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The testing of bacterial strains used in the inoculation of legumes

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THE TESTING OF BACTERIAL STRAINS USED IN THE INOCULATION OF LEGUMES

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APART from their value as fodder plants, legumes such as peas, beans, clovers, vetches and lucerne play an important role in increasing soil fertility. Their value in the soil-enriching sphere has been recognised for centuries, but it is only comparatively recently that the reason for it has been fully understood.

Legumes are the only class of plants which develop bacterial nodules on their roots (see Fig. 1). These nodules are formed by bacteria known as *Rhizobia,* which convert nitrogen from the atmosphere into compounds which can be used by the plant.

This means that although the soil may be lacking in nitrogen—which is a very important plant food—the presence of the bacteria ensures that the plant obtains adequate supplies, and can live and grow healthily. The atmosphere contains almost unlimited quantities of nitrogen but, without the assistance of the bacteria, this would remain unavailable to the plants.

When the plant remains of the legumes decay in the soil, the nitrogenous compounds produced by the bacteria are freed and become available for the nutrition of other plants. Thus the growing of legumes enriches the soil by increasing the quantity of nitrogen available to plants.

BACTERIAL STRAINS

Each group of legumes has its own particular type or strain of bacterium which is capable of infecting its roots. For example, a strain of *Rhizobium* which gives excellent results on subclover will be completely useless on peas, etc. When a group of legumes is grown in soil in which they have not previously been grown, the specific *Rhizobium* group strain (or in some cases specific strain) is not usually present, and consequently ways of introducing the bacteria have been developed.

Years ago, the practice was to broadcast loads of soil from an old paddock on to the new one and so introduce the bacteria, but this method is very cumbersome and is also dangerous because diseases or insect pests present in the old paddock may be spread in this way.

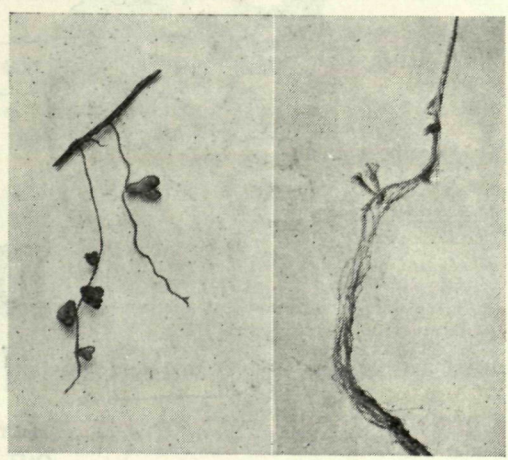


Fig. 1.—Nodules formed by the bacteria on roots of pea (left) and clover (right).

A far more convenient method has been devised whereby the seed is artificially inoculated with the requisite bacteria which have been grown in the laboratory on agar media, a jelly-like base containing food material on which the bacteria thrive. For this purpose a number of strains are propagated in pure culture by the Department of Agriculture. The origin of these strains is varied some having been isolated locally and others obtained from England, America, and other countries.

Some strains have been grown on agar for many years and under these conditions they may lose their effectiveness or become contaminated. Therefore, frequent testing is necessary to ensure that the strains retain their effectiveness, so that the farmer will be assured of reliable cultures.

NEW STRAINS OF BACTERIA

Frequent attempts are made to secure even more effective cultures than those issued commercially. For this purpose, isolations are made from the nodules on outstandingly vigorous plants which have been grown either from sterilised seed or uninoculated seed. A preliminary screening of the new strains thus obtained is done in a similar way to the testing of type strains. Any promising

