Pesticide — Plant Disease Interactions: Effect of Cycloate on Growth of Rhizoctonia solani

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ABSTRACT

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Linear growth of *Rhizoctonia solani* after 72 hr at 20 C was significantly less on all nutrient concentrations of potato-dextrose agar amended with cycloate (S-ethyl N-ethylthio-cyclohexanecarbamate) from 10 to 100 μg/ml, than in controls without cycloate. Rate of growth of mycelium of *R. solani* was less in Czapek-Dox broth with 1 g yeast extract/liter amended with 4 or 8 μg/ml cycloate, than in controls. In raw soil amended with 4 and 8 μg/g cycloate, and in steamed soil amended with 4 μg/g cycloate, colonization of 5-mm-long mature bean hypocotyl segments by *R. solani* was slightly greater than in soil without cycloate. At 16 and 32 μg/g cycloate, colonization of hypocotyl segments was significantly less than in nonamended controls in both steamed and raw soil. Colonization of sterile sugar beet seeds by *R. solani* also was less with 8, 16, and 32 μg/g cycloate than in controls.

Herbicides are becoming more important in modern agriculture. Although much research is done to evaluate the phytotoxicity of herbicides to economic crop plants, questions remain concerning the effects of herbicides on soil microorganisms including soil-borne plant pathogens.

Rodriguez-Kabana et al. (11) using rates ranging from those "considered likely to accumulate in soil under field applications to others much higher than would be expected," found that in liquid culture, diuron had little effect on the mycelial production of *Rhizoctonia solani* Kühn. EPTC generally had little effect on the growth of *R. solani*, although the fungus initially was inhibited by this herbicide. Atrazine inhibited mycelial production throughout the 22-day trial period, but paraquat increasingly inhibited the fungus as herbicide concentration increased.

Ebner (5) found that *R. solani* was sensitive to diuron at 125 μg/ml and to linuron at 60 and 125 μg/ml. In media in which a carbon or nitrogen source was absent and in a complete medium, Antonopoulos (3) found that picloram and dicamba at 5 μg/ml inhibited growth of the fungus. Growth of *R. solani* also was suppressed with increasing concentrations of benefin, trifluralin, and isopropalin, whereas nitralin was only slightly fungitoxic (6). Millikan and Fields (8) reported a 93% reduction in growth of *R. solani* in Fries' nutrient solution amended with simazine. Bone and Kuntz (4), however, found no inhibition of growth of the fungus in pure culture in the presence of Dacthal.

Altman (1) and Altman and Ross (2) observed both in field and greenhouse work that an interaction, leading to increased disease incidence, existed between sugar beet (*Beta vulgaris* L.) seedling damping-off caused by *R. solani* and certain herbicides used in sugar beet culture which included cycloate, pebulate, and pyrazon. The authors concluded that this interaction could in part explain poorer stands of plants in certain herbicide-treated fields compared with stands in nontreated fields. The purpose of this work was to investigate the effect of cycloate (S-ethyl N-ethylthiocyclohexanecarbamate) on *R. solani*. This herbicide currently is recommended for use as a preplant treatment for sugar beets in Colorado and in all other intermountain states in which sugar beets are grown.

MATERIALS AND METHODS

Inoculum.—The isolate of *R. solani* used in all experiments was RR-9 (assignable to AG2), originally isolated from a rotted sugar beet root in eastern Colorado by Pierson and Gaskill (10) and supplied by E. G. Ruppel, USDA-ARS, Ft. Collins. For in vitro tests, the fungus was maintained on potato-dextrose agar (PDA) at 20 C. Inoculum for soil tests was prepared after the method of Pierson and Gaskill by growing the *R. solani* on sterile barley grain for 21 days at 20 C. The inoculum was then air-dried, ground in a Wiley mill, and stored at 2-4 C.

