INJECTION-EXPERIMENTS ON PLANTS

BY

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It struck me why injection-methods being more direct were not largely tried to combat physiological or fungus diseases of plants or to help their growth. In March 1922, I began injection-experiments on a number of chlorotic pot plants of *Crinum asiaticum* by injecting a 0·5% solution of ferrous sulphate into their bulbs with a hypodermic steel needle. The basal end of the needle was connected by means of a rubber tube with a graduated glass tube fixed on a stand at some height from the ground (see fig.), so that the level of the solution in the tube was kept higher than the point of insertion of the needle and thus the solution could enter by means of a continuous upward pressure for a number of days. The quantity absorbed daily was noted. The results were not quite conclusive and the work was interrupted for a time as I had to leave India for Europe. After my return, I was repeating the experiment in December 1924, when my attention was directed to the work of Dr. C. B. Lipman of the University of California, (Journal of General Physiology May 20, 1924, Vol. VII, No. 5, pp. 615—623), who cured a number of chlorotic and diseased *Citrus* trees by injecting a large quantity of ferrous sulphate solution into their trunks; the method adopted was identical to that used by me; it took about three months to restore the normal green appearance of the trees.

In April 1925, I noticed some yellow *Mimosa* plants (*Mimosa pudica*) growing in the open among a large number of green healthy plants and apparently suffering from chlorosis, in the Bose Research Institute at Calcutta. All leaves (with their leaflets) of affected plants looked yellow. Evidently, there was no deficiency of iron in the soil as the neighbouring *Mimosa* plants so close by were completely green. Possibly, it was a case of injury to the roots and root-hairs of affected plants by coming in contact with hard bricks underground, as has been found by Dr. R. Marloth in the course of his investigations into the causes of the chlorotic condition of fruit trees in the Wellington District (Univ. South Africa. Dept. Agric. Sci. Bull. 29. 21p. 6 pl.—1924).[1] Marloth has recorded that about 5,000 trees in

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[1] I have seen this paper in abstract in Bot. Abstracts, July–Nov. 1926.

the region were affected, the roots alone indicating a diseased condition, some of which might be due to mechanical injury in cultivation.

Very dilute solution of ferrous sulphate (·25%) was injected into the stem of the plant in its own habitat by means of a hypodermic steel needle attached to a graduated glass tube by means of rubber

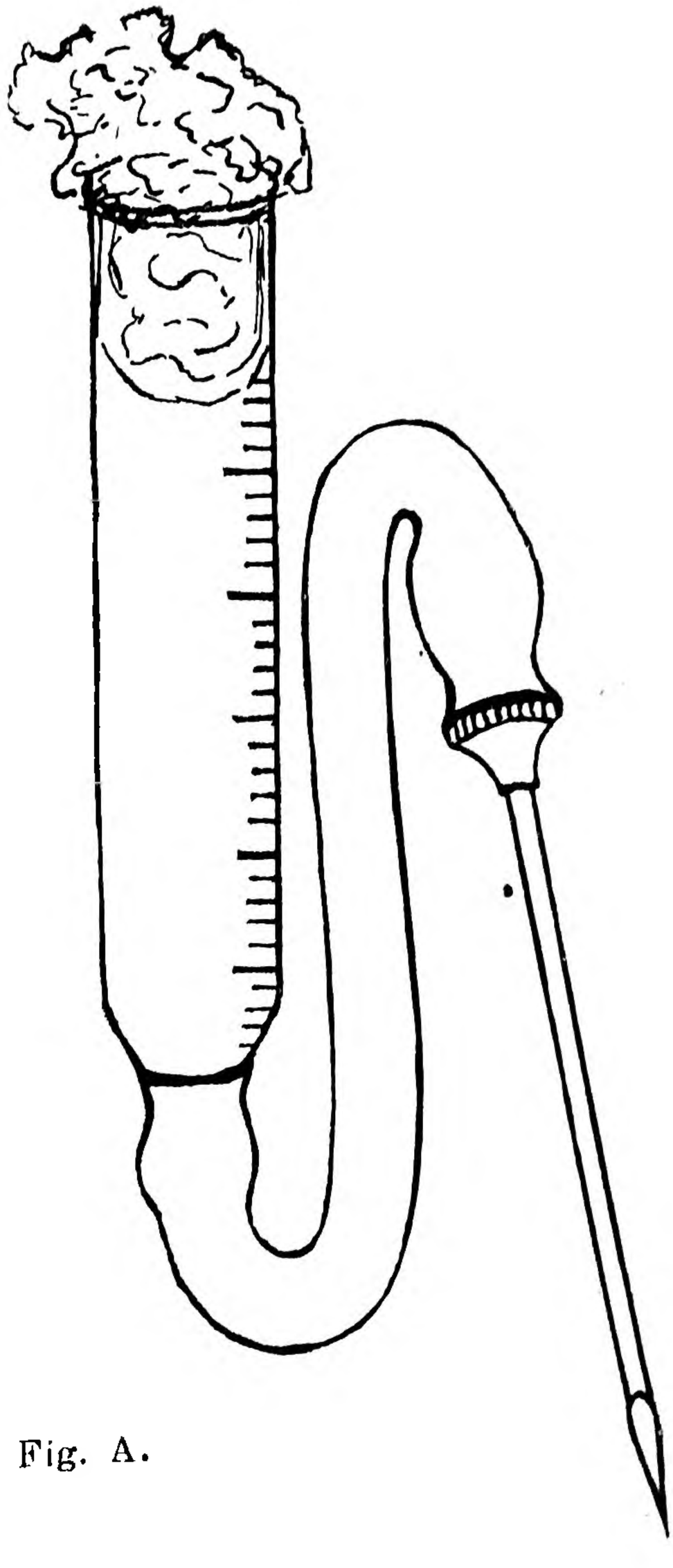

Fig. A.

