Seed Mycoflora of Soybeans Relative to Fungal Interactions, Seedling Emergence, and Carry Over of Pathogens to Subsequent Crops

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ABSTRACT

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Quantitative estimates were made of fungi frequently found on soybean (*Glycine max*) seed in 175 seed samples of the cultivars Amsoy, Corsoy, Wayne, and Hodgson grown in Iowa in 1977. The genera studied were *Chaetomium*, *Paecilomyces*, *Alternaria*, *Fusarium*, *Penicillium*, *Aspergillus*, *Cladosporium*, *Cercospora*, and *Phomopsis*. Significant negative correlations were obtained between the amount of seed infected by *Cercospora kikuchii* and that infected by *Phomopsis*, *Fusarium*, or *Alternaria*, and a significant positive correlation occurred between seed infection by *Phomopsis* and *Fusarium*. *Phomopsis* seed infection was correlated with reduction of seedling emergence in laboratory and field tests. Seed infection by *Fusarium* was correlated with reduction in laboratory emergence only. No one of the other fungi was associated with reduced laboratory or field emergence. Cultivars did not differ significantly with regard to relationships between fungi on the seed or between seedborne fungi and seedling emergence. Infection levels for *C. kikuchii* and *Phomopsis* on planted seed were not correlated with those on seed harvested from these plants. Statistically significant correlation coefficients obtained in the study ranged from 0.3 to 0.6.

Additional key words: seed diseases.

More than 30 fungi are listed as being seedborne on soybeans (*Glycine max* [L.] Merr.) (2), ranging from major pathogens of the seed to nonpathogens. The *Phomopsis* spp. associated with soybean pod and stem blight and soybean seed decay (8) generally are recognized as a major cause of lowered soybean seed quality (2). *Cercospora kikuchii* (Mat. and Tomoy) Gardner, is ubiquitous on soybean seed in the midwestern USA, causing purple staining on seeds and damage to seed coats (11), but there is conflicting evidence regarding the effect of this fungus on seed germination (9,11,12,14). Other seedborne fungi such as *Peronospora manshurica* (Naoum.) Syd. ex Gäum. and *Colletotrichum truncatum* (Schw.) Andrus and W. D. Moore, the causal organisms of downy mildew and anthracnose, respectively, are known to reduce seed germination and to infect seedlings, but seed infection usually is not considered to be a major source of inoculum. *Fusarium* spp. and *Alternaria* spp. can be seedborne on soybeans (1,13), but the significance of seed infection with respect to establishment of disease has not been determined. Various other fungal pathogens of soybeans, although associated with soybean seeds, have never been shown to act as pathogens as a result of their presence on seeds. In the remainder of this paper, the abbreviation "sp." will not be used after generic names unless a particular species is being designated.

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Soybean seed mycoflora can be influenced by factors such as planting date (6), harvest date (18), geographic location (15), environment (11,12), and cultivar susceptibility to pathogens (11,12). Roy and Abney (12) also suggested that interactions may occur on seed between *Cercospora kikuchii* and either *Diaporthe* or *Alternaria*. The manipulation of interactions between seedborne fungi of soybeans could have considerable potential value as a means of controlling seedborne pathogenic organisms.

Carry-over of pathogens from planted seed to seed from the resulting plants is a concern of seed growers. Wilcox and Abney (17) showed that the percentage of *C. kikuchii* on planted seed had little effect on the percentage of infected seed in the resulting crop, but such information is lacking for other seedborne pathogens of soybeans.

The present study examined relationships among fungi present on soybean seed grown in Iowa, between seedborne fungi and seedling emergence in laboratory and field tests, and between fungi on planted seed and that on seed harvested from the resulting crop.

