

Relationships Between Seedborne Soybean Fungi and Altered Photoperiod

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Cooperative investigations of USDA-ARS and the Purdue University Agricultural Experiment Station. Journal Paper 10059.

Accepted for publication 26 February 1985.

ABSTRACT

Wilcox, J. R., Abney, T. S., and Frankenberger, E. M. 1985. Relationships between seedborne soybean fungi and altered photoperiod. *Phytopathology* 75:797-800.

The objective of this study was to evaluate seedborne pathogens of soybean cultivars in maturity groups I through IV when all cultivars were forced to mature as group II cultivars by manipulating photoperiod. Twenty cultivars were grown at Lafayette, IN, under a normal and an imposed 14-hr dark period. The extended dark period effectively shortened the maturation time for all cultivars. Mature seeds harvested from each cultivar under the two photoperiod treatments were plated on potato-dextrose agar to determine incidence of microorganisms in the seeds and to assess seed germination. Forced early maturation increased the incidence of

seed infected by *Phomopsis* spp. from 5.3 to 31.5% and decreased seed germination from 89.4 to 61.4% across all cultivars. The results demonstrated that low incidence of *Phomopsis* spp. seed infection in late-maturing cultivars was not due to inherent resistance to these microorganisms but to escape from infection and disease development. *Cercospora kikuchii* and other microorganisms including *Alternaria*, *Chaetomium*, *Fusarium* spp., and bacteria occurred in seeds at very low levels and were not affected by photoperiod.

Additional key words: *Diaporthe* spp., *Glycine max*, pod and stem blight.

Poor quality soybean [*Glycine max* (L.) Merr.] seed has often been associated with infection by *Phomopsis* sp., *P. sojae* Lehman, *Diaporthe phaseolorum* (Cke. and Ellis) Sacc. var. *caulivora* Athow and Caldwell, and *D. phaseolorum* var. *sojae* (Lehman) Wehm. High incidences of these fungi in soybean seed have been

associated with high temperatures and humidity during seed maturation (4,7,10,11,13). Since these weather conditions are more prevalent in late summer and early fall than during the late fall, poor seed quality has been related to date of planting and maturity date of soybean cultivars. Kilpatrick and Hartwig (3) reported that *P. sojae* was isolated most frequently from seed produced by early plantings and only rarely from seed produced by late plantings of soybeans.

Reports by Ross (10), Shortt et al (11), and Wilcox et al (14) have shown that the incidence of *Diaporthe* and *Phomopsis* spp. is highest among early maturing cultivars in a specific area and decreases progressively as the maturity dates of cultivars increases. Tekrone et al (12), in a study that included cultivars differing

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widely in maturity and planting dates spanning a 2-mo period, found that the incidence of *Phomopsis* spp. in seed was closely related to the date a cultivar reached harvest maturity. If an early maturing cultivar was planted late and reached harvest maturity late in the season, incidence of *Phomopsis* spp. in the harvested seeds was lower than if the same cultivar was planted early and matured early in the season. However, late-maturing cultivars had consistently low incidences of seedborne *Phomopsis* spp. in the seed because they matured during the cooler and often drier weather of mid-fall.

Identifying sources of resistance to *Phomopsis* spp. is difficult because of the confounding effects of maturity and weather. Early maturing cultivars mature seed during the warm, often humid, weather of late summer and characteristically have high incidences of seedborne *Phomopsis* spp. Late-maturing cultivars mature seed during the cooler and often drier weather of mid-fall and usually have very good seed quality. The objective of this study was to determine if photoperiod could be manipulated so that cultivars varying widely in maturity could all be forced to mature at the same time, in late summer. This would avoid confounding genotypic disease resistance with escape from disease due to late maturity. It would permit an accurate assessment of sources of resistance to *Phomopsis* spp. and to other seedborne fungal pathogens.

MATERIALS AND METHODS

The experiment was conducted in 1981 and 1982 in a split-plot design with two replications each year. Main-plots were twenty cultivars, three in maturity group I, five in group II, seven in group III, and five in group IV. Each main-plot consisted of paired rows of each cultivar, 50 cm in length and spaced 75 cm apart. Twenty seeds were planted per row, then thinned to 10 plants per 50-cm row shortly after the seedlings emerged. Plots were established on a field that had been in soybeans continuously at the Purdue Agronomy Farm, West Lafayette, IN for the past 10 yr.

