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Semiportable Laminar Flow Hood for Tissue Culture and Microscope Use for Research and Teaching

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terile rooms or reach-in boxes were used to prevent contamination of cultures in early work with plant tissue and embryo culture. The new sterile hoods, which are a spinoff of the aerospace program (2), make continuous work at transferring tissue cultures comfortable and reduce contamination. The laminar flow hood, evaluated by Coriell and McGarrity (1), is particularly comfortable and effective. These hoods are large, heavy, and expensive pieces of equipment and not entirely suitable for protoplast manipulation and other microscope work. This report will describe a sterile hood, which can be built easily with readily obtainable components and has advantages over many of the available commercial units. The laminar flow hood described is reasonably portable and minimizes vibration of the work surface, making it ideal for microscope work.

The basic components are a High Efficiency Particle (HEPA) filter, a fan to pump air through this filter at 30.5 m (100 ft)·min^{-1} (1) and a containment chamber for the nearly particle-free air. Figure 1 shows a diagram of the hood and Fig. 2 shows the completed hood. The separation of the blower unit from the plenum to the HEPA filter provides a vibration-free work surface. This separation also means the system can be uncoupled and have some degree of portability.

The hood is assembled with off-the-shelf components. The HEPA filter should be purchased in a tested condition (i.e., probed for leaks), and should be protected during construction. Inadvertent punctures in the filter can be sealed with silicone rubber caulking material. Since the hood will be located in moderate temperatures and dry conditions, an ordinary HEPA filter with a particle board frame, aluminum separators, rubber-based adhesive, and foam rubber gaskets can be used. Air passes into the filter from behind by the use of a plenum. A plenum 15-cm deep attached to the filter gave adequate air distribution in the chamber, as measured with a hot-wire anemometer. The HEPA filter plenum and prefilter blower unit are connected with 10-cm dryer vent hose.

The blower unit should be capable of moving 30.5 m (100 ft)·min^{-1} air through the HEPA filter. Care should be taken to use a foreward curve blower as the other styles would be too noisy for most laboratory purposes. The air volume required can be determined by multiplying the surface area of the HEPA filter by air flow rating. Thus, a 1 × 2 m (3 × 6 ft) filter will require a 61 m^3·min^{-1} (1800 cfm) blower. The HEPA filter has about 2.5 cm (1 inch) water gauge static pressure (SP) drop, so the blower must be rated to move 61 m^3·min^{-1} at 2.5 cm SP. The direct-drive blower used had a split-capacitor 4-speed motor. The necessary volume of air was moved at a lower speed, which allowed considerably longer use of the HEPA filter. As the filter loads with particles, there is increased resistance that can be overcome by increasing the motor speed to give the

