

Improved Techniques for Initiation of Bacterial Brown Spot of Bean in the Greenhouse

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ABSTRACT

The atomizing technique for initiating bean bacterial brown spot infections in the greenhouse was superior to four other inoculation methods investigated, including pinching and rubbing with cheesecloth, Q-tip, or pipe cleaner. Placing bean plants in a moist chamber, or covering them with plastic bags, for 24 hr before and after inoculation was not significantly better than allowing them to remain on the greenhouse bench. *Phytopathology* 61:1310-1311.

Additional key words: *Phaseolus vulgaris*, *Pseudomonas syringae*.

Bacterial brown spot (*Pseudomonas syringae* van Hall) of bean (*Phaseolus vulgaris* L.) was first described in New York by Burkholder in 1930 (1). The disease has become serious in Wisconsin (2, 4) during the last few years, especially in sprinkler irrigated beans. During extensive screenings of many bean plant introductions (P.I.'s) and commercial cultivars for resistance to bacterial brown spot, it became apparent that improved techniques were needed for artificially initiating the disease. Of particular interest to us was the development of techniques which would give reproducible infections and be less laborious and more efficient. The pathogenic isolate Y59 of *P. syringae* from bean was stored on nutrient agar glycerol medium (NAG) containing 0.8% nutrient broth, 2.0% glycerol, and 2.0% agar at 4 C. Inoculum was prepared by growing the bacteria on nutrient dextrose agar (NDA) medium (5) for 24 hr at 28 C. Bacteria were washed from the medium with sterile distilled water and diluted to a concentration of about 1.5×10^8 cells/ml ($A = 0.3$ at 600 nm using a Bausch & Lomb Spectronic 20 colorimeter).

Beans, *Phaseolus vulgaris* 'Tenderwhite', were sown in Vermiculite, then transplanted after 2 weeks (one/pot) into 3:1 soil-sand mixture in 5-inch pots and grown in a greenhouse at 21 C.

To establish a reliable, yet efficient, method for inoculating plants, a number of techniques were compared. In the simplest method, the ends of forefinger and thumb were dipped in the inoculum and the first set of young trifoliate leaves was gently pinched when

TABLE 1. Comparison of techniques for inoculating *Phaseolus vulgaris* with *Pseudomonas syringae* in the greenhouse

Technique used for inoculation	Disease index ^a	Remarks
Atomizing	100 a	Single and separate distinct lesions; no injury.
Rubbing with:		
Cheesecloth	55 b	Single and separate distinct lesions which varied in number; little injury.
Q-tip	65 b	Single and separate distinct lesions which varied in number; little injury.
Pipe cleaner	50 b	Single and coalesced lesions which varied in number; some injury caused puckering and desiccation.
Pinching	25 c	Too much injury; leaves puckered and desiccated; difficult to count lesions.

^a Data are the average of four trials; each treatment replicated 10 times. Means followed by a letter in common do not differ significantly at the 1% level of significance according to Duncan's multiple range test.

they were one-third expanded. A second method involved the introduction of bacteria by rubbing the upper and lower leaf surfaces with either a cheesecloth, a pipe cleaner, or a Q-tip soaked in the bacterial suspension. In a third method, bacteria were atomized under 15 psi at 10-12 inches onto both leaf surfaces.

In order to determine the necessity of pre- or post-inoculation misting of plants, one set of plants was placed in a moist chamber and misted constantly at 98-100% relative humidity for 24 hr before and after inoculation. Another set was kept on the greenhouse bench and covered with plastic bags 24 hr before and after inoculation, and a third set had no treatment. Plants were classed 7 days after inoculation as healthy or slightly, moderately, and severely blighted (7). Classes carried the respective weightings: healthy = 0;

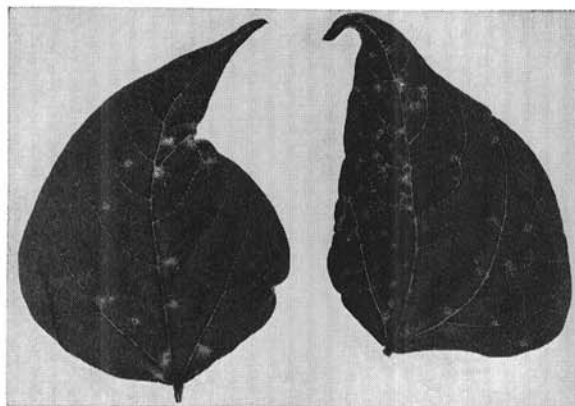


Fig. 1. Typical singular bacterial brown spot (*Pseudomonas syringae*) lesions on bean leaflets, cultivar Tenderwhite, 10 days after inoculation by atomization technique using (Left) 10^4 ; (Right) 10^8 cells/ml.

slight = 25; moderate = 50; severe = 100. Disease indices were calculated using Sherwood & Hagedorn's formula (6).

Differences among the disease indices for the various inoculation techniques were compared statistically using Duncan's multiple range test (Table 1). No significant differences were found among the three rubbing techniques; however, pinching and atomizing were either significant or highly significant from the rubbing. Although pinching was the most efficient technique, a high degree of injury made accurate lesion counts impossible. The various methods of rubbing did not produce a uniform number of lesions when using a constant level of inoculum. Therefore, we could not obtain a reliable and accurate disease rating. In addition, rubbing often caused excessive puckering and injury of the tissues, which varied in magnitude from one technique to the other. Atomizing resulted in distinct lesions (Fig. 1) well distributed over the leaf surfaces which could be reproduced in repeated tests, thus permitting the calculation of an accurate disease index. Since there was no significant difference in the disease indices obtained for misted plants and those inoculated in situ with or without the use of plastic bags, it is more efficient to eliminate misting. Thus, contrary to previous reports (3, 7), there is no need to main-

tain plants under a high level of moisture before or after inoculation in order to initiate the bacterial brown spot disease. The "inoculation in situ", using the atomizer, is a rapid technique for screening P.I.'s and commercial cultivars of bean to *P. syringae*.

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