# Design of an Acoustical Particle Counter and Its Use in Phytopathological Research

Martin W. Imhoff and S. R. Coover

Graduate research assistant, Department of Plant Pathology, North Carolina State University, Raleigh 27650, and research assistant professor, Department of Environmental Sciences and Engineering, School of Public Health, University of North Carolina, Chapel Hill 27514.

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## ABSTRACT

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An acoustical particle counter and rotorod samplers were used to monitor spores of *Uromyces phaseoli* var. *typica* on snap bean during the course of four epidemics in controlled environment chambers. In the acoustical counter, airborne particles are drawn through a capillary section of a tubular acoustical element, where particles larger than 5 $\mu$m in diameter shed vortices as they are accelerated less rapidly than the surrounding air. Pressure changes caused by these disturbances in the laminar flow cause sound waves detectable with an electret microphone. Acoustical counts and rotorod counts were highly correlated (r = 0.98), with approximately one acoustical count for every 26 rotorod counts. Disease increase as measured by a visual rating scale was highly correlated with both acoustical counts (r = 0.94) and rotorod counts (r = 0.96). Immediate digital readout, simplicity of design, and large sampling volume give the acoustical counter advantages over other particle counting methods for some phytopathological investigations.

*Additional key words:* bean rust, epidemiology.

Many phytopathological investigations employ particle counting methods, usually for the purpose of volumetrically determining numbers of fungal spores in the ambient air over time. Valuable information can be obtained from such studies with applications ranging from disease prediction to simpler methods of disease rating (1,5,13). Devices commonly used for monitoring air flora include rotorod samplers and Hirst and Burkard spore traps, both of which depend on particle impaction. All such methods sample air flora nearly isokinetically at moderate wind speeds so samples are not biased by wind direction (9), but they all require tedious and time-consuming microscopic examination for spore counting, making it difficult to obtain results quickly or from a large number of samples.

Continuous direct readout, sizing, and counting of airborne particles are performed almost exclusively by optical techniques, primarily light scattering (6). Passage of an airborne particle through a light-scattering counter produces a pulse of scattered light, the intensity of which is related to the size of the particle. Most commercial light-scattering counters utilize highly sensitive, sophisticated (and expensive) electronics and generally exhibit an effective upper particle aerodynamic diameter cutoff of 10–15 $\mu$m because sedimentation and impaction prevent larger particles from negotiating the baffled sample entry lines required to prevent spurious responses from the entrance of ambient light. As such, the light-scattering counters are generally unsuitable for monitoring large particles such as pollens and many spores.

In the early 1960s Langer (12) reported that passing a large airborne particle through a specially designed capillary at high velocity would produce an audible acoustical pulse. Langer's data indicated that the acoustical sensor exhibited a lower particle detection threshold diameter of 5–15 $\mu$m, but Langer's device provided no mechanism for sizing particles, nor was the mechanism of acoustical pulse production clearly delineated. As such, the acoustical particle sensor remained a laboratory curiosity.

In the late 1970s, however, Coover (2) discovered that the threshold size for the acoustical particle sensor could be varied by varying the flow rate through the capillary. More specifically, it was discovered that threshold size gas directly related to the airflow Reynold's number in the capillary of the acoustical sensor (3). Airflow Reynold's number is a dimensionless quantity describing the degree of turbulence in a flow stream and is related to flow rate by the following relationship: $Re = 4Q/\pi dn$, in which Re = Reynold's number, Q = flow rate, $\pi$ = pi, d = capillary diameter, and n = kinematic viscosity of air. Transformation from flow rate to Reynold's number provided a particle threshold diameter relationship that was independent of capillary diameter (Fig. 1). This discovery provided a mechanism by which the acoustical particle sensor could size (in an integral sense) as well as count particles. Details about the development of the acoustical particle-sizing device, as it was now called, performance testing, and the theoretical basis for operation will be reported elsewhere (4).

