Performance of a Cyclone Spore Trap

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ABSTRACT

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A multipurpose, volumetric, cyclone spore trap was designed and evaluated under controlled conditions and in the field. The trap detected spores in small concentrations because of its high sampling rate of 340–450 L/min and efficient spore separating ability. For the majority of dry spores tested, retention by the inlet walls of the trap did not exceed 1.6% except with ascospores of *Monilinia fructicola* which were impacted in the trap volute and had to be recovered by rinsing. Losses in spore viability during operation were prevented by collecting spores directly in water, by reducing sampling rate to 340 L/min, and by limiting the period of operation to 30 min. The cyclone spore trap is suitable for atmospheric sampling in the field or from aircraft or automobiles and can be used in wind tunnel studies.

Additional key words: air sampling, spore collector.

The cyclone principle used in industry for removing dust particles from atmospheres lends itself to efficient collection of biological particles such as pollen and fungal spores. Miniature cyclone traps have a high atmospheric sampling rate, compared with impaction spore traps, and high separation efficiency (12). This makes them very suitable for bulk collection of pollen or fungal spores from herbage (2,16,19,20). Since spores are separated in the absence of sticky surfaces or filters, they are immediately ready for viability testing, dry inoculation, or other studies. Miniature cyclone traps also are suitable for atmospheric spore sampling at stationary sites (4,12) and with their high sampling rates are adaptable for use in miniature wind tunnels in spore-release studies (15) or for high-speed atmospheric sampling from aircraft. They are potentially capable of detecting minute concentrations of biological particles in the atmosphere when used with a suitable culture medium (4,12). A cyclone trap designed for bacterial aerosol sampling in the field has been described (4). The objective of this investigation was to further develop the cyclone trap method started by Ogawa and English (12) into an efficient multipurpose volumetric spore trap designed for fungal spores and pollen.

MATERIALS AND METHODS

The cyclone spore trap (Fig. 1) was designed to efficiently separate spores of 3–5 μm or larger diameter (3,8,11,12,17). Early models were constructed at the University of California, Davis, in two sections with brass volute and brass or glass cone. The later model (Fig. 1A–C) manufactured by Richards Engineering Ltd., 7 Coventry St., Levin, New Zealand, was made from anodized aluminum with interchangeable nozzels for different applications and a push-fit plastic collection vial attached to a removable cone. In collection efficiency tests, a standard nozzle of 56 mm diameter was used to provide isokinetic sampling at 340 L/min in a 2.3 m/sec air stream. Air was drawn through the trap by a centrifugal air blower-cleaner or household vacuum cleaner connected to the outlet tube. Different sampling rates were obtained by varying air flow rates with a variable transformer. A stand (Fig. 1C) with wind vane, rain shield, and suction fan powered from a portable generator was built for field use.

The technique used to measure the air flow rate through the cyclone was designed to eliminate any effect of the measurement equipment on the flow rate during normal operation of the unit. A larger centrifugal type blower was substituted for the calibration procedure. The cyclone was connected to the inlet of the blower and a metering tube was fitted to the outlet. The flow rate was varied in selected steps with a variable voltage controller input to the blower. The basic calibration of the flow rate through the cyclone was determined by measuring the cyclone pressure in a straight section of pipe between the cyclone and the blower, and the associated flow rate through the metering tube. Finally, the original blower was connected to the cyclone but without the metering tube. The cyclone pressure was then measured and the flow rate taken from the calibration curve.

The metering tube was constructed according to the American Society of Mechanical Engineers (ASME) standards (1) with 3.81-cm (1 ½-in.) tubing and pressure taps one diameter (1 D) and 0.5 D from the sharp edge orifice. The upstream length of straight tubing was 25 D and downstream length was 5 D, which are recognized as minimum lengths for uniform flow. Flow coefficients were

TABLE 1. Separation efficiency of the cyclone spore trap

Test spores	Sampling rate (L/min)	Separation of spores (%)	Spores not separated
Lycopodium clavatum spores (32 μm diameter)	170	100.6 ± 1.2	...
	340	99.7 ± 1.2	...
	520	99.5 ± 1.2	...
Monilinia fructicola conidia (10 × 15 μm)	100	99.9	1 in 935
	250	99.9	1 in 1,885
Rhizopus stolonifer sporangiospores (9 × 12 μm)	200	100.0	1 in 2,414
	340	100.0	1 in 6,482
Penicillium sp. conidia (3 × 4 μm)	340	99.9	1 in 4,156

