Controlled Droplet Applicators for Inoculation with Plant Pathogens

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ABSTRACT

Perry, J. B., and Williams, P. H. 1984. Controlled droplet applicators for inoculation with plant pathogens. Plant Disease 68:107-108.

The MicroULVA controlled droplet applicator was evaluated as a tool for inoculating plant pathogens in the field and greenhouse. Diseases caused by *Albugo candida* race 2, *Uromyces phaseoli, Pseudomonas lachrymans*, and *Colletotrichum orbiculare* were severe and uniformly distributed when large spray droplets (60–70 μm) were used in the field and greenhouse. Incidence of *A. candida* and *U. phaseoli* in the greenhouse decreased when small droplets (30–40 μm) were used; however, severe disease of *Xanthomonas campestris* in field plots of cabbage occurred after inoculation with small droplets. Small volumes of inoculum were used, making this inoculator a potentially valuable tool for field use.

Uniform inoculation of plant materials with limited quantities of inoculum is difficult under many greenhouse and field conditions. Controlled droplet application (CDA) sprayers developed to deliver low volumes of pesticides to spray targets may become effective inoculation tools for plant pathologists. Liquid is dispensed through a spinning disk nozzle (rotary atomizer), which disperses the stream as droplets (Fig. 1). Droplet size falls within a narrow range determined by rotational speed of the disk. Droplet size and flow rate can be adjusted to deliver the desired droplet density on the target (2).

During 1980–1982, we evaluated the MicroULVA CDA sprayer (Micron Corporation, P.O. Box 19698, Houston, TX 77024) as an inoculation device. This is a lightweight hand-held sprayer powered by D-cell (1.5V) batteries. The pathogen suspension is gravity-fed to the spinning disk nozzle from a 0.5-L bottle. Experiments were designed to access the potential of this sprayer for inoculation of a variety of pathogens in the greenhouse and the field.

MATERIALS AND METHODS

Pathogen-host combinations studied in the greenhouse and field were *Albugo candida* race 2 on Giant Long Standing mustard (*Brassica juncea*), *Uromyces phaseoli* on Topcrop bean (*Phaseolus vulgaris*), and *Pseudomonas lachrymans* and *Colletotrichum orbiculare* on SMR 18 cucumber (*Cucumis sativus*). The MicroULVA was also used to inoculate cabbage (*B. oleracea* var. *capitata*) field plots with *Xanthomonas campestris* pv. *campestris*. All plants for greenhouse experiments were seeded and grown in steam-sterilized greenhouse soil mix in plastic multipak trays (48 cells per flat) in a greenhouse at 24 C under natural light.

A suspension of *A. candida* zoospores and sporangia was prepared by incubating frozen sporangia in glass-distilled water for 2–3 hr at 16 C. Inoculum was adjusted to 10^5–10^6 sporangia per milliliter and kept chilled on ice to maintain zoospore motility (7). Mustard plants were inoculated at the three-leaf stage. Inoculated plants were incubated overnight in a moist chamber at 20 C and disease incidence (number of plants with lesions) was recorded 7–10 days later.

Inoculum of *U. phaseoli* was prepared by suspending 1 mg of refrigerated urediospores per 2 ml of 10^{-4} M nonanol. Bean plants were inoculated when the first pair of unifoliolate leaves was fully expanded. Inoculated plants were incubated 24 hr in a moist chamber at 20 C and disease incidence was rated 7–10 days later.

Inoculum of *P. lachrymans* was prepared from a 1-day shake culture grown in H-K medium (3) at 28 C. One milliliter of suspension was added per 19 ml sterile 0.1 M phosphate buffer, pH 7.1. SMR 18 cucumber plants were inoculated when the first true leaf was fully expanded. Inoculated plants were incubated 48 hr in a moist chamber at 20 C and disease incidence was rated 7 days after inoculation.

Inoculum of *C. orbiculare* was prepared from a 5- to 9-day-old culture on a green bean baby food agar slant (three jars, each 128 g, of strained green beans, dH_2O to 1 L, 30 g agar). Spores were suspended in water and the suspension was centrifuged at 3,000 *g* for 10 min. The pellet was resuspended in water at a concentration of 8×10^5 spores per milliliter. SMR 18 cucumber plants were treated as for *P. lachrymans*.