MATERIALS AND METHODS

Samples of soybean seeds grown in 1977 were obtained from growers in 56 counties in Iowa. Cultivars obtained were Amsoy, Corsoy, Wayne, and Hodgson; the numbers of samples of each cultivar were 50, 59, 35, and 31, respectively. The kinds of fungi present in each seed sample were quantitatively estimated in a modified blotter test. The seeds were surface-sterilized in 1% sodium hypochlorite for 30 sec, rinsed in sterile water, and placed on autoclaved blotters in boxes (25 cm × 15 cm and 4 cm deep). The blotter was moistened with 80 ml of distilled water containing 500 μg/ml of 2,6-dichloro-4-nitroaniline (Botran 75W) to suppress the growth of *Rhizopus*. Two boxes, each containing 50 seeds, were prepared for each seed sample. These were incubated at 25 C for 10 days in the dark. The number of seeds from which each kind of fungus grew was counted. If more than one fungus grew from the same seed, each was counted. The fungi were identified under a dissecting microscope. Relationships between fungi present on seeds were determined by using correlation coefficients.

Percentage emergence was determined 7 days after seeds were planted, 30 mm deep, in sterilized sand beds maintained at 25 C under constant light. Four lots of 100 seeds were tested for each sample. All seed samples also were planted in the field near Ames on 22 May 1978. Each seed lot was sown in plots consisting of two rows 0.75 m apart and 5 m long, with 100 seeds per row, in two randomized complete blocks. The number of emerged seedlings in each plot was counted on 7 June. Relationships between seed infection, laboratory emergence, and field emergence were determined by calculating correlation coefficients. Because the presence of *Phomopsis* might mask the effects of the other fungi, the correlation coefficients were recalculated by using only seed lots in which the level of *Phomopsis* seed infection was less than 6%.

Fifteen field plots of each cultivar, grown from seed lots that represented a range in laboratory seed infection levels of the two major seedborne pathogens, *C. kikuchii* and *Phomopsis*, were selected from the emergence experiment. These were harvested on 7 October and the seeds were tested quantitatively for *C. kikuchii* and *Phomopsis*, as previously described. Correlation coefficents were calculated for both *C. kikuchii* and *Phomopsis* between infection levels on planted seed of the selected plots and that on harvested seed. Inherent in the experimental design was the possibility that results might be affected by seedborne inoculum spreading into adjacent plots. Mean infection levels for *C. kikuchii* and *Phomopsis* on harvested seed, therefore, were calculated for groups of plots according to the total amount of *C. kikuchii* or *Phomopsis* infection on the seed planted in the two plots on either side of each plot. For this analysis, the two replicate plots of each seed lot were treated as individual plots. To test for interplot interference, mean infection levels were compared in relation to the amount of seedborne inoculum in adjacent plots.

TABLE 1. Correlations[a] between soybean seed infection[b] by *Cercospora kikuchii* and seed infection[b] by other fungi

Cultivar	*Phomopsis* spp.	*Fusarium* spp.	*Alternaria* spp.
Amsoy	−0.58***	−0.37**	−0.33*
Corsoy	−0.48***	−0.49**	−0.37**
Wayne	−0.60***	−0.51**	−0.46**
Hodgson	−0.46**	−0.31	−0.41
All Cultivars	−0.52***	−0.41***	−0.33***

[a]Statistical significance is indicated by: *, $P = 0.05$; **, $P = 0.01$; and ***, $P = 0.001$.
[b]Number of seed infected of 100 examined on modified blotter test.

RESULTS

Statistically significant negative correlations occurred between the amount of seed infection by *C. kikuchii* and that by *Phomopsis*, *Fusarium*, and *Alternaria*, respectively (Table 1). Similar correlation coefficents were obtained when calculated over all cultivars and for each cultivar, with the exception of Hodgson, in which *C. kikuchii* seed infection was not significantly correlated with that for *Alternaria* or *Fusarium*. Significant positive correlations occurred between seed infected by *Phomopsis* and by *Fusarium* when calculated over all cultivars (r = 0.32) and for Amsoy (r = 0.33) and Corsoy (r = 0.43), but the coefficients were not significant for Wayne and Hodgson. No other significant correlations were found between *C. kikuchii* or *Phomopsis* seed infection and that for any of the other of the commonly found fungi.

