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Effects of Nematicides and Herbicides Alone or Combined on *Meloidogyne incognita* Egg Hatch and Development[1]

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Abstract: The effects of nematicides carbofuran (C) and fenamiphos (F) and herbicides metribuzin (M) and trifluralin (T), alone and in combination, on hatching, penetration, development, and reproduction of *Meloidogyne incognita* race 3 were determined under laboratory conditions. To study hatching, entire egg masses were exposed to nematicides (6 μg/ml), herbicides (0.5 μg/ml), and their combinations over a period of 16 days; the hatched juveniles were extracted and counted every 48 hours. Second-stage juveniles that hatched from day 6 to day 8 were used as inoculum to determine the effects of the chemicals on penetration, development, and reproduction of *M. incognita* on tomato 4, 16, and 32 days after inoculation. F, F + T, and F + M inhibited hatching; whereas, C, T, M, C + T, and C + M did not affect hatching, penetration, development of females, or reproduction. Since so few juveniles hatched from the fenamiphos treatments, we were not able to use them for the postinfection development study. There was no apparent reduction in the effect of the nematicides by the herbicides.

Key words: carbofuran, fenamiphos, hatching, herbicide, life cycle, *Meloidogyne incognita*, metribuzin, nematicide, pesticide interaction, root-knot nematode, survival, trifluralin.

Nematicides and herbicides are used alone or in combination on many crops to manage nematodes and weeds. Even though nematicide–herbicide combinations are generally successful (4,13), herbicides may enhance nematicide efficacy (13,16), have no effect (1), reduce nematicidal activity (19), or enhance nematode populations (2,3).

The influence of oximecarbamate and organophosphate nematicides on *Meloidogyne incognita* can be attributed to one or a combination of the following effects: 1) inhibiting egg hatch, 2) restricting the migration rate of the juveniles without killing them, and 3) inhibiting the development of the nematode within the roots. Efficacy of nonfumigant nematicides varies with the chemical, the life stage of the nematode, and the nematode species (18). The effects of herbicides on nematodes have been grouped into four categories: 1) negligible influence, 2) indirect reduction of nematode populations through control of various weed hosts, 3) slight nematicidal activity, and 4) enhance nematode reproduction (3).

Our objective was to determine, under laboratory conditions, the effects of the nematicides carbofuran and fenamiphos and the herbicides metribuzin and trifluralin, when used alone or in combination, on egg hatch, penetration, development, and reproduction of *M. incognita*.

Materials and Methods

Egg hatch: Two egg masses (ca. 700 eggs/mass) of *Meloidogyne incognita* (Kofoid & White) Chitwood race 3 were hand picked from 50-day-old galled tomato roots (*Lycopersicon esculentum* Mill. cv. Rutgers), and placed in each of 36 30-μm-pore microsieves (1.9-cm-d) which were enclosed in 20 × 60-mm petri dishes partially filled with either water or with chemical solutions to form an aqueous film around the egg masses. Treatments included carbofuran and fenamiphos at 6 μg/ml (6.7 kg a.i./ha) alone, metribuzin and trifluralin at 0.5 μg/ml (0.56 kg a.i./ha) alone, combinations of carbofuran with metribuzin and trifluralin, combinations of fenamiphos

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TABLE 1. Total number of *Meloidogyne incognita* juveniles hatched after 48 hours over a period of 16 days of exposure to nematicides (6.7 kg/ha) and herbicides (0.5 kg/ha) (hatching experiment) and recovered 4, 16, and 32 days after inoculation (postinfection development experiment).

		Number per plant							
		4 DAI	16 DAI			32 DAI			
Treatment	Juveniles hatched	J2	J2	J2–J4	Fem	J2	J2–J4	Fem	Eggs (× 1,000)
Fenamiphos (F)	49								
Carbofuran (C)	854	11	23	29	64	3,725	6	178	31.6
Trifluralin (T)	517	27	14	11	73	618	12	87	10.8
Metribuzin (M)	631	23	6	6	59	1,029	0	112	17.7
F + T	87								
F + M	70								
C + T	583	19	0	10	27	3,962	19	237	26.8
C + M	719	32	8	20	52	1,584	0	100	13.2
Water (W)	606	19	10	21	52	1,210	6	87	15.2
Selected linear comparisons†	A, B, C	NS	NS	NS	NS	NS	NS	NS	NS

DAI = days after inoculation. J2 = second-stage juvenile; J2–J4 = swollen stage juveniles (late second, third, and fourth stages); Fem = females.

Means are the average of 12 replications for three hatching experiments and 4 replications for postinfection experiments.

