

# Internal Seed-Borne Nature of *Sclerotinia sclerotiorum* and *Phomopsis* sp. and Their Effects on Soybean Seed Quality

J. F. Nicholson, O. D. Dhingra, and J. B. Sinclair

Graduate Research Assistants and Professor, respectively, Department of Plant Pathology, University of Illinois, Urbana 61801.

Supported in part by the Illinois Agricultural Experiment Station; USDA Regional Project S-72; and the U.S. Agency for International Development Grant csd-1922.

The authors thank R. L. Rogers, Louisiana State University; C. H. Andrews and J. C. Delouche, Mississippi State University; C. W. Blackmon, Clemson University; L. S. Bird, Texas A&M University; and D. Egli and D. Tekrony, University of Kentucky, for providing seed lots. The Commonwealth Mycological Institute confirmed the identification of our isolates of *Phomopsis* sp.

Accepted for publication 15 May 1972.

## ABSTRACT

*Sclerotinia sclerotiorum* and *Phomopsis* sp. (*Diaporthe phaseolorum* var. *sojae*) are internally seed-borne in soybean (*Glycine max*), and may inhibit seed germination in vitro and field emergence. *S. sclerotiorum* was internally seed-borne in: 30 of 39 lots of Lee 68 seed harvested from six states in 1969 and 1970; eight lots of 1971 Cutler seed from Kentucky; and one lot of Beeson and 17 lots of Amsoy 1971 seed from Illinois. When recovery was more than 25%, in vitro germination and field emergence were reduced. Recovery of *S. sclerotiorum* from Lee 68 seed was reduced when stored at room temperature ( $22 \pm 3$  C) after 6 and 24 months, when compared to samples of the same seed lots stored for 18 months at  $3 \pm 1$  C. Recovery from Cutler seed increased 26% between normal

and late harvest periods 30 days apart, but harvest method (machine or hand) appeared to have no effect on percent occurrence of the fungus. *S. sclerotiorum* was first isolated from seed and pods of field-grown plants at 16 weeks after planting.

*Phomopsis* sp. was internally borne in the following seed lots: 12 of 20 Lee 68 lots harvested in 1970 from five states; eight lots of 1971 Cutler seed from Kentucky; one lot of 1971 Beeson seed; and 17 lots of 1971 Amsoy seed from Illinois. *Phomopsis* sp. was recovered from less than 25% from any single lot assayed, except for two lots. A reduction in in vitro germination or field emergence was not detected.

Phytopathology 62:1261-1263.

*Additional key words:* pod and stem blight, stem rot.

Sporadic outbreaks in the U.S. of *Sclerotinia* stem rot of soybean (*Glycine max* [L.] Merr.) caused by *Sclerotinia sclerotiorum* (Lib.) d By., has been reported (1, 4, 5, 6, 9). *S. sclerotiorum* was reported to be seen-borne in 26 genera of plants (12), with particular emphasis being made on sunflower (2, 8) and bean (3). Several workers (4, 5, 6) reported that *S. sclerotiorum* was associated with soybean seed. Chamberlain (4) presented evidence that *S. sclerotiorum* might be seed-borne in soybean, and Nicholson & Sinclair (10) showed that *Sclerotinia* sp. was seed-borne in Lee 68. Factors affecting seed-borne aspects and the effect of the fungus on seed quality have not been studied. *Phomopsis sojae* is seed-borne in soybean (7, 13, 15, 16). We report here additional information on factors affecting seed-borne incidence of *S. sclerotiorum* and *Phomopsis* sp. and their effect on in vitro germination and field emergence.

**MATERIALS AND METHODS.**—Thirty-four Lee 68 seed lots were assayed in 1970 and 1971 for the presence of internally borne microorganisms. The lots were harvested, in 1969, in Illinois, Louisiana, Mississippi, South Carolina, and Texas; and in 1970, in Kentucky, Louisiana, Mississippi, South Carolina, and Texas. Planting date, harvest date, and method (hand or machine) of harvest varied for the different lots. Results from an assay for the presence of *Pseudomonas glycinea* in the 1969 seed has been reported (10, 11). Portions of each lot of 1969 seed were stored at either room temperature ( $22 \pm 3$  C) or

in a cold room ( $3 \pm 1$  C and 60% relative humidity) for 24 months, and then assayed again using Difco lima bean agar (LBA). Seed produced in 1970 was assayed on LBA (pH 7), Difco potato-dextrose agar (PDA) (pH 5.6 or 7), and Difco cornmeal agar (pH 5.6). Twenty-five seed from each lot were placed on each medium. All seed were surface-sterilized in a 1.73% sodium hypochlorite solution for 30 sec. In vitro germination and percent recovery of various microorganisms were recorded after 10 days.

Other seed lots (50 seed/lot) harvested in 1970 were assayed on LBA (pH 7) for *S. sclerotiorum* and *Phomopsis* sp.: eight lots of Cutler, either machine (6 lots) or hand harvested (2 lots) at two dates (30 days between each harvest) from Kentucky; one lot each of Beeson and Amsoy mechanically harvested from Ogle County, Illinois; 16 lots of Amsoy mechanically harvested from the University of Illinois Agronomy South Farm; and two lots of Lee 68 and one lot of Amsoy hand harvested from the South Farm. In vitro germination and percent occurrence of the two fungi were recorded after 10 days.

