A Device for Uniform Deposition of Liquid-Suspended Urediospores on Seedling and Adult Cereal Plants

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ABSTRACT

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A device that uniformly deposits liquid-suspended urediospores on seedling and adult cereal plants consists of a pneumatic cylinder which moves an atomizer upward at a constant, regulated speed. During the upward movement of that atomizer, air at a constant pressure from another pump is directed into the atomizer and a uniform cone-shaped cloud of urediospores suspended in liquid is sprayed onto the surface of the plants. The efficiency of the inoculation device was tested by spraying a range of concentrations of urediospores of *Puccinia hordei* suspended in light mineral oil onto double-stick plastic tape, primary leaves of seedlings, and flag leaves of adult barley plants. Uniform inoculations were achieved; standard errors of the mean were small in all experiments. The device is equally well suited for seedlings and adult plants, inexpensive to construct, and easy to use.

Quantitative studies on the expressions of the host-parasite interaction require that inoculum be uniformly applied in known amounts. This is especially important in experiments to test components of resistance such as receptivity and latent period and small differences in resistance. A number of procedures have been developed to provide uniform deposition of urediospores of the rust fungi on cereal plants (1,2,4–8), most of which are satisfactory for seedling or adult plants, but not for both. For this reason, we developed a device that uniformly deposits urediospores on both seedling and adult cereal plant parts.

MATERIALS AND METHODS

The inoculation device (Fig. 1) is mounted on plywood and consists of a pneumatic cylinder with a 30-cm stroke (Clippard air cylinder, model UDR-12; Air Engineering Co., Minneapolis, MN 55406), a steel guide rod 6 mm in diameter and 30 cm long that steadies an aluminum bar $4 \times 20 \times 90$ mm that holds the inoculator described by Browder (3). A gelatin capsule attached to the atomizer contains the inoculum suspension. Two regulatory valves A and B control the air supply for the air cylinder. The air is supplied by a pressure/vacuum pump (35 kPa, portable combination pressure/vacuum pump; Fisher Scientific Company, Pittsburgh, PA 15219). Valve A controls the air supply to the cylinder and valve B regulates the pressure of the air admitted into the cylinder and thereby the speed with which the atomizer rises during the inoculation process.

When pressurized air is admitted into the cylinder, the atomizer slides upward at a constant speed. During the upward movement of the inoculator, metered air from another source (35 kPa, portable pressure/vacuum pump) is supplied to the atomizer and a uniform coneshaped cloud of urediospores is sprayed onto the plants.

The device (Fig. 2) is mounted on a cart with locking wheels. The steel frame of the cart has the dimensions of $76.5 \times 139 \times 105$ cm. There are three platforms. The top platform bears the inoculation device as illustrated in Fig. 1. The metal plate (Fig. 1) is movable over a horizontal 20×40 cm opening in the platform. This opening provides easy access to the middle platform that holds a board (25×20 cm) that is vertically adjustable. This board holds the pot containing the plant to be inoculated. The bottom platform provides space for the pressure/vacuum pumps.

The device can be used to inoculate seedlings and flag and other leaves as well as stems of adult cereal plants. When inoculating seedlings, the square opening in the top platform in front of the metal plate is closed with a thin board on which a pot containing seedlings is placed. Each leaf is held on the metal plate by means of two strips of magnetized plastic tape with the upper side of the leaf blade facing the atomizer. One seedling leaf is inoculated at a time. Flag- and other secondary leaves as well as stems are inoculated by placing the pot containing the plant on the vertically adjustable board of the middle platform. The plant part to be inoculated is then attached to the metal plate as described for seedlings.

The efficiency of the inoculation device was tested by spraying double-stick plastic tape, primary leaves of seedlings, and flag leaves of adult barley (*Hordeum vulgare* L.) plants with urediospores of *Puccinia hordei* Otth. suspended in light mineral oil (Soltrol 170; Phillips Chemical Company, Specialty Chemicals Division, Borger, TX 79007). The urediospores used in all experiments were produced on greenhouse-grown seedlings of the barley cultivar Manker.