Three specific applications of the acoustical particle-sizing device have been explored: as a gross particle clean room monitor, as an outdoor ambient pollen monitor (10), and as a device to count and/or monitor planting of small seeds such as tobacco (2). An acoustical particle counter seemed particularly well suited for monitoring spore concentrations in the air during disease development in artificially induced growth chamber epidemics. This paper describes the operation of the acoustical counter as a spore counter and compares its performance in relation to rotorod spore counts of bean rust urediospores during disease increase in controlled environment chambers.

## DESCRIPTION AND PERFORMANCE OF THE ACOUSTICAL COUNTER

A diagram of the acoustical particle-counting element utilized in this study is presented in Fig. 2. The polyester plastic element consists of a 1.0-cm-diameter × 15.0-cm-long entry section that tapers to a 1.5-mm × 6.0-cm capillary section via a 10° half-angle conical contraction. Figure 3 represents a schematic diagram of the entire acoustical apparatus. The acoustical pulse triggered by the

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passage of a large airborne particle through the acoustical element is detected by a miniature electret microphone located near the mouth of the entry section. The microphone signal is amplified by a linear amplifier. A pulse height analyzer following the amplifier is used to discriminate between acoustical pulses and "noise." Discrimination against noise is readily achieved as the signal-to-noise ratio (S+N)/N is of the order of 100:1. The analyzer is immune to ordinary room noise. A trigger with presettable dead time, set to slightly longer than the acoustical pulse duration, prevents multiple triggering of the sinusoidal wave form of the acoustical pulses. A digital scaler records the acoustical pulses received during a selected time interval.

Flow rate through the acoustical element is measured and regulated with a rotameter and needle valve. Selection of flow rate and hence capillary Reynold's number, is based on the desired lower particle-size threshold that is desired. High flow rates (high Reynold's numbers) provide small particle-size thresholds. A flow rate of 17.2 L/min (Reynold's number = 15,000 for a 1.5-mm capillary) was used in this study.

A 47-mm-diameter, 0.8 $\mu$m mean pore size membrane filter located approximately 2 cm from the exit of the acoustical element prevented contamination of the subsequent flow system with particulates. Particles impact on the filter at such high velocity (approximately 160 m/sec, under the conditions used in this study) that they either shatter or bounce off, making quantitative particle counts on the filter impossible. Suction is supplied by a mechanical vacuum pump capable of producing a stable flow at a suction of at least 380 torr. Both rotary vane and high-vacuum, oil-sealed vacuum pumps have been used successfully with the acoustical particle-sizing device.

Knowledge of the detection efficiency of the acoustical particle-sizing device is essential if the device is to be used for absolute quantitative analyses. Detection efficiency was determined for five species of monodisperse pollens and spores (6-$\mu$m-diameter wheat smut spores, 19-$\mu$m-diameter ragweed pollen, 28-$\mu$m-diameter Lycopodium spores, 36-$\mu$m-diameter timothy pollen, and 45-$\mu$m-diameter beech pollen). Milligram quantities of the pollens or spores were dispersed in a 1-m$^3$ acrylic plastic chamber by a blast of

