Environmental Factors Influencing Infection of Soybean Seeds by *Phomopsis* and *Diaporthe* Species During Seed Maturation

A. J. BALDUCCHI, Research Associate, and D. C. McGEE, Professor, Department of Plant Pathology, Seed and Weed Science, Iowa State University, Ames 50011

ABSTRACT

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The effect of moisture and temperature on infection of soybean seeds during seed maturation (growth stages R7 to harvest maturity) by *Phomopsis longicolla, Diaporthe phaseolorum var. sojae,* and *D. phaseolorum* var. *caulivora* (collectively referred to as PD) was investigated. Pods, grown in the greenhouse, were detached from plants at the R8 growth stage (full maturity), inoculated with a conidial suspension of *P. longicolla*, and exposed for different times to high (100%) or low (40–60%) relative humidity at 25 C. At least three continuous days at high humidity were needed for extensive seed infection to occur. Periods of high humidity could be interrupted after 1 day by up to 3 days at low humidity, and extensive seed infection would still occur. When 1-day periods at high humidity were continually alternated with low humidity, no significant seed infection took place. At temperatures of 20 and 15 C, it took 4 and 5 days, respectively, at 100% relative humidity to reach the level of seed infection attained within 3 days at 25 C. Higher seed infection occurred in pods incubated at 85–100% relative humidity for 7 days when they were detached at R7 (beginning maturity) than at R8. Field-grown soybeans, either inoculated with *P. longicolla* or naturally infected with PD, were overhead-irrigated at different growth stages between R6 and R8 in 1982 and 1983. Irrigation and inoculation both were associated with increased seed infection at harvest maturity. High positive correlations (*r* values ranged from 0.58 to 0.98) were obtained between seed infection and average temperature during irrigation. Lower negative correlations (*r* values ranged from 0.32 to 0.56) were obtained for relationships between seed infection and plant age during irrigation.

Present knowledge of the epidemiology of Phomopsis seed decay of soybeans (*Glycine max* (L.) Merr.) indicates that pods are a pathway for infection of seeds by the causal organisms *Phomopsis longicolla* Hobbs sp. nov. (4), *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *sojae* (Lehman) Wehm. & Sacc., and *D. phaseolorum* var. *caulivora* Athow & Caldwell (5–7) (collectively referred to as PD). Pods can be infected from flowering time onward, but extensive seed infection will not occur before the R7 (2) (beginning maturity) growth stage (2,7), and then, only under certain weather conditions. These conditions are not well understood. Various workers have associated increased seed infection with wet weather late in the growing season (13), with irrigation applied to field plots (9), and with exposure to high relative humidity in growth chambers (3,10). There also is evidence that high temperature during seed maturation favors infection (10,12). Two predictive methods developed for Phomopsis seed decay determine whether foliar fungicides should be applied at the R6 growth stage (full seed) to control seed infection. One is based on a point total determined by the existence of cultural and climatic factors known to be associated with increased severity of disease (11). The other uses the incidence of pod infection at R6 as the predictive measurement (7,8). Neither of these methods can account for the effects of weather between R6 and harvest on seed infection. Hepperly et al (3) (1980) showed that seed infection increased linearly in soybean pods detached from plants and held at 95% relative humidity for 4–10 days. This work was carried out at only one temperature (25 C). Other data (9,10,12,13) related environmental factors over extended periods (e.g., R6 to harvest maturity) to seed infection. The objective of this study was to define temperature and humidity conditions, over periods of 1 wk or less, that cause seed infection. These data could have value in improving predictive methods by incorporating short-range weather forecasts.

Present address of first author: Garst Seed Co., P.O. Box 500, Slater, IA 50244.

