Influence of Soybean Genotype on Rate of Seed Maturation and Its Impact on Seedborne Fungi

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ABSTRACT

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Soybean (*Glycine max*) maturation rate, measured as the length or duration of late-season growth stage intervals and rate of moisture loss, was studied in a diverse group of soybean genotypes (plant introductions and adapted cultivars) to determine the role of plant and seed dry down on *Phomopsis* spp. and *Cercospora kikuchii*. A modified scale for late-season growth stages was developed and used to identify pod and seed maturation intervals after physiological maturity ($R7_1$, $R7_2$, and $R7_3$, identified by 1, 25, and 50% of all pods with mature pod color, respectively). Length of the $R7_1$–R8 period during major pod and seed dry down was associated consistently with the incidence of seed infection. Among genotypes with near-identical maturities, soybeans resistant to seedborne diseases had shorter $R7_1$–R8 intervals and a greater rate of moisture loss than susceptible soybeans. Incidence of pod infection by *Phomopsis* spp. or *C. kikuchii* was similar for soybean entries that matured under similar environmental conditions, regardless of their seed susceptibility under natural field conditions or to inoculation with *P. sojae* or *C. kikuchii*. Only susceptible genotypes showed a rapid increase in seed infection between $R7_1$ and R8, when pod and seed moisture decreased from 30–35% to 15–18%.

The most common diseases of soybean (*Glycine max* (L.) Merr.) seed in Indiana are caused by *Phomopsis* spp. (*P. sojae* S. G. Lehman and *P. longicolla* T. W. Hobbs) and *Cercospora kikuchii* (Matsumoto & Tomoyasu) M. W. Gardner (2,9). Identification of cultivar resistance to these seed pathogens has been difficult because the time of maturity (R8) and environmental conditions between the yellow pod and mature pod stages (R6–R8) influence fungal infection (3,10). Despite these limitations, differences in susceptibility to seed diseases have been reported for cultivars of several maturity groups (2,5,10). Although resistance or tolerance has not been characterized genetically, differences among cultivars suggest that some mechanism that limits fungal invasion of seeds is associated with genotypes that show a low incidence of infected seeds. Attempts to associate soybean plant features (growth habit, pubescence, hard seededness, etc.) with resistance to seed infection (2,5,10) have not given evidence for a direct influence of such features on the development of seed diseases in genotypes that mature in the same environment. However, the observation of seed maturation effects on fungal infection (T. S. Abney and L. D. Ploper, *unpublished*) and results (1) from studies on the effect of growth regulators suggest that modifications in the rate of late-season maturation affect seed infection.

Our objectives in this study were to describe the rate of pod and seed maturation and determine if it is a characteristic that influences the development of seed diseases after inoculation with *P. sojae* or *C. kikuchii*. Two preliminary reports have been published (6,7).

MATERIALS AND METHODS

Field experiments were conducted in 1985 and 1986 at Lafayette, IN. Twelve selected soybean entries representing diverse germ plasm were paired in six sets of genotypes of similar maturity (days to R8). Each member of the pair gave an opposite reaction to seed infection by species of *Phomopsis* and *C. kikuchii* in preliminary tests at Lafayette. Genotypes with consistent disease reactions in 1984 and previous years (6) (T. S. Abney, *unpublished*) were included and initially classified as susceptible if seed infection incidence in 1983 and 1984 was higher than 10% for *Phomopsis* spp. and 15% for *C. kikuchii*. Based on this preliminary classification, resistant entries (PI 404169A, PI 416946, PI 417274, PI 417460, Gnome, and PI 80837) and susceptible entries (PI361093, Miami, Amsoy 71, PI 417520, PI 361065B, and PI 361095) were paired for maturity and represented maturity groups I, II, II, II, II, and IV, respectively. The group II pairs differed in subgroup maturity (e.g., pair 2 was an early group II, whereas pair 5 was a late group II).

Soybeans were planted in a randomized complete block with three replications on 15 May 1985 and on 23 May 1986 at a seeding rate of 25–30 seeds per meter. Each plot consisted of four rows, each 2 m long and 0.76 m apart. A split-split plot design was used, with paired entries as main plots, disease reaction of entries as subplots, and treatments as sub-subplots. The three treatments included were a control and inoculation with *P. sojae* or *C. kikuchii*. Inoculum for each treatment was applied to stems and leaves until runoff with a hand-held, CO_2-powered sprayer. Inoculum of *C. kikuchii* ($1.5–2.0 \times 10^5$ conidia per milliliter of water) prepared from cultures grown on V8 juice agar was applied to plants at the R2 (full bloom) growth stage. A composite of five highly virulent seed isolates (9) of *C. kikuchii* (IN-C4, KY39, ATTC 36864, NC91, and DE9) was used. Inoculum of *P. sojae* (5×10^6 conidia per milliliter of water) prepared from cultures grown on potato-dextrose agar (PDA) was applied to plants first at the R5 and again at the R6 growth stages. A single-spored seed isolate of *P. sojae* (83-MS3) was used. This seed isolate of *P. sojae* was used in preliminary pathogenicity tests (T. S. Abney and L. D. Ploper, *unpublished*) and is characterized by its early, dense pycnidial development and abundant conidia, which distinguished it from the majority of the isolates of *P. sojae* or *P. longicolla* recovered from soybean seeds in Indiana. *P. longicolla* was not used in the inoculation treatments because a highly virulent isolate had not been identified in our pathogenicity tests. Also, *P. sojae* is the predominant *Phomopsis* species isolated from Indiana soybean seeds (T. S. Abney, *unpublished*).

