Growth Regulator Effects on Soybean Seed Maturation and Seedborne Fungi

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ABSTRACT

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Growth regulators (chlorflurenol [methyl 2-chloro-9-hydroxyfluorene-9-carboxylate], a morphactin, and ethephon [2-chloroethylphosphonic acid] or ethrel) were applied to soybeans (*Glycine max*) at midseason to alter the rate of maturation and incidence of seedborne fungi. Maturation rate, measured as the length of late-season growth stage intervals, was studied in diverse soybean genotypes to determine the role of plant and seed drydown on seed infection by *Phomopsis* spp. and *Cercospora kikuchii*. Chlorflurenol delayed maturity, extended the length of late-season growth stage intervals, and increased seed infection in entries susceptible or moderately resistant to seed diseases. Ethrel tended to hasten maturity, shorten late-season growth stage intervals, and decrease the percentage of seedborne fungi. Growth regulator effects on soybean genotypes that matured under the same environmental conditions indicate that initial levels of seed infection are directly related to the rate of pod and seed maturation (soybeans with low levels of seed infection have shorter drydown periods than those with high levels of seed infection). The growth stage interval consistently associated with seed infection was from physiological maturity ($R7_1$) to harvest maturity (R8). Modification of the $R7_1$–R8 interval of susceptible and moderately resistant entries by means of growth regulators was directly associated with changes in levels of seed infection.

Seed infection caused by *Phomopsis* spp. and *Cercospora kikuchii* (Matsumoto & Tomoyasu) M. W. Gardner is a major concern in most areas where soybeans (*Glycine max* (L.) Merr.) are grown. Diseases caused by these pathogens reduce the appearance, seed health, and germination of soybean seeds regularly (12,14, 15). The diseases are prevalent and widely distributed, but high levels of seed infection occur only in years when high temperatures and humidity coincide with seed maturation (2,8,9,14,15). In these environments, highly susceptible soybeans may have more than 25% infected seed, whereas, in other environments, only trace amounts occur (15).

Although extensive research has been done on features (morphological, physiological, etc.) of the soybean plant that might be associated with resistance or tolerance to seedborne diseases, environmental conditions during final stages of seed maturation (physiological maturity) are now recognized as the most dominant influence (2,8,9,11). Early-maturing cultivars in Indiana produce poorer quality seeds more frequently than late-maturing cultivars because of temperature and moisture conditions that favor seed diseases (15). Length of the period from physiological maturity to harvest maturity (R7–R8) influences seed infection by either increasing or decreasing the chances of conditions favorable for seed infection to occur (2,11,12). The importance of the R7–R8 interval in resistance to seed infection for soybean cultivars that mature in the same environment has not been identified conclusively because isolines that differ with respect to drydown (long vs. short drydown interval) are not available. However, growth stage evaluations from our preliminary studies (1) on growth regulators applied late in the season (after flowering) suggested that the rate at which maturity proceeded during late seed maturation could have an effect on seed infection by *Phomopsis* spp. and *C. kikuchii*. Colony appearance and growth rate of the fungi in vitro and in isolations from pod tissue were not affected by the growth regulators. Previous studies (4,5) indicate growth regulators modify soybean maturation but have not included seed disease information.

Our objectives were to determine if late-season applications of growth regulators modify the rate of pod and seed maturation and, consequently, to ascertain if rate of maturation, when modified by the growth regulators, alters the development of seed diseases. Preliminary reports have been presented (11,12).

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MATERIALS AND METHODS

Field experiments were conducted at Lafayette, IN, in 1985 and 1986. Cultivars Amsoy 71, Miami, and Gnome and nine plant introductions were selected because of their different levels of seed infection by *Phomopsis* spp. and *C. kikuchii* and harvest maturity observed in 1983 and 1984 field tests. These 12 entries were grouped in six pairs of genotypes that were similar in maturity (days planting to R8) but dissimilar in seed disease reaction. Entries were classified as susceptible if seed infection levels in 1983 and 1984 were higher than 10% for *Phomopsis* spp. and/or higher than 15% for *C. kikuchii*. Hereafter, the entries will be referred to as resistant or susceptible based on these seed infection criteria. 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 and represented maturity groups I, II, II, II, II, and IV, respectively.

