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# Interaction of *Heterodera glycines* and *Glomus mosseae* on Soybean[1]

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*Abstract:* The effects of the arbuscular mycorrhizal (AM) fungus *Glomus mosseae* on *Heterodera glycines*-soybean interactions were investigated in greenhouse experiments. Mycorrhizal and nonmycorrhizal soybean cultivars that were either resistant or susceptible to *H. glycines* were exposed to initial nematode population densities (Pi) of 0, 100, 1,000, or 10,000 eggs and infective juveniles. Soybean growth, nematode reproduction, and AM fungal colonization were determined after 35 (experiment I) and 83 (experiment II) days. Soybean shoot and root weights were reduced an average 29% across *H. glycines* Pi but were 36% greater overall in the presence of *G. mosseae*. Analyses of variance indicated that root colonization and stimulation of soybean growth by *G. mosseae* were inhibited at high *H. glycines* Pi, while the combined effects of the nematode and fungus on soybean growth were best described as additive in linear regression models. No evidence for increased nematode tolerance of mycorrhizal soybean plants was observed. Nematode population densities and reproduction were lower on a nematode-resistant soybean cultivar than on a susceptible cultivar, but reproduction was comparable on mycorrhizal and nonmycorrhizal plants. Root colonization by *G. mosseae* was reduced at high nematode Pi. The results suggest that nematode antagonism to the mycorrhizal symbiosis is a more likely consequence of interactions between *H. glycines* and AM fungi on soybean than is nematode suppression by the fungus.

*Key words:* arbuscular mycorrhizae, *Glomus mosseae, Glycine max, Heterodera glycines,* nematode-mycorrhizal interaction, soybean, soybean cyst nematode, vesicular-arbuscular mycorrhizae.

The soybean cyst nematode *Heterodera glycines* is the leading cause of disease losses in soybean (*Glycine max*) in the United States and worldwide (Wrather et al., 1997). Damage by the nematode results from increased moisture and nutrient stress due to the disruption of root vascular tissues at nematode feeding sites and the deleterious effects of nematode parasitism on $N_2$ fixation by *Bradyrhizobium japonicum* (Endo, 1964; Kennedy et al., 1999). Arbuscular mycorrhizal (AM) fungi have equally dramatic, but stimulatory, effects on P nutrition and water transport (Khalil et al., 1999; Ross, 1971; Safir et al., 1972), $N_2$ fixation (Zhang et al., 1995), and growth and seed production in soybeans (Tylka et al., 1991). An additional benefit of mycorrhizal symbiosis often reported for soybean and other crops is the suppression of sedentary nematode parasites (Carling et al., 1989; Hussey and Roncadori, 1982; Smith et al., 1986a; Tylka et al., 1991), typically accompanied by improved host tolerance to the nematode. Because AM fungi are abundant in soybean production soils (Khalil et al., 1992; Winkler et al., 1994), maximization of their role as nematode antagonists offers a theoretical strategy for management of *H. glycines* in soybean (Tylka et al., 1991).

Population suppression of, and (or) improved host tolerance to, the root-knot nematode *Meloidogyne incognita* by AM fungi is well documented for a variety of host plants (Carling et al., 1989; Habte et al., 1999; Smith et al., 1986a, 1986b), although this phenomenon may vary with fungal species (Habte et al., 1999; Smith et al., 1986b) and P nutrition (Carling et al., 1989). In contrast, few studies have examined the role of AM fungi in *H. glycines*-soybean interactions. Tylka et al. (1991) observed transient suppression of *H. glycines* by AM fungi, accompanied by increased soybean tolerance to the nematode under greenhouse conditions, but no nematode suppression and only additive effects of the nematode and fungi on soybean yield in field microplots. Winkler et al. (1994) found no evidence for mycorrhizal suppression of *H. glycines* field populations but did report that colonization of soybean roots by natural populations of AM fungi was reduced in *H. glycines*-susceptible cultivars compared to resistant cultivars, and only in the presence of the nematode. The objective of the current study was to characterize the interaction of *H. glycines* and AM fungi on soybean, using cultivars with differential susceptibility to the nematode.

