Quality Losses in Phomopsis-Infected Soybean Seeds

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ABSTRACT

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Several measurements of seed quality were used to compare soybean (*Glycine max* 'Wells') seeds with and without symptoms or signs of *Phomopsis sojae* infection. Seeds with symptoms contained more oil, protein, and recoverable *P. sojae* than symptomless seed. In addition, symptomatic seeds were smaller in size and volume, were lower in density, produced lower quality oil and flour, and had lower viability and durability than did symptomless seeds. Quality losses in symptomatic seeds were associated with premature death and the breakdown of seedcoat tissues. Thiram seed treatment reduced recovery of *P. sojae* from both symptomless and symptomatic seeds on PDA, but more effectively controlled the fungus in symptomless seeds than in symptomatic seeds. Seed quality losses attributed to "weathering" were due in part to *P. sojae* infection.

Additional key words: Pod and stem blight.

Prior to 1960 *Phomopsis sojae* Leh., causal agent of pod and stem blight of soybean (*Glycine max* [L.] Merr.), was considered of little importance to soybean production (1,13,19,22). Since that time, significant germination losses have been reported in heavily infected seedlots in the United States and Canada (9,10,27). Presently, *P. sojae* is recognized as a major cause of moldy, poorly germinating soybean seeds in Brazil, Canada, and the United States (4,5,7,27). *Phomopsis* seed infection often exceeds 50% on susceptible cultivars when harvest is delayed (9,23,28). Increasing concern over the quality of soybean seed has resulted in the printing of "percent Phomopsis-moldy seed" on soybean seed certification tags by the Illinois Crop Improvement Association, Urbana. Much is known about the ability of this pathogen to reduce seed viability and vigor, yet little is known about how *P. sojae* influences other seed quality factors. We report on the effect of infection by *P. sojae* on soybean grade components and composition as well as on the quality of soybean flour and oil.

MATERIALS AND METHODS

A seed lot of Wells soybeans was harvested in mid-October 1975 at 12% moisture from the University of Illinois Agronomy South Farm, Urbana, and stored at 0–2 C until used. The seed lot averaged over 50% infection with *P. sojae,* with many seeds showing discoloration, fissuring, flattening, and the presence of greyish-white fungal mycelium. The seed lot was divided into sublots of seeds with and without these symptoms.

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To measure the effect of *P. sojae* on soybean seed quality, the following tests were conducted on four or more 100-seed samples for each seed type: germination and recovery of *P. sojae* before and after seed treatment with thiram, 100-seed weight, volume and density, protein and oil content, simulated grain handling, and flour and oil quality.

Four 100-seed samples of each seed type were surface disinfested by submerging them in 0.5% sodium hypochlorite (Clorox®) for 4 min. Similar seed lots were treated with 125 g/100 kg of thiram as Arasan 50 Red (50% bis-dimethylthiocarbamoyl disulfide) (E. I. du Pont de Nemours & Co., Inc., Wilmington, DE 19898). Seed lots then were plated immediately on potato dextrose agar (Difco PDA) and incubated for 7 days at 25 C. The percentage germination and number of recoveries of *P. sojae* were recorded. A seed was considered germinated if the radicle was 2.5 times the length of the cotyledons. The percentage control (C) by seed treatment of internally seedborne *P. sojae* was determined by the following formula:

$$C = \frac{R_{nt} - R_{st}}{R_{nt}} \times 100$$

where R_{nt} was the number of *P. sojae* recoveries from nontreated seed and R_{st} was the number of *P. sojae* recoveries from thiram-treated seed.

To determine seed density, 12 100-seed samples each were randomly selected from seed lots with and without symptoms. Each sample was weighed and poured into a 100-ml graduated cylinder. The volume of each seed sample was recorded at the edge of the highest seed. Weight and volume data were used to determine density.

Protein and oil content were determined using the Dickey-John Infrared Analyzer (14). Before analysis, flours were adjusted to 6% moisture. Five determinations were made for each of four 100-seed samples.

To test susceptibility of grain to splitting, five 100-seed samples of nonsplit seed from each seed type were placed in cloth bags and tumbled for 1 hr in a soil mixture to simulate grain handling. The number of split seeds was recorded after treatment.

