Soybean Bacterial Blight: Flower Inoculation Studies

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ABSTRACT

Soybean flowers were inoculated with *Pseudomonas glycinea*, and the resulting pods were surface-sterilized, opened, and assayed in vitro for the pathogen. With greenhouse and field inoculations, *P. glycinea* was detected in the interior of 15% of the pods, usually at the proximal end. In the field tests, the pathogen was not associated with seeds in infected pods, but in the greenhouse it was associated with seeds in 25% of these pods. Other types of bacteria were isolated from within pods of inoculated and control plants. Pod set was not reduced by flower inoculation.

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Additional key words: Pseudomonas glycinea, seed.

The present work was undertaken to investigate seed infection by the incitant of bacterial blight of soybean [*Glycine max* (L.) Merr.], *Pseudomonas glycinea* (Coerper) Stapp. This disease is common and widespread in the North Central soybean region of the United States. *P. glycinea* does not appear to enter the vascular system of the stem (14), by which it might reach the seeds within the pod. The direct invasion of the seed from overlying pod lesions (4) is not common in Ohio. If the bacterium is to enter the seed during its development, the flower appears to be the remaining portal through which this could take place. A study of that entry route is the subject of this article.

MATERIALS AND METHODS.—Open soybean flowers were inoculated with cells of *Pseudomonas glycinea*. Pods developing from these flowers were surface-sterilized, opened, and placed on an agar medium to detect the pathogen. In some experiments, the seeds were aseptically removed from the pods and assayed separately. Details of these procedures follow.

Inoculation.—Each flower was inoculated once with a 0.02 to 0.05-ml drop of a water suspension of *P. glycinea* (ca. 10^8 cells/ml) applied from a 1.0-ml syringe fitted with a blunted needle. The drop was inserted between the two lateral petals, which served to hold it in place. We presume the drop was dispersed among the flower parts and eventually evaporated. Care was taken not to injure flowers. Inoculum was obtained from 40 to 48-h-old tube slants containing SNA agar (g/liter:nutrient agar (Difco), 23; sucrose, 10). Sterile water was similarly applied to control flowers.

Desiccation of the inoculum in flowers of field plants was reduced by inoculating between 1800 and 2000 h, when night dew was forming. In most of the greenhouse tests, plants were placed in a mist chamber for 18 h immediately following inoculation. Mist was formed from deionized water. In a few tests, plants were not placed in mist after inoculation.

The soybean cultivars, 'Harosoy 63' and 'Beeson', and two isolates of the pathogen were used. Both isolates produced the typical watersoaking symptom on leaves of both cultivars.

Greenhouse tests.—The greenhouse-grown soybean plants (Harosoy 63) were from a seed lot free of *P. glycinea*, as indicated by an in vivo assay method (11). Plants were grown singly in 15-cm diam pots containing a steamed mixture of Wooster silt loam, sand, and peat moss. Flowers developed 4-6 wk after the seedlings emerged. Greenhouse inoculations were made between 20 April and 20 June, when plants flowered well. In April and May, supplemental light was provided by fluorescent lights 16 h/day. The temperature was 22-30 C.

Field tests.—Field plants were derived from soybean seed (Beeson) produced in the greenhouse. Seed did not carry the bacterial blight organism (11). Single plants were grown in 15-cm diam pots, each of which was placed within a protective wire mesh cylinder 0.5 × 1.0 m high. Plastic film was attached to the circumference of the cylinder. Air movement near the plant was assured by omitting the film 15 cm from the ground and by not covering the cylinder top. The purpose of this device was to protect the plant from wounding. If the pathogen was introduced inadvertently, wounding would predispose the plant to infection and subsequent rapid increase of the pathogen (2, 3). Apparently these precautions were successful, because no blight symptoms were seen and *P. glycinea* was not found in control pods.

Assay technique.—Pods and seeds were assayed for the presence of *P. glycinea* 10-34 days (greenhouse tests) or 19-27 days (field tests) after the inoculation of flowers. Pods were 1.6 - 6.0 cm long and were not lesioned or otherwise blemished. Each contained two or three seeds. Pods were surface-sterilized by dipping in 95% ethanol, followed by a 1-min immersion in a continuously stirred solution of 20% Clorox (5.25% sodium hypochlorite). Pods then were rinsed in flowing, sterile water. The ethanol treatment served to minimize the formation of air bubbles around pod hairs when pods were disinfested in Clorox. These procedures were deemed necessary for complete sterilization of the pod exterior surfaces.