Evaluation with plastic tape. Double-stick plastic tape (3×15 cm, Scotch, Magic Transparent Tape; 3M Co., St. Paul, MN 55101) was placed on the metal plate 12 cm from the automizer. The inoculum, at a concentration of 8 mg of urediospores per milliliter of oil, was sprayed onto the tape for 1 sec as the atomizer rose 20 cm. The plastic tape was then subdivided into three equal sections, 3×5 cm. The urediospores deposited per section were counted in eight randomly chosen 20-mm^2 areas. The experiment was repeated once.

Evaluation with seedlings. Fully expanded primary leaves of 7-day-old seedlings of barley cultivar Larker were inoculated. The seedlings were grown in a greenhouse at ~20 C in square plastic pots ($7 \times 7 \times 5$ cm) in soil. The plants were illuminated by daylight extended with fluorescent lighting (11,000 lux) when the day length was <12 hr. Three concentrations of inoculum (8, 2, and 0.5 mg of freshly collected urediospores per milliliter of oil) were sprayed onto the upper surface of seedling leaves. The air pressure was adjusted in the inoculation device, so that the inoculator rose 20 cm/sec. Each concentration of inoculum was tested on six pots, each containing four plants. The experiment was arranged in a randomized complete block design. After inoculation, the plants were put in a dew chamber for 16 hr at 18 C and then in a growth chamber at 20 ± 1 C with fluorescent illumination at 10,000 lux for 14 hr daily. Fourteen days after inoculation, uredia per leaf were

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counted and the leaf area was measured with a portable area meter (model LI-3000; Lambda Instruments Corp., Lincoln NE 68504). Comparisons were made on the number of uredia per square centimeter of inoculated leaf surface.

Evaluation with flag leaves. Plants of cultivar Larker were grown in soil in 10-cm-diameter pots (one plant per pot) in a greenhouse at about 20 C and illuminated with daylight supplemented by fluorescent light. On the day before inoculation, tillers were removed leaving only the main culm. Either 4 or 2 mg of freshly collected urediospores of *P. hordei* per milliliter of oil were sprayed onto the upper surface of flag leaves of nine plants in the boot stage of growth. The experiment was arranged in a completely randomized design. The air pressure was adjusted in the inoculation device so that the atomizer rose at 13 cm/sec. After inoculation, the plants were kept in a dew chamber at 20 ± 2 C for 16 hr and then transferred to a growth chamber at 20 ± 1 C with fluorescent illumination at 10,000 lux for 14 hr daily. Fourteen days after inoculation, the number of uredia per square centimeter of leaf surface was assessed by using methods described for seedlings.

RESULTS

Evaluation with plastic tape. There were no significant differences in the number of urediospores deposited on the three sections of the plastic tape (Table 1). The comparison of the corresponding sections of the tape used in the two trials also showed no significant differences in deposition of urediospores. The standard error of the mean ranged from three to seven urediospores per 20 mm^2 for all tape sections in both trials.

Evaluation with seedlings. Inoculum concentrations of 8, 2, and 0.5 mg of urediospores per milliliter of oil resulted in an average of 39 ± 2, 10 ± 1, and 3 ± 1 (mean and standard error of mean) uredia per square centimeter of leaf, respectively. Differences due to concentrations of inoculum were statistically significant ($P = 0.005$). The standard error of the mean was small for the tested inoculum concentrations, and the uredia were uniformly distributed over the entire leaf blades.

Evaluation with flag leaves. Inoculum concentrations of 4 and 2 mg of urediospores per milliliter of oil resulted in the deposition of

TABLE 1. Mean number of urediospores of *Puccinia hordei* deposited on plastic tape by the new inoculation device

Test	Tape section[a]	Urediospores[b]/20 mm^2
1	Top	86 ± 7
	Middle	81 ± 3
	Bottom	88 ± 4
2	Top	87 ± 5
	Middle	94 ± 3
	Bottom	88 ± 6

[a] Mean of eight areas (each 20 mm^2) and standard error of the mean.
[b] There were no significant differences within tests or among tests (analysis of variance, $P > 0.05$).

