Effects of DCPA, EPTC, and Chlorpropham on Pathogenicity of *Meloidogyne hapla* to Alfalfa[1]

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Abstract: Treatments with the herbicides chlorpropham (isopropyl *m*-chlorocarbinilate), DCPA (dimethyl tetrachloroterephthalate), and EPTC (S-ethyl dipropylthiocarbamate), alone or in combination with *Meloidogyne hapla* Chitwood, significantly reduced the growth of both nematode-resistant 'Nev Syn XX' and susceptible 'Ranger' alfalfa (*Medicago sativa* L.) seedlings. *M. hapla* infection of both alfalfas was reduced by all herbicides because of fewer available infective courts in the treated plants. EPTC, however, reduced resistance to *M. hapla*, as indicated by increased galling of 'Nev Syn XX' plants. *Key Words: Meloidogyne hapla, Medicago sativa,* DCPA, EPTC, chlorpropham, resistance, susceptible, temperature, phytotoxicity, pathogenicity.

The importance of the relationship between herbicides and soil-borne pathogens, including nematodes, on plant growth is well documented (2, 3). A combination of *Heterodera schachtii* and cycloate (S-ethyl cyclohexylethylthiocarbamate) reduced the growth of sugarbeet (*Beta vulgaris* L.) (1). Combinations of trifluralin (*a,a,a*-trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine) soil treatment and *M. hapla* inoculation reduced growth of tomato (*Lycopersicon esculentum* Mill) or alfalfa (*Medicago sativa* L.) more than either treatment alone (6). However, onion (*Allium cepa* L.) and bean (*Phaseolus vulgaris* L.) seedlings grown in DCPA (dimethyl tetrachloroterephthalate) treated soil have been shown to be less susceptible to infection by northern root-knot nematode, *Meloidogyne hapla* Chitwood, than seedlings grown in untreated soil (9).

Herbicides are used extensively in both seedlings and established alfalfa plantings. Many of these plantings are infested with northern root-knot nematodes. This study was conducted to determine whether herbicide treatments affected root-knot nematode infection on alfalfa and what effects herbicide and *M. hapla* combinations had on plant growth. The responses of nematode-resistant and susceptible cultivars to treatment combinations were also compared.

MATERIALS AND METHODS

General: The herbicides chlorpropham (isopropyl *m*-chlorocarbanilate), DCPA (dimethyl tetrachloroterephthalate) and EPTC (S-ethyl dipropylthiocarbamate) were mixed singly with methyl-bromide-treated Provo sand (moisture holding capacity = 22%) in a soil mixer to give respective chemical concentrations equivalent to 4.5, 9.0, and 6.7 kg/ha. Two alfalfas, *M. hapla*-resistant 'Nev Syn XX' selection and *M. hapla*-susceptible 'Ranger' variety, were used in all experiments. Seeds of these varieties were scarified, surface-sterilized for 2 min in 0.5% sodium hypochlorite, rinsed in distilled water, and germinated on filter pads saturated with captan (N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide). Germinated seeds (5–15-mm radicle) were planted and grown for experimental use in flats of herbicide-treated and untreated soil. Seedlings were transplanted from flats to 15-cm-diam containers (one seedling per container) and grown in the greenhouse at 22 ± 4 C. Each treatment was given to 20 single plants. *M. hapla* egg masses, collected from nematode-infected tomato plants, were surface-sterilized in 0.5% sodium hypochlorite and rinsed in sterile distilled water. The larvae used in inoculum in these experiments were hatched from these egg masses in sterile distilled water in an oxygenator at 25 C.

Effects on plant growth and galling: Fourteen-day-old seedlings were transplanted into containers of the same untreated or herbicide-treated soils in

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which they were grown initially. Half of the seedlings were inoculated with 500 *M. hapla* larvae and the remainder left as uninoculated controls. Fresh plant weights were determined 42 days after transplanting.

Effect of time of herbicide application on plant growth: Fourteen-day-old seedlings were transplanted from: 1) herbicide-treated soil into soil containing the same herbicide treatment, 2) herbicide-treated soil into untreated soil, 3) untreated soil into herbicide-treated soil, and 4) untreated soil into untreated soil. Half of the seedlings were inoculated with 500 *M. hapla* larvae at transplanting; the remainder were uninoculated controls. Fresh plant weights were determined 42 days after transplanting.

