# A Method for Uniform Infection of Seedling and Adult Cereal Plants by *Puccinia graminis* f. sp. *tritici*

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## ABSTRACT

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An inoculation chamber was constructed to inoculate seedling and adult plants of cereals uniformly with urediospores. Plants were rotated in one direction while revolving in the opposite direction in a cloud of spores of known concentration. Comparison of three liquid spore carriers and dry spores showed that an inert fluorochemical, FC-40, was the most satisfactory. Use of the inoculation chamber, the fluorochemical spore carrier, and standardized conditions for plant growth and incubation gave infection rates with standard deviations of 7–16% of the mean for seedlings and 10–36% of the mean for adult plants.

Detection and evaluation of plant characteristics that contribute to low disease incidence have required the development of techniques for uniform inoculation, incubation, and growth of plants (1,2,5,7,8,10). However, most techniques are developed for studying particular diseases on seedlings. In wheat stem rust, *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn., low receptivity and low spore productivity may be expressed only in adult plants. A new, precise method of inoculation suitable for adult plants as well as seedlings was required for the investigation of this disease.

## MATERIALS AND METHODS

A cylindrical settling tower, 91.5 cm in diameter and 152.5 cm high, was constructed of 24-gauge galvanized sheet metal (Fig. 1). The top and bottom of the cylinder were closed with tightly fitted panels of plastic-covered particle board (19 mm thick), and the chamber was mounted on three casters. Access to the chamber was through a side opening; the covering, a plexiglass sheet (3 mm thick), slid up and down in slots beside the opening.

A 75.5-cm diameter turntable (Fig. 2) made of 19-mm thick particle board and covered with galvanized sheet metal was mounted 28 cm from the bottom of the chamber and supported by a center shaft and three uniformly spaced peripheral supports on which ball bearings were mounted. A pulley (6.5-cm diameter) turned by a small electric motor and gear box (7 rpm) was connected by V-belt to a larger pulley (12.5-cm diameter) on the turntable center shaft, which turned four times a minute. The drive components were mounted on the bottom of the chamber under the turntable.

The pots containing plants to be inoculated are placed on 10 plates 11.4 cm in diameter mounted on uniformly spaced shafts 8 cm from the edge of the turntable (Fig. 1). The shafts pass through flanged bronze bushings in the turntable. Sprockets (No.25-19 teeth) are mounted on the shafts under the turntable and are driven by a chain that rotates the plates. An idler sprocket controls tension on the chain drive to the plates. Plate rotation is achieved by a chain drive from a second sprocket (No.35-24 teeth) on one plate shaft to a stationary sprocket (24 teeth) welded to the gear box and motor support (Fig. 2). The plates rotate in the opposite direction of the turntable but at the same speed.

Freshly harvested spores were introduced into the settling tower either dry or in liquid suspension. Dry spores were placed in the end of a piece of copper tubing that was bent at 90° so that the end pointed upward in the center of the chamber; the other end of the tubing extended through a hole in the side of the chamber where it was attached to the barrel of a Crosman carbon dioxide pistol (Crosman Arms Canada Ltd., Dunnville, Ontario) that dispersed the spores. The liquid spore suspensions were sprayed upward in a fine mist in the center of the chamber by a Paasche air brush, Type H (Passche Airbrush Co., Chicago, IL 60614), modified by replacing the ink cup with a 25-ml container (Fig. 1). About 97% of the spores sprayed into the chamber in Freon-113 settled to the bottom of the chamber in 5 min.

The liquids used to suspend the spores were FC-40 (3), Freon-113 (6), and Amsco odorless insecticide base oil (9).

Seedlings were grown in 1:4 soil/sand mixture in 10.2-cm pots. Pots 12.7 cm in diameter were used for adult plants. Seedlings were thinned to 12 plants per pot and adult plants to four. Plants were grown in growth cabinets (Controlled Environments Ltd., Winnipeg, Manitoba, R3H 0W9, Mod. P.G.W.-36) at 18 C and a 16-hr day at 18,300–22,500 lux at plant height. Plants were fertilized with 21-20-10 NPK as required. Seedling tests were performed when the primary leaf was fully developed. The emerging second leaf was cut off so that it did not interfere with spore deposition. Adult plants were trimmed to a single main tiller. The pustules that developed on the upper three leaves and stem internodes were counted separately and recorded as pustules per square centimeter of leaf surface or centimeter of stem internode.

