A Vacuum-Operated Settling Tower for Inoculation of Powdery Mildew Fungi

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ABSTRACT

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A vacuum-operated settling tower was developed for the inoculation of powdery mildew fungi. *Sphaerotheca fuliginea* race 1, which causes powdery mildew of cucurbits, was used as the test organism. Uniform conidial deposition within the tower was obtained in 120 sec; 89% of the conidia was deposited as a single conidium per site. The ease, rapidity, uniformity, and reliability of this technique indicate that it is of special use when inoculating large numbers of plants or excised plant parts that require quantitative evaluations.

Additional keywords: *Cucurbita pepo, Oidium, Sphaerotheca.*

Uniform inoculation of plant pathogens is necessary for quantitative evaluation of biological assays (26). This is extremely important in breeding plants for disease resistance. Screening systems, especially those developed for the detection of field or horizontal resistance, have suffered from the lack of rapid and reliable quantitative inoculation techniques (22).

The three methods traditionally used to artificially inoculate Erysiphaceae are leaf-to-leaf contact, dusting with dry conidia, and blowing over the test plant with human breath, electric blower, or air blast (28). The methods allow for the deposition of groups of conidia, not a single conidium per site. This promotes a general loss of uniformity, as well as accuracy, in the assays (17). Although the limitations in the different methods are known, they are still in use by breeding and other research programs (8,12,15,16,27). Other methods, such as the camel's hair brush and the cottonswab techniques, which were derived from the above methods and which provide a better uniformity in conidial deposition (13,17), still are not adequate for the quantification of inocula (22).

Techniques involving the use of suspensions of conidia in liquids, such as water or perfluorotributylamine (4,14), have not been widely adopted. Water has been reported to cause damage to conidia (5,19,25), and loss of viability and infectivity have been reported (4,7).

The development of settling towers (ST) was based on Stoke's law of sedimentation (9). Although more sophisticated, the system of conidial dispersion used in ST is comparable to the dusting method (10,11); Schein's quantitative inoculator (26) and different carriers (21,22) also have been used with ST, with better results. However, as pointed out by Rowell and Olien (24) and by Schein (26), the use of ST is considered a slow, cumbersome, and laborious technique for inoculating a large number of plants at frequent intervals.

The objective of this work was to develop an easy, rapid, and reproducible method of quantitative inoculation that would allow for uniform deposition of conidia of powdery mildew fungi of cucurbits.

MATERIALS AND METHODS

Construction of the settling tower. The 50 × 50 × 115 cm tower is made of 15-mm-thick plywood, necessary to support the negative pressure, and veneered with a flat sheet of formica, both inside and outside. A 15-mm-wide × 5-mm-thick, soft rubber band is glued to the perimeter of the base; this gasket improves the adherence of the tower to a basal tray without loss of vacuum. The tower sits over an acrylic or wooden formica-veneered 54 × 54 × 8 cm basal tray. Internally, an inoculum platform is immediately beneath the 10-cm-diameter top lid (Fig. 1). An external air valve, located 25 cm above the base, on one side, connects the tower through a reinforced rubber hose to a vacuum pump (Precision vacuum pump, model D 150, GCA Corporations, Chicago, IL).

Inoculation procedures. Inocula are placed as sporulating leaves or leaf parts in an open petri plate covered with a layer of tape-fastened cheesecloth. The dish is placed on the inoculum platform, and the plants or plant parts under test are placed on the tray. The air valve is opened for 10 sec; during this time, vacuum is built up in the chamber. Then the air valve is closed and the top lid is lifted up, sharply breaking the vacuum. Depending on the roughness of the material used for the bottom tray, it may be necessary to apply a thin film of vaseline or silicone grease to the rubber gasket at the base of the tower or to the tray. Sixteen 9-cm-diameter petri plates were placed on the tray each time.

Evaluation of sedimentation rate, uniformity of deposition, and dispersion of conidia. Squash (*Cucurbita pepo* L. var. *melopepo* 'Caserta') leaf disks (2.5 cm^2, nine per plate) placed in plates containing water-agar medium (1.5% agar) were used in preliminary experiments to evaluate the efficiency of the new technique. Once the technique was evaluated as efficient as characterized by fungal colony growth on leaf disks, trials were conducted only with water-agar plates in random positions, each of the 16 plates representing one replication.

Squash (cv. Caserta) plants infected with *Sphaerotheca fuliginea* (Schlecht ex Fr.) Poll race 1 (23) were maintained in a greenhouse and were the source of inocula.

To determine the sedimentation time, seven trials were run where plates were left for 30, 60, 120, and 180 sec in the tower after breaking the vacuum. Plates then were examined for number of conidia per field, using a 40X transmitted-light stereoscope. The fields were read per plate.

