Uptake and Translocation of Systemic Fungicides by Soybean Seedlings

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ABSTRACT

Five fungicides known to be systemic in plants other than soybean (*Glycine max*): benomyl [methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate, E. I. duPont's Benlate]; chloroneb (1,4-dichloro-2,5-dimethoxyl benzene, E. I. duPont's Demosan); DCMOD (2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin-4,4-dioxide, UniRoyal's Plantvax); and DMOC (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilido, UniRoyal's Vitavax) were fungitoxic to the following soybean pathogens: *Diaporthe phaseolorum* var. *caulivora; D. phaseolorum* var. *sojae; Cercospora kikuchii;* and *Cephalosporium gregatum* in vitro. TBZ [2-(4′-thiazolyl) benzimidazole], was fungitoxic to *D. phaseolorum* var. *sojae*. TBZ was not evaluated against the other three pathogens. Some fungitoxic component of benomyl, chloroneb, and TBZ moved systemically in soybean seedlings after seedling roots were exposed to the chemicals. It was not ascertained whether the specific fungicide or a compound related to it accounted for fungitoxicity in treated seedling tissues. DCMOD and DMOC were phytotoxic at the levels tested, and could not be bioassayed for systemic activity.

Benomyl and TBZ tended to accumulate in the cotyledons of treated seedlings, but not in the hypocotyl tissues. This may account, in part, for the general lack of success in using these fungicides as seed and soil treatments for disease control in soybean seedlings. Phytopathology 60:1486-1488.

Additional key words: Benlate, Demosan, Vitavax, Plantvax, Mertect, soybean pathogens.

A number of fungicides move systemically in cotton (1, 2, 3, 5), cucumber (4), wheat (9), and bean (7, 8). In some cases, systemic activity against certain plant pathogens was demonstrated (2, 3, 4, 9). This paper reports (i) in vitro activity of five systemic fungicides on four soybean pathogens; and (ii) root uptake and translocation of three systemic fungicides in soybean seedlings.

MATERIALS AND METHODS.—Soybean (*Glycine max* L. Merr.) 'Amsoy' was used throughout this study. Commercial formulations of systemic fungicides were used: benomyl [methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate, E. I. duPont's Benlate, 50 WP]; chloroneb (1,4-dichloro-2,5-dimethoxyl benzene, E. I. duPont's Demosan, 65 WP); DCMOD (2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin-4,4-dioxide, UniRoyal's Plantvax, 75 WP); DMOC (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilido, UniRoyal's Vitavax, 75 WP); and TBZ [2-(4′-thiazolyl)-benzimidazole]; and Merck & Co.'s Mertect, 60 WP). All rates are given in ppm of active ingredient.

In vitro and bioassay studies.—The techniques used for determining in vitro activity of the five fungicides against four soybean pathogens and bioassay studies were similar to those described by Sinclair and coworkers (1, 2, 3, 5, 8). The fungicides were incorporated separately into Difco potato-dextrose agar (PDA-D) at 1 and 10 ppm for benomyl and chloroneb, at 0.2 and 1 ppm for TBZ, and at 25 and 100 ppm for DCMOD and DMOC. Agar free of fungicide served as a control. An acetone extraction of soybean tissue from fungicide-treated plants was used for bioassay studies. Plant tissues were cut into small pieces and ground with 20 ml acetone in a Waring Blendor. The mixture was filtered through four layers of cheesecloth, and the volume reduced in a flash evaporator. Distilled water (7 ml) was then added and the extract autoclaved to remove the remainder of the acetone and prevent contamination. There was no apparent change in fungitoxicity of benomyl, chloroneb, or TBZ during autoclaving. Three ml of extract were added to 9 ml of freshly prepared potato-dextrose agar (PDA). Plates in both studies were inoculated with 6-mm discs of PDA or PDA-D containing *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *sojae* (Lehman) Wehm. There were two replications of each treatment. For each replicate, the mean of the two readings of radial growth of fungus colonies was recorded in mm at 4 days after seeding. Incubation was at room temp (24 to 26 C).

Root-uptake studies.—Bioassay techniques were used to determine the qualitative uptake of certain fungicides by soybean roots and the movement into aboveground parts of seedlings. Soybeans, germinated for 5 to 7 days in vermiculite (Terralite brand), were transplanted either into soil for greenhouse experiments or into vermiculite for laboratory experiments. Various concn of the fungicides were mixed with the growth media. In each case, growth media without fungicide served as a control.

