A Wind Tunnel for Controlled-Environment Studies of Ascospore Release by *Venturia inaequalis*

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ABSTRACT

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We designed and built a bench-top wind tunnel for the study of ascospore release by *Venturia inaequalis* in controlled environments. Compressed air was forced through a column of water to adjust relative humidity. Air then entered one end of a 102-mm-diameter tube at the rate of 20 liters/min, passed over a platform holding a leaf sample bearing pseudothecia of *V. inaequalis*, and exited the tunnel through a 2- × 10-mm orifice 20 cm downwind of the sample. Ascospores exiting the orifice impacted on a clear plastic tape borne on a clock cylinder that revolved once in 6, 12, or 24 h. Temperature of the leaf sample was monitored by a thermocouple imbedded in the sample platform and was recorded by a data-logger. Light was directed to the leaf sample through a fiber-optic bundle coupled to a 150-W quartz-halogen lamp. The quality and intensity of the light could be adjusted by colored or neutral density filters at the source. Simulated rain was applied to the leaf sample at the rate of 5.3 cm/h through a fine-spray nozzle located 50 cm above the sample. The temperature of the simulated rain, as well as a water jacket surrounding the tunnel, was controlled by passing the water supply through a controlled-temperature bath. The tunnel maintained temperatures as low as $1.0 \pm 0.2°C$ for 6 h, and could operate for up to 24 h continuously. Utility of the wind tunnel and reproducibility of obtained results was demonstrated using ascospores of *V. inaequalis* and *Guignardia bidwellii*.

Additional keywords: aerobiology, epidemiology, spore trap.

The ascigerous stages of many plant pathogens function in survival during overwintering and during periods between crops that can last several months. Ascospores released from fruiting structures such as perithecia, pseudothecia, or cleistothecia are the primary inoculum for several important plant diseases (20,22,33), and for such diseases, the effects of environmental factors on ascospore release are studied for the purpose of more precisely describing when the crop is at risk for infection (12,14,21,24,27,32).

Ascospore release can be monitored in the field using volumetric traps (5,8,13,19,25,28,32,35). However, simultaneous variations in rain quantity and intensity, air temperature, relative humidity, light intensity, wind speed and direction, and ascospore maturity can interact and, thereby, confound the interpretation of results (3,34). Numerous laboratory investigations have attempted to control variation in some of the above factors by examining ascospore release in wind tunnels (2,6,7,19,23), but we are unaware of any that have approximated the field environment with respect to rain, relative humidity, and light quality and intensity. The need for such an apparatus was especially acute in our research on ascospore release by the apple scab pathogen, *Venturia inaequalis* (Cooke) G. Wint. The seasonal distribution of ascospore release by *V. inaequalis*, the effects of various environmental factors on the rate of spore discharge, and the suppression of ascospore release during darkness have all been the subject of intensive research over a period of many years and in many countries (1–5,9,10,17,19,25,28,30,31,35,37,38,39). Most published field studies have produced similar results with respect to the relative release of ascospores during light and darkness (3,4,6,19, 25,35). Based upon the patterns of ascospore release, MacHardy and Gadoury (26) recommended that the Mills criteria (29) be revised to reflect the delay of ascospore release when rain begins during darkness. However, in some greenhouse (17,38) and field studies (38), unusually large releases of ascospores have been recorded during darkness. To better understand possible variation in the effects of light and darkness upon ascospore release by *V. inaequalis*, we constructed a wind tunnel in which we could simulate and control the above environmental factors. Although designed for studies of *V. inaequalis*, the tunnel was also evaluated using ascospores of *Guignardia bidwellii* (Ellis) Viala & Ravas and could be used for studies on many ascomycetes. Preliminary reports involving the use of the tunnel to determine the effects of various environmental factors on ascospore release by *V. Inaequalis* have been published (15,16).

MATERIALS AND METHODS

Construction. The body of the wind tunnel was made from various pieces of polyvinyl chloride (PVC) pipe and pipe fittings (Fig. 1 and Table 1). The overall shape of the tunnel was of an inverted T, the base comprised the intake and outflow ends of the tunnel, and the vertical stem consisted of a tube in which simulated rain was produced (Fig. 1). The pieces of the tunnel (Table 1) were assembled as shown in Figure 1. Initially, straight couplings were cemented to each branch of the T-coupling with PVC pipe cement. An end clean-out was glued to the straight coupling at the intake end of the tunnel. A 20-cm length of pipe was glued to the coupling at the outflow end of the tunnel (Fig. 1), and a reduction coupling was then glued to the opposite end of the pipe. The vertical stem of the apparatus was formed by gluing a 30-cm

length of pipe to the remaining straight coupling of the T, and a straight coupling and an end clean-out were glued atop the opposite end of the pipe. Both the end clean-out atop the vertical stem and the end clean-out at the intake end of the tunnel accepted threaded plugs (Fig. 1). The interior and exterior surfaces of the seams were further sealed with a silicone sealant (Dow Corning Corp., Midland, MI).

