The Iowa Soybean Pod Test for Predicting Phomopsis Seed Decay in Kansas

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ABSTRACT

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The Iowa pod test for predicting the need for foliar fungicide applications to control Phomopsis seed decay of soybeans (*Glycine max*) was evaluated under Kansas growing conditions. The mean incidence of pod infection by *Phomopsis longicolla* was 50, 15, and 50% in 1987, 1988, and 1989, respectively. No correlation was found between the incidence of *P. longicolla* on pods at soybean growth stage R6 and either the incidence of the fungus on seeds or the germination of seeds at harvest. For seeds from plants treated with benomyl, the percentage of pathogens detected was not significantly different from that of seeds from unsprayed plants in 1987 and 1988. In 1989, *Penicillium* spp. occurred at significantly lower levels and *Alternaria* and *Fusarium* spp. at significantly higher levels on seeds from benomyl-treated plants than on those from unsprayed plants. Seeds from plants treated with benomyl at growth stage R7 had a significantly higher level of seed germination than seeds from unsprayed plants following harvest in 1988 only. Under Kansas growing conditions, the model consistently predicted the need for foliar sprays when they were not needed.

In 1985 and 1986, heavy rains occurred throughout the harvest season for soybeans (*Glycine max* (L.) Merr.) in Kansas, delaying harvest and greatly reducing seed quality. The reduced seed quality, due mainly to infection by *Phomopsis longicolla* T. W. Hobbs (Kansas State University Plant Disease Diagnostic Clinic, *unpublished data*), had adverse affects on seed germination.

The application of benomyl to the growing seed crop to control *Phomopsis* spp. and other seedborne fungi, though effective (3,4,13), has not been routinely practiced in Kansas, because of the normally dry conditions at harvest. In wet years, point systems developed in other states (10,11) have been used to make spray decisions but have provided mixed results, since inoculum levels present in the field have not been taken into account.

A predictive method for the use of fungicide sprays was developed in Iowa (7,9), based on the assumption that soybean pods are a pathway for seed infection (6). This method uses the percentage of pods infected by *P. longicolla* at soybean growth stage R6 as a predictor of potential seed infection. McGee (8) reported good results with this test in Iowa. Since predictive methods are not always accurate outside the geographic area in which they were developed, this study was conducted to determine the usefulness of the Iowa pod test as a predictor for foliar fungicide applications on soybeans under Kansas growing conditions.

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MATERIALS AND METHODS

In 1987, tests were conducted on commercial farms at three locations in Kansas, with each location serving as one replicate. The fields were located in Brown, Cowley, and Osage counties. In 1988 and 1989, tests were conducted on one site at the Kansas State University Agronomy Research Farm, Manhattan, and two sites at the Kansas State University Mound Valley Research Field, in southeastern Kansas.

Soybean pods were collected at growth stage R6 (100 pods from each location) and processed as described by McGee and Nyvall (9). The pods were incubated in a moisture box at 23 C under constant light (16 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) for 7 days.

At each location a 1-ha area was divided in two. Benomyl (0.56 kg a.i. per hectare) was applied to plants in one half of the test plot during growth stage R7. Plants in the other half of the plot served as the untreated control. All chemical applications were made with a commercial ground-rig sprayer within 8–14 days after the initial sampling of pods. The actual timing depended on the weather.

Soybeans were harvested at maturity. A seed sample (1.0 kg) was collected from each of the sprayed and the unsprayed plots at each location and brought to the laboratory. For each plot, three subsamples of 100 seeds each were plated on potato-dextrose agar and incubated for 7 days at 25 C with a 12-hr photoperiod (50 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). The incidence of species of *Phomopsis, Diaporthe, Alternaria, Aspergillus, Cercospora, Fusarium, Penicillium*, and *Rhizopus* was recorded. The mean of the three subsamples was used as one replicate in the analysis.

