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Effect of Planting Date, Alachlor, and Fenamiphos on *Heterodera glycines* Development[1]

B. S. Sipes and D. P. Schmitt[2]

Abstract: The effects of alachlor (2.25 kg a.i./ha) and fenamiphos (2.25 kg a.i./ha) on the penetration and development of *Heterodera glycines* were examined on *Glycine max* cultivars Deltapine 105 planted 29 April, 29 May, and 29 June 1986 and Deltapine 105 and Centennial planted 15 May, 15 June, and 15 July 1987. Penetration was lowest on the third planting of soybeans and on fenamiphos-treated plants. Development from second-stage juveniles to adult females required 270 (1986) and 260 (1987) DD20/32 on roots from the first planting control and alachlor treatments. Fenamiphos, alone or with alachlor, retarded development in Deltapine 105 (1986) and in Centennial (1987). Males matured in roots from the second planting in 190 (1986) and 180 (1987) DD20/32 regardless of treatment or cultivar. No development occurred in roots from the third planting until 400 DD20/32 in 1986, but in 1987 development was similar to that in roots from the second planting. Nematode development was similar in alachlor-treated and control roots regardless of planting date. Fenamiphos restricted nematode penetration on most planting dates and slowed development. Simultaneous applications of alachlor and fenamiphos usually also inhibited development.

Key words: alachlor, development, fenamiphos, *Glycine max*, *Heterodera glycines*, pesticide interaction, soybean, soybean cyst nematode.

A successful harvest entails many management strategies and may involve the use of pesticides. Several pesticides may be applied to a single field for control of several pests. The effects of pesticides on their target organisms are generally well researched, but their effects on nontarget organisms often are unknown (16).

Some herbicides and fungicides stimulate nematode hatch (10,14,20). When such pesticides are used with a nematicide, nematode control is often enhanced; however, interactions between pesticides may also decrease the efficacy of one pesticide (antagonism) (16). Sequential applications of the herbicide alachlor and the nematicide fenamiphos resulted in less control of *Heterodera glycines* Ichinohe than when the nematicide was applied alone (4,20,21). Alachlor is associated with increased nematode penetration (4) and less inhibition of development by fenamiphos (21), both factors contributing to a late season nematode population resurgence (20).

The interaction among soybean (*Glycine max* (L.) Merr.), *H. glycines*, alachlor, and fenamiphos is influenced by climatic, edaphic, and cultural factors. Temperature is a primary factor affecting all organisms and chemical reactions (1,3,15,17,19). Nitrogen fixation, photosynthesis, respiration, water uptake, and translocation are a few of the physiological processes in soybean that are affected by temperature (17). Nematode movement (15), penetration (9), development (1), and reproduction (19) also are influenced by soil temperature. The solubility, longevity (as a function of microbial degradation), and volatility of a pesticide will also change with temperature (3). Warm temperatures stimulate microbial activity and thus, pesticide degradation. Volatility and solubility also increase as temperature increases.

Soybeans are planted from late April to early July in North Carolina; therefore, alachlor and fenamiphos activity should be characterized relative to *H. glycines* penetration and development under various soil temperature regimes. The objective of this research was to measure the effect of alachlor and fenamiphos on *H. glycines* penetration and development as affected by natural soil temperature at different planting dates.

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[2] Graduate Research Assistant and Professor, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

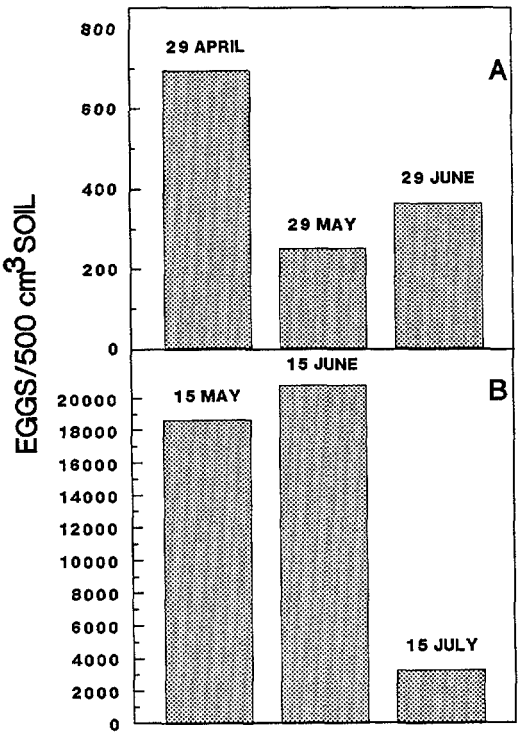

FIG. 1. Mean number of *Heterodera glycines* eggs per 500 cm³ soil recorded at three planting dates. A) 1986. B) 1987.

