Effect of Three Fungicides on Internally Seed-Borne Fungi and Germination of Soybean Seeds

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Supported in part by the Illinois Agricultural Experiment Station and the S-72 Regional Research Project.
Accepted for publication 4 December 1974.

ABSTRACT

Captan, thiram, and benomyl at .016, .033, and .033 g active ingredient/20 g seed, respectively, were used to treat three lots of soybean (*Glycine max*) seeds with high levels of internally seed-borne fungi and low percentage germination. Seeds treated with fungicides had a higher germination in vitro, and emergence in vermiculite and field soil than nontreated controls. The internally seed-borne fungi were primarily located in seed coat (testa) tissues and only occasionally were found in embryo tissues. Captan and thiram moved into seed coat tissues, but did not penetrate the embryo, therefore, were effective only against fungi in the seed coat. Benomyl penetrated the seed coat and embryo and was effective against fungi in seed coat and embryo, but was phytotoxic.

Phytopathology 65:553-556

Additonal key words: Diaporthe phaseolorum var. *sojae, Pencillium expansum,* systemic fungicides.

Several seed-borne fungi have been associated with reduced emergence of soybean seeds, with *Diaporthe phaseolorum* var. *sojae* being one of the most important (13, 17). Fungicide seed treatment of soybean can reduce the quantity of seed-borne fungi (7, 11, 16) and increase emergence of low quality seeds (germination below 70% and high incidence of seed-borne organisms) (4, 9, 10), but has little or no effect on high quality seeds (germination above 70% and low incidence of seed-borne organisms) (3, 6, 10). Captan and thiram, nonsystemic seed protectants, and benomyl, a systemic fungicide, were used to determine the extent to which these compounds enter soybean seeds and affect internally-borne fungi.

MATERIALS AND METHODS.—Three soybean [*Glycine max* (L.) Merr.] seed lots were selected from seeds produced in 1972 (2). Lot number, cultivar, state where grown, germination in vitro (at 25 C) and internally seed-borne fungi, respectively, were: lot #16 = 'Bragg', Louisiana, 70, 22; lot #18 = 'Dare', Louisiana, 57, 55; and lot #22 = 'Dare', South Carolina, 41, 37. All seeds used for in vitro studies were surface sterilized in 0.25% sodium hypochlorite solution for 4 minutes, followed by a 70% ethanol solution for 2 minutes, and rinsed in sterile distilled water (12). Seeds planted in vermiculite

(Terralite) and in field soil were not surface sterilized. All experiments were done three times at 25 C unless otherwise indicated.

Seeds from each lot were treated with a slurry of recommended amounts of either captan{(*N*-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide, Stauffer's Captan 80 WP),} thiram [bis(dimethylthiocarbamoyl) disulfide, du Pont's Thylate 65% WP] or benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, duPont's Benlate 50 WP] at .016, .033, or .033 g active ingredient/20 g seeds, respectively. One-hundred seeds per treatment per lot were plated (four seeds per each of 25 plates) on Difco potato-dextrose agar (PDA), or planted in vermiculite or field soil. Nontreated seeds served as controls. In vitro germination and percent incidence of fungi were recorded after 5 days. Seedling emergence was recorded after 15 days in the greenhouse.

To determine whether the seed-borne fungi in soybean were located in either the seed coat or embryo, 300 seeds from lots #18 and #22 were surface sterilized as previously described. They were then soaked for 1 hour in sterile distilled water to loosen the seed coat from the embryo. One-hundred seed each were treated in one of three ways: (i) plated directly on PDA culture plates; (ii) seed coats were aseptically separated from the embryo (remainder of the seed) and each plated separately on PDA culture plates; and (iii) the same as (ii), except the embryos were surface sterilized as previously described before plating on PDA culture plates. The total number of fungal colonies growing from each sample was recorded after 5 days.

