Literature Cited


Apparatus for Growing Plants With Aseptic Roots for Collection of Root Exudates & CO2

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Introduction

The aseptic culture of plant roots is required for certain studies on root metabolism and on the interrelations between root and soil microflora. The nature and activity of the rhizosphere population presumably is influenced greatly by materials which leak or are excreted from roots (24). Moreover, the apparent susceptibility to root-infecting pathogens of some plants only at certain stages of their development may be related to the type of materials excreted (3) and to the CO2 and O2 status within the rhizosphere. Inasmuch as some microorganisms, including certain plant pathogens, are tolerant to high pCO2 and low pO2 (30, unpublished results, Timonin, Stotzky, & Goos), enrichment of CO2-tolerant pathogens and suppression of CO2-intolerant, non-pathogenic, competitive microorganisms in the rhizosphere may be an important factor in pathogenesis. Progress on these problems requires a technique for growing, for extended periods of time, plants with aseptic root systems which will enable: A, collection of root exudates for quantitative and qualitative analysis; B, measurement of root respiration, in order that the CO2 concentration in soils may be partitioned between that released by roots and that by the soil and root microflora (16, 18, 26); and C, evaluation of the effects of added organic materials or microorganisms on root and plant development. Moreover, the technique should facilitate complete removal of exudates in a minimum volume, in order to eliminate carry-over from one collection to another and to minimize concentration for subsequent analysis. To ensure that the organic materials are derived only from intact roots, contamination with seed material and damage to the roots must be prevented.

An evaluation of procedures described in the literature for the aseptic culture of plants (1, 3, 5, 6, 7, 12, 13, 14, 17, 18, 20, 22, 25, 26, 27, 32, 33, 34, 35, 36, 38, 40, 41) indicated that these would not fulfill all the requirements. With some procedures (3, 12, 17, 20,
long periods must though several respiratory activities of the above-ground plant parts. Such a separation eliminates the elaborate equipment required to grow entire plants for extended periods of time in hermetically sealed systems (21).

Although several apparatuses (9, 10, 18, 26, 32, 37, 41) have been described to measure root respiration, few (18, 26, 32, 41) employed aseptic conditions. Many investigators (1, 10, 13, 14, 18, 20, 34, 36, 40, 41) used liquid media, but, as root activity in liquid probably differs from that in solid media, this was undesirable. Most apparatuses which utilized a solid rooting medium (2, 3, 5, 6, 7, 9, 12, 17, 20, 22, 25, 26, 27, 32, 33, 35, 37, 38) provided no easy means of removing exudates or of adding sterile irrigation solution when required. Only a few procedures (1, 13, 14, 18, 36, 40) separated the seed from the root zone, a requirement necessary to distinguish between materials released from roots and from seeds. Because of the large variability between replicated plants (41), it was desired to culture concomitantly a sufficient number of plants to satisfy the application of statistical methods.

The plant culture unit herein described fulfills all the necessary requirements and is adaptable to numerous studies. The apparatus incorporates various procedures described in the literature, and has been employed for several years with a variety of plants.

Materials & Methods

Description of Plant Culture Unit: The plant culture unit consists of several parts and is illustrated in figures 1 and 2.

The carrier consists of the body (125 x 95 x 390 mm) and the top (100 x 175 mm), both constructed from plywood (3⁄4 in.). The top section is cut to fit over the planting, air outlet, and auxiliary tubes, and is fastened to the body with two bolts and wing nuts. A wooden housing for the sterility check tube and the mercury valve is attached to the side. The carrier is treated with sealer and varnish to withstand repeated autoclaving.

The planting tube consists of two sections of glass tubing (40 mm O.D., 60 mm long): the lower section, covered at one end with plastic-coated glass-fiber screening, fits into and is flush with the bottom of a No. 13½ rubber stopper; the top section is fastened to the bottom section with tape². The screening is cut randomly to facilitate root penetration and is secured to the tube by pressure of the rubber stopper. Vermiculite is placed in the planting tube to a depth of approximately 35 mm.

