

An Apparatus for Accurate Control of Atmospheric Water Potentials in Studies of Foliar Plant Pathogens

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ABSTRACT

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An apparatus for precise and accurate control (± 10 bars) of atmospheric water potentials (Ψ) is described. Atmospheric Ψ of $\leq -1,000$ to 0 bars at temperatures in the range of 10–50 C can be produced and maintained in the apparatus for up to 5 days. Atmospheric Ψ may be changed and equilibrated at a different level within 10–15 min. In the apparatus,

Peronospora parasitica sporulated on infected cabbage seedlings only when atmospheric Ψ was > -60 bars. The apparatus is suitable for studies of atmospheric Ψ in relation to various stages of the infection cycles of foliar pathogens.

Additional key words: *Brassica oleracea* var. *capitata*, downy mildew, relative humidity.

Foliar plant pathogens require atmospheric moisture for growth and development. Effects of low atmospheric humidities and of free water on foliar pathogens have been researched widely (1,5,11,14). Studies of atmospheric humidities near saturation, however, present special experimental difficulties and rarely have been conducted critically (14,16). Slight temperature fluctuations (± 0.5 C) in near-saturated air may produce condensation on samples making it impossible to discern whether the observed results are due to free water or atmospheric humidities (16).

Control of atmospheric water potentials (Ψ) has been achieved by using various chemical solutions (6,18) or humidified airstreams (2,4). Each chemical solution provides only a single atmospheric Ψ , and no controlled changes are possible. Systems that utilize humidified airstreams may allow controlled changes in atmospheric Ψ during experiments, but accurate control of airstream temperatures is essential for critical control of atmospheric Ψ . Temperature control in most of the apparatus described in the literature has been inadequate (4,13). Systems combining the use of chemical solutions and airstreams also have been reported (10,15), but had limited range of atmospheric Ψ and were sensitive to slight temperature fluctuations.

In the present study, a special apparatus was developed to investigate relations of atmospheric Ψ and development of foliar pathogens in intact hosts. The apparatus is accurate over a wide range of temperatures and atmospheric Ψ . The instrument was used to examine effects of atmospheric Ψ on sporulation of *Peronospora parasitica* (Fr.) Tul. in cabbage seedlings (*Brassica oleracea* var. *capitata*).

MATERIALS AND METHODS

A diagram of the apparatus for controlling atmospheric water potentials is shown in Fig. 1. Components of the apparatus with the exception of the dew-point hygrometer and recorder, are located in a growth chamber (Model EF-7, Conviron Ltd., Winnipeg, Canada R3H 0W9).

Air supply, airflow and air pressure. A clean airstream (free of hydrocarbons or other contaminants) at 700 kPa pressure is supplied by a "dry" compressor (Model VC 350, Canadian Broomwade Ltd., Mississauga, Canada L5K 1A4). The airstream is regulated by a flow meter and microvalve assembly (Cat. No. F-1200; Roger Gilmont Instruments, Inc., Great Neck, NY 11021) to provide a flow rate of 0.5–0.7 L/min entering the apparatus. All tubing for conducting air through the apparatus has an internal diameter of 0.4 cm. Pressure of the humidified airstream is monitored with a manometer attached by a "T" junction to the air line leading from the first bath to the second bath. Airstream pressure deviated from atmospheric pressure by ≤ 0.3 –0.5 kPa.

Air temperature. Airstream temperature is regulated ($\pm \leq 0.05$ C) by passage through two copper coils (each 8 m long) set in two precision water baths. The baths are heated with heater circulators (Tempunit Tu-14; Techne Inc., Princeton, NJ 08540; and Lauda B-1, Brinkmann Instruments Inc., Clarkson, Canada M9W 4Y5). The baths are cooled below ambient temperatures by passing cold water (8–10 C) through coils of copper tubing (8 m long) immersed in each of the baths. Bath temperatures are kept below ambient to prevent condensation from the airstream as it passes from the first bath to the second bath.

