Efficacy of Polyethyleneglycol and Organic Solvents for Infusing Fungicides into Soybean Seeds

B. J. Shortt and J. B. Sinclair

Graduate research fellow and professor, respectively, Department of Plant Pathology, University of Illinois, Urbana 61801.
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ABSTRACT

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Soybean (cultivar Bonus) seeds treated with one of six contact or six systemic fungicides in acetone, dichloromethane (DCM), or aqueous polyethyleneglycol (PEG) were assayed for fungicide activity. Activity was detected in seeds treated with the systemic fungicides benomyl, carboxin, sisthane, and thiabendazole. Solubility of the fungicides in the solvents was not related to uptake by the seeds. Benomyl, sisthane, and thiabendazole were detected in seeds treated with acetone, DCM, or PEG, however, carboxin was detected only in seeds treated with PEG. Larger inhibition zones and a greater reduction of seedborne *Phomopsis* sp. occurred when benomyl and sisthane were infused with PEG than with either acetone or DCM. No treatment was 100 percent effective. Acetone and, to a greater extent, DCM killed *Phomopsis* sp. borne within the seedcoat, however, they damaged exposed cotyledonary tissues. Increased cotyledonary damage was associated with increased recovery of *Bacillus subtilis*. The efficacy of any treatment depended on the fungicide and solvent-carrier combination.

Additional key words: Glycine max, pod and stem blight, *Diaporthe phaseolorum* var. *sojae.*

The infusion of fungicides into dormant seeds is a useful and efficient means of plant disease control. Acetone and dichloromethane (DCM) have been used to infuse systemic fungicides into seeds of a variety of crops including soybeans (*Glycine max* [L.] Merr.) (1,5,9–11,13,15). The infusion of systemic fungicides into soybean seeds with acetone has been shown to control damping-off caused by *Phytophthora megasperma* var. *sojae* (9). Ellis et al (1) reported that the infusion of benomyl into soybean seeds with DCM reduced the incidence of seedborne *Phomopsis* sp. Soybean seed decay caused by *Phomopsis* sp. is a major problem in Illinois (2).

Research on the use of water as a solvent has been limited. Seedborne fungal pathogens of several vegetables have been controlled by soaking seeds in water suspensions of thiram (7,8), however, the fungicide was not incorporated into seeds. Adding polyethyleneglycol to water (PEG) reduces the osmotic pressure of the solution and allows many types of seeds to be soaked for extended periods without damage. Seeds soaked in PEG do not fully imbibe water and do not germinate. Hepperly and Sinclair (4) immersed soybean seeds in PEG and found that germination and seedling vigor were not adversely affected and that water-soluble antibiotics, such as K penicillin G and streptomycin sulfate, could be incorporated into seeds during immersion.

We report the results of experiments comparing the efficacy of acetone, DCM and aqueous PEG for infusing six contact and six systemic fungicides into soybean seeds for the control of seedborne *Phomopsis* sp.

MATERIALS AND METHODS

Uptake of fungicides by seeds in three solvents. Soybean (cultivar Bonus) seeds were soaked for 24 hr at 25±3 C in acetone dichloromethane (DCM), or polyethyleneglycol 6000 (Carbowax 6000, Union Carbide Corp., New York, NY 10017) in water (29%, v/v) (PEG) containing one of the following fungicides: benomyl ([1-butylcarbamoyl-2-benzimidazole carbamate] Benlate 50W, E. I. duPont de Nemours & Co., Wilmington, DE 19898); ([*cis*-*N*-trichloromethylthio-4-cyclohexene-1, 2-dicarboximide] Orthocide 80W, Chevron Chem. Co. San Francisco, CA 94104); carboxin ([5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide] Vitavax HBM25, Union Carbide Corp.); ethazole ([5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole], Terrazole 35W, Olin Corp., New York, NY 10022); fentin hydroxide ([triphenyltin hydroxide], Du-ter, Thompson-Hayward Chemical Co. Kansas City, KS 66110); iprodione ([3-3,5-dichlorophenyl-*N*-1-methyl-2, 4-dioxo-1-imidazoleamine carboxamide), PR 26019W, Rhodia Inc., Monmouth Junction, NJ 08903); maneb-zinc [(zinc ion and manganese ethylenebisdithiocarbamate], Manzate 200 80W, E. I. duPont de Nemours & Co]; PCNB ([pentachloronitrobenzene), Terraclor 75W, Olin Corp.]; sisthane ([α-butyl-α-phenyl-1-*H*-imidazole-1-propanenitrile), RH 2161EC Rohm and Haas Co. Philadelphia, PA 19105]; thiabendazole ([2,4-thiadiazolyl-benzimidazole), Mertect 160 60W, Merck Chem. Div., Rahway, NJ 07065); thiram ([bis(dimethylthiocarbamoyl)disulfide), Arasan 50 Red, E. I. duPont de Nemours and Co.; or vinclozolin ([3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione], BASF Wyandotte Corp., Wyandotte, MI 48192). All treatments contained 1,000 μg active ingredient of fungicide per milliliter of solvent. Aqueous PEG treatment mixtures were slowly aerated with a pressurized air system. All seeds were rinsed twice for 1 min in the same solvent-carrier used to treat them to remove any surface-carried fungicide and then air dried at 25±3 C. Nontreated seeds and seeds soaked in the solvents alone served as controls.