Subplots consisted of two photoperiod treatments. One was the normal daylength at Lafayette, IN. The second was a photoperiod of 10 hr, imposed by covering one row of each cultivar with a light-tight box from 1700 hours until 0700 hours the following day. This treatment was imposed on the subplots from June 21 each year until all plants in a subplot had begun to flower. For group I cultivars, this was a period of 5–7 days, while the group IV cultivars were covered for a 12- to 15-day period.

The dates were recorded when the cultivar in each plot was mature (95% of pods with mature color). Each plot was harvested 3–4 wk after maturity. Fifty seeds from each subplot were surface sterilized in sodium hypochlorite (1% available chlorine) and were plated 10 seeds per plate on non-acidified potato-dextrose agar. After 5–6 days, incidence of all microorganisms growing from the seeds was recorded and classified into one of three categories: 1) *Cercospora kikuchii* (Mats. and Tomay) M. W. Gardner; 2) *Diaporthe phaseolorum* var. *caulivora*, *D.p.* var. *sojae*, and *Phomopsis* spp.; and 3) other fungi and bacteria. No attempt was made to separate *D.p. caulivora*, *D.p. sojae*, and *Phomopsis* spp.; these are collectively referred to hereafter as *Phomopsis* spp. The percent seeds infected with the above microorganisms was recorded for each subplot. The percent seeds germinated in these plates was recorded for each subplot by using germination standards recommended by the Association of Official Seed Analysts.

For statistical analyses, maturity dates were converted to days after 31 May. Data on percent incidence of microorganisms and seed germination were converted to angle = arcsin (percentage^{1/2}) prior to analysis. Subplot data were used in analyses of variance. Regression analyses were computed to evaluate relationships among cultivar maturity date, incidence of *Phomopsis* spp., and seed germination. All tests of significance were at $P = 0.05$.

RESULTS

Combined analyses of variance of the subplot data for both years indicated significant effects only for maturity date and normal

versus extended dark period. Averaged over all cultivars for both years, the extended dark period shortened the time required for the cultivars to reach maturity by 16 days. The interaction of cultivar × photoperiod treatment was significant for maturity date, indicating that the cultivars responded differently to the imposed photoperiod treatments. In the combined analyses, the interaction of cultivar × photoperiod was not significant for incidence of the various pathogens on the seed or for percent seed germination.

The combined analyses indicated a significant effect of years on maturity date, incidence of both *C. kikuchii* and *Phomopsis* spp. in the seed, and percent seed germination. Seed produced in 1981 averaged 1.2% *C. kikuchii*, 15.0% *Phomopsis* spp., and 77.7% germination while seed produced in 1982 averaged <0.3% *C. kikuchii*, 3.5% *Phomopsis* spp., and 91.0% germination. Environmental conditions were more conducive to infection of seed by *Phomopsis* spp. and to poor germination of harvested seed in 1981 than in 1982. The year × cultivar × photoperiod treatment interactions were significant for maturity date, percent seed infected with *Phomopsis* spp., and percent seed germination but not for infection with *C. kikuchii* or other organisms. For this reason separate analyses of variance were computed for each year of the study.

In the 1981 analyses, the interaction of cultivar × photoperiod treatment was significant for maturity date, percent *Phomopsis* spp. and percent germination, but not for either incidence of *C. kikuchii* or other microorganisms including *Alternaria*, *Chaetomium*, *Fusarium* spp., and bacteria, in the seed. In the 1982 analyses, the interaction of cultivar × photoperiod treatment was significant only for maturity date. Since the incidence of microorganisms in seed was very low and the interactions of cultivar × photoperiod treatment were not significant for incidence of microorganisms in seed or for seed germination in 1982, only 1981 data are presented.

Extending the length of the dark period was effective in shortening the number of days required for all cultivars to mature (Table 1). The 14-hr dark period reduced the time required to reach maturity by 6–14 days for those cultivars that matured in September and by 22–35 days for those that matured in October under the normal daylength. This resulted in all cultivars except Douglas reaching maturity between 1 and 16 September in 1981.