MATERIALS AND METHODS

A field at the Central Crops Research Station near Clayton, North Carolina, infested with *H. glycines* race 1 was planted with the *H. glycines*-susceptible soybean cultivar Deltapine 105 in 1986 and 1987. The *H. glycines*-resistant soybean cultivar Centennial was also planted in 1987. Soybeans were planted and pesticides applied on three dates each year to attain three soil temperature regimes: 29 April, 29 May, and 29 June 1986 and 15 May, 15 June, and 15 July 1987. A 2 × 2 factorial experiment in a completely randomized block design was used on each planting date in 1986. A split-plot design was used in 1987 with pesticides as whole plots and soybean cultivars as subplots. Plots were two 5-m-long rows spaced 1 m apart. Treatments were replicated six times in 1986 and four times in 1987. Alachlor 4E was broadcast over the plot at 0 or 2.25 kg a.i./ha and fenamiphos 3EC was applied in a 15-cm band at 0 or 0.23 g a.i./meter over the planting row. The plots were irrigated with the equivalent of 1.25 cm rain following treatment.

Each plot was assayed for nematodes immediately before planting each year and at harvest in 1987. Nematodes were extracted from a 500-cm³ soil sample using a combination of elutriation (6) and centrifugation (11). Cysts were crushed with a glass tissue grinder to release eggs (1).

Heterodera glycines penetration and development in soybean was determined at 4-day intervals in all plots until mature females were observed. Two root systems per plot were collected with a 5-cm-d soil coring device to a depth of 15 cm. Nematodes in the roots were stained (7) and numbers of penetrations were recorded. Twenty nematodes per root system were randomly selected and categorized as 1) second-stage juveniles (J2), 2) swollen J2 (SJ2), 3) third or fourth-stage juveniles (J34), 4) adult males (M), or 5) adult females (F) (18). Because of continuing infections by J2 in the field, the 5 (1986) or 10 (1987) most developed nematodes were used to calculate the mean stage of population development.

Population development (D) of *H. glycines* was calculated for each collection date based upon the mean number of individuals observed in each life stage weighted against the J2 (1,13,23). Each weighting factor is derived from half the time spent in a life stage divided by the total time of the life cycle from infective J2 to F (10 days) (12) plus the total time spent in all previous life stages, so that D = (% J2) + (% SJ2 × 2.33) + (% J34 × 4.25) + (% M × 6.33) + (% F × 6.67).

Soil temperature was monitored throughout the experiment with two thermograph probes placed 20 cm below the soil surface. Degree days were calculated from the mean daily high and low soil temperatures (2). Basal and upper developmental thresholds for *H. glycines* development used in the degree-day calculation were 20 C (9) and 32 C (19), respectively, and are reported as DD20/32 (1).

Plots were hand weeded and cultivated

TABLE 1. *Heterodera glycines* penetration (no./root) of soybean cultivars Deltapine 105 (1986) or Deltapine 105 and Centennial (1987) in response to treatment with alachlor, fenamiphos, or both on three planting dates during two growing seasons.

	1986			1987		
	29 April	29 May	29 June	15 May	15 June	15 July
Control	26 ax	14 ay	5 az	190 ax	159 ay	30 az
Alachlor	21 ax	12 aby	6 az	165 ax	137 ay	32 az
Fenamiphos	12 bx	14 ax	2 by	43 by	72 bx	5 bz
Combination	10 bx	7 by	1 bz	33 by	84 bx	3 bz

Means with the same letter within a planting date (a, b) and across planting dates (x, y, z) within each year are not significantly different ($P = 0.05$) according to the Waller–Duncan k-ratio *t*-test.

to provide uniform weed control throughout the season.

Regression analyses using linear, quadratic, exponential, and Gompertz models were performed on D with DD20/32 as the independent variable. The best fitting model was selected for each treatment based on r^2 values and level of significance. The mean number of root penetrations by nematodes in both growing seasons and RF values (Pf/Pi) were compared using a Waller–Duncan k-ratio *t*-test.

