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An Economical Laminar-flow Microbe-free Chamber for Culturing Small Plants[1]

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An economical plant growth chamber, 16 cm in diameter and 50 cm high, was made from two 4-liter glass beakers. The bottom was removed from one beaker, and a 1 cm hole cut in the bottom of the other. The bottomless beaker, when placed atop the other, telescoped slightly for firm support. Sand was placed in the bottom of the chamber as a growth substrate. A polyethylene bag was placed over the two beakers and filtered air (under pressure) and nutrient solution entered the chamber through rubber tubing. Six chambers, each with an open petri dish of nutrient agar in the bottom, were tested for 2 weeks, and all dishes remained microbe-free. The laminar flow of air between the plastic bag and the glass walls of the beakers prevented microbes from entering the chamber. The chambers supported good growth of corn and soybean seedlings for 4 weeks when a sterile nutrient solution was added to the sand as needed.
INDEX DESCRIPTORS: gnotobiology, axenic culture, sterile culture, germ-free culture.

Researchers concerned with relationships between plants and microorganisms found it necessary to develop a method for growing plants under microbe-free conditions. The design of chambers for growing plants under aseptic conditions was influenced by the objective of the researcher and usually was planned with a view of discontinuing the cultivation long before plant maturity. Culture vessels such as glass tubes (Bromfield, 1958; Tillet, 1966; Miflin, 1969), glass cylinders (Kathrein, 1951; Szember, 1960), Erlenmeyer flasks (Harris, 1956; Waris, 1958), Warburg flasks (Joy and Folkes, 1965), bottles with screw-type caps (Keim, 1953; Rovira and Bowen, 1966), and a bell jar (Sobieszczanski, 1963) were used for culturing plants from embryos, callus tissue, or seeds.

Various types of cabinets have been used as chambers for the growth of plants. Estey and Smith (1962) designed one of the first chambers that was gas sterilizable and grew plants from seed to maturity in a sterile environment. The chambers were constructed of wood and glass and were fitted with a front panel with armholes bordered by flanged aluminum rings to which rubber gloves were attached. Air and water were drawn into the chambers through a series of filters. There was no arrangement for the transfer of material into and out of the chamber.

In 1957, Trexler and Reynolds developed a flexible film chamber for rearing bacteria-free animals, and which could also be used for growing plants. Shoulder-length rubber gloves attached to the plastic walls allowed for mechanical manipulations within the chamber. A double-door lock was also built into the chamber wall for material transfer into and out of the chamber without contamination. Lindsey (1967, 1970) used this type of chamber for growth of peanuts, tomatoes, corn, and beans under aseptic conditions. Such chambers offer the advantages of reliable aseptic conditions, ample room for plant growth, mechanical manipulations, and excellent plant growth conditions. However, they have the disadvantages of being expensive, occupying considerable laboratory space, and being much too large for seedling work. The objective of this research was to devise an economic, relatively small plant growth chamber that would provide a reliable aseptic environment and support good plant growth.

MATERIALS AND METHODS

Materials used for chamber construction consisted of two 4-liter Pyrex glass beakers (16 cm diameter), four number nine rubber stoppers, one crystallizing dish (17 cm diameter), one polyethylene bag (45 cm long × 20 cm in diameter), aluminum foil, masking tape, rubber sheeting, two hose clamps (7 cm diameter), glass wool, one 1-liter Nalgene graduated cylinder (6 cm diameter), rubber tubing (6 mm ID), one tube household cement and one screw-type hose pinch clamp. The bottom was removed from one beaker and a 1-cm hole was cut in the center of the bottom of the other (Fig. 1). The bottomless beaker, when placed atop the other, telescoped slightly for firm support. Sand (10 cm depth) was placed in the bottom of the chamber as a growth substrate. A plug of glass wool placed over the 1-cm drainage hole in the bottom of the chamber before the sand was added, allowed for adequate drainage and prevented loss of sand. The crystallizing dish, aluminum-foil skirt, rubber stoppers, and rubber tubing were autoclaved before use. For autoclaving, the bottomless beaker was removed from the lower beaker containing the sand. The polyethylene bag was sterilized in 80% ethyl alcohol. Chamber components were assembled in a laminar-flow hood after seed was sown in the sand at a depth 1.5 cm. Masking tape was used to fasten an aluminum-foil skirt, 3 cm wide, to the outside of the lower beaker just above the crystallizing dish. This skirt excluded microorganisms from nutrient solution, which occasionally drained from the chamber and was a possible source of contamination.

Two openings 10 mm in diameter were cut on opposite sides of the plastic bag just above the top rim of the upper section of the chamber for entry of the air-supply line and nutrient solution line. These openings allow entry of the rubber tubing without tearing the plastic, but are not sufficiently tight to exclude outside air. The openings were made airtight by cutting two pieces of rubber sheeting 3 cm × 3 cm and by cutting in the center of each piece a 7-mm-diameter opening. Each 7 mm opening was then centered over a 10-mm bag opening and the sheeting glued to the plastic bag with household cement. Filtered air (under pressure) was brought to the surface of the sand in the chamber through the tubing, and the flow controlled with a screw-type clamp. Strips of tissue paper 6 cm × 3 cm were held directly below the plastic bag near the outer wall of the chamber to adjust chamber air flow. The clamp was tightened until air flow was reduced to the point that the paper strips moved only slightly. It is important that the air-supply line reaches the bottom of the chamber so that carbon dioxide does not accumulate. About 20 cm of nutrient solution line was left outside the chamber, and the end of the line was kept sterile by covering it with a piece of sterile aluminum foil held in place with masking tape.

