Soybean Seed Rot and the Relation of Seed Exudate to Host Susceptibility

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ABSTRACT

Pre-emergence seed and seedling rot of soybeans in the Mississippi Valley area of Arkansas and Mississippi was determined to be caused primarily by *Pythium ultimum* and *P. debaryanum*. More than twice the quantity of soluble carbohydrate exuded from seed of the susceptible cultivar Hood than from the resistant cultivar Semmes. A third cultivar Lee was intermediate, both in amount of carbohydrate in its seed exudate and in the amount of seed rot. No qualitative differences were detected in the exudates from seed of resistant and susceptible cultivars. A direct relationship between the amount of soluble carbohydrate exuded by a germinating seed and seed rot caused by *Pythium* spp. was shown in these studies.

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Additional key words: Pythium ultimum, Pythium debaryanum, seed exudate, resistance, carbohydrate.

Poor stands of Hood soybeans [*Glycine max* (L.) Merr.] are common in the Mississippi River Valley region of Arkansas and Mississippi. This problem appeared to be most severe when excess moisture was present following planting. The organism most frequently isolated from decaying seed and diseased seedlings was *Pythium ultimum* Trow. Some isolates of *P. debaryanum* Hesse were also recovered. Middleton's (10) description of these fungi was used as the basis of their identification. In most instances, germination of the seed was arrested at a very early stage, even before the radicle emerged from the seedcoat. Disease symptoms observed in this study agreed with those previously reported to be caused by *Pythium* spp. (4, 9, 12).

This study was made to verify that the *Pythium* isolated from the decaying seed caused the poor emergence of Hood soybeans, and to determine the nature of the susceptibility of this variety. A preliminary report has been published (6).

MATERIALS AND METHODS.—The soybean cultivars used in this study were: (i) Hood, which is very susceptible to seed and seedling rot; (ii) Semmes, which has appeared resistant under field conditions; and (iii) Lee, which is intermediate in its reaction. Seeds of these cultivars were obtained from Dr. E. E. Hartwig, Stoneville, Mississippi. The *Pythium* cultures were isolated from decaying seed and diseased seedlings growing in field plots at Stoneville, Mississippi. They were maintained on Difco lima bean agar.

The effect of glucose near germinating seed on the development of seed and seedling rot was determined by coating wet seeds with powdered glucose. These were allowed to dry, then planted in loam soil known to be naturally infested with *Pythium*. For this test, 25 coated seeds were planted in 30.5-cm (12-inch) diam clay pots. Noncoated seeds were planted in check pots.

The inoculum for pathogenicity or cultivar reaction tests were produced by culturing *Pythium* isolates in 100 ml of Difco cornmeal agar slush (2.5 g/liter) in 250-ml Erlenmeyer flasks for 3 days at 21 C. The pathogenicity tests were made by placing 10 soybean seeds on sterilized sand in 10-cm diam clay pots and evenly distributing 25 ml of the inoculum, diluted 1:1 with distilled water, over the seeds. The seeds and inoculum were then covered with 12 to 15 mm of sterile sand. A high moisture level was maintained in the pots until seedlings emerged. The percent emergence indicated the relative resistance of a cultivar. An emergence of 50% or more was considered a resistant reaction.

For paper chromatography of soluble carbohydrates in seed exudates, 50 seeds of each cultivar were surface-sterilized in 95% ethyl alcohol for 0.5 min and placed in sterile 250-ml Erlenmeyer flasks with 30 ml of sterile distilled water for 16 h at 25 C. The water was then recovered, and the seeds were rinsed with additional distilled water to make a total of 50 ml. The water containing exudates from the germinating seed was "deproteinized" by mixing one volume of the exudate solution with two volumes each of 0.3 N $Ba(OH)_2$ and 5% aqueous $ZnSO_4 \cdot 7H_2O$ and filtering through No. 2 Whatman filter paper (3). A standard volume (25 ml) of the water-exudate solution from each cultivar was then reduced to near dryness over calcium chloride under a partial vacuum and resuspended in 1.0 ml of distilled water. Twenty microliters were then spotted on 15 × 52-cm No. 1 Whatman chromatography paper and the components of the exudate separated by a descending overrun technique. The butanol-acetic acid-water (4:1:5, v/v) solvent was mixed, separated in a funnel and the lower fraction placed in the bottom of the chromatographic tank. The spotted paper was then placed into the tank and allowed to equilibrate for 3 hr. The upper protion of the solvent mixture was then placed in the trough through holes in the tank lid. The solvent was allowed to overrun the paper for 72 h at 21-23 C. Solvent was added as needed to maintain the level in the trough half full or more. After 72 h, the chromatogram was dried at room temp. To develop the spots, the chromatogram was drawn through a saturated aqueous solution of silver nitrate, diluted 200 times with acetone, and dried at room temp. It was then drawn through methanol containing sodium hydroxide, prepared by dissolving 2.0 g NaOH in a minimum amount of water, and making up to 100 ml with methanol (3). The chromatogram was dried at room temp.

TABLE 1. Soluble carbohydrate exudation from germinating seed of three soybean cultivars and their emergence when planted in sand infested with a mixture of *Pythium ultimum* and *P. debaryanum*

Cultivar	μg carbohydrate[a] per seed	emergence[b] (%)
Hood	500 A[c] (320-630)	6 A (0-10)
Lee	355 B (235-455)	55 B (30-70)
Semmes	205 C (115-250)	76 C (60-90)

[a]Mean and range of seven tests.
[b]Mean and range of two tests, five replications each.
[c]Means followed by a different letter in each column, differ significantly ($P = 0.01$) by Duncan's new multiple range test.