RESULTS

Heterodera glycines development was affected by environmental conditions and pesticides applied at planting in both 1986 and 1987. Nematode penetration was lowest at the third planting each year (Table 1) when preplant nematode population levels were also lowest (Fig. 1). Penetration was less in fenamiphos-treated plots with or without alachlor, except at the second planting in 1986. Alachlor did not affect penetration at any planting date during either growing season. Although penetration of susceptible and resistant cultivars was similar in 1987, reproduction was significantly lower ($P = 0.05$) on the resistant cultivar (Table 2).

The rate of development to maturity differed among planting dates and between pesticide treatments within planting dates (Tables 3–5). Development was slow on plants of the first planting (29 April 1986 and 15 May 1987). Males first developed on Deltapine 105 at 220 and 260 DD20/32 (12 and 16 days after planting) in 1986 and 1987, respectively. Females matured on Deltapine 105 at 270 DD20/32 in 1986 and 260 DD20/32 in 1987. Females did not mature on Centennial until 300 DD20/32. Males were found in roots of second-planting soybeans within 190 and 180 DD20/32 (16 and 12 days after planting) in 1986 and 1987, regardless of cultivar. Development in roots of final-planting soybeans was strikingly different between 1986 and 1987. Adults were not observed until 850 DD20/32 in 1986 but developed within 130 DD20/32 in 1987 (55 and 16 days after planting, respectively).

Environmental conditions following the first planting resulted in different rates of

TABLE 2. RF values (Pf/Pi) for *Heterodera glycines* on soybean cultivars Centennial and Deltapine 105 planted 15 May, 15 June, and 15 July 1987 in soil treated with alachlor, fenamiphos, or both.

	Centennial			Deltapine 105		
	15 May	15 June	15 July	15 May	15 June	15 July
Control	0.01 ax	0.02 ax	0.19 ax	1.28 ax	1.50 ax	12.62 by
Alachlor	0.01 ax	0.02 ax	0.57 ax	4.19 axy	1.44 ax	10.07 aby
Fenamiphos	0.01 ax	0.01 ax	0.03 ax	1.34 ax	1.17 ax	4.72 ax
Combination	0.02 ax	0.03 ax	0.06 ax	1.01 ax	1.05 ax	3.53 ax

Means with the same letter within a planting date × cultivar (a, b) and across planting dates (x, y) are not significantly different ($P = 0.05$) according to the Waller–Duncan k-ratio *t*-test.

TABLE 3. Mean stage of population development equations for *H. glycines* on soybean cultivars Deltapine 105 planted 29 April 1986 and Deltapine 105 and Centennial planted 15 May 1987 in soil treated with alachlor, fenamiphos, or both.

	Deltapine 105		Centennial
	29 April 1986	15 May 1987	15 May 1987
Control	$D = 0.443 + 0.029H - 0.00006H^2$ $r^2 = 0.92$	No significant model	$D = 0.80e^{0.0066H}$ $r^2 = 0.91$
Alachlor	$D = 0.189 + 0.029H - 0.00005H^2$ $r^2 = 0.99$	$D = 1.10e^{0.0055H}$ $r^2 = 0.75$	$D = -2.80 + 0.068H - 0.00012H^2$ $r^2 = 0.99$
Fenamiphos	$D = 1.19e^{0.0043H}$ $r^2 = 0.97$	$D = 1.23e^{0.0015H}$ $r^2 = 0.52$	$D = 0.61e^{0.0055H}$ $r^2 = 0.95$
Combination	$D = 0.271 + 0.020H - 0.00003H^2$ $r^2 = 0.81$	$D = 1.70e^{0.0074H}$ $r^2 = 0.98$	$D = 1.03e^{0.0019H}$ $r^2 = 0.79$

D = mean stage of population development; H = accumulated degree days based upon a 20 C basal and 32 C upper developmental threshold; e = natural logarithm.

TABLE 4. Mean stage of population development equations for *H. glycines* on soybean cultivars Deltapine 105 planted 29 May 1986 and Deltapine 105 and Centennial planted 15 June 1987 in soil treated with alachlor, fenamiphos, or both.