With aseptic techniques, surface-sterilized pods were halved laterally, each half was split longitudinally, exposing the seeds, and the four pod pieces, with seeds attached, were pressed, interior surface downward, upon the surface of a selective medium [M71 agar (5)] in petri plates. The agar surface and pod pieces were examined

40-48 h later for colonies of *P. glycinea,* which were reliably recognized when examined under a dissecting microscope with light directed tangentially from below the plate (7). In some experiments, seeds were aseptically removed from the pod sections and placed on the agar separately. Molten agar (42 C) was poured over the seeds to provide close contact between the agar and the seed.

The identity of *P. glycinea* was verified with a pathogenicity test, using subcultures of presumed *P. glycinea* colonies from most pods and from all seeds. Unifoliolate leaves of seedling soybean plants were wound-inoculated with ca. 10^7 bacterial cells/ml [the Q-tip test (6)]. All presumed *P. glycinea* isolates incited typical watersoaked blight lesions.

RESULTS.—*Greenhouse tests.*—Five hundred forty-three pods, derived from flowers inoculated on 12 different days, were assayed. *P. glycinea* was found within 88 of these pods, usually at the proximal end. The pathogen also was found in the center region in three pods, at the distal end in four pods, and in all regions in seven pods. *P. glycinea* was most often found associated with the dorsal (placental) and ventral regions of the pod. Other bacteria with varied colony types were found in most pods, often associated with the seeds.

In tests in which inoculated plants were not placed in mist after inoculation, 14% of the pods yielded *P. glycinea,* whereas 17% of the pods yielded the bacterium when plants were placed in mist.

Twenty-two of the 88 pods from which the pathogen was isolated also bore 28 seeds that carried the pathogen. Thirteen seeds bearing *P. glycinea* had been cultured separately from pods; the remainder had been assayed in situ within the pods.

Pods which developed from control flowers treated with sterile water did not carry *P. glycinea.* However, other types of bacteria were detected within many of these pods and were associated with seeds.

Pod set did not seem to be affected by inoculating flowers with *P. glycinea.* Pods developed from 77% of the 707 flowers inoculated with the bacterium and 80% of the 321 control flowers.

Field tests.—Pods that developed from flowers treated on 2 and 3 August, 1972, were cultured 19, 21, 22, 23, 26, or 27 days after treatment. Two hundred eighty-six flowers were inoculated, and all of the 265 pods that set were assayed. Of these, 39 bore *P. glycinea* within the pod. The pathogen was found at both ends within two pods and was confined to the proximal end in the rest. *P. glycinea* was not found associated with seeds, all of which were assayed separately from pods.

Thirty-nine of the 40 control flowers formed pods and all were assayed. The pathogen was not found within pods nor associated with seeds.

A varied bacterial flora was isolated from within most of the inoculated and control pods. On the other hand, nearly all of the seeds appeared to be sterile.

DISCUSSION.—This work has demonstrated that, under experimental conditions, *P. glycinea* can become associated with soybean seeds in healthy pods if the pathogen is introduced into the flower. The flower-to-seed transfer may take place with other bacterial pathogen-suscept combinations, but insofar as we are aware, the subject has not been investigated.

How often the flower-to-seed transfer takes place with *P. glycinea* in nature is not known, but the following data suggest that the opportunity for transfer is often present. *P. glycinea* was found in flowers of diseased field plants during two growing seasons. In one test, two of 51 open flowers and 12 of 35 pollinated flowers (petals were withering) carried the pathogen, as detected by the selective medium and verified by the pathogenicity test (Leben, *unpublished*). *P. glycinea* cells may have entered these flowers in water moving from diseased plant parts, by other agencies, or they may have arrived in the flower because the pathogen was living in the bud as the flower was forming (7). Once in the flower, *P. glycinea* may have multiplied, as has been suggested for *Erwinia amylovora* (9, 10). The complex of bacteria that was found in pods and associated with seeds may also have entered via the flower. This mode of entry could account for the bacteria that have been found inside of bean and other fruits (8, 13). These organisms appear to be important in fruit decomposition.

We do not know whether the *P. glycinea* associated with the seed was indeed within the seed. Possibly the bacterium was within the seed coat, which in beans is vascularized (1). Suggestive evidence for this location is found with cotyledon lesions. We have observed (3, 11) that lesions were initiated only on the cotyledon outer surface, where the seed coat and cotyledon were in contact. A related bacterium, *P. phaseolicola,* has been reported to be in the seed coat and on the cotyledon surface (12).

It is of interest to note that flower inoculation did not reduce pod set and that pods were healthy, as far as could be determined. It is possible that at higher inoculum levels, *P. glycinea* would reach the interior of more pods, provided that pods eventually form from such flowers. This subject needs further investigation.

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