The occurrence of reproductive growth stages (4) was recorded to identify the rate of maturation throughout the season. A modified version of the scale for the R7–R8 dry-down interval (critical to fungal invasion of seeds) was used. We subdivided the R7–R8 interval into three substages ($R7_1$, $R7_2$, and $R7_3$, with 1, 25, and 50% pods with mature pod color [i.e., brown or black], respectively). Only data from control (uninoculated) treatments were used to compare rates of

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maturation among the 12 entries. Moisture content of pods and seeds and seed dry weight were determined at growth stages R6, $R7_1$, $R7_3$, and R8. Pod samples (one sample per selected entry and replication) were taken at each growth stage mentioned above to establish the rate of moisture loss. The entries PI 404169A, PI 361093, PI 417274, Amsoy 71, Gnome, PI 361065B, PI 80837, and PI 361095 (maturity pairs 1, 3, 5, and 6) were used both years. Two additional entries (PI 417460 and PI 417520, maturity pair 4) were included in 1986. One hundred pods were collected from control plants at growth stages R6, $R7_1$, $R7_3$, and R8, immediately hand-shelled, and their moisture content and that of seeds was determined gravimetrically by drying at 105 C for 24 hr.

Fungal infection by *C. kikuchii* and *Phomopsis* spp. (*P. sojae* and *P. longicolla*) of pods and seeds at growth stages R6, $R7_1$, $R7_3$, and R8 was recorded to determine disease progress in pods and seeds of inoculated and uninoculated or control treatments. Single pods containing seeds were collected randomly from the main stems of 10 different plants in the middle two rows of each plot. Pod and seed samples were separated, surface-disinfested by immersing pods and shelled seeds in 95% ethanol for 20 sec and 1% sodium hypochlorite for 1 min, and then placed on PDA in 9-cm-diameter culture dishes. After incubation for 7 days at 24–26 C, the samples were evaluated for fungal growth. All pod and seedborne microorganisms were recorded; however, only infections by *C. kikuchii* and *Phomopsis* spp. will be presented because they were the predominant seed pathogens. The use of *Phomopsis* spp. includes both *P. sojae* and *P. longicolla* for the data in this study and should not be interpreted as a new or unnamed species of *Phomopsis*. A harvest (R8) seed sample (mechanically threshed) from the two center rows of each plot also was used for a bulk seed assay to enhance the R8 hand-shelled seed sample data. One hundred seeds per plot of the mechanically harvested sample were assayed for seedborne fungi as described above.

RESULTS

The R6–R8 growth stage interval and two subintervals ($R7_1$–R8 and $R7_3$–R8) differentiated the soybean entries initially classified as resistant and susceptible to seed infection by *Phomopsis* spp. and/or *C. kikuchii* (Table 1), although differences were not significant in all maturity pairs. There were differences in duration of the R6–$R7_1$ period between resistant and susceptible entries (Table 2), but for maturity pairs 4 (PI 417460 and PI 417520) and 5 (Gnome and PI 361065B), the susceptible entries had shorter intervals in both 1985 and 1986. Duration of the $R7_1$–$R7_3$ interval was approximately equal for resistant and susceptible entries.

The mean moisture contents of pods at growth stages R6, $R7_1$, $R7_3$, and R8 were 75, 64, 36, and 16%, respectively, for 1985 and 73, 62, 33, and 15%, respectively, for 1986. The average moisture content of seeds across all entries at the green bean stage (R6) was 70% in 1985 and 69% in 1986. At physiological maturity ($R7_1$), the mean moisture content had decreased to 55% in 1985 and 57% in 1986. At $R7_3$, the average moisture content had declined to 34 and 40% in 1985 and 1986, respectively. When plants reached the R8 growth stage, the mean moisture content for 1985 and 1986 was 15 and 18%, respectively. Moisture content in samples after $R7_1$ was higher in 1986 than in 1985.