The root chamber, constructed of glass tubing (75 mm O.D., 300 mm long) flared at both ends to provide a tight fit with No. 13½ rubber stoppers, is supported on a wooden platform fastened to the inside of the carrier. The pressure of the top section of the carrier holds the root chamber in place and maintains an air-tight seal with the rubber stoppers. To ensure against possible leakage, tape³ is wound around the junction of the glass and the rubber stoppers. A sheet of heavy aluminum foil is placed over the chamber to prevent exposure of the roots to light. Auxiliary tubes of glass (7 mm O.D.) are inserted in both the upper and lower stoppers and closed with a serum seal.

The irrigation sprinkler is a glass tube (7 mm O.D., 300 mm long) with a T-joint at the top to which rubber tubing, perforated at 1 cm intervals, is attached. The holes are positioned so that the irrigation solution sprays downwards at approximately a 45° angle. The straight portion of the sprinkler protrudes through the bottom stopper of the root chamber and is connected to the irrigation chamber.

The solution-return and aeration tube is an L-shaped glass tube (7 mm O.D., 40 mm long), covered

² Time Tape, Professional Tape Co., Inc., Riverside, Ill.

³ Scotch Electrical Tape No. 33, Minnesota Mining and Manufacturing Co., Minneapolis, Minn.
on one end with plastic-coated glass-fiber screening secured by a sleeve of rubber tubing. A section of glass tubing (9 mm O.D., 60 mm long), covered on the top with screening through which the L-tube is inserted, is held in the bottom stopper of the root chamber and covered with a pad of glass wool. Omitting the L-shaped piece or the glass wool results in air-blocks and plugging of the tube with the rooting medium, thereby preventing return of the irrigation solution. A screw-clamp fastened to the carrier controls the flow of air and solution.

The irrigation chamber is a glass tube (50 mm O.D., 130 mm long) flared at both ends to accommodate No. 10 rubber stoppers, which are held securely by wire and sealed with tape. Three glass tubes are inserted in the top stopper: one extends to within 5 mm of the bottom stopper and connects to the irrigation sprinkler; a second, terminating approximately 1 cm below the stopper, connects to the solution-return and aeration tube; and the third terminates just below the stopper and connects to the inlet air filter. Two glass tubes are inserted in the bottom stopper: one connects to the irrigation reservoir, and the other to the sterility check tube and mercury valve.

The sterility check tube is a dropping bottle (28 mm O.D., 90 mm long) containing several ml of a nutrient solution (1% glucose; 0.2% peptone; 0.3% yeast extract; 0.05% K\textsubscript{2}H\textsubscript{2}PO\textsubscript{4}). The dropping tube is inserted through the top of the rubber bulb, from which the tip has been removed, and is connected with rubber tubing to the irrigation chamber.

The mercury valve is a vacuum trap (outside tube: 12 mm O.D., 160 mm long; inside tube: 7 mm O.D., 120 mm long) containing approximately four centimeters of mercury. A piece of rubber tubing, containing a short glass tube, is attached to the side arm of the trap. A serum seal or a small test tube containing 70% ethanol is placed around the glass tube to prevent entrance of microorganisms. Both the sterility check tube and the mercury valve are connected to the irrigation chamber with a T-tube and rubber tubing. By manipulation of clamps, the irrigation solution is shunted either to the sterility check tube or through the mercury valve.

The irrigation reservoir is a 2- or 4-liter bottle fitted with a rubber stopper containing two glass tubes: one is attached to the irrigation chamber and the other to an outlet tube in the upper stopper of the root chamber. Hoagland’s solution No. 1 (11) is used routinely, but a reservoir containing sterile water may be attached in parallel, so that alternate irrigation with water will minimize the accumulation of salts in the root zone.