Seed of selected soybean genotypes were planted on 15 May 1985 and 23 May 1986 at 25–30 seeds per meter in randomized complete blocks in three replications. Each plot consisted of four rows 2 m long and 0.76 m apart. A split-split plot design was used, where maturity pairs were the main plots, seed disease reaction the subplots, and growth regulator treatments the sub-subplots. The treatments included were a control, chlorflurenol (CF 125), and ethrel. Each growth regulator was applied relatively late in the soybean life cycle (6,7) but before physiological maturity (R7). An aqueous solution of the morphactin (0.002%) CF 125 (chlorflurenol methyl ester) was applied when plants reached the R5 growth stage, and an aqueous solution of ethrel (0.1%) (a commercial formulation of ethephon) was applied when plants reached a mid- to late-R6 growth stage. Each growth regulator was applied to runoff in an aqueous spray to the two center rows of treatment plots with a hand-held sprayer powered by CO_2.

Rate of maturation. Reproductive growth stages were evaluated and recorded with a modified version of the scale proposed by Fehr and Caviness (6). To provide a more precise measurement of growth stage during the critical drydown period, the interval between R7 and R8 was subdivided into four substages ($R7_1$, $R7_2$, $R7_3$, and $R7_4$, identified by 1, 25, 50, and 75% of all pods with mature pod color, respectively). Each plot was monitored every 2 days, from

R1 (beginning bloom) to R8 (full maturity), to determine the date of occurrence of each specified stage or substage of development. The average stage assigned, based on a visual estimate, indicated that 50% of the plants in the two center rows of the plot were at or beyond a particular stage of development. The time interval between the occurrence of reproductive growth stages (using the modified scale for late-season growth stages) was used to determine the rate of soybean maturation. Changes in the number of days from planting to full maturity (R8) and modifications in the length of time between two growth stage intervals ($R6–R7_1$ and $R7_1–R8$) were used to determine the effect of treatments on maturation rate.

Disease progress in pods and seeds. Fungal infection of pods and seeds at growth stages R6, $R7_1$, $R7_3$, and R8 was examined to determine the disease progress. Single pods containing seeds were collected randomly from the main stems of 10 different plants in the middle two rows of each plot and stored at 4 C until processed within 24 hr. Pod and seed samples were separated, surface-disinfested in 95% ethanol for 20 sec and 1% sodium hypochlorite for 1 min, and plated separately on potato-dextrose agar in 9-cm culture dishes. After incubation for 7 days at 24–26 C, pods and seeds were evaluated for presence of pathogens, and all pod and seedborne microorganisms were recorded. Data for pathogens were summarized as the percentage of pods and seeds infected. A harvest (R8) seed sample (mechanically threshed) from the two center rows of each plot was also 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 and seed germination.

Regression analyses were used to study how alterations of maturation rates, within a given entry, affected the incidence of seedborne fungi at growth stage intervals $R6–R7_1$ and $R7_1–R8$. Initially, analyses of variance for seed infection by either *Phomopsis* spp. or *C. kikuchii* were run separately for each entry and for each of the two growth stage intervals considered, and significance of the linear component was tested. If the linear component was significant, a linear regression model was used to determine how maturity rate affects the incidence of seedborne fungi. The percentage of seed infection at harvest maturity was regressed on duration or length (days) of a growth stage interval.

Table 1. Mean number of days between reproductive growth stages $R7_1$ and R8 for six pairs of soybeans entries with similar maturity but dissimilar reactions to seed infection

Seed disease reaction[x]	Maturity pair[y]					
	1 (I)	2 (II)	3 (II)	4 (II)	5 (II)	6 (IV)
1985						
Resistant	11.7 a[z]	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
1986						
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

[x]Seed infection by *Phomopsis* spp. and *Cercospora kikuchii*.
[y]Number in parentheses is soybean maturity group classification.
[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 Waller Duncan Bayesian LSD test.

