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An Alternative Method for Evaluating Soybean Tolerance to *Heterodera glycines* in Field Plots[1]

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Abstract: Alternate planting dates and periodic destruction of the previous year's soybean crop as well as 1-year bare fallow were used to establish a range of population densities of *Heterodera glycines* for the subsequent year. Soybean cultivar Coker 156 (susceptible, moderately tolerant) was compared to cultivars Essex (susceptible, intolerant) and Bedford (resistant) to evaluate tolerance at different *H. glycines* population densities established through the previous year's treatments. Yield of Coker 156 was consistently intermediate between yields of Bedford and Essex in 1986 and 1987. Yield of Essex was negatively correlated ($P = 0.05$) with preplant egg numbers of *H. glycines* in 1987, whereas yield of Bedford and Coker 156 were not related to nematode density. Reproduction of *H. glycines* was greater ($P = 0.05$) on the moderately tolerant Coker 156 than on either of the other cultivars.

Key words: *Glycine max, Heterodera glycines,* nematode, soybean, soybean cyst nematode, tolerance.

Tolerance to several plant-parasitic nematodes has been identified in certain cultivars of soybean (*Glycine max* (L.) Merr.). Cultivars tolerant to *Heterodera glycines* (1,4,13), *Pratylenchus brachyurus* (15,22), and *Hoplolaimus columbus* (17) are available to growers. Tolerance of potato (*Solanum tuberosum* L.) to *Globodera rostochiensis* and *Globodera pallida* has been characterized (25,26).

Soybean cultivars resistant to *H. glycines* have been used extensively in the United States to alleviate yield suppression caused by this pest. Resistant cultivars, however, place selection pressure on the nematode population, resulting in an increase of populations that parasitize these resistant cultivars (21). Cultivars tolerant (14) to *H. glycines* may not yield as well as resistant cultivars in the presence of this nematode, but they can be integrated into cropping sequences to minimize selection pressure on *H. glycines.* Tolerant cultivars also may be a viable option where resistant cultivars are not available or where crop rotation is practiced. Wallace (28) recommends that breeders consider the incorporation into crops of tolerance to nematodes rather than resistance.

Although tolerance to nematodes has been recognized, several key aspects of this phenomenon need further study. The basic mechanisms involved in tolerance to nematodes are poorly understood, although rooting pattern and root-growth dynamics may play a role (18,25). Second, because tolerance depends on the preplant nematode population density (5), growers must be advised against using tolerant cultivars in situations where nematode densities are above damaging levels. Finally, a tolerant cultivar likely would maximize nematode reproduction. Thus, the use of tolerant soybean should be integrated with other tactics for management of *H. glycines.*

Measuring tolerance to nematodes remains problematic. Researchers commonly compare nematicide-treated plots with untreated plots (1,4,13,15), or they may use different nematicide rates and compare the slopes of the regression lines (26). These approaches have provided valuable information about tolerance to nematodes, but they have certain shortcomings. Nonfumigant nematicides such as aldicarb may stimulate growth and yield of soybean in

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the absence of nematodes (3) and may control nontarget insect or other pests. Fumigant nematicides may be phytotoxic and (or) affect other soil microfauna and microflora. Thus, perceived tolerance in experiments with nematicides may result from physiological interactions within the plant or effects on nontarget organisms. A better approach is to compare yields of different cultivars in response to a range of nematode population densities and compare the slopes of the regression lines (13,26). This approach was considered impractical for screening large numbers of genotypes, however (14). Rotations or other cultural management practices may be used to obtain the requisite population densities, but these approaches may require several years to achieve the desired effect (2).

Seinhorst proposed the model $y = m + (1 - m)z^{P - T}$ to measure the effects of nematode population density at planting and damage to plants, where y = yield; m = minimum yield or yield at the population density in which all available space is occupied by the nematodes; z is a constant less than 1; P is the preplant nematode density; and t is the nematode density below which no noticeable yield reduction occurs (23), referred to as the tolerance limit (24). The proposed model, while arbitrary (24), has been supported by other nematologists because it is essentially biological (11). The tolerance limit has been challenged (27); furthermore, it is difficult to establish such a tolerance limit with accuracy (10). The utility of the model is questionable because its nonlinear character does not facilitate hypothesis testing. Nevertheless, relating plant tolerance to the T value or tolerance limit of the model has practical implications.

The current research was undertaken to evaluate soybean tolerance over a range of *H. glycines* population densities in field plots. The objectives of this research were to: 1) establish different population densities of *H. glycines* by manipulation of the previous year's crop; 2) evaluate this method of adjusting population densities, which would take only 1 year's preparation; and 3) compare a susceptible, moderately tolerant soybean cultivar (Coker 156) with a susceptible intolerant cultivar (Essex) (1) and a cultivar (Bedford) resistant to SCN races 3 and 14 at different population densities in order to test the hypothesis that tolerance to soybean cyst nematode in field plots depends on density.