Altering maturity by extending the length of the dark period resulted in an increased incidence of *Phomopsis* spp. in the seeds of all cultivars. The increased incidence of *Phomopsis* spp. was significant for all group I cultivars, only for Nebsoy among the group II cultivars, for all group III cultivars, and for all group IV cultivars except Douglas. Under the normal photoperiod there was a higher incidence of *Phomopsis* spp. in the seed of Douglas than for the other group IV cultivars.

The relationship between days to maturity and incidence of *Phomopsis* spp. in seed was evaluated by regressing percent seed infected with *Phomopsis* spp. on days to maturity for each cultivar under both the normal daylength and the extended dark period (Fig. 1). Under the normal daylength as days to maturity increased there was a decreasing incidence of *Phomopsis* spp. in the seed. The slope of the regression line ($b = -0.299 \pm 0.225$, $R^2 = 0.30$) was significantly different from zero. Under the 14-hr dark period there was higher incidence of seed infected with *Phomopsis* spp. and the decrease in seed infection with progressively later maturity dates was more rapid than under the normal dark period. The slope of the regression line ($b = -1.887 \pm 1.37$, $R^2 = 0.32$) differed significantly from zero and from the slope of the regression line for cultivars grown under the normal daylength.

Germination of the seed was affected by extending the length of the dark period and inducing the cultivars to mature in early to mid-September (Table 1). All but four of the cultivars produced seed with significantly lower percent germination under the extended dark period compared to the normal daylength.

Percent seed germination was regressed on percent seed infected with *Phomopsis* spp. to study the relationships between these two characteristics (Fig. 2). The slope of the regression line for seed produced under normal daylength ($b = -0.932 \pm 0.169$, $R^2 = 0.69$) did not differ significantly from the slope for seed produced under

the extended dark period ($b = -1.293 \pm 0.428$, $R^2 = 0.88$), which indicated that the relationship between percent seed germination and incidence of *Phomopsis* spp. infection was very similar for the two treatments. In this study, a combined regression equation $Y = -1.023X + 94.4$ calculated across both treatments indicated an inverse one-to-one relationship between incidence of infection by *Phomopsis* spp. and percent seed germination.

DISCUSSION

Covering soybean plants with a light-tight box from 1700 hours until 0700 hours the following day altered the microenvironment surrounding the covered plants. It is doubtful that this in itself

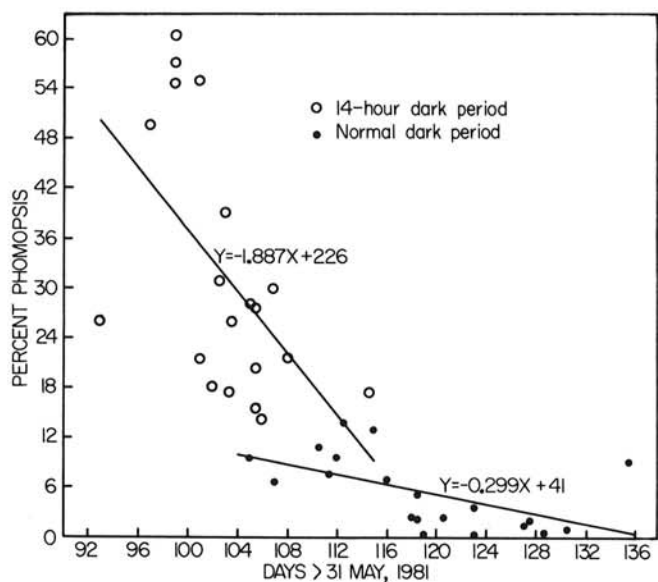


Fig. 1. Regression of percent seed infected with *Phomopsis* spp. on days to maturity for 20 soybean cultivars under normal and extended dark periods in 1981 (31 August = 92 days and 30 September = 122 days).

affected the response of the soybean plants to microorganisms affecting seed quality. Covered plants were completely dry when the boxes were removed each morning, and uncovered plants were covered with dew. One would expect that seeds of the dew-covered plants would be invaded more frequently by pathogen than those of the dry plants. In addition, the light-tight boxes covered each cultivar only until the plants began to flower. None of the plants were covered from the time of initial pod development through maturity. During the period of pod formation and development all

