Pod Protection Effects on Soybean Seed Germination and Infection with Diaporthe phaseolorum var. sojae and other Microorganisms

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ABSTRACT

Soybean seed was harvested when mature and 6 weeks after maturity from nonprotected pods and pods protected from 5 weeks before maturity until 6 weeks after maturity to compare germination and infection with *Diaporthe phaseolorum* var. *sojae* and other microorganisms. Protection prior to ripening did not affect germination or infection. Protecting pods for 6 weeks after maturity significantly increased germination and reduced infection with *D. phaseolorum* var. *sojae*, *Alternaria* spp., and miscellaneous microorganisms. The data do not clearly indicate the time of greatest infection with *D. phaseolorum* var. *sojae*, but infection with *Alternaria* and miscellaneous microorganisms progressively increased when protection was delayed 2 or more weeks after maturity. Pod protection or time of harvest did not influence infection with *Cercospora kikuchii*. Stem inoculation with *D. phaseolorum* var. *sojae* did not increase seed infection.

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Additional key words: pod and stem blight, purple seed stain, soybean seed quality.

Soybean seed quality, judged by germination and general appearance of the seed, has become of increasing concern in much of the soybean-growing area of the United States. In the midwestern U.S. and in Maryland and Delaware, *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *sojae* (Lehman) Wehm., the causal organism of pod and stem blight, is the predominant organism associated with lowered seed quality. The fungus generally has been considered a weak parasite. It overwinters in the seed and on diseased pods and stems in the soil. Pod and stem blight first appears as pycnidia on the petioles of lower leaves and upon broken lower branches. Pycnidia develop on both the main stem and upper branches following the death of the entire plant after maturity, or after death from other causes. Dead stems may be covered with pycnidia or the pycnidia may be limited to small patches usually near the nodes. Pycnidia frequently are found on dry, poorly developed pods. There is no proof that the fungus causes the failure of pods to develop normally. The most important aspect of the disease is its effect on the seed. Infected seed may exhibit various degrees of cracking of the seed coat and shriveling, and frequently are partially or completely covered with a white mold. Infected seeds may be smaller than healthy ones, but the fungus is sometimes present in seeds that are normal in size and appearance. We, and others (2, 3), have found that infected seeds usually do not germinate or, if they do, may produce weak seedlings. Wilcox & Abney (4) reported a correlation coefficient of 0.91 between seed infected with *D. phaseolorum* var. *sojae* and percentage of seed germination.

The time and site of infection with *D. phaseolorum* var. *sojae* is not well understood. The fungus apparently does not become systemic from infected seed. On the other hand, the fungus may remain passive in the plant for some time before its presence is expressed by slightly premature ripening and/or the appearance of pycnidia in conjunction with adverse conditions or normal ripening. Our unpublished data indicate that the fungus does not penetrate uninjured pods but may enter through abrasions, cracks, or other injuries. Seed infection increases with delayed harvest, particularly when accompanied by alternate wetting and drying, which is conducive to pod deterioration and splitting along the suture. Wilcox & Abney (4) found that seed infection with *D. phaseolorum* var. *sojae* on individual plants was higher on lodged or broken branches in contact with soil than on erect central stems.

To determine the time of seed infection and the possible systemic nature of infection, we compared the amount of infection in nonprotected pods with that in (i) pods protected for intervals prior to ripening, (ii) pods protected for intervals prior to and

after ripening with delayed harvest, and (iii) pods protected on inoculated plants with normal and delayed harvest.

MATERIALS AND METHODS.—All tests were with 'Amsoy 71' soybean, *Glycine max* (L.) Merr., planted 18 May 1971 at the Purdue University Agronomy Farm near Lafayette, Indiana. Amsoy 71 is susceptible to *D. phaseolorum* var. *sojae* and highly susceptible to *Cercospora kikuchii* (Matsumoto & Tomoyasu) Gardner, the causal fungus of purple seed stain. Plants were in rows 1 m apart and 2.4 m long, and thinned to ca. 5 cm between plants. Ten pods at various locations on each of 10 plants in each row were protected on the appropriate dates by enclosing them in a Central States No. 571 pollinating bag tightly stapled around the pedicels. The protected pods and an equal number of nonprotected pods were harvested from the same plants either when mature or 6 weeks after maturity. Plants were inoculated by inserting a toothpick tip overgrown with mycelium of *D. phaseolorum* var. *sojae* into the stem at the first or second trifoliolate leaf node and covering the wound with petrolatum. The plants were inoculated 9 August, 5 weeks before the plants were mature, and the protected pods on these plants were covered the same day. The tests were arranged in split-plot designs with four replications.

One hundred seed samples from each treatment were surface-sterilized with 1% sodium hypochlorite, and 10 seeds were placed on each petri plate of potato-dextrose agar. After 5-6 days, germination of the seed and all microorganisms growing from the seed were recorded. *Diaporthe phaseolorum* var. *sojae* and possibly some *D. phaseolorum* (Cke. & Ell.) Sacc. *caulivora* Athow & Caldwell were combined because of the difficulty of differentiating between them without further culturing. All other microorganisms except *Cercospora kikuchii* and *Alternaria* spp. (mostly *Alternaria tenuis* Auct.) were classed as miscellaneous. The data were analyzed statistically.