Moisture content for both pods and seeds decreased linearly after $R7_1$ (physiological maturity), as determined by regression analysis over time (days after planting). The regression coefficient *b* (in percent moisture per day) was used as a measurement of the rate of moisture loss between $R7_1$ and R8. Regression coefficients (Table 3) for pods and seeds of resistant and susceptible entries within maturity pairs were compared (using the *t* test for homogeneity) to determine if entries classified as resistant to seed infection had a higher rate of moisture loss than entries classified as susceptible. Within maturity group I (PI 404169A and PI 361093), pods and seeds of both entries lost moisture at approximately the same rate. Within maturity group II, entries with resistance to seed infection (PI 417274 and Gnome in 1985 and PI 417460 in 1986) had significantly higher rates of moisture loss in both pods and seeds than the susceptible entries with which they were paired (Amsoy 71 and PI 361065B in 1985 and PI417520 in 1986), except for pods of PI 417274 vs. Amsoy 71 in 1986. Within maturity group IV, resistant PI 80837 had higher regression coefficient values for both pods and seeds than susceptible PI 361095 both years, but these differences were significant only in 1986.

Under natural conditions (uninoculated control), *Phomopsis* spp. were the most commonly isolated microorganisms from either pods or seeds both years. Both *P. sojae* and *P. longicolla* occurred, but *P. sojae* was the predominant species infecting both pods and seeds. *C. kikuchii* also was prevalent on pods but did not cause a high incidence of seed infection. The 1985 data will be presented to emphasize the incidence of both pod and seed infection by these two fungal genera. Weather conditions during seed maturation were more favorable for fungal seed infection in early maturing soybeans in 1985 than in 1986. Climatological data for West Lafayette, IN, recorded at the Purdue Agronomy Farm show that precipitation during the period 15 August to 15 October in 1985 and 1986 was 21 and 23 cm, respectively; however, in 1985 precipitation occurred more uniformly throughout this period than in 1986. In 1986, precipitation occurred intermittently and with relatively high levels (≥1.6 cm) on four dates after mid-September. Precipitation in June, July, and August in 1985 was 27.5 cm, whereas for that same period in 1986, it was 20.6 cm. The precipitation average over several years for this 3-mo interval is 31.4 cm.

Frequency of seed infection by *Phomopsis* spp. or *C. kikuchii* at the R8 growth stage in control plants essentially substantiated the initial classification of the entries PI 404169A, PI 416946, PI 417274, PI 417460, Gnome, and PI 80837 as resistant and PI 361093, Miami,

Table 1. Number of days between reproductive growth stages for 12 soybean entries

Growth stage interval[x]	Mean[y]	Range	Resistant entries[z]	Susceptible entries
1985				
R6 – $R7_1$	20.3	12.0 – 26.3	19.2	21.4
$R7_1$ – $R7_3$	5.0	3.3 – 6.0	5.0	5.1
$R7_3$ – R8	6.7	4.3 – 12.7	5.2	8.2
$R7_1$ – R8	11.8	8.7 – 18.7	10.2	13.3
R6 – R8	32.1	23.7 – 38.3	29.4	34.7
1986				
R6 – $R7_1$	14.0	10.0 – 20.7	14.2	13.7
$R7_1$ – $R7_3$	4.2	2.3 – 7.3	3.4	5.0
$R7_3$ – R8	5.2	3.7 – 8.0	4.6	5.9
$R7_1$ – R8	9.4	7.0 – 13.7	7.9	10.9
R6 – R8	23.4	17.0 – 28.7	22.2	24.6

[x] R6 = Full green bean seed stage, $R7_1$ = physiological maturity (1% pods with mature pod color), $R7_3$ = intermediate maturity (50% pods with mature pod color), and R8 = full maturity (95% pods with mature pod color).

[y] Data are means of three replications for the control treatment.

[z] Seed infection reaction to *Phomopsis* spp. and *Cercospora kikuchii*. Resistant entries: PI 404169A, PI 416946, PI 417274, PI 417460, Gnome, and PI 80837. Susceptible entries: PI 361093, Miami, Amsoy 71, PI 417520, PI 361065B, and PI 361095.

Amsoy 71, PI 417520, PI 361065B, and PI 361095 as susceptible to *Phomopsis* spp. and *C. kikuchii* (Figs. 1 and 2, Table 4). This initial classification also was substantiated for most of the soybean entries by frequency of seed infection in inoculated plants with the exceptions of PI 404169A and PI 416946 and Gnome. Of these entries classified as resistant, PI 404169A and PI 416946 had the least resistance to seed infection by *Phomopsis* spp. (20 and 19%, respectively, with inoculation), but in each case, they had a lower incidence of seed infection than the susceptible entry with which they were paired (PI 361093 with 43% and Miami with 44%, respectively). The remaining resistant entries had a very low incidence of seed infection by *Phomopsis* spp. (<6%). Susceptible entries had natural incidence of seed infection by *Phomopsis* spp. ranging from 10% in PI 361065B to 37% in PI 361093. When inoculated with *P. sojae*, seed infection increased by an average of 20% and ranged from 6% in PI 361093 to a 35% increase in Amsoy 71. For *C. kikuchii*, the genotypes classified as resistant had less than 5% seed infection in uninoculated plants and less than 14% in inoculated plants, with the exception of PI 416946 and Gnome, which showed unexpected susceptibility to the 1985 inoculation (38 and 50%, respectively). Seed infection by *C. kikuchii* in susceptible entries averaged 6% in the control treatment and increased an average of 49% because of *C. kikuchii* inoculation.