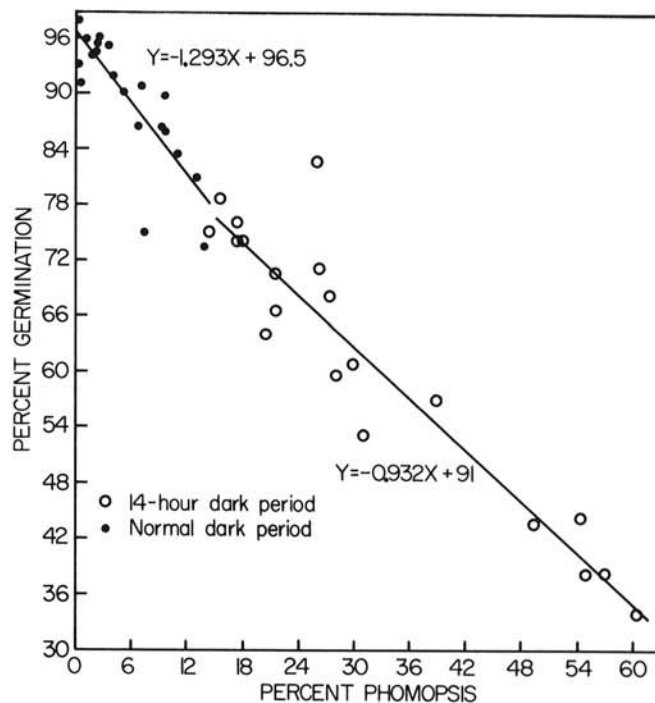


Fig. 2. Regression of percent seed germination on percent seed infected with *Phomopsis* spp. for 20 soybean cultivars under normal and extended dark periods in 1981.

TABLE 1. Effects of natural and 14-hr daily dark periods on maturity, incidence of microorganisms in seeds, and seed germination of 20 soybean cultivars in four maturity groups at West Lafayette, IN, in 1981

Cultivar	Maturity (mo.-day)		<i>Cercospora kikuchii</i> (%)		<i>Phomopsis</i> spp. (%)		Other (%)		Germination (%)	
	Natural	14-hr	Natural	14-hr	Natural	14-hr	Natural	14-hr	Natural	14-hr
Group I										
Hodgson 78	9-13	* ^a 9-7	2.5	1.9	9.6	* 54.6	0.5	8.4	89.7	* 41.8
Lakota	9-15	* 9-5	1.5	5.8	6.4	* 49.5	3.0	8.4	86.4	* 43.5
Hardin	9-18	* 9-7	2.3	5.0	10.9	* 57.0	1.5	7.5	83.6	* 38.0
Group II										
Wells II	9-20	* 9-9	1.0	3.3	9.5	* 21.4	1.0	4.4	86.0	* 66.6
Nebsoy	9-20	* 9-10	2.0	4.8	7.5	* 31.2	5.7	4.4	75.1	* 53.1
Amsoy 71	9-20	* 9-13	3.5	6.0	14.0	* 15.5	3.9	2.3	73.5	* 78.5
Century	9-24	* 9-12	0.2	2.0	6.9	* 17.4	0.5	1.9	90.8	* 74.1
Amcor	9-23	* 9-14	6.5	5.3	13.0	* 20.4	0.0	6.0	80.9	* 64.0
Group III										
Gnome	9-27	* 9-10	0.0	1.5	0.0	* 17.9	7.4	9.4	93.2	* 74.2
Will	9-26	* 9-12	0.0	5.2	2.6	* 25.9	3.5	1.5	96.1	* 71.2
Pella	9-26	* 9-15	0.0	0.3	2.3	* 30.0	1.0	0.0	94.4	* 61.0
Calland	9-26	* 9-16	0.0	1.0	5.0	* 21.4	1.0	2.9	90.0	* 70.6
Cumberland	9-28	* 9-14	0.3	3.5	4.1	* 27.5	1.0	1.9	92.1	* 68.1
Hobbit	10-1	* 9-7	0.0	0.5	0.0	* 60.5	1.0	1.9	98.0	* 33.5
Williams 79	10-1	* 9-9	0.3	2.0	3.5	* 55.0	1.5	1.9	95.0	* 38.0
Group IV										
PI 80837	10-6	* 9-1	0.0	0.0	0.3	* 26.0	5.6	4.0	91.4	* 83.5
Union	10-5	* 9-11	0.3	0.3	2.3	* 38.9	0.8	5.4	95.5	* 57.1
Cutler 71	10-5	* 9-13	0.0	3.3	1.5	* 28.3	3.7	1.8	94.0	* 59.6
Pixie	10-8	* 9-14	0.0	2.3	1.0	* 14.3	2.5	2.9	96.0	* 75.0
Douglas	10-13	* 9-20	0.0	2.5	9.2	* 17.3	1.5	2.6	86.6	* 76.2

^a Asterisk indicates paired values differ significantly, $P = 0.05$.

cultivars were exposed to naturally occurring environmental conditions.