A test plug with 30 1-mm-diameter holes drilled in an X-pattern was cemented to the inside surface of the threaded plug of the intake end to diffuse the air entering the tunnel. An 8-mm-diameter hole was drilled through the center of the threaded plug, and a connector for 6.35-mm-diameter plastic tubing, which supplied the air, was inserted and sealed to the threaded plug with silicone sealant (Fig. 1).

Light was supplied to the interior of the tunnel through a fiber-optic bundle connected to a 150-W quartz-halogen illuminator (Table 1). The terminus of the fiber-optic bundle was inserted through a 25-mm-diameter rubber stopper, which was in turn inserted through the wall of the trap (Fig. 1) and directed at the center of the sample platform (Fig. 1). Light quality was adjusted to approximate that of daylight, with the illuminator set at full intensity by inserting a daylight-correction filter (model FLB-1-65, Dolan-Jenner Industries, Inc., Woburn, MA) between the lamp and the fiber-optic bundle; light intensity was adjusted by use of neutral density filters (Table 1).

The sample platform consisted of a 5- × 5-cm piece of closed-cell polyurethane foam. In the case of *V. inaequalis*, 20 1-cm-diameter leaf disks were fastened to the platform using stainless steel pins. A 100-k-Ω unicurve thermistor (model UUT51J1, Fenwal Electronics, Inc., Framingham, MA) was imbedded in the platform and attached to a data-logger (model CR-21, Campbell Scientific Inc., Logan, UT). The thermistor was balanced with a 249-k-Ω 1% resistor.

Ascospores released from the sample were entrained in the airstream and exited the tunnel through a 4-cm-long brass tube 2 cm in diameter which tapered to a 2- × 10-mm orifice at the terminus (Johnson Machining, Phelps, NY). The tube was inserted through the center of a test plug and held in place with epoxy cement.

Spores exiting the orifice impacted on the surface of an uncoated Melinex tape (Burkard Manufacturing Co., Ltd., Rickmansworth, Herts., United Kingdom) borne on a cylindrical chart drive (Table 1 and Fig. 1). The distance from the rear aperture of the orifice to the impaction surface was 0.6 mm. The gearing of the chart drive could be changed to yield rotations of 6, 12, or 24 h. The chart drive was supported upon a section of PVC pipe inserted into and glued to a reduction coupling (Fig. 1). The upper three-fourths of the section of pipe that protruded beyond the coupling was removed to provide a flat surface for the installation of the chart drive (Fig. 1).

The temperature of the wall of the tunnel and the temperature of the distilled water supply used for simulated rain was controlled by a circulating refrigerated bath. Ethylene glycol was circulated at the rate of 100 ml/min through two coils of 3.18-mm plastic tubing wound around the outside of the tunnel at two locations (Fig. 1). The entire tunnel was insulated by a 25-mm-thick jacket of rigid fiberglass pipe insulation.

Simulated rain was produced by a brass, solid-cone spray nozzle (model 1/8-T-TG-0.3, Spraying Systems Co., Wheaton, IL). A 12.5-mm hole was drilled through the center of the threaded plug atop the vertical stem of the tunnel (Fig. 1). A threaded tubing connector bearing the spray nozzle was inserted through this hole and sealed in place with silicone sealant (Fig. 1). Distilled water was passed through a coil of 9.5-mm copper tubing immersed in the coolant reservoir of the refrigerated bath at the rate of 70 ml/

Fig. 1. Cross section of wind tunnel showing **A,** distilled water supply line, **B,** spray nozzle, **C,** threaded plug, **D,** end clean-out, **E,** coupling, **F,** rigid fiberglass insulation, **G,** coolant supply line, **H,** coolant return line, **I,** air vent line to reservoir, **J,** 101.6-mm pipe, **K,** windings of coolant lines, **L,** humidified air intake line, **M,** water drain line to reservoir, **N,** T-coupling, **O,** sample platform, **P,** test plug, **Q,** reduction coupling, **R,** orifice, **S,** clock cylinder, **T,** Melinex tape, **U,** 76.2-mm pipe, **V,** fiber-optic bundle, and **W,** thermistor and line to data-logger.

