

## Pesticide - Plant Disease Interactions: Effect of Cycloate on Sugar Beet Damping-Off Induced by *Rhizoctonia Solani*

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### ABSTRACT

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Seedling survival of sugar beet cultivars MSH 212, FC 901, and FC 702/5 was significantly reduced ( $P = 0.05$ ) in soil infested with *Rhizoctonia solani* and amended with cycloate (*S*-ethyl *N*-ethylthiocyclohexanecarbamate) at 4, 8, or 16  $\mu\text{g/g}$  soil, compared to infested, nonamended controls. The survival patterns for cultivars FC 701/5 and Mono Hy A1 were similar to those for MSH 212, FC 901, and FC 702/5, although differences for FC 701/5 and Mono Hy A1 were not

statistically significant ( $P = 0.05$ ). Increased sugar beet seedling hypocotyl exudates in the presence of cycloate may be implicated in the increased damping-off with cycloate added at 4, 8, or 16  $\mu\text{g/g}$  soil, whereas reduction in fungal growth rate and/or colonization ability caused by the herbicide at 32  $\mu\text{g/g}$  soil balanced with increased exudates, is offered to explain why damping-off rates in infested soil were not increased by cycloate at 32  $\mu\text{g/g}$  soil.

*Additional key words:* herbicide predisposition, *Beta vulgaris*.

The increasingly important role of herbicides in modern agriculture necessitates the evaluation not only of the phytotoxic potential of a given herbicide but also of its potential to alter the relationship between a susceptible and its pathogens. Predisposition to disease by a herbicide may be a direct effect on the physical structure or biochemical defenses of the susceptible (12), an alteration of the microclimate owing to the removal of weeds (6, 7), decrease in the "antiphytopathogenic potential" of a soil (11), or a direct stimulation of the pathogen (7, 10). None of these factors operates independently. Thus, when each is examined independently the results must be integrated to provide explanations for an interaction.

Heitefuss (5, 6) reported that herbicides could induce morphological and physiological changes in host plants, including reduction of wax formation on leaves, and changes in carbohydrate, nitrogen, and glucoside metabolism. He also stated that plant growth may be retarded or stimulated in the presence of a herbicide. Paul (8) and Paul and Schönbeck (9) found significant reduction of root infection caused by *Fusarium moniliforme* with the addition of 7 to 15  $\mu\text{g/ml}$  diallate (a thiocarbamate herbicide) in both soil and liquid culture, compared to nontreated controls. Disease reduction was attributed chiefly to an effect on the host plant in which lignin-containing substances and  $\beta$ -glucosidase activity were increased after application of diallate.

Romig and Sasser (12) have shown that disease due to *Rhizoctonia solani* Kühn increased in snapbean when

trifluralin or dinoseb was applied to soil. They concluded that disease increase was due to a herbicide-induced reduction of snapbean structural and biochemical defenses. Altman and Ross (2) reported increased seedling damping-off in sugar beets (*Beta vulgaris* L.) caused by *R. solani* in the field and in steamed and raw soil treated with prebulate and pyrazon in greenhouse tests. They suggested that predisposition of the seedling to disease by the herbicides may in part explain why the use of some herbicides results in poorer stands of plants in certain treated sugar beet fields compared with stands in nontreated fields. Altman (1) also found similar effects in another series of experiments with pyramin and cycloate. He found that glucose exudates from hypocotyls of sugar beets were greater at the soil-plant interface in the herbicide-treated soil and concluded that the increase in glucose exudates and an increase in mineral-containing hypocotyl exudates predisposed seedling to damping-off induced by *R. solani*.

The purpose of this work was to further investigate the effect of cycloate, a thiocarbamate herbicide, on sugar beet seedling damping-off induced by *R. solani*.

### MATERIALS AND METHODS

Sugar beet cultivars used included FC 701/5 and FC 702/5, *Rhizoctonia*-resistant breeding lines obtained from the USDA-ARS, Fort Collins, Colorado; MSH 212, a moderately resistant hybrid and Mono Hy A1, a *Rhizoctonia*-susceptible commercial cultivar, both obtained from Great Western Sugar Company; and FC

901, a highly susceptible cultivar obtained from the USDA-ARS. The relative resistance of these cultivars to *R. solani* damping-off has been reported previously (3).

Steamed (temperature  $\approx 80$  C) greenhouse soil mixture [top soil, peat moss, and unwashed sand (1:1:1, v/v)] was infested with *Rhizoctonia* inoculum at 100, 200, or 400  $\mu\text{g/g}$  soil in a twin-shell blender for 3 min. The various soil-*Rhizoctonia* treatments were amended with aqueous herbicide solutions to give, on an active ingredient basis, cycloate at 4, 8, 16, or 32  $\mu\text{g/g}$  soil. Five-hundred grams of cycloate-amended soil-inoculum mix was placed in each 9.0  $\times$  9.5 cm polyethylene plastic pot. Ten equally spaced sugar beet seeds were planted 1.5 to 2.0 cm deep. Noninoculated and nonherbicide-treated controls were included in each experiment. Pots were placed in growth chambers with a 12-hr photoperiod (31,200 lux) at 26 C constant temperature. Pots were irrigated as needed. Seedling survival was recorded 21 days after planting. A randomized complete block design with three replications was used. Upon repetition of the experiment, results were similar.