RESULTS AND DISCUSSION.—There were no significant differences in the percentage germination or infection with *Diaporthe, Cercospora, Alternaria*, or miscellaneous microorganisms whether the pods were protected 1, 2, 3, 4, or 5 weeks prior to ripening of the seed. Average infection from protected and nonprotected pods, respectively, was as follows: *Diaporthe*, 14% (range 13.8 - 15%) vs. 12%; *Cercospora*, 16.9% (range 14.3 - 22.8%) vs. 16.2%; *Alternaria*, 3.9% (range 2.8 - 5.5%) vs. 3%; and miscellaneous microorganisms, 1.4% (range 0.2 - 3.2%) vs. 1.1%. The only significant difference in the test was an 8% lower average germination of seeds from the protected pods, suggesting that the pollination bags provided a somewhat unfavorable environment for the maturation of the seed. Some bags contained moisture following rains, indicating less than total protection.

In test 2, pods were protected for 1 to 11 weeks (5 weeks before maturity until 6 weeks after maturity). There was no significant difference in germination or infection with *Diaporthe* or *Cercospora* associated with the precise length of time the pods were protected. There was a significant increase in *Alternaria* and miscellaneous microorganisms when the protective bags were put on 2 or more weeks after the seed was mature. Germination of seed from nonprotected pods (24%) was significantly lower than germination of seed from protected pods (65%), whereas infection with *Diaporthe* (46% vs. 26%), *Alternaria* (35% vs. 11%), and miscellaneous microorganisms (15% vs. 6%) was significantly higher from nonprotected than protected pods. Infection with *Cercospora* (16% vs. 18%) was lower from nonprotected pods. We believe this was due to our inability to detect all of the *Cercospora* when there was a high percentage of the seed infected with the much faster-growing *Alternaria* and miscellaneous microorganisms, particularly Fusaria. As had been observed before, infection with *Cercospora kikuchii* did not increase with delay in harvest as did infection with *Diaporthe, Alternaria*, and the miscellaneous microorganisms. Seed from protected pods harvested 6 weeks after maturity were 26% infected with *Diaporthe* as compared to 12-14% infection from protected or nonprotected pods harvested when mature, suggesting either that the pods were not completely protected, the fungus was present on the pods before they were covered, or the fungus entered the seed via the plant. The

TABLE 1. Soybean seed germination and infection with *Diaporthe, Cercospora, Alternaria*, and miscellaneous microorganisms from protected or nonprotected pods harvested when mature or 6 weeks after maturity on plants inoculated with *Diaporthe phaseolorum* var. *sojae* or noninoculated plants

Treatments			Germination and infection (%)				
Inoculated	Protected	Harvested	Germ. (%)	*Diaporthe* (%)	*Cercospora* (%)	*Alternaria* (%)	Miscellaneous (%)
No	No	15 Sept.	78.8	13.0	16.5	2.0	1.3
Yes	No	15 Sept.	84.3	11.5	13.3	4.3	1.5
No	Yes	15 Sept.	80.0	7.5	15.3	4.3	3.8
Yes	Yes	15 Sept.	80.8	8.0	16.0	3.0	1.8
No	No	25 Oct.	27.0	41.0	17.0	44.5	14.8
Yes	No	25 Oct.	18.5	46.3	13.5	41.0	14.3
No	Yes	25 Oct.	81.5	13.5	16.0	3.0	3.0
Yes	Yes	25 Oct.	68.8	20.3	16.3	7.5	4.3

TABLE 2. Analysis of variance for soybean seed germination and infection with *Diaporthe, Cercospora, Alternaria*, and miscellaneous microorganisms from protected or nonprotected pods harvested when mature or 6 weeks after maturity on plants inoculated with *Diaporthe phaseolorum* var. *sojae* or noninoculated plants

Source of variation	Germination	*Diaporthe*	*Cercospora*	*Alternaria*	Miscellaneous
Main plot					
Replication	NS[a]	*	NS	NS	*
Inoculation	*[a]	NS	NS	NS	NS
Subplot					
Protection	**[a]	**	NS	**	**
Inoc. × Prot.	NS	NS	NS	NS	NS
Sub-subplot					
Harvest	**	**	NS	**	**
Har. × Inoc.	*	NS	NS	NS	NS
Har. × Prot.	**	**	NS	**	*
Har. × Prot. × Inoc.	NS	NS	NS	*	NS

[a] NS = no significant difference; * = difference significant at the 5% level; and ** = difference significant at the 1% level.

protection was at least partially effective because the seed from the nonprotected pods harvested 6 weeks after maturity had 20% more infection with *Diaporthe* than seed from pods protected until 6 weeks after maturity, and 34% more infection than seed from pods protected until mature.

The results of the inoculation test are presented in Table 1 and the statistical treatment of the data is in Table 2. Inoculation with *D. phaseolorum* var. *sojae* did not significantly alter the amount of seed infected with *Diaporthe*, although infection was 7% higher on inoculated plants with the pods protected until 6 weeks after maturity. The nonsignificant difference does not suggest systemic infection. Protection and date of harvest significantly affected germination and infection with *Diaporthe, Alternaria*, and miscellaneous microorganisms but not infection with *Cercospora.*

Although infection with *Diaporthe* was higher in all tests than generally has been observed when seed is harvested as soon as mature, infection increased fourfold when harvest was delayed 6 weeks. Percentage increase in seed infected with *Alternaria* and miscellaneous microorganisms was even greater with delay in harvest without pod protection. It was not possible to determine when most of the infection with *Diaporthe* occurred, but it appeared to be long enough after the seed was mature to be associated with deterioration of the pod wall. Infection with *Cercospora kikuchii* did not increase with delayed harvest. Even protecting the pods up to 5 weeks prior to ripening did not affect the amount of infection, indicating that infection with this fungus takes place earlier. This agrees with our earlier report (1) that one or two well-timed inoculations during the full-flower period gave the maximum infection with *Cercospora kikuchii.*

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