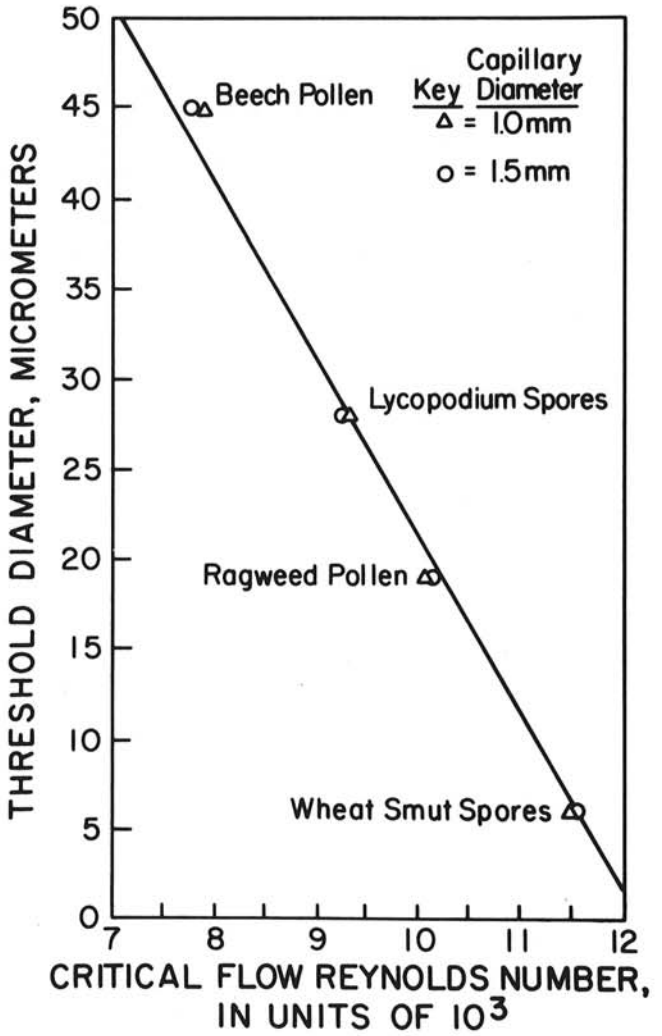


**Fig. 1.** Acoustical particle sizing device particle threshold diameter vs critical flow Reynold's number. The threshold diameter is required for an acoustical pulse to be produced in the acoustical element. Reprinted with permission from Environmental Science and Technology 14:951-954. Copyright 1980, American Chemical Society.

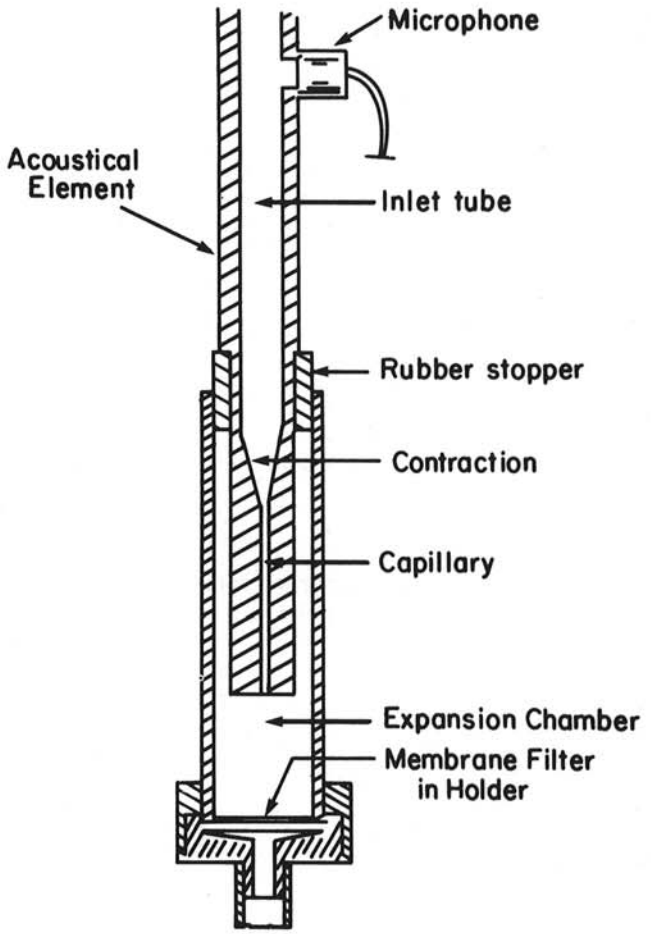


**Fig. 2.** Acoustical particle sizing element. Vacuum is applied beneath membrane filter so that particles are drawn from the inlet tube. Reprinted with permission from Environmental Science and Technology 14:951-954. Copyright 1980, American Chemical Society.