The uniformity of deposition was determined by using inocula with low, medium, and high sporulation densities. The densities were determined by following the inoculation procedures with 120 sec for sedimentation and counting conidia on plates as previously described and corresponding to approximately 2, 6, and 8 conidia/ 19.6 mm^2 field. Plates were set in a system of orthogonal

axes. Conidia were counted in three different fields per plate, and seven trials were conducted. Data were transformed to $\sqrt{x + 0.5}$ for analysis.

The dispersion of conidia, evaluated in seven trials, was rated using a quantitative rating scale with four classes, where class 1 = isolated conidium, 2 = conidia in pairs, 3 = conidia in triplets, and >3 = conidia in groups of four or more. Evaluation of conidial dispersion followed the methodology previously described, with three observed fields of 12.6 mm^2 each.

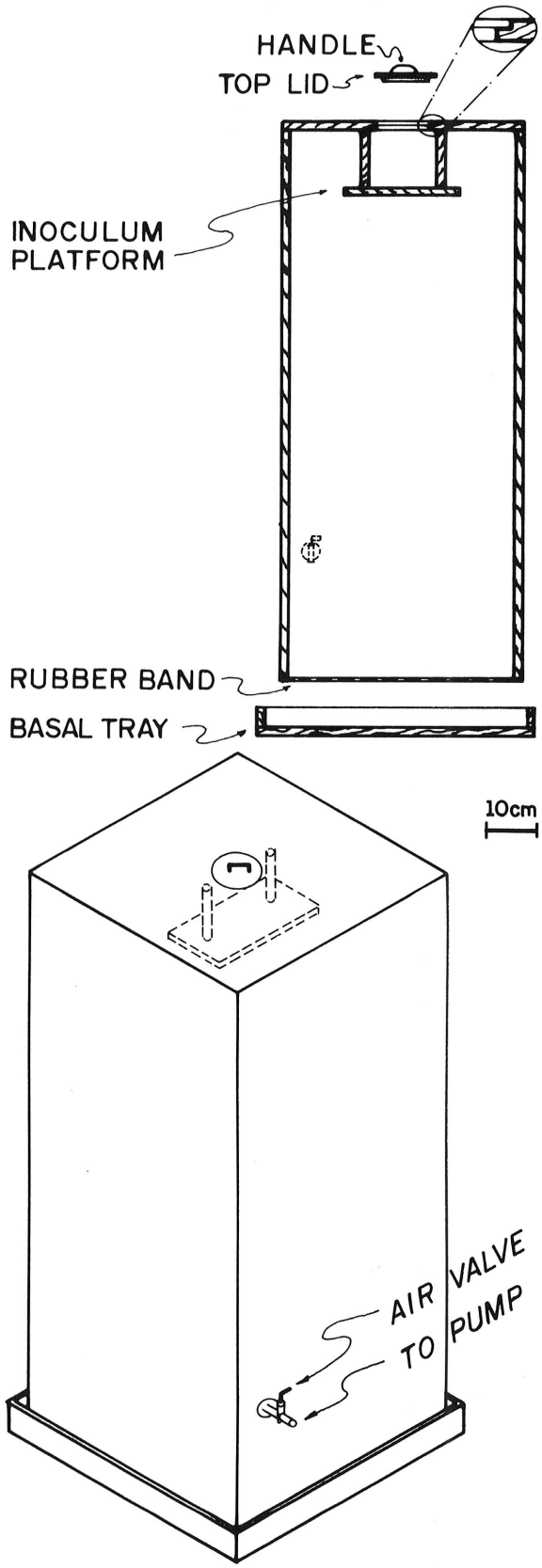

Fig. 1. Details of the vacuum-operated settling tower.

RESULTS AND DISCUSSION

Sedimentation rate. No differences were detected in the number of conidia sedimented at 120 and 180 sec (Table 1).

This indicates that 120 sec is sufficient exposure time. This time period, associated with the ease of operation when inoculating excised leaves or leaf disks, makes this ST rapid and easy to operate.

Uniformity of deposition. Uniform deposition, as indicated by nonsignificant differences ($P = 5\%$) between values at each position in the orthogonal axes, was detected with the three inoculum densities. Higher coefficients of variation (CV) were observed as densities decreased from 8 to 2 conidia/19.6 mm^2: CV = 9.49, 11.38, and 19.76%, respectively. This suggests that low inoculum densities (2 conidia/19.6 mm^2) should not be used. Nonuniform depositions have been reported as a common problem with traditional methods of inoculation (17).