## Materials and Methods

*Inoculum preparation: Heterodera glycines* inoculum was obtained from greenhouse cultures of Flyer soybean infested with a race 3 population of the nematode originally collected from a commercial soybean field in southeastern Kansas. Cysts were dislodged from the roots using a high-pressure water spray and collected on a 150-μm pore sieve. Inoculum consisted of eggs and second-stage juveniles (J2) that were released from cysts by mechanical grinding (Niblack et al., 1993) and collected on a 25-μm pore sieve. Spores of the AM fungus *Glomus mosseae* were collected from the soil of sudangrass (*Sorghum vulgare*) pot cultures by sieving and centrifugal flotation (Daniels and Skipper, 1982). *Glomus mosseae* was chosen as the fungal symbiont because it is one of the most abundant AM fungal species in soybean production soils (Khalil et al., 1992; Winkler et al., 1994).

*Experiment I:* Silt loam soil (36% sand, 40% silt, 24%

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clay; 1.6% organic matter; pH 7.2) was collected from an *H. glycines*-infested commercial production field near Columbus, Kansas. The soil was mixed 1:1 (v/v) with washed river sand and steam-pasteurized (2 hours at 80 °C). The final P content of the mixture was similar to the original soil (35 kg/ha vs. 32 kg/ha, respectively). A total of 60 2-liter plastic pots were filled with 1,800 $cm^3$ of soil mix. An egg suspension of *H. glycines* at a rate of either 100 or 1,000 eggs and J2 per 100 $cm^3$ soil, or an eggfree filtrate, as incorporated into the soil of each pot by manually mixing the nematode inoculum and the soil in a plastic bag. The soil was returned to the pot, and four *B. japonicum*-inoculated seeds of either the nematode-susceptible cultivar Bay or the resistant cultivar Forrest were planted to a depth of 2.5 cm. One-half of the pots of each treatment received a suspension of 300 *G. mosseae* spores, pipeted evenly over the four seeds, which were then covered with soil and watered.

The experiment was a 2 × 2 × 3 factorial arranged in a randomized complete block design with five replications. Pots were watered as needed, and plants were grown at ambient temperatures of 28 ± 5 °C day and 24 ± 5 °C night for 35 days. At termination of the experiment, eggs and J2 of *H. glycines* were collected from roots as described previously and counted at x 100 magnification. Plant shoots and roots were severed at the first internode and dried at 60 °C for 48 hours prior to weighing. After weighing, subsamples of the roots were placed in test tubes, covered with a 10% KOH solution, and heated at 90 °C for 1 hour prior to staining with trypan blue (Phillips and Hayman, 1970). Stained roots were placed in a petri dish scored in 1-cm squares to determine percent colonization by *G. mosseae* (Daniels et al., 1981).

*Experiment II:* The first experiment was repeated using the *H. glycines*-susceptible cultivar Hutcheson rather than Bay, and nematode inoculum levels of 0, 100, 1,000, and 10,000 eggs and J2 per 100 $cm^3$ soil. The second experiment was conducted under similar greenhouse conditions and was terminated after 83 days. All other methods were as reported above.

*Statistical analyses:* All data were subjected to analyses of variance using the general linear model (GLM) procedure of SAS (SAS Institute, Cary, NC). Nematode densities and fungal colonization were $\log_{10}$-transformed $(x + 1)$ prior to analysis to reduce heterogeneity of variances. Relationships between soybean growth and *H. glycines* initial population density (Pi), and between nematode final population density (Pf), reproduction (Pf/Pi), and Pi, were examined using linear regression analysis and compared across cultivar and mycorrhizal treatments.

## Results

*Plant dry weights:* The size of soybean plants at harvest was similar between experiments, with total plant dry weights averaging 5.0 g and 6.5 g/pot for Experiment I and Experiment II, respectively. The effects of *H. glycines* and *G. mosseae* on plant growth generally were consistent across experiments, despite differences in duration of the experiments, with a 29% average decrease in total plant weight for nematode-inoculated treatments vs. controls and a 36% average increase in total plant weight for mycorrhizal vs. nonmycorrhizal treatments (Table 1; Fig. 1). Absolute effects of both organisms were greater in the first experiment than in the second experiment, but relative trends were similar between experiments. Regression analyses of relative shoot and root weights, calculated by dividing absolute weights from each experimental unit within cultivar by the mycorrhizal, nematode-free treatment weights for that cultivar, and combined across experiments are presented in Figure 1.

