

Effects of Maturity and Determinacy in Soybean on Host-Parasite Relationships of *Heterodera glycines*¹

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Abstract: The effects of soybean maturity and determinacy on the host-parasite relationships of *Heterodera glycines* were investigated in a field microplot study over 2 years. Determinate and indeterminate isolines of the maturity group (MG) III cultivar Williams 82 and the MG V cultivar Essex were grown in microplots artificially infested with a race 3 isolate of *H. glycines* at three initial population (Pi) densities (0, 300, and 3,000 eggs/100 cm³ soil). Soybean seed yields, nematode final population (Pf) densities and reproductive index (Pf/Pi), and root colonization by *Macrophomina phaseolina*, the causal agent of charcoal rot, were monitored in each year. Seed yields were reduced ($P \leq 0.05$) in the presence of *H. glycines* in both years, but losses were greater in 1996 in the absence of drought stress. Yield loss was lower ($P \leq 0.06$) for the determinate isolate of Essex than for the other cultivar-isoline treatments across years. Nematode reproduction was density-dependent in the more conducive environment of 1996 but was unaffected by soybean maturity or determinacy traits. Root colonization by *M. phaseolina* increased ($P \leq 0.05$) in the presence of high *H. glycines* densities on determinate, but not indeterminate, isolines. Differences in *H. glycines*-induced yield loss among cultivar-isoline treatments were not related to nematode reproduction, *M. phaseolina* colonization, or environmental stresses. These results indicate that the effects of soybean maturity and determinacy on *H. glycines*-soybean interactions are not independent and that their combined effects must be considered in geographic regions where both traits vary.

Key words: *Glycine max*, *Heterodera glycines*, soybean cyst nematode, soybean determinacy, soybean maturity group.

The soybean cyst nematode *Heterodera glycines* is a major limiting factor to soybean (*Glycine max*) production in southeastern Kansas, with yield losses on susceptible cultivars commonly approaching 40% (Todd et al., 1995). Losses are variable across maturity groups (MG), with full-season MG V cultivars exhibiting lower levels of yield suppression than shorter-season MG III and IV cultivars in some environments (Todd, 1993). Differences in yield loss are not necessarily related to maturity, however, because the maturity trait is confounded with determinacy. Cultivars belonging to MG V are determinate, with flowering subsequent to vegetative growth, while most MG III and IV cultivars are indeterminate, with flowering and vegetative development concurrent (Fehr, 1987).

Cultivars in MG V are associated with lower egg densities of *H. glycines* than later-

maturing (MG VIII) cultivars in North Carolina (Hill and Schmitt, 1989), and their use has been advocated in nematode management programs (Koenning et al., 1996; Schmitt, 1991). In contrast, no differences in *H. glycines* reproduction were observed among MG III through V cultivars in southeastern Kansas (Todd, 1993). Soybean determinacy may provide a possible explanation for the conflicting results. All cultivars in the North Carolina study were determinate, while those in the Kansas study comprised both determinate (MG V) and indeterminate (MG III, IV) cultivars. The effects of determinacy on *H. glycines* host-parasite relationships have not been determined, but greater nematode reproduction has been reported on pod-producing than on vegetative soybeans (Hill and Schmitt, 1989), suggesting that the longer flowering period of indeterminate cultivars might offset any effects due to maturity.

Maturity group V cultivars flower later than MG III and IV cultivars in southeastern Kansas and typically experience fewer temperature and moisture stresses. Cultivars in MG V also exhibit less root colonization by *Macrophomina phaseolina* compared to early-maturing cultivars (Pearson et al., 1984; Todd, 1993), resulting in lower incidence

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and severity of charcoal rot, a stress-related disease of regional importance. Infection by *H. glycines* can enhance *M. phaseolina* colonization in some environments (Todd et al., 1987), but the role of *M. phaseolina* in the differential response of soybean cultivars to *H. glycines* is unclear. Champoux (1992) observed few differences in seed yield or root colonization by *M. phaseolina* between determinate and indeterminate soybean isolines, but the effect of determinacy on charcoal rot development under conditions of concomitant infection by *H. glycines* has not been determined.

The objective of this study was to determine the influence of soybean maturity and determinacy on the host-parasite relationships of *H. glycines*. Our hypothesis was that both determinacy and maturity affect *H. glycines*-induced soybean yield loss and nematode population response in southeastern Kansas.

