THE ETCHING OF MARBLE BY ROOTS IN THE PRESENCE AND ABSENCE OF BACTERIA.*

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In studies on plant nutrition[1] it is essential to investigate the reciprocal effects of the plant and of the soil bacteria upon one another. In a recent paper[2] the writers have shown that germinating legume seeds do not excrete substances in quantities sufficient to exert a toxic action upon the growth of the nodule bacteria of such plants. The present report makes it evident that soil bacteria may play an important part in the nutrition of plants.

It is a well known fact that roots in the presence of bacteria have the power to carry on active solution processes and thereby increase the available plant food in the soil. The means by which such a dissolving action of minerals takes place is a matter of considerable importance.[3] Unfortunately the problem has been complicated[4] by the failure of many investigators[5] to distinguish clearly[6] the factors[7] that are involved. Some interesting observations as to the causes for soil acidity have recently been made.[8,9]

That the acid excretion from roots has the power to etch marble has long been known. This power of roots to dissolve marble be-

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[1] Lyon, T. L., *J. Am. Soc. Agron.*, 1918, x, 313.

[2] Haas, A. R. C., and Fred, E. B., *Soil Science*, (in press).

[3] Haas, A. R., *Proc. Nat. Acad. Sc.*, 1916, ii, 561.

[4] Crocker, W., *Bot. Gaz.*, 1917, lxiii, 422.

[5] Czapek, F., Biochemie der Pflanzen, Jena, 1905, ii, 869.

[6] Kappen, H., *Landw. Vers. Sta.*, 1918, xci, 1.

[7] Johnson, H. V., *Am. J. Bot.*, 1915, ii, 250.

[8] Hoagland, D. R., *Science*, 1918, xlviii, 422.

[9] Gillespie, L. J., and Wise, L. E., *J. Am. Chem. Soc.*, 1918, xl, 796.

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comes of greater significance when we consider the observations of Hoffman[10] upon soil productivity. In twenty-seven out of thirty-two cases examined, it was noted that a greater number of bacteria were to be found adjacent to, rather than away from, the roots. The question therefore arises: Are the bacteria to any considerable extent responsible for the degree to which etching takes place? The results of controlled experiments have shown that roots, when growing in soil containing bacteria, possess a greater etching power upon marble than when growing in the complete absence of bacteria. General experiments were made using the apparatus described by Fred[11] for growing higher plants free of bacteria, as well as large Pyrex glass tubes.

Slabs of marble 4.2 cm. wide, 15 cm. long, and 2 cm. thick, were polished by rubbing the marble on cloth soaked with an aqueous solution of oxalic acid, and quickly removing any adhering acid by means of running water. It was arranged to have the polished surface of the marble slabs extend diagonally down the lower part of the culture vessel.

The same weight (250 gm.) of Miami silt loam soil from Madison was placed in each glass tube after the original soil sample was thoroughly mixed. The soil was shaken somewhat, in order to fill all the space about the marble. The tubes were plugged with cotton and sterilized in the autoclave at 15 pounds pressure for 2 hours on 3 successive days. The tubes were tested as to their sterility by observing whether or not growth took place after several days, when some of the soil was placed in beef peptone broth. Water was then added to each tube by means of a pipette, until the water content of the soil was 20 per cent by weight.

Canada field peas, that had been sterilized[11] with a 0.25 per cent mercuric chloride solution with reduced pressure, were placed in Petri dishes and covered with Ashby's agar. When the agar had solidified, the plates were inverted and incubated at 27°C. for 2 days. At the end of this period the seeds had germinated and short radicles were projecting through the seed-coats. This treatment

[10] Hoffman, C., *Kansas Univ. Sc. Bull.*, 1914, ix, 81.

[11] Fred, E. B., *J. Gen. Physiol.*, 1918–19, i, 623.

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made it possible to make a selection of the germinating seeds and to avoid the use of those which showed contamination. It should be pointed out in this connection that the uninoculated tubes were tested again at the close of the experiments for the presence of microorganisms.