Effect of herbicide treatment on galling of resistant and susceptible alfalfa: Since there is an inverse relation between age of resistant alfalfa plants at time of inoculation and the percentage of plants galled by *M. hapla* (8), 42-day-old plants were transplanted from herbicide-treated and untreated soil into containers of similarly treated soil, and each plant was inoculated with 1,000 *M. hapla* larvae. After 72 days the plants were harvested, and the percentages of plant roots galled and severity of root galling were determined.

Effect of herbicide treatment on M. hapla infection: Galling of nematode-resistant alfalfa is greater at 30-35 C than at lower temperatures (5, 7). Therefore, 14-day-old seedlings were transplanted into herbicide-treated and untreated soil, inoculated with 100 *M. hapla* larvae, and grown at 20, 24, 28, and 32 C in temperature-controlled water baths for 14 days. Seedling roots were carefully washed free of soil, and stained with an acid fuchsin-lactophenol solution. Numbers of nematodes per plant were determined using a stereomicroscope.

Effect of chlorpropham rate on M. hapla infection: Because of the extreme phytotoxicity of chlorpropham to alfalfa, 42-day-old plants were transplanted from soil without herbicide treatment into soil containing chlorpropham applied at different rates (0.56, 1.1, 2.2, or 4.5 kg/ha). Plants, including controls (transplants from untreated to untreated soil), were inoculated with 500 *M. hapla* larvae. Eighty-four days after inoculation, plants were harvested and percentages of plants galled and severity of galling were determined by visual observation.

RESULTS AND DISCUSSION

Effects of herbicides and nematodes on plant growth: Herbicide treatments with chlorpropham, DCPA, and EPTC, alone or in the presence of *M. hapla,* significantly reduced the growth of both nematode-resistant 'Nev Syn XX' and susceptible 'Ranger' alfalfa (Table 1). When compared with control plants, the growth of 'Nev Syn XX' alfalfa was reduced 47, 85, and 93% by DCPA, EPTC, and chlorpropham, respectively. The growth of 'Ranger' alfalfa was similarly reduced: 38, 80, and 94%.

Growth of both 'Nev Syn XX' and 'Ranger' alfalfa plants decreased significantly ($P = 0.01$) in the presence of *M. hapla* plus DCPA or chlorpropham, but not with *M. hapla* plus EPTC, when compared with plants grown in herbicide-treated soil only. Plant growth of 'Nev Syn XX' was reduced 26, 3, and 71% by *M. hapla* plus DCPA, EPTC, and chlorpropham, respectively, as compared with plants that were herbicide-treated only. Reductions in growth of 'Ranger' alfalfa were 15, 7, and 34%.

Effect of time of herbicide application on plant growth: M. hapla caused a reduction in plant growth of both 'Nev Syn XX' and 'Ranger' alfalfa in chlorpropham- and DCPA-, but not EPTC-treated soil, when alfalfa seedlings were transplanted from herbicide-treated to herbicide-treated soil (Table 2). However, when seedlings of the two alfalfas were transplanted either from untreated soil to herbicide-treated soil, or from herbicide-treated soil to untreated soil, *M. hapla* caused a significant reduction in plant growth in combination with each herbicide when compared with herbicide treatment only.

Effect of herbicide treatment on galling of resistant and susceptible alfalfa: Neither DCPA at 9.0 kg/ha nor chlorpropham at 4.5 kg/ha predisposed resistant 'Nev Syn XX' to galling by *M. hapla.* The extreme phytotoxicity associated with chlorpropham apparently affected the relationship between *M. hapla* and 'Ranger' alfalfa. Since only 70 to 75% of susceptible 'Ranger'

TABLE 1. Effect of herbicides on the pathogenicity of *Meloidogyne hapla*[a] to resistant 'Nev Syn XX' and susceptible 'Ranger' alfalfa.