Inoculation of plants with *P. sojae* significantly increased recovery of *Phomopsis* spp. from seed (27% compared with 16% in the control), whereas inoculation with *C. kikuchii* significantly reduced levels of naturally occurring *Phomopsis* spp. (6%). This pattern was evident in both resistant and susceptible genotypes, but the resistant entries as a group did not show a significant increase in seed infection attributable to inoculation. In susceptible entries, *P. sojae* inoculation significantly decreased natural seed infection by *C. kikuchii* to 2% compared with 6% in the control.

Incidence of pod and seed infection in 1985 by *P. sojae* and *C. kikuchii* at the R6, $R7_1$, $R7_3$, and R8 growth stages is presented in Table 4. Pod infection was extensive at the R6 and $R7_1$ growth stages, except for *C. kikuchii* in the control treatment. At $R7_1$, for both *Phomopsis* spp. and *C. kikuchii* (with and without inoculation), differences in pod infection between resistant and susceptible entries were not significant. At $R7_3$, differences in pod infection for uninoculated resistant and susceptible entries were not significant; however, at R8, significant differences between resistant and susceptible entries were noted for both pathogens.

The entries resistant to seed infection had a higher incidence of *Phomopsis* spp. in pods compared with entries susceptible to seed infection, whereas pod infection by *C. kikuchii* was higher in entries susceptible to seed infection compared with resistant entries. In the inoculated plants, susceptible entries had significantly higher pod infection by *Phomopsis* spp. than resistant ones at $R7_3$ but no significant differences for pod infection by *C. kikuchii*. At R8, both *Phomopsis* spp. and *C. kikuchii* were significantly higher in pods of the entries susceptible to seed infection than in resistant entries.

Under natural conditions, a marked decline in the incidence of pod infection by *Phomopsis* spp. occurred between growth stages R7 and R8 (from 59 to 36% in the resistant entries and from 61 to 29% in the susceptible entries). Only a small decrease was observed in the pods from inoculated plants. From the data presented, it is evident that the fungi (*Phomopsis* spp. or *C. kikuchii*) isolated from pods of resistant and susceptible genotypes were similar during the critical stage when the seed is infected and did not account for the differences in seed infection reaction.

Seeds were not infected substantially before physiological maturity ($R7_1$) in inoculated and uninoculated plants. Between growth stages R6 and $R7_1$, seed infection by *Phomopsis* spp. and *C. kikuchii* increased in susceptible entries, with the largest increase occurring in seeds from inoculated plants. At $R7_1$, seed infection by *Phomopsis* spp. in resistant entries ranged from 0 to 3% in uninoculated plants and 0 to 14% in inoculated plants, whereas susceptible entries ranged from 0 to 30% in uninoculated plants and 0 to 46% in inoculated plants. At the same growth stage, seed infection by *C. kikuchii* in resistant entries ranged from 0 to 1% and from 0 to 70% in uninoculated and inoculated plants, respectively; susceptible entries ranged from 0 to 11% and 17 to 79% in uninoculated and inoculated plants, respectively.

Maturation rate was measured either as the duration of selected growth stage

Table 2. Duration (days) of growth stage intervals for six pairs of soybean entries with similar maturity but opposite reaction to seed infection

