Recovery of Cephalosporium gregatum from Soybean Straw

L. E. Gray

Research Plant Pathologist, Plant Science Research Division, ARS, USDA, Urbana, Illinois 61801.

Cooperative investigations of the Plant Science Research Division, ARS, USDA, and the Illinois Agriculture Experiment Station. Publication No. 701 of the United States Regional Soybean Laboratory, Urbana, Illinois 61801.

Additional key words: brown stem rot, overwintering inoculum.

ABSTRACT

Cephalosporium gregatum was recovered from overwintered soybean straw when the straw was ground in a Wiley mill and the resulting material was incubated at 18 C for 5 days on water agar containing tetracycline HCl and streptomycin sulfate. Sporulation of the fungus on soybean stems in the field was noted up to mid-November but thereafter was observed rarely, presumably because saprophytic microorganisms disintegrated the cortical tissue. Spores of the pathogen were not detected with soil dilutions but the fungus was recovered frequently with stem fragments. The fungus survives in soil within the woody stem tissue which probably serves as the source of inoculum.

Phytopathology 62:1362-1364.

Cephalosporium gregatum (Allington & Chamberlain), the cause of brown stem rot of soybean, (*Glycine max* [L.] Merr.), is a serious vascular pathogen of soybean in central Illinois. No chlamydospores, sclerotia, or other resistant structures have been reported. How the pathogen overwinters and the nature of the inoculum that initiates infection of plants in the field are still uncertain. Allington & Chamberlain (1) failed to isolate the fungus from overwintered soybean straw from the field. Lai (2) and Lai & Dunleavy (4) reported that the fungus sporulates on soybean straw and can survive in soybean straw for as long as 10 months. They speculated that repeated sporulation of the fungus on buried straw provided an important source of inoculum (3, 4).

When Lai's technique (2) was used at Urbana, Ill., to detect *C. gregatum* associated with straw removed from the field throughout the winter, the fungus rarely sporulated on the straw. Close examination of the straw revealed obvious differences in the condition of straw collected in October and that collected during the winter.

The experiments reported in this paper were undertaken (i) to determine factors which influence sporulation of *C. gregatum* on soybean straw; (ii) to compare methods for consistently recovering the fungus from straw; and (iii) to determine the probable source of overwintering inoculum.

MATERIALS AND METHODS.–Unless specified otherwise, all of the soybean straw was collected from field plots which had a high incidence of brown stem rot in 1969, 1970, and 1971. Straw segments 30 cm long were collected in October 1970, placed in plastic bags, and stored in a refrigerator at 5 C for 3 months. Other soybean straw was collected from field plots during the winter months as needed for each experiment.

Recovery techniques.–C. gregatum was induced to sporulate on soybean straw by the technique reported by Lai (2). This technique was the standard procedure in experiments concerned with detection of the fungus in soybean straw.

A procedure designated the ground straw-plate technique (GS-P) was tested for its effectiveness in detecting *C. gregatum* in soybean straw. Straw samples were collected, air-dried 4 hr, and ground in a Wiley mill fitted with a 1-mm screen. The ground samples were stored in glass bottles under refrigeration for no longer than 24 hr. A small sterile spatula was used to sprinkle a 3-mg sample of the ground straw onto the surface of 1.6% water agar in 15- X 60-mm petri dishes. Two ml of an antibiotic solution (100 mg tetracycline HCl and 100 mg streptomycin sulfate/100 ml distilled H_2O) and then 10 ml of water agar at 45 C were added to each dish. The dishes were incubated at 18 C for 5 days; then the number of *C. gregatum* colonies in each dish was determined microscopically.

Straw treatment.–Lai's technique (2) and the GS-P technique were compared for their effectiveness in detecting the fungus in straw collected in October and refrigerated 3 months at 5 C and in straw collected from plots in December and used within 24 hr after collection. Ten 2.0-g samples of straw segments 1.0 cm long were removed from a composite sample for each source and used for each recovery technique. Duplicate plates for each 2.0-g sample were used for each technique. This experiment was repeated 3 times.

Both techniques also were tested for their effectiveness in detecting the fungus in straw which (i) had been buried in direct contact with a silt-loam soil in a trench 1 m long and 12 cm deep; (ii) placed on the surface of soil; and (iii) left standing in the field. Ten soybean stems, each 30 cm long, were exposed as indicated for the period October-December, after which they were sampled by the procedures described above.

Efficacy of the GS-P technique for detecting C. gregatum in soybean straw.–The efficacy of the GS-P technique for detecting *C. gregatum* in soybean straw which had overwintered in fields was tested in April 1971. All of the fields sampled either had been fall-plowed or the straw in them had been worked into the soil with a field cultivator. Straws collected randomly in each field were composited into 50-g samples. Three plates were used for the samples from each field. The experiment was repeated twice.