To determine the time of seed infection by *S. sclerotiorum* of soybeans in the field, seed of Amsoy and Lee 68 were planted at the South Farm and assayed weekly after pod set. The seed were planted in a randomized, replicated field plot on 27 May. Amsoy flowered on 11 July, and assay of seed began on 23 July. Lee 68 plants flowered on 30 August, and isolations began on 10 September. Pods were passed

through a flame, dipped in 70% ethanol for 30 sec, then teased apart, aseptically. Seed were plated on LBA. If lesions were present on the pods, isolations were made from their margins.

Field emergence of seed lots collected in 1969 (stored at  $3 \pm 1$  C) were planted in the Plant Pathology orchard plots (University of Illinois). The field emergence data used for the 6-month seed stored at  $22 \pm 2$  C was reported earlier (11). Lee 68 seed of the 1970 lots were planted at the South Farm in 1971. All field emergence studies at the University of Illinois were conducted using 100 seed/replication with four replications/seed lot planted in 6.1 m rows. Field emergence was taken 14 days after planting.

**RESULTS AND DISCUSSION.**—*S. sclerotiorum* was internally seed-borne in 14 of 17 lots of Lee 68 seed-harvested from six states in 1969 and 14 of 20 lots harvested in 1970. It was isolated from the following 1971 seed: the eight lots of Cutler grown in Kentucky; the Beeson and Amsoy lots from Ogle County (with an incidence of 1 and 2%, respectively); the 16 Amsoy lots from the South Farm; and the three hand-harvested lots from the South Farm.

Seeds infected with *S. sclerotiorum* were often discolored, flattened, and smaller than noninfected seed. When *S. sclerotiorum* grew from individual seed in vitro, the seed did not germinate. A seed was considered germinated when the radicle was 1.5 times the length of the seed. The white mycelial growth of *S. sclerotiorum* on soybean seed was similar to that described by Chamberlain (4).

When recovery of *S. sclerotiorum* was less than 25%, there was no apparent effect on the in vitro germination. Recovery was less than 25% in all Lee 68 lots harvested in 1969 and 1970, except for two lots (Louisiana) which had 34 and 87% recovery, respectively. The in vitro germination was 52 and 9%, respectively, whereas the lot from which *S. sclerotiorum* was not recovered had 95% germination. Recovery of *S. sclerotiorum* from Cutler seed ranged from 32 to 48% in five of eight lots, which had an average in vitro germination of 37%; the remaining three lots had a range of 12-20% recovery and an average of 80% germination. The ranges of recovery of the fungus and the in vitro germination for 16 Amsoy lots were 29-58% and 32-64%, respectively. The percent recovery of *S. sclerotiorum* and in vitro germination for the three hand-harvested seed lots from the South Farm in 1971 were: Amsoy 62 and 30%, respectively; Lee 68, 52, and 82%, and 40 and 90%, respectively.

Percent field emergence was always lower than the percent in vitro germination for all Lee 68 lots. Where recovery of *S. sclerotiorum* was less than 25%, there was no apparent effect. Where recovery was more than 25%, as in the case of the two lots from Louisiana, field emergence was reduced to 12 and 5%, respectively, whereas the remaining 19 lots of the 1970 seed had an average of 54%.

Other phytopathogens that can affect the germination and emergence of soybean seed and thus the seed quality are *Diaporthe phaseolorum* var. *sojae* (15, 16)

and *Pseudomonas glycinea* (11). The apparent lack of an effect on the in vitro germination and emergence when *S. sclerotiorum* occurred less than 25% may be due to the presence of *P. glycinea* in the same seed lots (11).

Recovery of *S. sclerotiorum* from Lee 68 seed was influenced by storage time and temperature. Survival of the fungus was reduced when seed was stored at room temperature. In the 1969 lots, the fungus was recovered from 10 of 17 lots after 18 months in cold storage and from 14 of 20 lots of the 1970 seed after 12 months. *S. sclerotiorum* was recovered from only five of the 1969 lots after 6 months and only one lot after 24 months at room temperature.

Recovery from Cutler seed was influenced more by harvest dates than by type of harvest. The average (four locations) in vitro germination of seed at the first and second harvest was 76 and 30%, respectively. The loss of germination may be due in part to the increase in the percent recovery of *S. sclerotiorum*. The average recovery of the fungus at the first harvest was 19%; and at the second, 45%. Hand-harvested seed at the first and second harvest showed 64 and 20% in vitro germination, respectively. Both measurements were lower than the average in vitro germination of machine-harvested seed at the first and second harvest, which were 80 and 24%, respectively. The increased recovery of *S. sclerotiorum* from late-harvested seed may possibly be correlated with rainfall, since a 19-cm rainfall was recorded between harvest periods. Hine & Wheeler (6) reported that heavy rainfall and low night temperatures in Arizona were significant factors in the disease outbreak of *S. sclerotiorum* on soybeans.

