Pesticide-Plant Disease Interactions: the Influence of Aldicarb On Growth of Rhizoctonia solani and Damping-Off of Sugar Beet Seedlings

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ABSTRACT

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Addition of aldicarb [2-methyl-2-(methylthio) propionaldehyde, O-(methylcarbamoyl) oxime] to soil infested with *Rhizoctonia solani* significantly increased damping-off of sugar beet seedlings in greenhouse and growth chamber studies. Seedling survival was lowest in steamed soil inoculated with *R. solani* barley grain inoculum at 400 μg/g and amended with aldicarb at 16 μg/g. In steamed soil inoculated with *R. solani* inoculum at 200 μg/g, colonization of mature bean hypocotyl segments after 72 hr was increased with aldicarb at 8 μg/g, but not with 32 μg/g. Linear growth of *R. solani* was reduced on potato-dextrose agar amended with aldicarb at 4, 8, 16, and 32 μg/ml.

Additional key words: nematicide predisposition, *Beta vulgaris*, aldicarb, *R. solani*.

In recent years, there has been concern about the influence soil-applied pesticides (such as herbicides, nematicides, and fungicides) may have on the relationship between a host crop and its indigenous soil pathogens. Altman and Ross (1) showed that the herbicide pyrazon increased damping-off caused in sugar beet seedlings by *Rhizoctonia solani* Kühn. Campbell (2), working with the herbicide cycloate, found that sugar beet seedling damping-off increased when rates of 4, 8, or 16 μg/g were added to *R. solani*-infested soil, but the addition of cycloate at 32 μg/g did not increase damping-off over the addition of inoculum alone. He also found that linear growth of *R. solani* was inhibited on cycloate-amended potato-dextrose agar (PDA). Other researchers also have reported increased disease when herbicides were used to control weeds in vegetables, cotton, and southern pea (5, 6, 10). Cole and Batson (3), however, reported a decrease in damping-off caused by *R. solani* in tomato seedlings when diphenamid was added to soil. Katan and Eshel (4) proposed four mechanisms by which a pesticide could increase or decrease disease in the plant. They postulated a pesticide could affect: (i) other microorganisms within the soil, (ii) pathogen virulence, (iii) pathogen growth, and (iv) host susceptibility.

Aldicarb is now used extensively in the Rocky Mountain Region of the USA as an at-plant treatment for nematode control. Steele and Hodges (9) reported that aldicarb at 3.5 μg/g was adequate to control *Heterodera schachtii* in California. The objective of this research was to test the effect of aldicarb on the damping-off caused in sugar beet seedlings by *Rhizoctonia solani*. Preliminary tests involving the in vitro growth and bean hypocotyl colonization by *R. solani* were carried out to determine the effect of aldicarb on the pathogen.

MATERIALS AND METHODS

A culture of *R. solani* (Isolate R-9, AG-2) was obtained from E. G. Ruppel at the U.S. Department of Agriculture, Agricultural Research Service, Fort Collins, CO 80521. Inoculum was prepared after the method of Pierson and Gaskill (8). The fungus was cultured on moist sterile barley grain for 3 wk at 20 C, air-dried for 72 hr, and ground in a Wiley mill. Steamed greenhouse soil [a mixture of topsoil, unwashed sand, and peat moss (1:1:1, v/v)] was mixed with 100, 200, and 400 μg of the inoculum per gram of soil in a twin-shell blender. *Rhizoctonia solani*-infested soil then was amended with 0, 4, 8, and 16 μg aldicarb (10% active ingredient)/g soil. These rates were used to approximate recommended aldicarb field rates of 4-8 μg/g soil.

Five hundred grams of infested, amended soil or noninfested, nonamended soil were placed in 9-cm-diameter × 9.5-cm-deep polyethylene plastic pots and 10 seeds of sugar beet cultivar Mono Hy A1 were planted, equally spaced 1.5-2.0 cm deep in each pot. Pots were placed in a growth chamber at 26 C with a 12-hr photoperiod. Pots were irrigated as needed. Percentage seedling survival was recorded after 21 days and compared to control. Damped-off seedlings were rinsed in 10% Clorox and plated on PDA to confirm the presence of *R. solani*. A randomized complete block design was used with four replications, and the experiment was repeated six times.