## RESULTS

**Effect of cycloate on sugar beet seedlings.**—In soil amended with cycloate at 16 and 32  $\mu\text{g/g}$  soil, leaf size, measured as blade width and blade and petiole length, was significantly reduced (Table 1). Both blade width and blade and petiole length were significantly less for plants grown in *R. solani*-infested soil treated with cycloate at 32  $\mu\text{g/g}$  soil than in noninfested soil treated with cycloate at 32  $\mu\text{g/g}$ . Severe stunting and fusion of cotyledons and primary leaves occurred with the cycloate amendment at 32  $\mu\text{g/g}$  soil. Number of seedlings surviving after 21 days in cycloate-treated soil did not differ from survival in nonamended control.

**Seedling damping-off.**—Survival of sugar beet seedlings decreased significantly ( $P = 0.05$ ) with addition of increasing amounts of *R. solani*-barley grain inoculum from 100 to 400  $\mu\text{g/g}$  soil compared to noninfested controls. The cultivar  $\times$  inoculum interaction was significant ( $P = 0.05$ ) and survival of individual cultivars

and response to inoculum dosage agreed with our previous report (3).

Cycloate at 4, 8, or 16  $\mu\text{g/g}$  significantly ( $P = 0.05$ ) increased damping-off compared to inoculated nonamended controls for cultivars MSH 212, FC 901, and FC 702/5 (Fig. 1). Rate of damping-off for these three cultivars at the 32- $\mu\text{g/g}$  treatment level in inoculated soil did not differ from inoculated, nonherbicide controls. Although differences were not significant ( $P = 0.05$ ), similar seedling survival patterns occurred for FC 701/5 and Mono Hy A1 as for cultivars MSH 212, FC 702/5, and FC 901. The cultivar  $\times$  inoculum  $\times$  herbicide interaction had a significance level of  $P = 0.18$ .

## DISCUSSION

Cycloate at all treatment rates affected growth of sugar beet seedlings as indicated by a deeper color of cotyledons and leaves. The herbicide at 16 and 32  $\mu\text{g/g}$  soil significantly ( $P = 0.05$ ) reduced both leaf blade width and leaf blade-petiole length (Table 1) and plants appeared stunted. Sugar beet seedling growth and vigor was generally reduced by the two higher cycloate rates.

In the sugar beet-*R. solani*-cycloate system, we do not believe, however, that the reduction in plant growth and vigor with increasing herbicide concentration is sufficient to explain the interaction. Increased sugar beet damping-off occurred at 26 C in greenhouse soil infested with *R. solani* and amended with cycloate at 4, 8, or 16  $\mu\text{g/g}$  soil compared to controls (Fig. 1); however, with cycloate at 32  $\mu\text{g/g}$  soil, the rate of damping-off was similar to nonamended, infested controls. Thus, damping-off did not increase when plants were most severely affected by the herbicide, but did increase with cycloate at 4  $\mu\text{g/g}$  when plant growth and vigor were not significantly ( $P = 0.05$ ) reduced.

Altman (1) reported increased hypocotyl glucose exudates from sugar beet seedlings grown in the presence of cycloate and pyramin. Hypocotyl extracts were analyzed spectrophotometrically for glucose using the Kornberg-Horecker method for true glucose. This increase in glucose was detectable at 2, 4, and 10 wk after herbicide treatment. Increases in electrical conductivity also were observed in double-distilled water containing sugar beet hypocotyls of plants grown in herbicide-treated soil compared with controls. This further indicated an increase in hypocotyl exudates after treatment with pyramin or cycloate.

Campbell and Altman (4) reported that percentage substrate (bean hypocotyl segment) colonization by *R. solani* in steamed soil treated with cycloate at 4 or 8  $\mu\text{g/g}$  soil was not significantly ( $P = 0.05$ ) different from colonization in nontreated steamed soil. In steamed soil with cycloate at 16  $\mu\text{g/g}$  soil, substrate colonization was significantly less than controls and cycloate at 32  $\mu\text{g/g}$  soil significantly reduced the percentage colonization over both controls and the 16- $\mu\text{g/g}$  treatment level. The reduction in substrate colonization was attributed to a reduction in the rate of growth and/or colonization ability of *R. solani* at the higher herbicide rates.