Dispersion of conidia. Figure 2 indicates that 89% of the conidia are deposited as single cells. This is highly desirable, especially in quantitative studies (18) where number of colonies per leaf is assessed and compared. This is considered a major advantage of this technique. The air mass that occupies the interior of the ST

TABLE 1. Number of conidia of *Sphaerotheca fuliginea* per field at different sedimentation times in the vacuum-operated settling tower

Time (sec)	No. conidia/19.6 mm^2
30	4.500 a[z]
60	7.500 b
120	9.464 c
180	10.321 c

[z]Values followed by the same letters do not differ according to Duncan's multiple range test, $P = 5\%$.

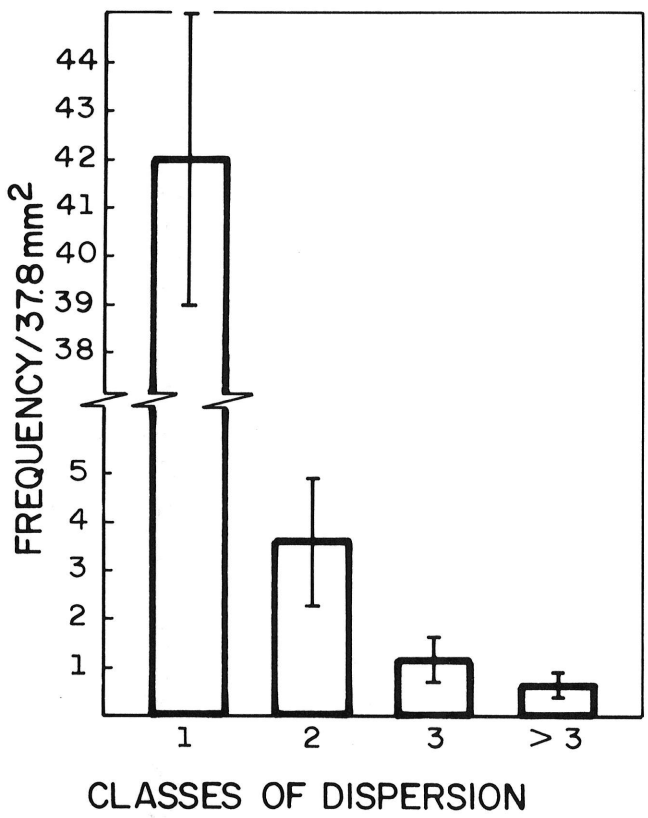

Fig. 2. Classes of dispersion of conidia of *Sphaerotheca fuliginea* as evaluated by frequency of 1, 2, 3, or >3 conidia per site. Vertical bars correspond to standard deviations.

when breaking the vacuum functions as a dispersal tool. This is thought to partially reproduce natural dispersal (3) in which suspensions and carriers may cause changes in the host-parasite relations (14,20).

This ST may become a useful tool for the detection of horizontal resistance where precise methods of inoculation are required (22). We consider that inoculation with dry conidia can be homogeneous and controllable, contrasting with Delon and Schiltz's statement that inoculation with dry spores is heterogeneous and inoculum density cannot be controlled (6). Thus, because this vacuum-operated ST is easy to construct and operate and uniformly distributes individual conidia, it is an alternative to the techniques employed for the inoculation of powdery mildews. It is now used in our pea (*Pisum sativum* L.) and melon (*Cucumis melo* L.) powdery mildew resistance breeding programs (1,2,18).

