Relationships Among *Cercospora kikuchii*, Other Seed Mycoflora, and Germination of Soybeans in Puerto Rico and Illinois

P. R. HEPPERLY, Assistant Professor, Department of Crop Protection, University of Puerto Rico, Mayaguez 00708, and J. B. SINCLAIR, Professor, Department of Plant Pathology, University of Illinois, Urbana 61801

ABSTRACT

Hepperly, P. R., and Sinclair, J. B. 1981. Relationships among *Cercospora kikuchii*, other seed mycoflora, and germination of soybeans in Puerto Rico and Illinois. Plant Disease 65:130-132.

Seeds from similar field plots planted to four soybean (*Glycine max*) cultivars in Puerto Rico and Illinois were assayed for seedborne mycoflora. The predominant fungus recovered was *Cercospora kikuchii* from Puerto Rico seeds and *Phomopsis* sp. from Illinois seeds. Purple-stained seed had higher germination at normal harvest than unstained seed. *C. kikuchii* from Puerto Rico-grown seeds was antagonistic to seedborne *Fusarium* spp. and *Phomopsis* sp., which were recovered six and three times more often, respectively, from unstained than from purple-stained seeds. *C. kikuchii* and *Phomopsis* sp. were not antagonistic in Illinois seeds except when incidence of *C. kikuchii* exceeded 10%. Recoveries of *Fusarium* and *Phomopsis* increased and germination and recoveries of *C. kikuchii* decreased when harvest was delayed in Puerto Rico. Multiple regression equations related the occurrence of *C. kikuchii*, *Fusarium*, and *Phomopsis* to reduced soybean seed germination in Puerto Rico. In Illinois, variation in the incidence of *Phomopsis* explained most of the variation in germination.

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

The role of *Cercospora kikuchii* (T. Matsu. & Tomoyasu) Gardner, the causal fungus of purple seed stain, in soybean (*Glycine max* (L.) Merr.) seed health is poorly understood. Murakishi (4) reported 19% reduction in germination of seeds infected with *C. kikuchii*, while Sherwin and Kreitlow (7) stated that the fungus did not affect seed germination. Lehman (3) noted that although *C. kikuchii* seed infection did not affect germination, it did result in postemergence damping-off. Wilcox and Abney (11) showed that soybean seeds infected with *C. kikuchii* had lower germination and field emergence than uninfected seeds.

Recently, Roy and Abney (6) reported reduced incidence of seedborne *Phomopsis* sp. (*Diaporthe phaseolorum* var. *sojae*) when field plants were artificially inoculated with *C. kikuchii*. With *Phomopsis* inhibited, seeds from *C. kikuchii*-inoculated plants had higher germination rates than uninoculated controls. For this reason, *C. kikuchii* was suggested as a biological control for *Phomopsis* seed infections. We investigated the interactions between naturally occurring *C. kikuchii* and other seedborne fungi and their combined effects on germination of soybean seeds produced in Puerto Rico and Illinois.

Supported in part by the Puerto Rico and Illinois Agricultural Experiment Stations and U.S. Agency for International Development (AID) contract TA/c-1294. The views and interpretations are those of the authors and should not be attributed to AID or any individual acting on its behalf.

0191-2917/81/02013003/$03.00/0
©1981 American Phytopathological Society

MATERIALS AND METHODS

Four soybean cultivars—Bonus, Clark 63, Pickett, and Williams—were grown in plots of four 3-m rows in a randomized complete block design replicated four times at the University of Puerto Rico (UPR) substation at Isabela in 1976 (9). One center row was harvested at maturity; the second row was harvested 1 mo later (delayed harvest). Seeds were separated into those with and those without symptoms of purple seed stain. Four samples of 100 seeds were randomly selected from each seed group for each cultivar at each harvest.

Seeds were disinfected by immersion in a 0.5% NaOCl solution (10% Clorox) for 4 min and then plated on potato-dextrose agar (Difco). Germination and incidence of *C. kikuchii*, *Colletotrichum dematium* (Pers. ex Fr.) Grove var. *truncata* (Schw.) Arx., *Fusarium* spp., and *Phomopsis* sp. were recorded after 1 wk at 25 C.

A similar field plot (but substituting the cultivar Wells for Pickett) was planted at the Plant Pathology Research Center, University of Illinois, Urbana, in 1977. Due to the low incidence of purple seed stain, the harvested seeds were not separated into groups. All plants were harvested at maturity.