Humidity. Atmospheric Ψ is regulated by saturating the air at a desired dew point temperature in the first bath and then adjusting, if necessary, the temperature of the airstream in the second bath. The air is saturated in the first bath by passage through two humidifiers. Each humidifier consists of a cylindrical brass chamber containing a coarse sintered-glass dispersion tube, immersed in glass-stored, double-distilled, deionized water. After humidification, the air passes through two similar (but empty) brass chambers that act as traps for water droplets. An increase in temperature in the second bath lowers the relative humidity (RH) to the desired level before the air passes into the specimen chamber.

Specimen chamber. The sides and bottom of the chamber were constructed of 0.5-cm-thick brass plate, and the lid of 12.7-mm-thick acrylic plastic sheet. The internal dimensions of the chamber are 42 × 15 × 15 cm. The lid attaches to a flange on the chamber sides and is secured with 42 brass bolts. A rubber gasket between the chamber lid and flange ensures a tight seal. The airstream enters the chamber through a 4-mm-diameter orifice located near the center of one side of the chamber and is expelled through a similar orifice at the opposite side.

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Measurement of temperature and calculations of humidity. Water temperatures in each bath are measured continuously with 0.8-mm-diameter (20-gauge) copper-constantan thermocouples referenced to ice. The measurements are compared at intervals with immersion type (± 0.1 C) mercury-in-glass thermometers shielded from radiation. The thermometers were kept continuously in the baths. Dew-point temperature of air expelled from the specimen chamber is measured continuously by an optical-type dew-point hygrometer (Model 440, E.G. and G., Environmental Equipment Division, Waltham, MA 02154). Output of the thermocouples and of the hygrometer are recorded continuously on a strip-chart recorder (Model 7100B, with 17505A input modules, Hewlett-Packard Inc., San Diego, CA 92127).

Relative humidities in the specimen chamber are calculated according to the psychrometric formula $RH = (e/e_0) \times 100$ in which e is the vapor pressure at the dew-point temperature of the air-stream, and e_0 is the saturated vapor pressure at the temperature of the air in the specimen chamber. Values for e and e_0 were obtained from a table of saturated vapor pressures over water. Relative

humidity values were converted to water potentials (in bars) using the formula: $\Psi = (RT/V) \ln (e/e_0)$ given that R is the gas constant, T is temperature in $^{\circ}\text{K}$, and V is the volume of a mole of liquid water.

Calibrations. Each instrument used in temperature measurement was calibrated. The thermometers were calibrated against a similar (but certified by the National Bureau of Standards) mercury-in-glass thermometer. The thermocouple junctions produced $< 1 \mu\text{V}$ when placed in good thermal contact with each other at a constant temperature. At 13 and 18 C, outputs for the copper-constantan thermocouples were 508 and 707 μV , respectively. Accuracy of the recorder was determined with a Dial-A-Volt (General Resistance Inc., New York, NY 10455).

To test the accuracy of atmospheric Ψ control in the specimen chamber, the temperature of the first bath was raised above that of the second bath, which resulted in condensation in the copper tubing leading to the specimen chamber. The voltage output by the dew-point hygrometer was then assumed to correspond to the dew-point temperature of air in the specimen chamber. This