Table 2. Mean number of days from planting to full (R8) maturity and seed maturation ($R7_1$ to R8) for soybean entries treated with CF 125 and ethrel in 1985

Maturity pair	Entry	Disease reaction[x]	Planting to R8[y]			$R7_1$ to R8[y]		
			Control	CF 125	Ethrel	Control	CF 125	Ethrel
1	PI 404169A	R	119.7 a[z]	125.0 b	120.7 a	11.7 a	14.0 b	12.7 ab
1	PI 361093	S	117.0 b	118.3 b	114.0 a	11.0 a	10.7 a	9.0 a
2	PI 416946	R	121.3 b	124.7 c	118.7 a	10.7 ab	11.3 b	9.0 a
2	Miami	S	125.0 ab	126.7 b	123.0 a	12.0 a	12.7 a	12.0 a
3	PI 417274	R	122.3 a	125.0 b	120.7 a	9.3 a	10.7 a	8.7 a
3	Amsoy 71	S	129.0 ab	131.3 b	127.0 a	13.0 a	15.3 b	12.7 a
4	PI 417460	R	130.0 b	130.0 b	127.3 a	8.7 a	9.0 a	8.0 a
4	PI 417520	S	127.7 ab	129.3 b	125.7 a	13.7 ab	14.7 b	12.3 a
5	Gnome	R	130.7 a	133.7 b	129.3 a	10.3 a	12.0 a	10.7 a
5	PI 361065B	S	132.7 a	138.0 b	131.3 a	18.7 a	24.0 b	19.3 a
6	PI 80837	R	140.7 a	143.7 b	140.0 a	10.7 ab	12.7 b	10.0 a
6	PI 361095	S	143.3 a	146.3 b	142.3 a	11.3 a	11.3 a	11.3 a

[x]Seed infection reaction to *Phomopsis* spp. and *Cercospora kikuchii*. R = resistant and S = susceptible.
[y]$R7_1$ = Beginning or physiological maturity (1% pods with mature pod color), and R8 = full maturity (95% pods with mature pod color). Control = untreated plants, CF 125 = plants treated with chlorflurenol at R5 growth stage to lengthen time of seed maturation, and ethrel = plants treated with ethephon at R6 growth stage to shorten time of seed maturation.
[z]Treatment means for each factor (planting to R8 or $R7_1$ to R8) within a row followed by the same letter are not significantly different (k-ratio = 100) according to the Waller Duncan Bayesian LSD test.

RESULTS

Rate of maturation. Mean growth stage intervals for all soybean entries in the control treatment were shorter in 1986 than in 1985. This was particularly evident for the intervals $R6–R7_1$ (20.3 days in 1985 vs. 14.0 in 1986), $R7_1–R8$ (11.8 days in 1985 vs. 9.4 in 1986), and R6–R8 (32.1 days in 1985 vs. 23.4 in 1986). Entries designated as resistant to seed diseases had shorter growth stage intervals than susceptible entries in both 1985 and 1986, except for the $R6–R7_1$ interval in 1986. There were differences in the duration of the $R6–R7_1$ period between resistant and susceptible entries in all maturity pairs. However, for maturity pairs 4 (PI 417460 and PI 417520) and 5 (Gnome and PI 361065B), the susceptible entries were the ones that had shorter intervals. The $R7_1–R7_3$ interval was approximately equal for resistant and susceptible entries. The $R7_1–R8$ growth stage interval (Table 1), the interval from physiological maturity to harvest maturity when seed infection normally occurs, also differentiated resistant and susceptible entries. These differences were significant in most maturity pairs.