The data were analyzed for variance, using Fisher's least significant difference method for mean separation. Correlation coefficients were determined by the Spearman Rand method. Stepwise selection of independent variables was used for multiple linear regression analysis. Germination was considered a dependent variable predicted by various mycoflora (independent variables). The independent variables for seeds grown in UPR-Isabela were *C. kikuchii*, *Colletotrichum dematium* var. *truncata*, *Fusarium* spp., and *Phomopsis* sp.; for seeds grown in Illinois, incidence of *Phomopsis* sp. infection (moldy seed) and total incidence of seedborne fungi were added to the model.

RESULTS

The fungi isolated most frequently from the soybean seeds grown in Puerto Rico were *C. kikuchii*, *Phomopsis* sp., and *Fusarium* spp. (mostly *F. semitectum*). Incidence of each fungus differed between purple-stained and unstained seed (Table 1). *C. kikuchii* was isolated more often from stained seed (90%) than from unstained seed (30%). Conversely, *Fusarium* and *Phomopsis* were isolated less frequently in stained than unstained seeds.

Germination and incidence of the three

Table 1. Mean germination and incidence of seedborne fungi in soybean seeds grown at Isabela, Puerto Rico, 1976

Seed sample	Germination (%)[a]	Incidence (%)[a]		
		C. kikuchii	*Fusarium* spp.	*Phomopsis* sp.
Purple-stained[b]	...[c]	90.6	2.8	2.7
Unstained[b]	...[c]	30.6	8.8	17.1
Harvested at maturity[d]	90.7	62.8	2.3	4.8
Delayed harvest (1 mo)[d]	32.5	52.5	10.6	16.6

[a] Numbers in each pair are significantly different ($P = 0.0001$).
[b] Combined over cultivars and harvest dates.
[c] Comparison of means is invalid because of highly significant interaction of seed stain × harvest date with respect to germination.
[d] Combined over cultivars and seed staining. Each mean represents 3,200 seeds from four cultivars (Bonus, Clark 63, Pickett, and Williams).

fungi changed when harvest was delayed. Germination and incidence of *C. kikuchii* were lower from delayed-harvest seeds; incidence of *Fusarium* and *Phomopsis* increased (Table 1).

Of the four soybean cultivars grown in Puerto Rico, Bonus had the least infection of *C. kikuchii* (Table 2). Incidence of both *Fusarium* and *Phomopsis* was greatest in Pickett seeds harvested 1 mo after maturity.

The fungi recovered most frequently from Illinois-grown seeds were *Phomopsis* sp., *C. kikuchii*, and *Colletotrichum dematium* var. *truncata*. Incidence of *C. kikuchii* ranged from 0 to 20%, and most lots averaged less than 10%. No overall correlation was found between the occurrence of *C. kikuchii* and *Phomopsis* sp. ($r = -0.13$), but in 12 seed lots where *C. kikuchii* incidence exceeded 10%, a significant ($P = 0.05$) negative correlation ($r = -0.61$) was found (Table 3).

In seeds from Puerto Rico, a significant ($P = 0.0001$) negative correlation was found between the incidences of *C. kikuchii* and *Phomopsis* sp. ($r = -0.59$) (Table 3). The occurrence of *Fusarium* and *Phomopsis* was highly correlated ($P = 0.01$) ($r = 0.87$). In both Illinois and Puerto Rico seeds, the occurrence of *Phomopsis* was negatively correlated ($P = 0.01$) with germination ($r = -0.87$ and -0.59, respectively). In Illinois seeds, low significant correlations ($P = 0.01$) were found between *Colletotrichum dematium* var. *truncata* or *C. kikuchii* and germination ($r = -0.26$ and 0.19, respectively).

In Illinois seed lots, incidence of *Phomopsis* sp. accounted for most of the variation in seed lot germination (Table 4). In Puerto Rico seeds, no single variable controlled the majority of variation in germination.

DISCUSSION

In temperate regions of the United States and Canada, soybean germination and quality have been closely related to the incidence of *Phomopsis* seed infection (1,2,8,10). Delay in normal harvesting increases incidence of *Phomopsis* and lowers germination (12). Linear correlation coefficients between germination rates and seedborne incidence of *Phomopsis* have varied from $r = -0.8$ to $r = -1.0$. That is, *Phomopsis* appears to account for over two-thirds of the variability in soybean germination in these studies.

