Mechanical Device for Rapid Inoculation with Bacterial Plant Pathogens

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ABSTRACT

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A device for rapid inoculation with bacterial plant pathogens consists of a fan nozzle and petcock valve attached to a cordless electric grass clipper. Inoculum is contained in a pressurized tank. The inoculum mist created by the nozzle wets the clipper blades, which introduce the bacterial cells into leaf wounds. The inoculator has been used successfully in the greenhouse and field for several host-pathogen combinations.

Often plants cannot be inoculated with bacterial plant pathogens by methods used with fungi because the bacteria must enter a wound or natural opening. During an investigation of halo blight of rye (*Secale cereale*), a method was needed to inoculate plants rapidly with *Pseudomonas coronafaciens* (Elliott) Stevens in field plots. Inoculating leaves by pricking them through a droplet of inoculum was difficult and slow. A scissors method (7) gave consistent results and was more rapid than the pinprick method but was laborious for use in field plots. Inoculation with a hypodermic syringe was quite successful in greenhouse studies (2,3) but was not suited to field work. This paper describes the design and applicability of an inoculator that may be used to inoculate many bacterial plant pathogens rapidly and efficiently.

METHODS AND RESULTS

Description of the inoculator. The inoculator consists of a cordless electric grass clipper (Disston, Inc., Danville, VA) on which a fan nozzle is mounted (Figs. 1 and 2). Grass clippers with rechargeable and interchangeable battery packs allow the operator to inoculate plants for several hours without interruption. The nozzle assembly (Fig. 1) can be removed from the clipper by removing the wing nut from the threaded rod. The assembly also can be transferred easily to another clipper.

A Hudson Climax 67335 garden sprayer (H. D. Hudson Manufacturing Co., Chicago, IL) was modified by replacing the hose and supply tube with flexible plastic tubing of appropriate diameter to fit over the exit port and to reach to the bottom of the tank. Alternatively, the standard hose can be disconnected from the spray wand and a section of tubing of appropriate diameter and length can be connected to the hose. The tank was pressurized by hand to 0.7–1.1 kg/cm^2 (10–15 psi) and held on the operator's back by two adjustable nylon web straps or carried by a single shoulder strap by a second person. The tubing leading from the tank to the clipper was 115 cm long. This length permitted the operator to hold the clipper at arm's length when the tank was carried on the operator's back and provided sufficient freedom of movement when another person carried the tank.

The fan nozzle directed a mist onto the cutter blades and up to 6–8 cm in front of them by adjustment of the nozzle angle. Therefore, leaves are wetted with a bacterial suspension before and during cutting. When closely spaced row plants such as rye were inoculated, heavy inoculum could be conveniently applied to leaves by directing the mist ahead of and onto the blades. When space-planted crops were inoculated, less inoculum was used by spraying the blades only.

Preparation of inocula. Inocula of all pathogens used in these studies were grown overnight in medium 523 (6), adjusted to 50 Klett units (Klett-Sumerson colorimeter), and diluted 10^{-2} in sterile distilled water or 0.85% NaCl. Tween 20 was added as a surfactant.

Field trials. In three trials during two seasons with *P. coronafaciens,* rye plants