	Deltapine 105		Centennial
	29 April 1986	15 May 1987	15 June 1987
Control	$D = 0.38e^{0.0159H}$ $r^2 = 0.90$	$D = -3.37 + 0.098H - 0.00023H^2$ $r^2 = 0.95$	$D = 0.84e^{0.0087H}$ $r^2 = 0.85$
Alachlor	$D = -1.47 + 0.04H - 0.00006H^2$ $r^2 = 0.82$	$D = 0.52e^{0.0155H}$ $r^2 = 0.85$	$D = 0.53e^{0.0099H}$ $r^2 = 0.78$
Fenamiphos	$D = 0.91e^{0.0051H}$ $r^2 = 0.60$	$D = 0.52e^{0.0155H}$ $r^2 = 0.87$	$D = 0.54e^{0.0117H}$ $r^2 = 0.85$
Combination	$D = 0.828 + 0.032H - 0.00006H^2$ $r^2 = 0.87$	$D = 0.43e^{0.0087H}$ $r^2 = 0.93$	$D = 0.75e^{0.087H}$ $r^2 = 0.93$

D = mean stage of population development; H = accumulated degree days at a 20 C basal and 32 C upper developmental threshold; e = natural logarithm.

TABLE 5. Mean stage of population development equations for *H. glycines* on soybean cultivars Deltapine 105 planted 29 June 1986 and Deltapine 105 and Centennial planted 15 July 1987 in soil treated with alachlor, fenamiphos, or both.

	Deltapine 105		Centennial
	29 June 1986	15 July 1987	15 July 1987
Control	No development	$D = -1.02 + 0.064H - 0.00014H^2$ $r^2 = 0.90$	$D = 0.99e^{0.0037H}$ $r^2 = 0.48$
Alachlor	No development	$D = -2.81 + 0.119H - 0.00040H^2$ $r^2 = 0.89$	$D = 0.78e^{0.0095H}$ $r^2 = 0.95$
Fenamiphos	No development	$D = 0.88e^{0.0038H}$ $r^2 = 0.74$	$D = 0.80e^{0.0061H}$ $r^2 = 0.95$
Combination	No development	$D = -0.14 + 0.036H - 0.00012H^2$ $r^2 = 0.90$	$D = 0.93e^{0.0017H}$ $r^2 = 0.51$

D = mean stage of population development; H = accumulated degree days at a 20 C basal and 32 C upper developmental threshold; e = natural logarithm.

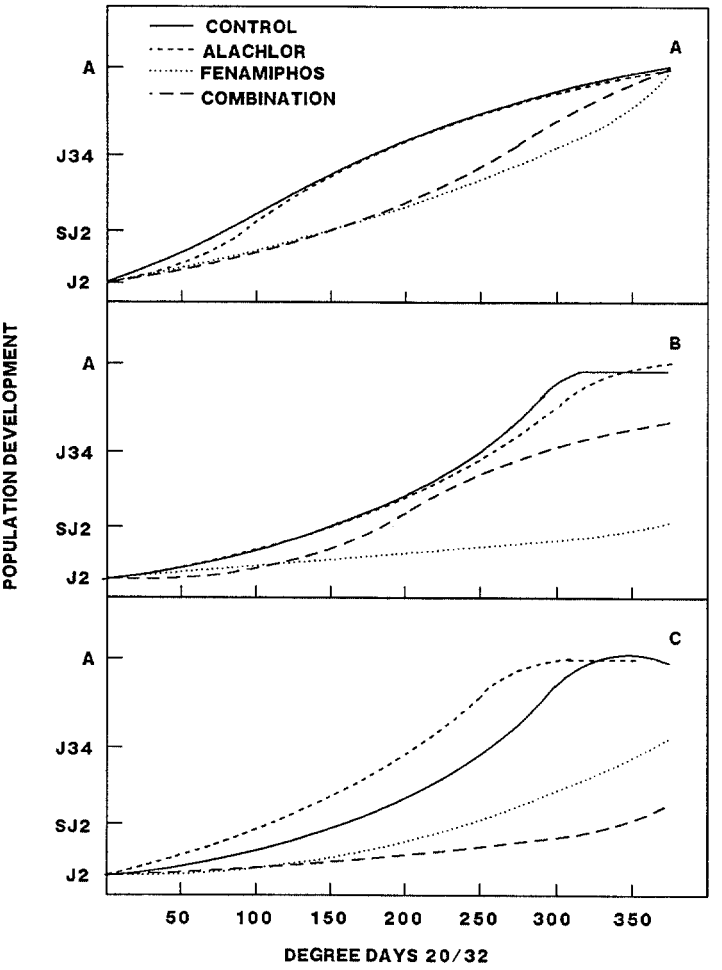

FIG. 2. *Heterodera glycines* mean population development, as affected by alachlor (2.25 kg a.i./ha) and fenamiphos (2.25 kg a.i./ha). A) Deltapine 105 planted 29 April 1986. B) Deltapine 105 planted 15 May 1987. C) Centennial planted 15 May 1987.

