A SIMPLE ASSEMBLY FOR USE IN THE TESTING OF CULTURES OF RHIZOBIA

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For the determination of nodule-forming ability and efficiency of species of *Rhizobium*, it is necessary to protect plant cultures from contaminating nodule organisms. This is usually accomplished by sterilization or disinfection of seed, containers, nutrient solution, sand and water and by maintenance of such sanitation in the laboratory and greenhouse as will largely forestall the entry or transfer of unwanted rhizobia. When only a small number of cultures are to be tested, elaborate assemblies may be used. However, when thousands of such determinations are to be made almost simultaneously in a small greenhouse, a simpler and more easily operated method is desirable. The method suggested in this paper, it is hoped will supply this need. It merely involves, outside of the usual disinfection of materials, the irrigation of sterile sand by capillarity and the use of extreme acidity in nutrient solutions as a lethal agent against foreign rhizobia; such a solution altered by the neutralizing materials in the sand, ultimately becomes the liquid food for use of the plant.

SAND AND NUTRIENT SOLUTIONS

Sand used in these assemblies is washed very thoroughly with tap water to eliminate soluble and fine materials. It is then dried in air or in a steam dryer and sieved to obtain grains which will pass through holes a millimeter in diameter. Before use the sand is mixed with neutralizing and fertilizing materials of about the same or smaller granular size to the extent of approximately 2% by volume. A stock mixture for use in supplying these materials consists of the following:

Dolomitic limestone	10 parts
Calcium carbonate	1 part
Granulated superphosphate	1 part
Potassium sulphate	$\frac{1}{10}$ part

The nutrient solutions are prepared in concentrated form for dilution before using. The ones employed at the present time are made according to the formulae given below:

Potassium phosphate KH_2PO_4 anhydrous	143.27 grams
Potassium nitrate, cryst	16.30 grams
Copper sulphate $CuSO_4 + 5H_2O$	0.80 gram
Manganese sulphate $MnSO_4 + 4H_2O$	.80 gram
Iron sulphate $FeSO_4 + 7H_2O$	.80 gram
Boric acid, cryst	.80 gram
Sulphuric acid, conc. sp. gr. 1.84	500.00 ml.
Water	3500.00 ml.

This solution is prepared out-of-doors in a 2-gallon crock in which the sulphuric acid is carefully added to the water.

The second formula is the same as that given except that potassium nitrate is omitted.

In the preparation of the dilute nutrient solution, 4 ml. of the concentrated acid stock solution is mixed with one liter of tap water. This solution has a reaction of about pH 2.0.

ASSEMBLY

Although larger or smaller containers may be used, an assembly employed in the laboratory of Soil Microbiology can be made mainly from easily obtained stock materials. The items included are (1) a 32-ounce Boston round, cork finish, narrow or wide mouth, flint glass bottle from which the bottom has been cut, (2) a 1-hole rubber stopper of correct size into which has been inserted a $2\frac{1}{4}$ inch tube which may be a capillary alone or some other type of tube containing a small piece of absorbent cotton or manufactured wicking, (3) a 100-mm. glass Petri dish top, and (4) a 14-ounce round, flint glass, screw-cap-finish cream jar approximately $4\frac{1}{2}$ inches high by $3\frac{1}{2}$ inches in diameter overall, with a 3-inch diameter mouth.

Having sand, nutrient solution and containers available, 350 ml. of nutrient solution are added to each cream jar. Bottles are then fitted with the stoppers and capillary tubes and set in the cream jar as shown in Plate I.

Using the rubber end of a rubber force pump (the kind used in plumbing) attached to a source of vacuum, suction is applied to the cut end of the bottle so as to draw liquid through the capillary and thereby remove air bubbles. About 730 grams of sand mixture are then poured into the inverted bottle and allowed to stand for an hour or until the sand has become completely moistened. It is then in condition to punch holes for receiving seed planted later. Petri dish lids are placed over the bottles as shown in Plate I. The completed assemblies may now be placed in the sterilizer and subjected to steam under 15 pounds pressure for one half hour. When withdrawn from autoclave they may be stored for future use or allowed to cool, and planted.