Potato-dextrose agar (Difco Laboratories, Inc., Detroit, MI 48232) (PDA) was seeded with either *Phomopsis* sp., causal fungus of pod and stem blight and seed decay (8) or *Rhizoctonia solani* Keuhn. Mycelial cultures of each fungus were grown in Luria broth (6) for 21 days at 25 ± 3 C.

Inoculum was prepared by macerating mats of mycelium of each fungus in a Waring blender with sterile distilled water for 1 min and standardizing the concentration of the suspension with a spectrophotometer at 450 nm. The inoculum concentration was 28 mg of mycelium per milliliter; propagule counts were not taken because of lack of homogeneity in propagule size. Seventy milliliters of mycelial suspension of each fungus then was added separately to 450 ml of warm (50 C) liquid PDA and mixed. Seven milliliters of the seeded PDA was pipetted into sterile, 9-cm-diameter culture plates and stored at 5 C until used.

Treated and nontreated seeds were halved axially and placed cut-side down one seed-half per plate on seeded PDA plates. Plates

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were incubated in the dark for 1 day at 5 C to allow for the maximum diffusion of fungicide and then for 3 days at 25 C to allow the test fungus to grow.

Solubility data was supplied by the manufacturers of the fungicides.

Efficacy of selected treatment combinations. Soybean (cultivar Wells) seeds were soaked for 24 hr in acetone, DCM, or PEG containing either benomyl, sisthane (each a systemic fungicide), or thiram (a topical fungicide). All treatments contained 1,000 μg active ingredient of fungicide per milliliter of solvent. Seeds that received PEG treatments were aerated as before.

All seeds were rinsed twice for 1 min in the same solvent-carrier used to treat them to remove surface-carried fungicide and then air dried at 25 ± 3 C. Nontreated seeds and seeds soaked in the solvents alone served as controls. Treated and nontreated seeds were surface sterilized in 0.5% NaOCl (10% Clorox, Clorox Co., Oakland, CA 94621) for 4 min, rinsed twice in sterile distilled water, and placed four seeds per plate on PDA in 9-cm diameter culture plates. Culture plates were incubated in the dark for 7 days at 25 C and the percent germination and occurrence of *Phomopsis* sp. and bacteria were recorded.

Seed samples also were placed on water-saturated blotter pads ([Kimpac] Graham Paper Co., St. Louis, MO 63178) in an incubator programmed for 100% RH, constant light (800 μEin/m^2/sec), and 25 C. After 6 days, the percent germinated seeds and vigorous seedlings were recorded. A seed was considered germinated if the radical was 2.5 times the length of the cotyledon and a seedling was considered vigorous if the cotyledons were lifted 1 cm or more above the surface of the blotter pad.

Damage to seeds by each treatment was determined by soaking imbibed seeds in 1% aqueous tetrazolium red ([2-3,5-triphenyl tetrazolium chloride] Sigma Chemical Co., St. Louis, MO 63178) for 11 hr at 5 C. Seeds were observed for differential staining of live and dead tissues (3).

TABLE 1. Effects of solvent–fungicide seed treatment combinations on zones of inhibition around Bonus soybean seeds incubated on potato-dextrose agar seeded with either *Phomopsis* sp. or *Rhizoctonia solani*

	Phomopsis sp.			*R. solani*
Fungicide	Acetone	DCM[b]	PEG[c]	PEG
Benomyl	12.3[a]	15.0	29.8	0.0
Carboxin	0.0	0.0	0.0	51.3
Sisthane	10.0	10.3	48.0	0.0
Thiabendazole	34.3	0.0	32.3	15.5
Thiram	0.0	0.0	5.3[d]	0.0

FLSD ($P = 0.05$) = 5.39

[a] Mean inhibition zone diameters (mm) based on four replications of one seed per treatment combination.
[b] Dichloromethane.
[c] Polyethyleneglycol.
[d] Inconsistent occurrence of zones.