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MATERIALS AND METHODS

Laboratory experiments. The effect of relative humidity on infection of seeds by *P. longicolla* was examined in pods detached from Amsoy 71 soybeans grown to the R8 growth stage in the greenhouse. This cultivar was used in all experiments described in this paper. Pods were inoculated by immersion in a suspension (1.5×10^8 conidia per milliliter) of a *P. longicolla* culture originally isolated from a soybean seed. Inoculum was produced on potato-dextrose agar (PDA) plates and conidia were washed off in sterile water. The concentration used was necessary to ensure substantial pod and seed infection (7). A series of treatments was prepared in which pods were subjected to different sequences of days in the dark at high or low relative humidity in incubators maintained at 25 C. Each treatment consisted of four replicated sets of 10 pods placed on a wire rack inside a plastic box (27 × 16 × 4 cm), the bottom of which was lined with a paper blotter. High relative humidity (100%) was obtained by saturating the blotter with water and enclosing the box in a plastic bag; the presence of free water on the blotter surface ensured that the relative humidity was 100%. Low relative humidity was obtained by using dry blotters, not enclosing them in bags, and exposing them to the ambient relative humidity (40–60%) of the incubators. In all experiments, ambient relative humidity and temperature in incubators, growth chambers, and field were continually monitored with hydrothermographs. Transfers between humidity regimes were made by moving the rack of pods. Treatments were arranged in four randomized blocks. All seeds were removed from pods at the end of the treatment period, surface-sterilized in 1.3% sodium hypochlorite for 5 sec, rinsed in sterile water, and plated onto PDA adjusted to pH 4.5 with lactic acid (APDA). After incubation for 7 days at 25 C in the dark, the seeds from which *P. longicolla* colonies grew were counted. Seed infection was measured in this way in all laboratory experiments.

Effects of temperature on seed infection were examined by using three sets of soybean seeds naturally infected

by PD and one set grown in the greenhouse and inoculated with *P. longicolla*. The naturally infected pods were obtained from plants growing in soybean seed production fields in Iowa in which pod infection values, determined in pod tests (8) at the R6 growth stage, were 10, 36, and 56%. The pods were stored at 10 C and 50% relative humidity for 3 mo after harvest. Each set of pods was placed in 100% relative humidity environments in plastic boxes as described. The boxes were placed in incubators maintained at 15, 20, or 25 C. Each incubator contained three replicated blocks. Blocks were divided into four sections, and each contained eight boxes of each set of pods. Every day for 7 days, pods in one 100% relative humidity box for each set of pods in each block, in all incubators, were transferred to a low-humidity (40–60%) box. When transferred, pods were placed in the incubator in the same position as the original box and kept there for the balance of the 7-day period. After 7 days, all seeds were removed from the pods and tested for infection.

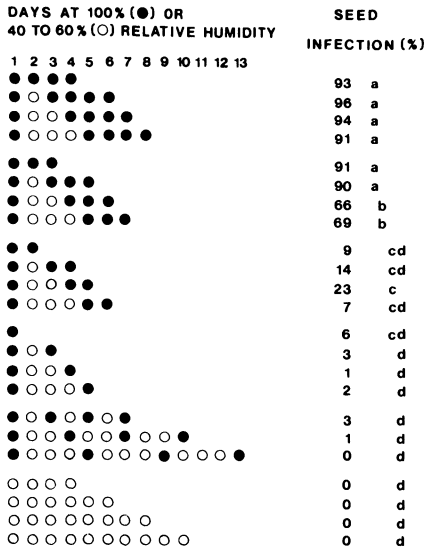

Fig. 1. Seed infection by *Phomopsis longicolla* in soybean pods detached from plants at growth stage R8, inoculated with an isolate of *P. longicolla*, then exposed to different combinations of days at 100 or 40–60% relative humidities. Means followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

The effect of high relative humidity on seed infection by *P. longicolla* was compared at the R7 and R8 growth stages. Two groups of Amsoy 71 soybeans were planted 2 wk apart and grown in a growth chamber maintained on a cycle of 14 hr of light at 28 C and 50% relative humidity and 10 hr of darkness at 22 C and 80% relative humidity. Each group consisted of 12 pots with four plants per pot. At the R6 growth stage in each planting, all pods in six pots were inoculated with *P. longicolla* by spraying with a suspension containing 1.5×10^8 conidia per milliliter. When the two groups were at growth stages R7 and R8, respectively, all pods were detached. Pods that had not been inoculated now were immersed in a suspension (1.5×10^8 conidia per milliliter) of the same *P. longicolla* isolate. Four replicates of 10 pods for each inoculation method/growth stage combination were tested immediately for seed infection. Corresponding sets of pods were placed on wire racks in a growth chamber maintained at the same lighting and temperature conditions as described before, but relative humidities were increased to 85 and 100% in the light and dark cycles, respectively. After 7 days, all seeds were removed from pods and tested for infection.