The average number of days to R8 was 126.8 days in resistant entries and 127.7 days in susceptible entries (Table 2). Chlorflurenol (CF 125) delayed the maturity date by an average of 2.7 days and a range of 0–5.3 days. There was less delay in resistant (average of 2 days) than in susceptible entries (average of 3.4 days). Plants treated with ethrel matured an average of 1.6 days earlier than the controls (126.7 vs. 128.3 days). Resistant and susceptible entries averaged an advance in maturity of 2.2 and 1.1 days, respectively. In both resistant and susceptible entries, ethrel significantly decreased the length of the late seed filling and early drydown period ($R6–R7_1$) compared with the control, whereas CF 125 significantly extended this period. Again, no significant interaction for the major drydown period ($R7_1–R8$) was observed between seed disease reaction (susceptible vs. resistant) and treatment (Table 2). Soybeans treated with ethrel tended to have lower values for the $R7_1–R8$ interval than the control, although the difference was not significant. The CF 125 treatment significantly extended

this interval by approximately 2 days, compared with the control.

Disease progress in pods and seeds. Numerous fungi infected pods and seeds, but species of the *Diaporthe/Phomopsis* complex (2,9), referred to as *Phomopsis* spp., were the most prevalent both years and will be emphasized with the 1985 data. Levels of pod and seed infection by fungi were similar in 1985 and 1986. *C. kikuchii* and *Alternaria* spp. were also more prevalent as pod pathogens than other common soybean pathogens, but they did not cause high levels of seed infection either year. A marked decline in pod infection by *Phomopsis* spp. occurred between growth stages $R7_3$ and R8 (from 59.4 to 35.6% in the resistant entries and from 61.0 to 29.4% in the susceptible entries).

At R6, pod infection by *Phomopsis* spp. was similar for all three treatments, and resistant entries had significantly higher pod infection than susceptible entries (Table 3). Pod infection by *C. kikuchii* was similar across treatments and seed disease reactions. At $R7_1$, no significant differences were observed in pod infection between resistant and susceptible genotypes by *Phomopsis* spp. or *C. kikuchii* in any of the treatments, except CF 125. This indicates that the levels of pod inocula in resistant and susceptible genotypes were similar at the time when seed infection normally begins. At the $R7_3$ and R8 growth stages, plants from some of the treatments exhibited significantly higher or lower pod infection by *Phomopsis* spp. than the control, but there was no definite pattern that would correlate with seed infection. Seed infection by either *Phomopsis* or *Cercospora* was low before physiological maturity and no differences were evident between resistant or susceptible genotypes (Table 4). However, from the $R7_1$ through the R8 growth stage, differences between resistant and susceptible entries in all treatments were observed for seed infection by *Phomopsis* spp. but not by *C. kikuchii*. Only the control treatment of the susceptible group had significantly higher seed infection by *C. kikuchii* than the resistant group. Plants in resistant and susceptible entries treated with CF 125 had consistently higher levels of seed infection by *Phomopsis* spp. than the control, but these differences were not always significant for the resistant entries. No conclusive trend was observed for seed infection by *C. kikuchii*.

The quality (appearance, seed health, and germination) of R8 seeds (pod or bulk harvested sample) was reduced by *Phomopsis* spp. both years. Results of seed infection at harvest maturity in control plants (Table 5) confirmed the initial seed disease classification of the entries PI 404169A, PI 416946, PI 417274, PI 417460, Gnome, and PI 80837 as resistant and PI 361093, Miami, Amsoy 71, PI 417520, PI 361065B, and PI 361095 as susceptible to *Phomopsis* spp. No significant correlation was found between the number of days to R8 and percentage of seed infection by either *Phomopsis* spp. ($r = -0.103$) or *C. kikuchii* ($r = -0.090$). Resistant and susceptible reactions were independent of maturity. Of the entries classified as resistant, PI 404169A and PI 416946 had the lowest levels of resistance to seed infection by *Phomopsis* spp. (12 and 19%, respectively), but in each case they had lower levels of infection than the susceptible entry with which they were paired (PI 361093, 37%, and Miami, 28%, respectively). The rest of the resistant entries had very low levels of *Phomopsis* spp. (below 6%). Susceptible entries had seed infection by *Phomopsis* spp. ranging from 9.7% in PI 361065B to 37% in PI 361093. Seed infection by *C. kikuchii* averaged 4%. The genotypes classified as resistant had levels of seed infection that were below 5% in the control plants, whereas susceptible entries averaged 6.1% seed infection, ranging from 2.3% in PI 417520 to 11.0% in PI 361095.