Spore recovery from soil.–Soil was passed through a sieve in an attempt to recover spores of the fungus from soil. Preliminary experiments in my laboratory demonstrated that when conidia of the fungus were mixed with autoclaved soil, the spores could be recovered by soil-sieving techniques (L. E. Gray, *unpublished data*). Forty soil samples of 20 g each (wet wt) were collected from a soybean plot in March, April, and May 1971. Twenty samples were taken from within the row from the top 2-cm depth, and 20 samples, from the 2 to 8 cm depth. Sterile water (400 ml) was added to each sample, and the soil suspension was mixed in a Waring Blendor for 45 sec. The suspension then was poured onto a series of sieves with openings of 140, 88, and 37 μ; material retained on the sieves was washed with 25 ml of water into separate beakers. Material $< 37\ \mu$ in size was allowed to settle, and the water was decanted until 25 ml remained in the beaker. We prepared duplicate plates for each fraction by transferring 0.1 ml of suspension from a beaker to the surface of stem extract agar (1) and distributed the suspension over the agar surface by manipulating the plate. The plates were kept at 18 C for 5 days and then examined microscopically.

RESULTS.–*Time of sampling on sporulation of C. gregatum.–C. gregatum* sporulated on 24 of 80 stem samples from soybean straw collected in October. The fungus did not sporulate on any of the straw samples collected from the field in December. In contrast, the fungus was detected in 100% of the straw samples from both sampling dates by the GS-P technique (20 of 20 plates/sampling date). The average number of colonies per plate was seven for the October samples and eight for the December samples.

Effect of straw treatment on sporulation and recovery of C. gregatum.–The fungus did not sporulate on any of the straw samples collected in December.

The pathogen was detected by the GS-P technique in 16 of 20 plates, each from straw samples that had been buried in soil, placed on the soil surface, or left standing in the field. The surface of straw buried in the soil was badly deteriorated; outer cortical tissue on the stem sections was disintegrated.

Efficacy of the GS-P technique for detecting the fungus in soybean straw.—The fungus was detected by the GS-P procedure in straw samples from 23 of 24 fields, and was present in 55 of the 72 plates prepared for the material collected from the 24 fields. The mean number of colonies per plate was four; the maximum was 29. The straw recovered from the fields in April had undergone considerable disintegration over the winter. Outer cortical tissue of the stem had disintegrated, leaving only the woody tissue.

Spore recovery from soil.—*C. gregatum* was recovered from straw fragments retained on the 140-μ sieve. All colonies of the fungus originated from pieces of stem material. The fungus was observed on 16 of 80 plates prepared from the 140-μ-sieve fraction, but was not recovered from any of the fractions representing smaller sized particles.

DISCUSSION.—The poor sporulation of *C. gregatum* on soybean straw collected from the field after mid-November is inconsistent with the results reported by Lai (2). Close examination of the straw revealed marked differences in the condition of the straw used by Lai (2) and the straw I used. The straw used by Lai (2) and by Lai & Dunleavy (3) was collected in October when the outer cortical tissue of the stem was still intact and probably not overgrown with microorganisms. In all of Lai's illustrations (2, 4), the stem epidermal tissue appears still intact even on straw which was buried in soil. This generally is not the case under field conditions. Saprophytic fungi rapidly colonize the outer surface of soybean straw that remains in the field, and the outer cortical tissue rapidly disintegrates when in contact with soil or on stems standing in the field throughout the winter. The condition of the straw determines how readily the fungus will sporulate on the material. For example, *C. gregatum* sporulated on soybean straw which, when collected in October, still had the outer cortical tissue intact. On the other hand, the pathogen did not sporulate on stem material which, when sampled in December, was overgrown with contaminants and had most of the cortical tissue disintegrated.

The GS-P technique consistently made possible the detection of *C. gregatum* in the woody tissue of soybean stems that had overwintered in the field. Lai's technique permitted detection of the fungus only when conditions were such that the stem cortical tissue remained intact. Researchers have been deterred from undertaking survival and population studies of *C. gregatum* because of the serious handicap imposed by the lack of a method for effectively detecting the fungus. The GS-P technique fulfills this need.

Repeated attempts to recover the fungus from soil by sieving techniques were unsuccessful. Moreover, the fungus was recovered from soil only in association with organic debris. Thus, whether spores can serve as inoculum is still uncertain. My data suggest that overwintering inoculum of the pathogen consists principally of mycelium that is harbored in the woody tissues of the soybean stem.

LITERATURE CITED

1. ALLINGTON, W. B., & D. W. CHAMBERLAIN. 1948. Brown stem rot of soybean. Phytopathology 38:793-802.
2. LAI, P. Y. 1968. Isolation of Cephalosporium gregatum from soybean straw. Phytopathology 58:1194-1195.
3. LAI, P. Y., & J. M. DUNLEAVY. 1969. Sporulation of Cephalosporium gregatum on naturally infested soybean straw. Phytopathology 59:343-345.
4. LAI, P. Y., & J. M. DUNLEAVY. 1969. Growth and sporulation of Cephalosporium gregatum on various media and buried soybean straw. Phytopathology 59:1950-1953.