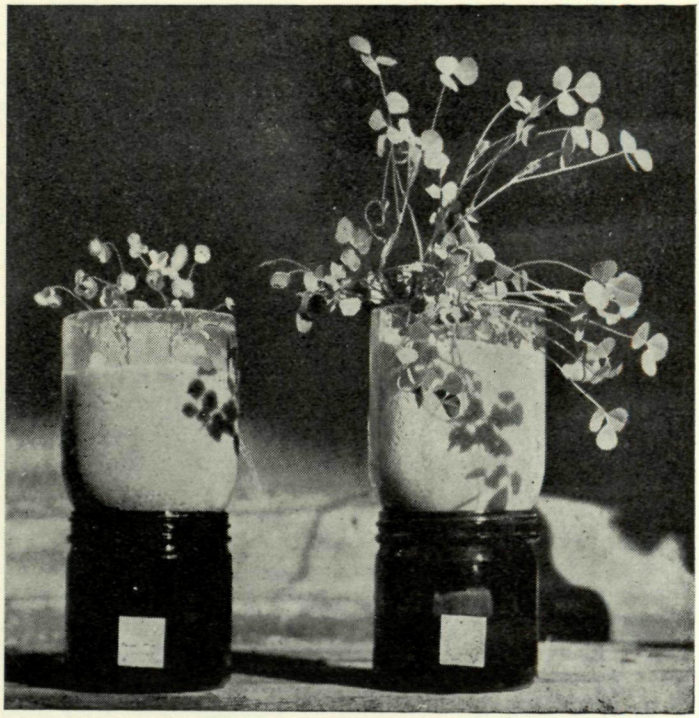


Fig. 2.—Culture jar method of testing strains. These plants are barrel clover and the sickly plant on the left is the control, grown without inoculation. The healthy plant on the right was inoculated with a good strain of bacteria.

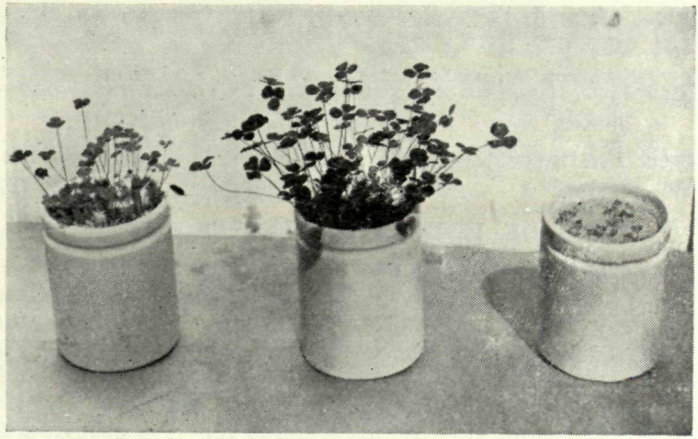


Fig. 3.—Glazed pot method of testing strains used with sub. clover plants. The left-hand pot contains plants inoculated with an old strain which was only partially successful. The controls in the right-hand pot received no inoculation. In the centre pot are plants inoculated with a new strain. Note the healthy growth and dark green colour.

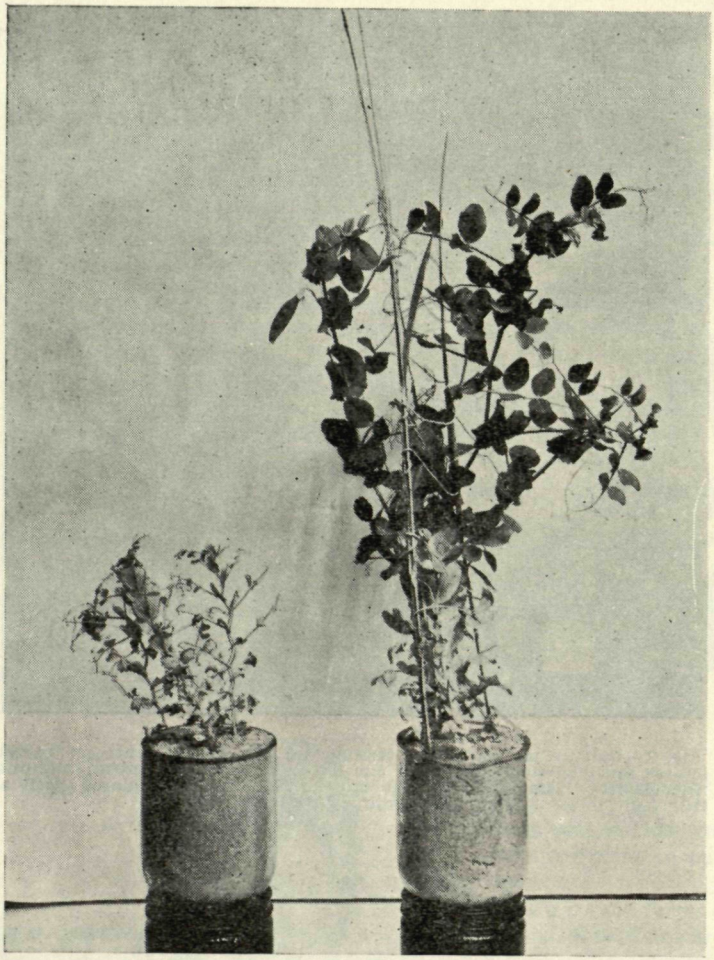


Fig. 4.—Culture jar, method using peas. Note the feeble uninoculated control on left and the strong plants on right which were inoculated with a good strain.

ones are later tested in the field (see Fig. 8).