Across all entries, CF 125 significantly increased the frequency of *Phomopsis* spp. (Table 5) recovered from seed (23.9 compared with 15.5% in the control); whereas seeds from the ethrel treatment had significantly lower levels of these pathogens (11.6%). This pattern was present in both resistant and susceptible genotypes, except for resistant entries,

Table 3. Percentage of pod infection by *Phomopsis* spp. and *Cercospora kikuchii* in six pairs of soybean entries treated with CF 125 and ethrel to alter maturation rates in 1985

		Growth stage[w]							
		R6		$R7_1$		$R7_3$		R8	
Treatment[u]	Entry reaction[v]	P[x]	C.k.[y]	P[x]	C.k.[y]	P[x]	C.k.[y]	P[x]	C.k.[y]
Control	R	29 a[z]	2 a	64 a	9 a	59 a	18 a	36 a	14 a
	S	14 b	2 a	62 a	13 a	61 a	21 a	29 b	19 a
CF 125	R	29 a	2 a	60 a	16 a*	65 a*	21 a	28 a*	16 a
	S	14 b	2 a	70 b	14 a	73 b*	15 a	34 b	16 a
Ethrel	R	29 a	2 a	62 a	9 a	61 a	9 a	29 a*	11 a
	S	14 b	2 a	59 a	9 a	59 a	16 a	34 b	16 a

[u] Control = untreated plants, CF 125 = plants treated with chlorflurenol at R5 growth stage to lengthen time of seed maturation, and ethrel = plants treated with ethephon at R6 growth stage to shorten time of seed maturation.
[v] Seed infection reaction to *Phomopsis* spp. and *Cercospora kikuchii*. R = resistant and S = susceptible.
[w] R6 = Full green bean seed stage, $R7_1$ = beginning or physiological maturity (1% pods with mature pod color), $R7_3$ = intermediate maturity (75% pods with mature pod color), and R8 = full maturity (95% pods with mature pod color).
[x] P = *Phomopsis* spp.
[y] C.k. = *Cercospora kikuchii*.
[z] Means within a column for each treatment followed by the same letter are not significantly different (k-ratio = 100) according to Waller Duncan Bayesian LSD test. * = Mean is significantly different than the mean of the respective control treatment based on Waller Duncan Bayesian LSD test.

Table 4. Percentage of seed infection by *Phomopsis* spp. and *Cercospora kikuchii* in six pairs of soybean entries treated with CF 125 and ethrel to alter maturation rates in 1985

		Growth stage[w]							
		R6		$R7_1$		$R7_3$		R8	
Treatment[u]	Entry reaction[v]	P[x]	C.k.[y]	P[x]	C.k.[y]	P[x]	C.k.[y]	P[x]	C.k.[y]
Control	R	0.3 a[z]	0.2 a	0.7 a	0.3 a	6.3 a	0.6 a	5.3 a	1.1 a
	S	0.3 a	0.0 a	13.3 b	3.6 b	28.6 b	9.3 b	26.0 b	6.3 b
CF 125	R	0.3 a	0.2 a	6.4 a	1.1 a	19.9 a*	2.2 a	13.1 a	2.8 a
	S	0.3 a	0.0 a	23.7 b*	1.6 a	47.1 b*	3.3 a*	46.4 b*	6.4 a
Ethrel	R	0.3 a	0.2 a	0.3 a	0.0 a	0.8 a	0.0 a	0.6 a	0.3 a
	S	0.3 a	0.0 a	6.5 a	0.9 a*	12.1 b*	2.6 a*	15.5 b*	2.6 a