The air filter is constructed from a dropping bottle (28 mm O.D., 90 mm long) from which the bottom is removed. The cut end of the bottle is forced into a hole (26 mm I.D., 20 mm deep) in a rubber stopper containing a glass tube (7 mm O.D., 40 mm long), and 2 cm of Silastic RTV 502 (29) is poured into the bottle around the tube. Placing a rubber plug over the cut end rather than inserting a tapered stopper (as shown in fig 1) and the addition of Silastic ensures an air-tight seal and prevents the air pressure from extruding the stopper. The glass dropping tube is inverted and forced through the rubber bulb, from which the tip has been removed, until the flared end is flush with the flat portion of the bulb inside the screw cap. A filter membrane between two pre-filter pads is placed inside the screw cap, and cotton or glass wool is placed inside the bottle. The flat portion of the rubber bulb acts as a gasket and prevents air leakage when the bottle and cap are screwed together. Tape is wound around the screw cap and bottle to ensure against possible leaks, and the filter is fastened to the side of the carrier with a tool clip (not shown in fig 1). When larger air flows are required, a baby nursing bottle and nipple which provides a larger filtering surface may be used in place of a dropping bottle.

The CO\textsubscript{2} collector is a glass tumbler containing NaOH and a glass-head bubble tower (28) and is attached to the root chamber with rubber tubing. An air filter and a one-way check valve are inserted in this line to prevent microbial contamination during replacement of the CO\textsubscript{2} collector. To facilitate replacement, a quick-disconnect is also inserted.

\begin{itemize}
\item Nalgene No. 6120, Nalge Co., Inc.
\item Nalgene No. 1276, Nalge Co., Inc.
\end{itemize}
Pyrex-brand glass, with the exception of the dropping bottles, and sulfur-free latex tubing, which did not deteriorate after repeated autoclavings and extended exposure in the greenhouse, are used throughout. All connections between glass and rubber tubing are bound with wire to prevent separation. The dimensions and materials presented for the various components are those routinely used in this laboratory; however, units differing in dimensions and construction materials have been used successfully.

► Aeration: Organic impurities and CO₂ are removed from the aeration stream by a series of scrubbers consisting of concentrated H₂SO₄, 4× KOH, and finally water to remove all traces of alkali and minimize drying of the roots (28). The air is distributed to individual units through a sterilized manifold. The scrubber solutions, however, do not remove all microorganisms, and an air filter is required.

Cotton or glass wool alone is not an adequate filter, as both are easily wetted, and microorganisms, especially fungi, grow through either. Polystyrene filter membranes⁵ shrink during autoclaving, resulting in loss of filtering efficiency. Membranes of cellulose esters⁶ remove air-borne contaminants from a dry air stream, but the surface tension of water films at the gas-liquid interface at each of the pores prevents air passage when the membranes are wet. Although the flow of air through wetted glass fiber membranes⁸ is not markedly impaired, some fungi can grow through and sporulate on the under surface of wetted membranes. When glass wool or cotton is combined with either cellulose esters or glass fiber membranes, efficient removal of air-borne contaminants is achieved. The cotton or glass wool absorbs moisture and keeps the filter membrane dry. Filters were aseptically replaced monthly, and no contamination entered the plant unit through the air stream.

A uniform rate of air flow through the manifold and into each unit is maintained with a needle valve and monitored with a flow meter and a manometer.

► Sealer: A sealing material is required to separate the photosynthetic and respiratory activities of the above-ground portions of the plant from the respiration of the roots, and to prevent microbial contamination of the root zone. The material must: A, be impermeable to gases and water; B, be non-toxic to plants; C, cause no physical damage to plants; D, be sufficiently pliable to permit normal enlargement of stems; E, withstand a continuous positive pressure of 0.5 to 1.0 psi; F, resist microbial and environmental deterioration; G, be autoclavable; H, be easily handled and applied, and I, be relatively inexpensive. Although a large number of materials were tested, some of which are shown in Table I, only one compound was suitable for use as a sealer. This material is a silicone fluid, which, upon the addition of a catalyst, is vulcanized at room temperature into a rubber-like solid. This material and its use have been fully described (29).