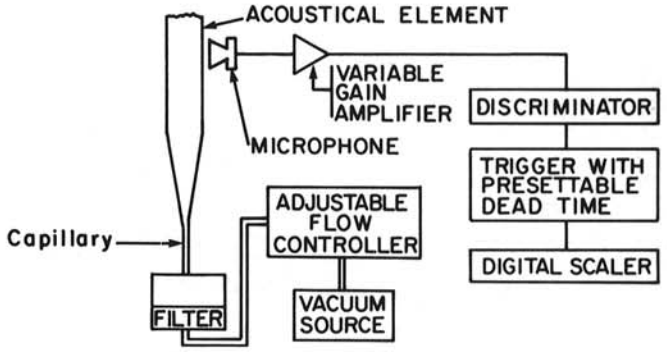


**Fig. 3.** Analyzer schematic. Vacuum source and counting electronics require 120 V. Reprinted with permission from Environmental Science and Technology 14:951-954. Copyright 1980, American Chemical Society.

air. The acoustical element of the particle-sizing device was placed in a rubber stopper in the neck of a 500-ml filtering flask containing 100 ml of ultrafiltered 2% saline solution. The flask containing the acoustical element was placed on the floor of the chamber with the inlet of the device pointing vertically upward. Particles exiting from the acoustical element were impacted and retained in the saline. Detection efficiency was determined by dividing the number of acoustical counts recorded by the total particle count in the saline as determined by analyzing an aliquot with a Coulter counter. Figure 4 presents a plot of detection efficiency versus Reynold's number.

## MATERIALS AND METHODS

Studies were conducted in four walk-in controlled environment chambers 2.44 × 3.66 × 2.13 m high at the Southeastern Plant Environment Laboratories (6). These chambers use a combination of cool-white fluorescent and incandescent lamps to provide an illuminance of 430 to 480 hlx. Day length was based on a light period of 13 hr. Air temperatures were maintained at ±0.25 C of the set point as measured with a #24, type "T" welded-bead copper-constantan thermocouple in a shielded, aspirated housing. Day/night temperatures were 18/18, 21/21, 24/21, 24/18 C for the four chambers. Top to bottom airflow was indicated by a Hastings air velocity meter (Hastings Co., Hampton, VA 23361) to average 20 m/min. Relative humidity was measured on a Weather Measure RO 21-10 hygrometer (Weather Measure Co., Sacramento, CA 95841) and maintained at 70% or more at all temperatures. Carbon dioxide concentrations were measured on a Beckman IR gas analyzer (Beckman Instruments, Atlanta, GA 30340) and controlled at 300–400 ppm by injection of commercial grade $CO_2$.