TABLE 1. Materials used in the construction and operation of the wind tunnel

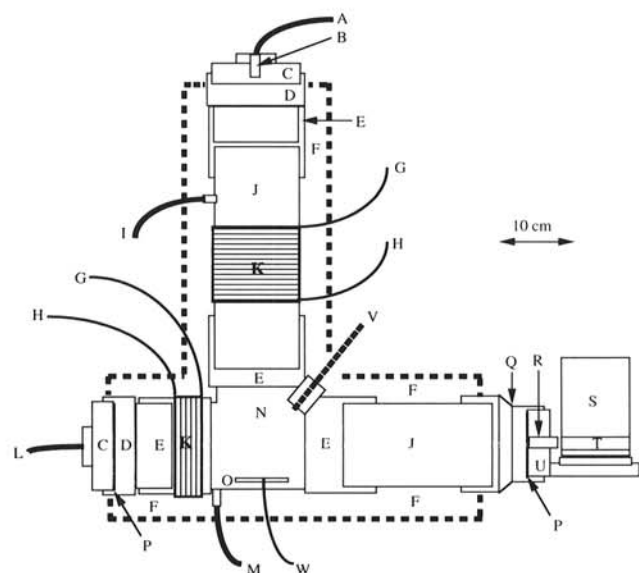| Section	Material	Supplier
Main body	Schedule 40 pipe and pipe fittings, white polyvinyl chloride (PVC) Type I, 101.6 mm (4 in.): two end clean-outs, two threaded plugs, five couplings, two test plugs; one 101.6- to 76.2-mm (4–3 in.) reduction coupling; one 20 cm length of 101.6-mm pipe; one 30 cm length of 101.6-mm pipe; one 15 cm length of 76.2-mm pipe; and one white PVC SDW 101.6-mm T-coupling	HEP Materials 446 Geneva-Waterloo Road Geneva, NY 14456
Water columns	Schedule 40 pipe and pipe fittings, white PVC Type I, 101.6 mm: one end clean-out, one threaded cap, one coupling, and one cap	HEP Materials
Light source	Fiber-Lite High Intensity Illuminator, series 180, 150 W	Dolan-Jenner Industries, Inc. Box 1020 Woburn, MA 10801
Color filters	Violet, green, daylight-balanced, yellow, and red	Dolan-Jenner Industries, Inc.
Neutral density (ND) filters	ND 0.1, 0.3, 0.6, 1.0, and 2.0	Omega Optical, Inc. 3 Grove Street Brattleboro, VT 05301
Orifice	2-cm-diameter brass tubing	Johnson Machining 22 South Wayne Street Phelps, NY
Chart drive and cylinder	PL-1452 chart drive with 9.29- × 13.20-cm cylinder	Belfort Instrument Co. 1600 S. Clinton Street Baltimore, MD 21224
Mounting platform for chart drive	PVC sheet, 6.35 mm thickness (0.25 in.)	McMaster-Carr Company 2828 Paulina Street Chicago, IL 60657
Spray nozzle	Brass Unijet solid-cone model 1/8-T-TG-0.3	Spraying Systems Co. Wheaton, IL 60188

min to adjust temperature, and then the distilled water was passed through insulated plastic tubing to the spray nozzle.

The humidity of the compressed air supply to the tunnel was regulated by passage through a column of water before entering the tunnel. The column consisted of a sealed 2-m length of 101.6-mm PVC pipe (Table 1) filled to a depth of 1.5 m with distilled water. Air was passed through porous diffusion stones at the base of the column and then drawn from the top of the column through insulated plastic tubing, adjusted by a flow meter (model RMA, Dwyer Instruments, Inc., Michigan City, IN) to 20 liters/min, and sent into the tunnel (Fig. 1). The temperature of the water in the column was maintained at 25 to 30°C by heating the base of the column in a water bath. Air entering the tunnel was cooled by the test plug and tunnel walls (Fig. 1) before reaching the sample platform.

Water exited the trap through a drain tube beneath the sample platform (Fig. 1) and was collected in a sealed 35-liter carboy. Air displaced by water accumulating in the carboy was routed to the vertical shaft of the tunnel through a second length of tubing (Fig. 1).

