Effect of Benomyl Applications on Soybean Seedborne Fungi, Seed Germination, and Yield

D. M. TeKRONY and D. B. EGLI, Professors, Department of Agronomy, R. E. STUCKEY, Extension Professor, Department of Plant Pathology, and T. M. LOEFFLER, Former Graduate Research Assistant, Department of Agronomy, University of Kentucky, Lexington 40546-0091

ABSTRACT

TeKrony, D. M., Egli, D. B., Stuckey, R. E., and Loeffler, T. M. 1985. Effect of benomyl applications on soybean seedborne fungi, seed germination, and yield. Plant Disease 69:763-765.

The effect of benomyl treatments during reproductive growth of soybeans (*Glycine max*) on fungal seed infection was evaluated in the field for 2 yr. Trends for improved seed germination and lower levels of seed infection by *Phomopsis* sp. and *Cercospora kikuchii* occurred for all benomyl treatments except the single application (1.12 kg/ha) near the R7 growth stage (first yellow pod). The greatest improvement in seed germination occurred with the single application (1.12 kg/ha) of benomyl at growth stage R6 (green pod, full seed). This treatment also had significantly lower seed infection by *Phomopsis* sp. for seven of nine planting dates in three experiments across 2 yr. There was little yield response to benomyl treatments except for a significant increase occurring in one (June 1978) of the nine planting dates after the split application (0.56 kg/ha at growth stages R4 and R6).

Additional key words: *Diaporthe phaseolorum* var. *sojae*

The adverse effects of seed infection with the pod and stem blight fungus (*Phomopsis* sp.) on soybean (*Glycine max* (L.) Merr.) seed germination are well documented (12,15,21,24). Environmental conditions, particularly moisture, strongly influence seed infection during seed development and maturation (17,19,22). Although the etiological aspects of the pod and stem blight disease have not been completely described, research indicates that little seed infection occurs before pod senescence (yellow to brown coloration) (7,14,23).

Yield response using labeled rates of foliar fungicides on soybeans is highly dependent on the presence of foliar diseases and has ranged from a significant yield increase (2,9,16) to little increase (10). Seed quality response to foliar fungicides has also been variable, although reductions in seed infection by pod and stem blight disease have been reported after two applications of benomyl (3,16).

The time of application for the labeled split treatment of foliar fungicides is at growth stages R3 and R5, which is well in advance of the time when maximum seed infection occurs near growth stage R7. However, a single application at the R5 or R6 growth stage has been approved in some states. This allows for an application time that is closer to the time of seed infection and provides additional time to determine if treatment is needed. This study was initiated to evaluate the effect of the time and rate of foliar fungicide applications closer to pod senescence on soybean seed quality and seed infection by fungi.

Contribution from the Departments of Agronomy and Plant Pathology, University of Kentucky, Lexington. Research supported in part by grant from Kentucky Seed Improvement Association. This paper (85-3-11-60) is published with the approval of the director of the Kentucky Agricultural Experiment Station.

Accepted for publication 3 April 1985 (submitted for electronic processing).

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MATERIALS AND METHODS

These investigations were conducted on Spindletop Experimental Farm near Lexington, KY, in 1978 and 1979. Soybeans (cultivar Williams) were planted on three dates, 20 May, 15 June, and 6 July, in 1978 at one field location and on 15 May, 15 June, and 5 July at two field locations in 1979. A split-plot design was used in all experiments with planting dates as main plots and fungicide treatments as subplots in a randomized complete block design with three replicates. Seed were planted at a depth of 4–5 cm in a Maury silt loam soil (fine, mixed, mesic Typic Paleudalf) at a rate of 30 seeds per meter in four rows 6 m long with 76-cm spacing. Stand counts were made 2 wk after planting. The 1978 experiment was planted in soil that had not been planted in soybeans for 3 yr, whereas both sites in 1979 had been in soybeans the previous year. Soybean straw was spread on the surface after seedling emergence in both years to provide an inoculum source for the pod and stem blight diseases.

Reproductive growth stages (4) were determined on 10 consecutive plants in a row of each plot starting at growth stage R2 (full bloom). The yellow pod growth stage used in 1979 was defined as when 50% of the plants evaluated had at least one normal pod at any one location on the plant, which was completely yellow. This stage occurred about 2 days before growth stage R7 (one mature [brown] pod on the main stem) and will be referred to as R7. Random samples of pods were collected at 2-day intervals after growth stage R7. Seed were removed and seed moisture was determined gravimetrically after drying at 105 C for 24 hr. The date of harvest maturity was determined to be the first time the seed dried to less than 14% moisture (wet basis).

Benomyl (Benlate 50WP) treatments were applied at the rates and growth stages shown in Table 1 at 2.8 kg/cm^2 of pressure and 617 L/ha of water using a single-row CO_2 plot sprayer that had a boom with two top nozzles and two drop (side) nozzles.