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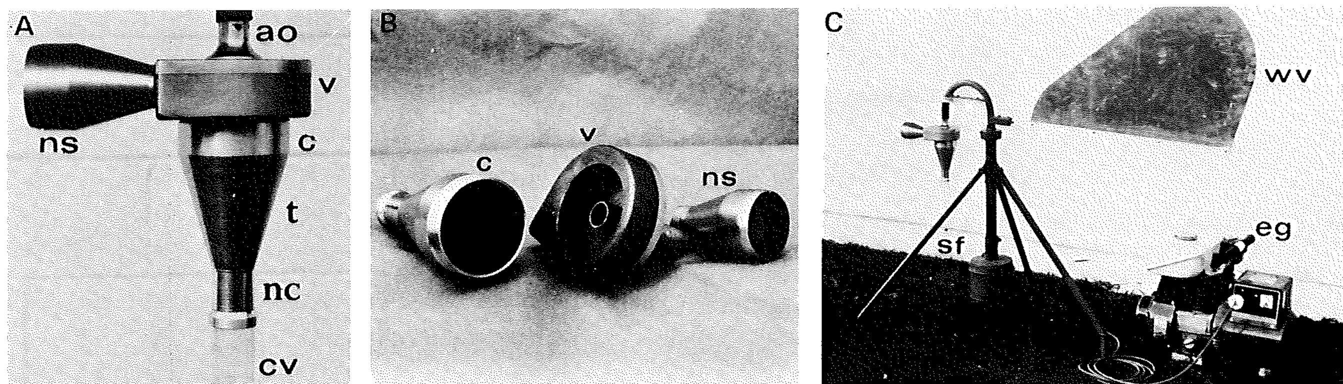

Fig. 1. A, Assembled cyclone spore trap. B, Dismantled cyclone spore trap. C, Prototype of field sampling stand with power unit. Critical dimensions (internal, diameters): air outlet (ao) = 19 mm, volute entry (v) = 90 mm, cone (cylindrical part) (c) = 74 mm, neck of cone (nc) = 16 mm, and collection vial (cv) = 29 mm. Other internal dimensions: length of transitional part of cone (t) = 105 mm, height of cylindrical part of cone (c) = 55 mm, and projection of outlet tube (ao) into cyclindrical part of cone (v and c) = 20 mm. ns = nozzle, sf = suction fan, wv = wind vane, eg = electric generator.

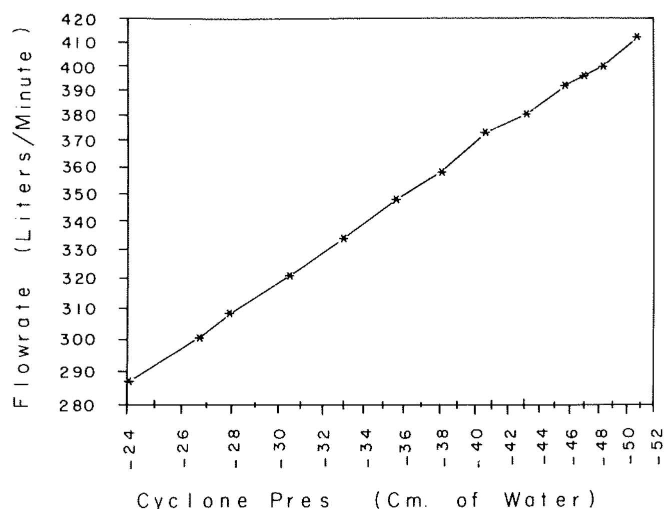

Fig. 2. Calibration curve relating the flow rate to various pressure values on the cyclone.

obtained from the ASME reference (1).

Fig. 2 illustrates the basic calibration curve relating the flow rate to various pressure values on the cyclone. The original Cadillac blower was attached to the cyclone and produced a pressure reading of −47 cm water at a line voltage of 115 V. Thus, from the calibration curve this pressure represents a flow of 390 L/min.