Flour and oil quality were tested by preparing flours from each seed type by grinding them in a Waring Blendor for 1 min. Prepared flours were organoleptically rated for "off" odor by 15 panelists in a blind study using the following ratings: (i) imperceptible, (ii) slightly perceptible, (iii) moderately perceptible, (iv) perceptible, (v) slightly pronounced, (vi) moderately pronounced, and (vii) very pronounced. Flour samples then were transferred to extraction thimbles and placed into Soxhlet extractors. Oil was extracted at 65 C with petroleum ether for 4 hr. The solvent was removed under vacuum overnight at 50 C. Four oil samples from each seed type were analyzed for peroxides using the standard American Oil Chemist's Society method for soybean (20). Oil was examined visually for pigmentation and organoleptically for off odor.

RESULTS AND DISCUSSION

With the exception of yield, grade is the major determinant of producer profit. Reductions in grade can cause sizable economic losses for growers. Seedborne *P. sojae* has not been examined for its effects on soybean grade or grade components. Criteria for soybean grade include damaged seed content, splits, and test weight. Seeds infected with *P. sojae* are distorted in size and shape and frequently are covered by a visible mycelial growth (moldy).

Since moldy seeds contribute to the damaged seed content, seed lots that are heavily infected with *P. sojae* may be downgraded. In U.S. No. I grade soybean seeds, moldy seed is limited to 2% or less; No. II to 3%; No. III to 5%; No. IV to 8%; and sample grade to above 8% (25). The grade and moldy seed content of Wells soybeans harvested at the University of Illinois Research Farms from four separate plots in 1975, 1976, and 1977 were: IV—6%, sample—11%; IV—8%, and sample—9%; I—0%, I—1%, I—1%, and 1—0%; and III—4%, IV—6%, III—5%, and sample—12%; respectively. The seed lots from 1975 and 1976 were harvested 1 month after maturity, while the 1977 seed lots were harvested at maturity.

Phomopsis sojae symptomatic seed were lower than symptomless seed in seed weight, volume, and density and higher in induced splitting (Table 1). *Phomopsis sojae* can lower grade by reducing test weight and by increasing the number of split seeds. Workers have considered *P. sojae* unimportant for soybean production (1,13,19) or important only with regard to germination (10,27). We found that symptomatic seed could lower grade directly by increasing numbers of moldy beans or indirectly by lowering test weight or increasing the number of split seeds.

Human consumption of cooked soybeans and soy flour may help alleviate a global protein deficiency (6). Tropical climates high in rainfall and temperature are favorable for rapid development of seedborne *P. sojae*. In such areas, the adaptability of this crop may depend on the ability to produce acceptable seed quality, especially for production of high-quality food products. In Korea, *P. sojae*-infected pods were found unfit for vegetable use (20). Results of organoleptic tests of crude flour showed that symptomatic seed yielded a poor quality flour that may be unacceptable for human consumption (Table 2). Food products from *Phomopsis*-infected seed are generally low in quality and acceptance.

In the United States, most soybeans are crushed for oil. Historically, the oil has been worth more than the residue meal. Soybean oil must be colorless for acceptance in food products. Unrefined oil is normally clear amber after the yellow carotenoid pigments have been removed (3). Oil from *P. sojae*-symptomatic seeds was visibly darker when compared with that from symptomless seed (Table 2). Besides being of low visual quality, oil from *P. sojae* symptomatic seed had an unpleasant, rancid, off odor, and a high peroxide value that indicated oil deterioration. Soybean oil quality also was shown to be lower in *Aspergillus*-infected soybean seeds than in noninfected seeds (17).

The soybean seedcoat accounts for only about 8% of the total dry weight of the seed and contributes little to oil and protein content. Nevertheless, the seedcoat structure is vital to preserve seed quality. Dehulling oil seed causes the rapid deterioration of their lipids (3). An intact seedcoat prevents atmospheric exposure and slows oxidation of lipids. The effects of *P. sojae* on seed quality dramatized the importance of the seedcoat in seed quality maintenance. *Phomopsis sojae* preferentially colonizes seedcoat tissues (15,23). Much of the fissuring and flattening of symptomatic seed probably is due to fungus breakdown of the seedcoat. *Phomopsis sojae* infection may remove the protective value of seedcoat tissues effectively, resulting in general loss of quality. Preferential use of seedcoat tissues by *P. sojae* may account for changes in protein and oil contents of symptomatic seeds (Table 2).

