different sugary corns was 10 μg per gram. The comparable average for crosses of T13, a high-niacin starchy corn, was 23 μg per gram. Furthermore, the coefficient of correlation for the concentration of niacin in sugary and starchy kernels from individual segregating ears of these crosses was 0.95 with 28 D/F. The su gene thus exerted a more or less consistent influence in increasing the niacin proportionally to the level otherwise established. That level presumably was determined by the multiple-factor system demonstrated by Richey and Dawson (2). The larger excess brought about by su when the underlying level was higher seems physiologically significant, requiring more than a difference in thickness of aleurone for explanation.

A DEVICE FOR CONTINUOUS AERATION AND NUTRIENT RENEWAL OF ENTIRE PLANTS UNDER STERILE CONDITIONS

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Several methods for the sterile culture of whole plants have been described in recent years. In some of these (2, 4, 6, 14) only the roots are maintained in a sterile medium. In others (3, 5, 7, 11, 12, 13, 15, 16) the entire plant is enclosed. None of these techniques for aseptic culture of enclosed whole plants provides concurrently for continuous flow of the nutrient solution and for forced aeration, although such a method for excised roots has been reported (9). A simple device which accomplishes both of these purposes is described here. It can be assembled from standard items of glassware and other supplies which are available in most laboratories or from supply houses. The apparatus has the further advantage of being usable either as a single unit or, by T-tube connections, as a battery of units interconnected to air line and nutrient solution reservoir. A set of as many as four assembled units may be sterilized in a reasonably large autoclave without dismantling. While the device is best suited to seedlings, small plants like Bacillus chrysostoma (13) may be grown throughout their life cycle. The technique has been developed to aid in a study of the effects of pure cultures of microorganisms on the uptake of mineral nutrients, but it is adaptable to many other types of cultures.

One culture-chamber unit (fig 1) includes the following parts: (1) a 500-ml tubulated pressure (filter) flask, (2) a 38 × 300 mm culture tube, (3) a 21-cm length of 6 mm O.D. glass tubing which interconnects the pressure flask and the culture tube, (4) an 80 × 30 mm open-ended glass cylinder containing 3 mm or 5 mm glass beads and resting on the rubber stopper which is used to close the culture tube, and (5) a 1/4 inch O.D. T-shaped glass connecting tube, with one arm of the "T" inserted through the 2-hole stopper which closes the culture tube, the other arm joined by rubber tubing to a short length of capillary glass tubing (nutrient solution inlet), and the leg of the "T" connected by rubber tubing to a second length of capillary glass tubing (air inlet). Because of the desirability of sterilizing the unit without disassembling it, all rubber stoppers and tubing should be resistant to steam sterilization and all glassware should be of pyrex or other heat-stable glass.

Several parts of the apparatus serve dual purposes. The heavy glass pressure flask is both a support for the culture device and a sump for the flowing nutrient solution. The glass tubing which elevates the culture tube above the pressure flask also provides an overflow for the nutrient solution in the culture tube and an air outlet. The glass beads act both in promoting aeration of the nutrient solution and in anchorage of the roots. The T-shaped connecting tube permits simultaneous entry of air and nutrient solution into the culture tube, although rates of flow of air and nutrient solution may be independently regulated.

When the device is in operation, nutrient solution enters the T-shaped connecting tube from the lower arm of the "T," air enters from the leg of the "T," and both air and nutrient solution pass together through the other arm of the "T" into the culture tube. It has been found that a more even flow of solution into the culture chamber is obtained by this arrangement than if air enters from below. Sterile air and sterile nutrient solution enter the culture chamber at the base of the open-ended glass cylinder holding glass beads. When the nutrient solution has risen to a height just slightly below the rim of the cylinder holding the beads, it drains from the culture chamber

LITERATURE CITED


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With a multiple setup of several culture chambers, some difficulty may be experienced in obtaining a satisfactorily uniform rate of flow of nutrient solution through all culture tubes, because of varying resistances in the system. This can largely be overcome by using capillary tubing of slightly different internal diameters for the nutrient solution inlets. In preliminary flow tests using water, the capillary tubes are interchanged as necessary to equalize flow to the desired degree. During the course of an experiment, if differences in flow rate develop among culture units, they may be corrected by use of open-sided Hoffman clamps. The nutrient solution flow rate may be regulated by placing a clamp in the nutrient solution line of an individual culture unit. It may also be adjusted by using a clamp to increase or decrease the rate of air flow to the unit, since the air flow rate to some extent affects the rate of solution flow.

