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Development of *Heterodera glycines* as Affected by Alachlor and Fenamiphos[1]

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Abstract: A series of greenhouse experiments was conducted to elucidate the postinfection development of *Heterodera glycines* in response to applications of alachlor and fenamiphos. The rate of *H. glycines* maturation on a susceptible soybean cultivar was not altered by 1.0 μg alachlor/g soil but was completely inhibited by 1.0 or 1.5 μg fenamiphos/g soil. An alachlor–fenamiphos combination allowed development after an initial 300-degree-day delay. Nematode maturation on the resistant soybean cultivar Centennial with 1.0 μg alachlor/g soil was similar to that observed on an untreated resistant control. Twice as many females matured on Centennial plants growing in alachlor-treated soil as on untreated Centennial plants. Fenamiphos in combination with alachlor (1.0 μg a.i./g soil) allowed development on Centennial at half the rate of the resistant control. This antagonism between alachlor and fenamiphos on development may help to explain late season population resurgence of *H. glycines* observed with field application of these pesticides.

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

Current agronomic practices for the production of soybean (*Glycines max* (L.) Merr.) may involve the use of several pesticides during a single growing season. Application of two or more pesticides to a field may lead to interactions between any two or more of the chemicals (10,13,15,18,19) changing the efficacy of one or more of them. The nematicide dichloropropane-dichloropropene controls *Globodera tabacum* (Lownsbery & Lownsbery) Behrens and *Meloidogyne* spp. better when used with the fungicides maneb and nabam than when used alone (13). Vernolate, trifluralin, and metribuzin stimulated the hatch of *Heterodera glycines* Ichinohe, enhancing control with aldicarb (10). In contrast, the acid anilide herbicide alachlor reduced control of *H. glycines* with fenamiphos, resulting in a late-season population resurgence of the nematode (19).

The antagonistic effects of alachlor on fenamiphos are partially due to increased hatch, survival, and penetration of soybean by *H. glycines* (4). Fenamiphos at low rates (0.5 μg a.i./ml) in combination with alachlor (0.06–1.0 μg a.i./ml) resulted in increased in vitro hatch and penetration over an untreated control (4). Survival of the nematode is slightly enhanced by the pesticides in vitro (4). Development of *H. glycines* in the presence of alachlor and fenamiphos may not proceed in a normal manner. Altered rates of development could help to explain the dynamics involved in the late season population resurgence associated with field applications of these pesticides. The objective of this research was to determine the rate of *H. glycines* development on susceptible and resistant soybean cultivars as affected by alachlor and fenamiphos.

Materials and Methods

Four experiments were conducted in the greenhouse. *Heterodera glycines* race 1, maintained on 'Deltapine 105' soybean (susceptible to all races), was used as inoculum in all experiments. Eggs released from cysts with a glass tissue grinder were placed in a hatching chamber and incubated at 28 C for 48 hours. Second-stage juveniles (J2) that hatched in the first 12 hours were discarded. Those nematodes hatching in the remaining 36 hours were used immediately as inoculum.

Deltapine 105 or 'Centennial' (*H. glycines*

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races 1 and 3 resistant) soybean seeds were germinated in moist paper towels at 28 C. Seedlings with 1–2-cm-long radicals were planted into wooden flats (35 × 50 cm) filled with 250-μm-d sterile sand (8). Approximately 250 freshly hatched J2 were pipetted in 1-ml aliquots of water directly onto each soybean radical which was then covered with sand. After 60 hours at 28 C, two seedlings were transplanted in a 5-cm-d clay pot filled with 250-μm-d sterile sand.

The sand in each pot was then treated with alachlor, fenamiphos, or a combination of these pesticides. Stock solutions of alachlor and fenamiphos were prepared before each experiment by adding 1 ml alachlor 4EC or 1 ml fenamiphos 3E to 999 ml deionized water. Stock solutions were diluted to achieve the desired concentrations for delivery in 10-ml aliquots. Treated pots were transferred to a greenhouse maintained at temperatures ranging from 23 to 31 C and watered as needed. Mercury vapor lamps provided supplemental lighting for 12 hours daily, 15 September through 15 April, to minimize soybean etiolation and flowering.