Alfalfa selection and treatment	Plant weight (g)[b]			
	DCPA[c]	EPTC[d]	Chloropropham[e]	Control
Nev Syn XX				
Herbicide	8.39†	2.37†	1.16†	—
Herbicide + *M. hapla*	6.24*†	2.30†	0.34*†	—
M. hapla	—	—	—	16.29
Control	—	—	—	15.87
Ranger				
Herbicide	9.88†	3.16†	1.01†	—
Herbicide + *M. hapla*	8.42*†	2.94†	0.67*†	—
M. hapla	—	—	—	16.64
Control	—	—	—	16.07

*Significantly different from herbicide treatment at 1% level (P = 0.01).
†Significantly different from *M. hapla* and control plants at 1% level (P = 0.01).
[a]14-day-old seedlings transplanted from similar herbicide-treated or untreated soil and inoculated with 500 *M. hapla* larvae.
[b]Readings made 42 days after transplanting and inoculation.
[c]9.0 kg/ha.
[d]6.7 kg/ha.
[e]4.5 kg/ha.

plants transplanted from the herbicide-treated soil to herbicide-treated soil were galled, chlorpropham phytotoxicity evidently eliminated infection courts for *M. hapla* (Table 3).

EPTC increased the susceptibility of 'Nev Syn XX' plants to *M. hapla*. Forty, 50, and 25% of the plants were galled when seedlings were transplanted at the time of larval inoculation from herbicide-treated to herbicide-treated soil, from untreated to herbicide-treated soil, and from herbicide-treated to untreated soil, respectively. Previous studies have shown that EPTC

TABLE 2. Effect of herbicides on the pathogenicity of *Meloidogyne hapla* to alfalfa.[a]

Treatment	(kg/ha)	Plant weight (g)							
		'Nev Syn XX'				'Ranger'			
		Herbicide	*M. hapla*	Herbicide +*M. hapla*	Control	Herbicide	*M. hapla*	Herbicide +*M. hapla*	Control
Chlorpropham[b]	4.5	1.0	—	0.4*	—	0.9	—	0.5*	—
Chlorpropham[c]	4.5	2.3	—	0.2*	—	2.3	—	0.9*	—
Chlorpropham[d]	4.5	9.7	—	7.2*	—	12.9	—	9.0*	—
EPTC[b]	6.7	2.3	—	2.1	—	2.6	—	2.7	—
EPTC[c]	6.7	3.3	—	2.0*	—	4.7	—	3.3*	—
EPTC[d]	6.7	8.9	—	7.1*	—	10.4	—	7.0*	—
DCPA[b]	9.0	7.4	—	6.1*	—	10.9	—	9.6*	—
DCPA[c]	9.0	15.8	—	12.7*	—	14.8	—	10.9*	—
DCPA[d]	9.0	16.8	—	15.1*	—	16.6	—	14.6*	—
Untreated			16.98		17.27		16.68		16.31

[a]14-day-old seedlings inoculated with 500 *M. hapla* larvae and harvested after 42 days.
[b]Seedlings transplanted from herbicide-treated soil to herbicide-treated soil.
[c]Seedlings transplanted from untreated soil to herbicide-treated soil.
[d]Seedlings transplanted from herbicide-treated soil to untreated soil.
*Significantly different from herbicide-treated soil at 1% level (P = 0.01).

TABLE 3. Effect of herbicides on root galling by *Meloidogyne hapla* of resistant 'Nev Syn XX' and susceptible 'Ranger' alfalfa.[a]

Treatment	Rate (kg/ha)	Root-knot galling index[b]							
		Nev Syn XX				Ranger			
		A	B	C	D	A	B	C	D
DCPA	9.0	1.76	1.00	1.00	—	4.19	4.06	5.00	—
EPTC	6.7	3.34	3.19	1.39	—	5.00	5.00	5.00	—
Chloropropham	4.5	1.00	1.00	1.00	—	2.76	3.00	4.16	—
Control					1.00				5.00

[a]42-day-old transplants inoculated with 1,000 *M. hapla* larvae. Readings made 72 days after inoculation.
[b]1 = no galling, 2 = 1–10% of each root system galled, 3 = 11–30% galled, 4 = 31–70 % galled, 5 = 71–100% galled.
A = Plants transplanted from herbicide-treated to herbicide-treated soil.
B = Plants transplanted from untreated soil to herbicide-treated soil.
C = Plants transplanted from herbicide-treated to untreated soil.
D = Plants transplanted from untreated to untreated soil (control).

reduces foliar cuticular wax deposition of treated plants (4). It is significant that thiocarbamate herbicides, including EPTC, have been shown to alter epicuticular lipids, whereas the carbamate chlorpropham does not (10). It is also possible that EPTC alters the root epidermis composition, thereby producing susceptible infective courts in normally resistant tissue. All 'Ranger' alfalfa plants were galled, regardless of the EPTC dose. DCPA and chlorpropham reduced galling of *M. hapla* on 'Ranger' alfalfa, probably because herbicide toxicity reduced the amount of host tissue available. There was no reduction in the root-knot index of 'Ranger' in the presence of EPTC.