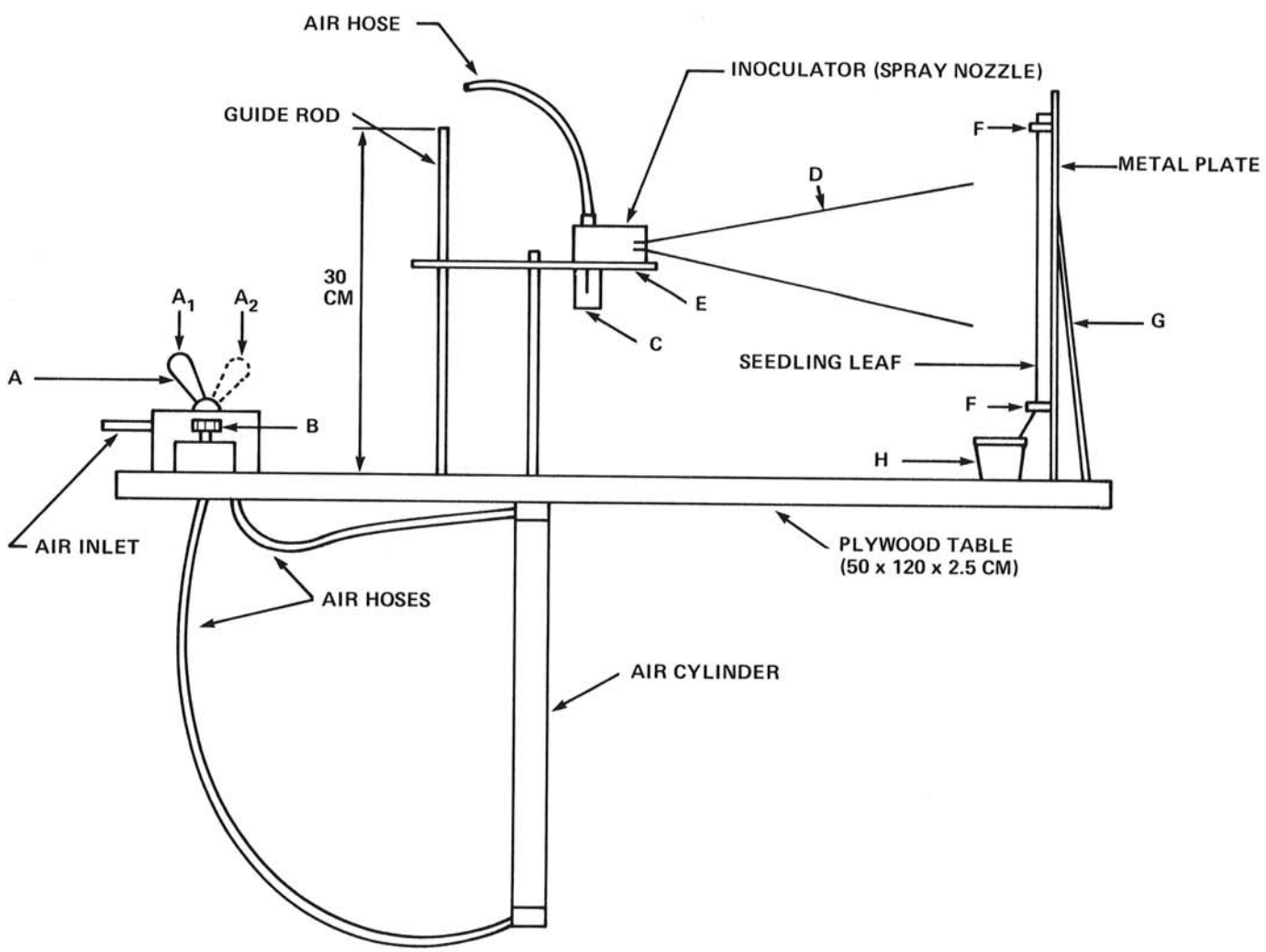

Fig. 1. Device for uniform deposition of liquid-suspended urediospores on cereal plant parts: A, control valve with two positions: A1, air is supplied and atomizer rises, A2, air is released and atomizer falls. B, Air pressure regulator, controls speed at which atomizer rises; C, Gelatin capsule containing inoculum suspension; D, cone-shaped spray of urediospores from atomizer; E, metallic bar for holding inoculator; F, plastic magnetic tape to hold plant leaf to metal plate; G, mounting brace for metal plate; and H, pot in which seedling is growing.