Inoculated plants were incubated in the dark at 20 C for 16 hr in a dew chamber like that described by Clifford (4). After incubation, seedlings were placed in a growth cabinet at 18 C under caps of sisal glaze vinyl that were removed when the next dark period began. The caps slowed the drying of the leaf surfaces and increased infection.

## RESULTS

**Spore deposition**. The distribution and concentration of spores deposited on a flat surface in the inoculation chamber was determined by placing vaseline-coated microscope slides horizontally on alternate rotating disks on the turntable. Urediospores were counted in each of 30 microscope fields (×100, 0.3564 $cm^2$ per field) on each of the five slides exposed to each treatment. Spore counts were adjusted for differences in the

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number of spores dispersed in the chamber by calculating the correction factor:

$$\frac{\text{spores counted per cm}^2 \text{ of slide area}}{\text{spores used for inoculation}} \times 10^6$$

and applying this factor to the mean number of spores counted per square centimeter for each treatment.

The number of urediospores used for each treatment was determined by counting the spores in the suspensions with a haemocytometer. Ten milligrams of spores were used for the dry spore application or suspended in 10 ml of Freon-113, but only 5 mg were suspended in 10 ml of FC-40 and mineral oil. Because Freon-113 is very volatile, it was necessary to evaporate it in a measured amount of suspension and then resuspend the spores in the same quantity of mineral oil in order to count them with a haemocytometer. The number of dry urediospores used was determined by suspending weighed quantities of spores in mineral oil. The spores counted were not, therefore, a measured portion of the spores used in the inoculation chamber but were a second sample of the same spore lot.

The corrected values for the numbers of spores counted for the liquid suspensions were similar (Table 1). The standard deviations were small; Freon-113 had the smallest. More dry spores than liquid-suspended spores were deposited on the slides. Dry spores had the highest standard deviation, and in addition, there were many clumps in the dry spores (6.1% of the spores counted were associated with other spores). FC-40 and mineral oil were intermediate (2.5%), and no clumping occurred with Freon-113. We concluded that the liquid spore suspensions, especially in Freon-113, were superior to dry spores for uniform spore deposition.

**Spore infectivity**. The number of pustules produced per square centimeter on seedling leaves by a standardized quantity of urediospores was determined about 12 days after inoculation by counting the number of pustules on each leaf, measuring the length of each leaf, multiplying by the average leaf-width, and dividing the number of pustules by the area (number of square centimeters). The same correction factor used for the spores deposited on slides was used to compensate for differences in the size and weight of spores collected at different times and for spores of different physiologic races.

Inoculation trials were done with 3 mg of urediospores of race C33 (15B-1L) and four methods of suspension in: (i) FC-40, (ii) Freon-113, (iii) mineral oil, and (iv) dispersed dry in the inoculation chamber. Fresh spore suspensions in FC-40 and Freon-113 produced about the same number of pustules on seedlings of Little Club wheat (Table 2). Dry spores produced considerably more infections, and spores in mineral oil produced considerably fewer, than did the first two methods. The infectivity of spores suspended in mineral oil and of dry spores was essentially unchanged after 26 hr, although the number of infections from dry spores decreased insignificantly after 2.5 hr. After 2.5 hr in Freon-113, spores produced about half as many infections and the level of infectivity did not change in the next 23.5 hr. The number of infections produced by spores in FC-40 increased sharply after 2.5 hr in suspension; 23.5 hr later more than twice as many infections were produced. The harmful effect of Freon-113 and mineral oil was observed previously (6,9), but the reason for the increased number of infections produced by urediospores suspended in FC-40 for 2.5 and 26 hr is not clear. Apparently, the chemical does not stimulate spore germination, which was already high. It seems more likely that it favorably affects other aspects of the infection process.