To study the absorption of fungicides by soybean seeds, 30 seeds of lot #22 were treated with either captan, thiram or benomyl as previously described. Treated and nontreated seeds were placed on moist Kimpac cellulose pads (Graham Paper Co., St. Louis, Missouri). After 24 hours, 10 seeds from each treatment were: (i) plated separately on PDA culture plates seeded with a spore suspension of *Penicillium expansum* Link; (ii) seed coats were aseptically separated from the embryo and each plated separately on *P. expansum*-seeded PDA culture plates; or (iii) the same as (ii), except that the embryos were washed for 2 minutes in running tap water before being plated on *P. expansum*-seeded PDA culture plates. Nontreated seeds and seed coats and embryos from nontreated seeds served as controls. Zones on inhibition of *P. expansum* were measured at the widest points from the plated tissues after 48 hours.

To determine if the test fungicides were absorbed by the seed coats, 20 seeds each were either left nontreated or were treated with one of the test fungicides and placed on moist Kimpac cellulose pads in 15-cm culture plates. After 24 hours, seeds were aseptically cut in half and the seed coats removed. The cup-shaped detached seed coats were placed on PDA culture plates so that the inside of the "cup" faced upward. Agar plugs (5 mm in diameter) were cut, using a sterile cork borer, from either 7-day-old PDA cultures of *Diaporthe phaseolorum* (Cke. & Ell.) var. *sojae* (Lehman) or from 24-hour-old cultures of *P. expansum*. The plugs were then cut with a sterile scalpel into quarters, one of which was placed on the inner surface of a seed coat "cup". There were 10 seed coat "cups" with each fungus for each fungicide. Observations were made after 48 hours for the extent of mycelial growth on the agar plugs and seed coats.

TABLE 1. The average percent incidence of fungi by genera recovered from either nontreated (control) or fungicide-treated soybean (*Glycine max*) seeds from three seed lots

	Percentage fungi by genera[b]								
Treatment[a]	*Alt.*	*Asp.*	*Cer.*	*Clad.*	*Diap.*	*Fus.*	*Pen.*	*Rhiz.*	Total
Seed lot 16									
Captan	0.3	0.6	0	0	1	1.0	0	0	2.9
Thiram	0	6.6	0	0	0	1.6	0	0	8.2
Benomyl	0.6	0	0	0	0	0.3	0	0.3	1.2
Control	1.6	0	1	0	4	7.6	0	0	14.2
LSD (P=0.05)									5.5
LSD (P=0.01)									2.1
Seed lot 18									
Captan	1.3	0	0	0	0.6	1.3	0	0	3.2
Thiram	0	11.6	0	0	2.3	0.3	0.3	0	14.5
Benomyl	1.3	0	0	0	0	0	0	2.6	3.9
Control	2.6	19	0	0.3	3.6	2.6	0	0	28.1
LSD (P=0.05)									9.4
LSD (P=0.01)									3.6
Seed lot 22									
Captan	1.6	0	0	0	5	3	0.3	0	9.9
Thiram	1.0	1.6	0.6	0	6	8	0	0.6	17.8
Benomyl	4.6	0.3	0.3	0	0	0.6	0	0	5.8
Control	6.0	0	1.0	0.3	23.3	8.3	0	1.3	40.2
LSD (P=0.05)									2.8
LSD (P=0.01)									7.4

[a]Compound and rate in grams active ingredient per 2 grams of seeds: captan, .016; thiram, .033; and benomyl, .033.

[b]Based on 300 seeds per treatment. *Alt.* = *Alternaria, Asp.* = *Aspergillus, Cer.* = *Cercospora kikuchii, Clad.* = *Cladosporium, Diap.* = *Diaporthe phaseolorum* var. *sojae, Fus.* = *Fusarium, Pen.* = *Penicillium,* and *Rhiz.* = *Rhizopus.*

RESULTS.—Eight genera of fungi were identified from seed of the three seed lots (Table 1). All fungicide treatments reduced the percentage incidence of total detectable fungi in the three seed lots below the controls (Table 1). Frequency of recovery of fungi with captan- and benomyl-treated seeds was less than thiram-treated seeds. There were no significant differences between benomyl- and captan-treated seeds.

All fungicide-treated seeds had a higher percent germination than the controls (Table 2). There were no significant differences in germination between fungicide treatments for lot #18. In lot #16, the germination of captan-treated seeds was higher than those treated with thiram or benomyl. In lot #22, the germination of thiram-treated seeds was higher than those treated with captan or benomyl.