Field experiments. Amsoy 71 soybeans were planted 8 June 1982 at Ames, IA. The planting was divided into four blocks, each consisting of five plots 3 × 3 m. At growth stage R5, pods in half of each plot (a subplot) were inoculated with a *P. longicolla* suspension containing 1.5×10^7 conidia per milliliter. Pods in the other half were not inoculated. In the week that plants reached growth stage R7 and in each of the three following weeks, separate plots in each block were continuously irrigated with overhead sprinklers for five consecutive days between 8 and 16 hr. One plot per block received no irrigation. Twenty-five pods were sampled from each subplot at harvest maturity on 21 October. All seeds were removed from pods and tested for infection by PD by surface sterilization in 1.3% sodium hypochlorite for 1 min, rinsing in sterile water, and plating on APDA. Twenty-five pods also were sampled from each subplot immediately before irrigation was applied. Infection was determined the same way as for seeds, except that the surface sterilization period was for 3 min. The experiment was repeated in 1983 with Amsoy 71 soybeans planted on 29 April. Irrigation and inoculation procedures and measurements of pod and seed infection were the same. The experimental design was altered to include both an irrigated and a nonirrigated plot for each irrigation treatment. Irrigation was applied during 5 instead of 4 wk. Temperature was measured at the experimental sites each year and the average daily mean temperature computed for each irrigation period. Growth stages were measured in randomly selected areas within each planting every week during the irrigation period. Plots were considered to be at a particular growth stage when at least 50% of the plants were at that stage of development.