Effect of herbicide treatment on M. hapla infection: The number of 'Nev Syn XX' plants infected by *M. hapla* larvae was significantly greater in EPTC-treated soil than in chlorpropham- or DCPA-treated soil. Resistant 'Nev Syn XX' alfalfa seedlings in DCPA-treated soil did not differ in infection at 20, 24, and 28 C, but were significantly less infected at 32 C (Table 4). Results differed with susceptible Ranger alfalfa seedlings; infection was significantly less at 28 and 32 C than at 20 and 24 C. Results were similar in EPTC-treated soil. The differences in infection between resistant and susceptible alfalfa seedlings, confirming previous data (7), are probably caused by differences in plant attractiveness to the nematode.

M. hapla infection of both 'Nev Syn XX' and 'Ranger' was reduced by all herbicides, which was correlated to a corresponding reduction in root growth and infection courts. However, the importance of the reduced infection of 'Nev Syn XX' by *M. hapla* larvae in EPTC-treated soil was minimized by the increased galling due to

TABLE 4. Effect of soil temperature on infection of resistant (Nev Syn XX) and susceptible (Ranger) alfalfa by *Meloidogyne hapla* in herbicide-treated and untreated soil.[a]

Treatment (kg/ha)[b]	Larvae per seedling								LSD
	Resistant (Nev Syn XX)				Susceptible (Ranger)				
	20C	24C	28C	32C	20C	24C	28C	32C	0.05
Chlorpropham—4.5	0	0	0	0	6	5	1	0	2
DCPA—9.0	6	10	5	2	20	24	12	6	6
EPTC—6.7	18	20	14	5	24	28	16	11	7
Control	23	30	18	12	31	40	24	20	6
LSD 0.05	6	7	6	5	9	8	6	6	

[a]14-day-old transplants inoculated with 100 *M. hapla* larvae per seedling. Readings made 14 days after inoculation.
[b]Seed sown and transplanted into identically treated soil.

the increased host susceptibility. No larvae were found in 'Nev Syn XX' plants and few larvae in 'Ranger' plants growing in chlorpropham-treated soils. This was due to the severe phytotoxicity, which resulted in few or no feeder roots on the treated plants. The seedlings can undergo initial infection by *M. hapla* under such circumstances, but the larvae would subsequently either die or migrate from the root tissue, especially in 'Nev Syn XX.'

Effect of chlorpropham rate of M. hapla infection: The resistance of 'Nev Syn XX,' as measured by the percentage of plants galled, varied with the rate of chlorpropham. Galls were apparent on 15 and 20% of the plants grown in 1.1 and 0.56 kg/ha chlorpropham, respectively, after 12 weeks, while no plants grown in 2.2 or 4.5 kg/ha were galled. These differences can be attributed to the phytotoxicity of chlorpropham, which eliminated feeder roots at the higher rates. All 'Ranger' plants were galled at chlorpropham rates of 0.56, 1.1, and 2.2 kg/ha. However, because infection courts were destroyed by the chemical, only 75 were galled at 4.5 kg/ha. Root-knot galling indices based on an index of 1 = no galling, 2 = 1–10% of root system galled, 3 = 11–30% galled, 4 = 31–70% galled, and 5 = 71–100% galled, were also more severe at the lower rates. Indices for 'Nev Syn XX' were respectively 1.0, 1.0, 2.15 $\pm$ 0.26, and 3.79 $\pm$ 1.37 at 4.5, 2.2, 1.1, and 0.56 kg/ha chlorpropham. Root-knot indices for 'Ranger' at similar rates were 2.39 $\pm$ 0.19, 3.97 $\pm$ 0.19, 3.97 $\pm$ 0.42, 4.57 $\pm$ 0.42, and 5.0.

From this study it is evident that certain herbicides can predispose alfalfa plants to *M. hapla* galling, and growers should be aware that the hazard of root-knot nematode infection may be enhanced when certain herbicides are used for weed control in nematode-infested alfalfa plantings.

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