Interval[x] and disease reaction[y]	Soybean entries paired for maturity (maturity group)					
	1(I)	2(II)	3(II)	4(II)	5(II)	6(IV)
1985						
R6 – $R7_1$						
Resistant	12.0 a[z]	13.0 a	19.0 a	26.0 b	26.3 b	19.0 a
Susceptible	16.0 b	23.0 b	26.0 b	20.7 a	19.7 a	23.3 b
$R7_1$ – $R7_3$						
Resistant	5.3 a	5.0 a	4.7 a	4.3 a	5.7 a	5.0 a
Susceptible	4.7 a	3.3 a	5.0 a	5.7 a	6.0 a	5.7 a
$R7_1$ – R8						
Resistant	11.7 a	10.7 a	9.3 a	8.7 a	10.3 a	10.7 a
Susceptible	11.0 a	12.0 a	13.0 b	13.7 b	18.7 b	11.3 a
$R7_3$ – R8						
Resistant	6.3 a	5.7 a	4.7 a	4.3 a	4.7 a	5.7 a
Susceptible	6.3 a	8.7 b	8.0 b	8.0 b	12.7 b	5.7 a
R6 – R8						
Resistant	23.7 a	23.7 a	28.3 a	34.7 a	36.7 a	29.7 a
Susceptible	27.0 b	35.0 b	39.0 b	34.3 a	38.3 a	34.7 b
1986						
R6 – $R7_1$						
Resistant	10.0 a	10.0 a	14.7 a	14.3 b	20.7 b	15.7 a
Susceptible	10.0 a	14.0 b	15.3 a	12.3 a	14.3 a	16.3 a
$R7_1$ – $R7_3$						
Resistant	3.3 a	3.3 a	2.3 a	4.0 a	3.7 a	3.7 a
Susceptible	4.0 a	4.0 a	5.3 b	5.3 a	7.3 b	4.0 a
$R7_1$ – R8						
Resistant	7.0 a	7.7 a	7.3 a	8.7 a	8.0 a	9.0 a
Susceptible	7.7 a	9.0 a	11.0 b	13.3 b	13.7 b	10.7 a
$R7_3$ – R8						
Resistant	3.7 a	4.3 a	5.0 a	4.7 a	4.3 a	5.3 a
Susceptible	3.7 a	5.0 a	5.7 a	8.0 b	6.3 b	6.7 a
R6 – R8						
Resistant	17.0 a	17.7 a	22.0 a	23.0 a	28.7 a	24.7 a
Susceptible	17.7 a	23.0 b	26.3 b	25.7 b	28.0 a	27.0 b

[x] R6 = Full green bean seed stage, $R7_1$ = physiological maturity (1% pods with mature pod color), $R7_3$ = intermediate maturity (50% pods with mature pod color), and R8 = full maturity (95% pods with mature pod color).

[y] Seed infection reaction to *Phomopsis* spp. and *Cercospora kikuchii*. Resistant entries (PI 404169A, PI 416946, PI 417274, PI 417460, Gnome, and PI 80837) and susceptible entries (PI 361093, Miami, Amsoy 71, PI 417520, PI 361065B, and PI 361095) were paired for maturity.

[z] Means of resistant and susceptible soybean entries, within each maturity pair and year followed by the same letter do not differ significantly (k ratio = 100) based on the Waller-Duncan Bayesian LSD test.

intervals or as the rate of pod and seed moisture loss between growth stages. Duration of early dry-down (R6–$R7_1$ or $R7_1$–$R7_3$) intervals was not associated with seed infection by either pathogen (Table 5). However, duration of the other three selected intervals (R6–R8, $R7_1$–R8, and $R7_3$–R8) was positively correlated with the incidence of both *Phomopsis* spp. and *C. kikuchii*. Rates of moisture loss (percent moisture per day) in pods and seeds at growth stage intervals $R7_3$–R8, $R7_1$–R8, and R6–R8 was negatively correlated with incidence of both *Phomopsis* spp. and *C. kikuchii* in seeds. With one exception, the rate of moisture loss from pods or seeds during the early dry-down intervals (R6–$R7_1$ and $R7_1$–$R7_3$) was not correlated with incidence of the fungi in seeds. Moisture loss from pods during the R6–$R7_1$ interval was negatively correlated with incidence of *Phomopsis* spp. in seeds. The critical intervals again were $R7_1$–R8 and $R7_3$–R8, when the rate of dry down and incidence of the pathogens were correlated. Correlations with the higher significance ($P = 0.01$) were those that included the $R7_1$–R8 interval, which, as mentioned earlier, is the period when dry down of pods and seeds is most rapid (for pods, from 62–64% to 15%; for seeds, from 54–56% to 15–18% moisture).

Table 3. Regression coefficient (b)[x] between pod and seed moisture content and the number of days after planting

Maturity pair	Disease reaction[y]	Entry	Pods		Seeds	
			1985	1986	1985	1986
1	R	PI 404169A	−4.474 NS[z]	−5.787 NS	−3.561 NS	−5.060 NS
1	S	PI 361093	−5.199	−5.733	−3.778	−4.572
3	R	PI 417274	−5.715**	−5.540 NS	−4.645**	−5.212*
3	S	Amsoy 71	−3.539	−4.629	−2.912	−3.683
4	R	PI 417460	···	−6.100**	···	−5.193**
4	S	PI 417520	···	−3.831	···	−3.171
5	R	Gnome	−3.972*	−4.646*	−3.549**	−4.775**
5	S	PI 361065B	−2.202	−3.240	−1.860	−2.421
6	R	PI 80837	−3.949 NS	−5.119 NS	3.493 NS	−4.279**
6	S	PI 361095	−3.691	−3.591	−2.785	−2.730

[x]Linear regression coefficient b (in percent moisture per day) indicates rate of moisture loss between physiological maturity ($R6_1$, 1% pods with mature pod color) and full maturity (R8, 95% pods with mature pod color).

[y]Seed infection reaction to *Phomopsis* spp. and *Cercospora kikuchii*, R = resistant and S = susceptible.

