Uptake of Three Systemic Fungicides by Germinating Soybean Seed

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ABSTRACT

Soybean seed were either nontreated or treated with benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate], chloroneb (1,4-dichloro-2,5-dimethoxybenzene), and DMOC (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) at 0.125, 0.25 or 0.5 g/100 g (2, 4, or 8 oz/100 lb.). Uptake was demonstrated by bioassay of extracts from germinating seed using an isolate of *Rhizoctonia solani* sensitive to the three fungicides. Benomyl, or a compound related to it, becomes localized in the cotyledons. Phytopathology 60:1373-1375.

Additional key words: Benomyl, chloroneb, DMOC, *Rhizoctonia solani*, seed dressing.

The uptake and partial translocation of three systemic fungicides by germinating soybean seed and the potential use of benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, duPont's Benlate 50 WP], chloroneb (1,4-dichloro-2,5-dimethoxybenzene, duPont's Demosan 65 WP), and DMOC (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide, UniRoyal's Vitavax 75 WP) as soybean seed dressings for control of pre- and postemergence damping off of soybean seedlings are reported.

The methods used were similar to those of Allam et al. (1). An isolate of *Rhizoctonia solani* Kuehn from diseased soybean seedlings was maintained on potato-dextrose agar (PDA) and used throughout this study. Uniform discs (0.6 cm in diam) were cut from margins of culture plates with a sterile No. 2 corkborer and used to inoculate PDA assay plates. Soybean seed was nontreated or treated with the three fungicides at 0.125, 0.25, or 0.5 g/100 g (2, 4, or 8 oz/100 lb.) seed. Seed were germinated in sterile, moist vermiculite (Terralite brand) in closed plastic containers (germinators) at room temp (ca. 25 C).

For the first experiment, approximately 40 seed of Wayne cultivar, nontreated or treated with one of the three fungicides at one of the three rates, were exposed for either 12, 24, 36, 48, or 96 hr in the germinator.

Seeds were taken aseptically from the germinator and prepared for bioassay by removing the seed coat. Four g of seed from each treatment were then rinsed 3 times with sterile distilled water and allowed to air-dry in a positive-pressure transfer hood, or were blotted dry between layers of paper toweling.

Tissue homogenate was prepared by grinding in a sterile mortar and pestle with 10 ml of sterile distilled water, filtering through sterile cheesecloth, and adding the filtrate to a flask containing 50 ml of sterile PDA cooled to 50-55 C. Finally, five drops of 25% lactic acid were added, and all ingredients were thoroughly mixed. Four 9-cm culture plates were poured from each flask with approximately equal amounts of agar.

For the second experiment, approximately 100 g of seed of Amsoy cultivar were treated with benomyl at one of the three rates and incubated for either 12, 24, 36, 48, 60, 72, or 84 hr in the germinator.

Each plate was inoculated with *Rhizoctonia solani* and incubated at room temp, and radial growth was measured.

The experiment was repeated 3 times and statistically analyzed, using a factorial arrangement of treatments (four times × four rates × four periods) in a completely randomized design with four plates/treatment combination. The data from each set of four experiments per fungicide were combined for statistical analysis.

Growth of the test fungus on all plates containing seed extracts was significantly lower (0.01) than control plates, except for that on extracts from seed treated with benomyl at 0.125 g/100 g seed after 12-hr exposure period and DMOC at 0.25 g after 96 hr. Growth on these plates were equal to control plates. This is indirect evidence that the three fungicides were absorbed through the seed coat and entered the seedling tissues (Table 1).

The pattern of fungus inhibition by extracts from treated seed differed among the fungicides used. Increased concn of benomyl on seed, in general, resulted in greater inhibition of *R. solani* in bioassay plates at the four exposure times with the widest differences between rates occurring at 48 hr. When exposure time to the three rates was increased, greater inhibition occurred at 24 than at 12 hr. At 48 hr, however, greater inhibition was noted for the 0.25- and 0.5-g rates, but not for the 0.125-g rate. At 96 hr, less growth inhibition was recorded for all three rates.

When chloroneb concn was increased from 0.125 to 0.25 g/100 g seed, there was greater inhibition of the test fungus in bioassay plates at the four exposure times and at all three rates at 12 hr. At the 0.5-g rate, however, more fungus growth occurred after 24, 48, and 96 hr than at 0.25 g for the same time periods. There was no explanation for these results. Increasing the length of the germination period within each concn resulted in a greater inhibition of *R. solani* in bioassay plates. Inhibition increased from 12 to 96 hr. Chloroneb, in general, reached levels high enough to control *R. solani* earlier when a rate of 0.5 g was used than when 0.125- and 0.25-g rates were used. This was similar to the performance of DMOC on cottonseed (2).

