# Detection of Pseudomonas glycinea in Soybean Seed Lots

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ABSTRACT

We detected *Pseudomonas glycinea* in lots of 500 soybean seeds by predisposing germinating seeds and seedlings to conditions favoring the formation of typical water-soaked lesions on seedling cotyledons. These conditions included the wounding of cotyledons of partly germinated seeds and the emergence of seedlings into water-saturated air.

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The soybean blight bacterium, *Pseudomonas glycinea*, is dispersed widely with seed. Since seed carrying the pathogen usually is normal in appearance, special methods are needed to identify seed lots that may give rise to the disease in the field. Nicholson & Sinclair (6) detected the pathogen within the seed by placing surface-sterilized seed on agar media and by noting growth of the pathogen on seed. Kennedy (3) detected the pathogen by inoculating seedlings with a suspension made from crushed seeds. In this paper we describe a simple method that makes use of the seedling as an enrichment medium to detect small numbers of *P. glycinea* in or on seeds in lots of 500. The method is based in part on the observations of Daft & Leben (1), who found that the number and area of cotyledon lesions on seedlings were increased when cotyledons were wounded as they were drawn toward the soil surface.

After a series of preliminary tests, the following method was adopted. A lot of 500 seeds was soaked in 250 ml of sterile deionized water for 2 hr, then planted in water-saturated vermiculite ("medium grade", W. R. Grace & Co., Cambridge, Mass.) in a polyethylene pan 31 X 36 X 14 cm high. Depth of the vermiculite was 8 cm. Pans were sealed with plastic film and kept at 24 C for 2 days. The germinated and nongerminated seeds were removed and shaken for 30 min with 7 g of sterile sharp sand in 250 ml of sterile water in a 4-liter Erlenmeyer flask. A shaker with a 14-cm horizontal stroke and 70 cycles/min provided a gentle mixing and abrasion of seedlings. With most cultivars, the radicle was 2 to 4 cm long. During the shaking process, seed coats usually became partly or completely detached; evidence suggests that coat removal and exposure of the cotyledon surface to wounding during shaking is essential. After shaking, seeds were replanted in the vermiculite, and water was added to replenish that which had been lost.

Pans were placed in polyethylene film-covered chambers, 90 X 40 X 47 cm high. Air within the chambers was 25 to 27 C, and the relative humidity was >95%. Chambers were lighted with fluorescent tubes 16 hr/day (600 ft-c at plant height), and a small amount of fresh air was introduced 2 out of each 12 min. Numbers of plants with cotyledon lesions incited by *P. glycinea* were recorded 8 to 10 days after seed was replanted. *Pseudomonas glycinea* lesions characteristically were dark green and had a water-soaked appearance. Older lesions consisted of pitted necrotic areas with water-soaked edges (Fig. 1). Lesions were formed only on the outer (lower) surface of cotyledons. To verify the presence of *P. glycinea* in representative lesions on plants from nearly all of the seed lots tested, each of 10 lesions was ground separately in 0.1 ml sterile water with a sterile spatula. The grindings were tested by the Q-tip method for pathogenicity on leaves of greenhouse seedlings (5). The appearance of typical water-soaked lesions demonstrated that all except two cotyledon lesions were incited by *P. glycinea*. These two produced a necrotic reaction (see below).

Using these methods, 28 seed lots (18 cultivars) were tested. Eleven seed lots did not appear to bear the pathogen. Seven seed lots had 0.4 to 5% of the plants with cotyledons bearing one or more typical *P. glycinea* lesions. Five seed lots had 5 to 15% diseased plants, and two had 72 and 79%, respectively. Plant emergence varied from 41 to 90%. Three seed lots had many broken or discolored seeds; no results

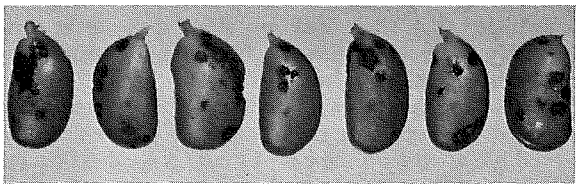

**Fig. 1.** Lesions incited by *Pseudomonas glycinea* on cotyledons of soybean seedlings from seed contaminated with the pathogen. Predisposing conditions for lesion formation included the wounding of cotyledons of partly germinated seedlings and the emergence of seedlings into water-saturated air.

could be obtained because fungi overgrew the cotyledons.

To obtain information on variation that could be expected by the detection method, seven of the 28 seed lots mentioned above were tested a number of times. Lots with different levels of contamination by *P. glycinea* were selected. Two lots which originally had produced only healthy seedlings again produced only healthy seedlings when they were each tested three times (1,500 seeds). Three lots with 5.5, 1.6, and 2.4% diseased seedlings in the first test produced an average of 4.6, 0.5, and 6.5% diseased seedlings, respectively, when they were each retested 3 times. The two heavily contaminated lots which had produced 72 and 79% diseased seedlings in the first test also were retested with similar results. For example, 79, 69, 77, 42, 68, and 68% diseased plants were found when one of the lots was tested 6 times. The variation may be linked to the fact that seed emergence also varied. For example, in the six tests noted above, 415, 380, 322, 307, 204, and 338 plants, respectively, emerged from the 500 seeds planted in each test.