TABLE 2. Effects of solvent–fungicide seed treatment combinations on percentage germinated seeds and recovery of *Phomopsis* sp. and bacteria from soybean seeds incubated on potato-dextrose agar

			Occurrence of:[a]	
Solvent	Fungicide	Germination[a] (%)	*Phomopsis* sp. (% of seeds)	Bacteria (% of seeds)
Acetone	Benomyl	87.0	3.5	48.0
	sisthane	83.8	15.8	18.8
	thiram	75.3	22.0	52.3
	none	78.0	27.8	26.5
DCM[b]	benomyl	86.5	0.5	41.0
	sisthane	81.3	10.5	44.5
	thiram	83.8	14.5	31.0
	none	81.3	16.5	49.8
PEG[c]	benomyl	89.0	1.3	26.0
	sisthane	91.0	0.3	15.5
	thiram	81.3	26.3	21.8
	none	69.3	35.3	18.0
	Nontreated	71.0	43.8	29.8
FLSD ($P = 0.05$)		5.58	4.43	9.82

[a] Mean percentages of five replicates of 80 seeds per replicate.
[b] Dichloromethane.
[c] Polyethyleneglycol 6,000.

RESULTS AND DISCUSSION

Phomopsis sp. was inhibited by all the fungicides tested at 1,000 μg/ml active ingredient using a paper disk assay (12). Only the systemic fungicides were effectively incorporated into soybean seeds with the solvent-carrier tested. Seeds treated with benomyl, sisthane, and thiabendazole produced zones of inhibition on PDA plates seeded with *Phomopsis* sp. (Table 1). This is the first report of fungicides being infused into soybean seeds with PEG. Zones of inhibition were not formed around seeds treated with captan, ethazole, fentin hydroxide, iprodione, maneb-zinc, PCNB, or vinclozolin regardless of the solvent-carrier used. Seeds treated with thiram in PEG had zones of inhibition around half of the seeds tested.

The solvent-carriers tested were not equally effective in incorporating fungicides into soybean seeds. Seeds soaked in PEG with benomyl or sisthane produced larger inhibition zones than when acetone or DCM were the solvent-carrier. Carboxin was detected in seeds infused with PEG but not acetone or DCM. Conversely, thiabendazole was infused equally well in acetone or PEG but was not incorporated at effective levels in DCM. However, the incorporation of thiabendazole infused into seeds with DCM was detectable if *Penicillium expansum* was the test fungus (1). The absence of zones of inhibition may be due to either the lack of fungicide incorporation into the seeds or incorporation at levels insufficient to inhibit the test fungus. No inhibition zones formed around nontreated seeds or seeds soaked in the carrier solvents alone.

In general, the solubility of each fungicide in each solvent-carrier was not related to the size of inhibition zones. Although benomyl and sisthane are relatively insoluble in water they were effectively incorporated into seeds in aqueous PEG.

Based on these results the systemic fungicides, benomyl and sisthane, and the contact fungicide, thiram, were selected for

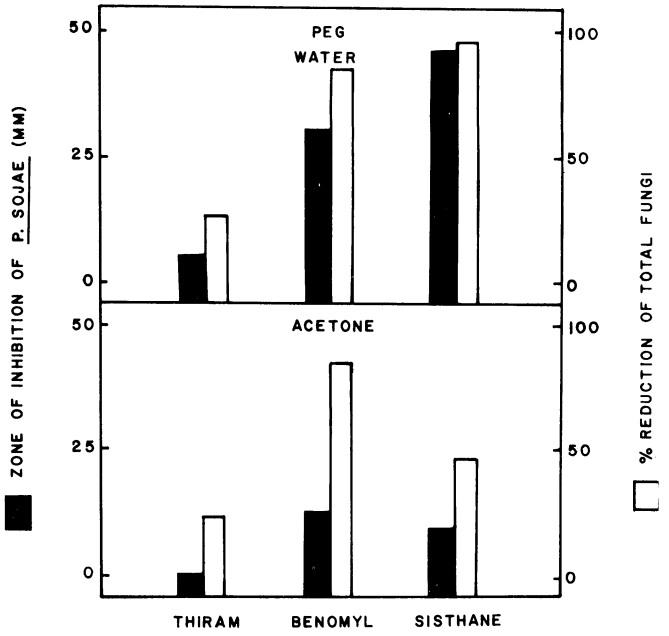

Fig. 1. Comparison of the zones of inhibition of *Phomopsis* sp. around soybean seeds (cultivar Bonus) treated with a fungicide in either aqueous polyethyleneglycol 6,000 (PEG) or acetone and the percentage reduction of total fungi recovered from treated seeds (cultivar Wells).

studies comparing the efficacy of acetone, DCM, and PEG as solvent-carriers for the infusion of fungicides into soybean seeds. The germination data from blotter pads for all treatments were similar to that recorded on PDA and therefore, only the data from the PDA test will be presented and discussed. Data from the vigor rating study were inconclusive.

