For investigating the effects of ethylene gas on plant material (e.g., fruit ripening or morphological responses) known concentrations in the test atmosphere of less than 1,000 parts per million (0.1%) are usually wanted. These atmospheres have been variously obtained. Some workers have used ripe fruit or cultures of *Penicillium digitatum* Sacc. as sources of a low (but unknown) concentration of ethylene. Others have used flowmeter combinations, bubble counting systems, or pressure cylinders made up to specified concentrations. In closed systems, low known concentrations of ethylene may be achieved by pipetting small amounts of gas into the system or (for very low concentrations) by pipetting portions of ethylene-saturated water or dilutions thereof (8).

For systems in which a continuous flow of the test atmosphere is desired, accurate regulation of the trace component is difficult. Combinations of capillary flowmeters, such as have been used for modified atmosphere studies (1, 2), are not sufficiently sensitive for regulation of the trace component, although quite satisfactory for major constituents of a mixture. (Capillary flowmeters are to be preferred over rotameters, because they can be made cheaply in any laboratory for any flow rate; they also give more stable readings). If a Mariotte bottle is used to regulate the flow of the trace constituent, the desired accuracy can be achieved in a system that will remain stable and can be calibrated and adjusted easily. This method was used and described by Huelin (6), but when his thesis was published it was not described (7). Griffiths and Potter refer briefly to the technique (5).

The Mariotte bottle (4, 11) should be better known to plant physiologists. Its theory has been described by Schwertz (12) in an application to flowmeter calibration, and applications in post-harvest physiology have been shown by Platenuis (9) and Pratt (10). The Mariotte bottle will maintain a constant very small flow of liquid under a constant pressure; hence, it can be used to displace a small flow of gas from a reservoir through a flowmeter into a larger air flow. Adjustments of the relative rates of flow of the trace gas and the air make a wide range of concentrations readily available. Huelin's basic method (6) is described in this note with the inclusion of modifications which extend its range of usefulness. In our laboratory, the method has been used to maintain concentrations ranging from 0.003 to 1000 ppm.

### Materials

Figure 1 presents the basic method with its useful modifications. The volume of air required for a particular plant sample or group of samples is provided from a supply of pure compressed air which is humidified (H) and metered through a calibrated flowmeter (J). Stable and accurate regulation of this main air stream, over a wide range of flow rates, is accomplished by use of a barostat tower (I). Note that many air supplies are not pure enough for critical investigations or for making reliable concentrations of ethylene in air of the order of one part per million or lower. Air compressor intakes are often located where they may pick up automobile exhaust or other fumes. Hence, for investigating the physiological effects of ethylene, the purity of the control air supply must be investigated.

The central part of figure 1 shows how a Mariotte bottle regulates the addition of the desired trace component to the above air supply through a fine capillary flowmeter which is joined to the main system through a tee. The Mariotte bottle (A) provides the regulation of a steady flow (dropwise) of liquid into the gas reservoir (C). The effective pressure (h) is independent of the volume of liquid in (A) and is adjusted by raising or lowering either the capillary air-inlet tube (B) or the entire Mariotte bottle. (The connection B-G is not needed in a simple system without back-pressure). The liquid displaces gas from the reservoir (C) through the capillary (D), and the rate of gas flow is proportional to the pressure indicated by the manometer (E). The gas then flows into the metered air stream at the tee (F).

The volumes of the Mariotte bottle and gas reservoir are selected in relation to the volume of gas to be dispensed between refills. For instance, to obtain a concentration of 1,000 ppm, 20 ml of ethylene per hour can conveniently be added to an airflow of 20 liters per hour, using a 500 ml graduated cylinder as the gas reservoir and a 4 liter Mariotte bottle; renewal will be required only once a day. For the same concentration in an air flow of 200 liters per

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hour, proportionally larger reservoirs and Mariotte bottles are desirable.

This apparatus may be calibrated by observation of either the volume of displacing liquid or the volume of gas delivered. It is best to calibrate it under the conditions of use by observation of the volume of dis-
will vary and uneven flows result. Another hazard of fluctuating temperature is condensation of water in the capillary flowmeter; for accuracy, the capillary must remain clean and dry.

If any back-pressure exists in the system at (F), due to attachment of further flow controls (fig 1, K), absorbing towers, etc., the simple system described thus far will not work. The Mariotte bottle dispensing system and the main air stream are usually operated at different head pressures, so that a back pressure at (F) will affect the two gas flows disproportionately. In fact, the air pressure is usually so great that any back pressure will cause the Mariotte system (A–F) to be blown out by a strong back flow. If the connection (B–G) is made to the Mariotte bottle air inlet, this difficulty is eliminated.

When the same gas mixture is to be used on several samples of tissue, the complete system shown in figure 1 allows one Mariotte bottle and trace gas supply to serve the entire system. An air supply slightly larger than required for all samples is humidified by the water bubbler (H), measured by the flowmeter (J), and regulated by the barostat tower (I). To this known air supply, ethylene (or other gas) is added as already described. After leaving the mixing tee (F), the dilute gas mixture enters a manifold and flowmeter board (K) of the sort described by Claypool and Keefer (3). Each individual sample is supplied a known total air-ethylene flow which is measured by a calibrated capillary, and these flows are regulated by the barostat (L). The small excess flow (from L) and the flows from all samples should be exhausted to the outside to minimize the ethylene contamination of the storage room.

**Summary**

Traces of ethylene or other gases can be metered accurately into larger air streams by the use of a Mariotte bottle and simple combinations of flowmeters. Special applications are described.

**Literature Cited**