Spores of the following species were used to determine performance of the spore trap: ascospores and conidia of *Monilinia fructicola* (Wint.) Honey, *Cladosporium fulvum* Cke., *Penicillium* sp., and *Alternaria alternata* (Fr.) Keissl.; sporangiospores of *Rhizopus stolonifer* (Fr.) Lind; pollen of *Pinus radiata;* and spores of the club-moss *Lycopodium clavatum*. Before each test the cyclone spore trap interior was surface-sterilized by rinsing with 70 or 95% ethyl alcohol. Where spore weights were required they were first dried in weighing bottles in a desiccator with anhydrous calcium chloride granules at 22 C for 30 min and reweighed immediately after collection. Weighing accuracy was ±0.5 mg.

The number of viable fungal spores trapped by the cyclone trap in different tests was determined as follows: Spores were washed from each cyclone section into a container with 10 ml of sterile distilled water plus nonionic wetting agent (alkylaryl polyglycol ether) at 0.03%, v/v. The spore concentration was estimated with a haemocytometer and adjusted when necessary by dilution to 100 spores per milliliter. Three 0.5-ml samples of this suspension were then spread evenly on three petri plates of either Monilinia selective medium (MSM) (14) for *M. fructicola*, or potato dextrose agar (PDA) for *R. stolonifer* and *Penicillium* sp. On MSM, colonies

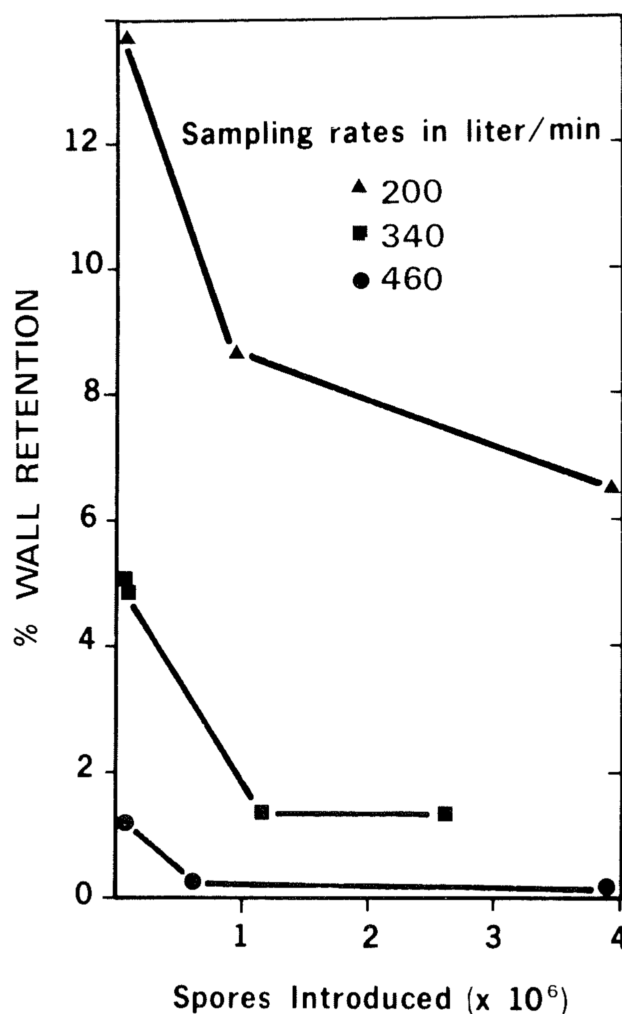

Fig. 3. Effect of *Rhizopus stolonifer* sporangiospore load and sampling rates during 5-sec runs on internal wall retention of spores in the cyclone spore trap.

from germinating spores were counted after 5 days' incubation at 24 C and on PDA within 1 day using the low power objective of a microscope. It was assumed that each colony represented a single spore since mycelial fragments were absent from haemocytometer preparations and care was taken to keep all spores in suspension by

frequent shaking. Results for each test were the means of five or more repeated samplings.

Collection efficiency tests. Collection efficiency of the cyclone spore trap is defined as the ratio of spores caught per unit volume of air to the absolute number of spores per unit volume of sampled air. Alternately, it is the percentage of spores per cubic meter of air caught by the trap (7).