Effect of cycloate on Rhizoctonia solani in

vitro.—Difco potato-dextrose broth, rehydrated according to directions (24 g PDB/1,000 ml distilled water) plus 17 g agar was taken as 100% PDA. Concentration of 5, 10, 25, and 50% PDA were obtained by using 1.2, 2.4, 6, and 12 g PDB/1,000 ml, respectively. Water agar (17 g agar/1,000 ml distilled water) served as 0% PDA. Media were autoclaved, cooled to 50 C, and amended with an aqueous cycloate solution to give herbicide concentrations of 10 to 100 μg/ml active ingredient. An equivalent amount of sterile distilled water was added to the control medium in each test. A 3- to 4-mm-diameter agar plug, 7 mm thick, cut with a sterile cork borer from the edge of an actively growing colony of *R. solani*, was placed in the center of each agar plate containing 25 ml of medium. Colonies were incubated under continuous fluorescent light (31,200 lux) at 20 C for 72 hr. Measurements of linear growth were made after 72 hr. A randomized complete block design was used with four replications.

In a second test, sterile Czapek-Dox broth amended with 1 g of yeast extract per liter was used as a test medium. Cycloate solution was added to 25 ml of the cooled, sterile medium in 125-ml Erlenmeyer flasks to give concentrations of 4 and 8 μg/ml active ingredient. Sterile distilled water equivalents were added to cooled control medium. Broth was inoculated with agar plugs of mycelium and incubated under continuous fluorescent light (31,200 lux) at 20 C. Mycelial mats were removed from the flasks and excess medium was removed by suction filtration. Growth as dry weight was measured after drying mats in open petri dishes at 105 C for 24 hr. Dry weights were determined every 48 hr. A randomized complete block design was used with three replications.

Substrate colonization by Rhizoctonia solani in cycloate-amended soil.—A modified technique of Papavizas and Davey (9) was used in substrate colonization experiments. Thirty 5-mm-long mature bean hypocotyl segments, or 100 sterile sugar beet seeds (cultivar Mono Hy Al) (approximately 1 g), were mixed with 100 g soil containing 200 μg/g (w/w) barley-grain inoculum and cycloate at concentrations of 0, 4, 8, 16, and 32 μg/g. In all but one colonization experiment, a steamed greenhouse potting mixture composed of top soil, peat moss, and unwashed sand (1:1:1, v/v) was used. In one test, bean hypocotyl segments only were mixed with raw soil or steamed greenhouse soil. Distilled water was added to bring soil to 20 to 25% of water holding capacity after the soil was placed in 5.3-cm-deep × 10.5-cm-diameter plastic dishes. Dishes were stacked to reduce moisture loss during a 3-day incubation at room temperature (23 ± 3 C). Water content did not differ within the stack. Each treatment was replicated three

