Effects of Cycloate on Development of *Heterodera schachtii* and Growth of Three *Beta* Species[1]

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Abstract: Greenhouse tests were set up to evaluate the effects of the herbicide, cycloate (S-ethyl cyclohexylethylthiocarbamate), on development of *Heterodera schachtii* and growth of three *Beta* species. Cycloate added to infested soil enhanced cyst development/gm root on *B. vulgaris* and larvae/gm of root in *B. patellaris* and *B. procumbens* at 4, 16, and 16 μg(a.i.)/gm of soil, respectively. Total numbers of nematodes/individual root system decreased because of poor root growth of seedlings in cycloate-amended soil. Penetration and larval development through stage three did occur in the wild *Beta* species in any treatment. Thus, resistance of *B. patellaris* and *B. pocumbens* to development of *H. schachtii* was not altered by cycloate. Cycloate also retarded growth ($P = 0.05$) of the sugarbeet cultivars and *B. patellaris* at 4 μg(a.i.)/gm and *B. procumbens* at 16 μg(a.i.)/gm of soil. Higher concentrations of nematodes/gm root in plants growing in cycloate-amended soil may be attributed to factors such as fewer roots available for penetration, possible effects of cycloate on egg hatch, greater attraction of nematodes to roots, and increased susceptibility of roots to larval penetration. Suppression of seedling growth in cycloate-amended soil may be attributed in part to higher nematode density and in part to direct root damage from cycloate. *Key Words: Beta vulgaris* (cv. 'Mono Hy Al' and 'Mono Hy D2'), *B. procumbens, B. patellaris,* nematode development, phytotoxicity, predisposition.

Cycloate (S-ethyl cyclohexylethylthiocarbamate) is effective as a selective herbicide when it is incorporated into the soil immediately before planting (15). This compound comes closest to providing season-long control of all sugarbeet (*Beta vulgaris* L.) weeds at 4 kg(a.i.)/ha, and beets show a wider margin of tolerance to this chemical than to other preplant herbicides (1).

The effects of cycloate on plant pathogens were reviewed by Campbell (4), but no report is available on its effects on the sugarbeet nematode, *Heterodera schachtii* Schmidt, or other plant-parasitic nematodes. However, the effects of two other thiocarbamates [pebulate (S-propyl butylethylthiocarbamate), a herbicide, and nabam (disodium ethylene bisdithiocarbamate), a fungicide] on *H. schachtii* were reported (3, 5, 12).

Because of the extensive use of cycloate in sugarbeet fields and the importance of *B. procumbens* Chr. Sm. and *B. patellaris* Moq. as sources of germplasm resistant to *H. schachtii* (11, 13), information of cycloate effects on nontarget organisms and hosts is vital. Previous studies on phytotoxicity of cycloate on sugarbeets (*Beta vulgaris* L.) have been conducted in the absence of *H. schachtii* (4, 6, 14, 18).

Our purpose was to investigate the effects of cycloate on the development of *H. schachtii* and on the growth of three *Beta* species in the presence of this nematode.

MATERIALS AND METHODS

Field soil infested with the sugarbeet nematode, *H. schachtii,* was passed through a 5-mm screen and mixed thoroughly with steamed soil (1:3 v/v) with a concrete mixer for 10 min to obtain 60 viable cysts per 600 gm soil. The homogenized soil was a sandy loam (with a pH of 7.7) consisting of 69% sand, 19% silt, 12% clay, and 2.9% O.M. (organic matter).

Sugarbeet cultivars 'Mono Hy Al' and 'Mono Hy D2', and the wild species *B. procumbens* and *B. patellaris* were sown in flats of steamed sandy loam soil (74% sand, 16% silt, 10% clay, and 2.4% O.M.; pH 7.8). The sugarbeet seeds were sown without scarification, and the seeds of wild *Beta* species were scarified by treatment with concentrated H_2SO_4 for a total of 6 h at three intervals of 2 h each. Seeds were rinsed with tap water at the end of each interval, and after the third acid treatment, they were soaked in a saturated solution of sodium bicarbonate for 10 min, rinsed again with tap water, air dried overnight, and planted on the succeeding day.

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Infested soil was mixed in a twin-shell blender with aqueous solutions of cycloate to obtain 4, 8, 16, or 32 μg/gm of the active ingredient. Controls included infested and noninfested soil without cycloate.

Six hundred gm of soil were placed in aluminum cylinders (6.5 cm diam and 17.5 cm long) (7). The soil was packed and single 2-week-old seedlings were transplanted into each cylinder. Cylinders containing *Beta* species were then placed in a greenhouse without supplemental light where temperatures ranged from 18-24 C, whereas those containing Mono Hy A1 seedlings also were placed in growth chambers with a 12-h photoperiod (31,200 lux) at 16 or 26 C constant temperatures. Cylinders were irrigated as needed. A standard greenhouse nutrient solution was substituted for tap water for one day at 2 weeks and 4 weeks after transplanting.