The MicroULVA was adjusted to deliver large spray droplets (60–70 μm). This size range was achieved by using the medium feed rate (about 60 ml water per minute) and by using six D-cell batteries (9V DC) as the power source (1). Plants were misted with deionized water, then inoculated by holding the spray nozzle about 25 cm above the foliage for 5 sec.

The effect of droplet size on inoculation performance was determined by comparing disease incidence on plants inoculated with large droplets with that resulting from inoculation with small droplets (30–40 μm). Small droplets were achieved by using a reduced feed rate (30 ml water per minute) and 10 D-cell batteries (15V DC) as the power source.

The effect of inoculum concentration was explored in a third set of experiments by inoculating plants with the concentrations described previously or with 10-fold dilutions of those concentrations. Large droplets were used in these experiments.

Field application of *A. candida, U. phaseoli, P. lachrymans*, and *C. orbiculare* to mustard, bean, and cucumber plants, respectively, with the MicroULVA was tested in 1982. Plants were direct-seeded in 1-m^2 plots, thinned to stand on 10-cm centers, and inoculated at the two-leaf stage in late evening as dew formed. Inoculum was prepared as for greenhouse tests and was delivered in large droplets (60–70 μm) at a walking rate of 1 m/sec with the MicroULVA held about 25 cm above the foliage.

In 1980, 1981, and 1982, cabbage breeding lines were inoculated in the field with *X. campestris* pv. *campestris* using the MicroULVA. A 48-hr shake culture of potato-dextrose broth incubated at 28 C was sprayed (without dilution) directly over 6-wk posttransplant plants early in the morning in still air and before

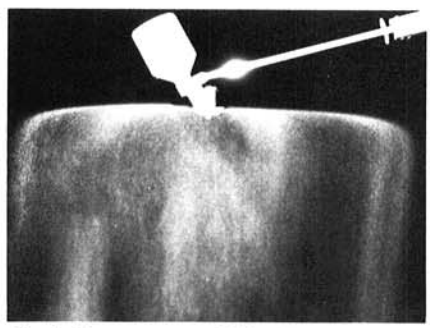

Fig. 1. Spray pattern of MicroULVA adjusted to deliver large droplets.

Accepted for publication 7 August 1983.

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Table 1. Effect of droplet size and inoculum concentration on disease incidence in greenhouse tests

Pathogen	Droplet size (μm)	Inoculum concentration	Plants diseased[a] (%)
Albugo candida race 2	60–70	5×10^5 Sporangia/ml	90 (12)
	30–40	5×10^5 Sporangia/ml	83
	60–70	5×10^4 Sporangia/ml	40
Uromyces phaseoli	60–70	1 mg Urediospores/2 ml	98 (3.5)
	30–40	1 mg Urediospores/2 ml	95
	60–70	0.1 mg Urediospores/2 ml	86
Pseudomonas lachrymans	60–70	1 ml Culture/19 ml buffer	99 (0.9)
	30–40	1 ml Culture/19 ml buffer	100
	60–70	0.1 ml Culture/19 ml buffer	100
Colletotrichum orbiculare	60–70	8×10^5 Spores/ml	100 (0)
	30–40	8×10^5 Spores/ml	100
	60–70	8×10^4 Spores/ml	100

[a] Data are means of replicates where standard deviation of the mean is given in parentheses; other data are from a single replicate of 35–56 plants.