Emergence in vermiculite was higher than the controls for seeds treated with either captan or thiram but not benomyl for the three seed lots (Table 2). There was no significant difference in emergence between seeds treated with captan and thiram from lots #16 and #18, but in lot #22, the difference was significant.

Emergence in soil was significantly higher than the controls of seeds treated with either captan or thiram. Captan-treated seeds had a higher emergence than

thiram-treated seeds ($P = 0.01$) in lot #22. There was no significant difference in emergence between nontreated seeds and those treated with benomyl in lot #16. Seeds treated with benomyl in lots #22 and #18 had significantly lower emergence than the controls at $P = 0.05$ and 0.01, respectively.

All fungicide-treated seeds, as well as the seed coats and embryos from fungicide-treated seeds produced zones of inhibition on *P. expansum*-PDA. The mean zones of inhibition in millimeters about seeds, seed coats alone, embryos alone, and washed embryos for each fungicide, respectively, were: benomyl 12.4, 10.1, 9.2, 8.2; captan, 8.4, 7, 3.3, 0; and thiram 4.7, 4.8, 1.9, 0. No zones of inhibition were noted either about embryos or washed embryos from nontreated seeds.

After 48 hours, mycelium of *D. phaseolorum* var. *sojae* and *P. expansum* had completely colonized the excised seed coat "cups" from nontreated seeds. There was no growth on any of the seed coats from fungicide-treated seeds. There was no growth on the agar plugs within the seed coat cups from benomyl-treated seeds, but there was growth on plugs in the seed coats from captan- and thiram-treated seeds.

Location of fungi within seed tissues.—The same genera of fungi with approximately the same percent incidence were noted for lots #18 and #22 as previously described (Table 1). The average total incidence of fungi for lots #18 and #22 was, respectively, for whole seeds, 25 and 41; for seed coats, 25 and 35; for nonsurface-sterilized embryos, 2 and 2; and for surface-sterilized embryos, 3 and 4.

TABLE 2. Mean percent in vitro germination, emergence in vermiculite and field soil, and total fungi isolated on potato-dextrose agar (25 C) from three lots of soybean (*Glycine max*) seeds, either nontreated or treated with one of three fungicides

Treatment[a]	Germination in vitro[b]	Emergence (Mean %)[b]		Average[b] percentage fungi
		Vermiculite	Field soil	
Seed lot 16				
Captan	89	85	82	2.9
Thiram	86	86	66	8.2
Benomyl	82	59	30	1.2
Control	67	55	32	14.2
LSD(P=0.05	3	7	18	2.1
LSD(P=0.01)	9	17	46	5.5
Seed lot 18				
Captan	65	47	35	3.2
Thiram	68	44	37	14.5
Benomyl	70	17	17	3.9
Control	44	15	7	28.1
LSD(P=0.05)	6	3	1	3.6
LSD(P=0.01)	17	8	3	9.4
Seed lot 22				
Captan	67	41	54	9.9
Thiram	71	36	17	17.8
Benomyl	68	16	1	5.8
Control	34	12	5	40.2
LSD(P=0.05)	2	3	2	2.8
LSD(P=0.01)	3	7	5	7.4

[a]Compound and rate in grams active ingredient per 20 seeds: captan, .016; thiram, .033; and benomyl, .033.

[b]Based on combined data from three replications of 100 seeds in three experiments.

DISCUSSION.—Poor germination is one of the criteria of poor quality seeds. One of the factors that can reduce germination and emergence of soybean seeds is seed-borne fungi. The in vitro germination, emergence in vermiculite and field soil of three lots of poor quality seeds were increased with fungicide seed treatment. Similar results have been reported from field studies (3, 4, 10). Captan and thiram are classified as protectant seed treatment fungicides being effective only against soil-borne organisms, or organisms on seed surfaces (15). Our results suggest that these two fungicides and benomyl are also active against seed-borne organisms located within the seed coat of soybeans.