**Variation in numbers of infections**. FC-40 was the best spore carrier. Spores suspended in it produced many infections

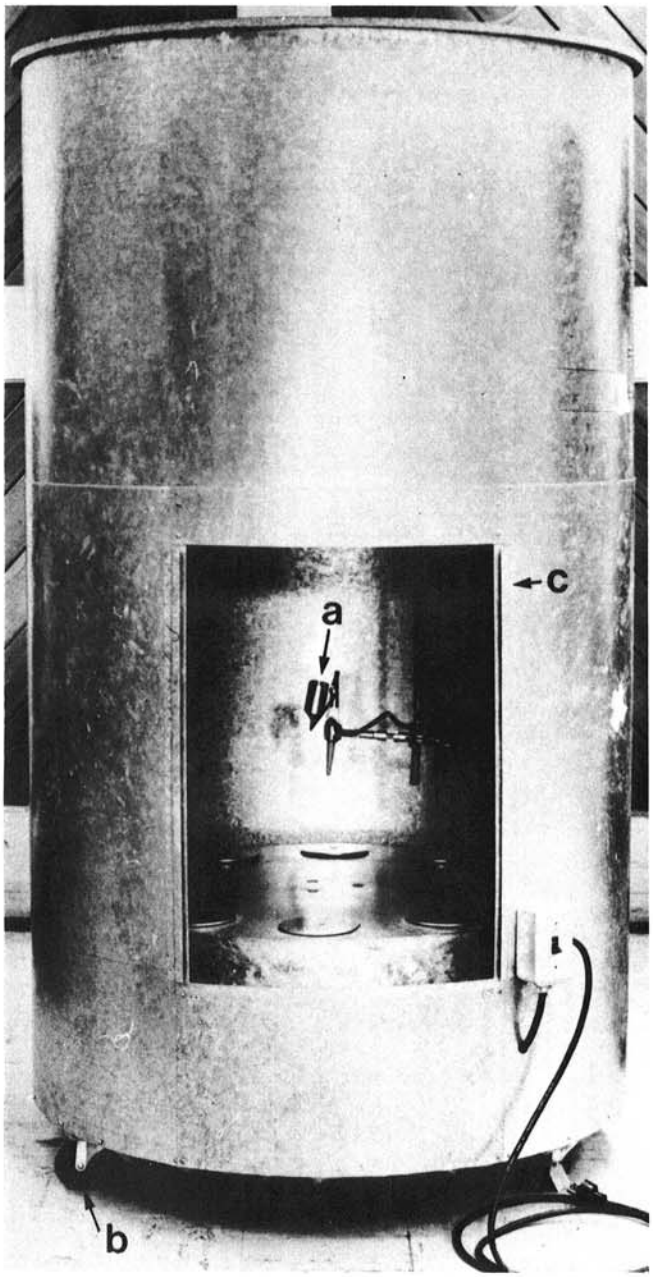


**Fig. 1**. Settling tower with plates mounted on turntable. **a**, modified air-brush; **b**, casters; and **c**, slots for plexiglass sheet.