Table 2. Incidence and severity of four diseases after field inoculation with a controlled droplet applicator

Pathogen	Number of plants in disease severity category[a]					
	0	1	3	5	7	9
Albugo candida race 2	0	0	0	0	0	93 (4.9)
Uromyces phaseoli	0	0	0	0	0	99 (0.9)
Pseudomonas lachrymans	0	0	0	0	16 (5)	79 (3.4)
Colletotrichum orbiculare	0	0	0	0	44 (5.4)	52 (5.4)

[a] Disease severity expressed on a scale of 0–9, where 0 = absent, 1 = very little disease, 5 = moderate disease, and 9 = very severe disease; each value is the mean of three replicates. Standard deviation of the mean is given in parentheses.

dew and guttation droplets had evaporated from the leaves (7). The MicroULVA was held about 25 cm above the cabbage rows. Droplets were 30–40 μm and inoculum was delivered at a walking speed of 1 m/sec. The field plot contained a row of susceptible cabbage cultivar Sanibel alternating with two rows of test lines. Plants were spaced at 0.5-m intervals in rows 1 m apart.

RESULTS AND DISCUSSION

In greenhouse applications of *P. lachrymans, C. orbiculare*, and *U. phaseoli*, 100% incidence was achieved using droplets in the range of 60–70 μm (Table 1). When applied at this size for 5 sec/flat of 48 plants, inoculum dosage per plant was about 0.1 ml. Lesions were discrete and well-scattered over the leaf surfaces. Inoculations with *A. candida* were more variable from test to test in the greenhouse (Table 1). This may have been due to damage from shearing or impaction forces generated by the technique on the delicate zoospores. Inoculation with droplets of 30–40 μm reduced incidence of *A. candida* and produced fewer lesions of *U. phaseoli* per plant. Sporangia of *A. candida* are 12–18 μm diameter (6) and urediospores of *U. phaseoli* are 20–30 × 20–26 μm (4). Perhaps, fewer of these large propagules were carried by the small droplets. Ten-fold dilutions of inoculum from concentrations normally used for inoculation of the pathogen (7) greatly decreased the incidence of *A. candida* and slightly reduced *U. phaseoli*. Overall severity of diseases was reduced at the 10-fold inoculum dilution.

Field applications of *A. candida, U. phaseoli, P. lachrymans*, and *C. orbiculare* gave 100% disease incidence with severity ratings of 7–9 (scale of 0–9) (Table 2). Lesions were typical for the individual diseases and well-distributed over the foliage.

Field applications of *X. campestris* also gave 100% disease incidence with severity ratings of 7–9 (scale of 0–9) in 11,000 known susceptible plants. Symptoms of black rot were the characteristic V-shaped marginal lesions, which coalesced to produce severe marginal scorching and eventual systemic spread into the cabbage head. Delivery rates of a suspension of *X. campestris* were about 0.5 ml/m of row or 0.25 ml/plant. At this rate, 1,250 ml of culture was adequate to inoculate a 0.25-ha field plot.

Field inoculation in 1982 of *Didymella bryoniae* onto cucumber cultivars, plant introductions, and breeding lines produced discrete, uniform symptoms of gummy stem blight throughout a plot of about 1,200 plants. Small droplets (30–40 μm) were used; resulting disease spread uniformly onto young tissues and allowed reliable identification of resistant and susceptible lines (A. J. Wyszogrodzka, *personal communication*).

Droplet size is controlled by the rotational speed of the spinning disk, which is affected by the voltage produced by battery output. The battery voltage drops with age and use. The rate of decline of battery voltage output depends on the state of the motor and the quality of the batteries and the extent of their use. At low nozzle rotational speeds, atomization ceases and sheet formation occurs (1). Spray droplet size becomes extremely variable and uniform inoculation is impossible. Therefore, to spray for long periods on a single day, excessive voltage reduction must be avoided by using sets of high-quality batteries in sequence for no more than 2 hr of spraying at one time (5).

Compared with various compressed-air inoculators with portable compression tanks and conventional spray or air-blast nozzles, the MicroULVA was a more convenient, lightweight device that provided levels of infection equal to those used previously (7). It provided an efficient method to uniformly inoculate large numbers of plants with a small volume of inoculum. We feel that CDA sprayers deserve attention as practical alternatives to inoculation with conventional compressed-air or carbon dioxide sprayers.

ACKNOWLEDGMENTS

This work was supported partly by the College of Agriculture and Life Sciences, University of Wisconsin, Madison, and partly by gifts from the National Kraut Packers Association, Project 118. We are grateful to M. J. Palmer and S. A. Vicen for technical assistance.

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