It is generally easier to maintain uniform flow of nutrient solution among culture tubes at 100 ml or more per culture per day, although considerably lower rates of flow are adequate for plant needs. Rates of air flow have not been measured, but no more air is pumped through the cultures than the minimum necessary to permit adjustment for uniform flow rates among cultures.

With a continuous flow of nutrient solution, it is possible to maintain quite low concentrations. One fifth strength Hoagland (8) solution has been found to give excellent plant growth. A stable form of iron is essential both to avoid the necessity of adding iron to the nutrient solution during the course of an experiment and to reduce the formation of precipitates which tend to clog the capillary nutrient solution inlet. The chelated EDTA iron complex of Jacobson (10) is satisfactory, although use of an equivalent amount of iron as ferrous ammonium sulfate instead of ferrous sulfate in preparation of the complex makes a much more stable stock solution, and the iron seems less precipitable by the pH changes caused by contact with roots. One of the newer sequestrans, diethylentriamine penta acetic acid (Alrose Chemical Company, Providence, R. I.), is also preferable to the more commonly used ethylenediamine tetra acetic acid (EDTA).

The sterilization of the culture chambers, either singly or in a battery, is accomplished as follows. A Koby air filter is connected to the air inlet of the culture chamber, a tubular fritted glass filter of ultrafine porosity (Corning No. 35000) joined to the nutrient solution inlet, and all air outlets are plugged with cotton or glass wool. The assembled apparatus is then autoclaved at 15 pounds pressure for 30 minutes. The air filter may then be hooked to an air line, and the bacterial filter submerged in a reservoir of nutrient solution. The nutrient solution filter is not necessary if the nutrient solution itself is sterilized and care is then taken in aseptically joining the nutrient solution inlet of the culture chamber to the nutrient solution reservoir. As a double check against penetration of the filter by microorganisms, the nutrient solution is passed through an 8 inch length of quartz-glass tubing
exposed to continuous ultraviolet illumination of principally 2537 Å wave length.

The chief possibility of contamination in this device, as with most others, is through introduction of fungal spores or bacteria into the culture chamber along with the seed or seedling. Fungal contamination is more readily controlled than is bacterial contamination. If absence of growth of microorganisms on selective bacterial or fungal media inoculated with nutrient solution effluent is used as evidence of sterility, all cultures in some later tests were apparently free of fungi, but not more than 70% of these later cultures were free of bacteria. Their complete freedom from fungal contamination seems to indicate, however, that if bacteria-free seeds or seedlings are introduced into the culture chambers, the apparatus as described here will keep them sterile.

Two methods of starting plants in the culture tubes have been used with the aseptic culture setup described here. One method involves the surface sterilization of seeds with hypochlorite solution under reduced pressure, the thorough rinsing of the seeds with sterile water, and the aseptic transfer of the seeds to the culture chamber, where they are allowed to germinate while resting on the glass beads. This method has two distinct disadvantages, first that all seeds may not germinate, thus eliminating some cultures, and second that microbial contamination is not easily detected. The second method has been found to be more satisfactory. In this method, the seeds are surface-disinfected as before, but then are transferred aseptically to sterile nutrient-agar media in petri dishes where they are germinated. Only the healthy seedlings which show no evidence of contamination are then transferred aseptically to the culture chamber where their growth continues under sterile conditions. Care must be taken to orient the seedlings properly on the glass bead support. Otherwise the developing roots are delayed in reaching firm anchorage.

Checks for contamination during the progress of an experiment may be made either by plating out the effluent from the culture chamber on media which favor rapid appearance of bacterial or fungal colonies, or more conveniently, if specific organisms are not to be identified, by merely placing a small quantity of glucose in the bottom of the pressure flask before the apparatus is autoclaved. Nonsterile cultures quickly develop cloudiness in the overflow nutrient solution if glucose is present.

As with other sterile-culture devices in which the entire plant is enclosed, the apparatus described here poses the problem of growth of plants in an atmosphere of high humidity. An overhead bank of 40-watt fluorescent lamps, spaced at 2 inch intervals, with a ratio of five "soft white" to one "daylight" lamp, has been found to provide adequate illumination (about 1000 fc at the top of culture tubes) without evidence of excessive heating. But even in an air-conditioned room in which temperatures are closely controlled, it has been found that there is some condensation of water on the inner walls of the culture tubes, especially in tubes located at the border of the bank of overhead lights. However, this has not seemed to produce abnormal seedlings, even in cultures which are found to be contaminated. The high humidity might nevertheless be expected in some cases to complicate experiments in which pure cultures of microorganisms are deliberately introduced. Seedlings generally have been maintained in the growth chambers for two to four weeks in apparently normal condition, and culture for longer periods seems limited mainly by lack of growing space.

