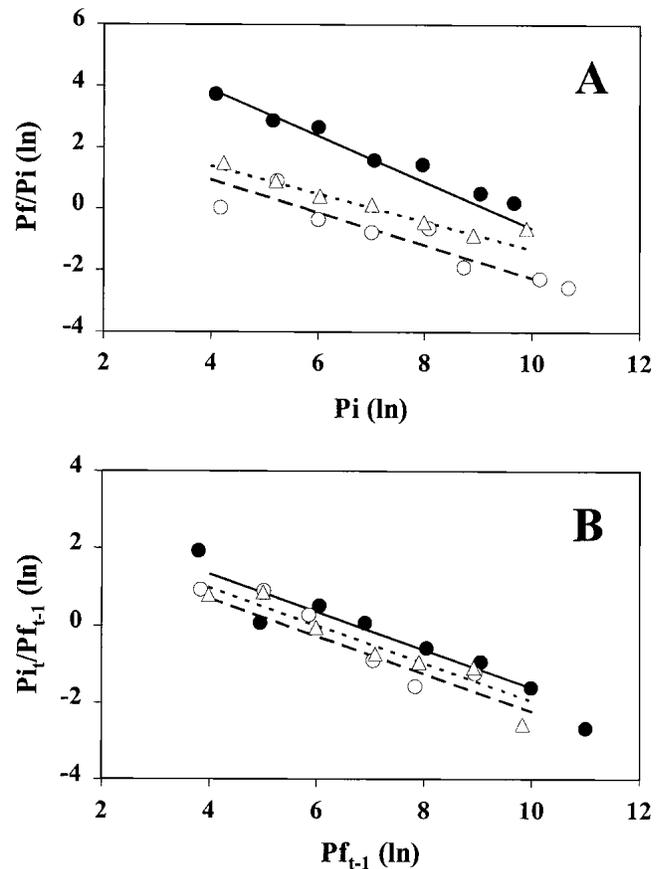


FIG. 2. Linear regression models for density-dependent growing season (A) and overwinter (B) change in *Heterodera glycines* egg densities for susceptible soybean (●), resistant soybean (○), and non-host crops (△). Data points depict mean multiplication or survival rates for a range of at-planting (P_i) or harvest ($P_{f,-1}$) population density classes, respectively. Slopes for growing season change differed ($P \leq 0.05$) between susceptible soybean and non-host crops; intercepts for overwinter change differed between susceptible and resistant soybean cultivars (see Table 2).

varied among years (Table 1). A $P_i \times$ crop \times year interaction ($P = 0.05$) indicated that there were years when the density-dependent overwinter survival relationship varied among crops (1992 and 1994), but these were exceptions. Intercepts in an equal-slope model did vary among crops ($P = 0.04$), with higher ($P \leq 0.05$) rates of survival predicted following susceptible soybean than

TABLE 2. Comparison of slopes and intercepts for density-dependent growing season and overwinter change in *Heterodera glycines* egg densities.

Crop	Growing season ^a		Overwinter ^b	
	Slope	Intercept	Slope	Intercept
Susceptible soybean	-0.75 a ^c	6.89 a	-0.49	3.29 a
Resistant soybean	-0.53 ab	3.08 b	-0.49	2.68 b
Non-host crop	-0.45 b	3.20 b	-0.49	2.95 ab

^a Egg density at harvest (Pf)/egg density at planting (Pi) vs. Pi.

^b Egg density at planting (P_i)/egg density at previous harvest ($P_{f,-1}$) vs. $P_{f,-1}$ (equal-slope model).

^c Estimates within a column followed by the same letter are not significantly different according to a paired *t*-test ($P = 0.05$).

following resistant soybean (Table 2; Fig. 2B). Survival rates following non-host crops were intermediate. Greater reductions in *H. glycines* populations have been observed for resistant cultivars compared to non-host crops (Chen et al., 2001a), and this pattern was suggested by our data. The common slope for density-dependent overwinter survival (-0.49) was similar to that observed for growing-season survival in the presence of resistant soybean or non-host crops (-0.45 to -0.53). Overall survival rates reported for *H. glycines* in this study are greater than those reported by Francl and Dropkin (1986), likely as a result of those authors' constraint of Pf/Pi values to an upper limit of 1.0.