Two bacteria-free pea seedlings were transferred into each vessel. The seeds were placed directly over the slanted marble slabs. This made it possible for the roots, as they grew downward, to come into intimate contact with the polished surface of the marble. The seeds were covered over with soil. Preliminary experiments showed that the roots of pea seeds, unless covered over with soil, have a tendency to grow along the surface and are tardy in penetrating the soil. This condition may be due in part to the effects of the sterilization upon the soil texture.

The tubes were placed in the greenhouse and were kept at about 60–70° F. When the seeds in the tubes had grown for 7 days, several of the tubes were inoculated. In the first experiments one-half the tubes were inoculated with a water suspension from field soil. Because of the great number of bacteria as well as the different types added in this inoculum, it is not surprising that the plants with bacteria far exceeded in growth the plants without bacteria. Perhaps the nitrifying bacteria caused this increased plant growth. To avoid the action of certain organisms, *e.g.* nitrifying bacteria, nodule bacteria, and molds, another experiment was carried out in which the soil was inoculated with a mixture of pure cultures of bacteria instead of soil extract. The nitrifying bacteria, therefore, were purposely omitted from this mixture. The following organisms were used: *Azotobacter*, *Bacillus aerogenes*, *Bacillus coli*, *Bacillus communior "C,"* *Bacillus communis "B," Bacillus hartlebii*, *Bacillus avaniensis*, *Bacillus mesentericus*, *Bacillus fluorescens liquefaciens*, *Bacillus fluorescens non-liquefaciens*, *Bacillus pyocyaneus*, *Bacillus proteus*, *Bacillus prodigiosus*, *Bacillus tumescens*, *Bacillus subtilis*, *Cladothrix dichotoma*, and *Cladothrix oderifera*.

5 cc. of sterile water were added to each of the uninoculated tubes in order to keep the moisture content of the soils similar in each tube. 25 days after the seeds had been placed in the tubes, the tubes were opened and the slabs and plants examined. A photo-

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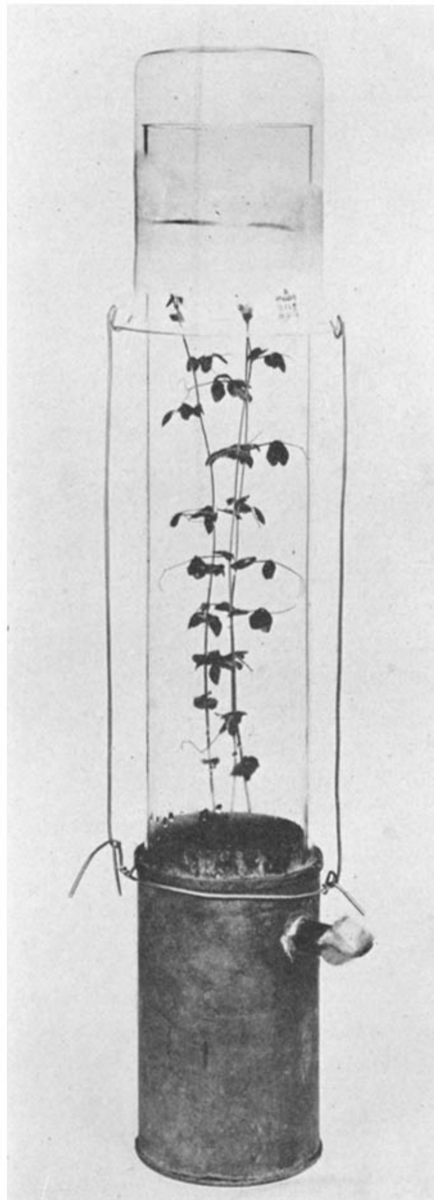

FIG. 1. Photograph of pea plant growing in soil with marble slab present.

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graph of one of the pea plants after a period of 42 days is shown in Fig. 1.

Immediately upon opening the uninoculated tubes, samples of the soil were inoculated in tubes of clear nutrient broth. After incubating for several days, the broth tubes were examined for the growth of microorganisms. Whenever any growth was observed, the contaminated tubes were included with the inoculated ones.

The plants were removed from the tubes by a gentle stream of water. It was found that in nearly every instance the plants in the inoculated tubes made a slightly better growth as regards the length of roots and tops than the plants grown under sterile conditions. An effort was also made to grow plants in agar, but it was found unsatisfactory.