Certain modifications of this device would be useful under special conditions. For very small seeds and seedlings, the filter paper cone support described by Audus (1) might advantageously be substituted even for the small (3 mm) glass beads. If the plant is to be introduced into the culture chamber as a seed rather than as a seedling, the seed could be inserted into the culture tube with less risk of contamination if dropped through the hole of a rubber stopper closing the top of a glass cylinder of the type used by Loo (13), than if the entire tube must be detached to permit insertion of the seed as is done here. However, the stopper at the top of the tube would reduce the effectiveness of overhead lighting.

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LITERATURE CITED


FIXATION OF N$_2^{15}$ BY EXCISED NODULES$^{1, 2}$

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Consistent fixation of nitrogen by excised soybean root nodules has been demonstrated recently by Aprison and Burris (2), although previous experiments (4, 7, 8) had yielded positive results only occasionally. The success of the present method is attributed to the short time lapse between picking the nodules and placing them under a gas mixture containing N$_2^{15}$, and the subsequent analysis of only the acid soluble portion of the nodules for N$_2^{15}$. Lincoln variety soybeans were used originally (2), and the present paper describes an extension of the testing method to excised nodules from other varieties of leguminous plants.

The methods used were modified only slightly from those already described (2). The nodules were picked and sized through a cascade of 4 plastic cups with perforated bottoms which would retain nodules of the following sizes: Size 1, over 6 mm in diameter; size 2, between 5 and 6 mm in diameter; size 3, between 4 and 5 mm in diameter; size 4, less than 4 mm in diameter. The screened nodules were washed in ice water, rapidly weighed and transferred to Warburg vessels containing 0.5 ml H$_2$O, and gassed with a mixture of 10 % N$_2^{15}$, 20 % O$_2$, and 70 % He. The N$_2^{15}$ contained 31 atom % excess N$_2^{15}$. The flasks were shaken for 1 or 2 hours at temperatures from 22 to 24°C. The nodules then were ground with 50 ml of 3 N HCl. The mortar was rinsed with two 5 ml portions of 3 N HCl, and the mixture was centrifuged. The supernatant was subjected to Kjeldahl digestion; the ammonia was distilled, converted to N$_2$ with alkaline hypobromite, and analyzed for N$_2^{15}$ concentration with a Consolidated-Nier mass spectrometer.

Table I records the fixation of N$_2^{15}$ by excised nodules from field grown legumes. Cowpeas and three commercial varieties of soybeans were tested. The best fixation was observed with Lincoln variety soybeans planted early in the season. The same variety planted late in the season under less favorable conditions showed inferior nodule growth and poorer fixation by the excised nodules. Only two results with Blackhawk approach the best fixation observed with Lincoln, and Hawkeye variety was inferior to both the other varieties. Cowpea nodules gave distinctly less fixation than any of the soybeans, although the nodules were well developed and red-centered. Decreased fixation.

Table I

<table>
<thead>
<tr>
<th>VARIETY OF PLANT</th>
<th>DATE OF PLANTING</th>
<th>AGE AT HARVEST, DAYS</th>
<th>SIZE OF NODULES (SEE TEXT)</th>
<th>ATOM % EXCESS N$_2^{15}$ OF REPPLICATES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine Max, var. Lincoln (soybean)</td>
<td>5/8</td>
<td>63</td>
<td>2</td>
<td>0.241; 0.248</td>
</tr>
<tr>
<td>Glycine Max, var. Blackhawk</td>
<td>5/13</td>
<td>59</td>
<td>2</td>
<td>0.077; .....</td>
</tr>
<tr>
<td>Glycine Max, var. Hawkeye</td>
<td>5/28</td>
<td>64</td>
<td>2</td>
<td>0.058; 0.093; 0.112</td>
</tr>
<tr>
<td>Vigna sinensis (cowpea)</td>
<td>5/28</td>
<td>44</td>
<td>2</td>
<td>0.016; .....</td>
</tr>
</tbody>
</table>

*1 to 3 gm wet weight of nodules per flask; temp., 22 to 24°C; nodules washed and 0.5 ml H$_2$O added per flask; time of exposure to N$_2^{15}$, 2 hours.
** Exposure for one hour.
† These nodules were sliced; no water was added to the slices.

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$^3$ Predoctorate fellow of the National Science Foundation.