A High Humidity Incubation Chamber for Foliar Pathogens

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ABSTRACT

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A chamber used to create and maintain a high humidity atmosphere for inoculations with foliar pathogens of plants is described. The chamber was constructed from polyvinyl chloride pipe, which is inert and rustproof. The clear polyethylene plastic cover on the chamber was replaced easily whenever necessary. Humid air piped into the chamber from two commercial cold-water humidifiers was evenly distributed along the sides of the chamber to provide uniform high humidity throughout the chamber. Relative humidity levels of 99 and 100% were maintained at various temperatures ranging from 14 to 36 C inside the chamber.

Many foliar pathogens require a saturated atmosphere or free water for germination of spores and penetration of host plant leaves (2,6–8). Methods ranging from the use of plastic bags to elaborate or sophisticated dew chambers are used to create and maintain a high humidity or saturated atmosphere conducive to development of plant diseases (6). The object of this report is to describe a humidity chamber that can be constructed easily to provide a uniform area of high humidity for inoculations with plant pathogens.

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MATERIALS AND METHODS

Polyvinyl chloride (PVC) pipe, 13 mm (1/2 in.), was used to construct the chamber (Fig. 1G). The base was constructed using eight straight tees, four 90° elbows, and eight straight sections of pipe. Three straight sections of PVC pipe were connected with two tees in a straight line for each long side. Four other tees were placed, one on each end of the long side pipes, and turned at a 90° angle from the two center tees. Two sections of PVC pipe were joined to the two straight sections of pipe by cementing the tees on the ends (Fig. 1A). A short length of pipe was inserted into the end tee so that a 90° elbow could be placed on the end (Fig. 1B). Glue for PVC pipe was used to seal the joints after they were fitted together and lined up. Rustproof wire could be tied between the two long pipes to strengthen the base.

Four long sections, 2.3 m (7 ft 6 in.), of PVC pipe (13 mm diam.) were arched from one side to the other to form the curved top (Fig. 1G). Small sections were cut from the side of a PVC pipe, 1.82 m (6 ft), so that it could be attached horizontally across the front of the arches for extra support. This provided a convenient attachment for the plastic cover, which could be opened for placement and removal of plant material (Fig. 1C and G). The chamber was then covered with clear polyethylene plastic. The plastic was attached with screws that were placed into small holes drilled into the PVC pipe. Sections of PVC pipe cut lengthwise provided a flexible strip to hold the plastic cover in place (Fig. 1D).

High humidity was provided by two centrifugal atomizing industrial humidifiers (model SW-2, Walton Laboratories, Moonachie, NJ 07074) placed at each end