Bean hypocotyl colonization experiments were set up

using a modification of the technique of Papavizas and Davey (7). Thirty, 5-mm-long, mature, bean hypocotyl segments were placed in 100 g of greenhouse soil inoculated with 200 μg inoculum/g soil and amended with aldicarb at 0, 8, or 32 μg/g soil. Twenty segments were recovered after 3, 4, and 6 days and rinsed in 10% Clorox for 1-2 min. Ten segments were placed on each of two plates containing 1.5% water agar amended with streptomycin sulfate and chlorotetracycline, each at 50 μg/ml. Numbers of segments colonized by *R. solani* were counted after 24 hr. Each treatment was replicated six times.

The effects of aldicarb on mycelial growth of *R. solani* also were evaluated in vitro. Aldicarb at rates of 4, 8, 16, and 32 μg/g was aseptically added to autoclaved PDA cooled to 50 C. The amended medium was poured into plates, allowed to solidify, and inoculated with 7-mm-diameter × 3-mm-thick mycelial plugs of *R. solani*. Linear growth of the mycelium was measured daily for 5 days. Mycelium grown in the presence of aldicarb was then replated on PDA to detect residual effects of the chemical. (Mycelium appeared to recover in 5 days and linear growth from aldicarb-treated plugs was similar to that from control plugs.)

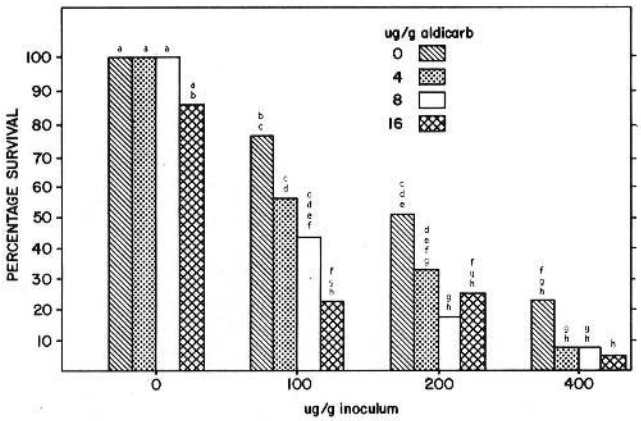

Fig. 1. Results of greenhouse studies involving the addition of *Rhizoctonia solani* inoculum to aldicarb-amended soil. Randomized Block Design. Bars not having similar letters were significantly different ($P = 0.05$) as determined by Duncan's multiple range test.

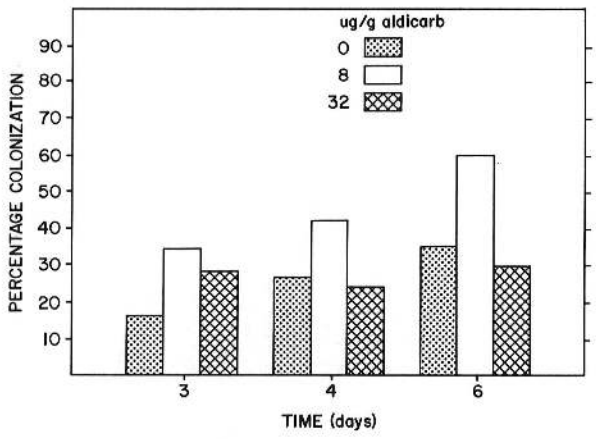

Fig. 2. Results of bean hypocotyl segment colonization by *Rhizoctonia solani* in aldicarb-amended soil.

RESULTS

Addition of aldicarb alone to soil did not reduce sugar beet germination (Fig. 1). At the highest rate of aldicarb (16 μg/g), some phytotoxicity was evident. Seedlings were stunted and leaf margins burned. Phytotoxicity was observed in both greenhouse and growth chamber experiments.

Addition of barley grain inoculum alone significantly ($P = 0.05$) decreased seedling survival compared to noninoculated controls. The inoculum rate of 200 μg inoculum/g soil resulted in 50% damping-off compared to 75% damping-off at double the inoculum level (Fig. 1). As rates of aldicarb increased from 4 to 16 μg/g, damping-off increased compared to inoculated nonamended controls. With 100 μg inoculum/g soil, the addition of aldicarb at 16 μg/g reduced seedling survival to the same level as that obtained by adding 400 μg/g alone. Lowest survival rates were recorded when the highest inoculum level (400 μg/g) was combined with high aldicarb concentrations (8 and 16 μg/g) in the soil. In several tests the addition of 4 μg aldicarb/g soil inoculated with *R. solani* at 400 μg/g reduced the number of surviving plants to such a low percentage that incorporation of additional pesticide did not result in greater increases in damping-off.