[z]Moisture data for maturity pair 4 not evaluated in 1985. Maturity pair 2 not evaluated for moisture content in 1985 or 1986. Homogeneity of regression coefficients within maturity pairs identified after b value of resistant entry based on t test. NS = not significant; * = significant at $P = 0.05$; ** = highly significant at $P = 0.01$.

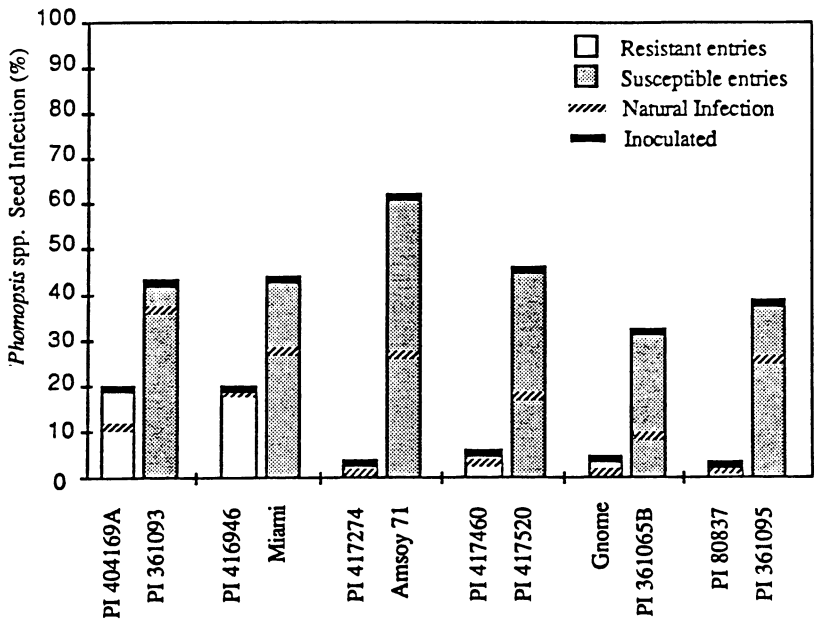

Fig. 1. Seed infection by *Phomopsis* spp. at maturity (R8) in 12 soybean entries paired for maturity. Soybean entries previously classified as susceptible if seed infection incidence in preliminary tests (6) was higher than 10% for *Phomopsis* spp. Resistant entries (PI 404169A, PI 416946, PI 417274, PI 417460, Gnome, and PI 80837) and susceptible entries (PI 361093, Miami, Amsoy 71, PI 417520, PI 361065B, and PI 361095) were paired for maturity. Inoculated = *P. sojae* conidia (5×10^6 per milliliter of water) applied to plants at growth stages R5 and R6.

DISCUSSION

Previous reports indicated that early-maturing cultivars produced poorer quality seeds than late-maturing cultivars because early-maturing cultivars matured under environmental conditions more favorable for seed infection (3,10,11). In the present study, resistant and susceptible genotypes were found among early-, as well as late-, maturing entries that were exposed to equal amounts of inoculum. Thus, genetic factors also influence incidence of seed infection. Bioassays of mature (R8) seeds confirmed the disease reaction of the genotypes tested (Figs. 1 and 2). Entries previously classified as resistant had a low incidence of fungal infection. Within this group, PI 417274, PI 417460, Gnome, and PI 80837 had the highest resistance to *Phomopsis* spp. and *C. kikuchii*, based on data from both inoculated and uninoculated plants. The only exception was the reaction of Gnome to inoculation with *C. kikuchii*. This entry had shown a low incidence of natural seed infection by *C. kikuchii*. The other two entries (PI 404169A and PI 416946) initially classified as resistant to seed infection were moderately resistant if compared with the soybean entry in their respective maturity pairing.

The enhanced or modified "growth stage scale" proved useful for estimating soybean developmental stages and duration of dry-down intervals after physiological maturity. With regard to duration of reproductive growth stages (Table 4), considerable variation exists among soybean genotypes of similar maturity and can be identified using the modified growth stage scale. The shorter dry-down period for resistant entries, as indicated by shorter $R7_3$–R8 and $R7_1$–R8 intervals and possibly the R6–R8 interval, appears important for seed infection. The $R7_1$–R8 interval is easy to determine because it starts when plants exhibit at least one normal pod with the mature pod color and ends when 95% of the pods attain that same color. Determining intermediate stages, such as $R7_2$, $R7_3$, etc., requires greater precision.

Variation in the intervals observed in 1985 and 1986 (Tables 1 and 2) was attributed primarily to differences in precipitation during August, when most of the late-season reproductive growth stages for maturity groups I and II entries occurred. Lower precipitation and higher temperatures occurred in August of 1986 than in 1985. Nevertheless, even with maturity dates advanced for most entries and with shorter growth stage intervals in 1986, it was possible to differentiate between resistant and susceptible genotypes of the same maturity, based on the duration of the dry-down period. Even

though duration of the dry-down intervals is influenced by weather (i.e., 1985 vs. 1986, Table 1), it is still a functional means of identifying genotypes that differ in resistance to seed infection by *Phomopsis* spp. and *C. kikuchii*.