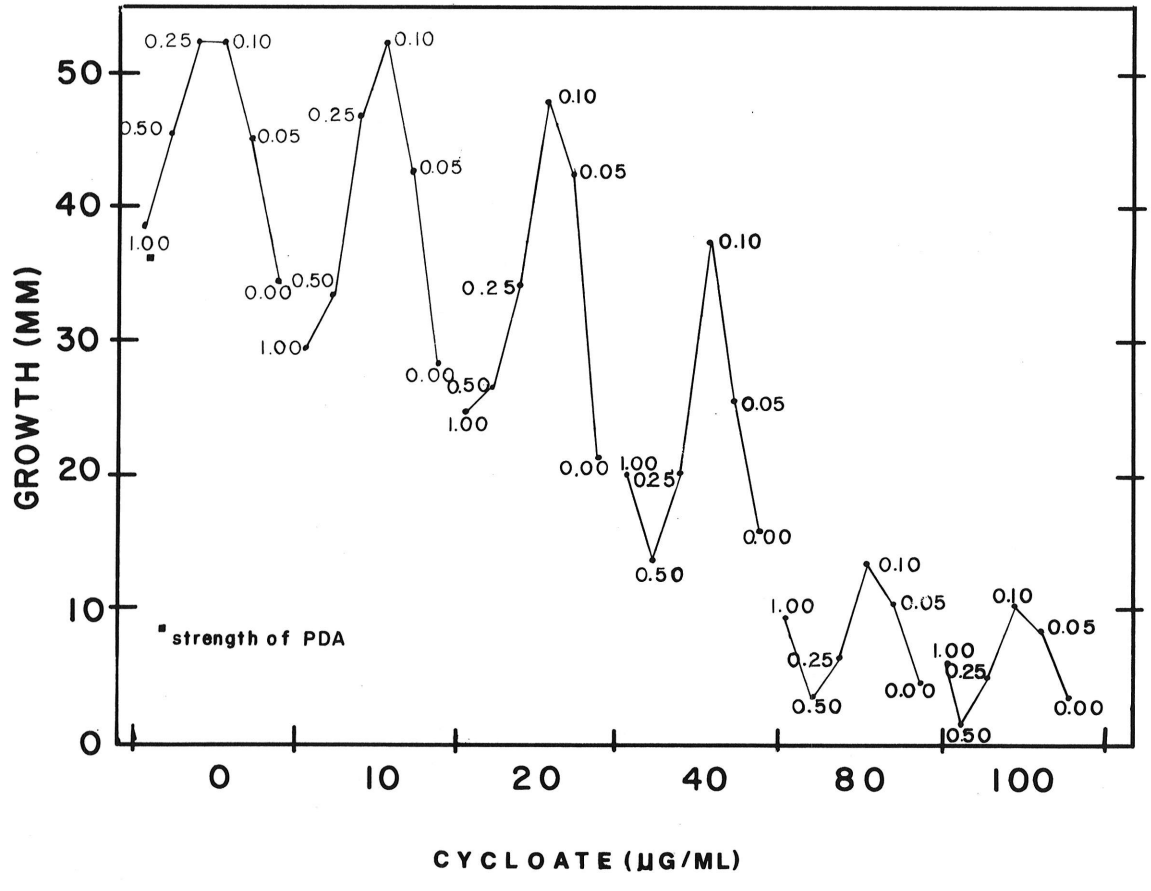

Fig. 1. Effect of cycloate on mycelial growth of *Rhizoctonia solani* on varying strengths of potato-dextrose agar amended with herbicide after 72 hr of growth at 20 C.

times. Twenty bean segments or 20 seeds were removed from each dish, washed 1-2 min under running tap water, surface-sterilized in 5% sodium hypochlorite solution, and either 10 segments or seeds placed in each of two petri plates containing 1.5% water agar amended with 50 μg/ml (w/v) each of streptomycin sulfate and chloramphenicol. The segments were incubated at room temperature for 24 hr and numbers of segments colonized by *R. solani* were counted using a dissecting microscope.

RESULTS

Linear growth on agar medium.—On all nutrient concentrations of PDA, amendment with 10 to 100 μg/ml cycloate significantly reduced linear growth of *R. solani* after 72 hr compared to nonamended controls (Fig. 1). Degree of inhibition increased with herbicide concentrations. Generally, linear growth in all herbicide treatments increased as concentration of PDA was reduced from 100 to 10%, but decreased when medium concentration was reduced from 10 to 0%. Visual observations indicated a decrease in mycelial density on the medium surface as medium concentration decreased.

Mycelial growth in liquid medium.—In Czapek-Dox broth amended with 1 g yeast extract per liter the autolytic point (AP = the point in growth where self-digestion begins due to exhaustion of nutrients) for *R. solani* occurred between 11 and 12 days after inoculation in controls, and after 13 and 15 days with 4 and 8 μg/ml cycloate amendment, respectively (Fig. 2, 3). Although the time required to reach the AP increased with the increasing amount of cycloate, total mycelial growth at the AP, measured on a dry weight basis, was not reduced.