After six days, colonization of bean hypocotyl segments in soil amended with aldicarb at 8 μg/g and *R. solani* at 200 μg/g was 25% greater than in soil amended with inoculum alone (Fig. 2). Colonization of hypocotyl segments in inoculated soil amended with aldicarb at 32 μg/g was similar to colonization of segments in inoculated soil without aldicarb. Highest level of colonization by *R. solani* of bean hypocotyl segments occurred in infested soil amended with aldicarb at 8 μg/g (Fig. 2).

Linear growth of *R. solani* after 72 hr on PDA amended with aldicarb decreased as nematicide concentration increased up to 16 μg/ml (Table 1). Linear growth on agar amended with aldicarb at 4 μg/ml was significantly ($P = 0.05$) less than the control. Aldicarb at rates of 8, 16, and 32 μg/ml also significantly decreased linear growth compared with the control or the 4 μg/g

TABLE 1. Linear growth of *Rhizoctonia solani* after 72 hr on potato-dextrose agar amended with aldicarb

Aldicarb (μg/g)	Mean linear growth (mm)[x]
0	59.00 a[y]
4	55.00 b
8	51.75 c
16	51.50 c
32	52.00 c

[y]Data given are the averages of 12 replicates.

[z]Numbers not followed by the same letter are significantly different at $P = 0.05$.

level. Mycelial plugs transferred from PDA plates containing aldicarb to nonamended PDA showed the same amount of linear growth per day as did mycelial plugs taken from nonamended controls. No morphological differences in the mycelium were observed. When treated or control plugs were transferred to nonamended PDA, growth rates for both isolates were similar.

DISCUSSION

Addition of aldicarb and *R. solani* inoculum to soil increased damping-off of sugar beet seedlings compared to soils inoculated with *R. solani* alone. Aldicarb at 16 μg/g was phytotoxic to the seedlings. Highest rates of damping-off also occurred with the addition of aldicarb to inoculated soils at 16 μg/g. Aldicarb phytotoxicity thus may have predisposed the seedlings to increased damping-off. Aldicarb is applied at planting time as a side dressed incorporation band treatment. Therefore, it is possible that the pesticide at rates as high as 16 to 32 μg/g can occur under field conditions. At these rates, it is conceivable that sugar beet seedlings could be more susceptible to increased damping-off provided that *R. solani* or other damping-off pathogens are present in the field.

In vitro test results showed that aldicarb inhibited mycelial growth of *R. solani*. Rates of aldicarb at 16 and 32 μg/ml, which were phytotoxic to sugar beet seedlings in greenhouse and growth chambers test also effectively inhibited growth of *R. solani* in vitro. This effect was not apparent in the bean hypocotyl experiment, where the addition of 8 μg/g to soil had little or no effect on numbers of segments colonized by *R. solani* compared to segments colonized by *R. solani* in soil without aldicarb. Hypocotyl colonization experiments may determine more accurately the effect of a pesticide on a soilborne pathogen than determination of linear growth on PDA. The mechanism of stimulation of hypocotyl colonization with field rates has not been determined. Since steamed greenhouse soil was used in all experiments, this limits the possibilities of any effect of the nematicide on other microorganisms normally found in nonsteamed soil.

In some tests fungi were inhibited by aldicarb at 16 μg/g. Similar pathogen inhibition has been observed with ×2 and ×3 rates of cycloate (a herbicide) compared to field rates of 4-6 μg/g. These higher rates which can be obtained under some field conditions caused a reduction in growth and reduction in pathogenicity both in vivo and in vitro (2).

The possible effects of the pesticide-microorganism interaction on pathogens other than *R. solani* or soil saprophytes have not been examined. A moot question arises: What would happen if other microorganisms either competed with *R. solani* for infection sites or degraded aldicarb–in one case reducing disease and in the second reducing phytotoxicity? Perhaps degraded aldicarb might also have an effect on the host crop or the pathogen. These latter questions warrant research in evaluating a complete pesticide-plant-pathogen interaction.

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