Materials and Methods

Experiments were conducted in 1985–87 at the Rhodes Farm of the University of Missouri, Delta Center, near Clarkton, Missouri. The soil type was a Brosley fine loamy sandy mixed thermic, axenic, hapludolf (86% sand, 11% silt, 3% clay; <1% O.M., pH 5.4). The sites were naturally infested with *H. glycines* race 14.

Soybean cultivar Essex was planted in 1985 and 1986 at various times with periodic crop destruction to establish a range of *H. glycines* population densities. Plots were initially 6.1 m long and 12 rows wide with 0.96-m row spacing. Alleys were 6.1 m wide to facilitate moving equipment between plots for planting and crop destruction. Treatments to establish population densities were 1) bare fallow; 2) short-season soybean planted June 15–21, 1985 and 1986; 3) full-season soybean planted May 13–19, 1985–1986; 4) May-planted soybean disked under 21 days after planting; 5) May-planted soybean disked under at 21 days after planting, then replanted and disked under again 21 days after the second planting; 6) May-planted soybean disked under at 45 days after planting; and 7) full-season soybean disked under at the R5 reproductive stage (9) about 1 September 1985 and 1986. Treatments were arranged in a randomized complete block design with five replications.

In the years following crop alteration treatments (1986 and 1987), each 12-row plot (main plots) was split into three four-row subplots to accommodate the cultivars randomly assigned to subplots. Seed of the three cultivars (Essex, Coker 156, and Bedford) were inoculated with *Bradyrhizobium japonicum* (Kirchner) Jordan and planted mid-May each year. Soil samples for nematode analyses were taken from the center two rows of each subplot. Composite samples consisting of 12 soil cores, each 2.5-cm-d, were taken to a depth of 20 cm at planting and at harvest. A 500-cm^3 subsample was processed by elutriation (6) and sugar flotation (20) to determine the number of *H. glycines* cysts. Cysts were crushed with a Ten-Broeck tissue homogenizer to free the eggs for counting. Preplant nematode data were analyzed with three observations per whole plot in a randomized complete block design to evaluate the effects of the previous year's treatments by analyses of variance (ANOVA). Nematode data at soybean harvest in mid October were analyzed as a split plot with cultivars as subplots. All nematode data were transformed ($\log_{10}[x + 1]$) to standardize the variance prior to analyses. Untransformed data are presented in tables for clarity. Soybean yield was determined by harvesting the center two rows of each plot. Yield data were subjected to ANOVA for a split-plot design. The Waller-Duncan k-ratio *t*-test was used to separate main effects means for both yield and nematode data. Plots were irrigated twice in 1986 and five times in 1987. A portion of the experiment was flooded by approximately 25 cm of rainfall for 1 week in 1986, 3 weeks after planting.

Results and Discussion

The numbers of eggs and cysts of *H. glycines* were affected similarly by soybean planting date and destruction sequence both years (Table 1). Late planting resulted in the greatest population density for the subsequent crop. One year of fallow was not sufficient to reduce the population density to zero. Highest population densities ($P = 0.05$) of *H. glycines* were achieved with late planting (June), and lesser densities by early planting (May), followed by other treatments, including fallow (Table 1). The crop destruction treatments employed in this research were so similar in their effects on *H. glycines* densities both years as to be redundant. Subsequent experiments might employ destruction at 2-week intervals, more planting dates, trap crops, or soybean cultivars of different maturity groups to provide a better range of *H. glycines* densities. Similarly, including a nonhost in selected plots for 2 years or a fumigant treatment should bring the population density to lower levels (near zero) (2) in order to estimate the *Y*-axis intercept with greater precision. Planting dates provided large differences in *H. glycines* densities (Table 1), but the influence of this factor was variable in

TABLE 1. Influence of planting date and various crop destruction sequences on the population density of *Heterodera glycines* race 14 cysts and eggs per 500 cm^3 soil prior to 1986 and 1987 soybean planting.

Previous year's treatment	1986 Cysts	1986 Eggs	1987 Cysts	1987 Eggs
Bare fallow	48 b	3,313 b	10 d	604 e
Late planted (June)	93 a	7,193 a	235 a	17,121 a
Full season (May)	43 bc	2,253 bc	70 b	4,095 b
Plant, disk at 21 days, replant, disk at 21 days	37 bcd	2,580 c	20 c	1,232 c
Plant, disk at 21 days	39 bcd	2,227 c	15 cd	1,330 bc
Plant, disk at 45 days	32 d	1,333 d	15 cd	773 de
Plant, disk at R5	35 cd	1,967 cd	15 cd	931 de

All data are means of five replicates with three observations per replicate. Means followed by the same letter do not differ by the Waller-Duncan k-ratio *t*-test (k-ratio = 100). Treatment means were different ($P = 0.01$) both years. Treatment × year interaction was not significant (ANOVA).

other research (16). The use of soybean cultivars with different maturities may provide more consistent population ranges of *H. glycines* population densities (12,16) to better estimate yield at high Pi.