Laboratory emergence values over all cultivars ranged from 77.0 to 98.0% and averaged 93.8%, whereas field emergence ranged from 37.5 to 93.5% and averaged 82.1%. *Phomopsis* and *Fusarium* seed infections both were correlated with reduced laboratory emergence over all cultivars and for each cultivar with the exception of Wayne (Table 2). This relationship also occurred between *Phomopsis* seed infection and field emergence over all cultivars and for each cultivar, but *Fusarium* seed infection was not correlated with field emergence. *Cercospora kikuchii* seed infection was correlated with increased laboratory emergence over all cultivars and for Amsoy and Wayne, but this effect was not so clear in the field emergence values. Seed infection by the other fungi were not correlated with either laboratory or field emergence. When analyses were made of seed samples with *Phomopsis* infection levels of less than 6%, *Fusarium* seed infection again was correlated with reduced laboratory emergence over all cultivars ($r = -0.37$, $P = 0.01$), but no other significant correlations were detected between fungal seed infection and emergence.

Mean infection levels for *C. kikuchii* on harvested seed ranged from 47% on Corsoy to 57% on Wayne, with a value over all cultivars of 51%, while those for *Phomopsis* ranged from 3.0% on Wayne to 5.2% on Amsoy, with an overall mean of 4.1%. Mean infection levels over all cultivars for seed planted in these plots were 46 and 15.6% for *C. kikuchii* and *Phomopsis*, respectively. No significant correlations were obtained, however, between infection

TABLE 2. Correlation[a] of seedborne fungi[b] on laboratory germination[c] of soybean seed and field emergence of seedlings[d]

Fungus genera	Correlation coefficients[a] between seed infection[b] by each fungus and laboratory germination[c] and field emergence[d] for seed samples of the cultivars									
	All cultivars		Amsoy		Corsoy		Wayne		Hodgson	
	Lab.	Field	Lab.	Field	Lab.	Field	Lab.	Field	Lab.	Field
Chaetomium	0.06	−0.01	−0.04	0.03	−0.07	−0.14	−0.19	0.11	−0.12	−0.22
Paecilomyces	0.07	0.09	0.05	0.03	0.28	0.24	0.22	0.18	0.18	0.23
Alternaria	−0.16	−0.12	−0.13	−0.02	−0.14	−0.05	−0.15	−0.13	0.13	−0.24
Fusarium	−0.43***	−0.19	−0.44**	−0.11	−0.47**	−0.29	−0.27	−0.29	−0.56**	−0.02
Penicillium	0.16	0.05	0.21	0.06	0.25	0.28	0.01	0.02	−0.27	0.11
Aspergillus	0.05	−0.03	−0.15	−0.09	0.12	−0.07	−0.13	0.05	0.06	−0.24
Cladosporium	0.06	0.02	0.13	0.06	0.14	0.20	0.05	0.23	0.02	−0.01
Cercospora	0.41***	0.13	0.50**	0.03	0.29	0.18	0.57**	0.45**	0.31	−0.16
Phomopsis	−0.53***	−0.35***	−0.52**	−0.27*	−0.65***	−0.42***	−0.24	−0.36*	−0.41*	−0.37*

[a]Level of significance indicated by: *, $P = 0.05$; **, $P = 0.01$; and ***, $P = 0.001$.
[b]Number of seed infected of 100 examined on modified blotter test.
[c]Four lots of 100 seeds per sample were grown in sand benches, and the number germinated after 7 days were counted.
[d]Two two-row plots of each sample were seeded in the field, and percentage of plants emerged after 14 days was calculated.

levels on harvested seed and that on planted seed when calculated either over all cultivars or for each cultivar. Mean infection levels of either fungus on harvested seed also were not related to infection levels on the seed planted in adjacent plots (Table 3).

DISCUSSION

The association between *Phomopsis* seed infection and reduced seedling emergence, detected for all cultivars, confirmed previous knowledge about this fungus (2). Laboratory emergence data for three of the four cultivars tested has provided, however, new evidence that *Fusarium* seed infection may be related to reduced soybean seedling emergence in the USA. Seedling blights of soybean caused by *Fusarium* have been described in Iowa (3), Ontario (5), and the Far East (1), and a collar and pod rot caused by *Fusarium semitectum* occurs in India (13). Some of these workers indicated that the pathogens were seedborne (1,13), and were associated with reduced seed germination (13).