► Surface-Sterilization of Seed: Although Ca(OCl)₂ was the most effective surface-sterilant tested, it did not remove deep-seated contaminants present in some seeds (8). Benzalkonium chloride (1:1,000 dilution of a 12.8% solution) removed most of these microorganisms and also increased germination percentages, but the resultant seedlings were stunted and abnormal in appearance. Use of HgCl₂ was discontinued, inasmuch as seeds apparently retain considerable quantities of mercury, even after extensive washings (4, 17). All seeds, therefore, are surface sterilized by agitation on a wrist-action shaker for 45 minutes in a Ca(OCl)₂ filtrate [10 g Ca(OCl)₂, 30-35% active chlorine, in 150 ml water] (39). Individual seeds, without rinsing, are then partially submerged in a semi-solid nutrient solution (same composition as that used in the sterility check tube plus 0.5% agar) contained in test tubes. The tubes are placed in the dark, and the seeds allowed to develop into small seedlings. Vigorously growing aseptic seedlings are selected, washed in several aliquots of sterile distilled water, and transferred to the growth units.

► Rooting Medium: Although soil is the natural rooting medium, water logging, poor aeration, adsorption of root exudates by soil colloids, solubilization of soil organic matter, etc., negate its use, and an inert porous medium is desired.

To determine the recovery of root exudates from various rooting media, vermiculite, perlite, and quartz sand were separated into different particle sizes, placed in root chambers, and rinsed until the wash water was free from turbidity. Standard solutions of glucose and glutamic acid were then added to the top of the media, and 100 ml water perfused through the

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⁵ Microsorban filters, Gelman Instrument Co.
⁶ Millipore Filter Corp., pore size: 0.45μ; Gelman Instrument Co., pore size: 0.40μ.
⁷ Gelman Instrument Corp., pore size: 0.25μ; H. Reeve Angel & Co., Inc., pore size: ultra fine.

![Fig. 3. The sequential (S) and cumulative (C) recovery of glucose and glutamic acid from various rooting media.](https://example.com/fig3.png)
media from the irrigation chamber via the irrigation sprinkler. The solution was allowed to return to the irrigation chamber, removed, and analyzed for glucose (23, 31) and glutamic acid (19). A fresh 100 ml of water was added to the irrigation chamber, and the entire process was repeated eight times. The recovery of glucose and glutamic acid was considerably better from sand than from perlite or vermiculite (fig 3). Seven to eight washings of perlite, but only three washings of sand, were required to achieve 100% recovery. The recovery of glucose was greater than that of glutamic acid from either perlite or vermiculite, suggesting that adsorption of glutamic acid but not of glucose (31) occurred. Adsorption was most marked with vermiculite and with the perlite fraction which passed a 40 mesh sieve. Perlite is essentially inert6, and any adsorptive capacity apparently is associated with the smaller size particles, inasmuch as adsorption of glutamic acid increased as particle size decreased. The exchange capacity of commercial vermiculite, however, ranges from 30 to 90 meq/100 g, and adsorption of polar materials was expected. Although sand is best suited for the root chamber, vermiculite is used in the planting tube to absorb any materials released from the seed and prevent their leaking into the root chamber.

To determine the best methods of removing exudates in the smallest possible volume, the previous

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Table I
Suitability of Various Materials as Sealers for Use in Plant Culture Unit