Cultivar × *H. glycines* interactions ($P \leq 0.05$) occurred in both experiments (Table 1). Steeper slopes occurred with regressions of relative shoot and root weights against *H. glycines* Pi for the susceptible cultivar than for the resistant cultivar, regardless of mycorrhizal status (Fig. 1). Additionally, only the regressions associated with the susceptible cultivar consistently explained a significant ($P \leq 0.05$) amount of variability in the data as indicated by the $R^2$ values. In contrast, a cultivar × *G. mosseae* interaction ($P \leq 0.05$) was observed only once (Table 1), and the relative effects of mycorrhizal symbiosis were similar for both cultivars across all *H. glycines* Pi levels (Fig. 1).

*Heterodera glycines* × *G. mosseae* interactions ($P \leq 0.05$) were observed for shoot dry weights in the first experiment, and for root dry weights in both experiments (Table 1), with differences in plant growth due to mycorrhizal symbiosis generally decreasing with increasing Pi. The same trend was suggested by regression analyses, although slopes were not significantly different between mycorrhizal treatments (Fig. 1). Multiple regressions of plant weights vs. numbers of *H. glycines* eggs and J2 per g root and % root colonization by *G. mosseae*

Table 1. Analysis of variance for the effects of *Heterodera glycines* (Hg) and *Glomus mosseae* (Gm) on shoot and root dry weights of Hg race 3-resistant and susceptible soybean.

| Source of variation | Mean squares | | | |
|---|---|---|---|---|
| | Experiment I | | Experiment II | |
| | Shoot | Root | Shoot | Root |
| Cultivar (C) | 47.76[a] | 2.37[a] | 42.30[a] | 0.01 |
| Hg | 30.28[a] | 4.63[a] | 12.24[a] | 0.35[b] |
| Gm | 51.95[a] | 3.19[a] | 5.89[b] | 0.87[a] |
| C × Hg | 16.95[a] | 3.01[a] | 1.33 | 0.47[b] |
| C × Gm | 2.90[b] | 0.27 | 0.39 | 0.22 |
| Hg × Gm | 3.91[a] | 0.37[b] | 0.60 | 0.58[a] |
| C × Hg × Gm | 0.78 | 0.05 | 1.98 | 0.19 |
| Error | 0.68 | 0.10 | 1.03 | 0.13 |

[a] $P \leq 0.01$.
[b] $P \leq 0.05$.