Soybean roots were harvested at predetermined intervals for nematode fixation and staining (7). Nematode life-stages were determined for the first 20 nematodes observed per root. Life-stage identification was based upon tail shape, gonad development, and rectum position (16). Life-stage categories were J2, swollen J2 (SJ2), third-stage juvenile (J3), fourth-stage juvenile (J4), adult male (M), or adult female (F).

A mean stage of population development (D) was calculated for each harvest date where D = (% J2) + (% SJ2 × 2.33) + (% J3 × 3.67) + (% J4 × 5.83) + (% M × 6.33) + (% F × 6.67). This equation uses the mean percentage of nematodes recorded in each life-stage category weighted against the J2 to determine a relative stage of development for the nematode population beginning after the infection period (1,12,20). Each life-stage weight is calculated by taking half of the time spent in a life stage plus the total amount of time spent in preceding life stages divided by the total length of the life cycle (10 days) (11).

Accumulated degree days were calculated for each harvest date using a triangulation method (2) with 20 C as the basal developmental threshold (1) and 32 C as the upper developmental threshold (17) and are represented as DD20/32 (1).

Experiment 1: Deltapine 105 soybeans were grown in soil treated with alachlor (1.0 μg a.i./g soil), fenamiphos (1.5 μg a.i./g soil), both, or water. Treatments were arranged as a 2 × 2 factorial in a randomized complete block design with 12 pots per treatment and replicated six times.

Experiment 2: Development of *H. glycines* on Deltapine 105 in response to fenamiphos at 1.5, 0.75, 0.38, and 0.0 μg a.i./g soil was determined. Treatments were arranged in a randomized complete block design with 15 pots per treatment and replicated four times.

Experiment 3: Deltapine 105 was used as a host for *H. glycines* in a 2 × 5 factorial test conducted in a randomized complete block design, five pots per treatment, and replicated four times. Alachlor was used at 1.0, 0.50, 0.25, 0.13 and 0.0 μg a.i./g soil. Fenamiphos was applied at 1.0 or 0.0 μg a.i./g soil.

Experiment 4. A randomized complete block design with treatments arranged in a 2 × 2 factorial, eight pots per treatment, and replicated six times was conducted using *H. glycines* on Centennial soybean. Soil was treated with alachlor (1.0 μg a.i./g soil), fenamiphos (1.0 μg a.i./g soil), both, or a water control.

Statistical analyses: D was analyzed for pesticide treatment differences. The first analysis of variance utilized DD20/32 as a covariate. Each experiment was then analyzed as a split-plot experiment with DD20/32 as subplots and treatments as whole-plots. Finally, rate of D equations were generated using regression analysis with DD20/32 as the independent variable. D was fitted to linear, quadratic, exponential and Gompertz models. The best fitting model, based upon highest level of signif-

icance and greatest r^2 values, was selected for comparing treatments.

Results

Experiment 1: Adult *H. glycines* males matured in 44 DD20/32 and adult females in 88 DD20/32 on untreated Deltapine 105 (Fig. 1A, B). With 1.0 μg alachlor/g soil, adult males and females did not mature until 88 and 101 DD20/32, respectively (Fig. 1A, B). Adult males matured by 147 DD20/32 in soil treated with 1.5 μg fenamiphos/g (Fig. 1A); the first female matured in 172 DD20/32 in this treatment (Fig. 1B). On soybean treated with both alachlor (1.0 μg) and fenamiphos (1.5 μg), males and females matured in 111 DD20/32 (Fig. 1A, B).

The rate of *H. glycines* development fit a Gompertz model for all but the fenamiphos treatment which responded linearly (Fig. 1C). Equations ($P = 0.01$) representing D for each treatment were

Control

$$D = 6.67e^{-1.85e^{-0.028DD20/32}}$$
$$r^2 = 0.85$$

Alachlor

$$D = 6.67e^{-2.52e^{-0.026DD20/32}}$$
$$r^2 = 0.87$$

Fenamiphos

$$D = 1$$

Combination

$$D = 6.67e^{-4.08e^{-0.016DD20/32}}$$
$$r^2 = 0.75$$

where e is the base of the natural logarithm. Alachlor did not differ from the control ($P = 0.01$). The combination of alachlor and fenamiphos slowed the rate of development to 50% of the control (slopes of the lines or regression coefficients of 0.016 vs. 0.028). In contrast, maturation in the fenamiphos treatment as given by the model did not proceed beyond J2.

