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Phomopsis Seed Decay of Soybeans—A Prototype for Studying Seed Disease

Every population of soybean (*Glycine max* (L.) Merr.) seeds potentially carries a wide variety of microorganisms. This potential microcosm of microorganisms includes fungi and bacteria, some of which can cause disease in soybean plants and seeds. Seed populations and individual seeds within a population may vary among themselves in the type and number of microorganisms they carry. Most pathogenic fungi, such as species of *Alternaria, Cercospora, Colletotrichum, Fusarium, Nematospora*, and *Phomopsis*, colonize or infect soybean seeds prior to harvest (31). Generally, we consider the seed diseases caused by *Phomopsis* to result in greater loss in seed quality in Illinois than any of these other field-infecting fungi.

Phomopsis seed decay is one of several ubiquitous soybean diseases caused by members of the *Diaporthe*/*Phomopsis* complex (12,22,28,31). Although all members of the complex are seedborne in soybeans, the primary cause of Phomopsis seed decay is *P. longicolla* T.W. Hobbs (12). Endemic throughout all soybean-growing areas in the United States and the world, this complex probably has been studied more thoroughly than many other seed diseases of large-seeded legumes caused by field fungi. Pathogens in this group also can infect hosts other than soybeans. *Phomopsis* isolates pathogenic to soybeans have been isolated from seeds of other large-seeded legumes, including azuki bean (*Vigna angularis* (Willd.) Ohwi & H. Ohashi), broad bean (*Vicia faba* L.), cowpea (*Vigna unguiculata* (L.) Walp.), French bean (*Phaseolus vulgaris* L.), jack bean (*Canavalia ensiformis* (L.) DC.), lima bean (*Phaseolus lunatus* L.), mung bean (*Vigna radiata* (L.) R. Wilcz.), pea (*Pisum sativum* L.), and scarlet runner bean (*Phaseolus coccineus* L.) (1,4,5,17). Phomopsis seed decay in soybeans can thus serve as a prototype for studying *Phomopsis* diseases in seeds of these and other crops.

Research on Phomopsis soybean seed decay began at the University of Illinois at Urbana-Champaign in 1969 and has involved personnel in the departments of Plant Pathology, Agricultural Engineering, Agricultural Economics, Agronomy, and Food Science and the Illinois Crop Improvement Association, Inc. (ICIA), Champaign. This article summarizes the research done primarily in Illinois on the disease and its causal agent.

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Symptoms

Severely infected soybean seeds are shriveled and elongated, and their coats are cracked and appear whitish and chalky (Fig. 1) (30,31). Severely infected seeds may not germinate or may have delayed germination. Seeds may be infected and not show symptoms (17,29). A paraquat treatment cannot induce symptoms of *P. longicolla* infection on asymptomatic seeds as it can on other infected soybean tissues (29). Both symptomatic and asymptomatic infections can result in pre- and postemergence damping-off and, under environmental conditions favoring early disease development, can result in significant reductions of yield (23). The fungus can also become systemic but fail to induce symptoms in seedlings from infected seeds (17,29).

Occurrence and Estimated Losses

Phomopsis seed decay is endemic in Illinois, with incidence varying from year to year. We have estimated the occurrence of Phomopsis seed decay each year since 1969 (Fig. 2). From 1969 to 1974, the occurrence of *Phomopsis* from surface-sterilized soybean seeds was recorded from seed lots collected at random from various locations within the state (3). Beginning in 1975, and every year since, the ICIA has recorded the occurrence of *Phomopsis* on over 8,000 soybean seed lots eligible for certification each year. A detailed study was done during 1975–1977 using ICIA data on the occurrence of Phomopsis seed decay in Illinois by county (Fig. 3) (26). The incidence of Phomopsis seed decay varied between years and among counties for any single year. Levels of disease were always highest in counties bordering major waterways, such as the Illinois, Mississippi, Ohio, and Rock rivers, regardless of rainfall, and throughout soybean-growing areas with abundant rainfall. These findings supported reports that abundant moisture and heavy dews were important in disease development (15,35). We found that incidence of the disease was higher in soybeans grown in river or stream bottomland or near large lakes in Illinois, where prolonged heavy dews and fogs are common.