MATERIALS AND METHODS

Site description: The experiment was conducted in 1995 and 1996, using 60 fiberglass-enclosed (perimeter only) microplots (0.81 m² × 0.5 m deep) established with minimal disturbance of the soil profile on the Kansas State University Research Farm near Columbus, Kansas. The soil was a shallow silt loam (27% sand, 49% silt, 24% clay), typical of upland soils in the area. Average rainfall at the site is 693 mm for the April-to-September growing season.

Experimental design: Two near-isogenic lines (isolines) of the soybean cultivars Williams 82 (MG III) and Essex (MG V) were used in this study. The isolines of each cultivar differed in determinacy (determinate or indeterminate), and all four isolines were susceptible to *H. glycines*. The isolines were planted into microplots that were infested with *H. glycines* race 3 at either 300 or 3,000 eggs/100 cm³ soil. Uninfested plots served as a control. All microplots contained natural soil infestations of *M. phaseolina*, with background levels in a previous study averaging 43 propagules/g soil (Todd, unpubl.). The experiment was arranged as a 4 × 3 factorial with five replications of each isolate × nematode infestation level treatment.

Immediately prior to seeding in 1995, designated microplots were infested with eggs and second-stage juveniles (J2) of a greenhouse-reared, race 3 population of *H. glycines*. The nematode was not detected in any of the microplots prior to infestation. Inoculum was obtained from 'Flyer' soybean by spraying the roots with pressurized water and collecting the cysts on a 150-µm-pore sieve. Cysts were mechanically ground to release eggs and J2 (Niblack et al., 1993). Inoculum for each microplot was mixed in 1 liter of pasteurized sand and incorporated into the microplots to a depth of 15 cm to achieve initial population levels (Pi) of 300 or 3,000 eggs/100 cm³ soil. Noninoculated pasteurized sand was incorporated into control plots. In 1996, background levels of *H. glycines* in each microplot were amended as needed to maintain the same Pi levels as in 1995. The isolines of Essex or Williams 82 soybean, provided by R. S. Nelson (USDA Soybean Germplasm Collection, Urbana, IL) were seeded in a row in the center of each microplot at the rate of 24 seeds/plot (8 seeds/ft row). Soybeans were planted between microplots to maintain normal field canopy conditions (Yen et al., 1995). Planting dates were 20 June 1995 and 5 June 1996.

Sampling procedures: Eight 2.5-cm-diam. × 15-cm-depth soil cores were collected from each microplot prior to inoculation and planting in 1996 and adjacent to the plant row at harvest in each year. Cysts from 100-cm³ subsamples were suspended in 4 liters of tap water, collected on a 150-µm-pore sieve, and mechanically ground as described for inoculum preparation. Eggs and J2 were counted at ×40 magnification.

Roots from two soybean plants were collected from each microplot at growth stage R6-7 (Fehr et al., 1971) each year for determination of *M. phaseolina* colonization levels. Roots were surface-sterilized in 0.8% NaOCl, air-dried, and ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) as described by Pearson et al. (1984). Colonies of *M. phaseolina* were counted from 100-mg subsamples of milled roots plated on chloroneb-rose bengal agar.

Soybeans were harvested in October of

each year by hand-collecting plants from each microplot and separating the seeds with a stationary threshing machine. Measurements included stand count and total seed weight. Relative yield was calculated to obtain an estimate of yield loss for each nematode-infested plot by dividing the seed yield for that plot by the seed yield for the noninoculated plot within year, cultivar, isolate, and replicate.

Statistical analyses: The data were analyzed using a strip-plot model (Milliken and Johnson, 1992), with treatment and year as strip factors, and the general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC). Nematode and fungal densities were \log_{10} -transformed ($x + 1$) prior to analysis to reduce heterogeneity of variances. Relationships among soybean yield, *H. glycines* egg densities (Pf) and reproduction (Pf/Pi), and *M. phaseolina* colonization were examined using regression and correlation analyses.

RESULTS

Total precipitation was greater than the long-term average in both years of the study (5% and 24% greater in 1995 and 1996, re-

spectively), although precipitation patterns varied substantially between years (Fig. 1). Excessive rainfall in May and June 1995, which delayed planting, was followed by below-average precipitation during the remainder of the growing season. In contrast, the pattern of rainfall during the 1996 growing season included a relatively dry May and June followed by an exceptionally wet August. Differences in precipitation pattern likely resulted in large year effects for soybean and *H. glycines* response variables (Table 1), manifested as lower soybean seed yields and nematode reproduction in 1995 than in 1996 (Table 2).

