Effects of Benomyl on the Colonization of Soybean Leaves, Pods, and Seeds by Fungi

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ABSTRACT

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The incidence of most fungi in soybean (*Glycine max*) leaves, pods, and seeds was reduced by benomyl treatment. Significant reductions occurred for *Diaporthe phaseolorum* var. *sojae, Colletotrichum dematium* var. *truncata, Glomerella glycines*, and *Cercospora kikuchii*. Several fungi, including *Alternaria alternata* and *Fusarium* spp., increased in frequency. Significant reductions and increases in the incidence of fungi in leaves, pods, and seeds were greater in lower than in intermediate and upper positions on benomyl-treated plants.

Currently there is widespread use of fungicides, particularly benomyl, to control foliar and seedborne diseases of soybean (*Glycine max* (L.) Merr.). Although the effects of benomyl on fungal diseases and on yield of soybeans have been investigated (2,4), its effects on colonization of soybean leaves, pods, and seeds by fungi have received little attention.

Our objective in this study was to determine the effects of benomyl on colonization of soybean leaves, pods, and seeds by fungi.

MATERIALS AND METHODS

Experiments were conducted on the Mississippi State University Plant Science Farm, Mississippi State. The soybean cultivar Forrest, maturity group V, was planted in a randomized block design in May 1978 and 1979. Plots consisting of a treated and control row, each separated and flanked by buffer rows, were replicated three times in 1978 and four times in 1979. Rows were 1 m apart and 7.5 m long in 1978 and 12.2 m long in 1979.

Two applications of benomyl were made on soybeans each year. The first application was made during the R3 stage of growth (4), which occurred 78 days after planting in 1978 and 82 days after planting in 1979. The second application was made 91 days after planting in 1978 and 96 days after planting in 1979. A hand-held sprayer, powered by carbon dioxide, was used to apply the fungicide over the entire plant canopy at 1.1 kg/ha in 234 L of water per hectare.

Each year, pods in the bottom, middle,

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Table 1. Effects of benomyl on the frequency of isolation of selected fungi from soybean leaves, pods, and seeds in 1978 and 1979[a]

	Frequency of isolation (%) in 1978		Frequency of isolation (%) in 1979	
Fungus	**Control**	**Benomyl**	**Control**	**Benomyl**
Leaf				
Diaporthe phaseolorum var. *sojae*	50.2	7.5*[b]	26.8	21.4*
Alternaria alternata	66.7	83.3*	16.4	20.5*
Phoma spp.	33.0	17.5	14.3	17.1
Glomerella glycines	10.2	1.1*	23.9	24.8
Nigrospora spp.	11.6	1.9	20.0	20.6
Colletotrichum dematium var. *truncata*	0.2	0.2	30.0	29.0
Colletotrichum sp.	5.4	0.4	22.5	10.8
Pod				
D. phaseolorum var. *sojae*	40.1	8.1*	42.9	10.3*
A. alternata	30.4	43.6*	15.1	31.1*
Phoma spp.	13.1	10.6	9.7	10.8
C. dematium var. *truncata*	0.2	0.2	13.5	4.9*
Colletotrichum sp.	2.2	0.3	1.6	0.3
Cercospora kikuchii	18.6	0.4*	4.9	0.2
Fusarium spp.[c]	4.8	4.3	25.9	29.8
Seed				
D. phaseolorum var. *sojae*	1.6	0.6	9.8	1.6*
A. alternata	6.5	3.9	4.1	8.1*
C. kikuchii	0.9	0.2	0.2	0.0
Fusarium spp.[c]	0.5	0.3	21.0	28.1*

[a] Figures were obtained by pooling data from different sampling dates. Data from three and two leaf samples were pooled in 1978 and 1979, respectively. For pods and seeds, data from five and four samples were pooled in 1978 and 1979, respectively.
[b] Asterisk indicates a significant difference from the control as determined by the t test ($P = 0.05$).
[c] Predominantly *F. semitectum* and *F. equiseti*.

and upper one-third portions of randomly selected plants in the R3 stage of growth (early pod development) were tagged. Twenty tagged pods and leaves were sampled per position and replicate. In 1978, leaves were sampled 1 day before and 6 and 21 days after the second application of benomyl; in 1979, leaves were sampled 7 days before and 18 days after the second application; in 1978, pods were sampled 1 day before and 12, 25, 49, and 62 days after the second application; and in 1979, pods were sampled 3 days before and 7, 18, and 38 days after the second application.

