Effects of Humidity, Temperature, Fertility, and Cultivar on the Reduction of Soybean Seed Quality by *Phomopsis* sp.

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ABSTRACT

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Effects of different combinations of high (33 C day, 24 C night) and low (26 C day, 16 C night) temperature, and high (90%) and low (43%) relative humidity imposed during plant maturation stages R6–R8 on the development of pod and stem blight symptoms, the occurrence of visibly moldy seed, Phomopsis seed infection, and seed germination were investigated for plants of two soybean cultivars maintained in growth chambers at high and low fertility. The temperature-humidity interaction was significant for all parameters studied. High humidity-high temperature resulted in seed with the most Phomopsis infection (49%) and the poorest germination (32%); high humidity-low temperature resulted in fewer Phomopsis-infected seed (33%) and better germination (62%). Both low humidity-low temperature and low humidity-high temperature resulted in the fewest Phomopsis-infected seed (4 and 4%) and seed with the best germination (95 and 96%), respectively. Percentage of moldy seed was also highest (28%) at high humidity-high temperature, lower (9%) at high humidity-low temperature and less than 1% at both low humidity and low temperature and low humidity-high temperature. There were no cultivar or fertility effects.

Additional key words: potassium.

In recent years, infection of soybean seed by pod and stem blight pathogens (*Phomopsis* sp. and *Diaporthe phaseolorum* var. *sojae*) has become a major cause of low seed quality in Ohio (8–10). Colonization by these fungi may lower the quality of seed used for processing and reduces germination of those used for seed. The percent occurrence of *Phomopsis* in mature seed may vary widely in different seasons and locations. *Phomopsis* has been isolated more frequently from seeds of early maturing soybean cultivars (6) and from seed of early planted soybeans but only rarely from seed of late planted soybeans (7). Kmetz et al (9) recovered *Phomopsis* from symptomless immature soybean pods and suggested it was a latent pathogen (sensu Verhoeff, 15) in soybeans under most field conditions. Kmetz et al (9) reported a dramatic increase in seed infection by *Phomopsis* sp. between the yellow pod stage (R7) and maturity (R8). Therefore, weather conditions during ripening may be most critical. Several authors (2,11,13) have suggested that high temperature and wet conditions either alone or occuring together late in the growing season probably favor seed colonization by fungi. Parts of soybean plants have been shown to have a late-season depletion of potassium (5). Two studies (1,12) report decreased Phomopsis seed infection after potassium was added to soil; however, a third study (14) showed no effects of potassium on disease.

The objective of this research was to determine the influence of fertilization and of environmental conditions, during soybean maturation, on seed infection by *Phomopsis*, and on the subsequent seed quality.

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MATERIALS AND METHODS

Plot design. Effects of four factors on infection of soybean seed *Phomopsis* sp. were evaluated in tests conducted in growth chambers (Model M 15, Environmental Growth Chambers, Chagrin Falls, OH 44022) by using a split-split-plot randomized complete block design. Two temperature levels were assigned to the main plots, two humidity levels to the subplots, and two cultivars and two fertility levels to the sub-subplots. Each fertility level-cultivar combination (sub-subplot) was repeated three times with each replication (three subsamples) and treatments were replicated three times, resulting in nine subsamples per treatment. Statistical significance was determined by analysis of variance of the complete experiment. Significant differences between effects were found by using Duncan's new multiple range test, $P = 0.05$.

Environmental factor treatments. Each subsample consisted of cultivar Wells (maturity group 2) or Wayne (maturity group 3) planted in a soil mix of high or low fertility in $20 \times 20 \times 40$-cm-long fiberglass containers. Twenty seeds per container were planted in a row and later the plants were thinned to 10. Plantings were maintained in growth chambers illuminated at 250 klux and regulated at 26 C day (16 hr) and 16 C night (8 hr) temperatures until plants had developed 10–12 nodes. Then, the daylength was

shortened and maintained at 12 hr to induce flowering. Plants were staked at stage R4 (3) to prevent lodging. At this stage, pods and stems were sprayed to runoff with a suspension containing 10,000 alpha spores of tap water by using an atomizer. Spore suspensions were prepared from 14- to 21-day-old acidified potato-dextrose agar cultures (APDA) of a *Phomopsis* sp. (sensu Kmetz et al, 9) obtained from soybean seed.