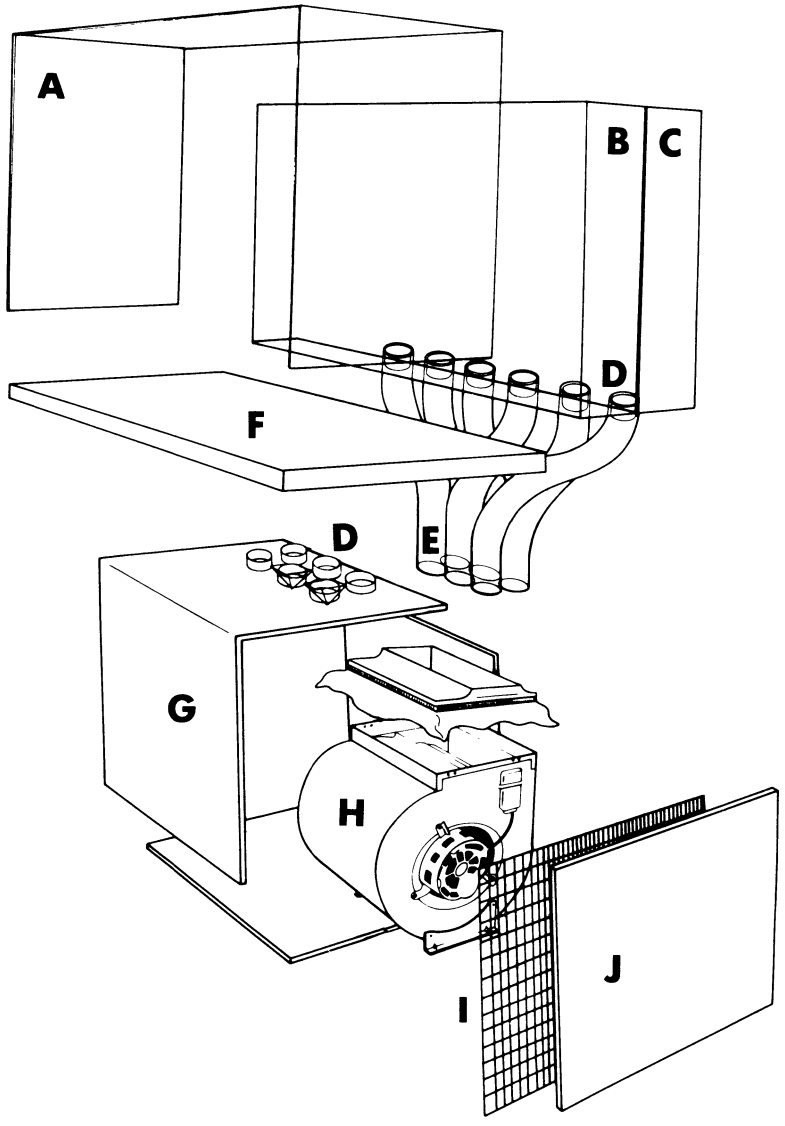

Fig. 1. Diagram on construction of laminar flow hood. **A.** Sterile air enclosure made with 4.8 mm plexiglass set into 12.7 × 12.7 mm aluminum channel 3.2 mm thick, fastened to HEPA filter frame with screws. Channel also added to top front edge to prevent sagging. **B.** A 76 × 122 cm HEPA filter (Part no. 459984, Mine Safety Appliances Co., Pittsburgh, Pa.). **C.** Plenum 15-cm deep made with 1.9-cm plywood and connected to filter frame with metal straps and woodscrews. **D.** Nipples made from 10-cm aluminum irrigation pipe 6 cm in length, epoxy-glued into plywood frame. **E.** Dryer vent hose 10 cm diameter. **F.** Table top or work bench. **G.** Prefilter motor enclosure, prefilter both sides. **H.** Forward curve high pressure blower (Model 4C686, Dayton Elec. Mfg. Co., Chicago, Il. connected to nipple area with Ventglas flexible air fabric. **I.** Filter holder 2.5 × 5 cm fence wire material set in 2.5 cm channel. **J.** Polyurethane prefilter material 40 pores per centimeter.

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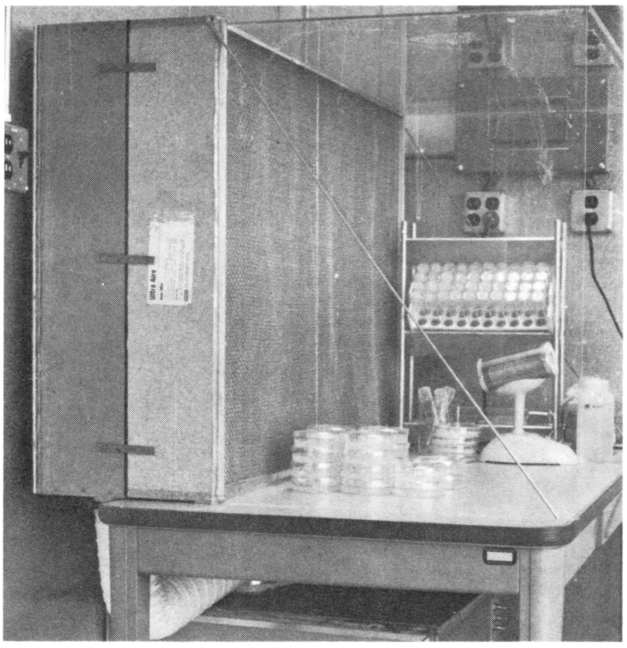

Fig. 2 Photograph of constructed laminar flow hood in operation.

same volume of air. The blower was mounted in a separate box arrangement to allow for complete prefiltration of the air by a 40 pore·cm^{-1} (100/inch) polyurethane foam filter, which adds considerable life to the HEPA filter.

