Germination of Phyllosticta maydis Conidia in An Incubation Chamber with Control of High Relative Humidities

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ABSTRACT

Control of relative humidity (RH) to within ± 0.6% was achieved in the range from 94% to saturation, in a 1-liter chamber maintained in the dark and at 20 C for 3 days by means of two continuous air streams saturated with water vapor at different temperatures. An accuracy of ± 0.15% at 98% RH was obtained over 5-10 hours. The system was designed to incubate conidia of *Phyllosticta maydis* on detached maize leaf segments or glass slides, but is adaptable for studying various host-parasite combinations on attached leaves or entire plants. Germination of *P. maydis* conidia on glass slides was similar to that on maize leaf segments and percent germination decreased from a maximum at 100% RH to zero at 94% RH.

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An atmospheric relative humidity (RH) in excess of 90% is required by many fungal parasites for successful infection of host leaves and stems (3, 4). Because of difficulties in controlling and measuring RH near saturation, the influence of high RH on spore germination and host penetration by fungi has seldom been closely defined.

In studies on spore germination, RH has usually been controlled in closed chambers by the presence of acid or salt solutions (3, 5). Among the disadvantages of this method are the prolonged equilibration period that occurs after the introduction of the test material to the chamber and the difficulty of accurately measuring the RH inside the chamber. Marked gradients of RH may arise near living plant materials used as surfaces for spore germination in closed chambers.

Open chambers in which the RH was controlled by passage of a continuous air stream of known vapour pressure deficit have given approximate RH control. Delp (5) regulated the vapour pressure deficit of air flowing continuously through an open chamber to within ± 0.5 mm Hg, equivalent to an accuracy of ± 3% RH near saturation at 20 C. In a controlled

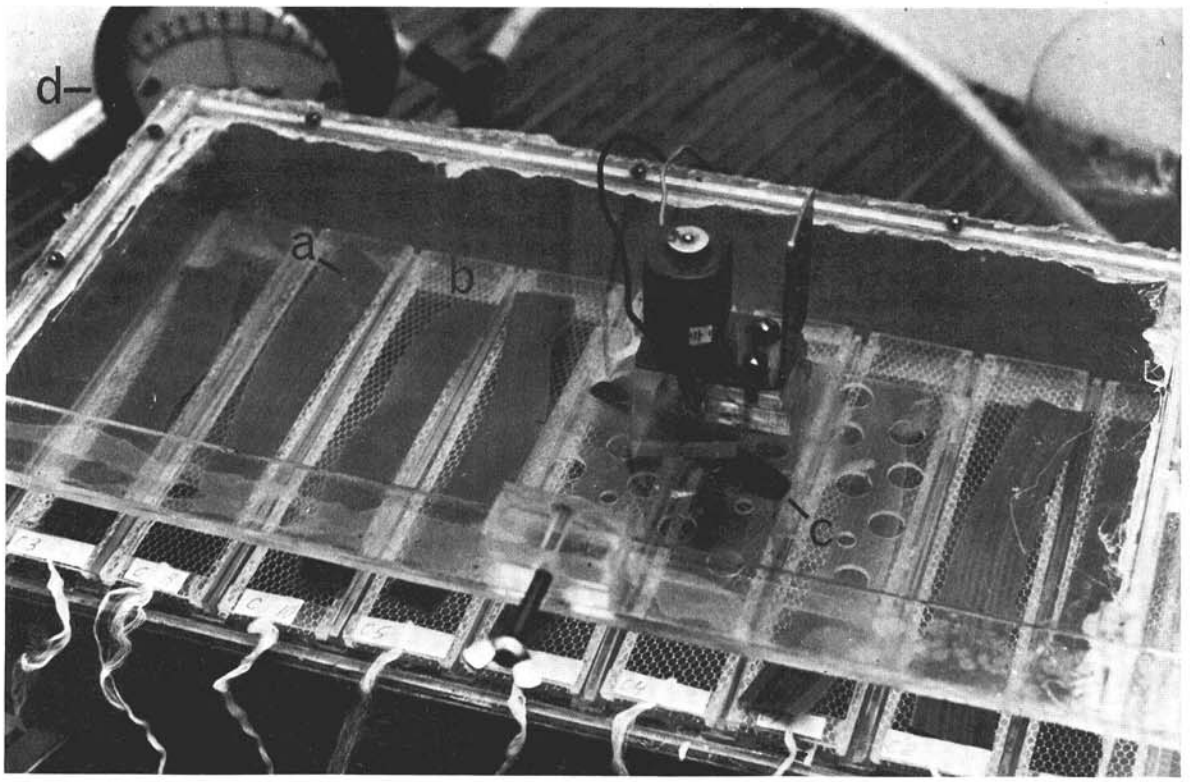

Fig. 1. Chamber used to control relative humidity near saturation, (a) leaf segments, (b) removable trays, (c) fan to provide mixing of air, and (d) pressure gauge.