During 1970, 1972–1977, and 1984–1987, the incidence of Phomopsis seed decay exceeded 2.1%, a threshold at which grain buyers discount the price paid for soybeans and at which losses to the disease can be measured economically. This threshold was used to estimate the losses for 1984–1987 (Table 1). This threshold is low, since significant effects on germination and emergence are apparent only when levels of *Phomopsis* exceed 20% (3,33). An economic threshold has not been established for the level of infected seeds used for value-added products. In addition to reducing grain quality, *Phomopsis* was the only one of nine fungal genera isolated from soybean seeds shown to reduce seedling emergence under both laboratory and field conditions (21,24).

Epidemiology

P. longicolla overseasons as dormant mycelia in infected seeds and in soybean and other host debris (31). Symptoms and pycnidia (fruiting structures) form on overwintered debris and on petioles of the current year's abscised leaves. Conidia produced by the pycnidia are spread by splashing water. These conidia germinate, and the fungus colonizes seedling and plant tissues within 2 cm of the point of infection without inducing symptoms (17,29). When plants begin to senesce or are under stress, the fungus spreads to tissues about 5 cm from the point of infection and induces symptoms. Infection rarely goes systemic in the vascular system.

Only infections initiated on pods can result in infections of seeds and cause seed decay, although the level of pod infection may not predict the amount of seed infection (14). Most seed infection occurs during or after the yellow pod stage (R7) (11). Prolonged wet periods during pod development and maturation and warm weather (temperatures above 20 C [68 F]) favor the spread of the fungus from infected pods into seeds. *Phomopsis* is more prevalent in seeds from pods at the bottom of the plant than from pods at the top (11), but when harvest is delayed by wet conditions, seeds may be infected throughout the plant. Alternating periods of wet and dry weather favor pod deterioration and splitting and also facilitate infection of mature seeds. More seed decay occurs in plants deficient in potassium (31), infected by a virus (9), or heavily attacked by insects (27).

The relationship among weather conditions, crop maturation, and the incidence of seedborne *Phomopsis* was demonstrated from seeds of 16 soybean cultivars representing four maturity

Fig. 1. Soybean seeds covered with growth of *Phomopsis longicolla*, the cause of Phomopsis seed decay.

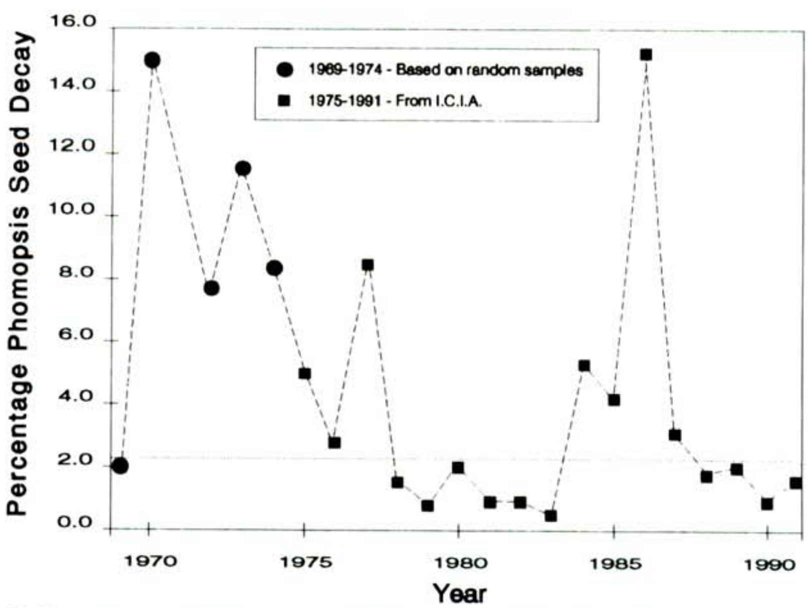

Fig. 2. Percentage occurrence of Phomopsis seed decay from 1969 to 1991. I.C.I.A. = Illinois Crop Improvement Association, Inc., Champaign.

groups harvested from 30 disease-monitoring plots at 12 locations in Illinois during 1978–1981 (Fig. 4) (15,16). Moisture, rather than temperature or geographic area, was the dominant environmental factor in determining disease development. In addition to the association of Phomopsis seed decay with the proximity of major waterways, other moisture factors influence the level of seed decay. Phomopsis seed decay generally is greatest during high rainfall seasons and least during low rainfall seasons. The recovery of *Phomopsis* is higher from seeds grown in northern areas of Illinois than in southern areas, partly because of heavier rainfall and prolonged high moisture periods in the north (33). Weather conditions during crop maturation stages affect disease development and result in differences among maturity groups. Within any growing region in Illinois, seeds of early-developing cultivars are exposed to the highest temperatures and most rainfall and are more likely to be infected by *Phomopsis* than later-maturing cultivars.

