

An Apparatus for Precise Control of Humidity, Temperature, Air Flow, and Light in Spore Discharge Studies

C. M. Leach

Professor, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331.
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ABSTRACT

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An apparatus for experimental studies on spore release by plant pathogenic and other fungi is described and illustrated. The apparatus provides precise control of atmospheric humidity, air temperature, light,

and air velocity. A precision spore trap that monitors the release of spores by the minute is part of the apparatus.

Spore release in many fungi can be triggered by environmental factors (1), particularly changes in atmospheric humidity, light, and air movement (2,4). To study the relationship of these factors to liberation of conidia by *Drechslera turcica* and other Fungi Imperfecti, a special spore release apparatus was developed from a simpler and less precise prototype (2). The new apparatus has been used to study the effects of changes in atmospheric moisture, exposure of specimens to red-infrared radiation, and vibration on spore release under constant air temperature and air velocity.

DESCRIPTION OF APPARATUS

A schematic drawing of the spore release apparatus is shown in Fig. 1.

Air supply. Compressed air (supplied by a paint sprayer compressor, Sears, 689 kPa/m² [100 lb/in²], 1 hp, twin cylinder) regulated at 68.9 kPa/m² (10 lb/in²) enters the apparatus after passing through an oil filter (Catalog No. 463, Matheson Gas Products, Will Ross, Inc., Lyndhurst, NJ). The oil filter is needed only if the compressed air is contaminated with oil vapor.

Humidity. Precise humidity control is obtained by mixing dry and saturated air. After entering the apparatus, the air divides into two streams: one passes through a drier and the other through a humidifier. The drier is a 102 × 6 cm glass tube filled with silica gel (indicating silica gel, 4.2–1.2-mm [6–20 mesh], J. T. Baker Co., Phillipsburg, NJ) that dries air to less than 20% RH. Saturated silica gel (pink) is easily redried (blue) in an oven for a few hours at 155 C. The humidifier is a series of bottles (modified, screw-capped household preserving jars) containing evaporators that are partially immersed in water. The cylindrical 8 × 10 cm fluted evaporators are modified automobile oil filters ("Purolator" P141). Bottles are alternated with 100W incandescent lamps on a rheostat

to control temperature and to simulate solar evaporation.

Dry and saturated air are combined in different proportions by a mixer valve which is a modified domestic bathtub-shower mixer faucet ("Aqua-line," U.S. Brass Works, Great Neck, NY). The calibrated valve is operated manually or automatically. Manually, the relative humidity can be lowered or raised within the range of 20 to 100% in ~2 min. When operated automatically with motor driven cams and gears, the relative humidity can be programmed to different repeating cycles ranging from minutes to hours per cycle. A check valve is included in the humidifier line to prevent reverse surging of air. A condenser and an adjustable (thermoswitch) temperature control unit prevents the apparatus from becoming filled with condensed water when the air is near saturation.

Another humidifier is located just ahead of the specimen chamber. This single unit is manually switched into the system by a diversion valve when saturated air is needed. Without this humidifier, maximum relative humidity obtainable is ~95–98%.

Air temperature. Air temperature is first regulated at the condenser; however, precise control is provided by a special regulator with its own adjustable (thermoswitch) temperature control unit. In addition, the apparatus is operated in a temperature-controlled room in which ambient temperature is slightly lower than air entering the specimen chamber.

Air flow. Air entering the apparatus is controlled with a pressure regulator and gauge set at 68.9 kPa/m² (10 lb/in²). Precise control is obtained with a low pressure regulator and a gauge set at 9.8 kPa/m² (1.42 lb/in²) to provide a 0.5 m/sec air velocity in the specimen chamber. The setting on this regulator (low pressure series Model 70; Matheson Gas Products) is naturally dependent on the size of tubing used in the apparatus and the desired air velocity. Air velocity is measured precisely with a "hot wire" anemometer (type 8500, "thermo-anemometer"; Alnor Instrument Co., Niles, IL) placed in the specimen chamber when needed. A flow meter indicates the volume of air flowing through the system

and serves as a leak detector. Variation of air velocity set at 0.5 m/sec is ± 0.01 m/sec.