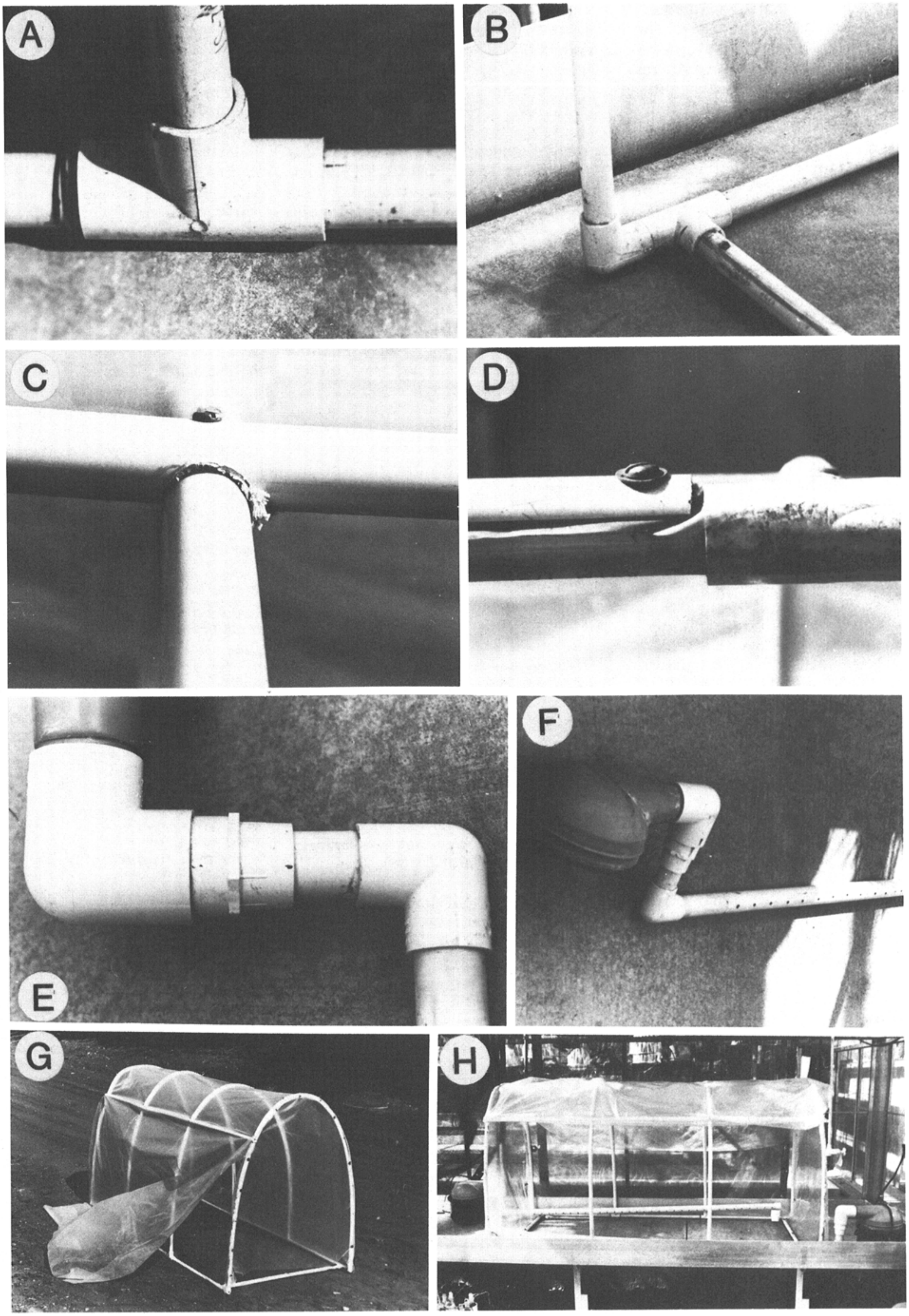

Fig. 1. (A) Straight tee used in construction of the long side pipe, connecting the sides and providing an attachment for the top. **(B)** End straight tee with 90° elbow. **(C)** Cut out on upper pipe so that it can be attached to the front of the chamber. **(D)** Strip of pipe used to hold plastic cover to main frame. **(E)** Pipe connections used to direct humid air from the humidifier into the chamber. **(F)** Same as E with dome and main pipe attached. **(G)** Main frame of chamber with plastic cover attached. **(H)** Completed chamber with humidifiers in place.

of the chamber (Fig. 1H). A water supply was connected to the unit, and water was provided to the humidifier continuously by a self-contained float valve. The humidifiers were wired into a ground-fault electrical circuit with an on-off safety switch located near the chamber. The humidifiers were allowed to run continuously when the chamber was in operation. A humidistat control used to turn the humidifiers on and off in earlier tests failed to function at such high levels of relative humidity.

The humid air was discharged from a directional dome located on top of the humidifier. The main pipe carrying the humid air was directed to the bench surface with two 90° elbows. A 90° elbow of PVC pipe (i.d. 60 mm) was fitted over the end of the humidifier discharge vent (o.d. 60 mm) (Fig. 1E). A reducer (62–52 mm) was inserted in the other end of the 90° elbow and fitted over a short length of 52-mm PVC pipe. Another 90° elbow was attached, and a straight length, 1.9 m (6 ft 6 in.), of 52-mm PVC pipe with a cap on one end was attached. Six-millimeter (1/4-in.) holes were drilled every 5 cm along the pipe (Fig. 1F). This pipe was placed in the chamber at a slight gradient to promote drainage of condensed water (Fig. 1H). Drainage holes were drilled at the lowest point.

During evaluation of the unit, the relative humidity was measured with two wet-dry bulb units (1,5) and a relative humidity probe (model 5120, Weathertronics, West Sacramento, CA 95691), which were ventilated by electrically operated fans. Each wet-dry bulb unit contained a dry-bulb thermocouple to measure ambient temperature and a two-junction wet-dry bulb thermopile to measure the wet-bulb temperature depression. Five low-millivolt signals were integrated and recorded with a CR5 digital data system (Campbell Scientific Inc., Logan, UT 84321). The relative humidity probe was removed from the chamber after 1 hr because of condensation on the sensor chip. It was then used to monitor the relative humidity in the greenhouse in which the high humidity chamber was being evaluated. Two units containing the dry-bulb thermocouples and the wet-dry bulb thermopiles were used in the chamber at all times. Signals for ambient temperatures and wet-bulb temperature depressions were integrated and recorded every 10 min for a total of 92 hr. Percent relative humidity was determined by the depression of the wet-bulb temperature and the ambient temperature (3). The greenhouse in which the chamber was evaluated was maintained at a night temperature of 19 ± 2 C and a day temperature of 29 ± 2 C. On two different days the temperature in the greenhouse was decreased and then increased to determine whether rapid fluctuations in temperature would affect the relative humidity inside the chamber.