Host-Parasite Relationship

We consider *P. longicolla* as one of the most aggressive fungal pathogens of soybean seeds (19). Microscopic studies on the penetration and colonization of soybean pods and seeds by *Phomopsis* have shown that the fungus enters either indirectly through the microcycle and hilar region, through pores and cracks in the seed coat (18,32,34), or directly through pod walls and then through seed coats (32). After penetration, the pathogen colonizes seed coat tissue (Fig. 5), following which it invades the embryo and endosperm, causing tissue disintegration (13,32). The fungus can be distinguished microscopically from other fungi in soybean seed coat tissues by its large (3.8–8.7 μm wide), hyaline mycelium that contains oil globules and by the formation of hyphal aggregates and mycelial mats in the tissues (Fig. 5) (19,31,32). Because *Phomopsis* colonizes first the seed coat tissues and then the embryo, problems arise in the measurement of soybean seed viability. In Illinois, variation in seed germination has been correlated with the incidence of *Phomopsis* (2,6,15,16).

Germination Potential

Problems in evaluating the viability of soybean seeds infected with *Phomopsis* occur when various methods for measuring germination are used, such as rolled paper towels, moist cellulose (Kimpac) pads, sand or a sand/soil mixture, agar plates, etc. For example, germination of soybean seeds placed in moist rolled paper towels was 33% below that of seeds from the same lot but sowed in sand (8). Such problems led to the concept of "germination potential" for soybean seeds (6,7).

Germination potential is the maximum seed germination that can be obtained in the absence of seedborne microorganisms or when the effect of such microorganisms on germination is minimized (e.g., as by use of seed treatment fungicides) and was developed using Phomopsis seed decay as the prototype. The germination potential of soybean seeds is broadly defined as the maximum level of germination by a seed sample under low stress conditions. The germination potential of soybean seeds can decrease with age or from phytotoxic treatment or deterioration by seedborne microflora, but it cannot be "increased" by any treatment. When the germination or emergence of seeds is greater after some treatment than in an untreated control, the stress factor that reduced the germination potential of those seeds has been mitigated, allowing their germination potential to be expressed.

Seedborne *Phomopsis* can mask the germination potential of soybean seeds by killing young embryos during water inhibition and germination. If fungal activity can be restrained by some means, however, the germination potential of such seeds can be measured (Table 2) (36). The germination potential of soybean seeds colonized or infected with *Phomopsis* can be realized by the application of fungicides to the seeds (2,25), thermotherapy (36), control of the environment during the germination process (8), or other means.

For many years in Illinois, the germination of soybean seeds sometimes has been lower immediately after harvest than after several months in storage. We have attributed this, in part, to the reduction in the quantity of *Phomopsis* asso-