Experiment 2: The rate of *H. glycines* development was affected by different concentrations of fenamiphos ($P = 0.05$). Adult males matured in 66 DD20/32 (Fig. 2A), and females in 76 DD20/32, in the absence of fenamiphos (Fig. 2B). Fenamiphos at 0.38 μg did not delay the appearance of the first females. At 0.75 μg fenamiphos, female maturity was delayed by 50 DD20/32 and no females and males developed by 145 DD20/32 at 1.5 μg fenamiphos (Fig. 2A, B).

Heterodera glycines development fit a Gompertz model for all concentrations except the 1.5 μg a.i./g soil rate. Equations ($P = 0.05$) for D are

Control

$$D = 6.67e^{-1.52e^{-0.025DD20/32}}$$
$$r^2 = 0.88$$

Fenamiphos (0.38 μg)

$$D = 6.67e^{-1.45e^{-0.009DD20/32}}$$
$$r^2 = 0.22$$

Fenamiphos (0.75 μg)

$$D = 6.67e^{-1.98e^{-0.009DD20/32}}$$
$$r^2 = 0.37$$

Fenamiphos (1.50 μg)

$$D = 1$$

where e is the base of the natural logarithm.

At the highest concentration of fenamiphos, 1.50 μg a.i./g soil, D did not proceed beyond SJ2 (Fig. 2C). The 0.38-μg and 0.75-μg levels of fenamiphos suppressed the rate of nematode development as determined by the regression models by 36%.

Experiment 3: Heterodera glycines population development was not altered by alachlor at any concentration tested. Adult females matured in 62 DD20/32 with all concentrations and fit the Gompertz model ($P = 0.01$)

$$D = 6.67e^{-1.99e^{-0.023DD20/32}}$$
$$r^2 = 0.99$$

The rate of nematode development with fenamiphos (1.0 μg a.i./g soil) was altered by 0.5 and 1.0 μg alachlor (Fig. 3). Fenamiphos alone and with 0.13 or 0.25 μg alachlor/g soil prevented nematode development. The simultaneous application of 0.50 μg alachlor with 1.00 μg fenami-