TESTING OF RHIZOBIAL STRAINS

As the main function of the *Rhizobia* is to transform atmospheric nitrogen into a form equivalent to a nitrogenous fertiliser, which is then available to the leguminous plants with which they are associated, the testing is done in a sterile medium, free of nitrogenous fertilisers. This ensures that the only source of nitrogen for the plant is that which the nodule bacteria obtain from the air. The effectiveness of the test is assessed by comparing the growth of inoculated plants with that of control plants grown from uninoculated seed, but otherwise under identical conditions. This is of course a far more rigorous test than would ever apply in the field, for no natural soil would be completely devoid of nitrogen.

METHODS

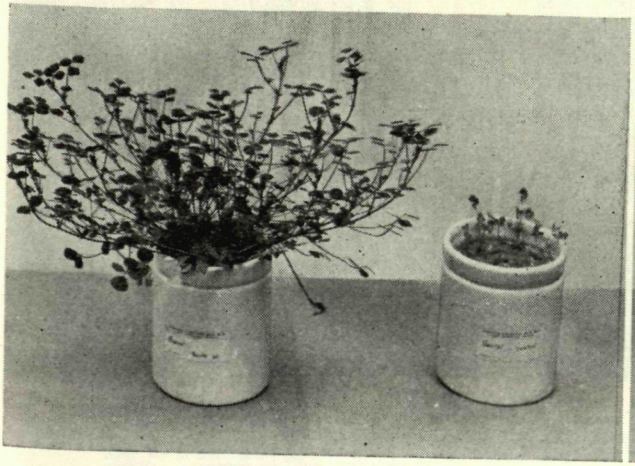
Inoculated seed previously sterilised is planted in sterilised sand which has also been thoroughly washed to remove any nutrient material, particularly nitrogen. This sand is watered with a sterile dilute solution also devoid of nitrogen. (The seed, sand and culture solution are sterilised to ensure that no bacteria are present except the ones being introduced for test.) Two types of jars can be used:—

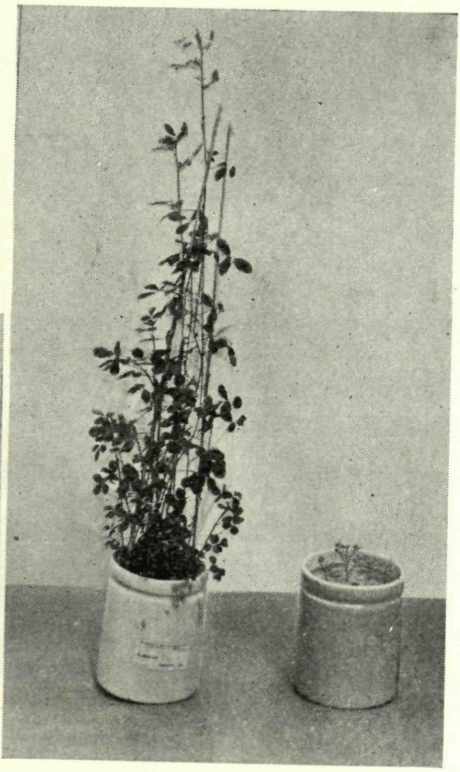
(1) **Culture Jars,** consisting of a bottle from which the base has been removed inverted over a jar containing the nutrient solution. The neck of the upper bottle is plugged and the bottle filled with sand. A wick allows the fluid to pass up from the reservoir into the sand—see Fig. 2. With this method the sand is kept almost saturated continuously.