Six weeks after treatment, plants were harvested and weighed, and the number of penetrated larvae in *B. procumbens* and *B. patellaris* and white females and cysts in *B. vulgaris* (cv. Mono Hy A1 and Mono Hy D2)/gram of roots was determined. Roots of wild species were stained in an acid-fuchsin lactophenol solution, cleared in lactophenol, and then subjected to a combination blender-sieving technique to determine the number of penetrated larvae (8). The white females and cysts in the sugarbeet cultivars were determined as described by Jatala and Jensen (9). A randomized block design with four replications was used, and the experiment was repeated three times. Data were subjected to analysis of variance and mean separations were performed by Duncan's multiple range test.

RESULTS

Effects of cycloate on Heterodera schachtii: In the greenhouse experiments, cycloate enhanced the development of *H. schachtii* ($P = 0.05$) on the basis of the number of white females and cysts/gram roots in the Mono Hy A1 and Mono Hy D2 seedlings. Although mature females did not develop on the wild *Beta* species, larvae readily penetrated the roots. In comparison to controls, an increase ($P = 0.05$) in the number of larvae/gm of root tissue was observed in *B. procumbens* and *B. patellaris* seedlings grown in cycloate-amended soil. These increases occurred at the lowest concentration, 4 μg(a.i.)/gm in Mono Hy A1 and Mono Hy D2, and at the higher concentrations, 16 and 32 μg(a.i.)/gm in *B. patellaris* and *B. procumbens* (Table 1).

In the growth chamber studies, cycloate resulted in greater numbers of white females and cysts/gm root at 16 μg/gm concentration, at 16 C (Table 2). Because plants grew poorly at 26 C, probably an unsuitable temperature for early growing stages of the cultivar Mono Hy A1, there was little or no root formation and evaluation of nematode development was not possible.

The total number of nematodes/root system was suppressed in amended soil. However, this suppression was not always significant. In addition, the increase in number of nematodes/gram of root did not always correspond to the total number of nematodes/root system in similar treatments (Tables 1 and 2).

Both males and gravid females developed in all sugarbeet seedlings growing in amended and nonamended soil at 16 C in the growth chamber and at 18-24 C in the greenhouse.

Effects of cycloate on the plants: In the greenhouse experiments, cycloate suppressed ($P = 0.05$) shoot and root growth of *B. vulgaris* (cv. Mono Hy A1 and Mono Hy D2) and *B. patellaris* at 4 μg(a.i.)/gm treatment (Table 1). The 32 μg(a.i.)/gm treatment retarded the development of new leaves so that the plants remained in the cotyledonary stage for 2 weeks after transplanting. Because true leaves did not develop or were suppressed, plants had a rosette appearance. *Beta procumbens* seedlings exhibited more resistance to the adverse effects of cycloate. Deep green color, brittleness, thick leaves, and fusion of cotyledons reported earlier (4, 6, 14) for Mono Hy A1 and other sugarbeet cultivars were also noticed in Mono Hy D2. *Beta patellaris* seedlings were also stunted ($P = 0.05$) in nematode-infested soil without cycloate in comparison with *B. patellaris* grown in nematode-free soil without cycloate. *Beta procumbens,* however, was not affected under similar conditions (Table 1).

In growth chamber studies, Mono Hy A1 seedlings grown at 16 C were slightly

TABLE 1. Effects of cycloate on *Heterodera schachtii* and three Beta species growing under greenhouse conditions.[y, z]

Beta species and treatments	Beta vulgaris						Beta procumbens			Beta patellaris		
	Mono Hy A1			Mono Hy D2								
	Top	Root	Nematodes	Top	Root	Nematodes	Top	Root	Nematodes	Top	Root	Nematodes
Noninfested-greenhouse soil												
(Nonamended)	2.63a	1.82a	None	1.93a	1.49a	None	3.47a	0.86a	None	4.33a	1.88a	None
Infested-field soil												
(Nonamended)	1.05b	0.56b	126a (70)a	0.58b	0.30b	277a (83)a	2.15ab	0.67ab	149a (100)a	1.68b	0.69b	162a (112)a
Infested-field soil												
4 $\mu g/g$ cycloate	0.24c	0.13c	186b (24)bc	0.14c	0.09b	500b (45)ab	1.80ab	0.40bc	168a (67)ab	0.90c	0.35c	325a (114)a
8 $\mu g/g$ cycloate	0.33c	0.11c	391c (43)ab	0.07c	0.03c	1,100c (33)b	1.45bc	0.37bc	211ab (78)ab	0.62cd	0.21cd	167a (35)ab
16 $\mu g/g$ cycloate	0.14c	0.05c	344c (17)c	0.23c	0.07c	531b (37)b	1.06bc	0.19c	248b (47)bc	0.23dc	0.13d	569b (74)a
32 $\mu g/g$ cycloate	0.03c	0.015c	340c (6)c	0.10c	0.01c	286a (28)b	0.18c	0.08c	155a (12)c	0.09e	0.005d	212a (1)b