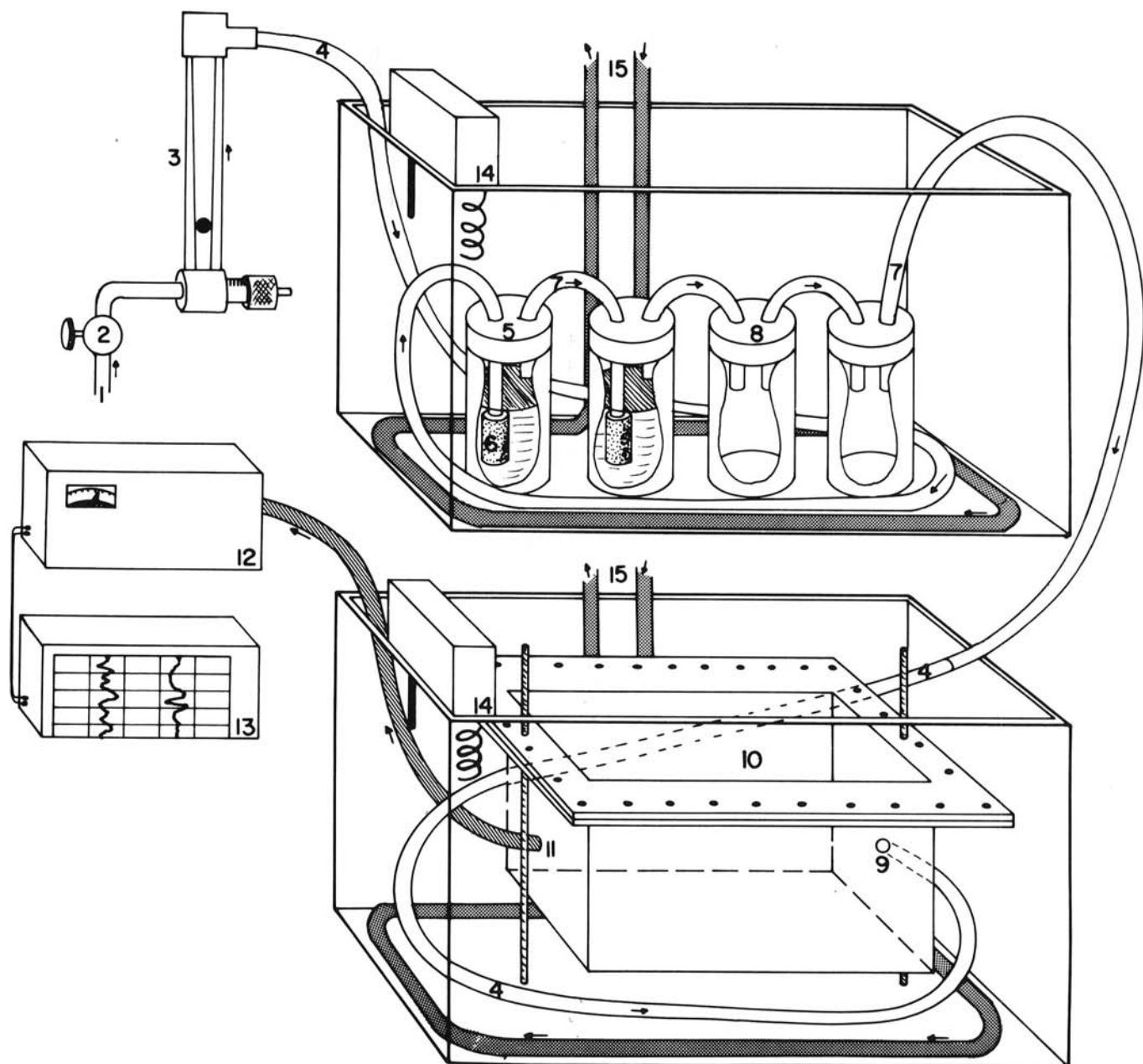


Fig. 1. A schematic diagram of the apparatus for accurate control of atmospheric water potentials: 1, compressed air inlet; 2, needle valve; 3, airflow meter and microvalve assembly; 4, copper tubing; 5, humidifier (in first water bath); 6, gas dispersion tube; 7, tygon tube; 8, water droplet trap; 9, air-inlet orifice; 10, specimen chamber (in second water bath); 11, air-outlet orifice; 12, dew-point hygrometer; 13, strip-chart recorder; 14, heater-circulator; 15, cooling coil.

TABLE 1. Sporulation of *Peronospora parasitica* on cabbage seedlings exposed to atmospheric humidities or to free water at 15 C

Humidities, atmos. water potentials, or free water		Sporangia per mm ²
RH ^a	Ψ ^b	
Free water	0	45
100	0	30
97.7	-30	55
95.5	-60	0
93.4	-90	0

^aRelative humidity (%).

^bAtmospheric water potential (bars).

procedure was repeated for a range of chamber temperatures and a calibration curve was derived. During operation of the apparatus, values from this curve differed from calculated values by < 0.1 C.