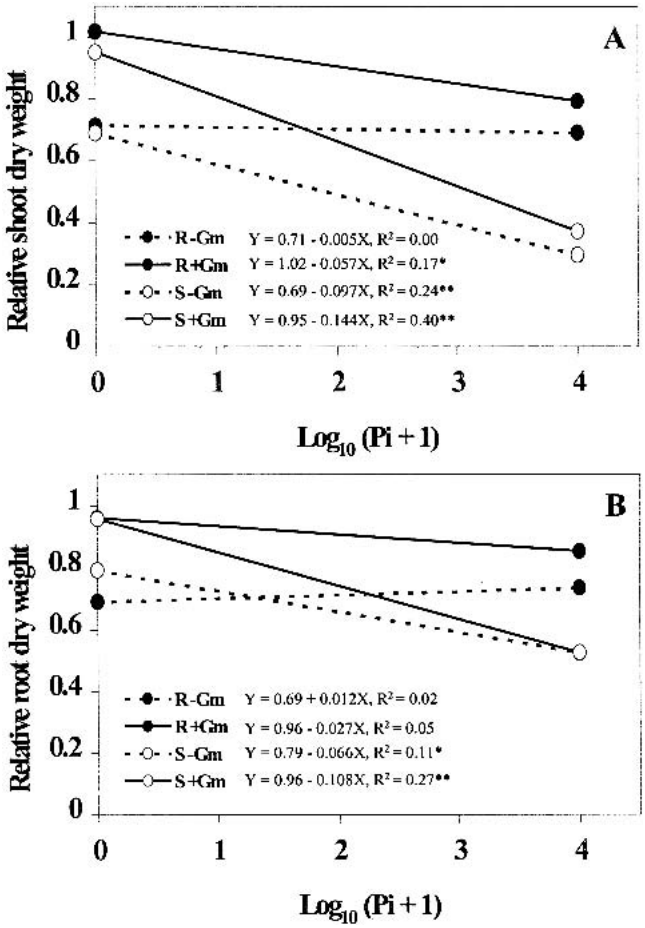


FIG. 1. Linear regressions of relative shoot (A) and root (B) weights of *Heterodera glycines* (Hg) race 3-resistant (R) and susceptible (S) soybean cultivars vs. Hg initial population density (Pi = number of eggs and second-stage juveniles per 100 $cm^3$ soil) on mycorrhizal (+Gm) and nonmycorrhizal (–Gm) plants. Intercepts and slopes were different ($P \leq 0.05$) for mycorrhizal treatments and cultivars, respectively. Significance of regressions indicated by * for $P \leq 0.05$ and ** for $P \leq 0.01$. Symbols designate cultivars and do not represent data points.

indicated that variation was best explained by an additive model for shoot weight ($R^2 = 0.42$, $P < 0.001$) and a multiplicative (antagonistic) model for root weight ($R^2 = 0.23$, $P < 0.001$).

*Nematode population densities:* The number of nematodes recovered from soybean roots and overall population increase (Pf/Pi) of *H. glycines* exhibited strong cultivar × nematode Pi interactions ($P \leq 0.01$) in both experiments (Table 2). Nematode numbers on the roots of the resistant cultivar averaged only 5% of those on roots of susceptible cultivars across all Pi levels (Table 3). Maximum final population densities were observed at intermediate Pi levels on susceptible cultivars, but densities at the two highest Pi levels did not differ significantly in either experiment. Nematode reproduction on susceptible cultivars was density dependent, with Pf/Pi inversely related to Pi ($R^2 = 0.42$ to 0.92, $P < 0.001$). Actual rates of nematode population increase varied noticeably between experiments. At the low Pi (100 eggs and J2/100 $cm^3$ soil), the average Pf/Pi value for the first experiment was 10-fold that measured for the second experiment (Table 3). Similarly, a density-dependent reduction in the rate of population increase was observed at an order of magnitude lower Pi for the first than for the second experiment. These results are contrary to those expected, given the longer duration of the second experiment, but are consistent with the greater suppression of soybean growth by the nematode in the first experiment.

No consistent effects of mycorrhizal symbiosis on *H. glycines* populations were observed in the present study (Table 2). Numbers of eggs and J2 per g root were 40% greater ($P = 0.03$) across Pi levels on mycorrhizal than on nonmycorrhizal plants in the second experiment, but Pf/Pi levels were not different in either experiment.

*Root colonization by G. mosseae:* Colonization of soybean roots by *G. mosseae* was comparable to, or higher than, levels observed for soybeans in the field, averaging 49% across cultivars, Pi levels, and experiments.

TABLE 2. Analysis of variance for the effects of soybean cultivar, *Heterodera glycines* (Hg), and *Glomus mosseae* (Gm) on final numbers of Hg eggs and second-stage juveniles (J2) per g root, nematode reproduction, and % Gm root colonization.

| | Mean squares | | | | | |
|---|---|---|---|---|---|---|
| | Experiment I | | | Experiment II | | |
| Source of variation | Pf[a]/g root | Pf/Pi[a] | Gm root colonization | Pf[a]/g root | Pf/Pi[a] | Gm root colonization |
| Cultivar (C) | 30.39[b] | 10.53[b] | 0.05[c] | 33.55[b] | 7.74[b] | 0.00 |
| Hg | 15.67[b] | 8.42[b] | 0.05[c] | 52.90[b] | 1.35[b] | 0.37[b] |
| Gm | 0.78 | 0.01 | 44.23[b] | 2.52[c] | 0.00 | 46.35[b] |
| C × Hg | 7.29[b] | 4.97[b] | 0.01 | 3.83[b] | 1.09[b] | 0.04 |
| C × Gm | 0.07 | 0.25 | 0.05[c] | 0.25 | 0.01 | 0.07 |
| Hg × Gm | 0.34 | 0.02 | 0.05[c] | 0.33 | 0.13 | 0.28[b] |
| C × Hg × Gm | 0.03 | 0.15 | 0.01 | 0.61 | 0.04 | 0.04 |
| Error | 0.42 | 0.08 | 0.01 | 0.54 | 0.04 | 0.06 |

[a] Pf = final number of eggs and J2 per pot; Pf/Pi = final number of eggs and J2 per pot/initial number of eggs and J2 per pot.
[b] $P \leq 0.01$.
[c] $P \leq 0.05$.