The fungi isolated included *Phomopsis* sp., *Alternaria* sp., *Cercospora kikuchii* (T. Matsu & Tomayasu) Chupp., *Macrophomina phaseolina* (Tassi) Goid., and *Cladosporium* sp. Ninety percent of all fungal isolates were *Phomopsis* sp. There was a negative correlation between seed germination and the recovery of *Phomopsis* sp. on PDA (Table 2). Benomyl and sisthane were highly effective in reducing seedborne *Phomopsis* sp. and thus controlling Phomopsis seed decay. Benomyl effectively reduced seedborne *Phomopsis* sp. regardless of the carrier solvent used. Sisthane was more effective when infused in PEG than when either acetone or DCM was used. Reduction of *Phomopsis* sp. by thiram in acetone or DCM was no greater than that by acetone or DCM alone; thiram in PEG however, was more effective than was PEG alone. Thus, the efficacy of the fungicides depended in part on the solvent-carrier used. In general, fungicides applied in PEG most effectively reduced seedborne *Phomopsis* sp.

Fungicide infusion in PEG resulted in a closer correlation between the size of inhibition zones and the percent reduction of seedborne fungi than when acetone was used (Fig. 1). Although less benomyl was infused in acetone, the effect was comparable to the benomyl in PEG treatment, indicating that the toxicity of acetone, and probably DCM, may augment the effect of the fungicides.

Acetone and (to a greater extent) DCM alone provided some *Phomopsis* sp. control which resulted in a higher seed germination. Both solvents are toxic to living tissues and probably act against *Phomopsis* sp. within the seed coats of infected seeds. Germination of seeds immersed in PEG was not significantly different from that of nontreated seeds, but the incidence of *Phomopsis* sp. was reduced.

A Gram-positive, spore-forming bacterium resembling that described by Tenne et al (14) as *Bacillus subtilis* Cohn frequently was observed on PDA around treated and nontreated seeds. Treating seeds with PEG reduced the recovery of this bacterium below that of nontreated seeds, but treating seeds in DCM increased its recovery (Table 2). Each fungicide had varying effects on the recovery of the bacterium. Incidence of the bacterium was reduced by sisthane infused with acetone or PEG, but not with DCM. Benomyl and thiram in acetone increased the recovery of the bacterium, possibly by enhancing the phytotoxicity of acetone.

Results from the tetrazolium test indicated that DCM and (to a lesser extent) acetone killed cotyledonary tissues exposed in seeds with cracked seed coats. These solvent-carriers also appeared to penetrate the seeds through fungal lesions in the seed coat and subsequently increased the level of damage associated with those lesions. Damage associated with acetone or DCM treatment was responsible in part for the saprophytic growth and the increased recovery of the bacterium from seeds that had been immersed in these solvents. The activity of seedborne bacteria in solvent-damaged seeds reduced germination and vigor of seedlings under conditions favorable to the bacterium. Heat stress of soybean seeds also has been shown to increase the occurrence of *B. subtilis* in soybean seeds (14). Damage of this type contributes to poor seed viability by providing locations for the establishment and growth of microorganisms present in the soil.

The role of solvents in the infusion of soybean seeds with fungicides is more complicated than previously reported. The close association of fungicides, solvents, and the seed allows for a variety of interactions. We showed that while soaking seeds in solutions of a nonsystemic fungicide (such as thiram) slightly reduced the incidence of seedborne fungi, this does not usually result in the infusion of the fungicide. Systemic fungicides, however, are infused easily into seeds and thus, are more effective for longer periods of time. The ability to diffuse across the inner layers of the seedcoat and the cellular membranes is probably responsible for their uptake by seeds. Field studies (9,11,13) have shown that systemic fungicides often are more effective when incorporated than when surface applied. In addition, much less fungicide is used and exposure and loss of fungicide into the environment is less when it is infused into seeds.

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