In the preliminary experiments, water-proof drawing ink was rubbed with a smooth cloth into the etchings to bring out the details better in the photograph. Fig. 2, A, shows the effect of the roots upon the marble in the absence of bacteria; Fig. 2, B, shows the effect when the bacteria are present. In the later experiments the etchings were better defined for photographic purposes by carefully filling the etchings with lamp black. Fig. 3, A, shows the effect when the bacteria were absent; Fig. 3, B, shows the effect when the bacteria were present.

The slabs were placed in a row with those from the uninoculated scattered among those from the inoculated tubes. A person unacquainted with the numbers of the slabs was then called upon to arrange the slabs in the order of their etching. In choosing a criterion of etching, the emphasis was put upon the depth to which the etching had taken place. The amount of etching upon the entire polished surface of the slabs was also taken into consideration although here the possibility of some roots not coming into close contact with the marble becomes a factor.

It was found that in every case the marble slabs that had the greatest degree of etching were those taken from the tubes that had been inoculated. It was also found that in many cases it was possible to predict from the order of the slabs whether or not an uninoculated tube had become contaminated before the conclusion of an experiment, for in this event such slabs would usually be classed among the pieces of marble from the inoculated tubes.

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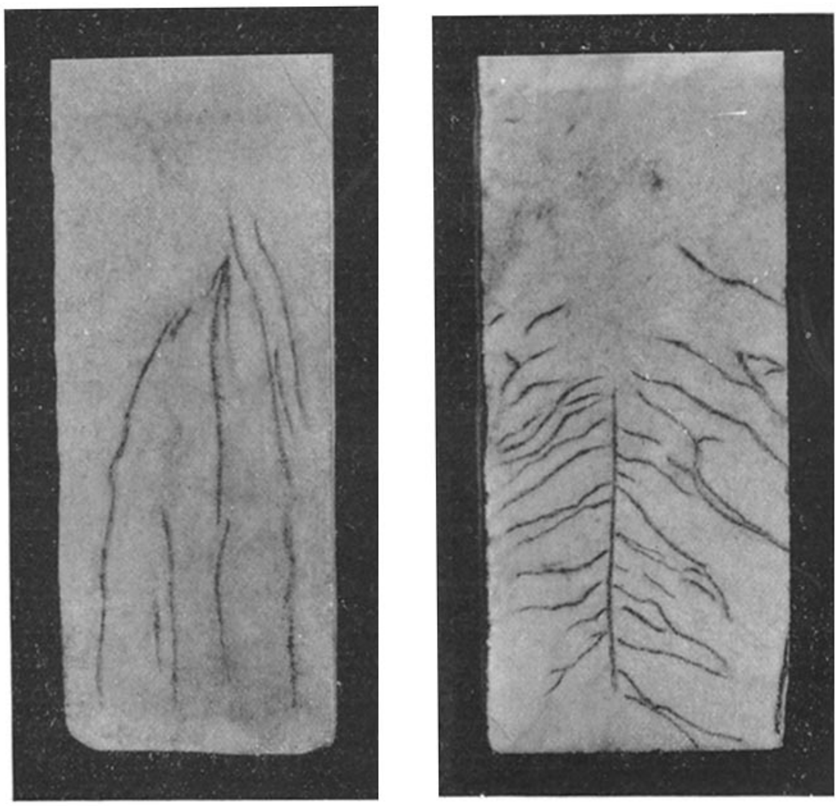
It seems probable that the greater etching power of the roots of pea plants in the presence of soil bacteria can be attributed to the normal carbon dioxide excretion of the living root cells, together with carbonic and other acids set free from dead or dying root cells whose decomposition is accelerated by the presence of the bacteria. While



A. B.

Fig. 2. A, plant roots without bacteria; B, plant roots with bacteria.

the results indicate that the dissolving power of roots is in part due to the action of the bacteria, it must be admitted that there are many factors involved. For example, the food supply of a plant must be different in the presence of bacteria from that of a plant in soil free of bacteria.

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A. B.

Fig. 3. A, plant roots without bacteria; B, plant roots with bacteria.

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SUMMARY.

In a study of the effect of soil bacteria upon the etching power of the roots of Canada field peas upon polished marble, it has been shown that the presence of the bacteria increases the etching power of the roots.

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