Seed from each treatment was tested for germination. Four subsamples of 100 seeds each were planted in sterile masonry sand with a moisture level of approximately 10%. They were incubated for 16 hr in the dark at 20 C and then 8 hr in the light at 30 C, for 7 days. To be counted as germinated, a seed had to have a normal taproot or enough secondary roots to be able to establish itself, the hypocotyl had to be extended, and there could be no lesions extending into the vascular tissue. The mean of the

Table 1. Mean incidence of fungi on soybean seeds harvested from plants treated with benomyl at soybean growth stage R7 and from unsprayed plants at three locations in Kansas

		Infected seed (%)[a]			
Fungus	**Treatment**	**1987**	**1988**	**1989**	**Mean (%)**
Alternaria spp.	Benomyl	19.9	18.3	41.3*	25.9*
	No fungicide	15.3	11.7	35.1	19.8
Aspergillus spp.	Benomyl	0.0	5.5	0.8	1.2
	No fungicide	0.1	10.0	0.6	2.1
Cercospora kikuchii	Benomyl	0.1	0.0	0.4	0.1
	No fungicide	0.1	0.0	0.1	0.1
Fusarium spp.	Benomyl	0.1	8.0	20.6*	6.8
	No fungicide	0.0	9.4	11.7	4.8
Penicillium spp.	Benomyl	5.9	1.1	25.8*	17.2
	No fungicide	19.2	1.6	48.1	9.6
Phomopsis spp.	Benomyl	0.2	0.4	5.9	1.4
	No fungicide	6.1	1.1	7.1	4.2
Rhizopus spp.	Benomyl	0.4	0.4	1.3	0.6
	No fungicide	0.3	1.4	1.7	1.1

[a] Asterisk denotes significant difference between seeds from benomyl-treated plants and those from unsprayed plants ($P = 0.05$), as determined by a *t*-test with paired observations.

four subsamples was used as one replicate in the analysis.

The percent infection for each pathogen was transformed by the arcsine square root transformation prior to analysis. Statistical analysis was performed with the MSTAT microcomputer statistical program (Department of Crop and Soil Sciences, Michigan State University, East Lansing).

RESULTS

The mean incidence of pod infection was 50, 15, and 50% in 1987, 1988, and 1989, respectively. The incidence and diversity of pathogens on the seeds differed from year to year and from one location to another. No statistical differences ($P = 0.05$) between the percentage of *Phomopsis*-infected seeds from unsprayed plants and the percentage from benomyl-treated plants were observed during the 3-yr test (Table 1). A correlation analysis indicated no significant correlation ($r = 0.53$) between the percentage of *Phomopsis*-infected seeds from untreated plants and the number of pods infected at soybean growth stage R6.

In 1987 and 1988, the incidence of pathogens on seeds from benomyl-treated plants was not significantly different from the incidence on seeds from untreated plants. In 1989, however, the incidence of *Penicillium* spp. was significantly lower and that of *Alternaria* spp. and *Fusarium* spp. was significantly higher in seeds from treated plants than in those from unsprayed plants.

The germination of seeds from unsprayed plants was not significantly different from that of seeds from benomyl-treated plants in 1987 and 1989. The germination rates for seeds from unsprayed and benomyl-treated plants were 93.6 and 93.4%, respectively, in 1987, and 91.7 and 88.7%, respectively, in 1989. A significant difference in germination was recorded in 1988, however, when the germination rates were 84.9 and 70.0%, respectively.

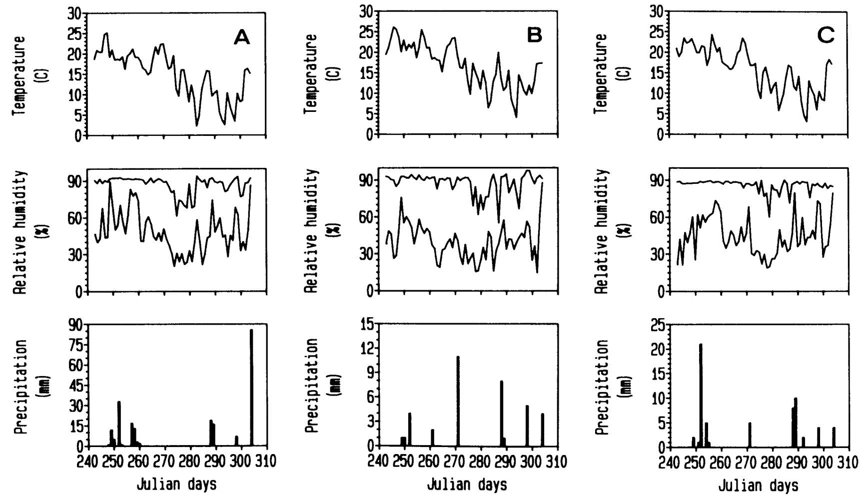

Fig. 1. Daily mean temperature, maximum and minimum relative humidity, and precipitation in **(A)** Brown, **(B)** Cowley, and **(C)** Osage counties, Kansas, in 1987.