Control of atmospheric Ψ in the specimen chamber was tested also with a range of solutions of certified reagent-grade NaCl and double-distilled, deionized water. Molalities of the solutions were calculated according to Lang (8). Sets of glass dishes containing the various solutions were placed in the specimen chamber for 24 hr. Atmospheric Ψ of the airstream was interpolated from the plot of weight change and molality of each solution after the exposure period. The derived Ψ differed from the theoretical Ψ by < 10 bars.

Sporulation studies. Plants of cabbage cultivar Brunswick were grown from seed in a soilless mix (Heco® #1; Heveco Ltd., Tabusintac, N.B., Canada E0C 2A0) for 14 days at 18 C (± 0.5 C) and in a 16-hr photoperiod (60 W/m²). The seedlings were then inoculated with a suspension of *P. parasitica* sporangia (10⁵/ml water) and incubated for 6 days in a mist chamber (16 C ± 0.5, 16-hr photoperiod, 60 W/m²). For each experiment, 80 plants with heavy sporulation of *P. parasitica* were transplanted into glass tubes (5 cm long, 1 cm in diameter) containing the soilless mix. A mixture of paraffin wax and petroleum jelly (6:4, w/w) was applied to the surface of the soilless mix around each plant to prevent evaporation of water. The cotyledons were washed gently in running water to remove sporangia and sporangiophores.

Forty of the plants were then placed in the specimen chamber and exposed in darkness to the desired levels of atmospheric Ψ and temperature. The other 40 plants were placed in a dark mist chamber at the same temperature (± 0.5 C) as the specimen chamber. After 14 hr, 15 plants were collected randomly from the specimen chamber. The freshly produced sporangia were collected by shaking each pair of cotyledons in 2 ml of water. Sporangia in the 2-ml samples were counted with the aid of a settling chamber. The surface areas of the cotyledons were measured with an MOP 3 digitizer (Carl Zeiss Inc., Oberkochen, Federal Republic of Germany), and the number of sporangia per square centimeter of cotyledon was calculated. Plants in which *P. parasitica* failed to sporulate under test conditions were placed for 24 hr in a mist chamber operating at the test temperature and with a 16-hr photoperiod.

RESULTS AND DISCUSSION

The apparatus is an accurate and versatile instrument for studying effects of atmospheric Ψ on pathogenic fungi. Atmospheric Ψ was controlled accurately (± 10 bars) through critical control of airstream temperature (± 0.05 C). A wide range of atmospheric Ψ (≤ -1,000 bars to 0 bars) can be produced and maintained at temperatures ranging from 10 to 50 C. Stable levels of atmospheric Ψ were maintained for periods up to 5 days. Atmospheric Ψ may be changed and equilibrated to a different level within 10-15 min.

The apparatus circumvented many difficulties and limitations encountered in other systems designed to control atmospheric Ψ.

Large fluctuations in atmospheric Ψ, long equilibration periods and changes in concentrations of atmospheric gases often encountered in closed systems were avoided. Controlled changes in atmospheric Ψ have not been achieved in closed systems, but are possible in open systems using humidified airstreams. In open systems, however, accurate temperature control is imperative for critical control of Ψ. Variations of airstream temperatures in most previous apparatus were ≥ 0.5 C, which would bring about fluctuations in atmospheric Ψ of ≥ 50 bars (2,4,10,13). Humidified airstreams can be utilized either as dual or single flow systems. In dual systems, "moist" or "dry" airstreams are mixed to produce various atmospheric Ψ (2,9). In these systems accurate control of atmospheric Ψ requires frequent adjustments of the airflow rates (17). Various valves and flow regulators used in dual airstream systems may interfere substantially with accurate measurement of Ψ. These difficulties were avoided in the single-airflow system we used.

In our apparatus *P. parasitica* sporulated only when infected cabbage seedling were exposed to atmospheric Ψ of > -60 bars (Table 1). Similar responses to atmospheric humidity were reported in other *Peronospora* spp. (3,7,12). The apparatus may be applied to studies of atmospheric Ψ in relation to the entire infection cycle of *P. parasitica* and other pathogenic organisms.

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