Effect of Benomyl Field Sprays on Internally-Borne Fungi, Germination, and Emergence of Late-Harvested Soybean Seeds

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ABSTRACT

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Soybean (*Glycine max* 'Beeson') plants were sprayed with 50, 100, 500, 1,000, or 5,000 μg/ml solutions of benomyl four times at 2-week intervals beginning 55 days after planting. Half the seeds were harvested at maturity (first harvest) and the other half 30 days later (late harvest). Seeds from sprayed plants from the late harvest had fewer seed-borne fungi, including *Diaporthe phaseolorum* var. *sojae* (*Phomopsis* sp.), higher germination in vitro, and greater field emergence than those from nonsprayed plants. Seeds from sprayed and nonsprayed plants from the first harvest had no significant difference in in vitro germination, field emergence, and occurrence of *D. phaseolorum* var. *sojae, Alternaria* spp., and *Fusarium* spp. Fungicide (methyl 2-benzimidazolecarbamate) activity was detected by bioassay approximately 6 months, after the first harvest, in seeds from plants sprayed with 500, 1,000, and 5,000 μg/ml benomyl and in seeds from late-harvested plants sprayed with 1,000 and 5,000 μg/ml benomyl.

Additional key words: seed quality, *Pencillium expansum*.

One of the important internally seed-borne fungi of soybean [*Glycine max* (L.) Merr.] is *Diaporthe phaseolorum* Cke. and Ellis var. *sojae* Wehm. (*Phomopsis* spp.) which causes pod and stem blight (4, 6, 7, 10). Seeds infected with this fungus rarely germinate. The occurrence of internally seed-borne *D. phaseolorum* var. *sojae* and other fungi, increase when harvest is delayed (11). A problem in producing high quality soybean seeds in Illinois is late harvest because of wet weather. Late harvests in 1972 and 1974 resulted in soybean seeds with low germination and a high percentage of seed-borne fungi (George Keith, Illinois Crop Improvement Association, *personal communication*).

Ellis et al. (2) and Prasartsee et al. (9) reported that foliar applications of benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, Benlate 50 WP, E. I. duPont de Nemours and Co., Wilmington, Delaware] on soybean plants significantly reduced the amount of internally seed-borne *D. phaseolorum* var. *sojae* and other fungi at maturity. We present data showing that foliar applications of benomyl reduces the incidence of seed-borne fungi at maturity, and partially suppress the increase in internally seed-borne fungi and the decrease in germination associated with late harvest.

MATERIALS AND METHODS

A plot of Beeson soybean was planted on the University of Illinois' Agronomy South Farm on 11 June 1974. Rows were 6.1 meters long on 76-cm centers and contained approximately 90 plants per row. Treated rows were sprayed four times with 2 liters of either a 50, 100, 500, 1,000, or 5,000 μg/ml solution of benomyl at 2-week intervals beginning 55 days after planting. There was a row of nonsprayed plants between each row of sprayed plants. Sprays were applied with a hand sprayer and directed at stems and pods. Nontreated plants served as controls. Each treatment was replicated three times in a completely randomized design.

Half of the seeds from each sprayed and nonsprayed row were harvested at maturity (first harvest), 17 October, and half 30 days later (late harvest). All seeds were stored at 4 C until assayed in the laboratory or planted in the field the following season.

Two-hundred seeds from each replication (600 seeds per treatment) were bioassayed for internally seed-borne fungi and their percentage germination in vitro recorded for both harvest dates. All seeds were surface-sterilized by soaking in a 0.25 percent sodium hypochlorite solution for four minutes, followed by 70 percent ethanol for 2 minutes, and finally rinsed in sterile distilled water (1). Seeds were plated on Gibco potato-dextrose agar (PDA) in culture plates. The percentage of germination and occurrence of fungi were recorded after 7 days at 25 C. A seed was considered germinated when the radicle was 1.5 times the length of the cotyledons.

On 16 May 1975, 100 seeds from each replication per treatment and harvest date were planted in a randomized replicated field plot in a split-plot design. Stand counts were recorded 15 and 30 days after planting.

Two days prior to planting, 100 seeds from each treatment and harvest date were bioassayed for fungicide activity. Bioassays were run using PDA seeded with *Penicillium expansum* Link. A 50-ml spore suspension which contained approximately 550,000 spores/ml, calculated using a Beckman spectrophotometer at OD = 0.5, was added to 1,000 ml of warm (50 C) PDA. Five milliliters of the bioassay medium was syringed into 100-mm diameter culture plates. Seeds were washed for 1

TABLE 1. Mean percentage germination and occurrence of various fungi[a] in Beeson soybean seeds from plants nonsprayed or sprayed with benomyl and harvested at maturity (first harvest) or 30 days later (late harvest[b])

Benomyl[c] rate (μg/ml)	First harvest[d]					Late harvest[d]				
	Germ	Total fungi	DSP	ALT	FUS	Germ	Total fungi	DPS	ALT	FUS
0	95.5	14.8	2.3	2.2	1.7	68.3	55.8	14.8	27.8	6.4
50	99.8	3.3	0.2	1.2	0.3	92.8	33.7	1.7	29.7	1.7
100	99.3	3.3	0	2.0	0.2	92.0	29.8	0	28.0	1.7
500	99.3	3.2	0.2	1.5	0.2	94.2	30.0	0	29.3	0.7
1,000	99.3	2.7	0.2	1.7	0.7	95.2	29.3	0	28.3	0.8
5,000	99.8	2.3	0	1.7	0.2	95.8	25.3	0	24.8	0.2
LSD ($P = 0.05$) (within each harvest)	6.9	8.2	3.4	10.2	4.7					
LSD ($P = 0.05$) (between harvests)	2.5	3.0	0.9	5.4	2.1					