The moistened sand prepared according to the foregoing method contains from 200 to 300 parts per million soluble salt as determined by the electrolytic bridge. The salt content apparently does not change greatly in a month or two under greenhouse conditions. The reaction of the sand is about pH 7.0 which, of course, may be changed by varying the neutralizing materials in the sand.

PLANTING

Disinfected seed or seedlings may be planted in the moist sand in the bottles and allowed to grow, at least for a while, under the protection of the Petri dish lid. When they reach the top of the bottle it is desirable to remove the lid, and in the case of fast-growing plants such as soybeans, some type of support is added. This is ordinarily accomplished by replacing the top by a hardware cloth cylinder covered at one end with cellophane, thereby confining the plants

and giving them partial protection from falling dust. If small plants become pale it may be of value to remove the Petri dish lid to allow better light conditions. In the latter case, the plants are left unprotected at the top.

After planting, the only attention needed is the placing of protective supports for the large growing plants and the replenishment of the nutrient solution. All additions of nutrient solution except the first, do not contain potassium nitrate. If sand gets too wet, a condition which rarely occurs, it may be remedied by removing the bottle from the liquid and placing it in an empty cream jar to drain. Sterilized gravel added to the top of the sand immediately after planting is useful in impeding evaporation.

The acidity of the nutrient solution is effective in killing rhizobia but certain molds grow in it. *Paecilomyces* sp. and even *Trichoderma* sp. occasionally appear, mostly as submerged mycelia. The fungal growth does not appreciably alter the reaction.

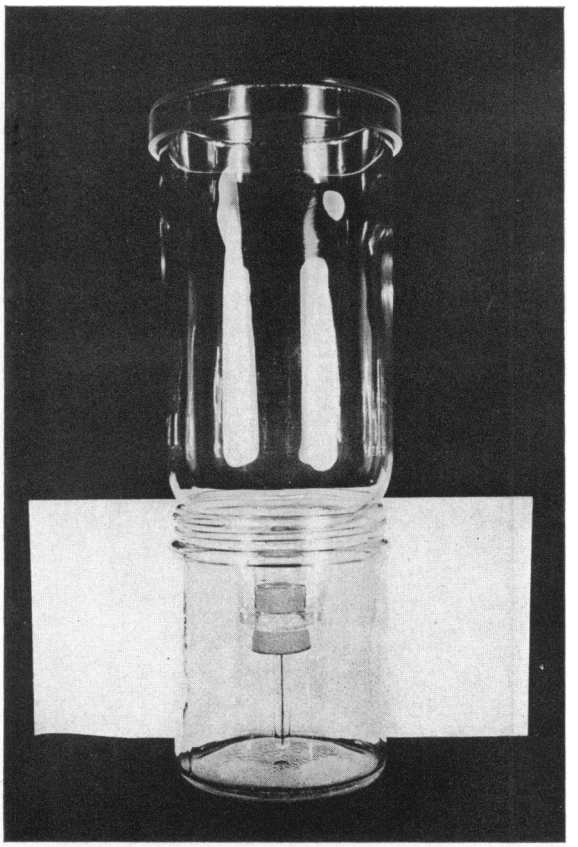
The method just described has been in use more than a year and in the case of large growing plants such as peas and soybeans, it has been very effective in indicating efficiency as well as nodule-forming ability of cultures of rhizobia. Small growing plants such as clover and alfalfa, on the other hand, under the more confined conditions, have not responded as well particularly with reference to the demonstration of efficiency.

SUMMARY

In brief, a method and apparatus are described for use in the testing of *Rhizobium* cultures which depends upon the self irrigation of disinfected sand mixtures through capillary action, with solutions so acid as to preclude the survival of any of the common nodule bacteria. This protective acid nutrient is neutralized when in contact with the calcium in the sand and carried further into the mixture makes a favorable artificial substratum for the growing of some plants under controlled conditions.

PLATE I

GLASSWARE ASSEMBLY WITHOUT SAND OR NUTRIENT SOLUTION



(Lewis T. Leonard: Assembly for Culture Testing)