Growth rate and/or colonization ability of *R. solani* in soil is influenced by cycloate (4) and is expected to be influenced by the amount of seedling exudate present in

TABLE 1. Effect of cycloate and *Rhizoctonia solani* on leaf size of sugar beet cultivar Mono Hy A1, 21 days after planting in the greenhouse

Herbicide rate ( $\mu\text{g/g}$ )	Leaf size <sup>x</sup>			
	Blade width (mm)		Blade and petiole length (mm)	
	Without <sup>y</sup> inoculum	With inoculum	Without inoculum	With inoculum
0	40.6 ab <sup>z</sup>	42.4 a	167.6 ab	158.4 b
4	35.4 bc	43.4 a	185.8 a	148.4 b
16	26.6 d	29.2 cd	121.2 c	101.0 c
32	24.0 d	15.0 e	99.6 c	64.2 d

<sup>x</sup> Average of five leaves/replication.

<sup>y</sup> Inoculum = 200  $\mu\text{g}$  *R. solani* inoculum per gram of soil.

<sup>z</sup> Values not followed by a common letter within a measurement category (column) are significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

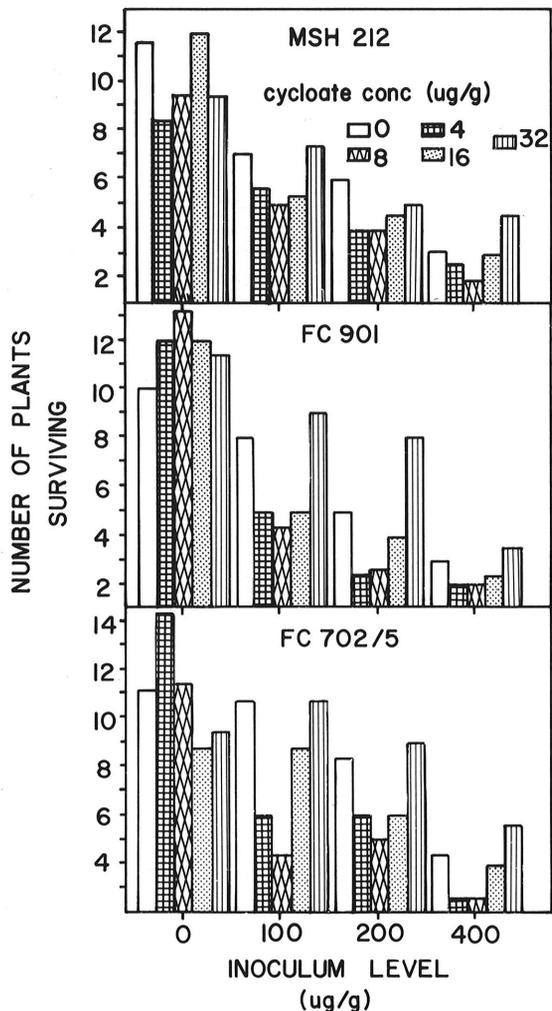


Fig. 1. Effect of cycloate at 0, 4, 8, 16, and 32  $\mu\text{g/g}$  soil and *Rhizoctonia solani* on survival of sugar beet seedlings (cultivars MSH 212, FC 901, and FC 702/5) in growth chamber experiments at 26 C with a 12-hr photoperiod (31,200 lux) after 21 days.

the soil or at the hypocotyl-soil interface (13, 14). We propose that there is a type of counterbalancing effect of these two influences acting in this pathogen-host-herbicide interaction.

With cycloate at 4 or 8  $\mu\text{g/g}$  soil, growth rate and/or colonization ability of *R. solani* is not inhibited by cycloate (4), but may be stimulated by increased exudates expected from sugar beet hypocotyls in the presence of cycloate. This leads to increased damping-off. Cycloate at 32  $\mu\text{g/g}$  soil, significantly ( $P = 0.05$ ) inhibits growth and/or colonization ability of *R. solani* (4). It is hypothesized that the seedling exudates induced by cycloate treatment cannot totally overcome this growth inhibition of *R. solani* or at least do not increase pathogen growth rate and/or colonization ability compared to nonherbicide controls. This results in a balancing effect between inhibition vs. stimulation which results in damping-off rates similar to infested, nonherbicide

controls. With cycloate at 16  $\mu\text{g/g}$  soil, inhibition of growth and/or inhibition of colonization ability is less than with cycloate at 32  $\mu\text{g/g}$  soil (4), thus the exudates may be overcoming the pathogen growth inhibition and even stimulating the fungus which leads to increased damping-off rates compared to controls.

An unexpected result was that seedlings grown in *R. solani*-infested soil amended with cycloate at 32  $\mu\text{g/g}$  soil had significantly ( $P = 0.05$ ) reduced leaf blade width and blade-petiole length compared to seedlings in noninfested soil similarly amended with cycloate at 32  $\mu\text{g/g}$  soil. This suggests that *R. solani* stressed the seedlings beyond the level obtained with the herbicide at 32  $\mu\text{g/g}$  soil alone. Since *R. solani* usually induces damping-off of sugar beet seedlings when it infects plants at this growth stage, this stressing without eventual damping-off may be unique and should be further investigated.

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