Specimen chamber. The Pyrex glass specimen chamber has been designed and tested for uniform air flow past the specimen. Air enters the 4×30 cm cylindrical chamber at right angles where it strikes a perforated, angled baffle plate to reduce jet effect. Air then passes through a 3-cm, lightly-packed, fiberglass diffusion filter held between wire mesh disks.

To insert specimens, the chamber is opened at one end by a large rubber coupling. Specimens are inverted and attached by rubber bands to a 42×107 mm plastic holder in the chamber. The holder is supported on two plastic rods that pass through stoppered openings at both ends of the specimen chamber. The chamber has other miscellaneous stoppered openings to insert thermocouples and other probes.

Spore trap. Air from the specimen chamber flows through a brass tube to a specially designed precision spore trap. Electrical grounding of this tube eliminates static electricity. Air enters the spore trap through a 31×1 mm slit orifice and strikes a Vaseline-coated 11×75 mm glass slide. Usually two slides are placed end to end on a platform that moves past the orifice at a precisely controlled speed (usually 1 mm/1.1 min). Slide speeds can be varied from a few millimeters per second to a few millimeters per hour by combining different synchronous motors and gears. The long slit orifice has been designed for electrical studies on charged spores (3) in which special electrodes (10 mm wide) are attached to either side of the slide. Smaller orifices would be satisfactory in most other studies. The spore trap is shielded and grounded for electrical studies.

Light. A lamp housing with a source of red-infrared radiation is built into the apparatus. Specimens are irradiated from below with a 250 W infrared lamp. A 20 cm deep water filter may or may not be used as a heat filter. For light quality studies, filters are inserted between specimen and light source.

Measurement of environmental factors. During experiments temperature and relative humidity are measured continuously; air velocity and light intensity are measured periodically. Relative humidity is monitored at two locations by shielded (radiation shields) wet-bulb thermocouple psychrometers located in the air stream before and after the specimen chamber. Details of the psychrometers are shown in Fig. 2 (modification of a design by K. Young, DSIR, Auckland, New Zealand). By connecting the wet and dry thermocouples in series (Fig. 2C), the differential temperature between the two thermocouples can be recorded. These are later converted to relative humidity readings by standard psychrometric conversion tables. Air velocity past the psychrometers must be greater than 3 m/sec to function properly. This is achieved by placing the psychrometers in tubing of smaller diameter than the specimen chamber. The wick of the psychrometer is a piece of finely braided, hollow core nylon fishing line (20.4-kg [45-lb] test) washed in warm acetone, followed by methanol, hot water, and a long rinse in distilled water.

Air temperatures (± 0.25 C) are measured at four locations by thermocouples (Chromel Alumel, Size 30, Code TT-K-30, Omega Engineering, Inc., Stamford, CT) that are similar to those in the psychrometers. Temperature and humidity are recorded continuously on a multichannel recorder using ice bath references between thermocouples and recorder ("Multipoint" recorder,