Cultivar \times isolate interactions ($P \leq 0.05$) were observed for both absolute and relative soybean seed yields (Table 1). This interaction resulted from a lower ($P \leq 0.05$) absolute yield for the determinate isolate of Williams 82 compared to all other isolines, but a higher ($P \leq 0.06$) relative yield (*H. glycines*-infested microplots only) for the determinate isolate of Essex compared to all other isolines. This pattern was consistent across *H. glycines* Pi levels and years. Relative yields of each cultivar \times isolate combination are plotted against *H. glycines* Pi in Figure 2.

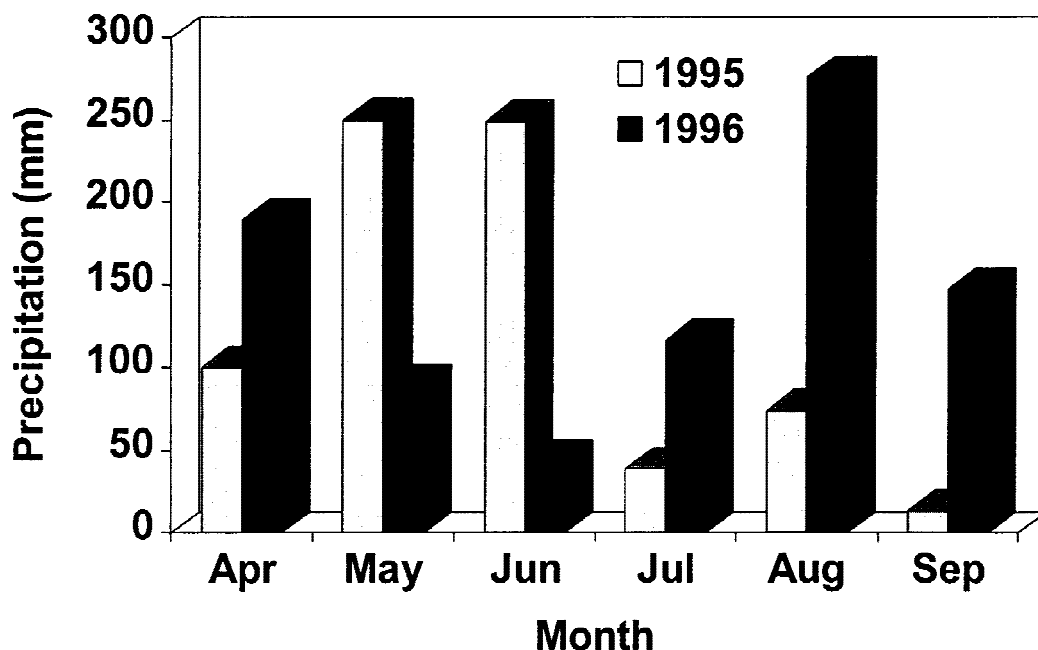


FIG. 1. Monthly growing-season precipitation amounts for the Kansas State University Research Farm near Columbus, Kansas, during 1995 and 1996.

TABLE 1. Analyses of variance in soybean seed yield, *Heterodera glycines* egg density and reproduction, and *Macrophomina phaseolina* root colonization across 2 years (1995–1996).

Source of variation	D.f.	Mean squares				
		Seed yield	Relative yield ^a	<i>H. glycines</i>		<i>M. phaseolina</i> colonization ^c
				Pf ^b	Pf/Pi ^b	
Year (Yr)	1	36,965**	0.493**	59.43**	6.44**	4.22
Error within Yr	4	59	0.019	0.67	0.05	1.89
Cultivar (C)	1	1,848	0.029	0.56	0.05	18.06**
Isoline (I)	1	4,701*	0.115	0.06	0.06	1.33
C × I	1	12,986**	0.188*	0.20	0.14	2.41
<i>H. glycines</i> Pi	2	11,041**	0.487**	61.14**	1.78**	1.65
C × Pi	2	517	0.038	0.33	0.00	0.79
I × Pi	2	1,251	0.045	0.24	0.00	3.10*
C × I × Pi	2	329	0.000	0.65	0.00	1.03
Error within treatment	44	697	0.037	0.65	0.06	0.64
Yr × C	1	10,206**	0.036	3.48*	0.15	3.47
Yr × I	1	432	0.002	0.81	0.02	4.03
Yr × C × I	1	1,376	0.000	0.09	0.09	0.77
Yr × Pi	2	3,384**	0.000	12.77**	1.80**	0.24
Yr × C × Pi	2	89	0.030	1.35	0.06	0.76
Yr × I × Pi	2	324	0.005	0.15	0.01	0.63
Yr × C × I × Pi	2	110	0.005	1.15	0.03	0.03
Residual error	41	543	0.036	0.54	0.05	1.15