The relationship of *Phomopsis* to seed germination is less well documented in tropical climates. In Puerto Rico, Paschal and Ellis (5) used incidence of *Phomopsis* as an indicator of resistance to field deterioration of soybean introductions. Their results showed a lower correlation between *Phomopsis* and germination than those found in temperate regions: *Phomopsis* accounted for only slightly more than one-third of the variability in germination. Other factors may be important in determining germination of seeds produced in humid tropical conditions.

In addition to *Phomopsis* ($r = -0.59$), *Fusarium* spp. (mostly *F. semitectum*) also had high negative correlation ($P = 0.01$) with soybean germination ($r =$

Table 2. Mean germination and incidence of seedborne fungi in soybean seeds grown at Isabela, Puerto Rico, 1976, by cultivar, seed type, and harvest date[a]

		Harvest date							
		Harvested at maturity				Delayed harvest			
Cultivar	Seed type	G	Ck	F	P	G	Ck	F	P
Bonus	Purple-stained	95.8	76.3	1.3	0.3	42.1	70.3	2.8	2.0
	Unstained	94.0	14.5	2.3	1.5	56.3	6.0	6.3	6.8
Clark 63	Purple-stained	94.8	95.8	1.0	1.0	37.3	95.0	1.5	2.8
	Unstained	88.3	46.5	4.5	13.5	52.7	38.3	9.8	18.0
Pickett	Purple-stained	88.3	88.3	0.8	1.5	9.0	80.3	10.8	18.5
	Unstained	76.0	28.8	1.8	12.3	4.3	15.5	26.0	55.0
Williams	Purple-stained	92.8	99.0	1.0	1.8	39.5	95.5	3.8	3.0
	Unstained	91.5	47.0	5.5	7.3	52.0	26.0	9.5	12.3

[a] Each value represents the mean of four seed lots of 100 seeds each. G = germination (%), Ck = incidence of *Cercospora kikuchii* (%), F = incidence of *Fusarium* spp. (%), and P = incidence of *Phomopsis* sp. (%).

Table 3. Coefficients of linear correlation (r) between seed assay variables in soybean seed lots from Puerto Rico and Illinois

Variables	Illinois[a]	Puerto Rico[a]
Phomopsis sp. and *Colletotrichum dematium* var. *truncata*	0.00 NS[b]	ND[c]
Phomopsis sp. and *Cercospora kikuchii*	−0.13 NS[d]	−0.59**[e]
Phomopsis sp. and *Fusarium* sp.	ND	0.87**
Phomopsis sp. and germination	−0.87**	−0.59**
Fusarium spp. and germination	ND	−0.71**
C. kikuchii and germination	0.19**	0.12 NS
Colletotrichum dematium var. *truncata* and germination	−0.26**	ND

[a] Coefficients of correlation are based on 64 seed lots.
[b] NS indicates no statistical significance of the coefficient of determination at $P = 0.05$.
[c] When many seed lots had no recovery of a particular fungus, correlation was not done (ND).
[d] From 12 seed lots with *C. kikuchii* incidence of 10% or more, *Phomopsis* sp. was negatively correlated with *C. kikuchii* ($r = -0.61$) ($P = 0.05$).
[e] ** = highly significant ($P = 0.001$).

Table 4. Linear regression equations for predicting soybean seed germination from seedborne incidence of various fungi and production of moldy appearing seed

Seed sample	Regression equation[a]	R^2
	Illinois seeds	
All seeds	G = 99.8 − 1.08 P	0.86
	G = 97.7 − 0.80 P − 0.97 Ms	0.90
	G = 100.2 − 0.57 P − 0.81 Ms − 0.26 Tf	0.91
	Puerto Rico seeds	
Normal harvest	G = 95.2 − 0.93 P	0.53
	G = 94.0 − 1.14 P + 0.98 F	0.61
Delayed harvest	G = 46.6 − 1.33 F	0.31
	G = 104.6 − 3.36 F − 0.63 Ck	0.72
	G = 107.7 − 2.40 F − 069 Ck − 0.59 P	0.79
Purple-stained seed	G = 87.7 − 7.61 F	0.61
	G = 92.1 − 4.40 F − 5.06 F	0.68
	G = 162.8 − 5.54 F − 4.63 P − 0.75 Ck	0.71
Unstained seed	G = 93.9 − 1.66 P	0.82
	G = 96.3 − 0.89 P − 1.77 F	0.88
	G = 85.9 − 0.84 P − 1.66 F + 0.29 Ck	0.90

[a] G = germination, Ck = *Cercospora kikuchii*, F = *Fusarium* spp., P = *Phomopsis* sp., Ms = moldy appearing seed, and Tf = total seedborne fungi. All equations are significant at $P = 0.0001$.

