A Method of Inoculating Bean Plants in Breeding Trials with Specific Strains of *Pseudomonas syringae* pv. *syringae*

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The procedure commonly used to inoculate bean plants being evaluated under field conditions for resistance to bacterial brown spot caused by *Pseudomonas syringae* pv. *syringae* involves applying dry, finely ground diseased leaves to the surface of seeds prior to planting. To prepare the inoculum, diseased foliage is collected from naturally infected plants, dried, ground to a powder and stored in a plastic bag at 8 C (1). In our trials, seeds to be inoculated have been misted lightly with water and shaken in a plastic bag with the ground-leaf inoculum. We have used this method to inoculate seeds of plants being evaluated for resistance, or in some trials to inoculate seeds of a susceptible cultivar that are planted in "spreader rows" adjacent to plants being evaluated.

There are several disadvantages of using dried-leaf inoculum: 1) diseased plants must be found and leaves collected and ground manually to a powder, 2) other pathogens may be present but remain undetected until disease develops in the inoculated plants, 3) it is difficult to apply inoculum quantitatively and uniformly, and 4) specific strains of the pathogen cannot be used. For these reasons, we tested several materials as carriers to inoculate seeds with laboratory-grown cultures of the pathogen. The materials that worked satisfactorily were: class II peat powder, as used for legume inoculation with *Rhizobium* (obtained from the Nitragin Co., 3101 W. Custer Ave., Milwaukee, WI 53209), mixed with 0.09 g calcium carbonate/1 g peat; grade #5 Zonolite™ vermiculite (W.R. Grace & Co., Cambridge, MA); and Microcel E™, a synthetic calcium silicate (Manville Corp., P.O Box 5108, Denver, CO). These materials are lightweight, have a high water-holding capacity and therefore can absorb a large amount of bacterial suspension, adhere to the seedcoat, and prevent dessication of the bacterial cells under dry conditions in the soil.

A field trial was conducted to compare the three carrier-inocula with dried-leaf inoculum. Each carrier was mixed with a high and a low concentration of a bacterial suspension at the ratio of 1 g of carrier per 10 ml of suspension. The bacterial suspension was prepared from bacterial cultures grown on King's B medium for 48 hours, washed off with 50 ml sterile distilled water, and adjusted to 6 percent transmittance at 620 um with a spectrophotometer. This constituted the high concentration of the bacterial suspension; the low concentration was a 1:10 dilution of the high rate. Dilution plating of this low-concentration suspension showed the population to be 8.5×10^6 cells/ml. Inoculation was accomplished by mixing 180 g of seeds of the cultivar Bush Blue Lake 47 with 10 ml of the bacterial suspension contained in 1 g of the carrier. For the control at a high rate, 1 g of dried-leaf inoculum was added to 10 ml of water and mixed with 180 g of seeds. A lower rate was used to approximate that normally used in our field plots. For each treatment, seeds were planted ca. 3 cm apart in 20 ft rows with 4 ft per row. Treatments were replicated 3 times in a randomized complete block design with 10 ft alleys between replicates. Uninoculated seeds were planted in a row between all treatment rows.

Disease severity was recorded 2 weeks after planting when seedlings were still at the primary leaf stage. In all of the rows planted with inoculated seeds, some of the plants were symptomless and others had leaf spots and puckering symptoms characteristic of bacterial brown spot. No difference in type or severity of symptoms was noted in any of the treatments. None of the treatments resulted in severe disease at this early stage, but all caused either slight or moderate levels of disease. None of the uninoculated plants had any brown spot symptoms. Symptoms were not recorded later because of concern that they could have been induced by inoculum that had spread from plants inoculated by other means.

The peat carrier with the high level of bacterial suspension was used in a preliminary study of the comparative ability of four strains of the pathogen to cause disease on the susceptible and resistant snap bean cultivars Bush Blue Lake 47 and Bush Blue Lake 94, respectively. In this trial, the inoculum was applied to seeds of a susceptible cultivar that were planted in "spreader rows" between rows of the susceptible and resistant cultivars. The pathogen spread from inoculated plants to uninoculated plants in adjacent rows and induced disease. The results of this trial were inconclusive. However, the inoculation procedure worked extremely well. Therefore, we recommend using laboratory-produced inoculum instead of dried-leaf inoculum in breeding trials because it overcomes the disadvantages described earlier for leaf inoculum.

References:

1) Daub, M.E. and D.J. Hagedorn 1981. Epiphytic populations of *Pseudomonas syringae* on susceptible and resistant bean lines Phytopathology 71:547-550.

A Modified Procedure for Assaying Bean Seeds for the Pathogen Causing Bacterial Brown Spot and Results of Assays of Commercial Bean Seeds

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Bacterial brown spot caused by *Pseudomonas syringae* pv. *syringae* has been a problem in snap bean fields in New York state for the past several years. As part of our research program on this disease we have investigated possible sources of inoculum, including the possibility that contaminated bean seeds are being brought into the state. To determine if we could detect the pathogen on seeds to be planted in New York in 1989 and 1990, we assayed untreated snap bean seeds provided by three seed companies.

The first year we used a procedure described by Mohan and Schaad in 1987 (1). In this paper they described a selective medium, referred to as KBC, that we have found to be excellent to isolate the pathogen from seed and plant samples. After isolating and identifying colonies as putative *P.s.* pv. *syringae,* we used a pod assay (2) to determine if the strains were capable of causing bacterial brown spot because most strains of *P.s.* pv. *syringae* are not pathogenic on beans.

In 1989, 32 seedlots were assayed and none were found to contain the pathogen. Several methods were then tried to increase the sensitivity of the assay. These included soaking the seeds at temperatures from 5 to 30 C at 5 C increments (5 C was standard in Mohan and Schaad's assay); incubating the seeds in liquid for various lengths of time (standard was 20 hours); and