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# A PRESSURE INJECTION DEVICE FOR INOCULATION OF MAIZE WITH BACTERIAL PHYTOPATHOGENS

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A PRESSURE INJECTION DEVICE FOR INOCULATION  
OF MAIZE WITH BACTERIAL PHYTOPATHOGENS

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ABSTRACT

A pressure injection device (PID) was used to inoculate maize (Zea mays) with bacterial phytopathogens. The PID was evaluated for its speed, effectiveness, and precision relative to the needle-eye method of inoculation. Corynebacterium nebraskense, Erwinia stewartii, and Pseudomonas andropogonis were each used successfully as inocula to infect maize and produce symptoms typical of Goss's wilt, Stewart's wilt and bacterial stripe, respectively. Quantitative inoculation with the PID was faster and as statistically precise as using a calibrated needle-eye.

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Investigators who inoculate large numbers of plants with bacterial phytopathogens frequently must choose between tedious, time-consuming quantitative techniques and more rapid qualitative techniques that produce inconsistent results. This report evaluates a pressure injection device (PID) as a tool for the rapid and reproducible inoculation of maize (= corn).

MATERIALS AND METHODS

Bacteria and Media: Bacterial cultures used in this study (Corynebacterium nebraskense 298 (a Nebraska strain), Erwinia stewartii Z017 (obtained from D. Dye), and Pseudomonas andropogonis Pi3195 (a Nebraska strain)) were maintained on a nutrient broth-yeast extract



FIGURE 1. Pressure injection device (PID) in use for inoculation of maize with bacterial phytopathogens.

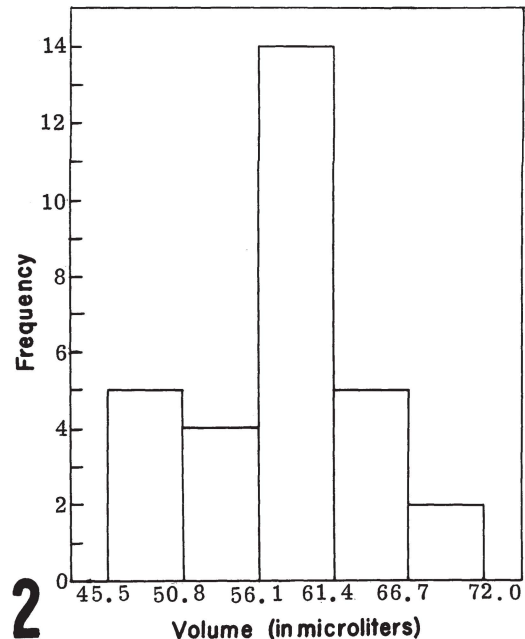


FIGURE 2. Distribution of sample volume from 30 consecutive samples delivered by the PID.

medium (NBY) agar (2) and also lyophilized to preserve the original cultures. The bacteria were grown on NBY agar for 48-72 hours at 25°C or overnight in NBY broth at 25°C. Single colonies from the agar plates or overnight cultures washed by centrifugation were suspended in 10 mM PO<sub>4</sub> buffer (pH 7.1). The A<sub>420nm</sub> (Spectronic 20) of the suspensions was adjusted to 0.1 (approximately  $1 \times 10^8$  colony-forming units (CFU/ml) and dilutions were made as necessary.

**Inoculation and Assay Procedures:** Maize seedlings (3-4 leaf stage) were inoculated by using the PID or needle-eye technique (3). The hand-held PID (Schuco injector (dental model), obtained from American Caduceus Industries, Inc., Williston Park, N.Y.) is equipped with a transparent 5-ml reservoir; inoculum is delivered by triggering a spring-loaded piston into a chamber with a small (152 μm) orifice for an exit. Pressure injection device inoculations were made at 1 to 2 cm above the soil line (Fig. 1). The sweet corn cultivar Golden Cross Bantam was used for evaluation of inoculation techniques and infectivity assays for the three pathogens. One hundred thirty-seven field corn varieties from a separate study (1) were inoculated with *C. nebraskense* in the greenhouse (25-30°C) to determine the usefulness of the PID as a rapid injection method for inoculating large (> 2000) numbers of plants.