* $P \leq 0.05$.** $P \leq 0.01$.^a Calculated as the seed yield for each *H. glycines*-infested plot/the seed yield for the noninfested plot within year, cultivar, isolate, and replicate.^b Pi = number of eggs and juveniles per 100 cm³ soil at planting; Pf = number of eggs and juveniles per 100 cm³ soil at harvest.^c Colonies/g root.

Year × cultivar interactions ($P \leq 0.05$) occurred for absolute seed yield and *H. glycines* Pf (Table 1) as a result of overall better performance of Essex vs. Williams 82, and because of poor nematode reproduction on the determinate isolate of Williams 82, respectively, in 1996 but not 1995 (data not shown).

All soybean and nematode variables displayed large effects due to *H. glycines* Pi, but year × Pi effects were also prominent (Table 1). Absolute seed yield was reduced in a stepwise fashion with increasing Pi in 1996, but only the highest Pi level reduced yield in 1995 (Table 2). Similarly, nematode reproduction (Pf/Pi) was inversely proportional to Pi level in 1996, but no difference in Pf/Pi was observed in 1995. Although the relationship between absolute seed yield and *H. glycines* Pi was greater in 1996 than in 1995 (Table 2), a year × Pi interaction did not occur for relative seed yield, indicating that the difference in yield between Pi levels of 300 vs. 3,000 eggs/100 cm³ soil was stable across environments (years).

Root colonization by *M. phaseolina* increased ($P \leq 0.05$) in the presence of high *H. glycines* densities on determinate, but not indeterminate, isolines (Table 1, Fig. 3). This relationship, in conjunction with higher levels of colonization on the indeterminate isolate of Essex, resulted in a higher ($P < 0.01$) overall level of colonization on Essex than on Williams 82 (data not shown).

TABLE 2. Effect of *Heterodera glycines* initial egg density (Pi) on soybean seed yield and nematode reproduction in 1995 and 1996.

Pi ^a	Seed yield (g/plot)		Pf ^b		Pf/Pi ^a	
	1995	1996	1995	1996	1995	1996
0	108 a ^b	165 a	2 a	0 a	—	—
300	108 a	140 b	42 b	3,583 b	0.14 a	11.99 a
3000	93 b	112 c	407 c	3,692 b	0.14 a	1.23 b

^a Pi = number of eggs and juveniles per 100 cm³ soil at planting; Pf = number of eggs and juveniles per 100 cm³ soil at harvest.^b Means within a column followed by the same letter are not significantly different according to Fisher's LSD ($P = 0.05$).