RESULTS AND DISCUSSION

The chamber described in this paper has been used for 2 yr with good results. The construction materials are inert, rustproof, and the clear polyethylene plastic cover is replaced easily whenever necessary. The curved top facilitates runoff of condensed water down the sides of the chamber and decreases dripping on enclosed plant material. The size and/or shape of the chamber can be varied easily to fit individual needs, available space, and locations, such as a bench located in a greenhouse or growth chamber. A chamber located in a greenhouse can be shaded with shade cloth or other material to prevent overheating by direct sunlight.

The same model humidifiers used with a similar chamber have been used in previous experiments in which the humidifiers were placed directly in the chamber with or without the directional dome (4). Although good disease development was obtained, the method allowed an uneven pattern of water droplets to develop on plant leaves. More water droplets were present on plants closest to the humidifiers than on those farther away and/or next to the sides of the chamber. With the present design, the humid air was blown uniformly along the sides of the chamber, preventing uneven distribution. The pattern of holes in the main pipe that distributes the humid air can be modified as desired. If the main pipe is not glued in place, it can be replaced with another pipe with different hole spacings or it can be rotated so that the direction of the humid air coming into the chamber can be modified. If a square-shaped or a wider rectangular-shaped chamber is constructed, the pipe may be run easily along the ends of the chamber with the use of a straight tee in the main pipe and another pipe at right angles to the main pipe. Thus, humid air may be directed to any portion of the chamber, toward the sides of the chamber, or to the base of the chamber, depending upon individual needs or particular uses.

When the humidifiers are turned on, it takes a few minutes for the chamber to acquire a saturated, "foglike" atmosphere, but once obtained, the saturated atmosphere is easily maintained. No problems have been encountered with the continuous operation of the humidifiers. Plants can be preconditioned before inoculation by placing them in the chamber for varying lengths of time.

The chamber has been used for 2 yr for incubation of several foliar pathogens on cereals and grasses in a greenhouse. The foliar pathogens used were *Septoria nodorum* (Berk.) Berk., *Septoria spraguei* Uecker et Krupinsky, sp. nov., *Helminthosporium sativum* P.K. & B., and *Pyrenophora trichostoma* (Fr.) Fckl. (Krupinsky, *unpublished*). The infections have been uniform with a 48-hr incubation time. No disease severity patterns due to uneven moisture on leaves of inoculated plants have been observed.

The relative humidity probe indicated a relative humidity of 100% during the first hour of chamber operation. In one wet-dry bulb unit, only five measurements out of 552 indicated a relative humidity below 99%. The second unit malfunctioned during the first 17 hr and indicated a relative humidity below 99% (94–98%) for that period, but for the remaining 75 hr it indicated a relative humidity below 99% only nine times out of 450 measurements. On one of the evaluation days, the chamber temperature was changed from 20 C at 0820 hours to 16 C at 0920 hours, to 24 C at 1020 hours, to 30 C at 1120 hours, and to 35 C at 1320 hours. During these 5 hr the relative humidity remained at 99 and 100% as indicated by both units in the chamber. Relative humidity measured by the relative humidity probe in the greenhouse went from 53% (0820 hours) to 51% (0920 hours), to 38% (1020 hours), to 38% (1120 hours), and to 35% (1320 hours). On another day the chamber temperature was changed from 20 C at 0800 hours, to 14 C at 0900 hours, and to 31 C at 1000 hours. During these 2 hr, the relative humidity measurements in the chamber remained at 99 and 100%. At the same time, relative humidity in the greenhouse varied from 60% at 0800 to 43% at 0900 hours and to 31% at 1000 hours. Thus a relative humidity of 99 to 100% was easily maintained in the chamber at operating temperatures from 19 ± 2 C at night to 29 ± 2 C at day and with fluctuations in temperatures from 14 to 35 C.

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