Collection efficiency was determined by comparing the cyclone system with other traps of known performance in a wind tunnel modeled after Gregory (5) to produce continuous spore clouds under turbulent airflow conditions. The tunnel was installed inside one glasshouse section and exhausted into still air in an adjacent section. Wind speed was controlled by a variable transformer and monitored by a thermoanemometer at 1.5, 3.5, 5.5, and 7.5 m/sec. Clouds of *Lycopodium clavatum* spores of 1 min duration were produced as described by Gregory (5) 1.4 m upwind of the spore trap position in the tunnel. Because the weights of successive spore clouds were not identical, all catches were initially corrected for the mean weight (0.015 g) of spores released.

To determine the absolute spore concentration passing the trapping position, six 1-min runs were made at each wind speed with sticky-surfaced glass rods (6 mm total diameter) in position. These runs were repeated with the cyclone spore trap in position, sampling at 340 L/min, with a 56-mm diameter nozzle to provide isokinetic sampling at 2.3 m/sec. Percent collection efficiency at each wind speed was calculated as mean spores per liter determined by the cyclone spore trap divided by mean spores per liter for the glass rod. Since glass rods tend to be inefficient at low wind speeds, the result was checked at 1.5 m/sec using a Cascade Impactor (C. F. Casella and Company Ltd., Regent House, Fitzroy Square, London. W. I.) with the fourth jet removed.

Total glass rod catch for each run was estimated from five equally spaced microscope traverses (1.25 mm wide) along 56 mm of rod length, equal to cyclone spore trap nozzle diameter. This was corrected for the estimated rod efficiency (5) and converted to spores per liter of air. Total Cascade Impactor catch per run was determined by counting the spores caught on glass slips (collecting surfaces) numbered 1, 2, and 3. The total was corrected for the estimated intake efficiency of the Cascade Impactor from instruction leaflet 3018/TE. The catch for each cyclone spore trap run was removed by rinsing the cone and collection vial with 4 ml water and the counts of suspended spores in six or eight haemocytometer slide preparations were averaged.

Comparisons with standard spore traps. Comparisons of the cyclone spore trap with the Cascade Impactor and Hirst spore traps (Burkard Manufacturing Company, Ltd., Rickmansworth, Herts., England) were made in a controlled environment and in the field. In a 2-m wide × 3-m high passageway, air streams of 0.5 or 3 m/sec (±0.5) were produced by manipulating ventilation fan speeds. The cyclone spore trap and Cascade Impactor trap were operated side by side 1.4 m downwind of a 1-m-high stand supporting a sporulating peach brown rot (*M. fructicola*) mummy. Distance between the trap intake nozzle and nozzle heights above floor level was 0.6 m. A small fan was aimed at the spore source to ensure spores were released into the airstream passing the spore traps. The Cascade Impactor with the fourth jet removed was operated at 17.5 L/min and the cyclone at 340 or 450 L/min with 15 ml tap water in the collection vial. Six 1-min trapping runs were made (each with a fresh spore source) for each wind speed and cyclone sampling rate. After each run, total spore catches were counted for both traps and converted to spores/m^3. Corrections were not made for collection efficiencies of the traps. The Cascade Impactor spore catches were counted by microscope and the cyclone spore trap catches were determined by plating samples of the spore suspension onto MSM and counting the resulting colonies.

In a field planting of Golden Queen peach trees (New Zealand) with brown rot disease (caused by *Monilinia fructicola*), a cyclone spore trap was operated alongside a Hirst spore trap for 20-min periods during which one to several passes were made along adjacent rows spraying with a water-filled airblast sprayer to produce spore clouds. Apart from momentary winds created by the sprayer, conditions were calm for all runs. The cyclone spore trap was operated at 340 L/min with 15 ml water in the collecting vial; the Hirst spore trap at 10 L/min with a stationary, petrolatum-coated slide. Spores/m^3 estimated by each trap were calculated as above.