Cercospora kikuchii, Colletotrichum truncatum, and *D. phaseolorum* var. *sojae* were shown to primarily colonize the tissues of soybean seed coats (1, 5, 14). Most of the fungi genera associated with seeds bioassayed in this study were located in the seed coat with a low incidence in embryo tissues. Captan and thiram moved (presumably by diffusion) into the seed coats of treated seeds, but not into the embryo tissues, while benomyl was taken up by both seed coats and embryo tissues. Benomyl was shown to be systemic in germinating soybean seeds and seedlings (8). All fungicides were effective in reducing the quantity of *D. phaseolorum* var. *sojae*. This suggests eradicant activity of the fungicides in the seed coat tissues. None of the fungicides tested controlled all the seed-borne fungi. Thiram is the least effective of the three fungicides against *Aspergillus* spp., and benomyl gave little or no control of *Alternaria* spp. Benomyl was effective in reducing fungi. However, it reduced germination in vermiculite and soil, but not on PDA. We cannot explain these differences. Benomyl may have been phytotoxic to poor quality seeds in our study, and it was shown to be phytotoxic to both good and poor quality seeds under field conditions (10).

LITERATURE CITED

1. DEUTSCHMANN, F. 1953. Uber die "purple stain" krankheit der sojabohne unde die forbstoffbildung ihres erregers Cercosporina kikuchii Mats. et Tom. Phytopathol. Z. 20:297-310.
2. ELLIS, M. A., M. B. ILYAS, and J. B. SINCLAIR. 1974. Effect of cultivar and growing region on internally seedborne fungi and Aspergillus melleus pathogenicity in soybean. Plant Dis. Rep. 58:332-334.
3. FENNE, S. B. 1949. Alfalfa and soybean disease in Virginia, 1948. Plant Dis. Rep. 33:90-91.
4. FENNE, S. B., and W. C. WHITE. 1950. Chemical treatment of soybean seed increased germination in laboratory tests. Plant Dis. Rep. 34:206-207.
5. ILYAS, M. B., O. D. DHINGRA, M. A. ELLIS, and J. B. SINCLAIR. 1975. Location of mycelium of Diaporthe phaseolorum var. sojae and Cercospora kikuchii in infected soybean seeds. Plant Dis. Rep. 59: (In press).
6. JOHNSTON, A. 1951. Soybean seed treatment. Soybean Dig. 11(7):17-20.
7. JOHNSTON, A. 1958. A note on fungicidal seed dressing of soybean. Malaya Agric. J. 41:152-155.
8. KIRKPATRICK, B. L., and J. B. SINCLAIR. 1973. Uptake of two systemic fungicides and their breakdown products by soybean seeds. Phytopathology 63:1532-1535.

9. KOEHLER, B. 1944. New developments in soybean disease studies. Soybean Dig. 4(8):6-7.
10. MENGISTU, A., M. A. ELLIS, D. J. ROYCE, and J. B. SINCLAIR. 1975. Soybean seed treatment studies. Fungicide-Nematicide Test Results of 1974. Vol. 30: (In press).
11. NENE, Y. L., V. K. AGARWAL, and S. S. L. SRIVASTAVA. 1969. Influence of fungicidal seed treatment on the emergence and nodulation of soybean. Pesticides 3:26-27.
12. NICHOLSON, J. F., and J. B. SINCLAIR. 1971. Amsoy soybean seed germination inhibited by Pseudomonas glycinea. Phytopathology 61:1390-1393.
13. PETERSON, J. L., and R. F. STRELECKI. 1965. The effect of variants of Diaporthe phaseolorum on soybean germination and growth in New Jersey. Plant Dis. Rep. 49:228-229.
14. SCHNEIDER, R. W., O. D. DHINGRA, J. F. NICHOLSON, and J. B. SINCLAIR. 1974. Colletotrichum truncatum borne within the seed coat of soybean. Phytopathology 64:154-155.
15. SHARVELLE, E. G. 1961. The nature of uses of modern fungicides. Burgess Pub. Co., Minneapolis, Minn. 308 p.
16. SINGH, O. V., V. K. AGARWAL, and Y. L. NENE. 1973. Seed health studies in soybean raised in the Nainital Tarai. Indian Phytopathol. 26:260-267.
17. WALLEN, V. R., and T. F. CUDDY. 1960. Relation of seed-borne Diaporthe phaseolorum to the germination of soybean. Proc. Assoc. Off. Seed Anal. 50:137-140.