temperature and RH apparatus, Ormrod & Woolley (6) observed fluctuations in dry bulb temperature of less than ± 0.5 C and in wet bulb temperature of 0.4 C within a 4.2-liter glass chamber, equivalent to fluctuations in RH greater than ± 3% near saturation at 20 C.

The system described here provided control of RH to within 0.6% near saturation at 20 C in a chamber using continuous air streams saturated at different temperatures. The chamber was designed for incubation of conidia of *Phyllosticta maydis* Arny & Nelson on detached maize leaf segments and glass slides (2).

MATERIALS AND METHODS.—A 1-liter plywood chamber (43 X 20 X 11.5 cm) was constructed with an acrylic plastic top (Fig. 1). A removable section on one side, which was backed with silicone foam rubber for an air tight seal, allowed the rapid insertion and removal of samples from the chamber. Rectangular trays for supporting leaf segments were made from a coarse nylon mesh and supported on racks built midway into the chamber. Air within the chamber was mixed by a plastic propeller (7.6 cm radius) attached to a 12v dc high speed motor mounted directly overhead and outside the chamber. To prevent disturbance of the leaf segments by air turbulence, a rectangular section of perforated plastic was mounted directly under the fan.

Two air streams saturated at different temperatures were required to raise the RH above 94% (or lower the dewpoint depression to less than 1 C). Frictional heat created by the rotating fan raised the air temperature inside the chamber more than 1 C above the dewpoint temperature when only one air stream was used. The two saturated air streams were pre-mixed in a small compartment built into the humidity chamber. Here, because of the exponential relationship between saturated vapour pressure and temperature, the vapour pressure after mixing of the two air streams was slightly higher than (rather than equal to) the saturated vapour pressure at the mixture temperature, resulting in some degree of supersaturation. The pre-mixing compartment was perforated to allow the supersaturated air to flow into the humidity chamber where the air temperature was raised by the fan and the RH lowered to a point below, but very close to, saturation.

The RH control apparatus (Fig. 2) was mounted in a commercial controlled-environment cabinet which regulated temperature to within ± 0.4 C. Rapid fluctuations of temperature in the cabinet were damped out to less than our measurement precision of ± 0.03 C within the high RH chamber. Two air streams were pumped with pressure-vacuum diaphragm pumps through a series of gas dispersion tubes which were immersed in distilled water to give finely divided air bubbles that were readily saturated.

Air stream I flowed through three flasks of distilled water connected in series to saturate the air. Both the vacuum and pressure side of the diaphragm pump were either directly or indirectly connected to the humidity chamber. A constricting valve on the vacuum side of the pump regulated the flow rate to 3 to 4 liters per minute (LPM) and maintained a positive pressure of 1 to 2 cm of water on the chamber to ensure that any chamber leaks would be directed outwards. Condensed water in the tubing was trapped by an empty flask to prevent it from entering the pre-mixing compartment. Temperature of thc controlled-environment cabinet and hence of air stream I was maintained at approximately 19 C. Air stream II flowed through an open system containing a series of four flasks of distilled water. The last flask in the series was located on a heater for temperature control of the water in the flask from 19 C to 40 C. With both air streams at 19 C, the RH inside the chamber was 94%, whereas this increased to 98-99% as the water temperature in the last flask in air stream II was raised to 40 C. Air temperature within the incubation chamber remained close to 20 C. Only the pressure side of the diaphragm pump in air stream II was indirectly connected to the chamber while the vacuum side was left open to the ambient air. Flow rate was regulated at 2.5 LPM with a valve. Tygon tubing was used for all air pathways shown in Fig. 2.