RESULTS

Separation efficiency of cyclone spore trap. A known weight of *L. clavatum* spores was drawn directly from the weighing bottle into the cyclone spore trap and the fraction caught in the vial was determined. In a sensitivity test, two cyclone spore traps were connected in tandem to collect a quantity of freshly produced

TABLE 2. Retention of spores by internal parts of cyclone spore

Test spores	Spore loads ($\times 10^6$)	Sampling rate (L/min)	% Total catch retained by:			
			Nozzle	Volute	Cone	Vial
Monilinia fructicola						
conidia (10 × 15 μm)[a]	1.05	150	···	2.5	1.1	96.4
	5.73	320	···	0.1	0.1	99.8
Rhizopus stolonifer						
sporangiospores (9 × 12 μm)[a]	0.05	200	···	0.7	9.0	90.3
	3.90	340	···	0.1	0.3	99.6
		450	···	0.1	0.3	99.6
Monilinia fructicola						
ascospores (10 × 6 μm)[b]	0.184	340	0.3	99.6	0.1	0.0
conidia[b]	0.756	340	0.1	0.0	0.1	99.8
Penicillium sp.						
conidia (3 × 4 μm)[b]	0.647	340	0.1	0.2	1.3	98.5
Rhizopus stolonifer	1.990	340	0.0	0.0	0.1	99.9
Lycopodium clavatum						
spores (32 μm diameter)[b]	0.056	340	0.0	0.0	0.0	100.0
Pinus radiata						
pollen (60 × 40 μm)[b]	0.038	340	0.0	0.0	0.0	100.0

[a]Viable spores counted by plating method. Older cyclone with fixed 19-mm nozzle.
[b]Total spores estimated with haemocytometer. Cyclone with detachable 56-mm nozzle.

fungal spores drawn into the system for 3–5 sec periods. The total number of viable spores retained by the cone plus those contained in the vial of each trap was determined. Separation efficiency was calculated as mean spores retained by first trap divided by mean spores retained by both cyclones.

Separation efficiency in both methods generally exceeded 99% for spore sizes 3–32 μm diameter at 100–500 L/min sampling rate (Table 1). Spore loads in these tests ranged from 1×10^5 (*R. stolonifer*) to 7.36×10^6 (*Penicillium* spores).

Retention of spores on internal walls. Freshly produced fungal spores were drawn into a single cyclone spore trap (19-mm nozzle) at different rates for 3–5 sec periods. The mean number of viable spores recovered from each section was determined. In further tests, airborne spores were collected for 20 sec by the cyclone (56-mm nozzle) containing 15 ml water in the vial and sampling at 340 L/min. With a wire loop 0.1-ml volumes of water were moved over all internal surfaces of the nozzle, volute, and cone to collect impacted spores, then removed to a haemocytometer to determine spore catch by each wall portion. Total vial catch was also estimated. Percent volute retention was calculated as spores retained by volute divided by spores retained by volute, cone, and vial.

Percentage of spores retained by internal wall parts decreased as sampling rate or spore load increased (Table 2 and Fig. 3, respectively) within a spore load range of 3.8×10^4 to 3.9×10^6. At 340 L/min in the second test, spore loads between 3.8×10^4 and 2×10^6 resulted in minimal wall retention for all spore types except *M. fructicola* ascospores, which were mostly impacted in the volute (Table 2). In additional tests, very low conidial loads (40–986 spores) of *M. fructicola* resulted in variable wall retentions ranging from 25 to 80%.

Spore viability in collection vial during operation. Spores from pure culture or diseased plant material were drawn into a single cyclone spore trap with or without 20 ml tap water added to the vial. At varying intervals the fan was stopped and a sample of spores was taken from the vial base with a fresh cotton wool swab or wire loop and streaked onto PDA. After 4–6 hr incubation at 24–25 C, the percent germination of three 100-spore counts was recorded.

Spores of five species of fungi representing the range from those easily killed (*Monilinia*) to rather robust spores (*Rhizopus*) lost viability after they were trapped in a dry collection vial. A selection of results is given in Table 3. Loss of viability was most rapid for delicate spores (particularly those freshly produced) and at higher air sampling rates. In one test, viability of *Monilinia conidia* was reduced in 10 sec. Viabililty of the most delicate spores was largely maintained, even at high sampling rates, by collecting directly into water.

TABLE 3. Effect of water and operating time of cyclone spore trap at 340 L/min sampling rate[a] on viability of trapped spores

Fungal species	Collection viable condition	Percent spore germination at the operating periods of: Seconds 0	10	60	Hours 1	6
Monilinia fructicola conidia	dry[b]	100	19	12		
	dry	94		85	57	
	wet	100			98	93
Cladosporium fulvum conidia	dry	81	59	67		
	wet	88		93		70
Rhizopus stolonifer sporangiospores	dry	98	86	92	93	92
Alternaria alternata conidia	dry	96	97	90		

[a]At higher sampling rates, viability was reduced below that presented above.
[b]These were freshly produced spores. In other runs, spores had aged for several days.