The nutrient solution used for plant growth was described by Howell and Bernard (1961). Stock solutions of components were autoclaved separately and added to sterile distilled water to obtain sterile nutrient solution, which was delivered to the chamber by a

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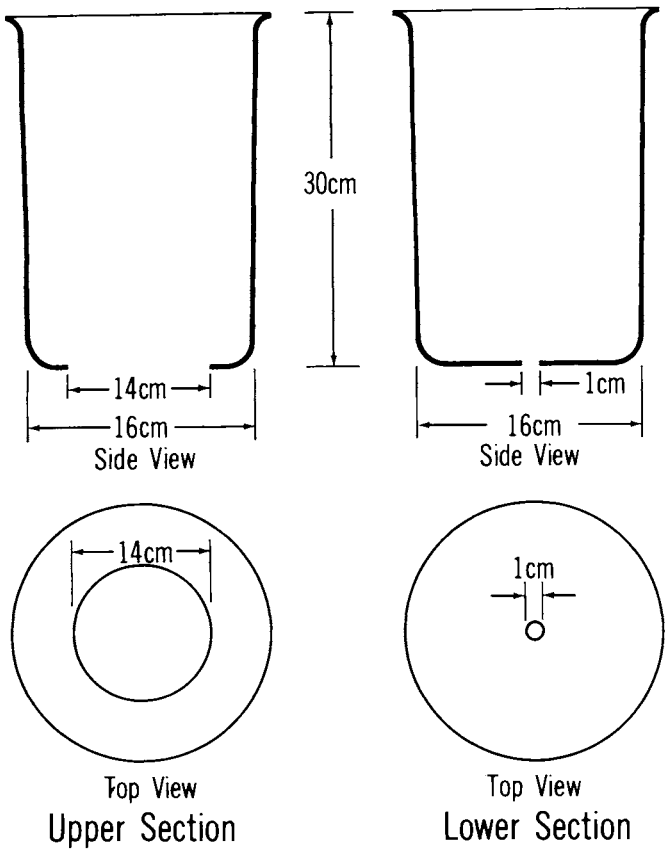

Figure 1. Side view and top view of upper and lower sections of a chamber for culturing microbe-free plants.

gravity-flow system. Plants were grown at 25C (±3C) with 30,000 lux of light.

The effectiveness of the chamber in excluding microorganisms from the chamber interior was tested by using six chambers, each with an open petri dish of nutrient agar in the bottom. A second dish of nutrient agar was opened outside each chamber, 30 cm from the base. The dishes outside the chambers were replaced each day to prevent bacterial colonies being overgrown by fungi. The covers were replaced on dishes after the exposure period and the dishes were stored at 26C. Bacterial and fungal colonies were marked and counted as they developed. The test was terminated after 2 weeks.

Possible growth reduction of plants caused by less light reaching the plants through the plastic bag was tested by growing 10 corn plants and 10 soybean plants in covered and uncovered chambers for 4 weeks. Each treatment was replicated six times. After the test was terminated, mean plant height and mean fresh weight of the plants in each chamber were determined.

Because the chambers were developed primarily for studies of seed transmission of bacteria in soybean seed, the effectiveness of the chamber in determining internal seed transmission of *Corynebacterium flaccumfaciens* (Hedges) Dowson, the causal bacterium of bean [*Phaseolus vulgaris* (L.)] wilt in seed was determined. Three bean cultivars, Red Kidney, Pinto, and Blue Lake, were grown in the field and were either inoculated with *C. flaccumfaciens* or left noninoculated. Seed was harvested at maturity and stored at 25C for 6 weeks before use. Before being planted, seed was placed in a 0.5% sodium hypochlorite solution for 15 minutes, removed, and rinsed six times with sterile distilled water. Ten plants of each of the three cultivars were grown separately in individual chambers. There were two treatments, 1) seed from inoculated plants was sown, and 2) seed from noninoculated plants was sown (controls). Each treatment was replicated four times for each cultivar. The test was terminated when the fourth trifoliate leaf had expanded. Any plants showing necrotic lesions on leaves that were typical of bean wilt were recorded as instances of seed transmission. Confirmation, by isolation of the bacterium, of presence of *C. flaccumfaciens* in leaf lesions was obtained from at least one diseased plant in each chamber in which seed transmission was recorded. The method of isolation of the bacterium and its identification has been described (Dunleavy, 1983).

RESULTS AND DISCUSSION

The laminar flow of air between the plastic bag and sides of the flasks was effective in eliminating bacteria and fungi from the interior of the chamber (Table 1). The population of microbes in the air was sufficient to adequately test the chambers. There was a slight reduction in mean plant height and mean fresh weight of both soybean and corn plants when the chambers were covered with plastic bags, but these reductions were not significant ($P<0.05$) (Table 2).