[a]Seeds plated on potato-dextrose agar and incubated 7 days at 25 C.
[b]Based on 600 seeds per treatment from each harvest time.
[c]Benomyl = methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (Benlate 50 WP).
[d]Germ = germination; DPS = *Diaporthe phaseolorum* var. *sojae* (*Phomopsis* spp.); ALT = *Alternaria* spp.; and FUS = *Fusarium* spp.

minute in running distilled water and cut in half at right angles to the embryo axis before being plated cut-side down on the medium. Zones of inhibition were measured from the edge of the plated tissue to the widest point after plates were incubated at 4 C for 18 hours, then at 30 C for 48 hours (3).

RESULTS

Germination in vitro.—There was no significant effect of any treatment on germination of seed from the first harvest (Table 1). However, germination of late-harvested seeds from nonsprayed plants was significantly less than from sprayed plants. There were no significant differences between the various fungicide treatments. Germination of seeds from all treatments of the late harvest was significantly less than that from the first harvest.

Internally seed-borne fungi.—The following genera of fungi were recovered from seeds at both harvests: *Alternaria* spp., *Aspergillus* spp., *Cercospora* spp., *Cladosporium* spp., *Diaporthe phaseolorum* var. *sojae*, *Fusarium* spp., *Nigrospora* spp., *Penicillium* spp., and *Rhizopus* spp.

There were significantly more fungi in seeds from the first harvest from nonsprayed plants than from sprayed plants, but not among seeds from plants sprayed with various concentrations of benomyl. There was no significant difference in the occurrence of *Alternaria* spp., *D. phaseolorum* var. *sojae*, and *Fusarium* spp. between seeds from nonsprayed and sprayed plants from the first harvest. The percentage of *D. phaseolorum* var. *sojae* increased significantly between the first and late harvest both in the control and 50 μg/ml benomyl treatment. The percentage total fungi and *Alternaria* spp. increased significantly for all treatments between the two harvests. There was a significant increase in *Fusarium* spp. between the two harvest dates only in seed from nonsprayed plants.

Diaporthe phaseolorum var. *sojae* was not recovered from late-harvested seeds from plants receiving higher rates of benomyl. There were significantly more total fungi, *Fusarium* spp., and *D. phaseolorum* var. *sojae* in seeds from nonsprayed plants than any other treatment from the late harvest. Late-harvested seeds of plants sprayed with 50 μg/ml benomyl had significantly more total fungi than those from any other sprayed plants from the late harvest. There were no significant differences in percentage of total fungi in seeds from the other benomyl-treated plants.

TABLE 2. Mean percent[a] emergence of Beeson soybean seeds from plants nonsprayed or sprayed with benomyl and harvested at maturity (first harvest) or 30 days later (late harvest). Counts were made 15 and 30 days after planting

Benomyl[b] rate (μg/ml)	First harvest		Late harvest	
	15 days	30 days	15 days	30 days
0	80	92	58	62
50	86	94	75	90
100	80	92	73	86
500	80	93	63	89
1,000	80	94	71	85
5,000	78	94	73	90
LSD ($P = 0.05$)	ns[c]	ns	13	11

[a]Based on three replications of 100 seeds each.
[b]Benomyl = methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate (Benlate 50 WP).
[c]ns = no significant differences.

Bioassay.—Zones of inhibition were produced around seeds of the first harvest only from plants sprayed with 500, 1,000, and 5,000 μg/ml benomyl. The percentage of seeds which produced zones was 44, 100, and 100, respectively. Zones of inhibition were produced around late-harvested seeds only from plants sprayed with 1,000 and 5,000 μg/ml benomyl with 16 and 80 percent of the seeds producing zones, respectively.

Field emergence.—There was no significant difference in emergence of seeds from the first harvest between any treatment at eigher 15 or 30 days (Table 2). At 30 days after planting, seedling emergence from seeds of nonsprayed late-harvest plants was significantly lower than that for seeds from any sprayed plants. At 15 days after planting, the 500 μg/ml treatment was the only treatment which did not have significantly higher seedling emergence than nonsprayed plants.

DISCUSSION

Our data agree with those in previous reports that a delay in harvest of soybean seeds results in an increase in seed-borne fungi and a reduction of seed germination (11); and that foliar applications of benomyl can reduce internally seed-borne fungi (2, 9). This report shows for the first time that foliar applications of benomyl can control the build-up of fungi in soybean seeds up to 30 days after maturity. There was an increase in *Alternaria* spp. in seeds from the late harvest. This fungus appears to have little effect on soybean seed germination and is tolerant to methyl 2-benzimidazolecarbamate (MBC), the breakdown product of benomyl (8).

Ellis and Sinclair (3) reported that the soybean seeds harvested from the same plants used in this study contained MBC, which could be detected in seeds from plants sprayed with 500, 1,000, and 5,000 μg/ml benomyl up to 43 days after the first harvest and up to 13 days after the late harvest. Fungicide activity, although reduced, was detected in some of the same seeds approximately 6 months after harvest. This suggests that seeds from benomyl-sprayed plants may be protected from colonization by certain fungi during storage and in the field.

Our data also confirmed that seed-borne fungi can reduce field emergence of soybean seeds (5). Benomyl sprays on soybean could be used to assure high quality seeds.

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