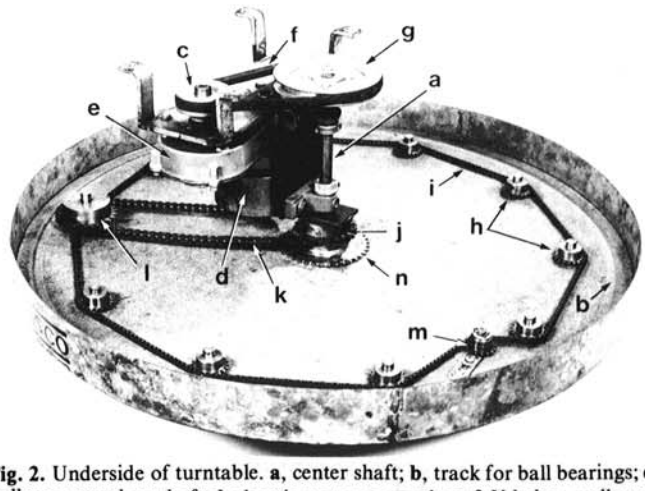


**Fig. 2.** Underside of turntable. **a**, center shaft; **b**, track for ball bearings; **c**, pulley on gear box shaft; **d**, electric motor; **e**, gear box; **f**, V-belt; **g**, pulley on center shaft; **h**, sprockets on plate shafts; **i**, chain that rotates plates; **j**, stationary sprocket welded to gear box and motor support; **k**, drive chain; **l**, second sprocket on plate shaft; **m**, idler sprocket; and **n**, platform support plate.

consistently over a 2-yr period and it was less volatile than Freon-113, permitting spore counts of the same suspensions used for inoculation.

Four experiments with race C17 (56) suspended in FC-40 were done to determine the variation in the numbers of infections (i) on each seedling in the same pot, (ii) on pots of 12 seedlings comprising a replicate, and (iii) on all seedlings in experiments done at different times. Four experiments with Little Club wheat, consisting of four replications of 12 seedlings, were performed on 20 January (Exp. I), 22 January (Exps. II and III), and 13 February (Exp. IV), 1975. A high inoculum concentration ($6.7 \times 10^6$ spores) was used in the first three experiments and a low concentration ($0.8 \times 10^6$s spores) in the last experiment.

The numbers of pustules on seedlings in the same pot varied between 30 and 70% of the mean for the 12 seedlings. The large variance may have been caused by differences in the position and shape of leaves in the same pot. The standard deviations for replicates of the same experiment varied from about 7 to 16% of the mean (Table 3). The standard deviation for the experiments performed with high inoculum concentrations was 15% of the mean. When the experiment with low inoculum concentration was included with the other three experiments, however, the standard deviation was 28% of the mean. The increased variance was caused by the relatively large number of pustules (Table 3) at the low concentration of inoculum, although the number of pustules actually counted was small. The large number of pustules may have resulted from reduced competition between pustules or from more accurate counting of the smaller number of infections. When 9–13 pustules are counted per square centimeter and single infections are distinguishable with difficulty, the error would be expected to be larger than when two to three pustules per square centimeter are counted.

The variance was greater when six races (C33 [15B-1L], C33 [15B-1L] orange, C17 [56], C45 [56A], C10 [15B-1], C50 [15B-5]) of *P. graminis* were used separately to inoculate adult plants of Little Club. Inoculum consisted of 3 mg of urediospores, ranging from $0.9 \times 10^6$ to $1.1 \times 10^6$ spores per inoculation, for each race. The distribution of pustules was more uniform on stem internodes than on leaves. Greater numbers of pustules developed on the flag leaves than on the two leaves below the flag leaves. The number of pustules on plants in the same pot varied between 15 and 65% of the mean for the four plants. The standard deviations for the number of pustules per centimeter of stem internode and per square centimeter of leaf area ranged from 9 to 36% of the corrected mean for three replicates, each consisting of four plants in one pot. The corrected mean for all races was $0.9 \pm 0.1$ pustules per centimeter of stem internode and $1.8 \pm 0.2$ pustules per square centimeter of leaf. The standard deviation for the six races was 23.1% of the corrected mean of all pustules per centimeter of stem internode and 20.6% of the corrected mean of all pustules per square centimeter of leaf. The standard deviations among replicates were, in most cases, as large as or larger than those among races, indicating that no significant difference in infectivity of races could be detected and that the spore carrier, FC-40, could not have differentially affected the races of stem rust.

## DISCUSSION

When standardized amounts of inoculum are used, consistency in numbers of infections is the essential requirement of any method of inoculation used to compare host cultivars for receptivity or pathogen races for infectivity. Our results demonstrate that an inoculation method in which plants revolve and rotate through a mist or air suspension of spores produces uniform spore deposition on both seedling and adult plants.