The chambers functioned well for the bacterial seed transmission test. *C. flaccumfaciens* was transmitted internally in bean seed of the three cultivars that were inoculated in the field, but not in control seed (Table 3). Mean percentage of seed transmission in seed from inoculated plants ranged from 13 in Pinto to 25 in Blue Lake. Chlorotic spots developed on unifoliate and trifoliate leaves shortly after these leaves unfolded from the bud and expanded. These spots then turned tan as the leaf tissue died. Bacteria isolated from such spots were all gram positive, produced a water-insoluble yellow pigment, and produced bean wilt symptoms when inoculated to bean. All isolates were identified as *C. flaccumfaciens*.

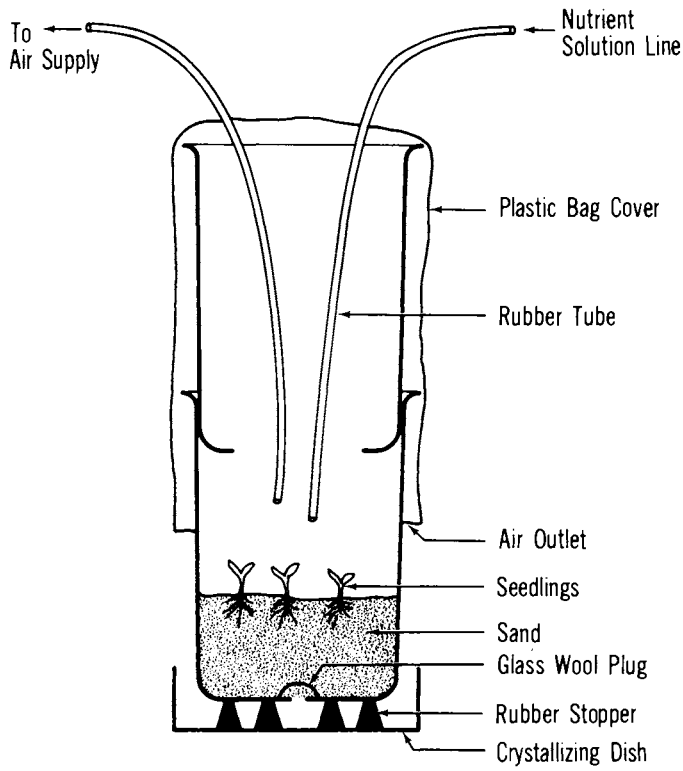

Figure 2. Side view of the completed chamber for culturing microbe-free plants.

Table 1. Mean number of bacterial and fungus colonies that appeared on nutrient agar plates placed outside and inside six chambers for 2 weeks.

Type of organism	Mean number of colonies	
	Outside[a]	Inside
Bacteria	308**	0
Fungi	140**	0

[a]Nutrient agar plates outside the chamber were replaced daily.
**Differs significantly from inside treatment ($P<0.01$) according to LSD.

Table 2. Mean height and fresh weight of 10 soybean and 10 corn plants grown separately in covered and uncovered chambers after 4 weeks of growth.

Crop	Mean[a] height (cm)		Mean[a] fresh weight (g)	
	Uncovered	Covered	Uncovered	Covered
Soybeans	12.6	11.2NS	30.2	28.7NS
Corn	15.7	13.9NS	35.6	33.8NS

[a]Mean of six replications.
NS = Not significantly different from the uncovered treatment ($P<0.05$) according to LSD.

Flexible film chambers described by Trexler and Reynolds (1957) have been widely used for growing plants in microbe-free environments, but these units are too large (97 cm × 61 cm × 97 cm) for small plant work. The chamber described in this study has several advantages not afforded by the Trexler and Reynolds units. The chambers are compact, and all available interior space can be utilized. For very small plants, the upper portion of the chamber can be removed. Laboratory bench space usually is at a premium, and the chamber occupies an area only 20 cm × 20 cm.

Additional advantages of the chamber are economy, simplicity, portability; also, each chamber (except plastic bag) can be autoclaved, and is essentially maintenance-free. The chamber can be disassembled and stored in a relatively small space when not in use. They are adaptable for a variety of plant studies. For example, they can be placed in a larger plant growth chamber where temperature can be controlled. The chief disadvantages of the chamber are that it can be used only for small plants or seedling growth of large plants, and there is no provision for access to plants during the period of plant growth.

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Table 3. Comparison of the mean percentage of internal seed transmission of *Corynebacterium flaccumfaciens* in three cultivars of beans (*Phaseolus vulgaris*). Plants were inoculated with *C. flaccumfaciens* in the field (control plants were not inoculated), seed was harvested and tested for bacterial seed transmission in the sterile environment of a growth chamber.

Plant treatment	Mean[a] seed transmission		
	Red Kidney	Pinto	Blue Lake
	%	%	%
Inoculated	15**	13**	25**
Control	0	0	0

[a]Mean of four replications
**Differs significantly from the control ($P<0.01$) according to LSD.

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