The results obtained during each year of the study were very different. In 1981, the incidence of seed infection by microorganisms was much higher than in 1982 when seed infection was so low that neither of the treatments, cultivars or photoperiods, could be accurately assessed. In both years, seeds of all cultivars were harvested 3–4 wk after maturity to permit extensive development of microorganisms in the seed. The increased incidence of seed infection associated with delayed harvest was noted by Wilcox et al (14). Delaying harvest after maturity has been used to increase the incidence of fungi in soybean seed to definitively assess reaction of cultivars to organisms affecting seed quality (2,4,6,10). In this study, however, that technique was not effective for seed harvested in 1982.

Weather conditions during seed maturation and after maturity were very different in each year of the study. In 1981, 18 cm of rain fell during the last week of August and an additional 5.7 cm fell during the first week of September. This corresponds to the period from 85 to 98 days after 31 May in Fig. 1. The highest incidences of seed infection by *Phomopsis* spp. were observed among cultivars forced, by photoperiod manipulation, to mature either at the end of or just after this period. Incidence of seed infected with *Phomopsis* spp. decreased as cultivars matured later in the season when rainfall was close to normal. High levels of moisture at the time of seed maturation and after harvest have been commonly associated with high incidence of fungi, particularly *Phomopsis* spp. and *D. phaseolorum* var. *sojae* in soybean seed (5,8,10,11,13). In contrast to 1981, rainfall during the last half of August through October 1982 was fairly close to normal.

The inverse relationship between percent seed infected with fungi (primarily *Phomopsis* spp. and *D. phaseolorum*) and seed germination has been well documented (1,9,11,12,14).

These data have implications for evaluating soybean genotypes for reaction to seedborne pathogens, primarily *Phomopsis* spp. As has been observed, late-maturing genotypes had lower incidences of seed infection by *Phomopsis* spp. than early maturing genotypes grown under normal photoperiods. When subjected to an extended dark period that forced all genotypes to mature by mid-September, all genotypes appeared susceptible to infection by *Phomopsis* spp. The correlation coefficient for percentage of seed infected by *Phomopsis* spp. for the 20 cultivars grown under normal versus short daylengths ($r = -0.068$) was not significant. This suggests that the superior seed quality of the late maturing genotypes was not due to physiological resistance but to escape from infection and disease development associated with late maturity.

Cultivars Gnome, Hobbit, Pixie, and PI 80837 (all of determinate growth habit) had the lowest incidences of seed infection by *Phomopsis* spp. when grown under normal daylengths. T. S. Abney (*unpublished*) has observed exceptionally low levels of *Phomopsis* spp. in seed of these determinate cultivars when they were grown in central Indiana. When these determinate cultivars were induced to mature during early September, similar to maturity group I or II cultivars, the incidence of seed infected by *Phomopsis* spp. increased to between 14.3 and 60.5%, equal to or higher than levels in group I or II cultivars grown under normal daylengths. This indicates that the low levels of *Phomopsis* spp. previously observed in seeds of these determinate cultivars were not

due to inherent resistance to the pathogen but to avoidance of conditions conducive to seed infection and pathogen development. Inherent characteristics, such as late maturity of a cultivar, that enable the plants to escape infection may be considered a form of resistance. However, this form of resistance could not be transferred to group I or II cultivars to improve their genetic potential for superior seed quality.

The data obtained in this study demonstrate that environmental effects, primarily moisture just prior to maturity, were of greater importance than genotype in determining reaction of soybean cultivars to *Phomopsis* spp. affecting seed quality. Due to differing maturity dates of the cultivars, their reaction to *Phomopsis* spp. was confounded with weather conditions that favored infection and development of *Phomopsis* spp. on early maturing genotypes but not on late-maturing genotypes. When this confounding effect was eliminated by manipulating photoperiod, all of the cultivars appeared to be susceptible to attack by *Phomopsis* spp. which is associated with poor seed quality and reduced seed germination.

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