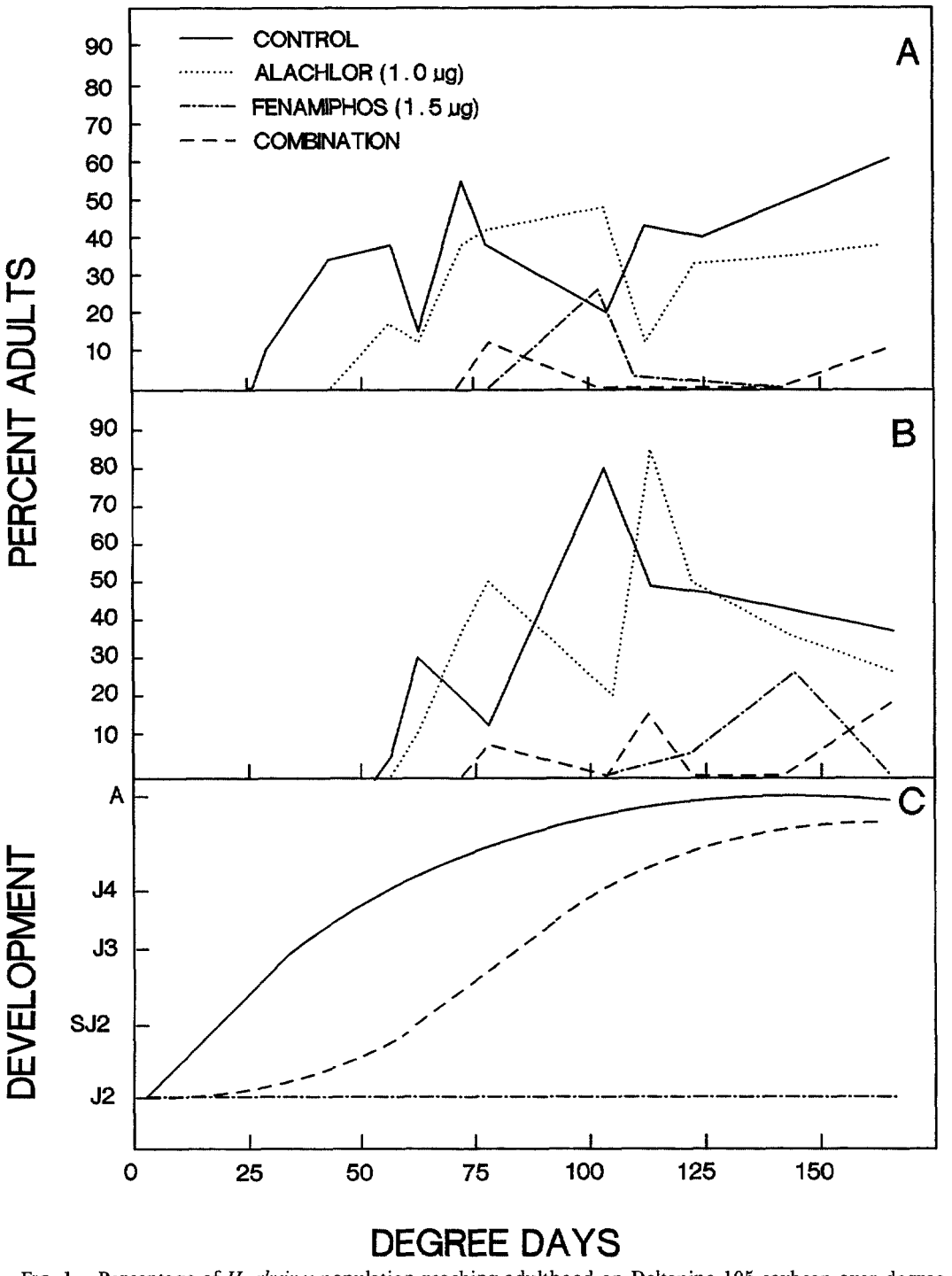

FIG. 1. Percentage of *H. glycines* population reaching adulthood on Deltapine 105 soybean over degree days (20 C basal and 32 C upper developmental thresholds) and rate of population development as influenced by alachlor, fenamiphos, or both. A) Males. B) Females. C) Rate of population development.

