3. Evidence of the long period the disease has been present in the trees themselves; *i. e.*, bare, weathered tops; *healed* cankers; thrifty branches, with bases diseased and hypertrophied, but living, etc.

4. Peculiarities of the bark; such as extensive development of a callus tissue, and the presence of a peculiar substance which is constantly associated with, and particularly conspicuous in cases of marked resistance.

5. The natural grouping of the trees in well-defined areas or "pockets," pointing to a genetic variation.

6. The manifestation by members of the same coppice group; and by branches, trunk and basal shoots of the same individual; of similar degrees of resistance, indicating an inherent condition.

If these facts and inferences are correct, they point the way clearly toward a reconstruction and a revival of our American chestnut. Many of the trees bloomed well, and this fall bore good fruit. A large number of nuts have been gathered and planted by Dr. Van Fleet, of the U. S. Department of Agriculture, at the trail grounds near Washington, D. C. If the resulting seedlings substantiate the inference that the disease resistance is a heritable character, the way lies open, both by inbreeding, and by crossing with the resistant oriental species (not good timber trees themselves) to develop an extremely resistant or perhaps practically immune strain of timber tree for the reforestation of our devastated chestnut woodlands.

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THE OCCURRENCE OF AZOTOBACTER IN CRANBERRY SOILS

SEVERAL papers have appeared recently in SCIENCE and elsewhere[1, 2] concerning the fact that the aerobic non-symbiotic nitrogen fixing organisms, namely the *Azotobacter* group, occur in the soil, when the concentration of the hydrogen-ion is not more than 10^{-6}, or the limiting exponent is 6.0.

Investigators[3] have gone so far as to use the presence of *Azotobacter* in the soil as an indication of the soil reaction. Gillespie,[2] interpreting the results of Christensen,[3] stated that they are in accord with those obtained by Gainey,[1] namely the limiting hydrogen-ion exponent for the presence of *Azotobacter* in the soil is 6.0.

The methods previously used in determining the soil acidity conveyed only a very indefinite idea about the true nature of the reaction of the soil. But only recently [4, 5] methods have been suggested which, either using the electrometric or an improved colorimetric method, have enabled us to get a better insight into the extent and nature of soil acidity. These studies have brought out the facts referred to above concerning the reaction limit for the existence of *Azotobacter* in the soil.

In the study of the microbial population of cranberry soils some interesting observations were made and of these only the occurence of *Azotobacter* will be reported here.

The cranberry soils are so distinctly different from ordinary soils that it was thought for a long time that no very large number of bacteria can exist in them and that the microbial population consists predominantly of molds. These soils are known to have a distinctly acid reaction and contain large quantities of undecomposed organic matter, namely the roots and the stubble of the dead plants. The existence of *Azotobacter* in cranberry soils would be of great practical importance, since the nitrogen of the air would thus be fixed and made available to the crops, which have to grow in soils rather poor in available nitrogenous constituents (particularly is this true of sandy bottom bogs). The undecomposed roots and stubble would supply the carbohydrates necessary for the activities of *Azoto-*

1 Gainey, SCIENCE, Vol. 48, pp. 139–140, 1918; *Jour. Agr. Res.*, Vol. 14, pp. 265–271, 1918.

2 Gillespie, SCIENCE, Vol. 48, pp. 393–394, 1918.

3 Christensen, *Soil Science*, Vol. 4, pp. 115–178, 1917.

4 Gillespie, *Jour. Wash. Acad. Sci.*, Vol. 6, pp. 7–16, 1916.

5 Sharp and Hoagland, *Jour. Agr. Res.*, Vol. 7, pp. 123–145, 1916.

bacter, particularly in the presence of cellulose decomposing organisms.

The high acidity of the cranberry soils would preclude the very idea of finding the *Azobacter* in these soils and the early students[6] of this group of organisms were of the opinion that they can not live in acid media at all but the reaction has to be adjusted first to neutrality before the conditions are made favorable for their activities.

A Savannah bottom cranberry bog situated at Whitesbog, N. J., was used for this work. A part of the bog was limed three years ago and the crop was almost double of the corresponding plot, unlimed. Samples of the soil from the two plots were secured under sterile conditions and used for this study. The soil is nothing more than some white sand interwoven with decayed and living plant residues.

The hydrogen-ion concentration of the two soils was determined by means of the colorimetric method, using the phenol-sulfon-phthalein indicators suggested by Clark and Lubs.[7] The method corresponds very closely with the electrometric determinations using the hydrogen electrode, as was shown by Gillespie.[2] A definite amount of soil was shaken with double its weight of distilled water, then centrifuged; the supernatant clear liquid was syphoned off and used for the determination of the hydrogen-ion concentration. The unlimed soil had an hydrogen-ion concentration of pH = 5.4 to 5.6 while for the limed soil pH was 6.2—6.4.

The two soils were added in 10-gram quantities to 100 cc.c. portions of a sterile faintly alkaline nitrogen-free mannite solution and incubated at 25°. The solution in the flasks containing the limed soils became turbid in four days and a pellicle characteristic of *Azotobacter* began to develop in some flasks. On microscopic examination the solution was found to contain an abundance of *Azotobacter* cells and *Actinomyces* filaments. The solution in all the flasks to which the unlimed soil was added remained clear as in the control, but has shown a profuse gas production. On microscopic examination no *Azotobacter* cells and no *Actinomyces* filaments were discovered.

The limiting reaction for the existence of *Azotobacter* in the soil, expressed in the hydrogen-ion concentration is thus found to fall between pH—5.4 to 5.6 and pH—6.2 to 6.4 and is probably nearer the latter. This will confirm the results of Gainey[1] and Christensen[3] that an hydrogen-ion concentration of the soil = pH—6.0 is the limiting reaction for the activities of *Azotobacter* in the soil.

The occurrence of *Antinomyces* filaments together with *Azotobacter* cells suggests a still more interesting and important possibility, association between these two groups of soil microorganisms. As will be soon shown elsewhere many *Actinomyces* decompose organic residues very rapidly. The association between these two groups of organisms, change of reaction, and the action of *Actinomyces* upon the nitrogen-fixation by *Azotobacter* is being studied at present in this laboratory.

The importance of *Azotobacter* in cranberry soils, which can be effected by changing the reaction of those soils, thus becomes apparent: these organisms, whether alone or in association with others, utilize the plant residues as a source of energy and this allows them to fix the atmospheric nitrogen and increase its supply in the soil, which goes towards an increased crop production.

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[6] Lipman, *Ann. Rept. N. J. Agr. Exp. Sta.*, pp. 262–268, 1904.

[7] *Jour. Bact.*, Vol. 2, Nos. 1, 2, 3, 1917.

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