Two thousand seeds of each of six of the seven lots also were planted without a predisposing treatment in steam-treated soil in the greenhouse to see whether cotyledon lesions were produced under these conditions. No typical blight lesions were observed, but 20 plants bore cotyledon lesions that were not water-soaked. These lesions were tested for pathogenicity by the Q-tip method. All incited a necrotic reaction in 2 to 3 days with no water-soaking. Since *P. glycinea* incited water-soaked lesions in 3 to 5 days, it was concluded that a pathogen other than *P. glycinea* produced these lesions.

It seemed possible that inoculum originating in pathogen-bearing seeds could be infecting pathogen-free seedlings of a given lot of seed during the shaking step of the procedure. A heavily contaminated seed lot and a slightly contaminated lot were germinated as usual, and the seedlings were mixed in differing proportions for the shaking step. Seedlings of one lot had been marked with a small amount of dye (cotyledons were pricked with a sharp needle previously dipped into 10 mg/ml of basic fuchsin in ethanol). After the shaking step, seedlings were separated into the original lots and planted as usual. Since the amount of disease was increased on seedlings from the lightly contaminated seed lot, we conclude that the shaking step may serve to "magnify" the incidence of disease by transferring inoculum to pathogen-free seedlings.

This method for detecting *P. glycinea* has provided evidence that the greenhouse can be used to produce pathogen-free seed from pathogen-contaminated seed. Thus, a lot of seed produced in the greenhouse did not appear to carry the pathogen when assayed 4 times (2,000 seeds), whereas the greenhouse plants used to produce this seed were derived from seed that produced 6.5% diseased seedlings when assayed. The rationale for producing foundation pathogen-free seed stocks in the greenhouse is based on (i) the probability that the pathogen is less likely to be spread in the absence of dew, rain, and storms that are common in the field (1, 2); and (ii) the hypothesis that a resident phase of the pathogen in the bud or on leaves is less likely to occur in the greenhouse than it is in the field (4). Recently the greenhouse was used by Schmitthenner et al. (7) to produce mung bean seeds that apparently were free of *P. phaseolicola*.

During the course of development of the detection method, a seed lot that later was found to carry a low level of contamination by *P. glycinea* was germinated for 2 days, shaken with a cell suspension from a pure culture of the pathogen, and planted in vermiculite. The following observations were made when conditions of tests were changed: (i) Soaking the seed in sterilized water for 2 hr before planting favored cotyledon lesion formation. (ii) The addition of sand during the shaking step greatly increased the number and size of cotyledon lesions. However, when seeds were shaken too long or with too much sand, cotyledons were injured excessively. (iii) When the cell suspension of *P. glycinea* was not added, seedling emergence was increased. (iv) There were more and larger cotyledon lesions on plants kept in chambers containing humid air than on plants produced in a growth room (27 ± 2 C, 16 hr of light/day, relative humidity ca. 35%). (v) It was necessary to keep vermiculite saturated with water for the best development of cotyledon lesions. Saturation was determined for a given lot of vermiculite by placing 100 g of vermiculite under water for 2 hr, draining well, and recording the weight of water retained.

This method for detection of *P. glycinea* in seed appears to be semiquantitative. Examples of variations in seed germination and disease results are noted above. The level of contamination would be underestimated when seeds were prevented from germinating by *P. glycinea* or other pathogens, or when plants emerged but the pathogen did not multiply sufficiently to produce a lesion. On the other hand, the method would overestimate the level of contamination when seedlings not bearing the pathogen became infected during the shaking step, as noted above. In any event, we feel that seeds indicated to be free or nearly free of the pathogen by the stringent predisposing conditions of this test would not be expected to produce diseased seedlings in the field, except under unusually severe conditions.

Since a number of leaf-spotting bacterial pathogens produce cotyledon lesions that may be a source of secondary infection, the method may be useful for detecting other bacterial pathogens in or on seeds of other plants.

## LITERATURE CITED

1. DAFT, G. C., & C. LEBEN. 1971. Mechanics of infection of soybean seedlings by overwintered and secondary Pseudomonas glycinea. Phytopathology 61:889 (Abstr.).
2. DAFT, G. C., & C. LEBEN. 1972. Bacterial blight of soybeans: epidemiology of blight outbreaks. Phytopathology 62:57-62.
3. KENNEDY, B. W. 1969. Detection and distribution of

Pseudomonas glycinea in soybean. Phytopathology 59:1618-1619.
4. LEBEN, C. 1971. The bud in relation to the epiphytic microflora, p. 117-127. *In* T. F. Preece & C. H. Dickinson [ed.]. Ecology of leaf surface micro-organisms. Academic Press, London and New York.
5. LEBEN, C., G. C. DAFT, & A. F. SCHMITTHENNER. 1968. Bacterial blight of soybeans: population levels of Pseudomonas glycinea in relation to symptom development. Phytopathology 58:1143-1146.
6. NICHOLSON, J. F., & J. B. SINCLAIR. 1971. Amsoy soybean seed germination inhibited by Pseudomonas glycinea. Phytopathology 61:1390-1393.
7. SCHMITTHENNER, A. F., H. A. J. HOITINK, & M. E. KROETZ. 1971. Halo blight of mung bean incited by a new strain of Pseudomonas phaseolicola. Phytopathology 61:909 (Abstr.).