TABLE 3. Effects of soybean cultivar and *Heterodera glycines* initial population density on nematode final population density and reproduction.

| | Experiment I | | Experiment II | |
|---|---|---|---|---|
| Cultivar/Pi[a] | Pf[a]/g root | Pf/Pi[a] | Pf[a]/g root | Pf/Pi[a] |
| Resistant | | | | |
| 0 | 397 c[b] | — | 91 e | — |
| 100 | 4,001 bc | 2.7 bc | 911 d | 0.6 bc |
| 1,000 | 4,558 b | 0.4 c | 2,735 c | 0.3 bc |
| 10,000 | — | — | 16,912 b | 0.2 c |
| Susceptible | | | | |
| 0 | 387 c | — | 73 e | — |
| 100 | 161,428 a | 134.1 a | 11,713 b | 13.1 a |
| 1,000 | 76,474 a | 1.9 b | 162,134 a | 17.2 a |
| 10,000 | — | — | 121,670 a | 1.0 b |

[a] Pi = initial number of eggs and second-stage juveniles (J2) per 100 $cm^3$ soil; Pf = final number of eggs and J2 per pot; Pf/Pi = final number of eggs and J2 per pot/initial number of eggs and J2 per pot.
[b] Means within a column followed by the same letter are not significantly different according to Least Squares Means of $log_{10}$-transformed data (P = 0.05).

Colonization was lower at high levels of root infection by *H. glycines,* with reduced ($P \leq 0.05$) colonization observed at the greatest Pi in both experiments, and in both nematode-resistant and susceptible cultivars (Table 2; Fig. 2). Colonization by *G. mosseae* was inversely related ($R^2 = 0.25$ to 0.65, $P < 0.01$) to *H. glycines* Pi across cultivars in both experiments. In the first experiment, this relationship was stronger for the susceptible cultivar, resulting in lower overall colonization of the susceptible compared to the resistant cultivar (Table 2).

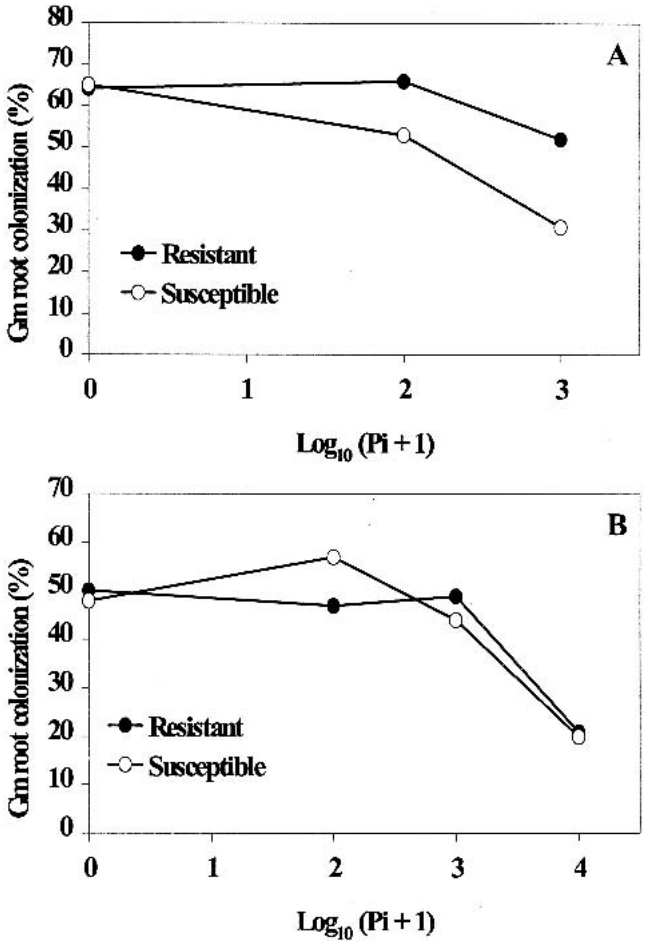


FIG. 2. Effect of *Heterodera glycines* (Hg) initial population density (Pi = number of eggs and second-stage juveniles per 100 $cm^3$ soil) on root colonization of Hg-resistant and susceptible soybean cultivars by *Glomus mosseae* (Gm) in greenhouse experiment I (A) and II (B).