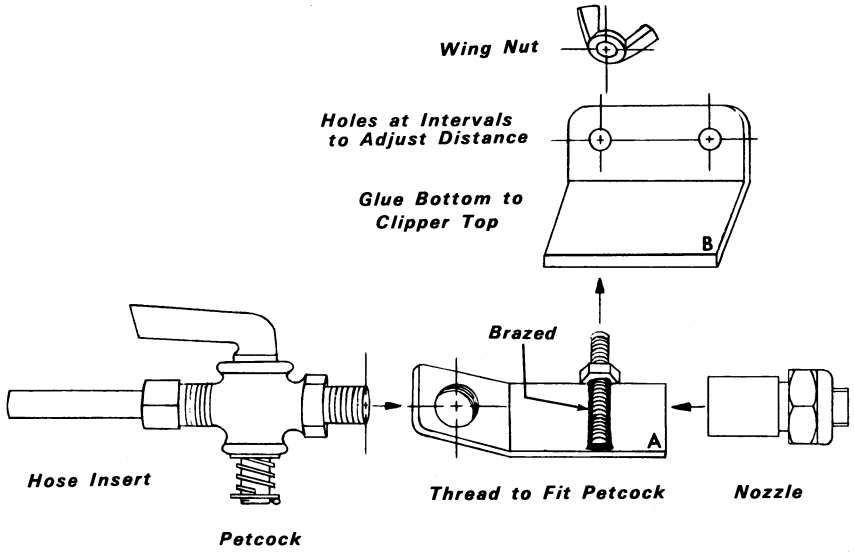

Fig. 1. Nozzle assembly. Hose insert 5 cm long, 8 mm (5/16 in.) diameter attached to petcock; 50 × 50 × 3 mm angle iron, 18 mm long; hole drilled and threaded in angle iron A; rod brazed to angle iron A to provide friction surface, nut screwed onto rod. Angle iron B 25 × 25 × 3 mm, 50 mm long, with two 6-mm diameter holes, drilled about one-third of distance from the top (holes provided to adjust position of valve and nozzle after assembly). Lower surface of angle iron B and top of clipper housing scored, then cemented together with filled epoxy resin. To assemble, angle iron A attached to petcock, then Tee Jet No. 1321 brass fan nozzle, 6.35 mm (1/4 in.) diameter attached to petcock. Threaded rod inserted into appropriate hole of angle iron B and secured with wing nut. Angular adjustment to direct the mist flow made by tilting the nozzle before tightening the nut.

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were inoculated at the end of a single-row plot or at the ends of two center rows of four-row plots; 100 rows were inoculated by cutting the leaves with scissors dipped in inoculum. Symptoms were abundant on all inoculated plants and secondary infections were observed on other plants in each plot 2 wk after inoculation. Eventually inoculum was dispersed throughout the plots and plants were infected uniformly. The clipper method was much faster than the scissors method and required less labor. These trials included more than 2,000 rows.

Five hundred rye lines planted in single rows 1.5 m long were inoculated at heading stage with inocula of *P. coronafaciens* and *Xanthomonas translucens* mixed together. One plant at the end of each row was inoculated, and within 2 wk symptoms of halo blight and bacterial blight were observed on susceptible plants at the end of the row opposite from the inoculation site. Secondary spread of both pathogens continued during the remainder of the season, resulting in uniform symptoms throughout the nursery. Lines susceptible to each bacterium were severely diseased at harvest time.

Six hundred rows of a triticale breeding nursery at Tifton, Georgia, were inoculated with *X. translucens*. One plant at the end of each 3-m row was inoculated. Plants were inoculated three times at 2-wk intervals beginning when early maturing lines were at boot stage. No infection resulted from the first inoculation because of cool weather. By the time most lines reached dough stage, severe leaf blight and abundant bacterial exudate were seen on inoculated plants and plants within 1 m of inoculated plants. Symptoms were rare on plants farther down the row. Definite differences in disease severity were noted among the lines, however, so meaningful evaluations could be made.

Table 1. Comparison of the injection and clipper methods for inoculating plants with bacterial pathogens in greenhouse test 1

Pathogen	Strain	Host	Cultivar	Infected plants (%)[a]	
				Injection	Clipper inoculator
Pseudomonas avenae[b]	C-11	Maize	Pioneer 3030	37	38
P. andropogonis	C-90	Sorghum	BR-54	56	94[c]
Erwinia chrysanthemi	5968	Maize	Pioneer 3030	27	67[c]
Xanthomonas campestris	B-24	Cabbage	Market Prize	63	84[c]
X. translucens	XT-10	Triticale	6TB 153	57	58

[a] Mean of four replicates with at least 15 plants per replicate.
[b] Equals *P. alboprecipitans.*
[c] Difference from the injection method is significant ($P = 0.05$).