LITERATURE CITED

1. Beek, M. A., Giordano, L. de B., Rossi, P. E. F., and Oliveira, C. R. B. 1985. Método de seleção parcial de ervilha (*Pisum sativum* L.) para oídio (*Erysiphe pisi* D.C.) Fitopatol. Bras. 10:288.
2. Beek, M. A., Giordano, L. de B., Rossi, P. E. F., and Oliveira, C. R. B. 1985. Resistência parcial de ervilha (*Pisum sativum* L.) para oídio (*Erysiphe pisi* D.C.) Fitopatol. Bras. 10:292.
3. Bulit, J., and Lafon, R. 1978. Powdery mildew of vine. Page 536 in: The Powdery Mildews. D. M. Spencer, ed. Academic Press, New York.
4. Bushnell, W. R., and Rowell, J. B. 1967. Fluorochemical liquid as a carrier for spores of *Erysiphe graminis* and *Puccinia graminis*. Plant Dis. Rep. 51:447-448.
5. Corner, E. J. H. 1935. Observations on resistance to powdery mildews. New Phytol. 34:180-200.
6. Delon, R., and Schiltz, P. 1982. Emploi d'une technique rapide de laboratoire pour l'étude de la résistance à l'oidium (*E. cichoracearum*) chez *N. tabacum* et des effets de quelques substances chimiques sur le dévelopment du parasite. Pages 157-169 in: La Sélection des Plantes pour la Resistance aux Maladies. Institut National de la Recherche Agronomique, Paris.
7. Domsch, K. H. 1953. Über den enfluss photoperiodischer behandlung auf die befallsintensitat beim gerstenmehltau. Arch. Mikrobiol. 19:287-318.
8. Dutt, B. L., Rai, R. P., and Harikishore. 1973. Evaluation of reaction of potato to powdery mildew. Indian J. Agric. Sci. 43:1063-1066.
9. Hausman, E., and Slack, E. P. 1947. Physics. 2nd ed. D. Van Nostrand Co., New York. 756 pp.
10. Jhooty, J. S., and McKeen, W. E. 1965. Studies on powdery mildew of strawberry caused by *Sphaerotheca macularis*. Phytopathology 55:281-285.
11. Jones, I. T. 1975. The preconditioning effect of day-length and light intensity on adult plant resistance to powdery mildew in oats. Ann. Appl. Biol. 80:301-309.
12. Kilpatrick, R. A., Baenziger, P. S., and Moseman, J. G. 1981. Multiple inoculation technique for evaluating resistance of single barley seedlings to three fungi. Plant Dis. 65:504-506.
13. Lim, T. M. 1973. A rapid laboratory method of assessing susceptibility of *Hevea* clones to *Oidium heveae*. Exp. Agric. 9:275-279.
14. Lumbroso, E., Fischbeck, G., and Wahl, I. 1982. Infection of barley with conidial suspensions of *Erysiphe graminis* f. sp. *hordei*. Phytopathol. Z. 104:222-233.
15. Mignucci, J. S., Lim, S. M., and Hepperly, P. R. 1977. Effects of temperature on reactions of soybean seedlings to powdery mildew. Plant Dis. Rep. 61:122-124.
16. Moseman, J. G., Baenziger, P. S., and Kilpatrick, R. A. 1981. Genes conditioning resistance of *Hordeum spontaneum* to *Erysiphe graminis* f. sp. *hordei*. Crop Sci. 21:229-232.
17. Nair, K. R. S., and Ellingboe, A. H. 1962. A method of controlled inoculation with conidiospores of *Erysiphe graminis* var. *tritici*. Phytopathology 52:714.
18. Occhiena, E. M., Reifschneider, F. J. B., and Pessoa, H. B. S. V. 1985. Avaliação da resistência de introduções de melão (*Cucumis melo* L.) ao oídio (*Sphaerotheca fuliginea*) Fitopatol. Bras. 10:284.
19. Peries, O. S. 1962. Studies on strawberry mildew, caused by *Sphaerotheca macularis* (Wallr. ex Fries) Jaczewski. I. Biology of the fungus. Ann. Appl. Biol. 50:211-224.
20. Rapilly, F., and Foucault, B. 1976. Premieres études sur la retencion de spores fungiques par des épiderme foliaires. Ann. Phytopathol. 8:31-40.
21. Reeser, P. W., and Hagedorn, D. J. 1981. A method for quantitative inoculation with powdery mildew (*Erysiphe pisi*) of pea. Phytopathology 71:901.
22. Reeser, P. W., Hagedorn, D. J., and Rouse, D. I. 1983. Quantitative inoculations with *Erysiphe pisi* to assess variation of infection efficiency on peas. Phytopathology 73:1238-1240.
23. Reifschneider, F. J B., Boiteux, L. S., and Occhiena, E. M. 1985. Powdery mildew of melon (*Cucumis melo*) caused by *Sphaerotheca fuliginea* in Brazil. Plant Dis. 69:1069-1070.
24. Rowell, J. B., and Olien, C. R. 1957. Controlled inoculation of wheat seedlings with urediospores of *Puccinia graminis* var. *tritici*. Phytopathology 47:650-655.
25. Sakurai, H., and Hirata, K. 1959. Some observations on the relation between the penetration hypha and haustorium of barley powdery mildew and host cell. V. Influence of water spray on the pathogen and host tissue. Ann. Phytopathol. Soc. Jpn. 24:239-245.
26. Schein, R. D. 1964. Design, performance, and use of a quantitative inoculator. Phytopathology 54:509-513.
27. Shanmugasundaram, S., Williams, P. H., and Peterson, C. E. 1971. Inheritance of resistance to powdery mildew in cucumber. Phytopathology 61:1218-1221.
28. Sitterly, W. R. 1978. Powdery mildew of cucurbits. Page 369 in: The Powdery Mildews. D. M. Spencer, ed. Academic Press, New York.