Increasing concn of DMOC on seed and length of germination period, or both, resulted in a greater in-

TABLE 1. Growth in cm of *Rhizoctonia solani* on potato-dextrose agar containing water extracts from soybean seed previously treated with one of three systemic fungicides exposed to favorable germination conditions

Fungicide[a]	g/100 g	Hr of exposure				
		12	24	36	48	96
Benomyl	0.0	9.0	9.0		9.0	9.0
	.125	9.0	6.1[b]		6.7[b]	6.8[b]
	.250	6.7[b]	5.8[b]		5.4[b]	6.1[b]
	.500	7.4[b]	5.3[b]		4.1[b]	5.6[b]
LSD (.01)		0.3	0.8		0.6	0.2
Chloroneb	0.0	9.0	9.0		9.0	9.0
	.125	7.1[b]	6.8[b]		6.0[b]	3.0[b]
	.250	6.7[b]	6.5[b]		5.5[b]	3.5[b]
	.500	6.1[b]	7.1[b]		6.1[b]	3.7[b]
LSD (.01)		0.3	0.2		0.2	0.2
DMOC	0.0		9.0	9.0	9.0	9.0
	.125		3.0[b]	0.6[b]	2.6[b]	8.8[b]
	.250		2.6[b]	0.6[b]	1.0[b]	9.0
	.500		1.9[b]	0.6[b]	1.0[b]	7.2[b]
LSD (.01)			0.8	0.3	0.6	0.2

[a] Benomyl = methyl 1 (butylcarbamoyl)-2-benzimidazolecarbamate. Chloroneb = 1,4-dichloro-2,5-dimethoxybenzene. DMOC = 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide

[b] Reading highly significantly (at the .01 level) below the control.

hibition of *R. solani* on bioassay plates at 24-, 36-, and 48-hr exposure times, but not at 96 hr. Inhibition increased when exposure time was increased from 24 to 36 hr, but not from 36 to 48 hr or from 48 to 96 hr. DMOC reached levels high enough to control *R. solani* earlier when a rate of 0.5 g/100 g seed was used than when 0.125- and 0.25-g rates were used at 24-, 36-, and 48-hr exposure time. No inhibition of the test fungus was noted by extracts from seed exposed for 96 hr at any rate. These results agree, in part, with those of Allam et al. (1), who reported that absorption of DMOC by cottonseed was proportional to the germination period within rates.

Seed samples from the second experiment were stored at −12 C. Samples were separated into cotyledon and hypocotyl tissue and bioassayed separately. Each sample was minced in a Waring Blendor with 10 ml of acetone. The tissue homogenate was filtered through a double layer of cheesecloth, and the filtrate was evaporated to near dryness under vacuum and resuspended in 7 ml of sterile, distilled water before autoclaving. Three ml of this suspension was mixed with 10 ml of molten PDA, poured into a 5-cm diam culture plate, and inoculated with the test fungus. The experiment was repeated 3 times, using a factorial arrangement of treatments (four times × four rates × seven periods) in a completely randomized design with two plates/treatment combination. Radial growth was measured after 48-hr incubation at room temp.

When extracts from cotyledons and hypocotyl tissues of benomyl-treated seed were bioassayed separately, there was little or no activity noted from hypocotyl extracts at any exposure time or at any rate (Table 2). But significantly greater inhibition of fungus growth was noted by extracts from cotyledons of treated seed than from nontreated seed. These results suggest that benomyl, or a compound related to it (4), becomes localized in the cotyledons and may not move systemically into other seedling tissues. Similar results were reported by J. C. White (*personal communication*). L. E. Gray & J. B. Sinclair (*unpublished data*) found a similar phenomenon when soybean seedling

TABLE 2. Growth in cm of *Rhizoctonia solani* on potato-dextrose agar containing acetone extracts from soybean seedlings originating from seed treated with one of three rates of benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate] and exposed to favorable germinating conditions

g/100 g	Hr of exposure						
	12	24	36	48	60	72	84
Hypocotyl tissue							
0.0	4.9	4.7	4.8	4.9	4.9	4.9	5.0
.125	4.8	4.7	4.6	4.7	4.9	4.9	4.9
.250	4.8	4.8	4.7	4.6	4.4	4.4	4.3
.500	5.0	5.0	4.6	4.4	4.2	4.8	4.5
LSD (.01)	ns	ns	ns	ns	ns	ns	ns
Cotyledon tissue							
0.0	4.5	4.5	5.0	5.7	4.8	4.9	4.9
.125	2.2[a]	0.5[a]	0.2[a]	1.7[a]	0.4[a]	0.2[a]	0.8[a]
.250	1.2[a]	0.0[a]	0.9[a]	0.0[a]	0.0[a]	0.3[a]	0.3[a]
.500	0.9[a]	0.1[a]	0.0[a]	0.0[a]	0.0[a]	0.1[a]	0.0[a]
LSD (.01)	1.6	1.3	2.1	0.4	0.7	0.6	0.9

[a] Reading highly significantly (at the .01 level) below the control.

roots were exposed to various systemic fungicides. In their results, benomyl remained active in hypocotyl tissue for a relatively short period of time, and thus offered only limited protection to soybean seedlings. These results may explain, in part, the general lack of success by us and others (3, 5) in increasing soybean stands using systemic fungicides.

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