Operation. Pathogen samples were loaded onto the sample platform, the thermocouple wire was placed at the level of the sample, and the platform was positioned directly beneath the spray nozzle (Fig. 1). The tunnel was sealed, and the air and coolant supply was turned on. Temperatures within the tunnel were allowed to stabilize to ± 0.5°C of the desired level before any ascospore release was initiated by turning on the water supply to the spray nozzle. Samples could be placed in the tunnel several hours before ascospore release was induced, exposing them to various preconditioning effects of light, temperature, or humidity. At temperatures below 10°C, the water supply to the spray nozzle was turned on before the sample was placed in the trap to speed cooling of the apparatus. Once the temperature had stabilized, the tunnel was opened, the sample was installed, and the tunnel was resealed; this operation required approximately 30 s.

Ascospores that collected on the Melinex tape (Burkard Manufacturing Co., Ltd.) were enumerated by mounting sections of the tape on microscope slides as described by Gadoury and MacHardy (11). The tape was scanned at right angles to the direction of rotation at 2-mm intervals, which corresponded to time intervals of 2.47, 4.95, and 9.90 min at 6-, 12-, and 24-h rotations, respectively. The number of ascospores counted in each transect was adjusted for the magnification used and converted to ascospores per cubic meter of air based upon the proportion of the interval examined and the volume of air sampled.

Temperature experiments. The apparatus was operated at 1, 2, 4, 6, 8, and 20°C for 6 h while temperature at the sample platform surface was recorded at 2-min intervals by the thermocouple and data-logger. In a separate experiment, the apparatus was operated at 10°C for 2 h with the illuminator at full intensity and the daylight-balanced filter in place, followed by 2 h during which the illuminator was alternately switched on and off at 6-min intervals.

Temperature was recorded at 2-min intervals, and the test was repeated two additional times.

Relative humidity experiments. Relative humidity of air entering the trap and exiting the orifice was measured by a wet-bulb/dry-bulb psychrometer (model 566, Bendix Inc., Baltimore). The air entering or leaving the tunnel was divided between two 2-cm lengths of plastic tubing, into which were inserted the thermometer bulbs of the psychrometer. Humidity was recorded at 15-min intervals for 3 h at 10 and 20°C.

Light intensity and quality experiments. Light intensity and quality at the sample platform was measured with a multispectral radiometer (model SR, Isco, Inc., Lincoln, NE). A remote sensor mounted at the level of the sample platform was connected to the radiometer by a fiber-optic bundle. Intensity was recorded at 25-nm intervals from 380 nm to 750 nm while the illuminator was operated at full intensity with daylight-correction, violet, and red filters. For comparison, we also measured light intensity as above during continuous rain on 24 May 1992 at 7:00 a.m. in an apple orchard in Geneva, New York.

Simulated rain experiments. Quantity of simulated rain and uniformity of wetting across the sample platform was measured by a 5 × 5 array of 1-cm-diameter glass tubes. Water was collected in the tubes at 15-min intervals for 2 h.

Reproducibility of results. Leaf samples were collected from beneath unsprayed apple trees just prior to bud break at three locations: Geneva, New York; Friedrichschafen, Germany; and Alnarp, Sweden. Samples from Germany and Sweden were air-dried, shipped via air to Geneva, and frozen at –15°C until needed. Twenty 1-cm leaf disks were placed in the tunnel at 5:00 p.m. and left in darkness at room temperature (22 to 25°C) and ambient humidity (30 to 55%) until 8:00 a.m. the following day. They were then exposed to simulated rain for 3 h in darkness, followed by 3 h of light. Two duplicate samples were tested from each of the above three leaf collections. The experiment was further repeated using three duplicate samples of 25 mummified grapes bearing numerous pseudothecia of *G. bidwellii* collected beneath 'Aurore' grapevines in Dresden, New York, just prior to the bloom stage of the vines.

Further experiments were performed to determine the ability of the apparatus to consistently shift from lower to higher temperatures. Leaf samples collected at Njøs, Norway, just prior to bud break of apple were collected and shipped to Geneva as described above. The leaf samples were exposed to light and simulated rain for 6 h at 1.0°C, followed by 3 h at 20°C. The illuminator was operated at full intensity with the daylight-correction filter in place. The test was repeated two additional times. A second ex-

TABLE 2. Stability of temperature within the wind tunnel during a 6-h period at six test temperatures between 1 and 20°C