<table>
<thead>
<tr>
<th>Material</th>
<th>Source</th>
<th>Chemical nature</th>
<th>Phytotoxicity</th>
<th>Sealing ability*</th>
<th>Pliability**</th>
<th>Suitability to unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household wax</td>
<td>Gulf Oil Corp.</td>
<td>Solid hydrocarbons</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Petrolatum</td>
<td>Fisher Scientific Co.</td>
<td>Semi-solid hydrocarbons</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Beeswax</td>
<td>Fisher Scientific Co.</td>
<td>Fatty acid esters</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Combinations:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household wax-beeswax-petrolatum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tackiwax</td>
<td>W. E. Clark &amp; Son</td>
<td>Mixed waxes</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Grafting wax</td>
<td>Advance Solvents &amp; Chemicals</td>
<td>Mixed waxes</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Advawaxes (M or P)</td>
<td></td>
<td>Polybutenes dispersed in microcrystalline wax</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Combinations:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Advawax-paraffin-petrolatum</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Combinations:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clay-wax-petrolatum</td>
<td>Fisher Scientific Co.</td>
<td>Bentonite or kaolin; paraffin; petrolatum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Duroplastic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Klingkote Liquid Cloth</td>
<td>Naugatuck Chemical</td>
<td>Latex; 68.5% solids; non-cured</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Latex NC-357</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhoplex B-15</td>
<td>Rohm &amp; Haas Co.</td>
<td>Acrylic resin emulsion</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Dow Corning 7 compound</td>
<td>Dow-Corning Corp.</td>
<td>Silicone release agent</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Myvacet 7-00</td>
<td>Distillation Prod. Ind.</td>
<td>Distilled acetylated monoglycerides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbowaxes</td>
<td>Union Carbide Chem.</td>
<td>Polyethylene glycols Co.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbolpol 934</td>
<td>B. F. Goodrich Chem.</td>
<td>Carboxy vinyl polymer Co.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Plastisol resin</td>
<td>Goodyear Tire &amp; Rubber</td>
<td>Polyvinyl chloride resin &amp; dioctylphthalate</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Adiprene L-100</td>
<td>E. I. duPont de Nemours</td>
<td>Liquid urethane elastomer</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mercury</td>
<td>Fisher Scientific Co.</td>
<td>Mercury; triple distilled</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Silastic RTV</td>
<td>Dow-Corning Corp.</td>
<td>Silicone rubber</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Ability to withstand a continuous positive pressure of at least 1 psi without leaking.
** Sufficiently pliable to permit normal development of the stem.

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6 Exchange capacity data supplied by the Perlite Institute and the Zonolite Co., respectively.
The experiment was repeated, except that the same 100 ml of solution was perfused eight times through the root chamber, an aliquot being removed for analysis after each perfusion. The recovery of both glutamic acid and glucose from perlite or vermiculite again was low (Fig 4). With sand, 85% of the material was recovered in the second perfusion, but subsequent perfusions reduced the recovery to approximately 50%. An equilibrium in the level of glucose and glutamic acid apparently was established between the solutions in the root and irrigation chambers. Although this method of removal would have limited the solution volume to 100 ml, the recovery, even before equilibrium was established, was not adequate, and a modification was tested.

The root chamber of a unit containing a pea plant was perfused once with 100 ml of irrigation solution, followed by three separate 100 ml volumes, each perfused three times. Aliquots were removed and analyzed for α-amino nitrogen (19) after each perfusion. Maximum recovery was obtained in the first washing (Fig 5). In the subsequent 100 ml volumes, recovery was highest in the first and decreased in the second and third perfusions, again indicating that an equilibrium was established. Similar results were obtained using standard solutions of glucose and glutamic acid.

To determine the number of washings necessary for maximum recovery of exudate from a plant, the same pea roots were washed with individual 100 ml volumes of irrigation solution 4 days after the previous experiment. Maximum recovery of α-amino nitrogen was achieved after the second washing, and additional washings increased recovery only slightly (Fig 6).

Based on these results, exudates are collected by washing the root chamber once with four individual 100 ml volumes of solution which are combined, concentrated in a flash evaporator, and stored at -6 C until analysis.

**Procedure for Operating Plant Culture Unit:**
The plant culture unit is assembled and all connections are tested. Sand, previously washed with concentrated HCl, 20% NaOH, and water, and heated in an oven at 170 C for several days, is placed in the root chamber. The sand is moistened, and the unit autoclaved for one hour at 121 C and 15 psi. The entire unit, including the irrigation reservoir, may be sterilized together, but it is easier to autoclave the unit and the reservoir separately and connect them aseptically immediately after removal from the autoclave.