Reproduction of *H. glycines* was greater ($P = 0.05$) on Coker 156 than on either Bedford or Essex soybean both years (Table 2). A susceptible-tolerant cultivar should support higher levels of nematode reproduction (7,8) than an intolerant cultivar such as Essex. The number of *H. glycines* cysts and eggs on cultivars Bedford and Essex were similar both years. Reproduction on Essex was limited because of damage caused by *H. glycines*, whereas reproduction on Bedford was limited because of its resistance to races 3 and 14 of this nematode.

As expected, the resistant cultivar Bedford consistently yielded more ($P = 0.05$) soybeans than either Coker 156 or Essex, regardless of year or SCN population density (Table 3). Coker 156 soybean was always intermediate in yield between Bedford and Essex within any given treatment, as would be expected for a moderately tolerant cultivar (Table 3). The previous year's treatments did not affect yield in 1986, but yields in 1987 were affected ($P = 0.05$) by the previous year's treatments (Table 3). Soybean yield was not consistently related to preplant population densities (cysts or eggs) in 1986. Flooding of part of the experimental area in 1986 may account for the lack of significant negative effects of *H. glycines* preplant density on soybean yield, although the cultivars were generally ranked as expected in response to nematode pressure (Tables 1,3). Yield of soybean cultivar Essex was negatively related to Pi ($r = 0.38$ $P = 0.02$) in 1986, but yields of Bedford and Coker 156 were not. The ranking of Coker 156 in relation to the other cultivars used in this experiment confirms other work (1,4) which considered Coker 156 to be tolerant. It was not feasible to evaluate Seinhorst's proposed model (23), as a tolerance limit could not be determined because of the lack of nematode population densities near zero or at intermediate Pi's. Future research on a tolerance to *H. glycines* should focus on greater range of population densities in order to better evaluate tolerance in terms of the model proposed by Seinhorst (23). The yield of Coker 156 tended to be lower at high *H. glycines* preplant densities, but yields were not significantly correlated with Pi. Wallace has suggested that tolerance evolved in response to physical stresses in the environment (28), and research indicates that tolerance may be related to rooting pattern (18). If tolerance to nematodes is a characteristic of cultivars tolerant to other stresses, between-plot variation in soil texture and (or) fertility would tend to confound the relationship between preplant nematode density and yield.

Although tolerance needs to be evaluated at different population densities, the method employed in this research is not efficient in terms of labor and land requirements. Preliminary tolerance evaluation should probably be conducted with

TABLE 2. Final numbers of cysts and eggs of *Heterodera glycines* per 500 cm^3 soil on three soybean cultivars in mid October—Bedford (resistant), Essex (susceptible, intolerant), and Coker 156 (susceptible, moderately tolerant)—in 1986 and 1987 near Clarkton, Missouri.

	1986		1987	
	Cysts	Eggs	Cysts	Eggs
Bedford	19 b	2,580 b	120 b	17,270 b
Essex	21 b	2,363 b	165 b	13,645 b
Coker 156	113 a	22,466 a	310 a	24,015 a

Data are means of 35 observations. Column means followed by the same letter do not differ by the Waller-Duncan k-ratio *t*-test (k-ratio = 100).

TABLE 3. Yield (grams per plot) of soybean cultivars Bedford, Coker 156, and Essex in response to different soil population densities of *Heterodera glycines* obtained by the previous year's treatments near Clarkton, Missouri in mid October of 1986 and 1987.

Previous year's treatment	1986				1987			
	Bedford	Coker 156	Essex	Treatment mean	Bedford	Coker 156	Essex	Treatment mean
Fallow	1,343	1,117	826	1,096 a	815	694	518	676 bc
Late planted	1,338	1,014	834	1,062 a	840	524	312	559 c
Full season	1,464	1,442	1,182	1,363 a	969	734	607	770 ab
Plant, disk at 21 days, replant, disk at 21 days	1,515	1,021	867	1,135 a	860	775	630	755 ab
Plant, disk at 21 days	1,683	945	740	1,123 a	835	662	604	700 bc
Plant, disk at 45 days	1,530	1,159	822	1,717 a	1,139	903	696	913 a
Plant, disk at R5	1,456	1,278	1,282	1,339 a	986	800	546	778 ab
Cultivar mean†	1,476 a	1,140 b	936 c		921 a	727 b	559 c	

Treatment means followed by the same letter do not differ (P = 0.05) by the Waller-Duncan k-ratio t-test (k-ratio = 100), with 15 replicates.

† Cultivar means followed by the same letter do not differ, Waller Duncan k-ratio t-test (k-ratio = 100), with 35 replicates.

nematicides in different environments to conserve resources (19). Cultivars showing moderate to high levels of tolerance should then be evaluated at different population densities.

Tolerance should prove to be a useful trait in improving soybean yield in the presence of *H. glycines*. The high level of nematode reproduction on tolerant cultivars, however, should be taken into consideration by private and public consultants making recommendations. The carryover *H. glycines* population may damage subsequent crops if tolerance is not integrated with other tactics for nematode management such as rotation and (or) the use of resistant cultivars.

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