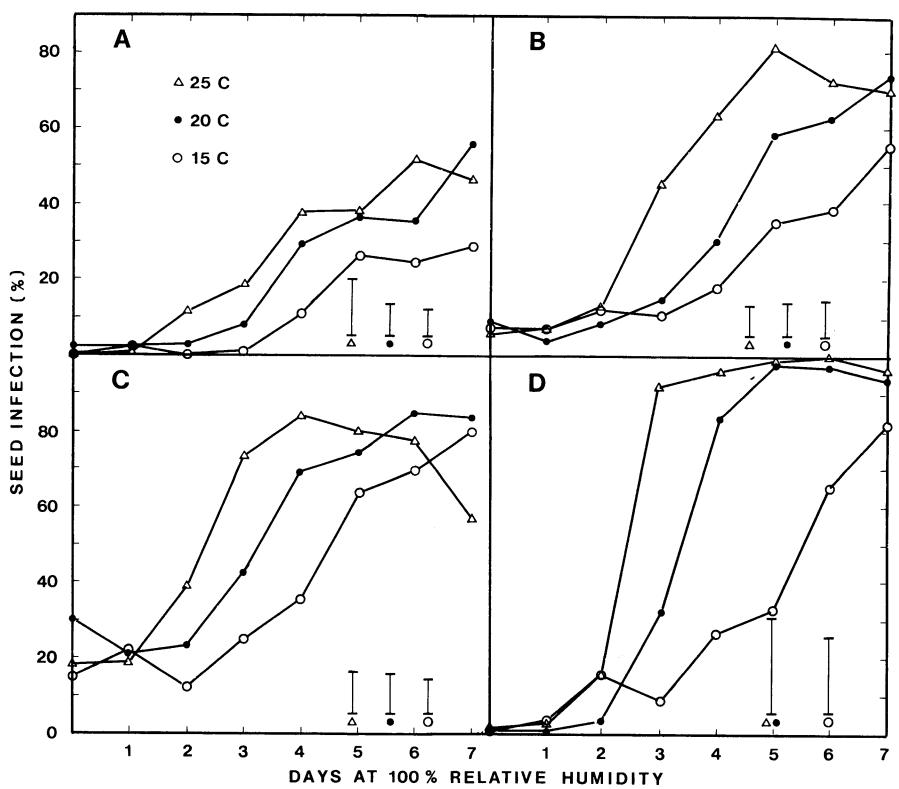

Fig. 2. Seed infection by PD in soybean pods detached from plants at growth stage R8 and held at three temperatures for different numbers of days at 100% relative humidity, then transferred to 40–60% relative humidity at the same temperature for the balance of 7 days. Bars represent LSDs ($P = 0.05$) within temperatures. Samples had **(A)** 10%, **(B)** 38%, and **(C)** 58% of the pods naturally infected by PD, as measured at the R6 growth stage in the previous growing season. **(D)** Sample was originally disease-free, then inoculated with an isolate of *Phomopsis longicolla* immediately before treatments were applied.

RESULTS

At least three continuous days of 100% relative humidity at 25 C were required to

obtain more than 90% of seeds infected in pods detached from plants at R8 and inoculated with *P. longicolla* (Fig. 1). Periods of 100% relative humidity could be interrupted after 1 day by up to 3 days at 40–60%, and extensive seed infection was still obtained. However, the longer the period of low humidity the longer was the subsequent time at 100% relative humidity needed to obtain 90% seed infection. No significant seed infection occurred when single days at 100% relative humidity were continually alternated with periods of low humidity. The percentage of seeds infected by PD increased with the number of days at 100% relative humidity in both naturally infected and inoculated pods held at three temperatures (Fig. 2). The rate of seed infection was influenced by temperature. For significant increases in seed infection to occur, it took about 3, 4, and 6 days for pods held at 25, 20, and 15 C, respectively. The original infection levels in naturally infected pods generally were related to the incidence of seed infection for all temperature treatments.

Increases in seed infection were greater in pods detached at R7 and exposed to a relative humidity range of 85–100% for 7 days than in pods detached and exposed at R8 (Table 1). This effect was evident when pods were inoculated at different times but at the same growth stage (R6) or inoculated at different growth stages but at the same time.

Seed infection, measured at harvest maturity, generally was greater in field plots irrigated for 5-day periods than in nonirrigated plots (Figs. 3 and 4). Seed infection also was greater in inoculated than in naturally infected plots. Pod infection, measured before irrigation, averaged 79.8 and 56.8% for inoculated and noninoculated plots, respectively, in 1982 and 88.2 and 48.9% in 1983. In both years, seed infection in irrigated plots was positively correlated with average daily mean temperatures during irrigation periods (Fig. 3). This temperature effect also was seen in the nonirrigated, inoculated plots in 1983. This can be explained by the experimental design in which nonirrigated subplots were immediately adjacent to irrigated subplots and presumably were subject to greater relative humidity at the time of irrigation than were other nonirrigated subplots. Seed infection was negatively correlated with plant age during irrigation (Fig. 4). Correlation coefficients were much lower than those obtained for temperature relationships.

DISCUSSION

Independent and combined effects of moisture and temperature on seed infection within PD-infected pods for growth stages R7 and R8 were elucidated in this study. The laboratory experiment in which the process of seed infection could be started and stopped by transferring pods between high- and low-humidity environments confirms previous knowledge (3,10) that high relative humidity is essential for seed infection to take place. Strong positive correlations between seed infection at harvest maturity and temperature during periods of high humidity in laboratory or field experiments showed that temperature influenced the rate of seed infection during wet periods. The age at which plants were exposed to high relative humidity also influenced prevalence of seed infection. This was evidenced by the greater amount of seed infection induced in the laboratory in pods detached at growth stage R7 than at R8. This effect was not as obvious in the field, where seed infection and plant age during irrigation were weakly correlated. It was probably masked, however, by the stronger influence of temperature on seed infection. The validity of using data obtained with detached pods to explain effects observed in the field has been demonstrated before (3,7). The agreement of laboratory and field data throughout this study supports this conclusion.