At stage R6 (full pod), different combinations of temperature (T) and relative humidity (RH) were imposed. They were: low T (26 ± 1 C day, 16 ± 1 C night) with low RH (43 ± 4%); low T with high RH (90 ± 4%); high T (33 C ± 1 C day, 24 C ± 1 C night) with low RH; and high T with high RH. High RH was obtained by placing a clear plastic (polyethylene, 0.076-mm, 3-mil) tent over the plants and intermittently hand-misting (replicate 1) or using an automatic humidifying system (replicates 2 and 3). For the latter, moist air was blown intermittently into the tent by a Herrmidifier (Model 500, Herrmidifier Co., Inc., Lancaster, PA 17604). The misting cycle was controlled by time clocks (Dayton time switch, Dayton Electric Mfg., Co., Chicago, IL 60648) to achieve humidity levels of 90% during both the day and the night. The low RH blocks were maintained at ambient humidity. The humidity in the chambers was initially measured with an Electric Hygrometer (Hygrodynamics, Inc., Silver Spring, MD 20910) using sensing elements for RH ranges of 29–43, 41–59, and 81–99%. Relative humidity was monitored throughout the experiment using Hygrothermographs (Belfort Instrument Co., Baltimore, MD 21224). High humidity varied between 86 and 94% and low humidity varied between 39 and 46%.

Fertility treatments. Fertility levels were varied by soil and foliar fertilization. For the low fertility treatment, soil from an unfertilized field plot was mixed with muck and Canadian peat (5:5:2, v/v), with 216 g lime and 162 g of Rhizobium inoculum (Legume-aid) per 100 L of soil. High fertility soil mix was recycled greenhouse soil mixed similarly. The low fertility soil mix tested P, 112 kg/ha; K, 135 kg/ha; with a pH of 6.1. The high fertility soil mix contained P, 150 kg/ha; K, 372 kg/ha; with a pH of 7.2. The high fertility treatments also received foliar applications of potassium (K) and phosphorus (P) as potassium polyphosphate (PPP), TVA 0-26-25, four times (at 7- to 10-day intervals) during pod filling stage, as recommended by Garcia and Hanway (4). The total amount of K applied was 24 kg/ha and that of P was 8 kg/ha. Spray solutions of PPP in tap water contained 0.1% Tween-80. The low-fertility treatment plants were sprayed with water plus surfactant only. Sprays were applied with an atomizer at 277 g/cm^2 pressure.

Data collection. Data were obtained on pod and stem blight (PSB) disease ratings, percentage visibly moldy seed, percentage Phomopsis-infected seed, and percentage seed germination. Foliar analysis for K and P were made at the full-pod stage. Mature soybean plants were evaluated for pod and stem blight severity immediately after harvest. The PBS rating system, proposed by Kmetz (*unpublished*), was used. It evaluates the severity of blotching and or speckling (pycnidia either scattered or in linear rows) as follows: 1 = no symptoms on stem or pods; 2 = slight symptoms on stem, no symptoms on pods; 3 = symptoms locally heavy but not covering entire system and pods generally without symptoms but occasionally one or two pods on plant with symptoms; 4 = symptoms on entire stem, pods generally without symptoms but occasionally one or two pods on a plant with symptoms; and 5 = symptoms on entire stem and on many pods. Percentages of visibly moldy seed, Phomopsis-infected seed, and seed germination were determined from 100 randomly selected seeds from each subsample. A visual count was taken of the number of moldy seed. The seeds were surface disinfested by soaking in 0.5% NaClO for 60 sec and then plated on APDA (10 seeds per plate). Plates were incubated at 24–26 C under continuous light from a 15-W cool-white fluorescent lamp. Seed germination was counted after 8 days and percentage seed infected with *Phomopsis* sp. was determined after 14–21 days, as described by Kmetz et al (9). Seeds were considered germinated if the radicle was 2 cm or longer.

Potassium and phosphorus determinations were made on leaf blade samples from fully developed, top-growth leaves (fourth to sixth nodes from top of plant) collected at full-pod stage (R6). The samples were rinsed twice for 15 sec in double-deionized water then oven-dried at 27 C for 12 hr. They were then ground to 0.5-mm (40-mesh) in a Wiley mill. Chemical analysis for K and P was by wet digestion using a modified version of the analytical procedure, described by Hanaway et al (5). A 0.5-g sample of the dried and ground tissue was digested in boiling concentrated nitric and perchloric acids until the solution became colorless. Phosphorus was determined colormetrically by a vanadomolybdate method. Total K was determined by flame photometry. All analyses are expressed as precentages of oven-dried material.

TABLE 1. Effect of humidity and temperture during soybean maturation on disease rating, percentage Phomopsis-infected seed, percentage visibly moldy seed, and seed germination[u]

Humidity level[v]	Temperature level[w]	Disease rating[x]	Phomopsis-infected seed (%)[y]	Moldy seed (%)[y]	Seed germination (%)[y]
High	High	3.1 a[z]	49 a	28 a	32 c
High	Low	3.0 a	33 b	9 b	62 b
Low	High	1.4 c	4 c	0 c	96 a
Low	Low	1.9 b	4 c	0 c	95 a

[u] Data from the two cultivars and the two fertility levels were pooled, because neither cultivar nor fertility significantly affected these characters.