† Letters are used to designate differences as determined by selected linear comparisons ($P = 0.01$): A = water vs. fenamiphos; B = water vs. fenamiphos plus metribuzin; C = water vs. fenamiphos plus trifluralin; NS = no significant differences.

with metribuzin and trifluralin, and distilled water as a control. These concentrations correspond to rates recommended for use on vegetable crops under field conditions (10). Each treatment was replicated four times. Dishes with eggs in distilled water or chemical solutions were maintained and randomized on a laboratory bench at 20 C with alternating 8-hour light and 16-hour dark periods. Every 48 hours, over a 16-day period, the distilled water and chemical solutions were changed and the second-stage juveniles (J2) that emerged were removed and counted. The experiment was repeated three times.

Penetration, development, and reproduction: The juveniles that hatched from day 6 to day 8 were removed from their respective treatments, rinsed four times in distilled water, counted, and inoculated separately on Rutgers tomato seedlings grown in pots containing 100% sand. Approximately 166 J2 per plant in 5 ml water were poured in two holes in the sand near the base of the seedling. Plants were randomized on a laboratory bench under a fluorescent lamp (280 $\mu E\ m^{-2}\ sec^{-1}$). Plants were watered daily with 10 ml distilled water or 50% Hoagland's solution (11). Four seedlings from each treatment were harvested 4, 16, and 32 days after inoculation. Roots were gently washed in tap water, cut into 1-cm-long pieces and macerated in water in a blender for 10 seconds. Nematode life stages recovered on a 25-μm-pore sieve were suspended in water, and their numbers were determined in 5-ml aliquots. The number of nematodes and the various developmental stages were obtained by staining the specimens in hot acid fuchsin (6) and counting with a microscope. Juvenile stages were separated on the basis of body swelling. All data were log-transformed and subjected to analysis of variance. In addition, data on nematode penetration, development, and reproduction were subjected to covariant analysis. The initial population was used as the covariate (9), thereby producing an equitable comparison of the means. Data were used to make nonorthogonal selected linear treatment comparisons (7) that were of interest to us. The comparisons included each of the treatments vs. the water control, each nematicide alone vs. the combination of that nematicide with the two herbicides, one nematicide vs. the other, and one herbicide vs. the other.

Results

Only treatments containing fenamiphos suppressed hatching ($P = 0.01$) (Table 1). Carbofuran, metribuzin, trifluralin, and their combinations did not suppress hatching. There was no apparent reduction in the effect of the nematicides by the herbicides. There was no difference in the effect of either of the herbicides on hatching. Since so few juveniles hatched from the fenamiphos treatments, we were unable to use them for the penetration, development, or reproduction study.

Based on the number of juveniles recovered 4 days after inoculation, carbofuran, metribuzin, and trifluralin, alone or in combination, did not adversely influence the penetration of juveniles (Table 1). The number of nematodes at different developmental stages found 16 days after inoculation and the number of females and eggs recovered from tomato roots 32 days after inoculation indicate that carbofuran, metribuzin, and trifluralin, alone or combined, did not affect the development or reproduction of *M. incognita* (Table 1). There was no apparent reduction in the effect of the nematicide by the herbicide in the postinfection development of *M. incognita*.

Discussion

In vitro exposure is an initial approach in determining the influence of nematicides and herbicides on nematode eggs and subsequent infectivity; however, the in vitro method does not show the variability in chemical concentration that occurs at the soil–nematode interface that takes place in the soil. In an experiment conducted on Lakeland sand (93.5% sand, 2.9% silt, and 3.6% clay), the half-life of fenamiphos in the 0–8-cm layer of soil was about 3 days (14). Concentrations of nematicides and herbicides alone or combined were constant in our experiments.

Our results agree with those in which concentrations of fenamiphos as low as 2 μg/ml inhibited hatch of *M. incognita* eggs (18). Concentrations above 1.5 μg/cm of soil were suggested to give adequate control of *M. incognita* in multiple crop systems (14,15), and concentrations of 10 μg/ml in the field prevented galling of tomato roots in a 20-cm soil layer when incorporated in the top 5-cm layer of Tifton sandy loam (5). No reports were found on the effect of the combinations of fenamiphos with metribuzin or trifluralin on nematodes, but the efficacy of aldicarb was improved with the application of metribuzin or trifluralin, and the same herbicides applied at recommended rates stimulated the population development of *Heterodera glycines* Ichinohe in the field (16).

Our results agree with those in which carbofuran at 8 μg/ml did not control *M. incognita* under field conditions (12); however, Di Sanzo reports carbofuran at 8 μg/ml controlled *M. incognita* in the zone of incorporaton (8). Carbofuran at 0.1 μg/ml stimulated hatching of *Heterodera schachtii*, Schmidt, but suppressed hatching at 50 μg/ml (21). Other reports indicated that carbofuran at 13.5 μg/ml controlled *M. graminicola* in the field (17) and at 0.9, 1.9, and 3.0 μg/ml reduced populations of *M. arenaria* Chitwood in the field (20).

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