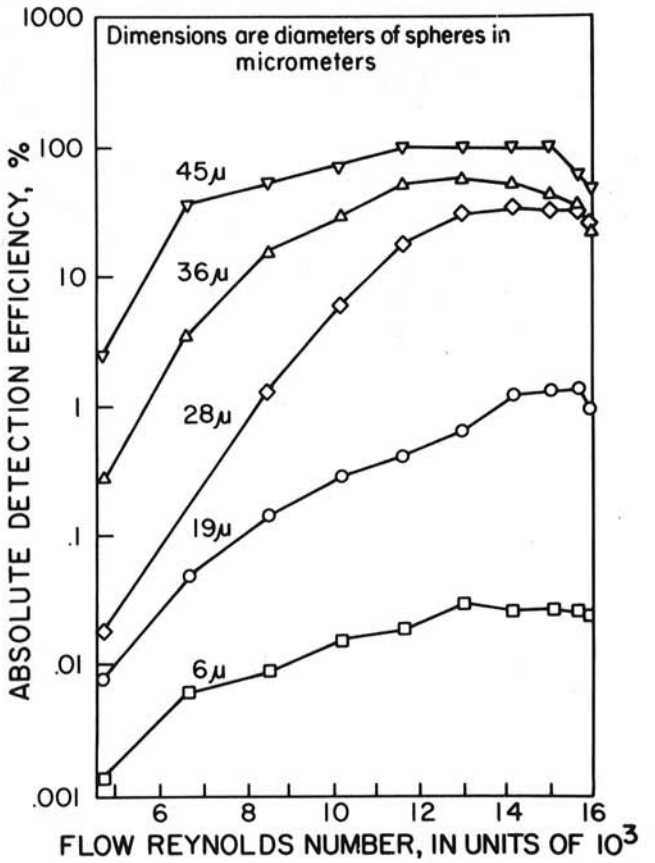


**Fig. 4.** Absolute detection efficiency for spheres (1.5-mm diameter acoustical element), determined as number counted acoustically divided by number caught in saline solution. Reprinted with permission from Environmental Science and Technology 14:951-954. Copyright 1980, American Chemical Society.

Plants were grown in 15.2-cm plastic pots containing gravel and peat-lite (2:1, v/v) substrate. Plants were irrigated twice each day with nutrient solution (7) and placed in two rows 1 m apart containing 30 plants each on perforated metal platforms 80 cm above the chamber floor. A fine mist of deionized water at a rate of about 10.6 L/hr from each of four sprayer nozzles was applied to the foliage three to five times per wk for 10 hr each night to simulate dew formation; 3–4 min of mist each hr was sufficient to keep leaves wet with little runoff.

*Phaseolus vulgaris* 'Bountiful' plants were inoculated 4–12 days after emergence by releasing spores of a single pustule isolate of *Uromyces phaseoli* var. *typica,* race 34 (11) into the chamber air and allowing them to settle onto the leaf surfaces. The bean rust spore is nearly spherical in water, approximately 21 × 22 μm, but when dry and airborne, it flattens to a more disklike shape of 21 × 13 μm as measured by an ocular micrometer. The spores are echinulate.

Two to three times each wk 90–150 individual leaflet disease ratings were made per chamber using blind samples chosen from top, middle, and lower strata of the canopy. Percent leaf area infected was rated with a visual rating scale consisting of photographs of leaves with varying proportions of tissue removed, percent removed tissue being measured on prewilted leaves by means of a planimeter. Both aging and density of pustules were accounted for in the total of 48 photographs. Diseased area consisted of both sporulating pustule area and yellowed halo area. Application of the rating scale was most accurate and precise below 10% diseased area as determined by replicated ratings of disease in the field.

The acoustical counter was located in the center of the chamber 50 cm from each plant row with the air intake facing upward, opening at approximately 100 cm above the floor. One rotorod sampler was set 15 cm above and another 15 cm below the acoustical counter air intake, both 30 cm distant horizontally. Plastic "I" rods, 60 × 1.59 mm, coated lightly with silicone grease, were used on the modified U-shaped rotor. The same two rotors and acoustical element were used in all four chambers for the duration of the study to avoid instrument variation. Background counts from both the rotorods and the acoustical counter showed

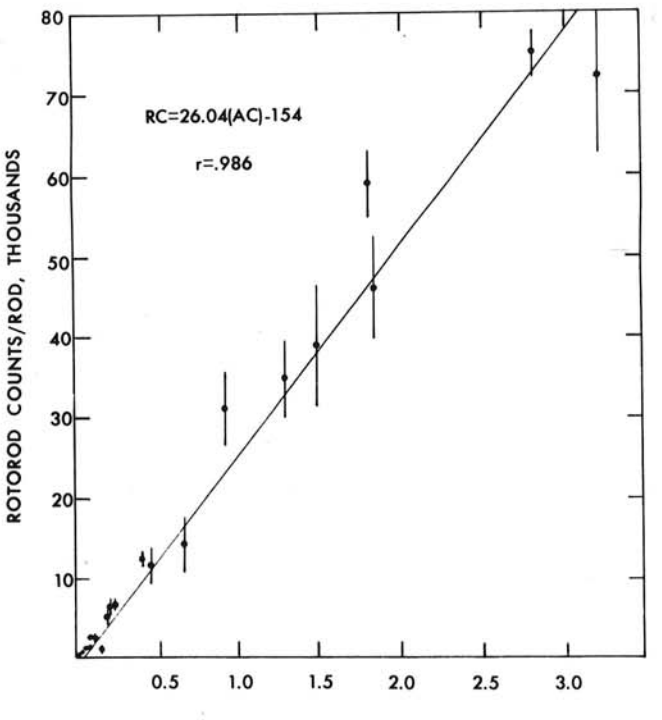


**Fig. 5.** Rotorod counts, average for four rods, vs acoustical counts. r = 0.98 corresponds to all 28 data points. Below 250 acoustical counts, RC = 28.97 (AC) - 379, r = 0.922, for 18 data points. Bars indicate one standard error.