Collection efficiency in wind tunnel tests. Cyclone spore trap collection efficiencies for spores of *Lycopodium clavatum* were compared to data obtained with glass rods and the Cascade Impactor (Fig. 4). Close agreement in collection efficiency between the Cascade Impactor and glass rod at the external wind speed of 1.5 m/sec was shown. Variability of results using the glass rod was relatively high at different wind speeds. The cyclone spore trap overestimates of atmospheric spore concentrations were higher by almost 200 percent between 1.5–5.5 m/sec external wind speed.

Comparisons with standard spore traps. The cyclone spore trap-MSM combination consistently estimated higher spore concentrations than the Cascade Impactor in the artificial airstream tests or the Hirst spore trap in the field (Table 4). With the cyclone spore trap, this difference increased with wind speed. In eight of the 32 runs, spores were detected by the cyclone spore trap but not by the slit impactor traps.

DISCUSSION

The cyclone spore trap combines higher efficiency with more flexibility of application compared with other spore traps. Separation efficiency of spores exceeded 99.5% at 340 L/min for

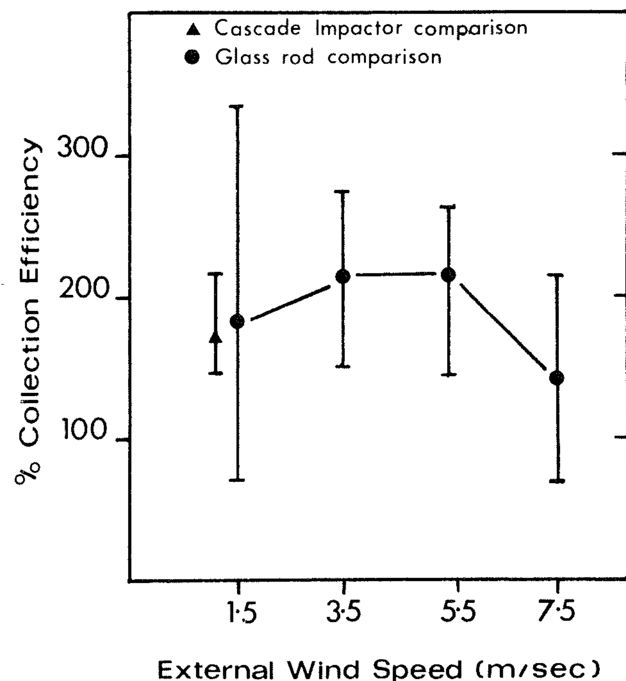

Fig. 4. Collection efficiency of the cyclone spore trap for *Lycopodium clavatum* spores sampled at 340 L/min and determined by comparison with glass rods and Cascade Impactor.

TABLE 4. Comparisons in efficiencies of cyclone spore trap with Cascade Impactor and Hirst spore traps in controlled environment and field

Cyclone compared with:	Wind speed (m/sec)	Cyclone sampling rate (L/min)	Ratio of cyclone/Cascade or Hirst for spores/m^3/min[a] (means of 6–8 runs)
		Controlled environment	
Cascade impactor	0.5	340	8:1
		460	6:1
	3.0	340	25:1
		460	174:1
		Field operation	
Hirst	0[b]	340	6:1

[a]Spore estimates not corrected for collection efficiency of respective traps.
[b]Calm conditions except during passage of air blast sprayer.

spores 3–32 μm diameter. This was slightly higher than that found by Ogawa and English (12) working with a similar cyclone. The spores tested here represent the medium to lower range of fungal spore sizes encountered in the atmosphere. For larger spores, such as pollen grains and certain *Alternaria* or *Helminthosporium* conidia, similar efficiencies would be expected.

Wall losses for most spores were less than 1% at sampling rates above 200 L/min provided that the cone contents were washed into the collection vial and spore numbers were not too low. The decrease in wall losses with increased spore load in the incoming air was attributed to the more efficient sweeping action of spore-laden air spiraling down the surface of the cone. A similar increase in efficiency with spore load was found by Mehta and Zadoks (10) and Teng and Close (18). Though this sweeping action was greatly reduced at very low spore numbers it could be improved by increasing the sampling rate. In addition, longer sampling periods could decrease wall losses due to the sweeping action of pollen, other spores, and dust particles in the incoming air.

