

per cent. of ash as given in column 3 by the per cent. of "original solids." In the same way the milligrams of total phosphorus pentoxide per 100 grams of original solids are reported in column 10.

Of these results, the quantity of ash, the alkalinity of the ash, and amount of soluble phosphates are of considerable uniformity in pure cider vinegar, and these, when considered with the other characteristics above enumerated, serve to differentiate this kind quite sharply from all other commercial varieties and to afford a basis for the approximate estimation of the quantity of spirit vinegar or of water, with which pure cider vinegar may have been adulterated.

[CONTRIBUTION FROM THE LABORATORY OF AGRICULTURAL CHEMISTRY,
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ROOT TUBERCLES IN WATER CULTURE.¹

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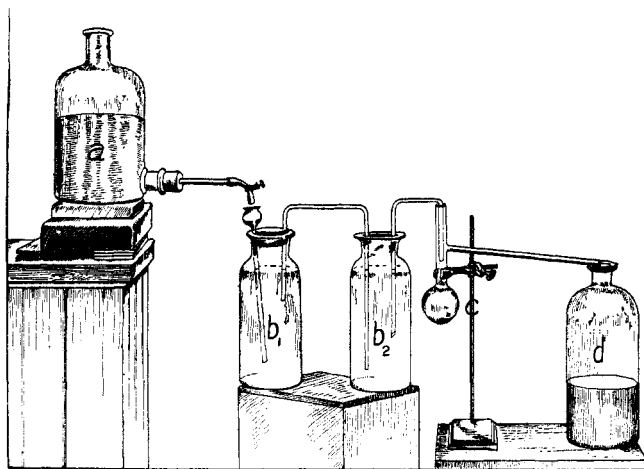
SINCE the discovery by Hellriegel of the connection between root tubercles and the fixation of atmospheric nitrogen by leguminous plants, the literature on this interesting and important question is being constantly enriched by painstaking experiments on the part of scientific investigators all over the world. The subject has thus far been studied only in connection with sterilized soil or sand. It occurred to the writer that it might be possible to produce root tubercles in water culture and thus be able to observe the process better than when the roots of the plants were buried in soil or sand. The work of conducting the experiments about to be described was entrusted to Mr. J. C. Britton, one of the writer's advanced students. The results more than met my expectations, and this preliminary notice is made public for the benefit of those who may feel inclined to employ the method in their investigations.

For the past four or five years the writer has employed in water-culture experiments an apparatus designed by himself, which presents certain advantages over the methods heretofore described. As this apparatus was employed in the experiments

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under consideration, a brief description of the same may not be out of place.

As will be seen from the accompanying illustration, *a* is an aspirator of four liters capacity; *b*₁ and *b*₂ are ordinary salt mouth bottles of two liters capacity, which serve as culture jars; *c* is a small flask with a side tube, as used in fractional distilla-



tion; *d* is a collecting bottle of four liters capacity. The culture jars are fitted with cork disks to hold the plants in the usual manner and also support the thistle tube and the siphons as shown in the illustration.

At the beginning of a vegetation experiment, the flask *c*, the culture jars, and the aspirator, are filled with the nutrient solution. The aspirator is closed with a rubber stopper carrying a glass tube, to the end of which a short piece of rubber tubing is attached. In this manner the solution is conveyed to the thistle tube, which extends to near the bottom of jar *b*₁, the flow being controlled by means of a Hoffman clamp placed on the rubber tubing. By means of a siphon, the short arm of which extends a short distance below the surface of the solution in jar *b*₁, and the long arm nearly to the bottom of jar *b*₂, the solution flows into jar *b*₂.

Another siphon with one arm extending a short distance

below the surface of the solution in jar *b*₂, and the other below the side tube in flask *c*, carries the solution into flask *c*, from which it runs through the side tube into the collecting bottle. The drop of the solution into the thistle tube is so regulated that the aspirator will empty itself in about twenty-four hours, when the solution in the collecting bottle is transferred back into the aspirator. This is done once a day until the vegetation experiment is completed.

The advantages of this arrangement are :

1. Two seedlings, or if, as the writer has frequently done, another culture jar be inserted between jar *b*₂ and the flask *c*, three seedlings can be grown in the same solution at the same time, so that, if an accident should happen to one of the seedlings, the experiment need not necessarily fail.

2. The solution is continually being aerated as it drops into the thistle tube and into the collecting bottle, as well as when it is transferred to the aspirator. By this means the roots of the seedlings are constantly supplied with free oxygen, a condition necessary for healthy growth.

3. Plants can be grown to maturity without removing them to other jars filled with fresh solutions.

4. The solution in the culture jars always remains at the same level, *i. e.*, on a level with the side tube of the flask.

Two solutions were prepared as follows :

1. COMPLETE NUTRIENT SOLUTION (Wolff's Solution).

	Grams.
Bone ash dissolved in nitric acid.....	20
Potassium nitrate.....	11
Magnesium sulphate.....	7
Potassium chloride.....	3
Ferrous sulphate.....	Minute quantity

The whole dissolved in distilled water, neutralized with sodium carbonate, and diluted to one liter.

2. COMPLETE NUTRIENT SOLUTION WITHOUT NITROGEN.

	Grams.
Bone ash dissolved in nine cc. sulphuric acid.....	20.0
Potassium sulphate.....	9.5
Magnesium sulphate.....	7.0
Potassium chloride.....	3.0
Ferrous sulphate.....	Minute quantity

The whole dissolved in distilled water, neutralized with sodium carbonate, and diluted to one liter. For the growing of plants

the apparatus was filled with a mixture of the respective solutions and distilled water in the proportion of five cc. of the solutions to one liter of water. Trials with fifteen cc., and even ten cc. of the solutions to one liter of water, failed on account of over-nutrition, the seedlings refusing to grow and finally dying.

The plants employed were dwarf peas. The seeds were made to germinate in sterilized sand, moistened with distilled water. When the seedlings were four or five inches in height, they were taken from the sand, the roots washed and the plants transferred to the culture jars.

Three sets of apparatus, as shown in the illustration, were provided. The first set contained the complete nutrient solution. The second set contained solution No. 2, as described above, without nitrogen.

The third set contained the same solution, but in addition the seedlings before being placed into the jars, were inoculated with tubercle germs, by immersing the roots for a few minutes in a cold water infusion of a soil, in which peas had been grown.

As might be expected, the plants in set No. 1, containing the complete nutrient solution, were healthy and vigorous, and produced flowers and fruit.

The plants in set No. 2 grew rapidly for a time, but were weak and sickly in appearance. In about ten days the leaves began to turn yellow and to show the effect of nitrogen starvation. A few small blossoms finally appeared, but this caused the speedy death of the plants. The roots developed very rapidly, completely filling the jars. They were doubtless in search of nitrogenous food.

The plants in set No. 3 grew normally for about ten days, when they began to show the effect of nitrogen starvation in a marked degree. No tubercles could be observed on the roots. About the thirteenth day the plants began to recuperate, the leaves assumed a normal green color, and from this time on the growth was vigorous and normal. On the fifteenth day the tubercles were first observed, but they had then attained considerable size.

The plants, like those of set No. 1, produced flowers and fruit. Investigations in this direction will be continued.