practically no spore release during ordinary air exchange in the chambers (rotorods estimated at 50–90% collection efficiency [8]). Therefore, to provide airborne inoculum, each day between 1400 and 1700 hours rotorods and acoustical counter were turned on and each plant shaken by rocking its pot back and forth by hand six to seven times. Sampling ended after 10 min or occasionally earlier if the acoustical counter indicated that counts had subsided to a background level again. Acoustical counts were read directly from the digital recorder and spores on rotorods were counted microscopically. For fewer than 500 spores/rod, the entire rod was counted. For increasingly larger spore concentrations, spores present on 10 increasingly smaller uniformly spaced representative areas were counted and averaged. All counts were adjusted to a whole-rod basis. The counts of spores on four rods were averaged to obtain a single mean value for each corresponding acoustical count when the upper rotor's counts showed no significant difference from that of the lower rotor.

All four epidemics were terminated 31–66 days after emergence, when disease severity ratings approached 10%.

## RESULTS

When the plants were shaken, acoustical counts rose rapidly and approximately 90% of the final number of counts occurred within 1 min after shaking ended. Rotorod counts below 10,000 showed more variation than counts above 10,000 (Fig. 5), while rotorod determination coefficient of variation remained nearly constant. The correlation between rotorod and acoustical counts was highly significant ($r = 0.98$), with one acoustical count produced for approximately every 26 spores caught on the rotorods. Application of linear regression to these data is invalid unless it is transformed to produce a random error structure. In this case, however, the regression is presented with untransformed data to emphasize this error structure and approximate rotorod/acoustical ratios.

All curves of disease vs time, acoustical counts vs time, and rotorod counts vs time could be fit to the linear form of the exponential growth model, $\log Y = \log Y_0 + kt$, at the 1% significance level ($r > 0.86$ in all cases), but each curve yielded different $Y_0$ and k parameters. However, disease was linearly correlated with acoustical counts and rotorod counts ($P = 0.01$) in each case. Figure 6 shows these results for one epidemic. Cumulative counts did not correlate as well with disease incidence as daily counts. The logit transformation, $\log(y/[1-y])$, as previously used in such studies (1,5,13) was not applied to either daily or cumulative counts, since no estimation could be made of a maximum value to substitute for 1.

## DISCUSSION

In this study, daily spore counts were better correlated with disease ratings than were cumulative counts. Other studies (1,5,13) have reported that cumulative spores counts were better correlated with disease severity. An explanation for this discrepancy may lie in the fact that all our disease ratings were below 12%, so relatively little competition for infection sites was occurring. The fungus would be expected to be growing and reproducing nearly at its genetic and environmental potential. Therefore, the great majority of all the pustules observed on any given day were less than one latent period old and would be expected to be in the peak of their spore production capacity (14). Because disease ratings were based on visual estimates of numbers of pustules, both disease ratings and spore counts would be expected to be proportional to those newest pustules which were always the great majority of all pustules present.

In Fig. 6, both disease severity and acoustical counts dip downward near days 56 to 65. Such downward dips in other bean rust disease progress curves have been explained by discrete flourishes of new host material accounting for more than the usual number of zero disease ratings (M. W. Imhoff, *unpublished*). In this epidemic, however, many heavily infected leaves died near day 60, thus accounting for the drop in spore counts, yet the dead leaves did not fall off until 2–3 days later. Thus, the day 61 ratings show the effect of lost sporulation and some leaves receiving ratings of 100% severity, while day 63 has a lower disease rating due to dropping of those 100% diseased leaves. Spore count ratings, meanwhile, kept pace with the most recently produced pustules.