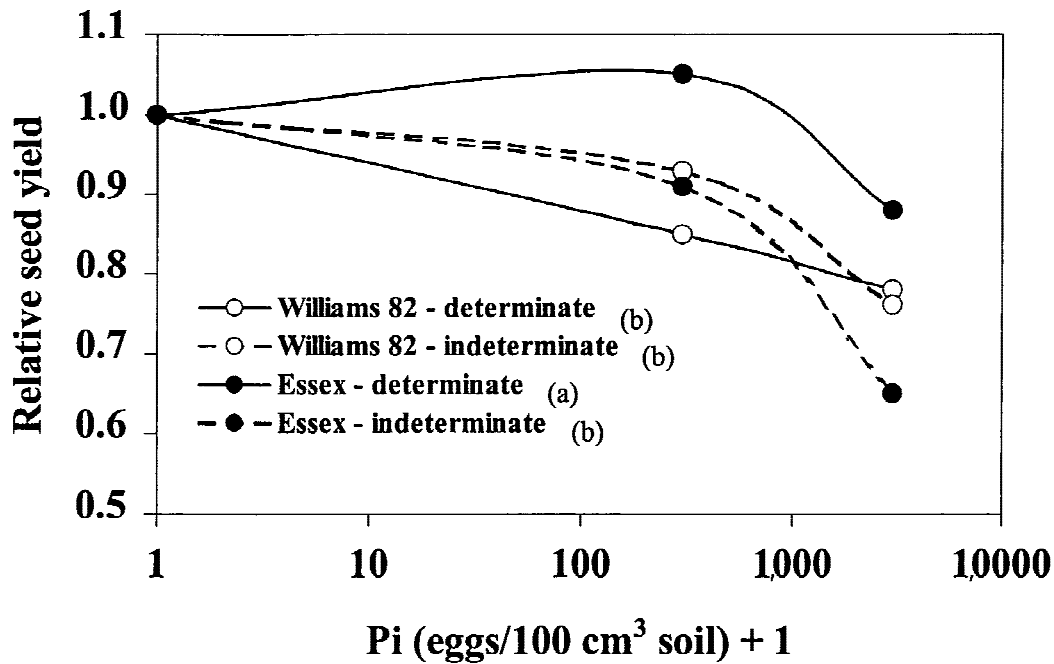


FIG. 2. Relative yields of cultivar-isoline treatments averaged across years and plotted against *Heterodera glycines* initial egg densities (Pi). Treatments followed by the same letter in parentheses are not significantly different across Pi levels according to least-squares means ($P < 0.06$).

Relationships among soybean yield, H. glycines egg densities, and M. phaseolina colonization: Soybean seed yield and nematode reproduction (Pf/Pi) were inversely related ($P < 0.01$) to *H. glycines* Pi across treatments and years, with Pi explaining 11 to 12% of the variation in responses. Seed yield in the determinate isolate of Essex was unrelated to Pi in either year of the study, but, in the more conducive environment of 1996, Pi explained 27 to 53% ($P \leq 0.05$) of the variation in seed yields of the remaining cultivar-isoline combinations. Nematode reproduction was unrelated to Pi in 1995, but 46% of the variation in Pf/Pi was explained by Pi in 1996. Root colonization by *M. phaseolina* was not correlated with seed yields or *H. glycines* egg densities.

DISCUSSION

Excessive rainfall in May and June, followed by below-average precipitation during the remainder of the growing season, is a common pattern in southeastern Kansas (Todd, 1993). Stresses associated with this

precipitation pattern in 1995 resulted in reduced soybean yields and apparent reductions in *H. glycines*-induced yield loss compared to 1996, which was characterized by a more stable precipitation pattern. The decline in 1995 egg densities after inoculation suggests, however, that lower levels of yield loss may actually reflect lower levels of root infection. In any case, the effects of Pi level and precipitation (year) were additive for *H. glycines*-infested treatments, as indicated by relative yield. Additive effects of drought and *H. glycines*-induced stresses have been documented previously for soybean (Young and Heatherly, 1988; Todd, 1993).

Full-season MG V cultivars display lower *H. glycines*-induced yield loss than earlier-maturing group III and IV cultivars under some southeastern Kansas environments (Todd, 1993). This study confirms this observation and attempted to examine the normally confounding effects of maturity and determinacy separately. Our results suggest that yield loss is not mediated by either effect independently, because lower levels of yield loss were observed only for the deter-