Although none of the other fungi commonly found on soybean seeds in this study had any adverse effect on emergence, some of them are pathogens of soybeans. *Aspergillus* can reduce seed viability in storage (16). *Penicillium* has been shown to cause disease when inoculated onto soybean seedlings (4). Nittler et al (10) suggested that *Alternaria alternata* is associated with low soybean seed quality. *Chaetomium* and *Cladosporium*, though seedborne on soybeans (7), have not been recognized as pathogens, and *Paecilomyces* has not been recorded previously on soybean seeds. Our data do not preclude the possibility, however, that, under more rigorous testing conditions such as laboratory cold germination tests or field planting in cold, wet soil, seed infection by some of these fungi might prove to be of more significance than indicated here.

Cercospora kikuchii has been associated with reduction in seed germination (9). In the present study, however, *C. kikuchii* seed infection was associated with increased seed germination. This effect can best be explained by relating it to the negative correlation obtained between *Phomopsis* and *C. kikuchii* seed infections. The increased germination correlated with higher *C. kikuchii* infection levels is most likely the result of lower infection levels of *Phomopsis*. These data, therefore, indicate that *Phomopsis* has a more severe effect on soybean seed germination than does *C. kikuchii*. This is an example of the complexity of the factors that influence both the seed mycoflora and seed germination. The low correlation coefficients generally obtained in this study when relating individual factors probably reflect the influence of several factors.

Roy and Abney (12) showed that *Phomopsis* seed infection could be reduced by inoculating soybean plants with *C. kikuchii* in field. The negative correlation between *C. kikuchii* and *Phomopsis* seed infections detected in this study is what would be expected if this interaction occurred under natural conditions. Although the relationship occurred consistently for all cultivars in the seed lots that were planted, it was not detected on seed harvested from the experimental plots. In the latter case, however, the overall sample size was reduced from 175 to 60 seed lots, and the range of infection levels for *Phomopsis* on the harvested seed (0–10%) was much narrower than that on the planted seed (0–54%). *Cercospora kikuchii* has limited value as a control agent of *Phomopsis* seed infection because it also is a pathogen of soybeans. However, other interactions between seedborne fungi, involving nonpathogenic fungi, may have potential in the biological control of pathogenic organisms.

Mean infection levels of *C. kikuchii* and *Phomopsis* on seed from harvested plots was not related to the amount of seedborne inoculum planted in adjacent plots, suggesting that interplot interference was a minor source of error in this experiment. The lack of correlation between *C. kikuchii* infection on harvested seed and that on planted seed is in agreement with the finding of Wilcox and Abney (17). *Cercospora kikuchii* seed infection levels were fairly high both on planted and harvested seed, and field conditions seemingly were favorable for infection by this fungus. On the other hand, *Phomopsis* infection levels on harvested seed were low, suggesting that environmental conditions may not have favored development of this disease from planted seed. Results obtained in 1979 (D. M. Garzonio and D. C. McGee, *unpublished*), however, from an experiment examining the relative importance of seed and soil as sources of *Phomopsis* inoculum, substantiate the conclusion of this study, that *Phomopsis* infection levels on planted seed are not related to that on harvested seed.

TABLE 3. *Phomopsis* and *Cercospora kikuchii* infection of harvested seed as related to seedborne inoculum of these fungi on seed planted adjacent to these plots in the field

Range of *Phomopsis* infection of seed planted adjacent to harvested plots[a,c]	Mean *Phomopsis* infection of harvested seed[b,c]	Range of *C. kikuchii* infection of seed planted adjacent to harvested plots[a,c]	Mean *C. kikuchii* infection of harvested seed[b,c]
0 – 20	4.3	0 – 80	50.7
21 – 50	3.9	81 – 120	50.8
50 – 100	3.5	121 – 160	52.1

[a] Total percentage infection of the fungus on the two seed-lots planted on either side of each harvested plot.
[b] Mean percentage infection of seed harvested from plots grouped according to amount of inoculum on the seed planted in adjacent plots.
[c] Infection levels for each fungus determined by testing 100 seeds per seed lot in a modified blotter test.

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