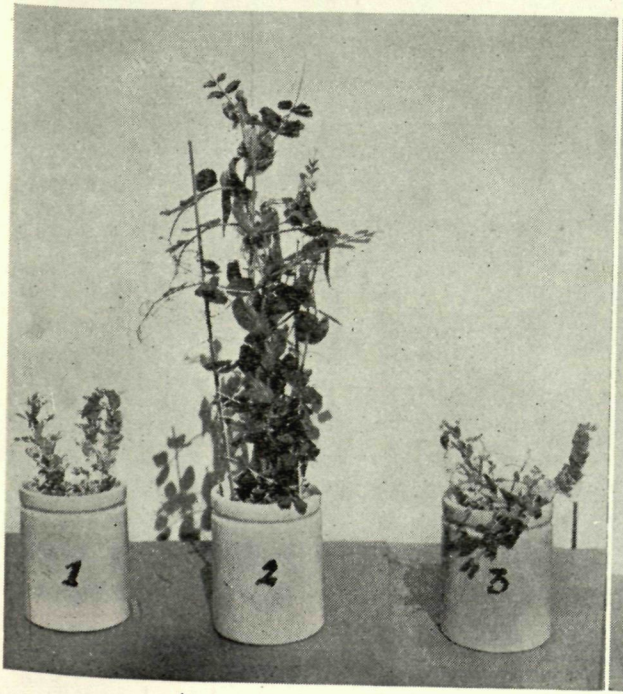
(2) **Glazed Pots.**—In this case a definite amount of nutrient solution is added to the sand in the pot—the whole being kept at constant weight by further additions of nutrient solution as required during the growing period. By this means marginal moisture conditions such as occur naturally in the field from time to time can be simulated—see Figs. 3, 5, 6 and 7.

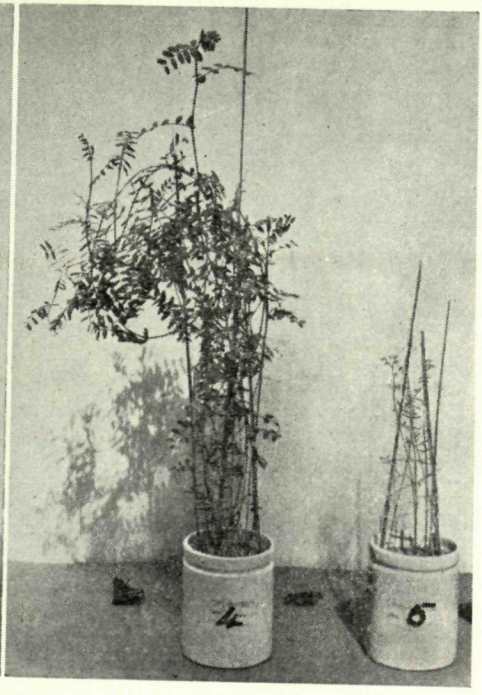
Fig. 5—Top photographs: Barrel clover (left) and lucerne (right). In each case the larger plants were inoculated and the smaller were the uninoculated controls.

Fig. 6.—Bottom photographs: Peas (left) and vetches (right). The left hand pot of peas (No. 1) received no inoculation. No. 2 was treated with a good strain and No. 3 with an ineffective strain. The vetches in No. 4 pot were inoculated, those in No. 5 were not.