Cotton and a rubber stopper are inserted loosely in the planting tube during autoclaving. After cooling, these are removed and approximately 125 ml of sterile irrigation solution is poured through the planting tube. A depression is made in the vermiculite, an aseptic seedling inserted, and the vermiculite is replaced around the stem. A thin layer of paraffin-sand (6, 35, 38) is placed over the vermiculite, and a sterile beaker containing a pad of sterile glass wool is inverted over the planting tube.

The unit is then tested for contamination by removing the sterility check tube from its housing, placing it below the level of the irrigation chamber, and
loosening the screw cap to allow air displaced by the entering irrigation solution to escape. The flow of solution into the tube is terminated by tightening the screw cap.

When the seedling has grown to the top of the planting tube, the beaker is removed and approximately 4 cm of sterile paraffin-sand is added. After 3 to 4 days, the top section of the planting tube is detached, the upper contaminated portion of the paraffin-sand is removed with a sterilized aspirator and replaced by a sterile layer, followed by a thin layer of a molten paraffin-petrolatum mixture (1:1) poured at 50°C. When the paraffin-petrolatum mixture has hardened, a thin layer of Silastic RTV 502, containing 0.25% catalyst (stannous octoate) and 50% thinner (Dow Corning 200 fluid, 20 centistokes), is added. The high concentration of thinner and low concentration of catalyst extends the curing time to approximately 12 hours and permits the plant to adjust to the sealer. Although the paraffin-petrolatum mixture alone is not suitable as a sealer (table I), it hardens rapidly and prevents the slower curing and diluted Silastic from leaking into the root chamber. After the Silastic has cured, a second layer, containing only 10% thinner and solidifying in approximately 30 minutes, is added.

The 3 to 4 day interim between removal of the beaker and application of the sealer allows the plant to survive the abrupt change in the ambient moisture content surrounding the above-ground portions. If the sealer is applied before this hardening period, the seedlings either wilt completely or subsequent development is retarded.

Collection of respired CO₂ may commence 24 hours after the sealer has solidified. The inlet tube of the air filter is flamed and attached to the manifold, the one-way check valve is attached to the outlet air filter, and the CO₂ collector is connected. Sterile, CO₂-free air, entering at the bottom of the root chamber through the solution-return and aeration tube (clamp open), flushes respired CO₂ through the outlet air filter into the CO₂ collector. The collector is replaced periodically, and absorbed CO₂ is determined titrometrically using an automatic titrator (28).

The irrigation chamber is filled by raising and inverting the irrigation reservoir and opening the appropriate clamps. The solution flowing into the irrigation chamber is replaced by sterile air through the connection between the root chamber and the irrigation reservoir. When irrigation is necessary, the clamp on the solution-return and aeration tube is closed, causing the air to force the solution through the irrigation sprinkler. The solution percolates through the sand and returns to the irrigation chamber when the clamp is opened. More rapid return of the irrigation solution may be obtained by briefly disconnecting the air filter from the manifold.

To collect exudates, the tubing leading to the sterility check tube is clamped, the mercury valve placed below the irrigation chamber, and the glass tube attached to the side-arm of the valve dipped in alcohol, flamed, and inserted into a sterile flask fitted with a 2-hole rubber stopper. One hole contains a cotton-plugged glass tube for escape of displaced air, and the other hole is covered with tape which is removed prior to insertion of the glass tube. The rate of flow of solution into the collection vessel is controlled with a screw clamp. Sterility of the unit is tested after each removal of exudate. If the unit is contaminated, microbial growth is usually evident in the sterility-check tube after approximately 24 hours.

Results & Discussion

A variety of plants (e.g., seeded banana, corn, squash, watermelon, cucumber, tomato, bean, pea, cotton, radish, cabbage) have been successfully grown in the unit. Some examples are illustrated in figure 7. Plants with non-sterile roots often were larger and greener and also flowered and fruited earlier than did plants having asptic roots. A preliminary analysis of the exudates is routinely made for organic carbon (15), α-amino nitrogen (19), and reducing sugars (23, 31), to obtain an estimate of the amount of organic material present. The exudates are then desalted, concentrated, and subjected to chemical and chromatographic analyses, as well as to various bioassays. Some typical preliminary analyses are given in table II, and the respiratory patterns that were obtained from the same plants are shown in figure 8.