At harvest maturity, the plants from a 5-m section of a bordered row were cut and threshed with a plot thresher, and after drying, the seeds were weighed for yield determination. Thirty to 40 consecutive plants from a randomly selected section of a bordered row were also removed from the field, air-dried, and hand-threshed, and the seeds were

Table 1. Rate and growth stage when benomyl treatments were applied in three experiments

Fungicide treatment	Rate (kg/ha)	Growth stages applied	Experiments (treated) 1978	1979a	1979b
Benomyl[a]	0.56	R4/R6	X	X	
	1.12	R6	X	X	X
	1.12	R7		X	
Untreated control			X	X	X

[a] Benomyl treatments were applied at the beginning of the growth stage.

stored at 10 C and 50% RH until seed-quality evaluations were made about 3 mo later. All seed samples were evaluated for standard germination following the procedures described by TeKrony et al (21) and for seed infection by fungi. The level of seed infection by *Phomopsis* sp. was determined by a modification of the procedure described by Kmetz et al (12). Fifty seeds were surface-sterilized by soaking in 0.5% NaOCl for 4 min, washed with 200 ml of sterile distilled water, and plated on acidified (pH 4.5) Difco potato-dextrose agar. Five seeds were placed on each culture plate and the plates were held under fluorescent light at room temperature (about 25 C) for 12–14 days before fungal identification based on visual colony morphology was made into the following groups: 1) *Phomopsis* sp. (both *Diaporthe phaseolorum* (Cke. & Ell.) var. *sojae* (Wehm.) and *Phomopsis* sp., thus both are grouped under *Phomopsis* sp.), 2) *Cercospora kikuchii* (Mat. & Tomoy), purple seed stain, and 3) total fungi (included those from 1 and 2 and *Alternaria* spp., *Cladosporium* spp., and other unidentified fungi).

RESULTS AND DISCUSSION

Higher levels of seed infection by *Phomopsis* sp. occurred in the untreated control in both experiments in 1979 than in 1978 (Table 2). Average seed infection by *Phomopsis* sp. across the three planting dates for the control was 16, 39, and 25% in 1978, 1979a, and 1979b, respectively, and ranged from 11% for the July planting date in 1978 to 51% for the June planting date in 1979a (Table 2).

When planting dates were averaged, there was a trend for lower levels of seed infection by *Phomopsis* sp. after benomyl treatments except for the single application of 1.12 kg/ha at the R7 growth stage in 1979a, when little reduction occurred (Table 2). The single application of 1.12 kg/ha of benomyl at growth stage R6 significantly (α = 0.05) reduced seed infection by *Phomopsis* sp. for seven of the nine planting dates across the three experiments. These reductions ranged from 11 percentage points in May 1978 and July 1979b to 27 percentage points in July 1979a. The split application of 0.56 kg/ha of benomyl at growth stages R4 and R6 significantly (α = 0.05) reduced seed infection by *Phomopsis* sp. for only two of the six planting dates compared in 1978 and 1979a (Table 2). Reductions in seed infection by *Phomopsis* sp. have been reported in Ohio (10) using the single application (1.12 kg/ha) of benomyl at a slightly earlier growth stage (R4–R5) than that used in this study. Ellis et al (3) reduced seed infection by *Phomopsis* sp. seed infection in Illinois with a single application (0.56 kg/ha) at a late stage of pod development or with a split application at midflowering and late pod fill; however, infection levels in the control were much lower (11.5%) than in either this study or the one in Ohio (10). Ross (16) used two or three foliar applications of benomyl at a rate of 0.68 kg/ha and reduced the percentage of seed with *Phomopsis* sp. infection, especially in irrigated plots.

Seed harvested from the control plots had much lower levels of seed infection with *C. kikuchii* than with *Phomopsis* sp. in 1979 but had similar levels in 1978 (Table 2). Seed infection with *C. kikuchii* ranged from low levels (1–5%) for all planting dates in 1979b and the July planting date in 1979a to 24% for the June and July planting dates in 1978. The split application of 0.56 kg/ha of benomyl at growth stages R4 and R6 significantly reduced seed infection with *C. kikuchii* for five of the six planting dates in 1978 and 1979a where greater than 10% infection occurred. The single application of 1.12 kg/ha of benomyl at growth stage R6 also significantly (α = 0.05) reduced seed infection by *C. kikuchii* for three of these planting dates. The single application of 1.12 kg/ha of benomyl at the R7 growth stage did not reduce seed infection by either *C. kikuchii* or *Phomopsis* sp.