The measurement of the rate of moisture loss was consistent with the modified growth stage scale. In all entries, seeds reached their maximum size near $R7_1$, which supported the accuracy of the growth stage visual assessment. Regression analyses of pod and seed moisture content with time provided further evidence that resistant entries lose moisture at a greater rate than susceptible entries, although no differences were detected for maturity group I entries and differences in maturity group IV entries were detected only in 1985. These results demonstrate that visual scales can provide accurate estimates of pod and seed moisture content, allowing a valid assessment of rates of dry down among soybean genotypes.

Evaluations within the $R7_1$–R8 interval indicated that extensive seed infection by *Phomopsis* spp. and *C. kikuchii* occurred in what was defined as the substage interval $R7_3$–R8, corresponding to the period in which pod and seed moisture values declined from 33.4–36.4% and 34.2–39.5% to 15.2–15.8 and 15.5–17.8% in 1985 and 1986, respectively. These results agree with those reported by Rupe and Ferriss (8). They also found that *Phomopsis* spp. grow well at water potentials as low as −45 bar where many other microorganisms are completely restricted. Because *Phomopsis* spp. can infect soybean seeds whenever environmental conditions maintain pods at water contents above 19%, it is not surprising to find high seed infection during the $R7_3$–R8 period or even after R8 during wet weather with delayed harvest.

Entries of similar maturity that differed in resistance to seed infection by *Phomopsis* spp. and *C. kikuchii* were equally susceptible to pod infection by *Phomopsis* spp. and *C. kikuchii* (Table 4). Maximum pod infection occurred between $R7_1$ and $R7_3$, followed by decline at full maturity (R8). Even with high pod infection, little seed infection occurred before physiological maturity. At this stage, the incidence of *Phomopsis* spp. and *C. kikuchii* in seeds began to increase in susceptible entries. However, levels of seed infection seldom rose above the highest incidence of pod infection. In summary, pod infections by *Phomopsis* spp. or *C. kikuchii* in resistant and susceptible genotypes that mature at the same time are quite similar.

Regression and correlation analyses in this study have associated the incidence of seedborne fungi with the rate of dry down ($R7_1$–R8 interval) under the environmental conditions in Indiana. Genotypes with shorter dry-down periods had less fungal seed infection than genotypes with longer dry-down periods, regardless of whether duration of late season growth stage intervals or rate of moisture loss was used to measure maturation and dry-down rate. Therefore, the use of visual scales, such as the one developed by Fehr and Caviness (4) or as modified in this study, to determine the date and duration of growth stages represents a workable tool to identify differences in seed dry-down rate.

Although other factors could determine or influence the levels of seed infection, e.g., additional resistance mechanisms may be present in entries classified as highly resistant, data from this study indicate that the rate at which soybean plants lose moisture during the final stages of maturation is critical to the infection process for seedborne fungi and could be used to identify cultivars with improved resistance to seed infec-

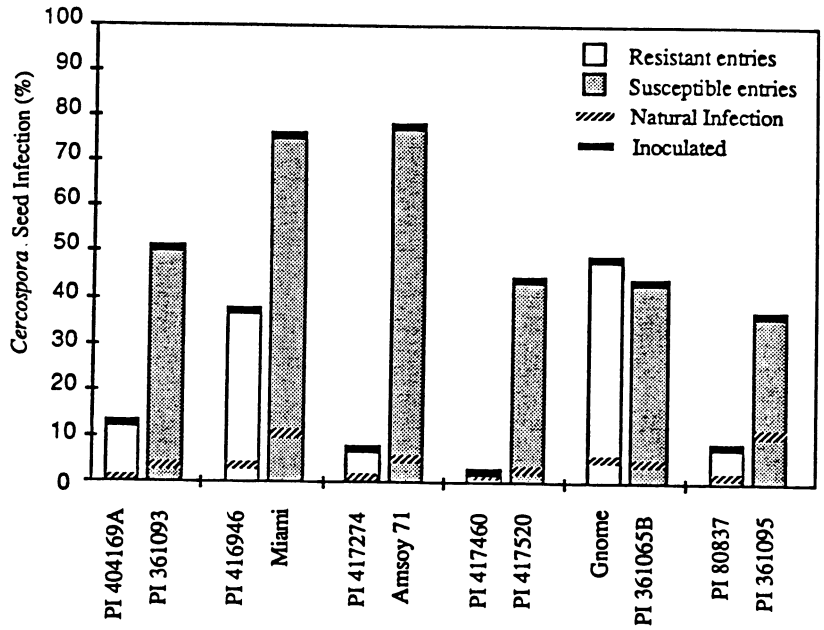

Fig. 2. Seed infection by *Cercospora kikuchii* at maturity (R8) in 12 soybean entries paired for maturity in 1985. Soybean entries previously classified as susceptible if seed infection incidence in preliminary tests (6) was higher than 15% for *C. kikuchii*. Resistant entries (PI 404169A, PI 416946, PI 417274, PI 417460, Gnome, and PI 80837) and susceptible entries (PI 361093, Miami, Amsoy 71, PI 417520, PI 361065B, and PI 361095) were paired for maturity. Inoculated = *C. kikuchii* conidia (1.5×10^5 per milliliter of water) applied to plants at growth stages R2.