Substrate colonization.—In steamed soil cycloate at 8, 16, and 32 μg/g significantly reduced colonization of sterile sugar beet seeds by *R. solani*; the 4 μg/g level had no significant effect (Table 1). In steamed soil amended with 4 μg/g cycloate and raw soil amended with 4 or 8 μg/g cycloate, numbers of bean stem segments colonized were slightly, but not significantly, higher than in controls not treated with cycloate; the 16 and 32 μg/g levels significantly reduced bean stem segments colonization by *R. solani* compared to controls.

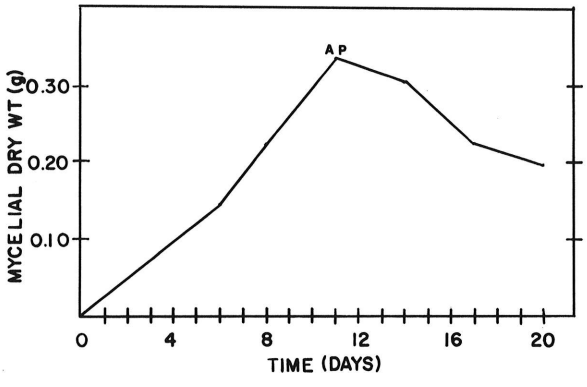

Fig. 2. Autolytic point (AP) of *Rhizoctonia solani* in yeast extract amended Czapek's-Dox broth at 20 C.

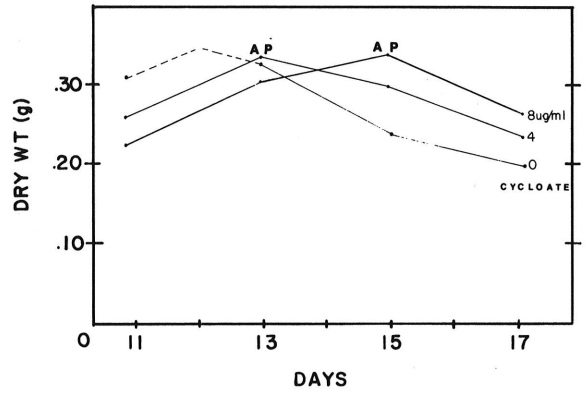

Fig. 3. Effect of cycloate at 0, 4, and 8 μg/ml on mycelial production by *Rhizoctonia solani* in yeast extract amended Czapek-Dox broth at 20 C.

DISCUSSION

Rate of linear growth of *R. solani* increased in amended and nonamended media and mycelial density decreased as the strength of PDA was reduced from 100 to 10%. It is

TABLE 1. Sugar beet seed colonization by *Rhizoctonia solani*[x] in steamed soil at 20 C

Cycloate concentration (μg/g)	Colonization (percentage)[y]
0	75.0 a[z]
4	66.6 a
8	40.0 b
16	28.3 b
32	21.6 b
C. V. = 32.5%	

[x]Barley inoculum level = 200 μg/g.
[y]Means of three replicates of 20 seeds each after 3 days.
[z]Means not followed by the same letter were significantly different at $P = 0.05$.

TABLE 2. Bean hypocotyl segment colonization by *Rhizoctonia solani*[x] at 20 C after 72 hr

Cycloate concentration (μg/g)	Colonization (%)[y] Soil type	
	Raw	Steamed
0	53.3 cd[z]	70.2 ab
4	56.6 bcd	73.3 a
8	60.0 abc	61.6 abc
16	26.6 e	43.3 d
32	20.0 e	23.3 e
C.V. = 23.2%		

[x]Barley inoculum level = 200 μg/g.
[y]Means of three replicates of 20 segments each.
[z]Means not followed by the same letter were significantly different at $P = 0.05$.

suggested that both effects were due to the decreasing total nutrient content of the medium.

Cycloate at 10 to 100 μg/ml significantly ($P = 0.05$) inhibited linear growth of *R. solani* on all strengths of PDA tested and reduced the rate of mycelial growth in Czapek-Dox broth at 4 and 8 μg/ml, although the latter differences were not significant. Evaluation of linear growth of *R. solani* on PDA amended with a herbicide may give an indication of the effect of a herbicide on a pathogen. Measurement of the total mycelial growth in yeast extract-amended Czapek-Dox broth may evaluate the effect of a herbicide on total fungal growth, however, both systems are highly artificial and may not indicate the effect of the herbicide on the fungus in vivo.