For the quantitative study of soluble carbohydrates in the exudate of germinating seed, an exudate sample was obtained and deproteinized as described above. The deproteinized exudate solution was analyzed with a Klett-Summerson photoelectric colorimeter by using the phenol-sulfuric acid method (5). A glucose standard was used.

RESULTS.—*Verification of pathogen and resistance.*—The organisms consistently isolated from decaying seed and diseased seedlings of Hood soybeans were determined to be *Pythium ultimum* and *P. debaryanum*. Tests in the greenhouse determined that both species of the pathogen were approximately equal in virulence to Hood and Semmes soybeans. However, they also showed that Semmes had a moderate level of resistance compared to Hood. The avg emergence of Hood from sand infested with *P. ultimum* and *P. debaryanum* was 10 and 3%, while the avg of Semmes was 40 and 50%, respectively.

Seed coated with glucose.—Semmes seeds, coated with glucose, emerged from naturally infested soil at an average rate of 60%, noncoated seed averaged 88%. The emergence from coated Hood seed averaged 44%, and from noncoated seed 64%. After 30 days, a growth depression was also observed. Plants from coated and noncoated Semmes seed averaged 31 and 42 cm, respectively, while those from Hood averaged 21 and 31

cm, respectively. These results indicated that a strong influence was exerted on disease development by the presence of glucose near the germinating seed. *Pythium* was isolated from abnormal plants.

Chromatographs of seed exudates.—Paper chromatographs of the exudate from germinating seeds of Semmes and Hood did not indicate a qualitative difference between the cultivars. However, an appreciable quantitative difference was indicated by the size and intensity of color development in the spots. Exudate from Hood seeds appeared to contain the same soluble carbohydrate components as that from Semmes, but in larger amounts. At least 13 different carbohydrates were indicated. The fastest-moving components were tentatively identified as glucose and fructose. The relative position of the components of the exudate are 0.90, 0.84, 0.78, 0.60, 0.54, 0.45, 0.35, 0.30, 0.26, 0.15, 0.12, and 0.07, using a reference point of 1.00 for fructose.

Amount of exudate and seedling emergence.—Quantitative determinations of the amount of soluble carbohydrate in the exudate from germinating seeds of Semmes, Lee, and Hood revealed that there was a great variation between cultivars. The greatest quantity was recovered from Hood (Table 1). Emergence tests indicated a relationship between the quantity of carbohydrate in the seed exudate and the percent emergence when the seeds were planted in sand infested with *P. ultimum* and *P. debaryanum*. Greater amounts of carbohydrate in the seed exudate were associated with reduced emergence (Table 1).

DISCUSSION.—Differences in the amount of soluble carbohydrates exuded by germinating seed of soybean cultivars Hood, Semmes, and Lee were determined to be quite large. Seed of Hood exuded more than twice that of Semmes, with Lee seed exuding an intermediate amount. The large amount of carbohydrate exuded by Hood seeds suggested that this stimulates the growth of the disease-causing organisms near the seeds, thus contributing to the very high rate of pre-emergence seed rot. Semmes and Lee cultivars showed much less damage. The disease is usually more severe when planting is followed by rain within 1-2 days. A high soil-moisture level may increase the amount of carbohydrate exuded by germinating seeds, possibly stimulating *Pythium* growth near the seeds, and leading to their rapid deterioration. The low rate of seed rot shown by Semmes is correlated with a small amount of carbohydrate in the exudate from its germinating seeds. The Lee soybeans were found to be intermediate, both for amount of carbohydrate in the seed exudate, and for the amount of pre-emergence seed- and seedling rot. Sugar exudates from peas (1, 2, 7), beans (11), and mung beans (8), which stimulate *Pythium* growth and disease incidence, have been reported.

Chromatographs of the exudates from germinating seed of Hood and Semmes revealed no indication of a qualitative difference between exudates from the two cultivars. The obvious difference was the quantity of soluble carbohydrates. The most prominent substances in the exudate from both varieties were glucose and fructose. A total of at least 13 different carbohydrate substances in the exudate were shown by the chromatographs, however.

Both the susceptible Hood and resistant Semmes showed stand reduction (24 and 20%) and stunted growth when plants were grown from seeds coated with glucose before planting in soil naturally infested with *Pythium*. The magnitude of the effect was similar for both cultivars. Under field conditions, a 20% stand loss might be compensated for by increased planting rate, or by extra plant growth which would fill gaps in the row. However, the fact that both varieties showed a growth reduction of 25% or more after 30 days indicates a problem in addition to partial stand loss. It should be noted that other naturally occurring organisms were also present in the soil used in this experiment, and their effects should not be overlooked. However, since *Pythium* was isolated readily from all abnormal plants in this study, there is little doubt that it was the primary disease-causing agent.

From these studies, it was concluded that the susceptibility of soybeans to pre-emergence seed and seedling rot caused by *Pythium* was associated with soluble carbohydrate exuded by the germinating seeds, and that differences in susceptibility between some cultivars were directly related to the amount of carbohydrate exuded by the seeds during germination.

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