Table 4. Incidence of pod and seed infection by *Phomopsis* spp. and *Cercospora kikuchii* at different growth stages in six pairs of soybean entries in 1985

Treatment[w]	Entry reaction[x]	Growth stage[y] R6 Pod	R6 Seed	$R7_1$ Pod	$R7_1$ Seed	$R7_3$ Pod	$R7_3$ Seed	R8 Pod	R8 Seed
Phomopsis (%)									
Control	R	29 b[z]	0 a	64 a	1 a	59 a	6 a	36 b	5 a
	S	14 a	0 a	62 a	13 b	61 a	29 b	29 a	26 b
Inoculated	R	42 b	1 a	80 a	4 a	80 a	8 a	75 a	9 a
	S	26 a	2 a	87 a	22 b	90 b	51 b	84 b	54 b
Cercospora (%)									
Control	R	2 a	0 a	9 a	0 a	18 a	1 a	14 a	1 a
	S	2 a	0 a	13 a	4 b	21 a	9 b	19 a	6 b
Inoculated	R	24 a	1 a	71 a	15 a	79 a	17 a	60 a	26 a
	S	27 a	2 a	77 a	46 b	83 a	63 b	72 b	71 b

[w] Control = not inoculated; inoculated = *P. sojae* inoculations for infection data by *Phomopsis* spp., and *C. kikuchii* inoculation for infection data by *C. kikuchii*.

[x] Seed infection reaction to *Phomopsis* spp. and *C. kikuchii*; R = resistant entries (PI 404169A, PI 416946, PI 417274, PI 417460, Gnome, and PI 80837) and susceptible entries (PI 361093, Miani, Amsoy 71, PI 417520, PI 361065B, and PI 361095).

[y] R6 = Full green bean seed stage, $R7_1$ = beginning or physiological maturity (1% pods with mature pod color), $R7_3$ = intermediate maturity (50% pods with mature pod color), and R8 = full maturity (95% pods with mature pod color).

[z] Means for pods or for seeds of six resistant and six susceptible within a column for each treatment followed by the same letter are not significantly different (k ratio = 100) according to the Waller-Duncan Bayesian LSD test.

Table 5. Correlations (r)[v] between seed infection by *Phomopsis* spp. or *Cercospora kikuchii* and duration of growth stages or rate of moisture loss (pods or seeds)

Growth stage interval[w]	Duration of growth stage (days)	Rate of moisture loss	
		Seeds	Pods
Phomopsis[x]			
R6 - $R7_1$	0.305[z]	−0.067	−0.396*
$R7_1$ - $R7_3$	−0.111	−0.121	0.049
$R7_3$ − R8	0.453**	−0.449**	−0.323
$R7_1$ - R8	0.358*	−0.543**	−0.396*
R6 - R8	0.459**	−0.334*	−0.324
Cercospora[y]			
R6 - $R7_1$	0.122	−0.016	−0.195
$R7_1$ - $R7_3$	−0.005	0.042	0.217
$R7_3$ - FR8	0.508**	−0.460**	−0.480**
$R7_1$ - R8	0.457**	−0.488**	−0.435**
R6 - R8	0.341*	−0.379*	−0.417*

[v] Dependent and independent variables each with 36 observations (N), three replicates each of 12 soybean entries in 1985.

[w] R6 = Full green bean seed stage, $R7_1$ = beginning or physiological maturity (1% pods with mature pod color), $R7_3$ = immediate maturity (50% pods with mature pod color), and R8 = full maturity (95% pods with mature pod color).

[x] Seed infection by *Phomopsis* spp. at harvest maturity for 12 soybean entries inoculated with *P. sojae* at growth stages R3 and R5.

[y] Seed infection by *C. kikuchii* at harvest maturity for 12 soybean entries inoculated with *C. kikuchii* at growth stages R2.

[z] For each correlation coefficient (r): * = significant at $P = 0.05$, and ** = highly significant at $P = 0.01$.

tion. Duration of growth stage intervals was influenced by environmental conditions during the 2 yr of evaluations; nevertheless, within each year, differences among the genotypes with regard to the length between the critical reproductive stages were still expressed and related to fungal seed infection.

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