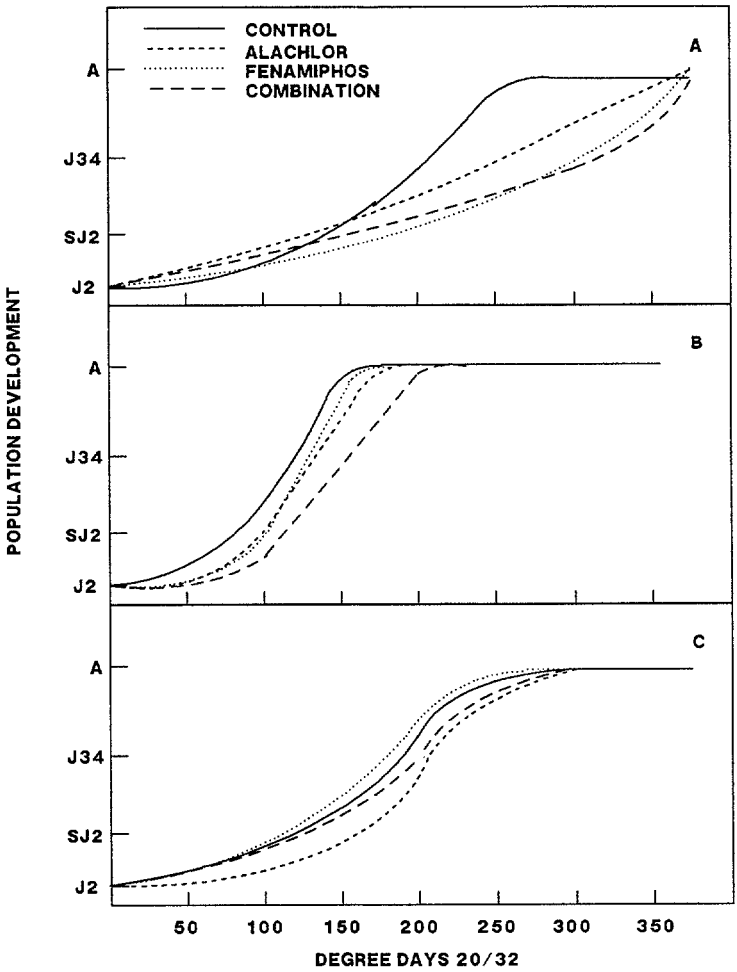

FIG. 3. *Heterodera glycines* mean population development as affected by alachlor (2.25 kg a.i./ha) and fenamiphos (2.25 kg a.i./ha). A) Deltapine 105 planted 29 May 1986. B) Deltapine 105 planted 15 June 1987. C) Centennial planted 15 June 1987.

D in response to the pesticide treatments. On alachlor-treated plants, D on the susceptible cultivar was not significantly different from D on the controls (Fig. 2A, B; Table 3). On Centennial, D was increased by the alachlor treatment, relative to the untreated controls (Fig. 2C, Table 3). Fenamiphos, alone or with alachlor, slowed D on Deltapine 105 in 1986 and on Centennial in 1987. In 1987, however, when fenamiphos and alachlor were applied sequentially, development occurred on Deltapine 105 (Fig. 2B).

Neither alachlor nor fenamiphos affected nematode development in the second-planting soybeans. In 1986 and 1987 development was similar among all pesticide treatments (Fig. 3A–C, Table 4). Development was slightly slower on Centennial than on Deltapine 105 (Fig. 3B, C).

Environmental conditions following the third planting differed greatly between years, resulting in significantly different D. Little development occurred until 400 DD20/32 in 1986 (Fig. 4A). In 1987 maturation proceeded to the adult stage on Deltapine 105 but did not continue past the J34 on Centennial (Fig. 4B, C). Development was fastest on Centennial treated with alachlor. The rate of D on the Deltapine 105 was similar to that on the second planting of Deltapine 105. Fenamiphos, alone or with alachlor, delayed nematode development on Deltapine 105.