The standard deviations obtained in both adult plant and seedling experiments are small. To obtain this degree of uniformity, however, especially for different inoculations, it is important that

TABLE 1. Number of spores of *Puccinia graminis* f. sp. *tritici* per square centimeter on five vaseline-coated slides placed horizontally on the turntable of the inoculation chamber when four methods were used to disperse spores in the chamber

| | Dispersal method | | | |
|---|---|---|---|---|
| Slide | FC-40[a] | Freon-113 | Oil | Dry spores |
| 1 | 120.7 | 303.0 | 154.3 | 384.4 |
| 2 | 95.4 | 275.0 | 162.7 | 406.9 |
| 3 | 103.8 | 252.5 | 126.3 | 415.3 |
| 4 | 115.0 | 280.6 | 126.3 | 275.0 |
| 5 | 123.5 | 300.2 | 134.7 | 333.9 |
| Total | 558.4 | 1,411.3 | 704.3 | 1,815.5 |
| Mean ± SE | 111.7 ± 5.3 | 282.3 ± 9.2 | 140.9 ± 7.5 | 363.1 ± 26.2 |
| Number of spores used $\times 10^{-6}$ | 1.7063 | 3.5750 | 1.8467 | 3.5750 |
| Corrected mean (spores/cm$^2$) | 65.5 ± 3.1 | 79.0 ± 2.6 | 76.3 ± 4.1 | 101.6 ± 7.3 |
| Standard deviation (percent of mean) | 10.6 | 7.3 | 11.9 | 16.1 |

[a] Fluoroinert chemical from 3M Company, St. Paul, MN.

TABLE 2. Mean number[a] of pustules of *Puccinia graminis* f. sp. *tritici* per square centimeter of seedling[b] leaf 0, 2.5, and 26 hr after uniform inoculation with race C33 (15B-1L)

| | Hours urediospores were suspended | | |
|---|---|---|---|
| Spore carrier [c] | 0 | 2.5 | 26 |
| FC-40[d] | 7.1 ± 0.3 | 9.7 ± 0.9 | 17.3 ± 1.1 |
| Freon-113 | 7.3 ± 0.8 | 3.1 ± 0.6 | 2.6 ± 0.1 |
| Mineral oil | 3.8 ± 0.4 | 4.4 ± 0.6 | 4.2 ± 0.3 |
| Dry spores | 11.4 ± 0.9 | 9.4 ± 0.7 | 8.9 ± 0.4 |

[a] Corrected mean ± the standard error of the corrected mean.
[b] Three pots contained 36 seedlings of the cultivar Little Club.
[c] Urediospores suspended in FC-40, in Freon-113, and mineral oil. Dry urediospores were stored in test tubes in the laboratory.
[d] Fluoroinert chemical from 3M Company, St. Paul, MN.

TABLE 3. Variation in the number of pustules of *Puccinia graminis* f. sp. *tritici* per square centimeter on seedlings of Little Club inoculated with 5 mg of urediospores of race C17 (56) suspended in 10 ml of FC-40, expressed as the standard deviation for replicates in percent of the experiment mean, and the mean corrected for differences in inoculum concentration

| Experiment | Standard deviation for replicates | Mean | Corrected mean |
|---|---|---|---|
| I | 7.3 | 13.01 ± .48 | 1.93 ± .07 |
| II | 16.4 | 9.83 ± .80 | 1.46 ± .12 |
| III | 12.9 | 12.86 ± .82 | 1.92 ± .12 |
| IV | 13.0 | 2.45 ± .14 | 2.82 ± .16 |

inoculum concentrations are uniform, that the inoculum is from the same source, that dew formation during incubation is uniform, and that plants are grown under uniform conditions of light and temperature before and after inoculation.

The level and consistency of infection varies with the method of introducing spores into the inoculation chamber. In our tests the fluoroinert chemical FC-40 routinely produced a high level of infection. The other liquid spore carriers also produced consistent results but were not as suitable as FC-40. Consistent results can therefore be obtained, but the method must be carefully standardized and executed.

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