The moisture content of the chamber air was monitored with a dewpoint hygrometer (Bendix Model DHGF-1P). Chamber air was directed into the hygrometer through a tygon outlet tube wrapped with heating cable to prevent condensation of moisture on the walls of the tubing and conserve the dewpoint of the sampled air. Tygon tubing may absorb and release moisture slightly, but under the steady state conditions in the system the moisture absorbed by the walls of the tubing would equilibrate with that in the air flowing through the tubing and would not be expected to change the dewpoint. The hygrometer automatically maintains a copper-constantan thermocouple at the dewpoint temperature of the sampled air and we referenced this to a 20-gauge junction mounted inside the RH chamber. The output of this thermocouple pair was thus a continuous record of the dewpoint depression (DD), that is the difference between air temperature and dewpoint temperature inside the incubation chamber. The absolute air temperature inside the chamber was measured by a five-junction 18-gauge copper-constantan thermopile referenced against ice (0 C). Because tests were carried out in the dark, the large thermocouples were free from radiation errors. RH was determined using a table of the saturated vapour pressure over water and the formula

$$RH = \frac{e}{e_s} \times 100\%$$

where e_s = saturated vapour pressure at the air temperature (T_A) of the chamber as measured by the five-junction thermopile and e = actual vapour

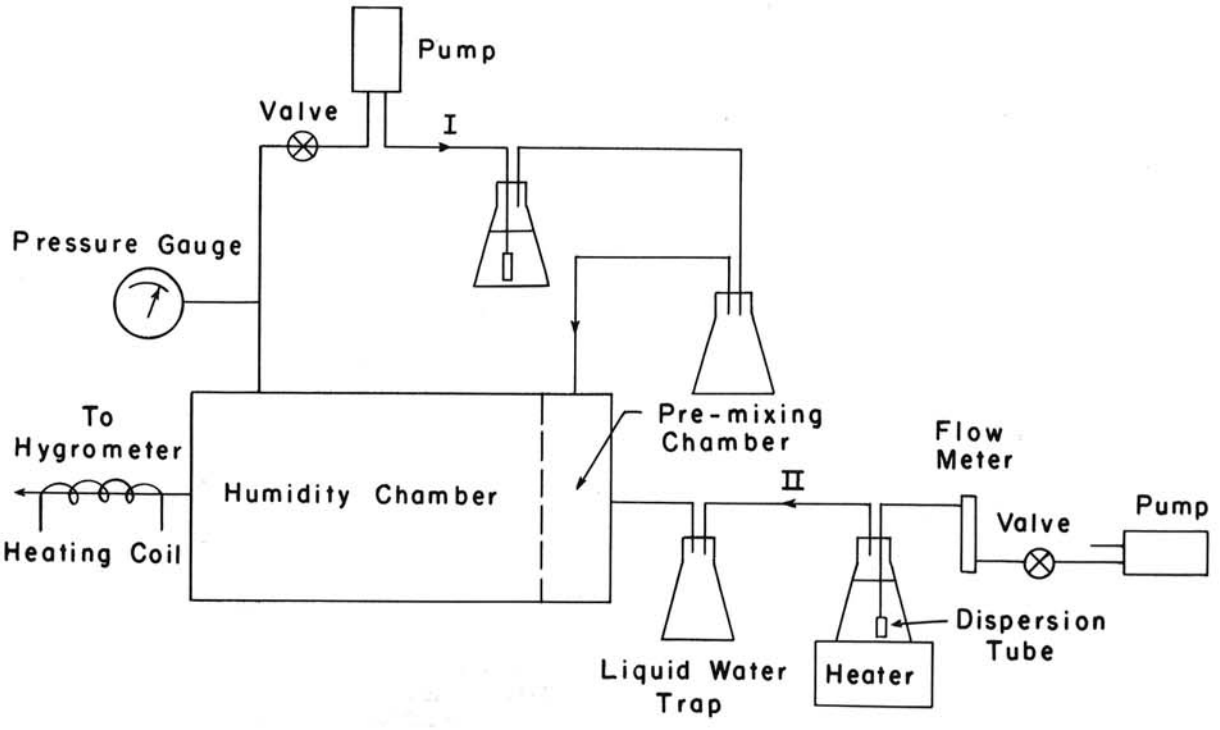

Fig. 2. Schematic diagram of the system used to control relative humidity near saturation. Only one of three distilled water flasks is shown for each air stream.

TABLE 1. Germination of *Phyllosticta maydis* conidia incubated at 20 C on maize leaf segments and on glass slides at various high relative humidities

Average percent relative humidity	Corn leaf segments		Glass slides	
	% Germination	95% CI[a]	% Germination	95% CI[a]
94.2	0		0	
96.2	32	18-46	30	22-38
98.5	50	37-63	52	42-62
100 (Saturation)	91	88-94	79	69-89

[a] CI = confidence interval.

pressure of the air, or the saturated vapour pressure at the dewpoint temperature (T_D) where $T_D = T_A - DD$.