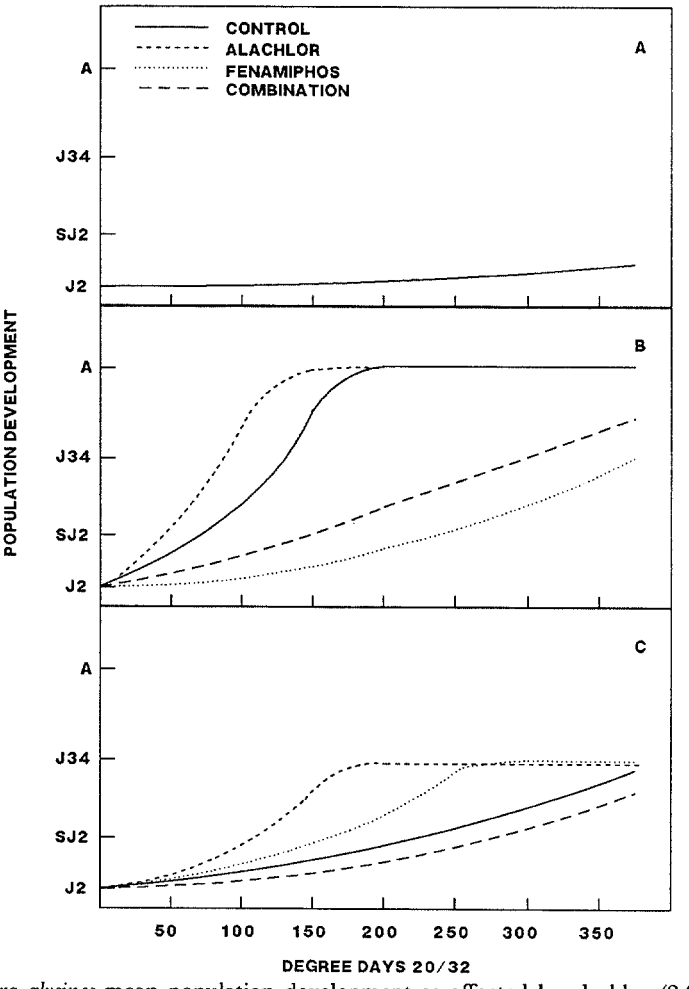

FIG. 4. *Heterodera glycines* mean population development as affected by alachlor (2.25 kg a.i./ha) and fenamiphos (2.25 kg a.i./ha). A) Deltapine 105 planted 29 June 1986. B) Deltapine 105 planted 15 July 1987. C) Centennial planted 15 July 1987.

DISCUSSION

Accumulated degree days (DD20/32) required for the first adults to mature were similar between the first and second plantings in 1986 and 1987 and between cultivars in 1987. The lack of development during the third planting of 1986 may have been due to a combination of prohibitive soil temperatures and inadequate soil moisture. Exposure to temperatures above 35 C increases mortality of *H. glycines* and is detrimental to its development (19). Temperatures above 32 C result in declining juvenile emergence (22). During the third planting in 1986, the soybean plants showed water stress at 8 and 12 days, between which only 0.7 cm of rainfall was recorded. Dry soil conditions do not favor nematode penetration and development (8).

Maturation of *H. glycines* may be delayed by fenamiphos in the field, but it is not prevented, even though similar concentrations of the nematicide arrest development in greenhouse experiments (21). Fenamiphos inhibition of *H. glycines* development in the greenhouse is negated by subsequent application of alachlor (21). This antagonistic action between pesticides as they affect nematode development was observed only during the first planting of 1987. During all other plantings, nematode maturation was similar on plants treated with fenamiphos alone or with alachlor. Although the rate of nematode development

was altered by the pesticide combination, no late-season population resurgence was observed. The lack of a resurgence was probably due to an initially high population of *H. glycines* (>14,000 eggs/500 cm^3 soil).

Microbial degradation could account for the reduced effect of fenamiphos on nematode penetration and for similar rates of development between treated and untreated plants during the second planting of 1986 and 1987. Microbial degradation of pesticides in the field is common (3). In addition, the phosphate group of fenamiphos can become ionized and bind with soil hydroxides, serving to further reduce the effective concentration of the nematicide (5).

Soil temperature and soil moisture appeared to have the most effect on *H. glycines* development, with pesticides playing a secondary role. Antagonism between alachlor and fenamiphos has been shown to increase *H. glycines* penetration in vitro over nematicide alone treatments (4) and to allow nematode maturation in greenhouse experiments (21). Differences in environments, however, may alter this antagonistic phenomenon in the field. Greater penetration and continued development in alachlor-fenamiphos treatments are a few of the biological interactions occurring in the soybean–*H. glycines* symbiosis and contributing to the late season population resurgence of *H. glycines* associated with alachlor and fenamiphos. Fecundity and embryogenesis of *H. glycines* are other biological functions not yet investigated, but alterations in these may also be important in the dynamics of the antagonistic interaction between alachlor and fenamiphos.

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