Disks of tissue 6 mm in diameter were excised from leaves and pods with a paper punch. In 1978, five disks were excised equidistant along the entire length of each leaf, between the leaf margin and midrib. Similarly, three disks were sampled per leaf in 1979. In both years, one pod disk was excised from the peduncle end, the middle, and the stylar end of each pod. Leaf and pod disks and seeds from the pods were surface sterilized for 5 sec in 95% ethanol and 1 min in 1% sodium hypochlorite, then plated on Difco potato-dextrose agar. Daily, during 5 days of incubation at 24 C, fungi growing from tissues and seeds were recorded or subcultured for later identification.

Table 2. Effects of benomyl on the frequency of isolation of selected fungi from leaves, pods, and seeds located at different positions on soybean plants in 1979

Fungus	Position of organ on plant[a]	Frequency of isolation (%)[b]	
		Control	Benomyl
	Leaf		
Diaporthe phaseolorum var. *sojae*	B	21.9	6.9*[c]
	M	51.2	46.9
	T	7.5	10.6
Alternaria alternata	B	8.7	15.0
	M	17.5	20.0
	T	21.1	26.9
	Pod		
D. phaseolorum var. *sojae*	B	47.2	8.4**[d]
	M	46.2	9.7**
	T	34.1	12.8**
A. alternata	B	4.1	20.6*
	M	14.4	26.2
	T	26.9	49.1*
Colletotrichum dematium var. *truncata*	B	23.4	10.0*
	M	10.3	2.2**
	T	3.7	3.1
	Seed		
D. phaseolorum var. *sojae*	B	17.2	2.5*
	M	34.1	5.9
	T	13.1	2.8*
A. alternata	B	3.4	8.4
	M	9.4	13.4
	T	10.0	18.1
Fusarium spp.[e]	B	17.5	38.1
	M	34.1	38.7
	T	30.0	29.1

[a] B = bottom third of plant, M = intermediate third, and T = top third.
[b] Figures were obtained by pooling data from different sampling dates. For leaves, data from two samples were pooled. For pods and seeds, data from four samples were pooled.
[c] An asterisk indicates a significant difference from the control as determined by the *t* test ($P = 0.05$).
[d] Two asterisks indicate a significant difference from the control as determined by the *t* test ($P = 0.01$).
[e] Predominantly *F. semitectum* and *F. equiseti*.

RESULTS AND DISCUSSION

Incidence of the majority of selected fungi (Table 1) was reduced by benomyl treatment. Reductions in incidence of *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *sojae* (Lehman) Wehm., *Glomerella glycines* Hori, *Colletotrichum dematium* (Fr.) Grove var. *truncata* Arx, and *Cercospora kikuchii* (Mat. & Tomoy.) Gardner were statistically significant, as were increases in incidence of *Alternaria alternata* (Fr.) Keissler and *Fusarium* spp. (primarily *F. semitectum* Berk. & Rav. and *F. equiseti* (Cda.) Sacc.). Reduced incidence of fungi supports the general view that delayed senescence and yield increases in benomyl-treated plants are primarily the result of decreased colonization of tissues by pathogens (2,7). The reductions (Table 1) seem significant in another sense, ie, that inoculum potential of fungi in the subsequent growing season may be reduced by benomyl.

Increases in incidence of *A. alternata, Fusarium* spp., and other fungi following benomyl treatment suggest that benomyl may have altered competition among fungi. Antagonism among fungi that colonize soybean tissues has been reported (9). The observation by Fokkema et al that *Cochliobolus sativus* infection of rye leaves increased following treatment of plants with benomyl suggested a reduction in antagonists of *C. sativus* (5). Unfavorable consequences could result from increases in the incidence of *Fusarium* spp. and *A. alternata* because the former can decrease germination of soybean seeds (1,9) and the latter causes leaf spots on soybean (1).

The relation of changes in incidence of selected fungi to the position of soybean organs on benomyl-treated plants in 1979 is presented in Table 2. Significant reductions and increases in incidence of fungi in response to benomyl were greatest in lower positions on the plants and progressively less in upper positions. This effect may be related to differences in the transpiration rate of leaves, and perhaps pods, in lower versus upper positions. Benomyl moves primarily in the transpiration stream, and its accumulation in plant organs appears dependent upon organ transpiration (6,8,10). High transpiration rates appear to result in an accumulation of benomyl out of the central leaf area and into margins (8). Generally, plant organs in lower positions transpire less than those in upper positions (3). In the present study, a more uniform distribution of benomyl over leaf and pod surfaces in lower plant positions, because of decreased rates of transpiration, may have been responsible for the greater activity of benomyl in lower than in upper plant positions.

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