The value of soybean seed treatment has been judged for its ability to increase stand without regard to control of seedborne microorganisms and their role in spreading disease (2). Tiffany (24) reported that seedborne *Colletotrichum dematium* var. *truncatum* not only caused damping-off in soybeans but also was transmitted to adult plants by symptomless seedborne infections.

TABLE 1. Grade-related components of Wells soybean seeds with or without symptoms of *Phomopsis sojae*

Seed lot	Weight[a] (g)	Volume[a] (ml)	Density[a] (g/ml)	Splits[b] (%)
With symptoms	14.2	22.4	.63	7.8**[c]
Without symptoms	16.7**[c]	25.2**	.66*[c]	1.4

[a]Means based on 12 replications of 100 seeds each.

[b]Mean number of split seeds after simulated grain handling on five replications. Data was transformed using square root transformation.

[c]** and * indicate statistical significance at $P = 0.01$ and 0.05, respectively, using FLSD test.

TABLE 2. Description of soybean flour and oil derived from Wells soybean seeds with or without symptoms of *Phomopsis sojae*

Seed lot	Sensory evaluation of flour for "off" odor[a]	Composition[b]		Oil	
		Oil (%)	Protein (%)	Peroxide value[c] (meq/kg)	Dark color and "off" odor
With symptoms	Moderately pronounced*[d]	25.6**[d]	43.3*	125**	+[e]
Without symptoms	Slightly perceptible	23.0	42.4	52	–

[a]Mean category based on 15 panelists.
[b]Mean based on 20 determinations.
[c]Mean based on four determinations.
[d]Indicates statistical significance at $P = 0.05$ and 0.01 level, respectively, using FLSD.
[e]Qualitative observation: +, with dark color and off odor; –, without.

TABLE 3. Percentage[a] seed germination and percent recovery of *Phomopsis sojae* from Wells soybean seeds with and without symptoms of *P. sojae*, nontreated or treated with thiram fungicide

	Treated[b]		Nontreated		Control with seed treatment[c]
	Germination	% Recovery	Germination	% Recovery	
With symptoms	20	34**	12	84**	62
Without symptoms	91**	4	86**[d]	30	86*[d]

[a]Based on four replications of 100 seeds each.
[b]Seed treatment consisted of application of Arasan 50 Red at 125 g/100 kg of seeds before planting.
[c]Phomopsis control (C) =

$$\frac{\left(\begin{array}{c}\textit{Phomopsis}\text{ recovery without}\\ \text{seed treatment}\end{array}\right) - \left(\begin{array}{c}\textit{Phomopsis}\text{ recovery with}\\ \text{seed treatment}\end{array}\right)}{\text{Phomopsis recovery without seed treatment}} \times 100$$

[d]** and * indicate statistically higher value at $P = 0.01$ and 0.05, respectively, using FLSD test.

Latent infections of *P. sojae* also have been found in soybeans (18). The importance of latent infections is unknown, but they likely contribute more to the disease development at the end of the season than do infections that kill young plants. In this study, low germination (12%) and high recovery of *P. sojae* on PDA were found for symptomatic seed. Seeds without symptoms had a high germination (86%) and 22% infection by *P. sojae* (Table 3). Seed treatment with thiram reduced *P. sojae* and increased germination in both seed types, but control of *P. sojae* was significantly greater in symptomless seeds. Soybean seed treatment has been considered necessary for "poor" quality seeds, ie, seeds with symptoms and germination below 80%, but not for "good" quality seeds, ie, seeds without symptoms and with a germination above 80%. Ignoring seed treatment of so-called good quality seed may work to perpetuate seedborne pathogens.

Agronomists, plant breeders, physiologists, and pathologists often describe soybean seed deterioration after a delayed harvest as "weathering" (3,8,12,21). Weathering is usually a problem under warm moist conditions, and the descriptions of "weathered" seed often are identical with symptoms caused by *P. sojae*. Little weathering was found after a delayed harvest when benomyl was applied at reproductive stages (8,11,16,26) and in cultivars resistant to seedborne infection (23,28). Therefore, we suggest that weathering is actually symptoms of infection by *P. sojae* and other seedborne fungi.

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