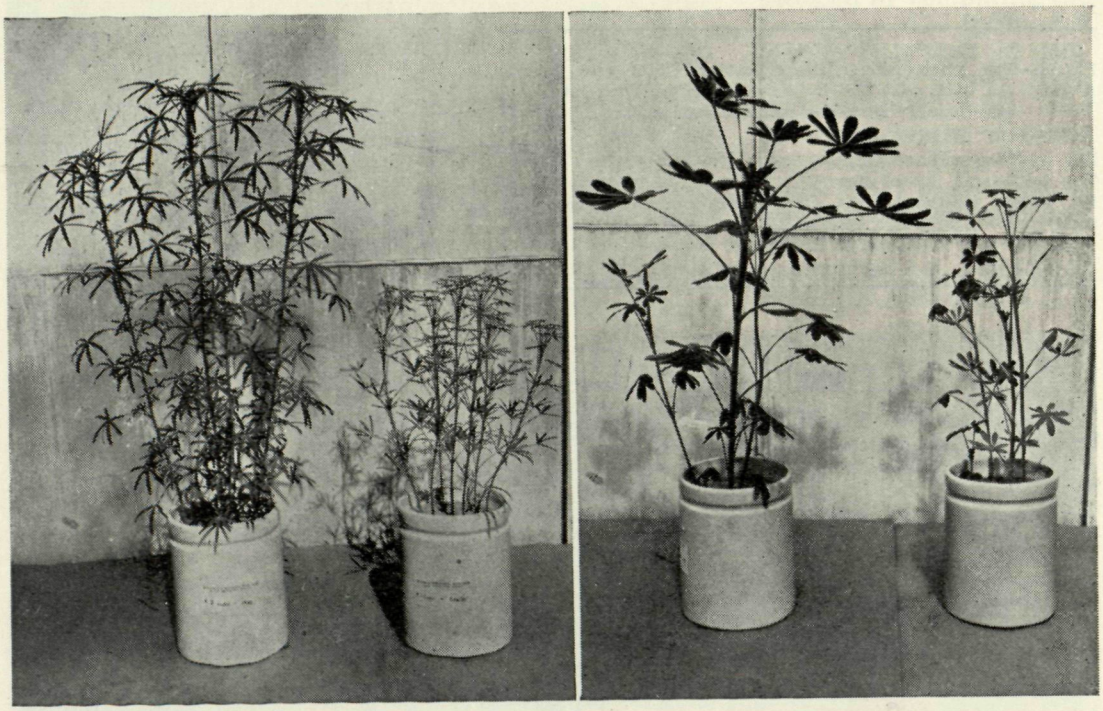


Fig. 7.—New Zealand lupins (left) and West Australian lupins (right). In each case, the large plants were inoculated and the small plants were not.

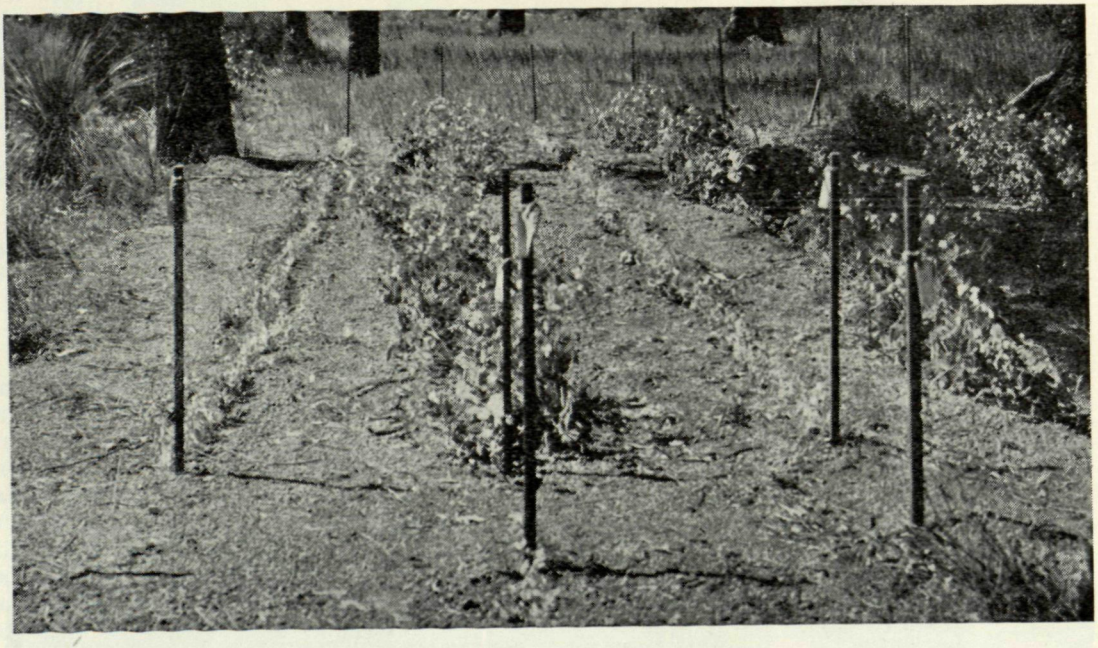


Fig. 8.—Field test on peas. Any strains which appear promising under glass-house conditions are tested out under field conditions. The uninoculated rows, at left and second from right, compare most unfavourably with the alternate rows which were inoculated.

It has been found that the level of moisture markedly affects the ability of strains to function. With optimum or high moisture levels even partially effective strains may give satisfactory results, but with the minimum amounts a better separation is possible. A strain which is effective under these rigorous conditions is more likely to be effective in the field than one which is grown under more pampered conditions. Consequently, method two has been found to give more satisfactory results.

The accompanying illustrations are reproduced from recent glasshouse experiments in which both type cultures and also several new strains were tested. Note the stunted, yellowed growth of the control plants grown in nitrogen-free sand without inoculation, compared with the vigorous, dark green growth of the plants inoculated with good effective strains of *Rhizobia*. Note also (Fig 6, Pot No. 3) that although the peas in this pot had been inoculated with a strain of *Rhizobium*, they gave no better result than the uninoculated control. This strain is, therefore, an ineffective strain for peas.

NOTE.—Inoculating the seed cannot make up for lack of fertility (except nitrogen deficiency) in the soil, or for exigencies of climate, etc.

Advice on purchasing and using nitrogen-fixing bacteria for the inoculation of legume seeds will be found on page 93 of this issue.