Figure 1 illustrates a single hood arrangement with a vibration-free work surface. This arrangement could be modified easily by making 3 or 4 smaller plenums and filters 50 × 50 cm (2 × 2 ft) and concurrently smaller plexiglass covers. Each of these units, could be fed with 2 dryer vent hoses from the prefilter fan unit to make sterile transfer areas for 3 or 4 students. The prefilter blower unit could be located outside the room to provide a slight positive SP to prevent dust or spore laden air from entering a culture room as well as providing a sterile laminar flow hood.

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Effect of Zeatin and 2iP on Shoot Proliferation of Three Highbush Blueberry Clones in Vitro

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N-(3-methyl-2-butenyl)-1*H*-purin-6-amine (2iP) has been used to promote multiple shoot formation in previous tissue culture studies with ericaceous plants (1, 3–7). Fordham et al. (3), however, found that (*E*)-2-methyl-4-(1*H*-purin-6-ylamino)-2-buren-1-ol (zeatin) was the most effective cytokinin for stimulating shoot proliferation of cultured Exbury azalea (*Rhododendron* sp.). This study was conducted to determine if highbush blueberry is similar to Exbury azalea in its response to zeatin.

Shoot tip cultures of 3 highbush blueberry clones (G-694, G-355, and G-224) were grown on a blueberry micropropagation medium (8) containing 0.025 mM 2iP. Then, from these cultures, unbranched axillary shoots about 1 cm in length were subcultured on WPM media (5) containing 0, 0.0185, 0.037, 0.074, or 0.148 mM zeatin (mixed isomers) or 2iP with 10 replications per treatment. Cultures were grown at 25°C under 16-hr photoperiods (55–60 μmol·s^{-1}·m^{-2} at the level of culture container) using warm-white fluorescent lights. The number of living shoots 0.5 mm or longer were counted at the end of 6 weeks.

Zeatin induced proliferation of 2 to 4 times as many shoots as 2iP (Fig. 1). Increasing the concentration of either cytokinin had a similar effect on shoot proliferation. The above experiment was repeated by subculturing one unbranched axillary shoot about

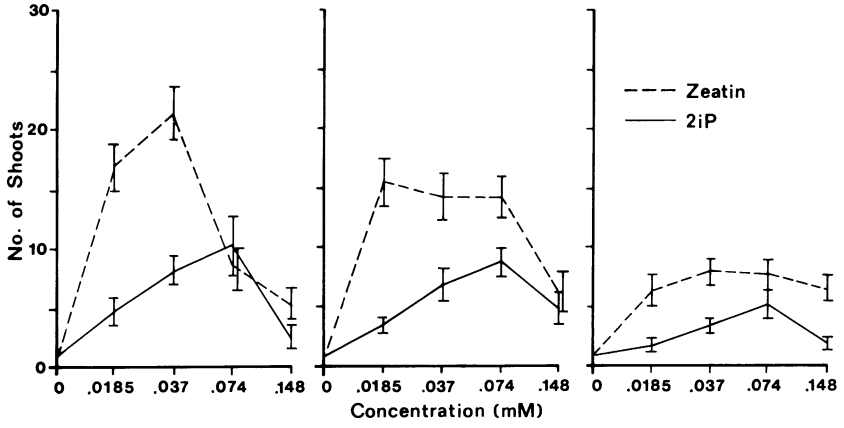

Fig. 1. Effect of zeatin and 2iP on the number of shoots produced by highbush blueberry clone G-694 (left), G-355 (center), and G-224 (right) shoot tips 6 weeks after subculture. Vertical bar represents ± SE.

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