## DISCUSSION

The independent effects of mycorrhizal fungi and *H. glycines* on soybean growth have been well documented. Arbuscular mycorrhizal fungi, such as *G. mosseae,* are known to have a stimulatory effect on the growth of many plants, including soybean, that is related to enhanced P nutrition and water transport (Khalil et al., 1999; Ross, 1971; Safir et al., 1972; Tylka et al., 1991), as well as increased $N_2$ fixation by *B. japonicum* (Zhang et al., 1995). Parasitism by *H. glycines,* in contrast, is associated with disruption of root vascular tissues (Endo, 1964) and, in susceptible soybean cultivars, reduced $N_2$ fixation (Kennedy et al., 1999), resulting in increased moisture and nutrient stress as well as growth suppression (Kennedy et al., 1999; Melakeberhan, 1998; Tylka et al., 1991). Studies of the concomitant effects of these organisms on soybean growth have not produced consistent results, however. While mycorrhizal symbiosis frequently is associated with increased tolerance to sedentary parasitic nematodes in greenhouse studies (Habte et al., 1999; Hussey and Roncadori, 1982; Tylka et al., 1991), only additive effects have been observed in field environments (Tylka et al., 1991; Winkler et al., 1994). In the present study, the effects of *H. glycines* and *G. mosseae* on soybean growth were additive, or the growth stimulation by *G. mosseae* was reduced in the presence of the nematode. No evidence for increased tolerance to *H. glycines* was observed for mycorrhizal plants.

Suppression of sedentary nematode populations is a commonly reported consequence of mycorrhizal symbiosis (Carling et al., 1989; Habte et al., 1999; Tylka et al., 1991). Although this phenomenon is well estab-

lished for *M. incognita,* Tylka et al. (1991) observed only transient suppression of *H. glycines* populations on mycorrhizal soybean roots. Similarly, numbers of *H. glycines* eggs on soybean roots were either unaffected or enhanced by mycorrhizal symbiosis in this greenhouse study and in a previous field study (Winkler et al., 1994). As was pointed out by Tylka et al. (1991), differences in carrying capacities between mycorrhizal and nonmycorrhizal plants can mask suppressive effects, and others have observed that nematode suppression can vary with fungal species and P nutrition (Carling et al., 1989; Habte, et al., 1999). Nevertheless, it seems clear that the potential for suppression of *H. glycines* populations by mycorrhizal fungi is much less than that observed for *M. incognita* on the same host plant. A possible explanation for this differential response is suggested by the observation that improved P nutrition is suppressive to *M. incognita* (Carling et al., 1989) but not *H. glycines* (Tylka et al., 1991).

The effect of nematode parasitism on mycorrhizal symbiosis has been less well characterized, but *M. incognita* apparently has no effect on fungal colonization of plant roots (Carling et al., 1989; Habte et al., 1999). In contrast, our study detected consistent suppression of root colonization by *G. mosseae* at high levels of *H. glycines* infection. This result corroborates evidence from a prior field study that, although more circumstantial, also suggested that *H. glycines* was antagonistic to mycorrhizal colonization (Winkler et al., 1994). The consequences of reduced colonization remain uncertain because level of colonization is not necessarily related to the size of the stimulatory effect on plant growth (Habte et al., 1999).

Promising demonstrations of mycorrhizal-induced nematode suppression and increased host tolerance under greenhouse conditions have inspired interest in manipulating this phenomenon for improved nematode management under field conditions. In the case of *H. glycines,* however, suppression has been either inconsistent (Tylka et al., 1991) or absent (this study). To the contrary, there is evidence that high levels of root infection by *H. glycines* may be antagonistic to mycorrhizal symbiosis. Although prior studies suggest that different species of arbuscular mycorrhizal fungi or different levels of P nutrition may have yielded different results, the conditions of our study, including *H. glycines* egg densities, fungal species and density, and soil P content, are representative of soybean production fields in Kansas (Winkler et al., 1994).

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