The volume of the inocula delivered was assayed by dilution plating, in duplicate, of bacterial suspensions collected by discharging the PID into test tubes. Any bacteria adhering to the interior walls of the test tubes were washed into the bottom with known volumes of buffer. Aliquots of the appropriate dilutions were spread on NBY plates and the colonies were counted after 3-5 days' incubation at 25°C. The volume of each sample was calculated from the total colony-forming units (CFU) in the sample versus the concentration of bacteria in the inoculum.

#### RESULTS AND DISCUSSION

With the use of bacterial suspensions of differing populations, we found that the number of bacteria discharged was directly proportional to the bacterial concentration in the inoculum (experimental  $t = 0.73$ ; tabular  $t = 0.05$ , 8 d.f. = 2.3065). Thus, the volume discharged was constant, and bacterial colony counts were a valid assay for volume.

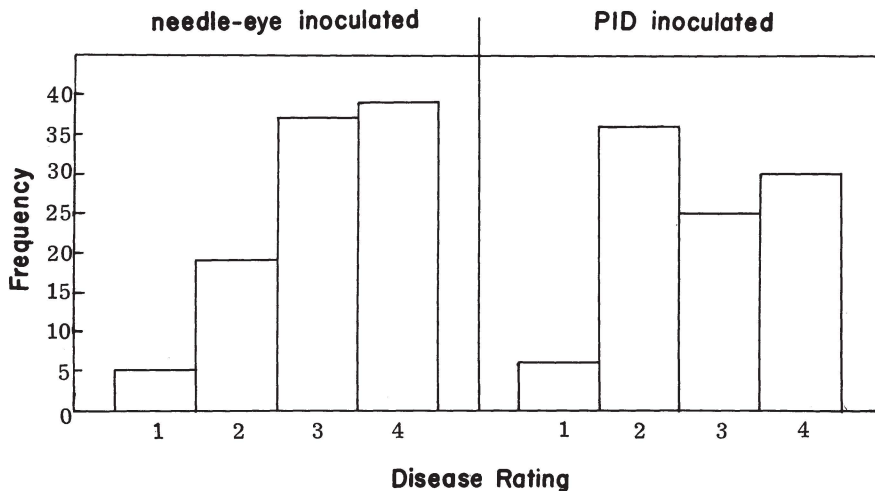


FIGURE 3. Distribution of disease severity in corn seedlings inoculated with Corynebacterium nebraskense.

To measure experimentally the degree of variation among delivered volumes, 30 consecutive samples were collected and plated (Fig. 2). The average sample volume was 57.6  $\mu$ liters with a standard deviation of 6.2  $\mu$ liters. Chi square analysis showed that the variation was within an acceptable range: The distribution was not statistically different from the theoretical normal distribution (experimental  $X^2 = 4.2341$ ; tabular  $X^2$  0.05, 2 d.f. = 5.99).

To determine reproducibility of bacterial infections in plants, 200 maize seedlings were inoculated with  $1 \times 10^8$  CFU/ml C. nebraskense: 100 by the PID method, and 100 by the needle-eye method. Two weeks later, each plant was rated for disease severity on a 0 (healthy) to 5 (dead) scale (Fig. 3). The variance of the disease ratings of the PID-inoculated seedlings was not statistically different from the variance of the needle-eye-inoculated seedlings (experimental  $F = 1.4248$ ; tabular  $F$  0.025, 100 d.f., 100 d.f. = 1.53). The data clearly show that the PID method is as consistent as the needle-eye method. The average disease rating, however, was somewhat lower for the PID than for the needle-eye technique (experimental  $t = 2.694$ ; tabular  $t$  0.05, 200 d.f. = 1.97). This may be due to the fact that some inoculum passes completely through the stalk of a maize seedling. Thus the effective inoculum may be lower, which would be reflected as lower disease ratings. (In addition to Goss's wilt (caused by C. nebraskense), the PID was also used to inoculate E. stewartii and P. andropogonis into corn, which subsequently developed infections typical of Stewart's wilt and bacterial stripe, respectively.)

Maize leaves cannot be inoculated effectively because insufficient inoculum is retained compared with the consistent and quantitative stalk inoculations. In fact, most of the inoculum will exit leaves with considerable force. This is not surprising because the PID was originally designed to penetrate animal tissue without a needle.

The PID has a 3 : 1 advantage over the needle-eye technique in inoculation speed (921 : 311 plants/hour), and can be used successfully to infect large numbers of maize plants rapidly.

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