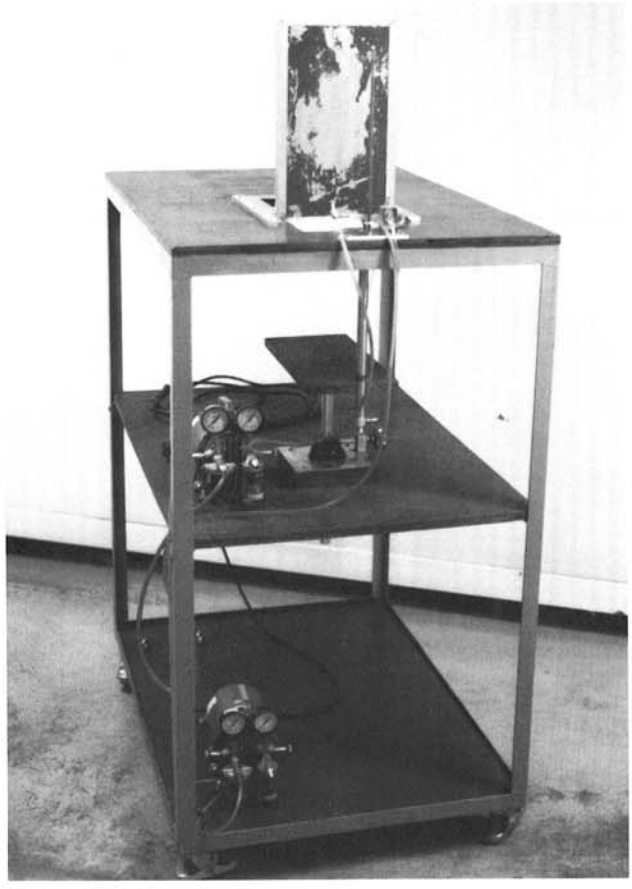

Fig. 2. Device for uniform deposition of liquid-suspended urediospores on cereal plant parts, mounted on a cart with locking wheels.

31 ± 2 and 13 ± 2 (mean and standard error of mean, respectively) urediospores per square centimeter of leaf surface. The difference was statistically significant ($P = 0.01$). The standard error of the mean was two uredia per square centimeter in both inoculum concentrations and the uredia were uniformly distributed over the entire blade of the flag leaves.

DISCUSSION

The inoculation device described above permitted uniform and precise inoculations. Experiments with plastic tape demonstrated that the distribution of urediospores was uniform and experiments with seedling and flag leaves showed that this resulted in uniform distribution of inoculum over entire leaves. Variations in the inoculum concentrations resulted in proportional variations in the number of uredia delivered. Since the inoculation device provides the required precision, it should enable studies on the nature of the relationship between inoculum concentrations and the number of infections.

The inoculation device also has been successfully used to inoculate barley stems with *P. graminis* f. sp. *tritici* (9) and wheat flag leaves with *P. recondita* f. sp. *tritici* (A. P. Roelfs, *personal communication*). These results suggest that the device should be suitable for studies of other diseases.

To achieve the highest possible degree of uniformity with the inoculation device, careful consideration should be given to a number of details. Viable urediospores should be thoroughly mixed with the carrier oil. Target plant surfaces should be in the center of the cone-shaped spray created by the device. The air pressure for moving the atomizer and for spraying the inoculum should be constant. To achieve uniform infection after the precise inoculation, the plants should be placed in a dew chamber where the environment is uniform for the infection process. Environmental conditions should be as uniform as possible after the plants are removed from the dew chamber.

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