Overestimation of multiplication and survival rates: Predicted and observed log-adjusted survival rates were greater than 100% ($P \leq 0.05$) at the lowest observed egg densities for both growing-season and overwinter periods (Fig. 2). This phenomenon has been reported previously (Riggs et al., 2001), and several hypotheses have been advanced to explain it. First, in the case of overwinter survival, there is evidence to suggest that *H. glycines* reproduction can continue following host senescence (Riggs et al., 2001). While the present study does not test this hypothesis directly, it is significant that survival rates greater than 100% were observed following non-host crops as well as susceptible soybean. It is possible that the slightly higher overwinter survival rates predicted for nematode populations following a susceptible crop compared with a resistant soybean crop reflect a measurable difference in post-senescence reproduction, but differences in survival simply may reflect differences in energy reserves of unhatched juveniles (i.e., the greatest percentage of juveniles with maximum reserves would be expected following a susceptible soybean crop). A second hypothesis proposes upward movement of cysts in the soil profile during freeze-thaw cycles (Niblack, pers. comm.). This scenario could account for high overwinter survival rates but does not explain similar rates of survival during the growing season.

The most plausible explanation for survival rates exceeding 100% is overestimation due to sampling errors resulting from spatial variability in nematode population densities or inconsistent extraction efficiencies (Riggs et al., 2001). Aggregated spatial patterns in nematode populations typically result in frequency distributions of population counts that are described by the negative binomial probability distribution (Francl, 1986; McSorley and Parrado, 1982; Noe and Barker, 1985). The negative binomial distribution is characterized by a positive skew, with low densities clustered near the mean and high densities extending far above the mean (Snedecor and Cochran, 1967). In the present study, frequency distributions of multiplication and survival values, in addition to Pi and Pf values, were positively skewed for all population density classes, resulting in mean estimates greatly exceeding median estimates

(data not shown). Log-transformation eliminated most of the skewness in the data, yet predicted survival rates remained unexpectedly high at low egg densities (i.e. > 1.0 , $P \leq 0.05$). Thus, we are unable to fully account for the anomalous estimates of survival rates and, based on the consistency of overestimation between oversummer and overwinter periods, differential extraction efficiency is unlikely to provide any additional explanation in this case. It is possible simply to constrain the upper limit of survival to 1.0 to minimize sampling errors as Francl and Dropkin (1986) did, but our data suggest that the phenomenon of survival estimates greater than 1.0 cannot be explained completely by sampling error and merits further attention.

Spring samples were collected post-tillage in the present study, and changes in spatial heterogeneity of cysts due to soil disturbance, which was minimal in this case, are therefore not taken into account. Francl (1986) found that the number of cysts in the plant row (our sampling site) were reduced following tillage, but only for cysts produced during the previous season. Cysts produced prior to the previous season were not stratified; thus, our estimates of overwinter survival rates are likely to be underestimates, and then only for plots following soybean. Regardless of discrepancies in estimates of actual survival rates, the present study substantiates the tendency for *H. glycines* populations in the north-central United States to exhibit high rates of overwinter survival.

Effect of environmental factors: Stepwise regression models incorporating environmental parameters into predictions of growing-season and overwinter population change in *H. glycines* egg densities are presented in Table 3 and Figure 3. Nematode population change during the growing season, averaged across all crops, was a function only of Pi and year. The model implies that density-adjusted rates of multiplication or survival declined during the duration of the study. This is consistent with the overall declines in egg densities across all rotations described above. The most likely explanation for this phenomenon is an accumulation of antagonistic organisms, such as egg parasites, but no data on nematode antagonists were collected in this study.