The high retention of *M. fructicola* ascospores by the volute walls was unexpected. These spores either have a sticky surface or are electrostatically attracted to the volute walls. This phenomenon may explain a previous failure of the cyclone spore trap to detect ascospores of *M. fructicola* in the field. Volute contents can be rinsed out and counted, but spore viability might be reduced after normal sampling periods. It is not known whether ascospores of other species behave similarly. Tests would be necessary to establish this before field sampling commenced.

Another unexpected phenomenon was the rapid mortality of delicate spores in the collection vial. This could explain why Ogawa and English (12) failed to detect *M. fructicola* conidia in orchard spore-trapping runs. Spore mortalilty was virtually eliminated by the addition of water to the collection vials and by redesigning the vial attachment and cone neck so that the latter was constantly splashed by the vortex of water created by the spiralling airstream. In previous models, impaction of spores about the cone and collection vial (out of reach of the water) had been a problem. If dry inoculation with delicate spores was intended, then collection time with the cyclone spore trap should not exceed more than a few seconds to avoid spore mortality. However, this time was sufficient to collect very large numbers of spores from sporulating peach mummies infected by *M. fructicola.* It was observed during spore trapping runs that 10–15 ml of water in the collection vial was necessary for maximum "washing" of the cone neck area. Since the rate of evaporation at 360 L/min was approximately 9 ml/hr, 20–30 min was the maximum time the trap could be operated before changing collection vials. Such durations did not affect the viability of delicate spores unless they were very fresh. The use of water to preserve microbe viability was also incorporated into the cyclone separator designed by Errington and Powell (4) for bacterial aerosols.

At the low wind speed of 1.5 m/sec, collection efficiency for the cyclone spore trap using the Cascade Impactor supported results obtained with the glass rods. The total curve resembled a mirror image of that obtained for the Hirst spore trap (7), with efficiencies approaching 100% at zero wind speed and 9 m/sec but much higher in between. This pattern follows the expected curve based on physical aspects of sampling (9) at 1.5 and 3.5 m/sec but departs from the expected at higher wind speeds. The large nozzle diameter and high sampling rate may explain the generally high efficiencies obtained. Nozzle impaction according to May (9) is least for small spores or large nozzles. In practice, high sampling rates became an advantage when attempting to detect low numbers of airborne spores.

In less controlled comparisons, the cyclone spore trap was more sensitive to low spore concentrations than the Cascade Impactor and Hirst spore traps. The high ratios obtained partly reflect the absence of corrections for collection efficiency. The increase in ratios with increased wind speed (0.5–3.0 m/sec) tends to support the collection efficiency results obtained at similar wind speeds with Lycopodium spores. The high sensitivity of the cyclone was unexpected because of its reduced efficiency at very low spore numbers, but can be explained by the increased "air washing" of the internal cyclone walls that occur when it is run for longer than the 3–5 sec periods of the wall retention tests, and the high sensitivity of the MSM plating method in detecting viable condidia. Thus, during 30-min spore trapping runs or brief spore-pollen collections from foliage, wall losses are probably insignificant for most spores. The presence of dust particles and the difficulty of detecting transparent conidia on Vaseline-coated slides could have led to underestimates by the slit impactor traps in these runs. These difficulties emphasize the usefulness of the cyclone-selective medium combination in detecting and identifying low concentrations of culturable spores.

The cyclone spore trap was also suitable for high speed air sampling by aircraft and automobile. Large volumes of air were screened with no problems of overloading, and many common airborne fungi (6) were detected in test runs. High effective sampling rates are possible in airplane sampling by increasing nozzle diameter and relying on particle momentum for their capture from airstreams into the nozzle. To provide the high air flow necessary (340 L/min) for efficient separation, the ram-air effect of a large nozzle as well as the venturi suction effect at an outlet tube sited in the aircraft slipstream is useful.

Other applications for miniature cyclones, such as bulk spore collection, are well known and their use as a miniature wind tunnel has been exploited by Ramsdell and Ogawa (15) and Ogawa, Hall, and Koepsell (13). We believe the cyclone spore trap to be an important tool both for general purpose stationary or airborne spore sampling and for special applications such as monitoring and detecting culturable spores of a single fungal species.

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