Temperature setting (°C)	Mean recorded temperature[a] (°C)	Standard error (°C)	Coefficient of variation[b] (%)	Range (°C)
1	0.90	0.012	1.34	0.78–1.10
2	1.90	0.033	1.63	1.77–2.03
4	3.92	0.054	1.38	3.50–4.03
6	5.96	0.043	0.72	5.63–6.05
8	8.00	0.009	0.11	7.96–8.04
20	20.20	0.077	0.38	19.70–20.39

[a] Mean of temperatures measured at 2-min intervals of three 6-h runs of the experiment.
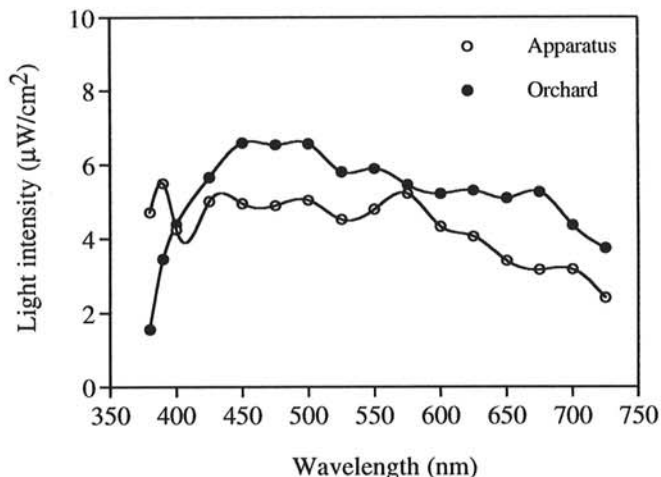
[b] (Standard error/mean) × 100.

Fig. 2. Light quality and intensity within apparatus compared with light quality and intensity in an apple orchard. The illuminator was operated at full intensity with a daylight-balanced filter in place. Orchard measurements were made during rain on 24 May 1992 at 7:00 a.m.

periment was then performed twice as above with the following changes: the sample consisted of mummified grape berries bearing pseudothecia of *G. bidwellii*, and the total duration of the test was 6 hr (3 hr at 2°C followed by 3 hr at 20°C).

RESULTS AND DISCUSSION

Temperature. Temperature fluctuation during operation of the apparatus was minimal. Temperature measured at the sample platform remained within 0.3°C at 1.0°C, and the range of variation was less than ± 0.2°C at 2°C, and less than ± 0.5°C at all other temperatures (Table 2). The coefficient of variation of temperature over the 6-h period of the test ranged from a maximum of 1.63% at 2°C to a minimum of 0.11% at 8°C (Table 2). Temperature measured at the sample platform during darkness did not differ significantly ($P = 0.01$) from that measured during illumination when the apparatus was operated at 10°C.

Relative humidity. The compression and subsequent decompression of the air supply provided a uniformly dry (10 to 15% relative humidity) air supply. Passage of the air supply through the heated water column raised its relative humidity to a level of 86 to 93%. Subsequent cooling of the air as it entered the apparatus resulted in the consistent production of a nearly saturated atmosphere within the apparatus, e.g., the relative humidity of air exiting the apparatus measured from 97 to 100%. Thus, the relative humidity of air within the apparatus approximated that observed during rain events and episodes of ascospore discharge by *V. Inaequalis* (16).

Lower relative humidities could be generated by filling the columns with various saturated salt solutions (13) and maintaining equivalent temperatures between the column and the apparatus. However, rainfall was required for ascospore release in *V. inaequalis* and *G. bidwellii* and, considering the confounding effects upon relative humidity of passing such an airstream through simulated rain, we used atmospheres that were close to saturation in an attempt to simulate humidity observed during rain events (16). Humidity control in the absence of simulated rain or as a preconditioning treatment for samples would be of value in the study of preconditioning effects or in the study of the direct impact of humidity on sporulation in pathogens such as downy mildews. Gottwald and Trocine (18) have recently described a method for the controlled injection of humidified air into the airstream of a wind tunnel to increase humidity. Their design, a refinement of a method described by Leach (23), incorporates a humidity sensor within the tunnel coupled to an external microprocessor-controller to modulate the injection of humid air, and could be readily adapted to our apparatus. The precise control of temperature obtained in our apparatus would be a requisite for reproducible control of relative humidity, especially as humidity nears saturation.

Light intensity and quality. The intensity and quality of light within the apparatus closely approximated that measured during rain at 7:00 a.m. on 24 May 1992 in an apple orchard in Geneva, New York (Fig. 2), when apple trees of the cultivar McIntosh were in bloom. Light levels at 7:00 a.m. during the period between bud break and petal fall of 'McIntosh' have been reported to be associated with elevated rates of ascospore release of *V. inaequalis* in New Hampshire (25). Therefore, light intensity and quality within the apparatus approximated that required to stimulate ascospore release by *V. inaequalis*.