In contrast to growing-season population change, overwinter survival of *H. glycines* was measurably affected by environmental variation. The density-dependent rate of overwinter survival was inversely related to average daily minimum temperature and monthly snowfall. As snow cover, presumably correlated with snowfall, serves to insulate the soil from temperature fluctuations, a similar mechanism is indicated for both environmental variables. There is evidence to suggest that survival of *H. glycines* is greater in colder compared to warmer climates, although an explicit comparison of survival by this nematode across latitudes failed to detect a definitive relationship (Riggs et al., 2001). The results of this study support the conten-

TABLE 3. Stepwise regression analysis of environmental effects on density-dependent multiplication and survival rates in *Heterodera glycines* egg densities.

Source of variation	Estimate	
	Growing season ^a	Overwinter ^b
Intercept	5.64 ($P = 0.013$)	4.83 ($P = 0.011$)
Year	-0.23 ($P = 0.014$)	NS
$P_x(\ln)^c$	-0.56 ($P = 0.079$)	-0.53 ($P = 0.009$)
Average daily maximum temperature (°C)	NS	NS
Average daily minimum temperature (°C)	NS	-0.28 ($P = 0.053$)
Average monthly precipitation (mm)	NS	NS
Average monthly snowfall (mm)	NS	-0.04 ($P = 0.005$)
R^2	0.61	0.91

^a Growing season multiplication or survival (P_f/P_i) = egg densities at harvest/egg densities at planting averaged by year.

^b Overwinter survival = egg densities at planting/egg densities at previous harvest averaged by year.

^c $P_x = P_i$ averaged across rotations within year for the growing season and P_{f-1} averaged across rotations within year for the overwinter period.

NS = Not significant

tion that higher rates of overwinter survival in northern compared to southern populations of *H. glycines* are partly a response to soil temperature.

Consideration of density-dependent effects on *H. glycines* multiplication and survival is integral to the development and evaluation of management strategies. In Kansas, for example, the probability of large population increases and associated yield loss on susceptible soybean cultivars following several seasons of non-host and resistant soybean crops limits the use of these cul-

tivars, even in long-term rotations (Long and Todd, 2001). The results of the present study also emphasize the importance of environmental mediation of these density-dependent relationships and suggest directions for future research. In particular, improved knowledge of the underlying biological mechanisms involved in density-dependent and environment-dependent changes in *H. glycines* populations is needed as a basis for further refinement of management recommendations.

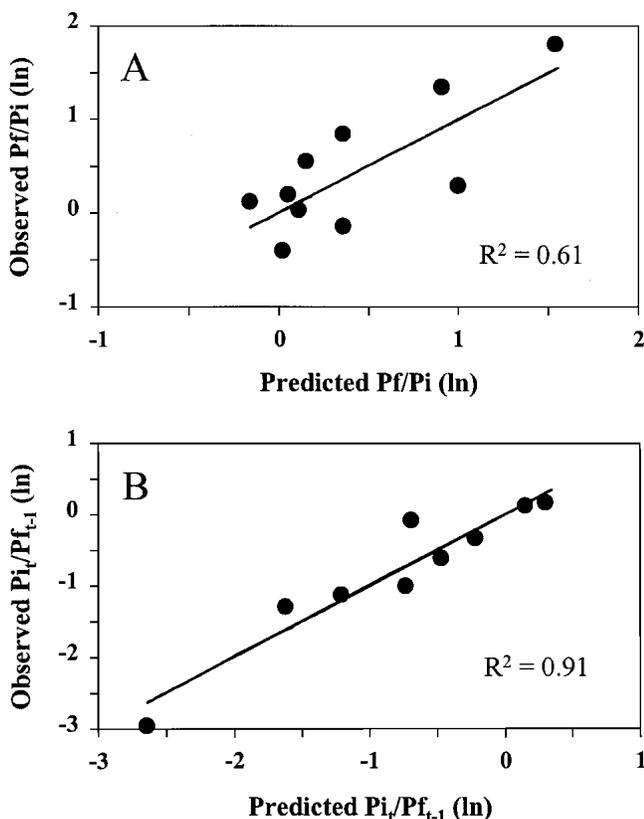


FIG. 3. Relationship between observed and predicted change in *Heterodera glycines* egg densities for growing season (A) and overwinter (B) periods. Predicted values are based on models described in Table 3.