Papavizas and Davey (9) used colonization of substrates to measure fungal competitive saprophytic ability in raw or natural soil. Katan and Eshel (7) used substrate colonization as a measure of the effect of diphenamid on growth of *R. solani* in soil. The substrate colonization method is perhaps the most accurate method for the appraisal of the effect of a herbicide on a soil-borne fungus.

In this study, colonization of autoclaved sugar beet seeds or living hypocotyl segments in steamed soil amended with cycloate is not a measure of the herbicide's effect on the competitive saprophytic ability of *R. solani*. It is a measure of the growth of *R. solani* through soil and/or colonizing ability of the pathogen in the presence of the herbicide, since a majority of competing organisms have been removed through steaming of the soil. With cycloate at 8, 16, and 32 μg/g, colonization of sugar beet seeds was significantly less than in controls and at 16 and 32 μg/g cycloate hypocotyl segment colonization also was significantly less than in nonamended controls after 72 hr (Tables 1 and 2). This indicates that the rate of growth and/or colonizing ability of *R. solani* was reduced in the presence of cycloate.

In raw soil amended with 16 and 32 μg/g cycloate, competitive saprophytic ability of *R. solani*, measured as numbers of bean segments colonized, was significantly reduced compared to nonamended controls (Table 2). No attempt was made to determine the effect of cycloate on the general soil microflora; however, on the basis of the experiments with steamed soil, reduction of colonization of bean segments by *R. solani* in raw soil probably is due to a reduction in rate of growth of the fungus.

LITERATURE CITED

1. ALTMAN, J. 1969. Predisposition of sugarbeets to Rhizoctonia solani damping-off with herbicides. Phytopathology 59:1015 (Abstr.).
2. ALTMAN, J., and M. ROSS. 1967. Plant pathogens as a possible factor in unexpected preplant herbicide damage in sugarbeets. Plant Dis. Rep. 51:86-88.
3. ANTONOPOULOS, A. A. 1969. Plant pathogen-herbicide interactions. Ph.D. Dissertation. Colorado State University, Fort Collins. 216 p.
4. BONE, H. T., and J. E. KUNTZ. 1968. The breakdown of dacthal in soil and its effect on certain processes of soil microbes. Proc. North Cent. Weed Control Conf. 23:34.
5. EBNER, L. 1965. Über die Nebenwirkung verschiedener Harnstoff-Herbizide auf einige Pilzarten. Z. Pflkrankh., Pflanzenpathol Pflanzenschutz Band 72:344-350.
6. ESHEL, Y., and J. KATAN. 1972. Effect of dinitroanilines on solanaceous vegetables and soil fungi. Weed Sci. 20:243-246.
7. KATAN, J., and Y. ESHEL. 1974. Effect of the herbicide diphenamid on damping-off disease of pepper and tomato. Phytopathology 64:1186-1192.
8. MILLIKAN, D. F., and M. L. FIELDS. 1964. Influence of some representative herbicidal chemicals upon the growth of some soil fungi. Phytopathology 54:901 (Abstr.).
9. PAPAVIZAS, G. C., and C. B. DAVEY. 1959. Isolation of Rhizoctonia solani Kuehn from naturally infested and artificially inoculated soils. Plant Dis. Rep. 43:404-410.
10. PIERSON, V. G., and J. D. GASKILL. 1961. Artificial exposure of sugar beets to Rhizoctonia solani. J. Am. Soc. Sugar Beet Technol. 11:574-590.
11. RODRIGUEZ-KABANA, R., E. A. CURL, and H. H. FUNDERBURK, JR. 1966. Effect of our herbicides on growth of Rhizoctonia solani. Phytopathology 56:1332-1333.