tubing, following exactly the same method as I did in case of the bulb of *Crinum asiaticum*. The glass tube was filled with a ·25% solution of ferrous sulphate, a few drops of machine oil were poured on the surface of the solution to prevent evaporation, and the needle was kept on for a number of days, the solution getting in by means of a continuous upward pressure. The daily record of the quantity absorbed was kept by noting the level of the liquid. In the course of

even three or four days I was surprised to find that all the leaflets of the plants turned completely green; the treated plant could hardly be distinguished from the normal green plant. I repeated the experiment on more than a dozen such chlorotic plants, and in each case the result was the same. In some plants the response was quicker, in two days the whole plant turned green. Of course, the rate of absorption varied depending on various external factors and individual requirements of plants. The quantity absorbed in the first twenty-four hours varied from ·3c.c. to 1·5c.c. in different plants. Two things had to be guarded against in such experiments,—the choking of the narrow bore of steel needles and the leaking out of the solution from the joints. The needle had to be inserted right into the wood of the stem—into the circulating current of the growing plant, taking care to exclude entrance of air. The corresponding strengths of the solution (Fe SO_4) were poured on the soil round the yellow *Mimosa* plants which were watched for a number of days; but the response was very feeble and almost negative.

While renewing the experiment of injection of very dilute solution of Fe SO_4 into stems of *Mimosa pudica* in June 1925, my attention was directed to the fact that most of iron in the solution was precipitated on the sides of the graduated glass tubes in the form of brownish deposits, being oxidised, and that what actually passed into the plants was thin clear acidulated water (H_2 SO_4 solution) containing a very minute trace of iron (much lower than ·25% solution). This led me to omit the iron solution altogether and to inject very faintly acidulated water (1 mim. of sulphuric acid in 100 cc. of water) with steel needles into stems of a new set of chlorotic *Mimosa* plants. In the course of five to six days (comparatively little longer time than that in the first case), the whole plant turned beautifully green; it was repeated on a number of yellow *Mimosa* plants; in each case the result was the same. The details of the rate of absorption in each case from day to day were recorded. The treated and cured plants were watched for more than a year; there was not a single case of reversion amongst them. It was found by the colorimetric method that the acidulated water in contact with the steel needle dissolved out a very minute trace of iron (0·00025% ionised iron), which was entering the plant along with the streaming solution.

As a sort of control, glass needles (hard and narrow capillary glass-tube ground to a fine point in the form of a needle) were used instead of the steel ones, and the experiments repeated on a fresh set of chlorotic *Mimosa* plants in the Bose Research institute in August and September 1926, with the same acid-water alone (1 mim. Merck's pure sulphuric acid in 100 cc. of water). It was found that the response

was very feeble and unsatisfactory, there was no perceptible change in the course of a fortnight though about 2 cc. of the acid-water were absorbed during the time.

Next, I tried similarly injection of the same acid-water with a steel needle into the stem of some stout medium-sized *Ixora coccinea* with yellow leaves, growing from the ground. In the course of eleven days the whole stalk with sub-branches turned green; of course, the total quantity of water absorbed was greater than that in the case of *Mimosa* plants.

A stray case of sudden conversion of a number of chlorotic *Mimosa* plants into normal green ones immediately after a heavy shower of rain was noticed in our college garden-fields (February 1926); possibly, the circulating stream of the rain-water had the effect of someway modifying the power of absorption of affected plants in this instance.

By using the very delicate test of Macallum for the detection of minute traces of iron, as described by Prof. Benjamin Moore in Proc. Royal Society, Vol. 87 (1914), pp. 556—571, it has been found that the amount of iron in a healthy leaf is much greater than that in a chlorotic leaf; and from the colorimetric method it appears that the quantity of iron in healthy plants (either in stems or roots) is almost double the quantity in chlorotic plants, and that the amount of iron is always greater in stems than that in roots. For this estimation I am indebted to my friend Dr. H. N. Mookherjee, B, Sc., M.B., D.I.C., of the Bio-Chemistry Department of our College.

Apart from the general scientific interest attached to the restoration of the green colour of chlorotic plants, the injection-method might be used with advantage in the case of feeding starved fruit-trees, and this direct method of feeding might prove a great improvement on the natural method of providing the tree with its sustenance through the soil by means of fertilizers.

Since writing most of this paper, my attention was directed to the work of Messrs. Hopkins and Wann (Bot. Gazette Vol LXXXIV, No. 4, December 1927, pp. 423—426) where it is stated that "it is not the total amount of iron in the culture medium, but the amount in the ionised form which is effective physiologically. Therefore, while we may have a larger total amount of iron present, we may have little or no growth because of a low concentration of the ferric ion." This probably explains that the iron-ion-concentration necessary for the conversion of chlorotic *Mimosa* plants into normal green ones is infinitesimally small.