RESULTS.—*Diaporthe phaseolorum* var. *sojae* was selected for the bioassay organism after it was determined that growth was inversely related to ratio of the five fungicides in comparative tests with the

three soybean pathogens: *D. phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora,* Athow & Caldwell; *Cercospora kikuchii* T. Matsu & Tomoyasu; and *Cephalosporium gregatum* Allington & Chamberlain. Benomyl was most active showing complete inhibition of the growth of the four fungi at 1 ppm. Chloroneb, DCMOD, and DMOC significantly reduced growth of all fungi at 10, 25, and 25 ppm, respectively. The in vitro growth of the test organism varied according to concn of fungicide. Fungus growth was completely inhibited by benomyl at 1 ppm, chloroneb at 4 ppm, TBZ below 1 ppm, DCMOD at 25 ppm, and DMOC at 10 ppm.

Soil incorporation of fungicides.—Three experiments were made to determine the influence of fungicide rate and exposure time on root uptake. Bioassays were made on whole seedlings, except roots, from four replicates at 1, 2, 3, and 4 weeks after transplanting using the following fungicide rates: benomyl and chloroneb at 500 and 1,000 ppm; DCMOD and DMOC at 100 and 500 ppm. Extracts from seedlings grown in soil containing benomyl at both rates completely inhibited growth of the test fungus at 4 weeks, whereas extracts from seedlings grown in soil with either chloroneb, DCMOD, or DMOC showed no inhibition. Phytotoxicity to seedling roots by DCMOD and DMOC probably limited root uptake; therefore, activity of the compounds could not be detected in bioassay plates.

In a succeeding experiment, benomyl at 50, 100, 200, and 500 ppm; chloroneb at 100, 1,000, and 5,000 ppm; and TBZ at 100 and 500 ppm were used as soil treatments with bioassays at 2, 3, 4, 5, 6, and 7 weeks. Uptake of fungicide appeared to be influenced more by rate than by time (Table 1). The pattern of results of bioassays at 2 and 3 weeks after transplanting was similar to that at 7 weeks. Extracts from plants exposed to 500 ppm benomyl showed almost complete inhibition of fungus growth at all assay dates. Extracts from plants treated with 200 ppm benomyl almost completely inhibited fungus growth beginning at 2 weeks, while significant inhibition by extracts from plants exposed to 100 ppm did not occur until 7 weeks. Extracts from plants treated with chloroneb at 100 and 1,000 ppm highly significantly inhibited fungus growth only at 7 weeks, but not those at 5,000 ppm. The higher concn of TBZ (500 ppm) produced highly significant inhibition of fungus growth at all dates except at 5 weeks, while at 100 ppm, complete inhibition was noticed only at 6 weeks.

Rates of benomyl, chloroneb, and TBZ were reduced to 100 and 200 ppm for the third experiment. Whole plants were bioassayed at 1, 2, 3, and 4 weeks, while hypocotyls and leaves were bioassayed separately at 5 weeks to determine fungicide distribution in treated seedlings. Extracts from whole seedlings or hypocotyls and leaves of plants grown in benomyl-treated soil at both rates completely inhibited fungus growth up to 5 weeks. No significant inhibition was noted by extracts from chloroneb-treated seedlings. Highly significant or complete inhibition was noted by extracts from 200 ppm TBZ-treated seedlings up to 4 weeks and by extracts from leaves at 5 weeks. At 100 ppm, however, highly significant inhibition was noted at 5 weeks from leaf extracts.

Phytotoxicity studies.—Phytotoxicity symptoms were observed in all greenhouse experiments. DCMOD and DMOC incorporated in soil at 100 ppm were phytotoxic to the extent that plants were killed within 1 to 2 weeks of continuous exposure. Chlorosis of the primary leaf margins spread inward on the blade, and was followed by necrotic spotting. Benomyl and chloroneb produced a slight marginal chlorosis on primary leaves when plants were exposed to rates of 500 and 1,000 ppm. At 1,000 ppm, the primary leaves of chloroneb-treated seedlings showed some necrotic spotting and leaf dwarfing. Seedlings exposed to TBZ at 500 and 1,000 ppm had white, interveinal discoloration spreading from the margins toward the midrib of primary leaves.