Table 2. Comparison of methods for inoculating plants with bacterial pathogens in greenhouse test 2

Pathogen	Strain	Host	Cultivar	Infected plants (%)[a]		
				Injection	Atomization	Clipper inoculator
Pseudomonas avenae[b]	C-13	Maize	Pioneer 3030	83	···	84
P. andropogonis	C-90	Sorghum	BR-54	93	···	95
P. phaseolicola	C-169	Bean	Top Crop	92	93	85
P. solanacearum	40	Tomato	Rutgers	100	···	75[c]
Xanthomonas campestris	B-24	Cabbage	Market Prize	87	···	96
X. translucens	XT-10	Triticale	6TB 059	···	68	47[c]
X. translucens[d]	XT-10	Triticale	6TB 059	···	62	40[c]

[a] Mean of four replicates with at least 20 plants per replicate.
[b] Equals *P. alboprecipitans.*
[c] Difference from the injection or atomization method is significant ($P = 0.05$).
[d] Plants kept in mist chamber 16 hr before and after inoculation, then returned to greenhouse.

Fig. 2. Nozzle assembly mounted on a cordless electric grass clipper. Inoculum is transported from a pressurized tank to the nozzle, which atomizes it onto the clipper blades.

Greenhouse trials. *P. coronafaciens* was inoculated onto 3-mo-old plants of 650 breeding lines of rye (one pot with five plants per line). The youngest leaves were cut off or injured by the clipper blades. The inoculation was completed in 90 min and required 5 L of inoculum. The plants were inoculated on two dates at 3-wk intervals. Typical halo blight symptoms developed at nearly all wound sites within 5 days, and secondary lesions were evident 12 days after inoculation.

Tests were conducted to assess the applicability of the inoculator with other bacterial pathogen-host combinations (Tables 1 and 2). Plants were 10–25 days old at the time of inoculation. One group of plants was inoculated by hypodermic injection, following published procedures (8,10,11,13). Plants were inoculated with the clipper by cutting the whorl of monocots and two or three of the youngest leaves of dicots. During inoculation the plants were thoroughly wetted. Triticale and bean also were inoculated by atomizing the plants with bacterial suspensions (5,9).

After inoculation, the plants were incubated in the greenhouse at 24–29 C until maximum expression of symptoms. Maize inoculated with *Erwinia chrysanthemi* was incubated for 4 days at 30–32 C in a dew chamber to insure infection. Control plants were inoculated by all methods with sterile distilled water plus Tween 20.

The amount of infection resulting from the two inoculation methods varied greatly depending on the bacterium (Tables 1 and 2). The clipper method was comparable to or better than the injection method for inoculation with *X. campestris,*

P. avenae (= *P. alboprecipitans*) (4), *E. chrysanthemi,* and *P. andropogonis.* Inoculation of plants by atomizing a cell suspension was comparable to the clipper method for *P. phaseolicola* but better for *X. translucens.* Although the injection procedure resulted in 100% infection of tomato with *P. solanacearum,* 75% of the plants showed typical wilt symptoms after inoculation by the clipper. No disease symptoms were observed on the controls.

DISCUSSION

The clipper inoculator has been used successfully in the greenhouse and field to inoculate *P. coronafaciens* and *X. translucens* and in the greenhouse to inoculate plants with other bacterial plant pathogens. The inoculator should be especially useful in field plot inoculations for evaluation of germ plasm for disease resistance.

In our field trials with *P. coronafaciens* and *X. translucens* on rye, infection foci were created early in the season. Natural spread from these foci created a uniform level of disease incidence throughout the nursery. Therefore, even when the inoculation efficiency is somewhat less than that of a hand method, the clipper method can create numerous infection centers in less time. Two methods described recently (1,12) for inoculation of *Erwinia stewartii* provide more uniform inoculation of individual plants than the clipper method does, but these methods are more time-consuming. When working with a pathogen that has little secondary spread or if a high inoculation efficiency is desirable on individual plants, the investigator must decide whether the clipper method is superior to other methods.

More inoculum is needed with the clipper method than with injection or similar methods, but the ease of growing most bacteria should not cause this to be a problem. In the field, concentrated cell suspensions can be stored on ice and diluted as needed.

The inoculator is lightweight, inexpensive, and easily constructed. It can be a timesaving device for inoculation with many bacterial plant pathogens.

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