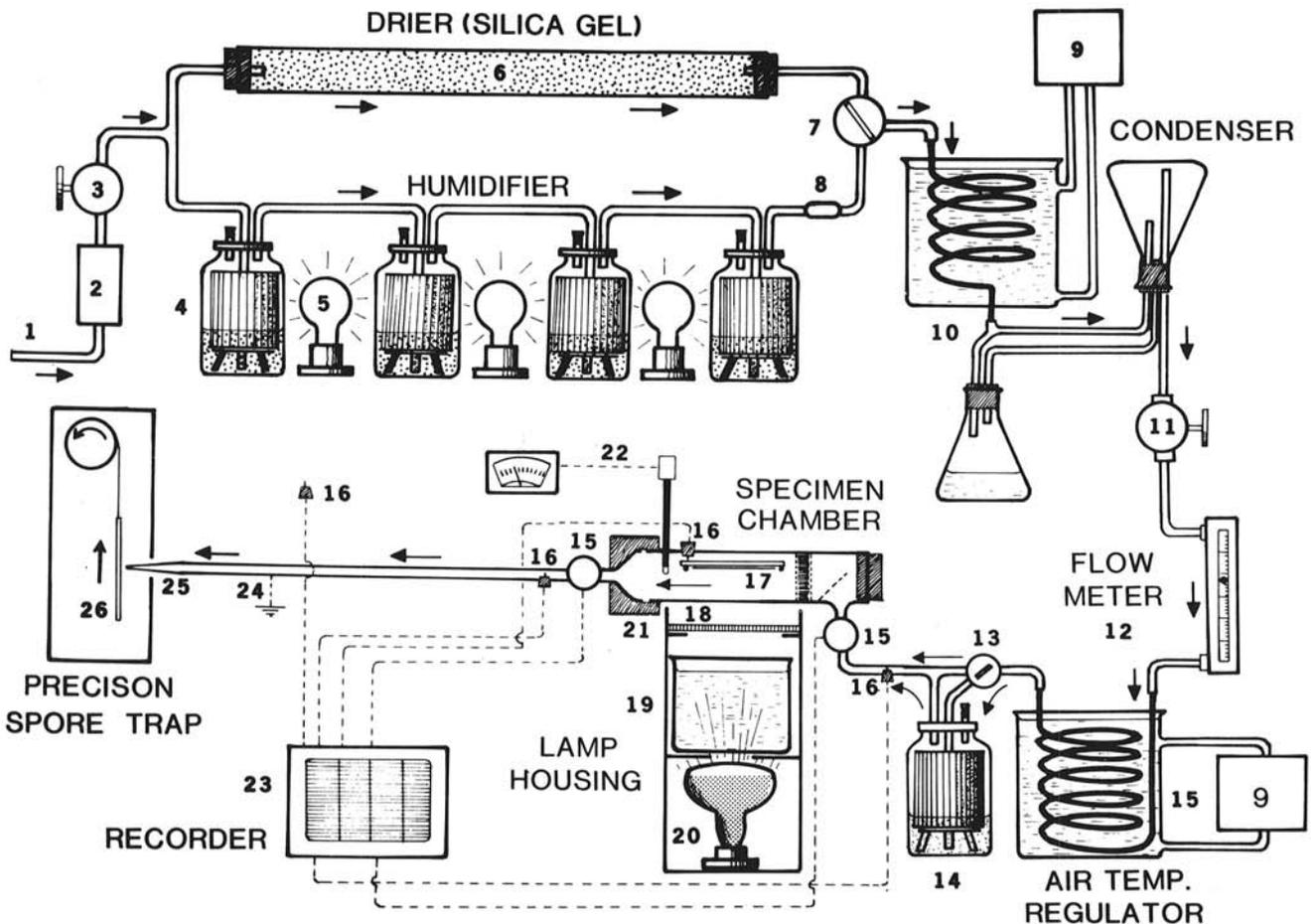


Fig. 1. A schematic diagram of the spore release apparatus: 1, compressed air inlet; 2, oil filter; 3, pressure regulator and gauge to control air entering apparatus at 68.9 kPa/m²; 4 and 5, humidifier; 6, drier; 7, mixer valve; 8, check valve; 9, waterbath temperature controllers; 10, condenser; 11, low pressure regulator and gauge set at 9.8 kPa/m²; 12, flow meter; 13, diversion valve; 14, humidifier; 15, wet bulb thermocouple psychrometers (two); 16, thermocouples for measuring air temperature (four); 17, specimen chamber with fiberglass diffusion filter; 18, light filter; 19, water filter; 20, 250W infrared lamp; 21, rubber coupling for insertion of specimens; 22, anemometer; 23, recorder; 24, electrical grounding; 25, slit opening to spore trap; 26, spore trap with Vaseline-coated glass slide.