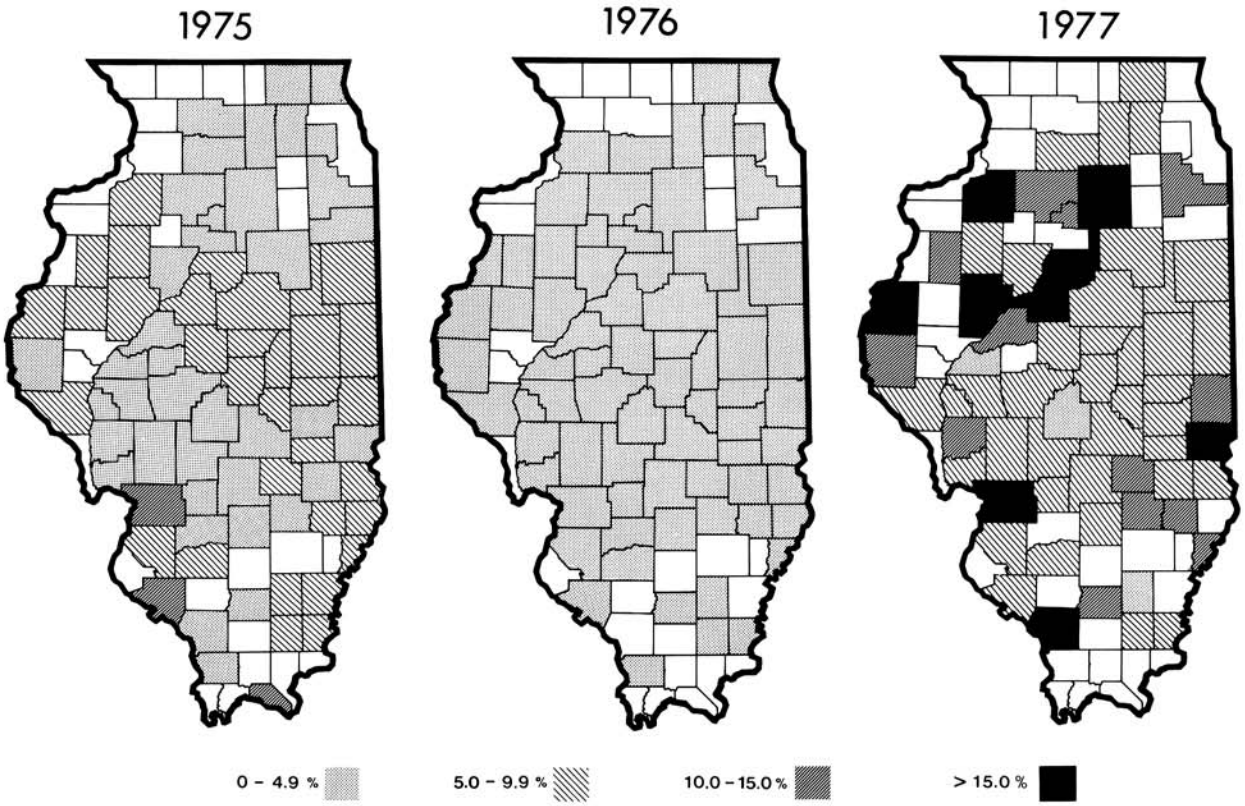

Fig. 3. Mean incidence of Phomopsis seed decay in Illinois by county during 1975–1977. (From Shortt et al [26])

ciated with such seed lots, assuming that superficial mycelia and spores of the fungus lose viability and thus may not survive well under storage conditions.

Seed Quality

Phomopsis not only reduces the quality of seeds used for planting by affecting germination potential but also can reduce the quality of seeds used for processing. Ideally, soybeans used for feed, food, and oil production should be high in protein and oil and low in fatty acid content. The infection of seeds by *Phomopsis* may reduce flour and oil quality, increase fatty acid content, change the composition of free fatty acids, and affect other quality factors (30). The quality of flour and oil derived from seeds infected with *Phomopsis* can be reduced to an unmarketable level (10). Similarly, soybean seeds with symptoms caused by *P. longicolla* were 4% lower in density and 13% lower in volume and weight and had a breakage susceptibility 20 times higher than asymptomatic seeds (20).

Table 1. Estimated losses due to Phomopsis seed decay in Illinois during 1984–1987

Year	Phomopsis seed decay[w] (%)	Discount[x] (¢/bu)	Production[y] (million bu)	Estimated loss[y] (million $)
1984	5.25	7	284.1	19.9
1985	4.15	4	382.5	15.3
1986[z]	15.24	62	360.0	223.2
1987	3.15	2	323.0	6.5

[w] From Illinois Crop Improvement Association, Inc., Champaign, based on over 8,000 soybean seed samples for each season.

[x] Calculated by Anderson's grain elevators, Champaign: $0.01 discount for each 0.5% *Phomopsis* from 2.1 to 5%, $0.02 discount for each 0.5% *Phomopsis* from 5.1 to 8%, and $0.03 discount for each 0.5% *Phomopsis* above 8%.

[y] From D. L. Good, professor of agricultural economics at University of Illinois at Urbana-Champaign.

[z] It is unlikely that grain buyers detected as much damage as reported, and discounts probably did not average $0.62/bu.

Significance and Outlook

This is the first report on disease loss estimates for 23 yr of a seed disease of a large-seeded legume. Research has led to a better understanding of the effects that fungi can have on seed quality and the measurement of these effects. The seed is a miniature plant, and the principles that apply to the study of diseases of full-size plants and seedlings also apply to diseases of seeds. Phomopsis seed decay is the result of the interaction among the seed, the pathogen, the environment, transmitting agent(s), and time, and that result often is the physical expression of symptoms. This seed disease is endemic in Illinois and has been studied more thoroughly than any other seed disease of large-seeded legumes caused by field fungi. The interaction, however, does not always result in symptom development. In its latent phase, *Phomopsis* must be considered as a seed colonizer that reduces germination and lowers the germination potential of asymptomatic seeds.