In addition to obtaining root exudates and respiration data, the unit is applicable to a variety of investigations which require aseptic roots. The influence on plant growth of single and mixed cultures of microorganisms in the root zone and the ability of aseptic intact roots to take up large organic molecules, such as would be elaborated by microorganisms, are being investigated. The release of volatiles other than CO₂ and O₂ from roots and rhizosphere microflora can be studied by periodically removing vapor samples through one of the auxiliary tubes for gas chromatographic analysis. The relative contribution of roots and the microflora to the CO₂ produced in the rhizosphere can be determined by comparing the respiration of aseptic and septic roots, and the effect of environment on diurnal respiration of roots is easily studied by replacing the CO₂ collectors with a
of the plants are separated spatially from the root zone, in order that products from the seed do not contaminate the root exudates. The photosynthetic and respiratory activities of the above-ground portions, which are allowed to develop in air, are separated from the respiration of the roots by a sealing material poured around the plant stem. A continuous sterile CO₂-free air stream aerates the roots and removes respired CO₂ for analysis. The roots are periodically perfused with a nutrient solution, and root exudates are collected aseptically. Aseptic sampling of the root-zone atmosphere for gas chromatographic analysis, and the introduction of microorganisms or other materials is possible. The apparatus is compact and is autoclaved as a unit. Mono- and dicotyledonous plants have been grown successfully in these units. Representative exudate and respiratory patterns are presented and potential applications are discussed.

Acknowledgments

The authors express their appreciation to the various companies which provided filter and sealing materials and seeds for use in these studies; to Mrs. Virginia S. Hessler for drawing the diagrams of the unit, and to Dr. E. R. Willis for photographs of the unit.

Literature Cited


** Table II **

<p>| Total Carbon, α-Amino Nitrogen, &amp; Glucose Excreted From Aseptic Roots of Bean &amp; Corn |
|----------------------------------------|-------------------------------------------|--------------------------------------------|-------------------------------|-------------------------------|</p>
<table>
<thead>
<tr>
<th>Plant</th>
<th>Time of sampling after germination (days)</th>
<th>Total carbon (mg)</th>
<th>α-Amino nitrogen (μg)</th>
<th>Glucose (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total*</td>
<td>Per day**</td>
<td>Total*</td>
</tr>
<tr>
<td>Bean (Bountiful)</td>
<td>25</td>
<td>lost</td>
<td>lost</td>
<td>15.6</td>
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<tr>
<td></td>
<td>31</td>
<td>16.4</td>
<td>2.7</td>
<td>67.5</td>
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<tr>
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<td>36</td>
<td>16.2</td>
<td>3.2</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>32.3</td>
<td>5.4</td>
<td>14.4</td>
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<tr>
<td></td>
<td>53</td>
<td>4.6</td>
<td>0.5</td>
<td>89.0</td>
</tr>
<tr>
<td>Corn (rd dwarf)***</td>
<td>11</td>
<td>17.7</td>
<td>1.6</td>
<td>8.3</td>
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<tr>
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<td>6.0</td>
<td>1.0</td>
<td>7.8</td>
</tr>
<tr>
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<td>27</td>
<td>17.5</td>
<td>1.8</td>
<td>9.2</td>
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<tr>
<td></td>
<td>47</td>
<td>22.6</td>
<td>1.1</td>
<td>7.0</td>
</tr>
</tbody>
</table>

* Amount of material released between sampling times.
** Total ÷ number of days between samplings.
*** Inbred corn with rd dwarf gene; sample CC-Stock 61131, Rogers Brothers Company, Idaho Falls, Idaho.

Fig. 8. Daily evolution of CO₂ from aseptic roots of bean and corn.