LITERATURE CITED

- Chen, S. Y., P. M. Porter, J. H. Orf, C. D. Reese, W. C. Stienstra, N. D. Young, D. D. Walgenbach, P. J. Schaus, T. J. Arlt, and F. R. Breitenbach. 2001a. Soybean cyst nematode population development and associated soybean yields of resistant and susceptible cultivars in Minnesota. *Plant Disease* 85:760-766.
- Chen, S. Y., W. C. Stienstra, W. E. Lueschen, T. R. Hoverstad. 2001b. Response of *Heterodera glycines* and soybean cultivar to tillage and row spacing. *Plant Disease* 85:311-316.
- Endo, B. Y. 1964. Penetration and development of *Heterodera glycines* in soybean roots and related anatomical changes. *Phytopathology* 54:79-88.
- Ferris, H. 1985. Density-dependent nematode seasonal multiplication rates and overwinter survivorship: A critical point model. *Journal of Nematology* 17:93-100.
- Francl, L. J. 1986. Spatial analysis of *Heterodera glycines* populations in field plots. *Journal of Nematology* 18:183-189.
- Francl, L. J., and V. H. Dropkin. 1986. *Heterodera glycines* population dynamics and relation of initial population to soybean yield. *Plant Disease* 70:791-795.
- Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS System for Mixed Models. Cary, NC: SAS Institute, Inc.
- Long, J. H., and T. C. Todd. 2001. Effect of crop rotation and cultivar resistance on seed yield and the soybean cyst nematode in full-season and double-cropped soybean. *Crop Science* 41:1137-1143.
- McSorley, R., and J. L. Parrado. 1982. Estimating relative error in nematode numbers from single soil samples composed of multiple cores. *Journal of Nematology* 14:522-529.
- Niblack, T. L., R. D. Heinz, G. S. Smith, and P. A. Donald. 1993. Distribution, density, and diversity of *Heterodera glycines* in Missouri. Supplement to the *Journal of Nematology* 25:880-886.
- Noe, J. P., and K. R. Barker. 1985. Overestimation of yield loss of tobacco caused by the aggregated spatial pattern of *Meloidogyne incognita*. *Journal of Nematology* 17:245-251.

- Riggs, R. D., T. L. Niblack, R. A. Kinloch, A. E. MacGuidwin, A. Mauromoustakos, and L. Rakes. 2001. Overwinter population dynamics of *Heterodera glycines*. *Journal of Nematology* 33:219–226.
- Ross, J. P. 1958. Host-parasite relationship of the soybean cyst nematode in resistant soybean roots. *Phytopathology* 48:578–579.
- Snedecor, G. W., and W. G. Cochran. 1967. *Statistical methods*. Ames, IA: The Iowa State University Press.
- Todd, T. C., W. T. Schapaugh, J. H. Long, and B. Holmes. 1995. Field response of soybean in maturity groups III–V to *Heterodera glycines* in Kansas. Supplement to the *Journal of Nematology* 27:628–633.
- Wang, J., P. A. Donald, T. L. Niblack, G. W. Bird, J. Faghihi, J. M. Ferris, C. Grau, D. J. Jardine, P. E. Lipps, A. E. MacGuidwin, H. Melakeberhan, G. R. Noel, P. Pierson, R. M. Riedel, P. R. Stellers, W. C. Stienstra, T. C. Todd, G. L. Tylka, T. A. Wheeler, and D. S. Wysong. 2000. Soybean cyst nematode reproduction in the north-central United States. *Plant Disease* 84:77–82.
- Yen, J. H., T. L. Niblack, and W. J. Wiebold. 1995. Dormancy of *Heterodera glycines* in Missouri. *Journal of Nematology* 27:153–163.