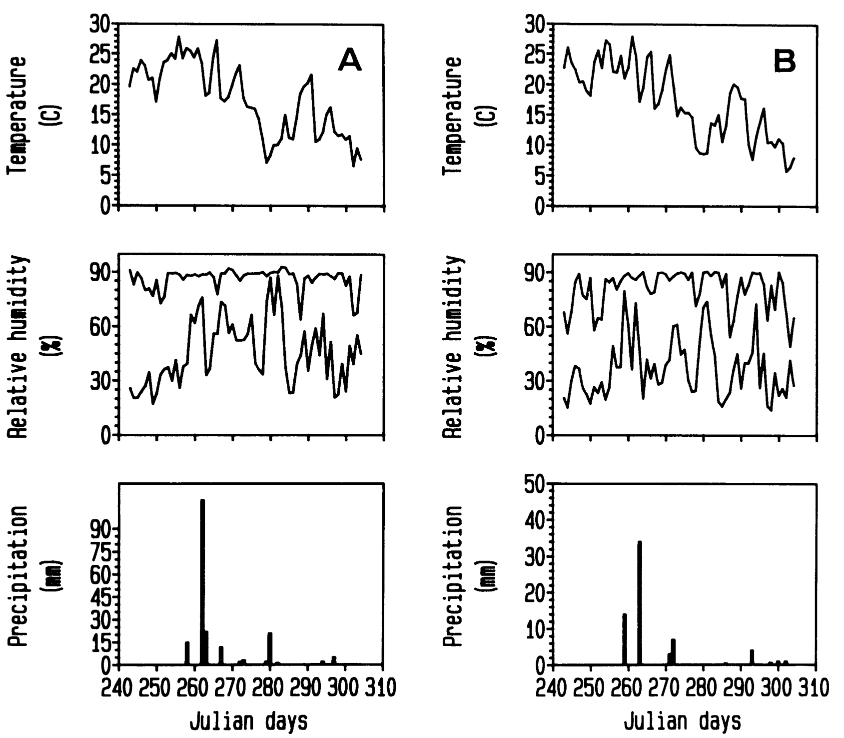

Fig. 2. Daily mean temperature, maximum and minimum relative humidity, and precipitation in **(A)** Manhattan and **(B)** Mound Valley, Kansas, in 1988.

DISCUSSION

A predictive method developed in Iowa (7,9), which uses inoculum of *Phomopsis* spp. on pods at soybean growth stage R6 as a correlative of the potential of the pathogen to enter the seeds, was tested under Kansas growing conditions. In addition to levels of *Phomopsis* spp. after harvest, the incidence of other seedborne pathogens was also measured.

In developing the Iowa method, McGee (7) often found it difficult to correlate pod and seed infection under conditions of natural infection in soybean fields. He suggested that this was due to the lack of prolonged periods of warm, wet weather, which are necessary for seed infection to occur within pods (1,6,12). This study supports his findings. At the time of year when soybeans are between R7 and harvest maturity (September and October), the relative humidity in Kansas seldom reaches 95% at night and regularly drops to the 25–40% range during the day (Figs. 1–3). In addition, there are frequently 7- to 10-day periods without rainfall, and average daily temperatures fall below 15 C by the beginning of October. Thus, even though pod infection levels were high enough (50% or more) to require spraying according to the model, weather conditions following sampling were such that the pathogen did not move to the seed.

Aspergillus spp. can cause significant seedling blight in Kansas (D. J. Jardine, *unpublished data*). A higher level of *Aspergillus* spp. was present in 1988, a year of extreme drought, than in 1987 or 1989. This is inconsistent with other research (2,5) in which the incidence of *Aspergillus* spp. was higher at high moisture levels. The higher level of seed germination following a benomyl spray in 1988 may be attributable in part to a reduction in levels of *Aspergillus* spp.