−0.71) in Puerto Rico (Table 3). When incidences of *Phomopsis*, *Fusarium*, and *C. kikuchii* were used in multiple regression equations, more of the germination variability was explained for Puerto Rico-produced seed (Table 4). Thus, more fungi must be considered in order to explain germination variability in seed from Puerto Rico than in seed from Illinois.

Roy and Abney (6) reported that artificial inoculation of *C. kikuchii* reduced incidence of *Phomopsis* seed infections. We found that natural *C. kikuchii* infection reduced both *Phomopsis* sp. and *Fusarium* spp. in Puerto Rico. Antagonism between *C. kikuchii* and other seed mycoflora was not apparent in Illinois seed, probably because of the low incidence of *C. kikuchii*.

Both *Phomopsis* sp. and *Fusarium* spp. increased in incidence after delayed harvest in Puerto Rico. Incidence of *C. kikuchii*, however, decreased when harvest was delayed, possibly because of increased competition from the other seedborne fungi. Although *C. kikuchii* is not known to cause seed deterioration in delayed harvest, germination of purple seed containing more than 90% *C. kikuchii* and few other fungi dropped appreciably in delayed harvest under tropical conditions. The reasons for delayed harvest losses in purple-stained seed deserve further study.

ACKNOWLEDGMENT

We thank F. D. Tenne for his cooperation in obtaining the soybean seeds grown in Puerto Rico.

LITERATURE CITED

1. ELLIS, M. A., C. C. MACHADO, C. PRASARTSEE, and J. B. SINCLAIR. 1974. Occurrence of *Diaporthe phaseolorum* var. *sojae* (*Phomopsis* sp.) in various soybean seedlots. Plant Dis. Rep. 58:173-176.
2. FOOR, S. R., F. D. TENNE, and J. B. SINCLAIR. 1976. Occurrence of seedborne microorganisms and germination in culture for determining seed health in soybeans. Plant Dis. Rep. 60:970-973.
3. LEHMAN, S. G. 1950. Purple stain of soybean seeds. N.C. Agric. Exp. Stn. Bull. No. 369. 11 pp.
4. MURAKISHI, H. H. 1951. Purple seed stain of soybean. Phytopathology 41:305-318.
5. PASCHAL, E. H., and M. A. ELLIS. 1978. Variation in seed quality characteristics of tropically grown soybeans. Crop Sci. 18:837-840.
6. ROY, K. W., and T. S. ABNEY. 1977. Antagonism between *Cercospora kikuchii* and other seedborne fungi of soybeans. Phytopathology 67:1062-1066.
7. SHERWIN, H. S., and K. W. KREITLOW. 1952. Discoloration of soybean seeds by the frogeye fungus, *Cercospora sojina*. Phytopathology 42:568-572.
8. TENNE, F. D., C. PRASARTSEE, C. C. MACHADO, and J. B. SINCLAIR. 1974. Variation in germination and seedborne pathogens among soybean seedlots from three regions in Illinois. Plant Dis. Rep. 58:411-413.
9. TENNE, F. D., and J. B. SINCLAIR. 1978. Control of internally seedborne microorganisms of soybeans with foliar fungicides in Puerto Rico. Plant Dis. Rep. 62:459-463.
10. WALLEN, V. R., and W. L. SEAMAN. 1963. Seed infection of soybean by *Diaporthe phaseolorum* and its influence on host development. Can. J. Bot. 41:13-21.
11. WILCOX, J. R., and T. S. ABNEY. 1973. Effects of *Cercospora kikuchii* on soybeans. Phytopathology 63:796-797.
12. WILCOX, J. R., F. A. LAVIOLETTE, and K. L. ATHOW. 1974. Deterioration of soybean seed quality associated with delayed harvest. Plant Dis. Rep. 58:130-133.