The peak transmission of the red filter measured at the sample platform was 31.9 µW/cm^2 at a wavelength of 675 nm with the intensity of the illuminator at maximum. Intensities measured at other wavelengths were 2.4, 19.4, 30.6, 28.5, 22.0, and 10.3 µW/cm^2 at 600, 625, 650, 700, 725, and 750 nm, respectively. The comparable values obtained for the violet filter were 5.5, 5.5, 4.6, 6.1, 6.4, 3.6, and 0.6 µW/cm^2 at 380, 390, 400, 425, 450, 475, and 500 nm, respectively. The red and violet filters could thus be used to exclude or accentuate the effects of far red light, which was

shown in previous studies to strongly influence ascospore release in certain ascomycetes (7). The use of narrow band-pass filters would increase the precision in selecting particular wavelengths for more detailed study.

Simulated rain. The mean rate of accumulation of simulated rain was 0.89 mm/min (± 0.12 mm/min, $P = 0.05$). Although this was an extremely high rate of rainfall (5.3 cm/h), it was within the range of observed natural rainfall. The large volume of water applied to the sample platform provided uniform wetting and had the added effect of producing extremely stable temperatures at the sample plane.

Reproducibility of results. Similar patterns of ascospore release were observed between scabbed leaf samples collected in New York, Germany, and Sweden (Fig. 3). At a constant tempera-
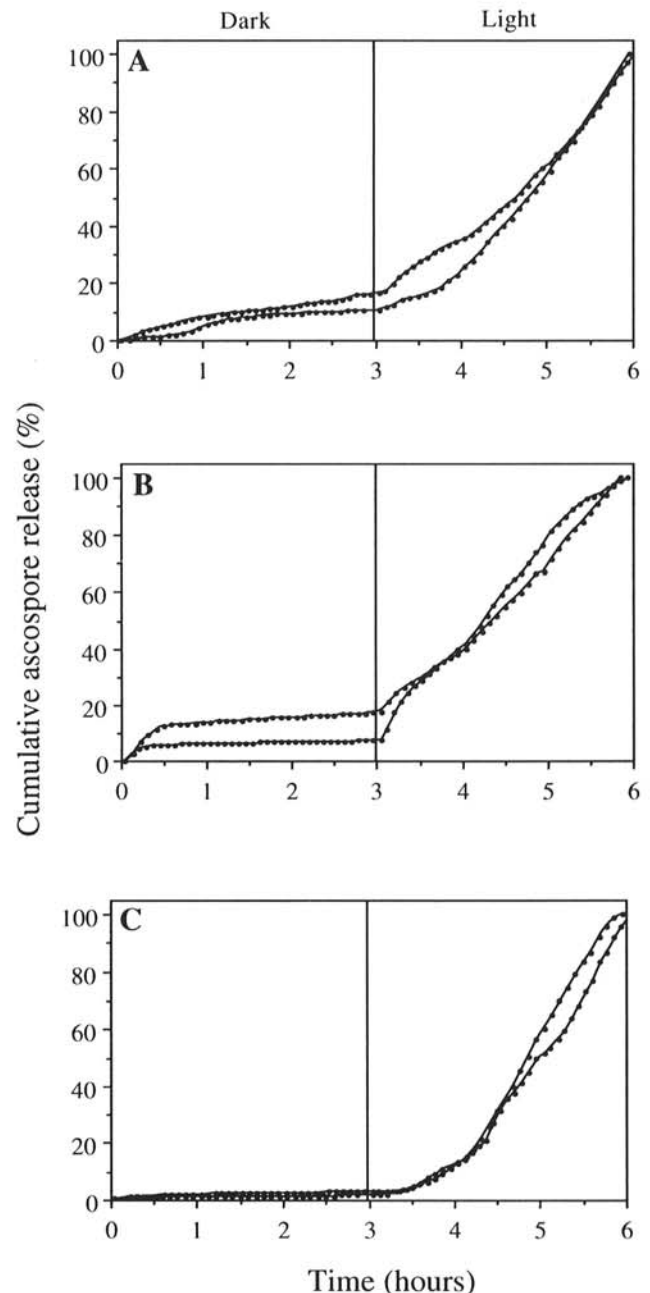