[u] Control = untreated plants, CF 125 = plants treated with chlorflurenol at R5 growth stage to lengthen time of seed maturation, and ethrel = plants treated with ethephon at R6 growth stage to shorten time of seed maturation.
[v] Seed infection reaction to *Phomopsis* spp. and *Cercospora kikuchii*. R = resistant and S = susceptible.
[w] 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).
[x] P = *Phomopsis* spp.
[y] C.k. = *Cercospora kikuchii*.
[z] Means within a column for each treatment followed by the same letter are not significantly different (k-ratio = 100) according to Waller Duncan Bayesian LSD test. * = Mean is significantly different than the mean of the respective control treatment based on Waller Duncan Bayesian LSD test.

which as a group did not show a significant decrease of seed infection attributable to ethrel (5.1 vs. 6.7% in the control). Lowest infection by *C. kikuchii* at harvest maturity across all entries were found in ethrel treated plants (1.4%) (Table 4). In susceptible entries, ethrel significantly decreased seed infection by *C. kikuchii* to 2.6% compared with 6% in the control. No significant increase with respect to the control (4.0%) was observed with CF 125.

In the majority of the genotypes, highest seed germination figures were found for seed from plants with the ethrel treatment (Table 5). As previously mentioned, seed from plants treated with ethrel had less *Phomopsis* spp. than seed from the control plants. In contrast, seed from the CF 125 treatment had lower germination than the control. This was also directly related to seed infection by *Phomopsis* spp., and it was more evident in the susceptible entries, which had a higher level of fungal infection.

Incidence of seedborne fungi as influenced by duration of selected growth stages. Increases in the length of a selected growth stage interval (as a result of the treatment) were almost always accompanied by increases in the incidence of a given pathogen (Table 6). For infection by *Phomopsis* spp., the length of the R6–$R7_1$ period appeared to have an effect on the incidence of these pathogens in seeds but only in four of the susceptible genotypes (PI 361093, Miami, PI 417520, and PI 361095). All of the susceptible entries, with the exception of PI 361065B, had a significant increase in incidence of *Phomopsis* spp. as a result of an increase in the duration of the $R7_1$–R8, the period of major drydown. On the other hand, soybean genotypes designated as resistant did not show any reaction to modifications of the R6–$R7_1$ interval (except PI 416946). Also, significant changes in seed infection by *Phomopsis* spp. did not occur as a consequence of changes in maturation rates during the $R7_1$–R8 interval of entries with the highest levels of resistance (PI 417274, PI 417460, Gnome, and PI 80837). However, the treatments had little impact on their drydown rates. But PI 404169A and PI 416946, with lower levels of resistance, reacted in a way similar to susceptible entries, that is, with variations in seed infection as a result of alterations in the drydown period. Only in very few cases was the variation in the level of seed infection by *C. kikuchii* associated significantly with variation in length of either the R6–$R7_1$ or the $R7_1$–R8 intervals (that occurred with PI 417520 and PI 80837 in the interval R6–$R7_1$, and PI 361065B in the interval $R7_1$–R8).

Table 5. Growth regulator effects on incidence of (%) seedborne *Phomopsis* spp. and seed germination at harvest maturity in 1985