[v] High humidity = 90 ± 4% RH, low = 43 ± 4% RH (ambient).

[w] High temperature = 33 ± 1 C day (12 hr), 24 ± 1 C night (12 hr); low = 26 ± 1 C day, 16 ± 1 C night.

[x] Mean of three replications of 120 plants, based on a scale of 1 to 5: 1 = no disease symptoms and 5 = entire stem and many pods blotched or speckled (pycnidia scattered on in linear rows).

[y] Mean percent based on 600 seed from each of three replications.

[z] Means followed by the same letter in each column are not significantly different, $P = 0.05$, according to the Duncan's new multiple range test.

RESULTS

Effects of humidity and temperature. The interaction of RH and T on the disease rating, percentage moldy seed, Phomopsis-infected seed and seed germination was highly significant (Table 1). There were higher disease ratings at high RH-high T and high RH-low T than at low RH at either high or low T. The combination of high RH-high T resulted in a significantly higher level of Phomopsis-infected seed than at high RH-low T. Seed infection at low RH with low or high T was minimal. Percentage moldy seed was higher at high RH-high T than at high RH-low T. Only trace amounts of moldy seed were found at low RH, regardless of temperature. Low RH-high T and low RH-low T resulted in seed with the highest germination percentage, the values not differing significantly. The lowest germination occurred in the seed produced at high RH-high T.

Cultivar and fertility effects. The two cultivars did not differ in disease rating, percentage Phomopsis-infected seed, moldy seed, or seed germination. Significantly higher levels of K and P occurred in foliage of plants from the high fertility treatment (1.4 and 0.25%, respectively) than in the controls (1.1 and 0.20%, respectively). Although foliar concentration of K and P was increased, there were no significant effects on disease ratings, levels of Phomopsis-infected seed, visibly moldy seed, or seed germination. None of the three- or four-way interactions were significant.

DISCUSSION

Soybeans that matured under high humidity conditions had severe disease symptoms, high percentages of Phomopsis-infected seed, and low germination rates at both temperatures that were studied. These results agree with previous reports (2,11,13) suggesting that moisture and high humidity late in the growing season influence seed infection by *Phomopsis* and other fungi. As a result of the increased infection, seed germination was significantly reduced. At low humidity, the percentage of Phomopsis-infected

seed was low at both temperatures and germination of the seed produced was excellent. The difference in percentage seed germination and Phomopsis-infected seed between the high and low temperatures was much less than the humidity effects. It was apparent from the interaction data that high temperature only affected the percentage Phomopsis-infected seed at high humidity. Wilcox et al (16) suggested that cooler temperatures later in the fall inhibit fungus development, in spite of humidity. Our data do not entirely support this conclusion. We found a small reduction in the percentage of Phomopsis-infected seed at low temperature as compared to high temperature, but only at high humidity. There was a somewhat larger reduction in the percentage of moldy seed at low temperature than at high temperature, again only at high humidity. In our experiment, however, we were dealing with infection of seed during maturation and not with delayed harvest of mature seed.

There is evidence that early maturing cultivars of soybeans have higher percentages of Phomopsis-infected seed than later maturing cultivars and that *Phomopsis* is isolated more frequently from seed of early than late soybean plantings (7,9). Both the late plantings and late maturing cultivars mature late in the growing season when temperatures in Ohio are commonly lower. In our experiment, cultivars Wells (early) and Wayne (later) had similar amounts of Phomopsis-infected seed when exposed to similar humidity-temperature conditions. It appears that low temperature conditions alone would not be sufficient to cause the low levels of *Phomopsis* in late-planted soybeans or late-maturing cultivars.

Variable results have been recorded on the effect of potassium on seed infection by *Phomopsis* sp. (1,12,14). In the present study, the percentage of potassium in the foliage was increased from deficient levels, in the low fertility treatments, to within the average range (sensu Hanway et al, 5) in the high fertility treatment, but the phosphorus levels in both treatments were within the established average range. Even though there were increased levels of potassium in the foliage of the high fertility treatment plants, potassium had no effect in this study.

It was concluded from the results of this study that humidity is the most important factor influencing seed infection by *Phomopsis* during soybean maturation. Temperature effects were minor. Soybeans that mature late in the season, either due to date of planting or cultivar maturity date, probably escape seed infection by *Phomopsis* sp. because relative humidity is not favorable at that time and not because of temperature conditions. Additional environmental information will be needed to verify this conclusion.

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