The acoustical particle counter was simple to operate, rugged, and very efficient in producing accurate spore count estimates, which were well correlated with the amount of disease present. Because we used it in a closed system, the counts almost exclusively reflected bean rust spores. At very low spore densities the rotorod method was more accurate, because the acoustical counter counted other particles in the air, such as large dust particles. Rotorods, however, become very difficult to count and probably are less efficient collectors and detectors at high spore densities, due to increasing rod surface saturation, whereas the acoustical counter can accept up to 20,000 counts per minute. The acoustical counter has the distinct advantage of the rapidity of obtaining data. Information on background counts and the rate of spore settling in the chamber could be obtained very quickly. We also learned quickly that plants distant from the center of the chamber contributed significantly to the counts within the chamber so that readings taken within the chambers did not simply reflect those spores being released from the nearest plants.

The acoustical counter has the disadvantage of detecting only particles greater than 5 $\mu$m in diameter, and its detection efficiency differs for different sized particles. At its present state of development, the acoustical counter may be more useful for indoor rather than field studies. However, if the ability to discriminate among different sized particles can be incorporated in the design, the applicability of this instrument can be extended to many field uses.

Other applications of the acoustical counter to phytopathological research might include the quantification of inoculum via counting spores settling in a settling tower. Spore sizing can now be estimated, although this capacity is not yet refined. Particulate air pollution can be monitored efficiently, and high or low count numbers could be used as a switching device for other monitoring or cleaning equipment. Also, the concentration of fine mist pesticide applications could be readily and quickly monitored, since mist particles are also detected by the acoustical counter. Similarly, pesticide drift may be detected through unusually high counts away from the spraying area. Sporulation from many wood decay fungi could be easily monitored by mounting a counter beneath a sporulating conk in an area with relatively little wind disturbance. Periodicity of spore production could be recorded by combining a stripchart recorder with the counter.

Acoustical particle counting and sizing technology can form the basis for an entirely new line of particle counting and sizing

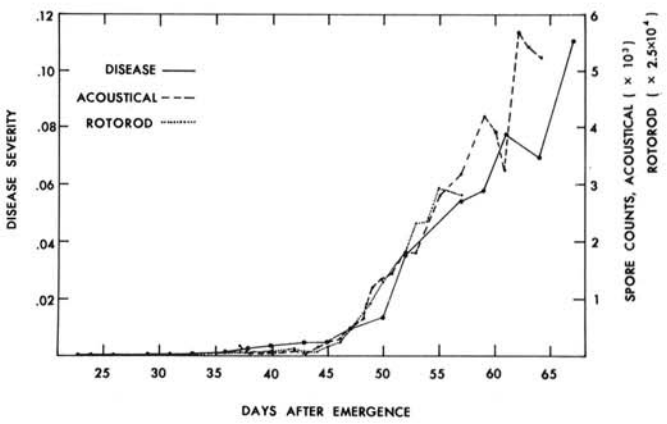


**Fig. 6.** Acoustical counts, rotorod counts, and disease severity vs time for 18 C, mist three-times-a-week epidemic. Spore count scales are adjusted to facilitate curve shape comparisons. $r = 0.94$ for acoustical counts vs disease severity, $r = 0.96$ for rotorod counts vs disease severity. Best fits for exponential growth models were $AC = 0.905\, e^{.193\, t}$, $r = 0.95$ for acoustical counts, $RC = 4.39\, e^{.248\, t}$, $r = 0.98$ for rotorod counts, and $Y = 0.000063\, e^{.156\, t}$, $r = 0.98$ for disease severity.

apparatus. Licenses for certain applications are still available. Licensing details may be obtained from R. L. Ely, Office of University Relations, Research Triangle Institute, Research Triangle Park, NC 27709.

Questions concerning pathological aspects of this study should be referred to the first author, while those concerning acoustical counter technology should be referred to the second author.

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