[y]In columns reporting "nematodes," the top number refers to mean number of nematodes/gm roots, and the number in parentheses refers to number of nematodes per root system in corresponding treatments.
[z]Numbers are means of four replications. Column means followed by common letters are not different according to Duncan's Multiple Range Test ($P = 0.05$).

TABLE 2. Effects of three concentrations of cycloate on the development of *Heterodera schachtii* and sugarbeet cultivar ('Mono Hy Al') under two different conditions.[y,z]

Treatments	Greenhouse (18-24 C)			Growth chamber (16 C)		
	Top	Root	Nematodes	Top	Root	Nematodes
Noninfested-greenhouse soil						
(Nonamended)	2.63a	1.82a	None	2.71a	2.65a	None
Infested-field soil						
(Nonamended)	1.05b	0.56b	126a (70)a	0.82b	0.40b	92a (37)a
Infested-field soil						
4 μg/g cycloate	0.24c	0.13c	186b (24)bc	0.84b	0.30bc	70a (22)ab
8 μg/g cycloate	0.33c	0.11c	391c (43)ab	0.49bc	0.15bc	92a (14)b
16 μg/g cycloate	0.14c	0.05c	344c (17)c	0.20c	0.11c	170b (19)b

[y]In columns reporting "Nematodes," the top number refers to mean number of nematodes/gm roots, and numbers in parentheses refer to number nematodes/root system in corresponding treatments.
[z]Numbers are means of four replications. Column means followed by common letters are not different according to Duncan's Multiple Range Test ($P = 0.05$).

affected when soil was amended with 4 and 8 μg(a.i.)/gm cycloate. However, at 16 μg(a.i.)/gm concentration, a suppression ($P = 0.05$) in shoot and root growth was observed (Table 2). At 26 C, Mono Hy Al seedlings grew poorly and the possible effect of the cycloate inhibition noted previously was masked.

DISCUSSION

An increase in populations of *H. schachtii* by a chemically related herbicide (a thiocarbamate) was reported by Altman and Ross (3). They found that *H. schachtii* cysts in fields treated with pebulate were double the number found in non-amended fields. Studies on another thiocarbamate (nabam), a fungicide, revealed that a 100 μg/ml solution of nabam, in contrast to tap water, increased hatching of sugarbeet nematode cysts. However, when infested soil was drenched with this chemical, nabam had no effect on cysts (12). According to Clarke and Shepherd (5), nabam solutions decompose into several compounds, some hatch-inhibiting and other hatch-stimulating.

Differences in the patterns of nematode increase in different *Beta* species suggest that the hatch-stimulating activity, if any, is not the only reason for the increased number of nematodes/gram of root (Table 1). Other factors that might be considered are fewer roots available for penetration; possible toxic effects of cycloate on roots, which predisposes them to increased penetration by the larvae; and an increased chemical and nutrient gradient which exudes from the roots and attracts more nematodes towards the roots. Several explanations for the increased numbers of nematodes/gm roots may also be inferred from the reports of: Wheeler (17) on delay in maturation of sugarbeet seedlings stressed by cycloate; Johnson and Viglierchio (10) on the increased penetration of the nematode to the young seedlings; Altman (2) on release of glucose to soil-plant-interface by seedlings growing in herbicide-amended soil; and Wallace (16) on hatch-stimulating activity of sugars.

The results of our experiments in a growth chamber at 16 C and in the greenhouse at 18-24 C indicated that the adverse effects of cycloate were more pronounced at the higher temperatures. The poor growth of the sugarbeet, Mono Hy Al, at 26 C masked the detrimental effects of cycloate because this temperature appeared to be detrimental to the growth of young seedlings of this cultivar. Suppression of plant growth in cycloate-amended soil at 16 and 18-24 C may also be attributed in part to a higher density of the nematode and in part to root damage from cycloate.

Considering our results on the adverse effects of cycloate on *B. patellaris* and both cultivars of *B. vulgaris,* which are extensively used in Colorado, and the increased density of *H. schachtii* population in roots of plants growing in cycloate-amended soil, field studies on the effects of this herbicide in nematode-infested soil deserve special attention.

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