A more precise definition of weather factors that favor infection of seeds by PD is now possible. Extensive infection occurred in pods exposed to 100% relative humidity, whereas virtually no infection occurred over the range of 40–60%. At the time of year (September and October) when soybeans are between R7 and harvest maturity, relative humidity regularly reaches 100% at night in Iowa but averages 60% during the day (1). In the present study, alternate 24-hr

Table 1. Seed infection (%) by *Phomopsis longicolla* in soybean pods inoculated at different times after 0 and 7 days and exposed to high relative humidity (RH)[a] at different growth stages

Growth stage when exposed to high RH[a]	Seed infection (%) in pods			
	Inoculated at R6[b]		Inoculated just before exposure to high RH[c]	
	0 Days	7 Days	0 Days	7 Days
R7	5	33	14	68
R8	0	5	0	17

[a] High RH by exposing detached pods to 85–100% RH in growth chambers.
[b] Pods inoculated at R6 with a *P. longicolla* suspension containing 1.5×10^8 conidia per milliliter.
[c] Detached pods at each growth stage inoculated by immersion in a *P. longicolla* suspension containing 1.5×10^8 conidia per milliliter.

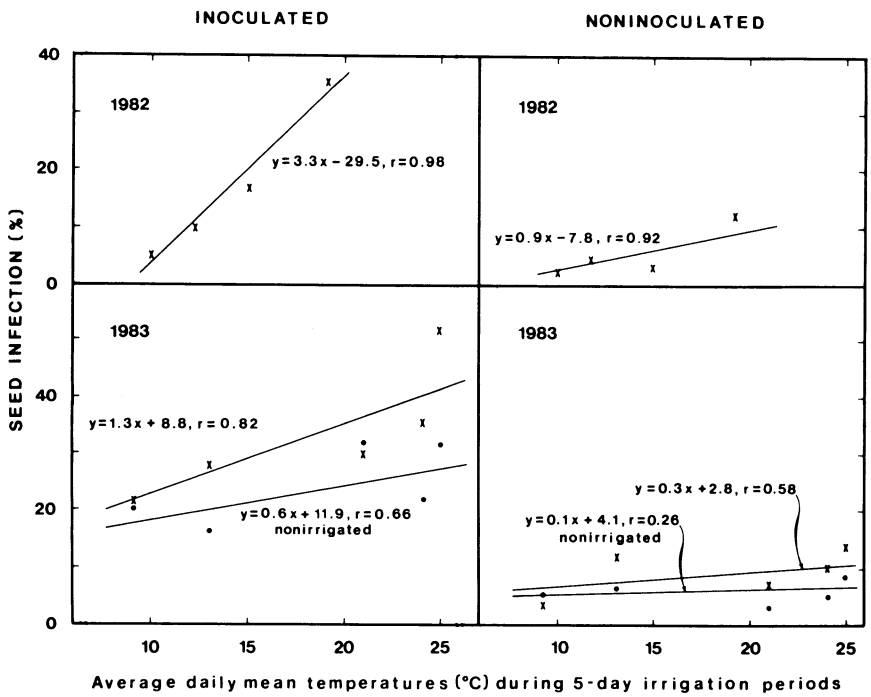

Fig. 3 Linear regressions between seed infection by PD at harvest maturity and average daily mean temperatures during 5-day irrigation periods of soybean field plots between growth stages R7 and R8. Data are presented for 2 yr for treatments in which pods were either inoculated at R5 with an isolate of *Phomopsis longicolla* or naturally infected with PD and either irrigated (x) or nonirrigated (•). In 1982, the nonirrigated treatment consisted of a separate plot in which seed infection values were 10 and 2%, respectively, for inoculated and uninoculated plots. Values are the mean of four replicates.