Fig. 3. Release of ascospores of *Venturia inaequalis* in repeated tests of leaves collected at bud break of apple in **A,** Geneva, New York; **B,** Friedrichschafen, Germany; and **C,** Alnarp, Sweden. Twenty 1-cm apple leaf disks bearing numerous pseudothecia of *V. inaequalis* were placed in the tunnel at 5:00 p.m., and left in darkness at room temperature until 8:00 a.m. the following day. They were then exposed to simulated rain for 3 h in darkness, followed by 3 h of light, at a constant temperature of 20°C. The two curves of each graph represent two runs of each test using duplicate samples.

ture of 20°C, a relatively low percentage of the ascospores were released in darkness during the first 3 h of wetting, but the rate of ascospore release increased sharply in light during the second 3-h period (Fig. 3). Linear regression was used to describe the rate of ascospore discharge from 20 to 80% release. Within leaf collections, slope coefficients between runs did not differ significantly at $P = 0.05$.

In three runs of an experiment with a regime of 6 h at 1.0°C followed by 3 h at 20°C, temperature within the apparatus remained stable for 6 h, increased within 15 min to 18 C, and increased within 30 min to 20°C (Fig. 4). Temperature variation during the initial 6 h at 1.0°C was minimal (Table 2 and Fig. 4), as was the rate of ascospore release by *V. inaequalis* (Fig. 4). A dramatically increased rate of ascospore release was consistently observed within minutes after temperature was increased above 1.0°C (Fig. 4). The time required to reach 50% release was more variable than the time required to reach 10 or 90% release (Fig. 4). The coefficients of variability of the time required to reach 10, 50, and 90% release were 1.5, 5.8, and 1.9%, respectively.

Ascospores of *G. bidwellii* were consistently released from pseudothecia during complete darkness at 20°C (Fig. 5A). Nearly identical patterns of ascospore release were obtained in two of three repetitions of the experiment in which ascospore release was nearly complete within 45 min (Fig. 5A). In the third repetition, the rate of ascospore discharge from 20 to 80% release, expressed as the slope coefficient of a linear regression, was significantly less ($P = 0.05$) than that observed in the first two repetitions (Fig. 5A). Nonetheless, in each repetition release was nearly complete within 120 minutes (Fig. 5A). Ascospore release was almost com-

pletely suppressed at 2°C, but the rate of ascospore release increased sharply within minutes when temperature was increased from 2 to 20°C (Fig. 5B). Although leaf wetness and temperature are used in all warning systems for infection by *V. inaequalis* (20,26,36) and *G. bidwellii* (33), the delay of ascospore release at low temperatures has not been used in gauging the risk of infection. We were using the apparatus to quantify the effects of low temperatures on ascospore release of these pathogens, with the intention of refining warning systems to account for this factor (15).

The apparatus used in our study had the following advantages over wind tunnels used in earlier laboratory studies of ascospore release by *V. inaequalis* and other fungi (2,6,19,23): i) light intensity and quality approximated that observed in a field environment and was completely independent of laboratory lighting; ii) internal temperature of the apparatus was independent of room temperature and was precisely controllable from 1 to 20°C; iii) relative humidity was adjusted to approximate that during rain events; iv) continuous simulated rain was applied to samples; v) precise timing of events during experiments lasting up to 24 h could be determined; and vi) trapping of spores was accomplished externally, thus avoiding the attendant problems of waterproofing the trapping surface and mechanism. The use of a nonhumidified air supply has been shown in our preliminary studies (15,16) to override the normal suppression of ascospore release by *V. inaequalis* during darkness, and may explain why this suppression was not observed in certain laboratory and greenhouse studies
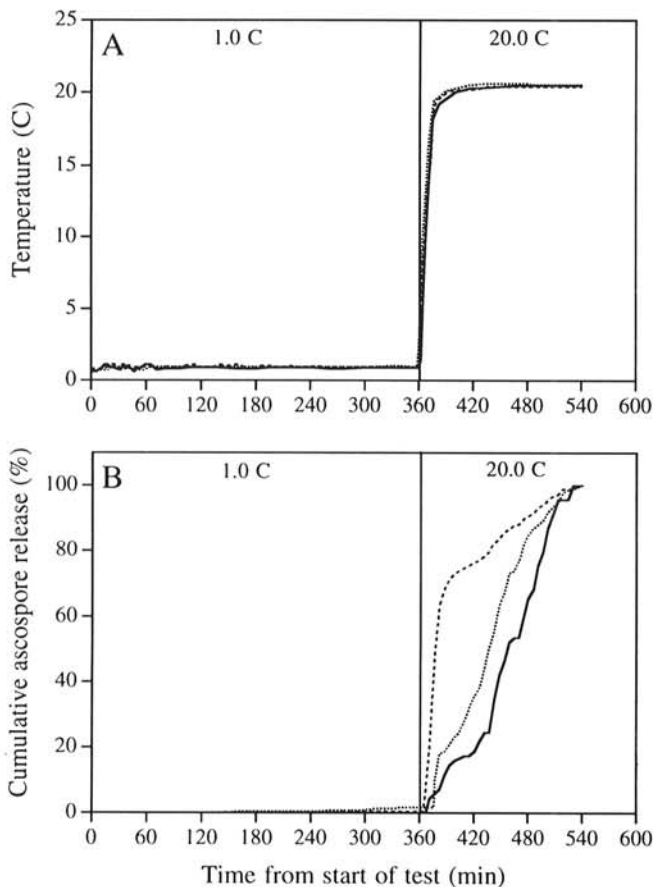