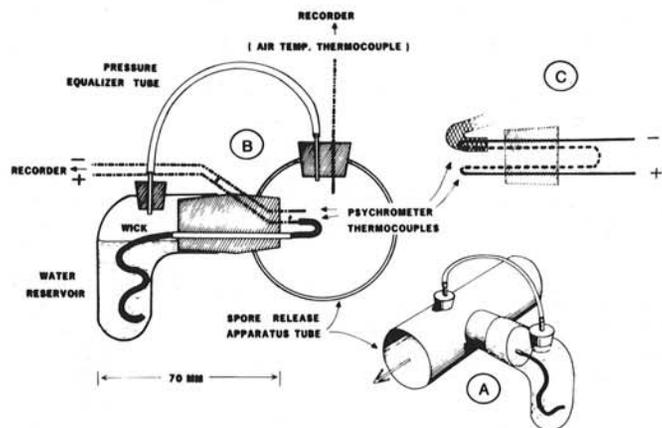


Fig. 2. Psychrometer system to measure air humidity in the spore release apparatus. A, General view of the psychrometer positioned in a tube of the spore release apparatus. B, Sectional drawing of a psychrometer. C, Arrangement of wet/dry bulb differential thermocouple for measuring wet bulb depression.

Model PM 8325, Philips, The Netherlands).

Light intensity at the plane of the specimen is measured with a compensated thermopile and galvanometer (Kipp und Zonen, The Netherlands). Air velocity is monitored periodically as mentioned.

Figure 3 shows an example of an experiment conducted in this apparatus.

DISCUSSION

The spread of most foliar diseases caused by fungi depends on the release and dispersal of airborne spores. Although mycologists and plant pathologists have been concerned with spore liberation for more than a century (1, 5), there are still gaps in understanding the mechanisms of spore liberation and the role of environmental factors in spore release. The apparatus described in this article has been developed to study spore release under controlled conditions. It has been used to study the influence of humidity, exposure to red-infrared radiation, and vibration at discharge of spore liberation by various dry-spored Fungi Imperfecti (*unpublished data*). The apparatus can also be used to study discharge of ascospores and basidiospores, and, with a few minor modifications, as a miniature wind tunnel for relating air velocity to spore removal under different humidity and light regimes.

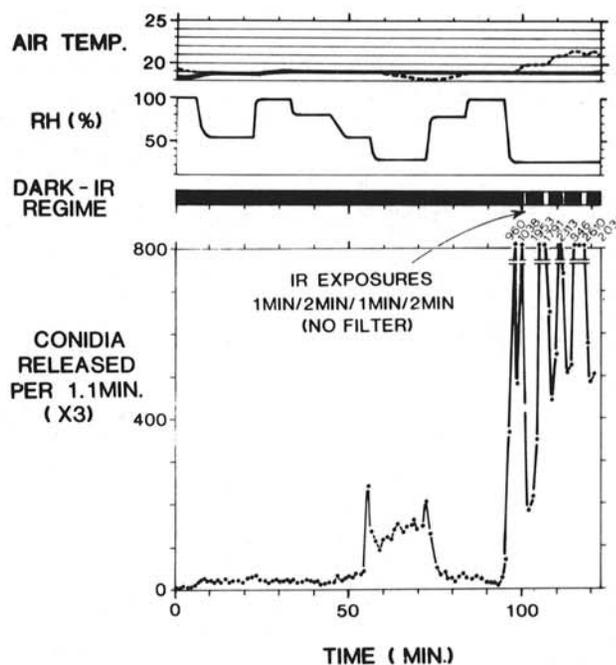


Fig. 3. Example of data plots from an experiment on conidial liberation by *Drechslera turcica* conducted in the spore release apparatus. Temperatures are for air entering specimen chamber (—) and air leaving the chamber (---). Only the relative humidity of air entering the apparatus has been recorded.

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