Wall et al (14) demonstrated that fungicide treatment has no effect on the germination of high-quality seeds. However, seeds from the same lot, with 15% mechanical damage, showed significant increases in germination percentages about half the time when treated with a fungicide, which suggests that mechanically damaged seeds may be more susceptible to seedborne pathogens. Seeds harvested in 1988 were also

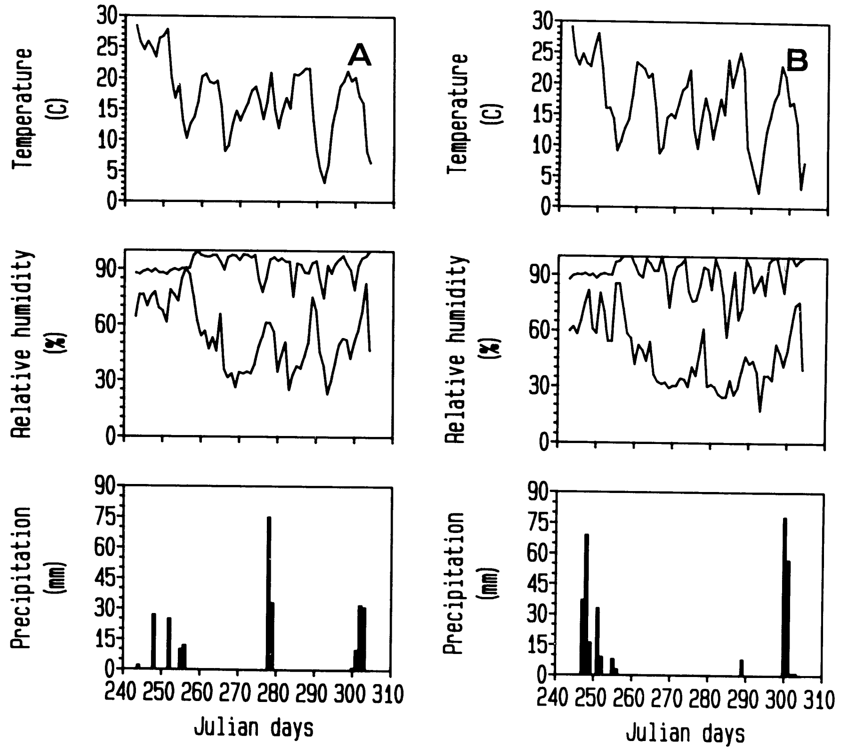

Fig. 3. Daily mean temperature, maximum and minimum relative humidity, and precipitation in **(A)** Manhattan and **(B)** Mound Valley, Kansas, in 1989.

low in quality as a result of mechanical damage related to low seed moisture (less than 10%) at harvest. The reduction of *Aspergillus* spp. on mechanically damaged seed from benomyl-treated plants may in part be responsible for the significant increase in germination observed in 1988.

Levels of *Alternaria* spp. were higher on seeds from benomyl-treated plants than on those from unsprayed plants in all 3 yr and were significantly higher in 1989. Following the suppression of disease organisms by the benomyl spray, species of *Alternaria* were apparently able to take advantage of reduced competition in recolonizing the seeds. The increased levels of *Alternaria* spp. did not affect the germination percentage, however.

Levels of *Penicillium* spp. were reduced each year following benomyl applications; the reduction was statistically significant in 1989. However, reductions in the level of *Penicillium* were not correlated with increases in germination.

The usefulness of this predictive method appears to be limited under normal Kansas growing conditions. Jordan et al (5) demonstrated that in Illinois, populations of seedborne pathogens varied from location to location and year to year, depending on environmental conditions. For instance, *Phomopsis* spp. were found at significantly higher levels most often under cool, wet conditions in northern Illinois, whereas *Cercospora kikuchii* (Matsumoto & Tomoyasu) M. W. Gardner, *Colletotrichum* spp., *Macrophomina phaseolina* (Tassi) Goidanich, and *Nematospora coryli* Peglion were associated with the hotter growing season in southern Illinois. Kansas is generally drier than Iowa and should have lower incidences of *Phomopsis* spp. This overall lower incidence could explain the lack of difference in levels of *Phomopsis* spp. between benomyl-treated and unsprayed plants. In years of excessive rainfall and high humidity, such as 1985 and 1986, the model may be useful. The lack of correlation between pod infection levels and both the incidence of *Phomopsis* spp. on seeds and the germination percentage in the 3 yr of this study would not warrant its general use by Kansas seed producers. As McGee suggested (7), the model's best use may be in identifying fields with low levels of pod infection, in which there would be very little risk of severe seed infection regardless of subsequent weather conditions. These fields would, therefore, not need to be sprayed. Because the autumn is normally drier in Kansas than in Iowa, the threshold of pod infection at which spraying becomes necessary may need to be adjusted upward. Further work on this possibility is needed.

ACKNOWLEDGMENT

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