Maturity pair	Entry	Disease reaction[w]	*Phomopsis*			Seed germination		
			Control	CF 125[x]	Ethrel[y]	Control	CF 125[x]	Ethrel[y]
1	PI 404169A	R	11.7 a[z]	37.3 b	8.0 a	57.7 b	31.3 a	66.0 b
1	PI 361093	S	37.0 b	44.0 b	27.0 a	51.0 ab	43.7 a	67.0 b
2	PI 416946	R	19.3 a	40.0 b	15.0 a	69.0 b	47.0 a	70.3 b
2	Miami	S	28.0 ab	32.0 b	20.3 a	56.3 ab	45.3 a	66.0 b
3	PI 417274	R	1.3 a	2.0 a	2.3 a	84.0 a	73.3 a	88.0 a
3	Amsoy 71	S	27.0 a	47.0 b	25.0 a	54.3 b	32.3 a	62.3 b
4	PI 417460	R	4.0 a	3.0 a	0.7 a	84.3 a	79.7 a	93.0 a
4	PI 417520	S	18.3 a	31.7 b	14.3 a	56.3 ab	47.3 a	73.7 b
5	Gnome	R	1.0 a	3.0 a	0.7 a	94.0 a	85.7 a	95.7 a
5	PI 361065B	S	9.7 ab	12.7 b	4.3 a	74.3 ab	61.3 a	81.0 b
6	PI 80837	R	3.0 a	3.0 a	3.7 a	86.7 a	75.7 a	90.3 a
6	PI 361095	S	25.7 ab	30.7 b	18.3 a	61.3 a	51.3 a	65.7 a
Resistant entries			6.7 a	14.7 b	5.1 a	79.3 b	65.4 a	83.9 c
Susceptible entries			24.3 b	33.0 c	18.2 a	58.9 b	46.9 a	69.3 c
All entries			15.5 b	23.9 c	11.6 a	69.1 b	56.2 a	76.6 c

[w] Seed infection reaction to *Phomopsis* spp. and *Cercospora kikuchii*. R = resistant and S = susceptible.
[x] Plants treated with chlorflurenol (CF 125) at R5 growth stage to lengthen time of seed maturation.
[y] Plants treated with ethephon (ethrel) at R6 growth stage to shorten time of seed maturation.
[z] Data are 1985 means of three replications (100 seeds each replication). Treatment means for each factor (seedborne *Phomopsis* spp. or seed germination) within a row followed by the same letter do not differ significantly (k-ratio = 100) according to the Waller Duncan Bayesian LSD test.

Table 6. Significance of the linear component and regression coefficients (b) for seed infection by *Phomopsis* spp. or *Cercospora kikuchii* with length of the two growth stage[v] intervals in soybeans treated[w] to alter maturation rates

Entry	Disease reaction[x]	R6-$R7_1$				$R7_1$-R8			
		Phomopsis		*Cercospora*		*Phomopsis*		*Cercospora*	
		F[y]	b[z]	F[y]	b[z]	F[y]	b[z]	F[y]	b[z]
PI 404169A	R	0.23	···	2.21	···	13.52*	11.346	1.79	···
PI 361093	S	14.48**	6.611	0.70	···	12.87**	4.521	0.97	···
PI 416946	R	28.63**	8.267	0.87	···	27.58**	9.511	3.78	···
Miami	S	47.93**	7.771	0.40	···	37.08**	26.250	0.04	···
PI 417274	R	0.08	···	0.01	···	0.67	···	0.28	···
Amsoy 71	S	1.00	···	2.65	···	20.33**	9.622	1.57	···
PI 417460	R	5.28	···	1.15	···	4.33	···	0.18	···
PI 417520	S	19.82**	3.898	10.90*	0.973	7.14*	4.355	1.87	···
Gnome	R	1.38	···	0.26	···	4.58	···	1.78	···
PI 361065B	S	0.46	···	1.37	···	2.62	···	9.44*	0.884
PI 80837	R	0.99	···	6.29*	−1.556	0.02	···	4.17	···
PI 361095	S	101.58**	7.006	2.56	···	52.00**	10.833	4.76	···

[v] R6 = Full green bean seed stage, $R7_1$ = beginning or physiological maturity (1% pods with mature pod color), and R8 = full maturity (95% pods with mature pod color).
[w] Untreated plants, plants treated with chlorflurenol (CF 125) at R5 growth stage to length time of seed maturation, and plants treated with ethephon (ethrel) at R6 growth stage to shorten time of seed maturation.
[x] Seed infection reaction to *Phomopsis* spp. and *Cercospora kikuchii*. R = resistant and S = susceptible.
[y] F test for the null hypothesis that variation in length of a given interval does not contribute to variation in percentage of seed infection by a given pathogen (test of linear component in ANOVA). * = Significant at $P = 0.05$; ** = significant at $P = 0.01$.
[z] Linear regression coefficients (b) are presented only when F is significant. Percentage of seed infection at harvest maturity was regressed on duration or length (days) of a growth stage interval using a linear regression model.