Laboratory studies.—Germinated seed were transplanted into plastic cups containing approximately equal amounts of vermiculite and fungicide mixture or distilled water (control) using three or four replicates/treatment. All experiments were carried out in an ISCO environmental chamber at 25 C, 62% rela-

TABLE 1. In vitro radial growth in mm of *Diaporthe phaseolorum* var. *sojae* on potato-dextrose agar (PDA) without and with extracts from soybean seedlings nontreated or treated with one of three fungicides at the rates and time of exposure indicated

Fungicide[a]	Rate ppm soil incorporation	Weeks after treatment[b]					
		2	3	4	5	6	7
Benomyl	50	27	24	50	28	20	21
	100	27	27	50	23	25	11**
	200	24	9**	6**	9**	6**	6**
	500	8**	10**	7**	15**	6**	6**
Chloroneb	100	30	26	50	29	32	17**
	1,000	29	27	49	28	21	16**
	5,000	29	22	50	25	28	22
Thiabendazole	100	25	26	50	23	6**	19
	500	8**	12**	7**	29	6**	6**
Control 1		24	24	50	28	30	22
Control 2		29	33	50	30	26	28

[a] Control 1, PDA with extracts from nontreated seedlings. Control 2, PDA without plant extracts.
[b] ** = Treatment means significantly different from control at the 1% level.

tive humidity, and approximately 5,000 ft-c. Roots, hypocotyls, leaves, and/or cotyledons of seedlings were bioassayed separately.

Extracts from roots, hypocotyls, and leaves of seedlings bioassayed separately at 4 days' exposure to benomyl or TBZ at 100 ppm completely inhibited fungus growth in bioassay plates. Root, hypocotyl, and leaf extracts from nontreated plants showed mean growth readings of 31, 32, and 31 mm, respectively. After 1 and 2 weeks' exposure to 100 and 200 ppm of each fungicide, extracts from hypocotyls, cotyledons, and leaves completely inhibited fungal growth. At 3 weeks, extracts from hypocotyl and leaves completely inhibited fungal growth. Cotyledons dropped from plants between 2 and 3 weeks. Extracts from plants treated with TBZ at the same rates significantly inhibited fungus growth below controls at 1 and 2 weeks. There was less inhibition of growth from leaf extracts (24 mm) at 2 weeks from plants treated with 100 ppm TBZ than from hypocotyl and cotyledons. Complete inhibition was noted by extracts from these tissues. These results suggested that fungicides might accumulate in the cotyledons. A series of experiments were conducted to test this suggestion.

Extracts from seedlings exposed continuously to 100 ppm benomyl or TBZ completely inhibited growth of the test fungus whether hypocotyl, leaf, or cotyledons were assayed. Bioassays made at 5 or 7 days after a 24-hr exposure to either fungicide showed that fungicidal activity remained high in extracts from cotyledons, but not in extracts from hypocotyl or leaf tissues (Table 2).

TABLE 2. In vitro radial growth in mm of *Diaporthe phaseolorum* var. *sojae* on potato-dextrose agar without and with extracts from hypocotyl (H), leaves (L), or cotyledons (C) of soybean seedlings nontreated or treated by exposing roots to 100 ppm benomyl and TBZ

Fungicide[a]	Tissue[b]	Exposure period to fungicides with no. of experiments in parentheses: Continuous days after 24-hr exposure		
		7 days (*4*)	*5 days* (*3*)	*7 days* (*1*)
Benomyl	H	6	25	25
	L	6	35	17
	C	6	11	9
Control 1	H	25	31	
	L	30	32	29
	C	36	32	
Control 2		37	36	29
		7 days (*3*)	*5 days* (*3*)	*7 days* (*1*)
Thiabendazole H	H	6	24	26
	L	6	33	
	C	6	15	6
Control 1	H	29	30	40
	L	29	28	42
	C	34	28	41
Control 2		39	35	45

[a] Control 1 = PDA with extracts from nontreated seedlings; Control 2 = PDA without plant extracts.

[b] Plants were exposed for 7 days to the fungicide, or for 2 days, then transplanted to nonfungicide medium for 5 or 7 days.

DISCUSSION.—Root uptake studies demonstrated that benomyl, chloroneb, and TBZ (or some fungitoxic compounds related to them) are taken up by the soybean seedling roots and translocated to hypocotyls, cotyledons, and leaves. A fungitoxic material was shown to be active in seedlings whose roots were continuously exposed to benomyl and TBZ for as long as 4 weeks. Benomyl is known to decompose either in bean plants or in aqueous solution within 5 days (7).

Results from bioassays showed that benomyl and TBZ tended to accumulate in cotyledons after root uptake. Other evidence cited by Thapliyal & Sinclair (8) shows that systemic fungicides also tend to accumulate in soybean cotyledons when used as seed treatments (J. C. White, *unpublished data*). These results suggest that the compounds remain active for only a relatively short period in the hypocotyl without continual root exposure, and thus offer only limited protection to soybean seedlings against attack of damping-off organisms. This might explain, in part, the general lack of significant differences in stand from experimental field plots in Illinois using systemic fungicides either as seed and/or soil treatments.

Testing for systemic activity of these compounds against specific soybean pathogens on seedlings was not entirely successful, possibly because of phytoalexin production (6) and because at least two of these fungicides tend to accumulate in the cotyledons after root uptake.

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