Total seedborne fungi found in the untreated control ranged from 29% for the June planting date in 1979b to 70% for the June planting date in 1978. Both the single benomyl application at growth stage R6 and the split benomyl applications at growth stages R4 and R6

Table 2. Fungal seed infection, soybean seed germination, and yield at harvest maturity after single and split applications of benomyl at three planting dates in 1978 and 1979

Year	Planting date	Treatment	Growth stages applied	*Phomopsis* sp. (%)	*Cercospora kikuchii* (%)	Total seedborne fungi (%)	Standard germination (%)	Yield (kg/ha)
1978	20 May	Control		16	14	47	90	2,822
		Benomyl	R4/R6[a]	20	2*[b]	39	86	2,957
			R6	5*	1*	16*	92	2,957
	15 June	Control		21	24	70	78	2,554
		Benomyl	R4/R6	9*	4*	14*	90*	3,091*
			R6	6*	6*	27*	91*	2,755
	6 July	Control		11	24	35	94	2,218
		Benomyl	R4/R6	5	4*	15*	93	2,486
			R6	8	20	49	94	2,285
1979a	15 May	Control		30	22	58	68	2,688
		Benomyl	R4/R6	25	0*	34*	75	3,024
			R6	14*	2*	27*	84*	2,822
			R7	24	29	55	61	2,688
	15 June	Control		51	12	68	60	2,621
		Benomyl	R4/R6	45	0*	53*	63	2,352
			R6	32*	9	45*	79*	2,352
			R7	45	17	69	62	2,218
	5 July	Control		37	1	44	66	1,613
		Benomyl	R4/R6	11*	0	27*	88*	1,747
			R6	10*	0	21*	94*	1,613
			R7	37	2	43	83*	1,814
1979b	15 May	Control		32	4	37	75	3,293
		Benomyl	R6	9*	0	15*	92	3,696
	15 June	Control		23	5	29	81	3,226
		Benomyl	R6	14	2	26	94	3,360
	5 July	Control		18	1	35	79	1,546
		Benomyl	R6	7*	1	23	86	1,882

[a] Benomyl applied at R4/R6 growth stages at 0.56 kg/ha; benomyl applied once at R6 and R7 at 1.12 kg/ha.
[b] * = Significant (α = 0.05) change from control treatment for the same planting date as determined by least significant difference test.

reduced total fungi seed infection for all planting dates except for the single treatment in July 1978 (Table 2). Two fungi, *Phomopsis* sp. and *C. kikuchii*, accounted for a large percentage of the total seedborne fungi infection in most of the control samples. The remaining fungi present included primarily *Alternaria* sp. and *Cladosporium* sp.

For six of the nine planting dates, the standard germination percentages for the untreated control did not reach the level (80%) recommended for certified seed (1) (Table 2). The germination of the control samples ranged from 60% for the June planting date in 1979a to 94% for the July planting date in 1978. After the single application of 1.12 kg/ha of benomyl at growth stage R6, significant increases in standard germination occurred for four planting dates and the germination of seed from all planting dates (except June 1979a) exceeded 80% (Table 2). The split application of 0.56 kg/ha of benomyl at growth stages R4 and R6 in 1978 and 1979a also resulted in acceptable germination ($\geq$80%) for four of the six planting dates compared. The single application of 1.12 kg/ha of benomyl at the R7 growth stage had little effect on standard germination except for the July planting date in 1979a. There was a significant ($\alpha = 0.05$) linear relationship ($r = -0.83$) between standard germination and *Phomopsis* sp. seed infection across all planting dates in the three experiments. There was little relationship, however, between seed infection by *C. kikuchii* and standard germination ($r = -0.27$). These data support other research that has shown significant, negative relationships between standard germination and *Phomopsis* sp. (12,21) but little association of standard germination with purple seed stain.

Even though there was a trend toward slightly higher grain yields after benomyl treatment, only one comparison (June 1978, split application at R4 and R6), showed a significant ($\alpha = 0.05$) increase over the control. There was little relationship ($r = -0.17$) between seed infection by *Phomopsis* sp. and yield across all planting dates. This supports other research in the North Central soybean production areas where little to no yield increase has occurred after benomyl treatments (6,10,11). It is in contrast, however, to significant yield increases that have occurred in more southern production areas after split applications of benomyl (2,9,16). Ross (16) related these yield increases to control of Septoria brown spot infection and foliar diseases other than those caused by *Phomopsis* sp. and *C. kikuchii*.

Much evidence has accumulated that shows that seed infection with *Phomopsis* sp. is directly related to soybean seed quality. The levels of seed infection by *Phomopsis* sp. are highly variable (10,21) and are dependent on both inoculum availability (5,13) and the field environment during seed development (17,22). Because of the uncertainty of seed infection and the economic investment involved when using foliar fungicides, several "point systems" have been developed to predict the occurrence of disease infection and advise growers when to use a foliar fungicide (18,20).

It has become evident that the maximum seed infection by *Phomopsis* sp. occurs at pod senescence (7,14,23). Hill et al (8) reported that benomyl provided protection against pod and stem blight disease in inoculated plants for at least 17 days after application and that the protective ability was completely lost by 21 days. In these experiments, the average time interval between growth stages R6 and R7 was 18 days, whereas the average time interval between R4 and R6 was 26 days. Thus, with the split application of 0.56 kg/ha at R4 and R6, one-half of the fungicide was applied more than 40 days before maximum seed infection occurred. This provides some explanation why the single application of 1.12 kg/ha of benomyl at the higher rate at growth stage R6 was more effective than the split application in reducing seed infection by *Phomopsis* sp. and improving seed germination.

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