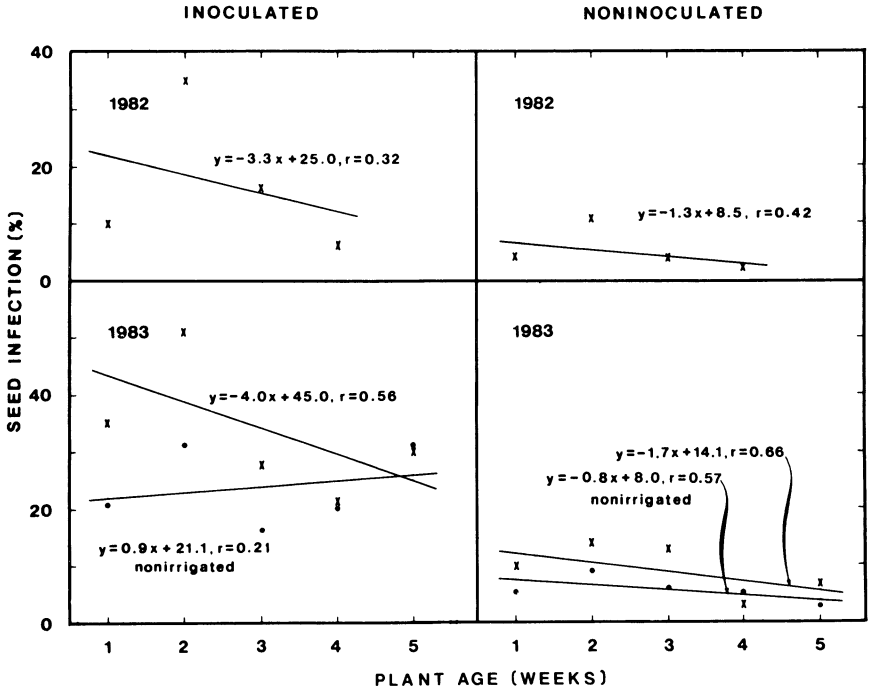

Fig. 4. Linear regressions between seed infection by PD at harvest maturity and plant age during 5-day irrigation periods of soybean field plots between growth stages R7 and R8. Week 1 represents the week during which plants reached R7. Data are presented for 2 yr for treatments in which pods were either inoculated at R5 with an isolate of *Phomopsis longicolla* or naturally infected with PD and either irrigated (x) or nonirrigated (•). In 1982, the nonirrigated treatment consisted of a separate plot in which seed infection values were 10 and 2%, respectively, for inoculated and uninoculated plots. Values are the mean of four replicates.

periods of high and low humidities did not result in significant seed infection; therefore, extensive seed infection may be expected only when there is precipitation (particularly during the day) that will maintain the relative humidity close to 100% for prolonged periods. Rainfall or fog would provide these conditions. The duration rather than the amount of precipitation will be the most important characteristic. It is not suprising that TeKrony et al (12) were unable to relate amount of rainfall between R7 and harvest maturity to seed infection but were able to correlate seed infection with minimum relative humidity over this period. The duration of the period of high relative humidity needed for seed infection to take place also will be affected by temperature. Our results suggest that at average temperatures lower than 15 C, there is little chance of severe seed infection in plants at R8 even when relative humidity remains close to 100% for 4 days, whereas at 20 and 25 C, this could be expected within 3 and 2 days, respectively.

Certain established patterns of Phomopsis seed decay may be explained by our findings. The disease tends to be more severe in early-planted or early-maturing cultivars (11). These crops usually are subjected to higher temperatures during the seed maturation phase than later maturing crops, and seeds are therefore more likely to become infected during wet periods. Harvest delays caused by wet weather have long been associated with increased severity of this disease (13). In the northern United States, harvest occurs in October and November, when average daily mean temperatures are usually lower than 15 C. As discussed, individual wet periods of as long as 4 days at such temperatures are unlikely to cause significant seed infection. However, we have shown that the process of seed infection can be resumed after periods of high humidity are interrupted by low humidity. There could therefore be cumulative effects of wet periods, and some increase in severity of seed infection might be expected if repeated wet periods occur at harvesttime.

The data obtained in this study provide a basis on which to develop a predictive model for this disease that could improve existing methods by incorporating short-term forecasts of temperature and precipitation.

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