The levels of incidence and economic loss are influenced by the presence of abundant moisture during maturation. A disease prediction model based on quantity and duration of moisture and growth stage could be developed. However, realistic economic thresholds for levels of disease on seeds to be used for processing must be determined separately from thresholds for seeds to be used for planting. Seeds in lots with approximately 20% Phomopsis seed decay can be used for planting without

Fig. 4. Location of Illinois soybean disease-monitoring plots during 1978–1981. (From Jordan et al [15])

significant losses of stand or yield. An arbitrary threshold of 2.1% has been set for grain to be used for value-added products, but a more realistic economic threshold needs to be developed.

Acknowledgments

The research conducted at the University of Illinois at Urbana-Champaign on which this article is based was supported in part by the Illinois Soybean Program Operating Board, Bloomington; the Illinois Crop Improvement Association, Inc., Champaign; the American Soybean Association, St. Louis; and the Archer Daniels Midland Foundation, Decatur, Illinois.

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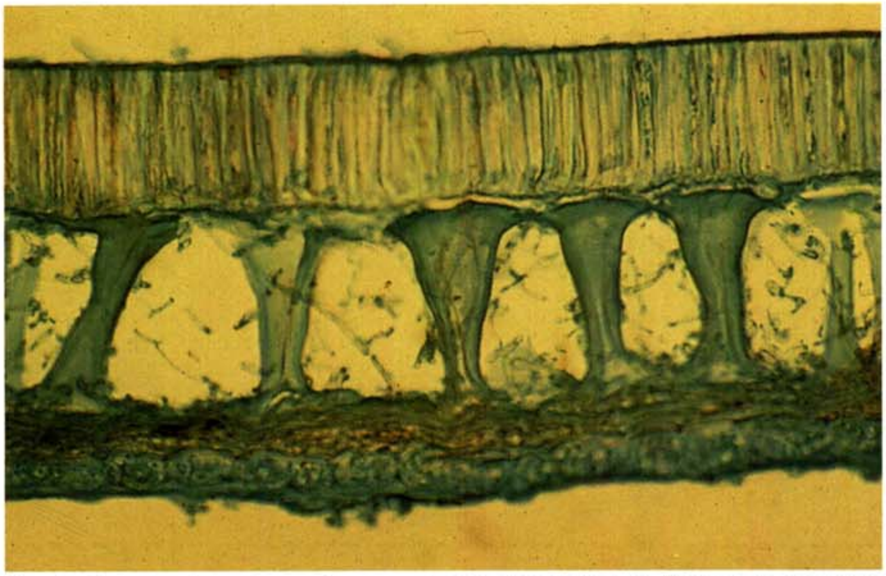

Fig. 5. Cross section of a soybean seed coat with hyphae of *Phomopsis longicolla* colonizing the hourglass cell layer and penetrating the upper palisade cell layer and the lower aleurone cell layer, in a bright field photomicrograph. (Courtesy M. B. Ilyas) (From Sinclair and Backman [31])

Table 2. Effect of soybean oil thermotheraphy on germination and recovery of *Phomopsis* from soybean cv. Wells seeds at 6.6% moisture content[w]

Treatment[x]	Time (min)	Germination[y] (%)	*Phomopsis* recovery[y] (%)
Unheated control	...	68 c[z]	70 a
Ethanol control	...	73 de	55 b
90 C	0.5	83 ab	19 c
	1.0	86 a	2 cd
	1.5	84 a	1 d
	2.0	83 ab	0.5 d
	2.5	83 ab	0.5 d
	3.0	85 a	0.5 d
	3.5	76 cd	0.8 d
	4.0	82 abc	0.0 d
	4.5	77 bcd	0.0 d
	5.0	82 abc	0.2 d

[w] From Zinnen and Sinclair (36).
[x] Neither control was placed in oil. Ethanol control was immersed 4 min in 95% ethanol before seeds of both controls were surface-disinfested by immersion in 0.05% NaOCl for 4 min, then rinsed at least twice in distilled water.
[y] All seeds were plated on potato-dextrose agar, incubated in the dark at 25 C, and observed 6–8 days after planting; a seed was considered germinated if its radicle was at least as long as its cotyledons. Mean of at least six replications of 50 seeds per replicate.
[z] Means followed by the same letter are not significantly different at $P = 0.05$ according to Fisher's least significant difference.

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