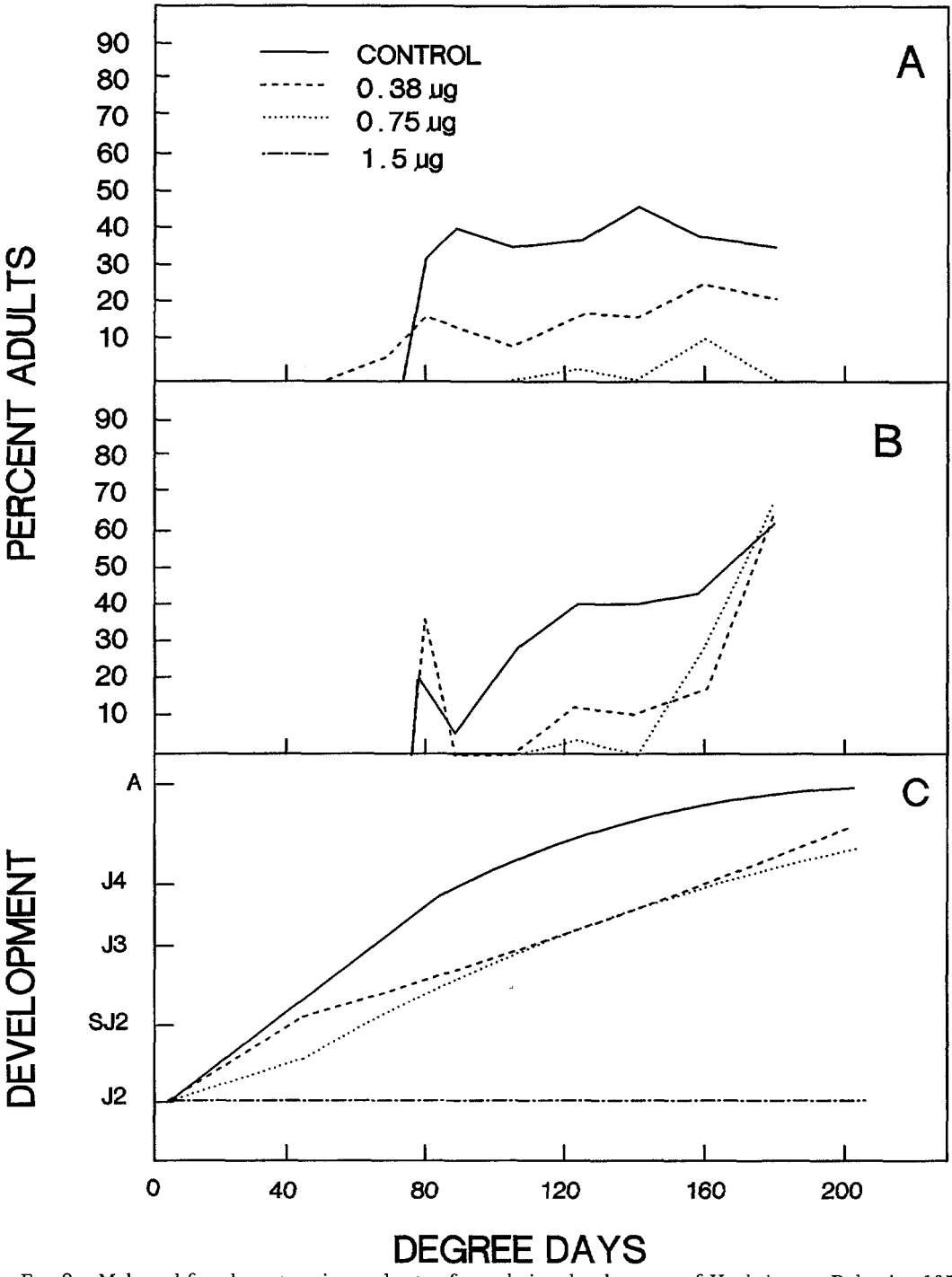

FIG. 2. Male and female maturation and rate of population development of *H. glycines* on Deltapine 105 grown in soil treated with fenamiphos over accumulated degree days (20 C basal and 32 C upper developmental thresholds). A) Males. B) Females. C) Rate of population maturation.

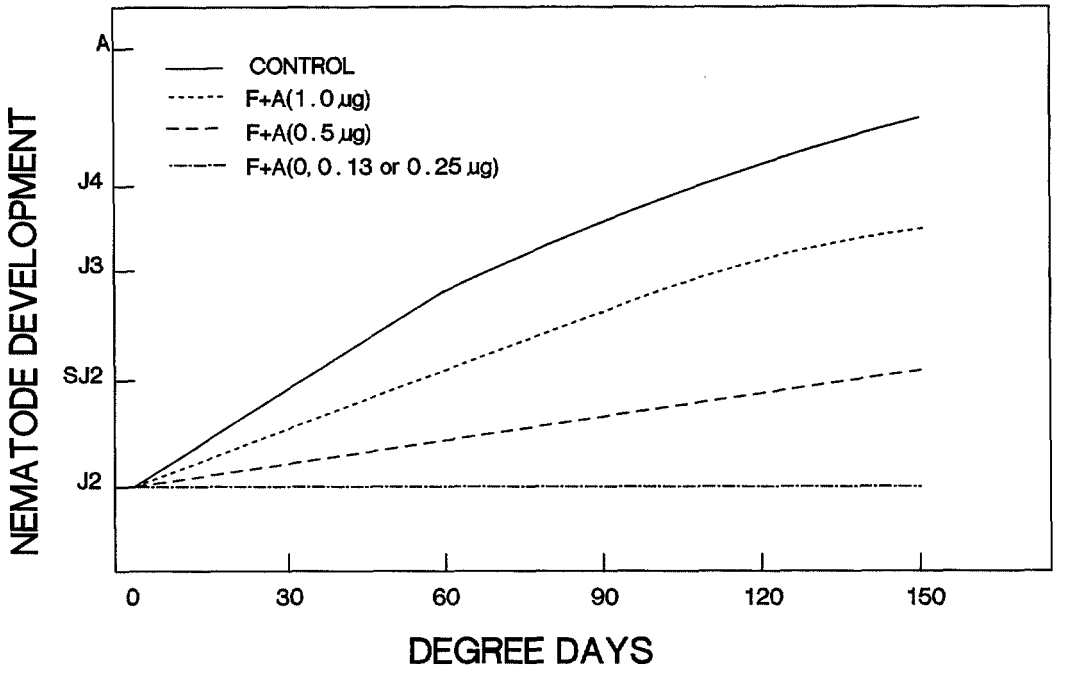

FIG. 3. Rate of *H. glycines* development on Deltapine 105 soybean treated with fenamiphos (F) and alachlor (A) (20 C basal and 32 C upper developmental thresholds).