*S. sclerotiorum* appears to infect soybean at least 16 weeks after planting. Isolations made from soybean pods and seeds of Amsoy and Lee 68 plants grown on the South Farm in 1971 showed that *S. sclerotiorum* was first isolated from Amsoy pods on 13 August, and from two of 40 seeds in September. The following week, none of 40 seeds contained the fungus. The fungus was first isolated from Lee 68 seeds on 7 October, when five of 40 seeds were infected.

*Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *sojae* (13, 15), the perfect stage of *Phomopsis sojae*, has been reported to inhibit germination of soybean seed (16). The fungus is pathogenic in the seed-borne phase, and causes severe losses to soybean by killing seeds and seedlings during germination (16). Kilpatrick (7) suggested that *P. sojae* invaded the plant systemically, and it was more frequently isolated from early maturing varieties than from later maturing ones. *P. sojae* was isolated frequently from seed nearest the peduncle and least from near the tip of the pod (7).

*Phomopsis* sp. was isolated from 12 of the 20 1970 Lee 68 lots with an average of 1-14% recovery from individual lots. The average of the 16 Amsoy lots from the South Farm had 15% of the seeds infected. Beeson and Amsoy seed from Ogle County had 68 and 82%, respectively. In contrast to *S. sclerotiorum*, the recovery of *Phomopsis* sp. from

Cutler seed from early and late harvests had 18 and 13% of the seed infected, respectively. In our studies, it appeared that *Phomopsis* sp. did not reduce germination or emergence to the same extent as *S. sclerotiorum*. Recovery was less than 25%, except for the seed from Ogle County. A greater percent occurrence may be necessary to measure these effects. The real effect of *Phomopsis* sp. may not have been apparent because of the presence of *S. sclerotiorum* and *P. glycinea* in the seed lots (11). The actual incidence of *S. sclerotiorum* and *Phomopsis* sp. in the seed may have been greater than these results indicate, since Tempe (14) found that surface-sterilization techniques often kill or inhibit growth of internally borne microorganisms.

## LITERATURE CITED

1. ABRAMOFF, I. N. 1931. Fungal disease of soybeans in the Far East, p. 3-84. Diseases and pests of soybeans in the Far East. Far-Eastern Plant Prot. Stat., Vladivostok, Russia. (Original in Russian, see Rev. Appl. Mycol. 11:87).
2. AUGAR, S. J., & H. F. NOME. 1970. Effect of age on the predisposition of sunflower to *Sclerotinia sclerotiorum*. Agricultura t c., Univ. Chile, Santiago. 30:161-165.
3. BLODGETT, E. C. 1946. The *Sclerotinia* rot disease of beans in Idaho. Plant Dis. Repr. 30:137-144.
4. CHAMBERLAIN, D. W. 1951. *Sclerotinia* stem rot of soybeans. Plant Dis. Repr. 35:490-491.
5. HILDEBRAND, A. A. 1948. Soybean diseases in Ontario. Soybean Digest 10:16-17.
6. HINE, R. B., & J. E. WHEELER. 1970. The occurrence of some previously unreported diseases in Arizona. Plant Dis. Repr. 54:179-180.
7. KILPATRICK, R. A. 1957. Fungi associated with the flowers, pods, and seeds of soybeans. Phytopathology 47:131-135.
8. KREXNER, R. 1969. Sclerotial disease of sunflower. Pflanzenarzt 22:20-22.
9. MOLNAR, B., & J. VOROR. 1963. Soybean stem rot caused by *Sclerotinia sclerotiorum* in Hungary. Nov nytermel s 12:51-56.
10. NICHOLSON, J. F., & J. B. SINCLAIR. 1971. *Thielavia basicola* and *Pestalotia* sp. internally seedborne in soybean. Plant Dis. Repr. 55:911-912.
11. NICHOLSON, J. F., & J. B. SINCLAIR. 1971. Amsoy soybean seed germination inhibited by *Pseudomonas glycinea*. Phytopathology 61:1390-1393.
12. NOBLE, M., & M. J. RICHARDSON. 1968. An annotated list of seedborne diseases. Commonwealth Mycol. Inst. Kew, Surrey, England. 191 p.
13. PETERSON, J. L., & R. F. STRELECKI. 1965. The effect of variants of *Diaporthe phaseolorum* on soybean germination and growth in New Jersey. Plant Dis. Repr. 49:228-229.
14. TEMPE, J. DE. 1968. The quantitative evaluation of seed-borne pathogenic infection. Int. Seed Test. Ass. 33:573-581.
15. WALLEN, V. R., & T. F. CUDDY. 1960. Relation of seed-borne *Diaporthe phaseolorum* to the germination of soybeans. Ass. Off. Seed Anal. Proc. 50:137-140.
16. WALLEN, V. R., & W. L. SEAMAN. 1962. Seed-borne aspects of *Diaporthe phaseolorum* on soybeans. Phytopathology 52:756 (Abstr.).