The influence of high RH on the germination of *P. maydis* conidia on maize leaf segments and glass slides was studied using the chamber described above. Conidia were washed with distilled water from *P. maydis* cultures grown on potato dextrose agar for two weeks under cool-white lights at 20 C. The resulting spore suspension was centrifuged and the spores were resuspended in distilled water to give 10^5 conidia/ml. This suspension was atomized onto leaf segments (4 cm × 10 cm) freshly cut from a healthy maize plant and droplets of the suspension were pipetted onto thoroughly cleaned glass slides (1). In four successive experiments, eight inoculated leaf segments and eight glass slides were incubated for 20 to 25 hr in the high RH chamber at average humidities of 100, 98.5, 96.2 and 94.2% (± 0.6%). The percent germination was determined for 25 randomly selected spores from each replication. A spore was considered germinated when the germ tube exceeded 2.5 microns in length.

RESULTS AND DISCUSSION.—The RH in the humidity chamber deviated only ± 0.6% (or ± 0.11 mm Hg at 20 C) during a period of 3 days for selected RH levels in the range of 94% to saturation. The small fluctuations in RH (± 0.6%) generally experienced over several hours are attributed to subtle changes in airflow, fan speed, and water temperature in the heated flask. The RH in the chamber was often maintained to within ± 0.15% of a mean of 98% RH for 5 to 10 hr (equivalent to ± 0.03 mm Hg vapour pressure deficit, or ± 0.025 C DD). Near saturation, DD could be resolved to within ± 0.013 C of its absolute value. The output of the thermocouple in the dewpoint hygrometer was less than 1 μV when both junctions were placed in good thermal contact with each other and maintained at constant temperature. Thus, the instrument error introduced in the measurement of DD is less than 0.025 C since the thermocouple output is 40 μV/Centigrade degree.

Deviations in RH greater than ± 0.6% occurred only when samples were inserted or removed from the chamber. Thus, the RH dropped as much as 5% when samples were inserted, but the equilibrium value (± 0.6%) was regained after 15-30 minutes. When samples were removed from the chamber, the RH dropped less than 1% and returned to equilibrium within 10 min.

The temperature in the humidity chamber was controlled to within ± 0.5 C over a period of 3 days. Temperature drift in the controlled environment cabinet probably caused the slow temperature fluctuations in the humidity chamber. Since the temperature of air stream I was similarly changed, the DD remained constant, and hence changes in RH were immeasurably small. RH changes only 0.25% per degree change in air temperature near 20 C if the DD is constant at 0.5 C.

For accurate control of RH near saturation, a high degree of control of air and dewpoint temperature is imperative. For instance, the RH of air with a constant dewpoint of 20 C changes from 100 to 94% as the air temperature rises from 20 to 21 C. In the system described here, a large lag in response to changes in external temperatures damped out rapid fluctuations of the controlled-environment cabinet and provided the necessary temperature control.

The percent germination of *P. maydis* conidia on maize leaf segments and on glass slides was similar (Table 1). Germination was maximal under free moisture conditions and declined with decrease in RH. At 94.2% RH there was no germination. Conidia kept at saturation germinated in less than 10 hr but with those kept at 98.5% and 96.2%, maximum germination required about 20 hr. Under free moisture conditions, the percent germination on glass slides was slightly lower than that on corn leaf segments. This is attributed to difficulty in maintaining a continuous water film on the glass which resulted in drying and abnormally low germination in some areas of the slides.

RH gradients at the leaf surface were avoided by high turbulent mixing of chamber air and by performing tests in the dark, thus preventing temperature gradients caused by absorbed radiation at the leaf surface and reducing transpiration by keeping leaf stomates closed. Because the rate of germination of *P. maydis* conidia incubated on leaf segments and on glass slides was similar, the measured RH of the air inside the chamber was considered similar to the RH at the leaf surface.

The apparatus described here controlled RH near saturation in an open system to an accuracy that has not previously been documented, thus making possible critical studies of the influence of high RH on fungi growing on many living and nonliving surfaces. Because the system may be modified to accommodate attached leaves or entire plants, the influence of high RH on various host-parasite combinations may be studied.

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