phos allowed some nematode development which fit the linear model ($P = 0.05$)

$$D = 1.08 + 0.009\ (DD20/32)$$
$$r^2 = 0.76$$

Development was faster with 1.0 µg alachlor and 1.0 µg fenamiphos than with the 0.50 alachlor and 1.0 µg fenamiphos treatment (Fig. 3). This rate of development fit a Gompertz model ($P = 0.01$)

$$D = 6.67e^{-2.22e^{-0.008DD20/32}}$$
$$r^2 = 0.94$$

The rate of development with alachlor and fenamiphos, with respective regression coefficients of 0.008 and 0.023, was only one-third that of the water control.

Experiment 4: The resistant cultivar Centennial greatly limited nematode development. On the control, only 12% of the infecting nematodes reached adulthood (Table 1), compared with 98% maturation on the susceptible cultivar control (Table 1). Fenamiphos (1.0 µg a.i./g soil) further suppressed development on Centennial (5% maturation). The percentage of individuals maturing in alachlor-treated plants was near that of the control, with twice as many females developing as on the control. Development with alachlor plus fenamiphos was similar to the control.

The first adult males and females developed within 76 DD20/32 on Centennial regardless of treatment. Quadratic models fit these observed rates of development on the resistant cultivar. Equations for D ($P = 0.05$) were

TABLE 1. Percentage of infecting *Heterodera glycines* maturing on Deltapine 105 or Centennial soybeans treated with alachlor (1.0 µg a.i./g soil), fenamiphos (1.0 µg a.i./g soil on Centennial and 1.5 µg a.i./g soil on Deltapine 105), or both pesticides.

	Deltapine 105			Centennial		
Treatment	Male	Female	Total	Male	Female	Total
Water	45	53	98	8	4	12
Alachlor	39	46	85	10	8	18
Fenamiphos	15	16	31	3	2	5
Combination	11	11	22	5	4	9
CV		86			44	

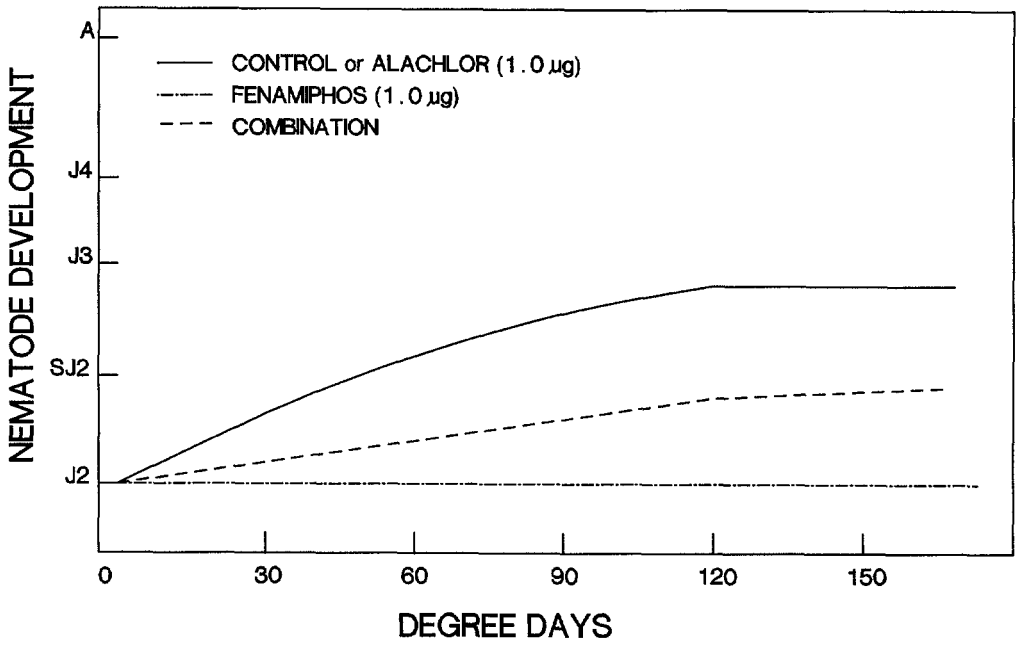

FIG. 4. Rate of *H. glycines* population development (from regression equations) on Centennial soybean treated with alachlor, fenamiphos, or the combination of both pesticides (20 C basal and 32 C upper developmental thresholds).