Fig. 4. A, Internal temperature of wind tunnel and **B,** cumulative release of ascospores of *Venturia inaequalis* in wind tunnel. Samples of 20 1-cm apple leaf disks collected at Njøs, Norway, were exposed to light and simulated rain for 6 h at 1.0°C. The apparatus was then readjusted to 20°C for an additional 3 h. The three curves of each graph represent three tests of duplicate samples.
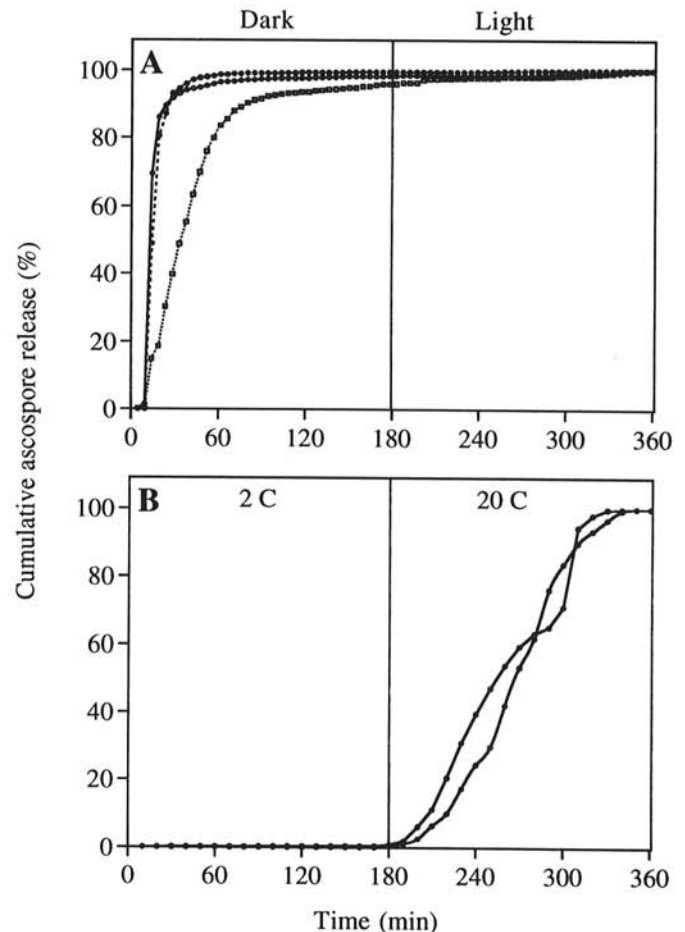

Fig. 5. Release of ascospores of *Guignardia bidwellii* in repeated tests of mummified berries collected during bloom of grapevines in Dresden, New York. **A,** Simulated rain was applied at 20°C to samples of 25 mummified berries maintained in darkness for 3 h, followed by 3 h in light. **B,** Release during simulated rain and continuous light at 2°C for 3 h, followed by 3 h at 20°C.

(17,38). The environmental factors that can be varied within the apparatus (temperature, light intensity and quality, relative humidity, rainfall rate and periodicity, and wind speed) may make it useful in investigations of a number of fungal plant pathogens. We have used the apparatus extensively in our studies of *V. inaequalis* to elucidate the influence of light intensity and quality on ascospore release, the interactions between population maturity and suppression of ascospore release during darkness, the threshold levels of light required to stimulate ascospore release, the duration of ascospore release at various temperatures, and the delay of ascospore release at low temperatures (15,16).

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