DISCUSSION

Resistant and susceptible disease reactions of seed as initially identified from preliminary data among soybean genotypes were confirmed based on levels of seed infection by *Phomopsis* spp. Seed disease reactions were associated with maturation characteristics that occurred among early- as well as late-maturing soybeans. Modifications in the rate at which maturity proceeded during later stages of pod and seed maturation by growth regulators further identify the drydown rate as critical to the incidence of seedborne fungi.

The drydown period, i.e. $R7_1$–R8, the time interval between physiological maturity and full maturity (identified by 1 and 95% pods with mature pod color, respectively), definitely appears to be

related to the way seed infection takes place. This and previous studies have indicated that symptomless fungal infections occur in young pods early in the growing season (8,9). These infections remain dormant in the pod wall and have no adverse effect until plants near maturity when seed infection occurs as the fungi grow from the pods into the seeds. Most seed infection occurs after physiological maturity ($R7_1$) (2,12). During and after the time of pod and seed ripening, the environment plays a major role in establishing levels of seed infection (9,14,15). Because of the critical influence of the environment, a longer interval between physiological maturity ($R7_1$) and full maturity (R8) increases the opportunity for seed infection. On the other hand, genotypes that exhibit fast drydown have a shorter time for potential seed infection and, hence, are likely to have fewer diseased seeds.

Weather conditions during seed maturation were somewhat different in each year of the study and for the seed maturation intervals for the soybeans of different maturity classifications. However, precipitation was adequate both years for soybean production and disease development. Also, because the resistant and susceptible genotypes were paired for similar harvest maturity (R8), the environmental differences were minimized. Precipitation during the period 15 August–15 October 1985 was 21 cm and 23 cm for the same period in 1986.

Growth regulators applied relatively late in the soybean life to preclude the growth abnormalities sometimes observed (1,3–5,10) provided an opportunity to show how modification of maturation rates was reflected in the levels of fungal seed infection within each genotype (Tables 4 and 5). A comparison of levels of infection with duration of late season growth stage intervals (regression analysis) indicated that delays in the $R7_1$–R8 interval were associated with increases in the frequency of *Phomopsis* spp. The importance of the length of the drydown period was evidenced by the significant regression coefficients in susceptible and moderately resistant entries for the $R7_1$–R8 period (Table 6).

The lack of significant regression coefficients with seed infection by *C. kikuchii* probably indicates that the natural inoculum of this fungus may have been restricted by the higher levels of the *Phomopsis* spp. (Table 3). Levels of pod infection by *Phomopsis* spp. were high, and seed infection levels (Table 5) were decreased or increased by growth regulator treatment of susceptible and moderately resistant entries.

The evaluations within the $R7_1$–R8 interval indicated that the majority of seed infections occurred during the $R7_3$–R8 substage interval when pod and seed moisture values decline. These results agree with those reported by Rupe and Ferris (13). Regression and correlation analyses in this study and others (L. D. Ploper and T. S. Abney, *unpublished data*) have associated the incidence of seedborne fungi with the rate of drydown, at least under the environmental conditions present in Indiana. Comparison of the 12 diverse germ plasm entries indicates that genotypes with shorter drydown periods have less fungal seed infection than genotypes with longer drydown periods (Tables 1, 5, and 6). Our results do not rule out other factors that could determine or influence the levels of seed infection. In fact, other resistance mechanisms may be present in entries classified as highly resistant, but the research presented here involving growth regulator effects on late season maturation rate presents substantial evidence that the rate at which soybean plants lose moisture during the final stages of seed maturation is critical in the infection process by seedborne fungi.

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