Control

$$D = 1.51 + 0.0023(DD20/32) - 0.00006(DD20/32)^2$$
$$r^2 = 0.77$$

Alachlor

$$D = 1.08 + 0.035(DD20/32) - 0.000013(DD20/32)^2$$
$$r^2 = 0.85$$

Fenamiphos

$$D = 1$$

Combination

$$D = 0.99 + 0.015(DD20/32) - 0.000004(DD20/32)^2$$
$$r^2 = 0.62$$

Development in alachlor was not significantly different from the control (Fig. 4). The fenamiphos treatment (1.0 µg a.i./g soil) suppressed development beyond SJ2 (Fig. 4). D was slightly greater with alachlor and fenamiphos than with fenamiphos alone yet much less than the control (Fig. 4).

DISCUSSION

Heterodera glycines development on the susceptible cultivar Deltapine 105 responded in a typical logistic fashion, an initially slow rate followed by a rapid rate of increase until most nematodes matured. Fenamiphos treatments increased the duration of the lag phase, and at high rates fenamiphos prevented the log phase. Development on the resistant cultivar Centennial was much slower than on the susceptible cultivar Deltapine 105, especially with fenamiphos alone.

Although *G. max* is tolerant of alachlor, physiological changes in the soybean take place as the herbicide is metabolized. Root morphology is altered (5), uptake of phosphates and sulfates decrease (6), and less glyceollin is produced in resistant soybeans in response to *H. glycines* infection (Huang, pers. comm.). On susceptible weed species, alachlor interferes with protein synthesis (3) and membrane lipid content (14), consequently altering membrane function (14). These physiological changes could affect the uptake and movement of chemicals such as fenamiphos.

Behavioral modifications in the nematode induced by fenamiphos are complex. The nematicide may interfere with nematode feeding (9), resulting in slowed de-

velopment (21). In this study, *H. glycines* juveniles stained intensely and had good internal organ integrity up to 40 days after treatment (end of experiment) with 1.0 or 1.5 μg fenamiphos/g soil. These doses of fenamiphos are not lethal to the nematode but seem to interfere with nematode development beyond SJ2.

Alachlor alters the behavioral modifications induced by fenamiphos, allowing nematode development past SJ2. Possible explanations for this antagonism are 1) alachlor alters nematode response to fenamiphos; 2) alachlor interacts with fenamiphos chemically or physically; or 3) alachlor alters the soybean and its response to fenamiphos. The ability of alachlor to alter nematode response to fenamiphos cannot easily be supported with currently available data. The most plausible explanation for the observed antagonism between alachlor and fenamiphos is an alachlor-induced alteration of the soybean plant. The antagonism may be the result of a decrease in the uptake and translocation of fenamiphos or greater metabolism of fenamiphos by the alachlor-treated soybean. Alachlor-treated soybean cell membranes may also be less permeable to the ambimobile organophosphate fenamiphos (5,6).

Heterodera glycines juveniles initiate host syncytia, specialized cells of increased membrane area which facilitate transport of cytoplasm between cells, upon which to feed and develop. Alachlor may alter these sites of extensive cytoplasm exchange (3,6,14). Alachlor alters the lipid content of morning glory (*Ipomoea hederacea* (L.) Jacq.) cells, making the cells more soluble to lipophilic compounds (14). Conversely, hydrophilic solubility may decrease in alachlor-treated cells. With the combination treatment of alachlor and fenamiphos, transport of the latter into the syncytia may be restricted, thus exposing the nematodes to less of the nematicide, and allowing development to proceed.

The ability of *H. glycines* to mature with the alachlor and fenamiphos treatment, coupled with the effects this pesticide combination has on hatch and penetration, helps to further explain the observed late-season field population resurgence. A complete elucidation of the dynamics of field population resurgence, however, will require further experiments with this pesticide combination to determine influences on penetration and development with prior pesticide treatment and the effect of this combination on fecundity.

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