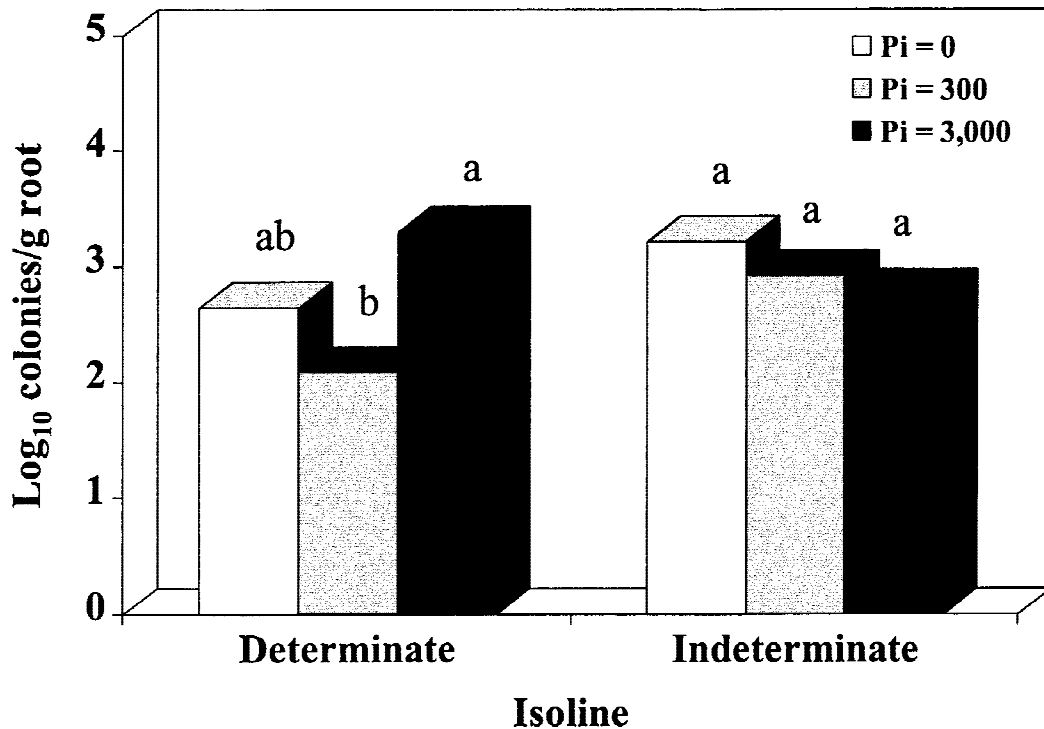


FIG. 3. Root colonization of *Macrophomina phaseolina* on determinate and indeterminate isolines at three *Heterodera glycines* initial egg densities (Pi = eggs/100 cm³ soil). Bars within isoline with the same letter are not significantly different according to least-squares means ($P < 0.05$).

minate isoline of the MG V cultivar Essex. The magnitude of difference in yield loss was impressive, with loss in the determinate isoline of Essex at a density of 3,000 *H. glycines* eggs/100 cm³ soil comparable to that exhibited by the other isolines at a density of only 300 eggs/100 cm³ soil (Fig. 2).

The standard determinate MG V isoline did not perform better than the standard indeterminate MG III isoline in the absence of *H. glycines* in the present study, even under the adverse conditions that occurred in 1995. It appears, therefore, that increased tolerance to *H. glycines* is not correlated with stress avoidance (i.e., late flowering). The hypothesis that the observed differences in yield suppression can be explained by differences in charcoal rot severity also must be rejected since *M. phaseolina* colonization of the roots of the determinate isoline of Essex was comparable to or greater than that in the other isolines. A previous attempt to relate differences in *H. glycines*-induced yield loss among cultivars of differing maturity to

M. phaseolina colonization levels resulted in the same conclusion (Todd, 1993).

Multiplicative interactions, with positive correlations between fungal root colonization and nematode densities, have been described for *M. phaseolina* and *H. glycines* under some environments in Kansas (Todd et al., 1987; Winkler et al., 1994), while additive effects, with no correlation between fungal root colonization and nematode densities, have been observed in other environments (Todd, 1993). Both types of interaction were observed in the present study. Determinate isolines tended to have lower levels of fungal colonization than indeterminate isolines in the absence of the nematode, but colonization increased in the presence of high *H. glycines* densities on determinate isolines while remaining stable on indeterminate isolines. This suggests that nematode stress may play a role in charcoal rot development in full-season, determinate soybean cultivars in southeastern Kansas, but that environmental stresses are more im-

portant for charcoal rot development in shorter-season, indeterminate cultivars.

Population increase in *H. glycines* is reported to be density-dependent (Alston and Schmitt, 1987; Todd et al., 1995; Wang et al., 2000) and related to soybean maturity (Hill and Schmitt, 1989; Wang et al., 2000). In the present study, *H. glycines* population increase (Pf/Pi) and Pi were observed to be inversely related under conditions conducive to nematode reproduction, but population densities were not influenced by soybean maturity—an observation supported by previous evidence from southeastern Kansas (Todd, 1993). The variability in results among studies examining soybean maturity effects on *H. glycines* population densities can be resolved, in part, by considering the effect of determinacy, since the former studies involved only cultivars within determinacy type and the latter Kansas studies involved cultivars across determinacy types. Greater nematode reproduction has been observed on flowering vs. vegetative soybeans (Hill and Schmitt, 1989), and the longer flowering period associated with indeterminate cultivars may translate into greater nematode reproduction, offsetting any effect due to maturity. Evidence for this hypothesis awaits further study, since no significant determinacy or maturity (cultivar) × determinacy (isoline) effects were observed for Pf/Pi in our study.

Our results suggest that host-parasite relationships between *H. glycines* and soybean are determined, in part, by the interaction of maturity and determinacy. Since these factors are not independent, predicted effects of maturity within determinacy type are not likely to reflect the effects of maturity across determinacy type.

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