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THE BACTERIA OF THE SOIL, WITH SPECIAL REFERENCE
TO SOIL INOCULATION. By R. STEWART MACDOUGALL,
M.A., B.Sc.

(Read 11th February 1897.)

Up till about twenty years ago the soil was looked upon as made up merely of so many bits of dead material—stone and lime, and clay, etc. Tillage was a mechanical operation, and changes that followed it were explained purely on chemical and physical grounds. In these last years, however, it has come to be recognised that the soil teems with myriad minute forms of life—useful, and harmful, and neutral; in a word, for the tiller of the ground the problem is not solely chemical, but also biological.

Many workers in soil bacteriology have given their attention to the exact numbers of germs present in the soil, and in different layers of it. It would weary the reader to give lists of the numbers which vary in a gramme (just over 15 grains) of earth, according to the circumstances, from only a few up to some millions. Let me rather give the general principles determining their number as enunciated by Maggiora:¹—(1) The number of bacterial germs, in otherwise resembling circumstances, is in forest soils less than in arable land, and in these less than in the soil of inhabited places. (2) In non-cultivated soils the number of bacteria changes with (*a*) the geological formation and the height above the sea; (*b*) with the imperviousness or the aeration of the soil, the germs being much less numerous in the former than in the latter; (*c*) with the nature of the soil,—sandy soil is less rich in bacteria than, say, humus soils. (3) In cultivated soils the number of germs increases with the culture activity and with the bringing of dung. Strongly dunged soils are much richer than poorly dunged or undunged. The greatest bacterial richness is at a depth of 8 to 20 inches; below this the number quickly decreases. This is true for both cultivated and non-cultivated soils.

If I say that Kramer, as an average of three experi-

¹ Maggiora, *Journal de la Agricole du Brabant*, 1888.

ments with the same soil, found in one gramme of earth—

At a depth of about 8 inches	650,000 germs,
" " 20 " . . .	500,000 "
" " 28 " . . .	276,000 "
" " 39 " . . .	36,000 "
" " 47 " . . .	5,600 "
" " 55 " . . .	700 "
" " 61 " . . .	only a few,

perhaps it will be asked how it is possible to make such exact calculations. A survey of the methods will show the problem not to be as difficult as it seems. If the earth sample be taken from the upper layer of the soil, we do it directly with a sterilised spoon, and then place the sample in a sterilised tube plugged with cotton wool. If the sample falls to be taken from the deeper layers, the above-mentioned method will not suffice, as in digging, the earth of the deeper layers will become mixed with parts from the upper layers, which will tumble in. To obtain an accurate sample from a layer of any depth, Frankel's borer is employed. The boring body of this instrument is about $1\frac{1}{2}$ inch in diameter. An inch or two above the boring point is an opening between 4 and 5 inches long and about 1 inch deep. This opening is destined for the earth sample. The borer, with a handle which can be lengthened out to 5 feet, is pressed into the soil, and the mechanism is so arranged that as long as the borer be twisted from right to left the opening mentioned above as in the side remains closed. When the desired depth has been reached, by turning the borer in the opposite direction the covering to the opening is pushed back and the opening exposed; the earth falls in, the opening is again closed, and the sample pulled up. One gramme of this earth is carefully weighed out and placed in a quart of water, which has been sterilised by boiling, the mixture being thoroughly shaken. Meanwhile one has ready a glass tube containing the gelatine nourishing material for the bacteria. The gelatine has been rendered germ free by repeated heating in a steam bath. This gelatine, when liquefied by a gentle warming, has added to it carefully by means of a sterilised pipette 1 ccm. of the above mixture of water and earth. After a thorough mixing, the entire contents are poured

out of the tube on to a gelatine plate. Within a very short time the gelatine grows stiff, and holds, caught at certain points, the organisms. The isolated bacteria begin to grow and multiply by fission, and soon each has given rise to a colony. It is taken for granted that each colony has arisen from one individual germ, and by counting the colonies one gets the number of germs present in the 1 ccm. of the earth mixed in the water, and can thus calculate the number in the quart, *i.e.* in the gramme of earth. The colonies differ in external appearance, and each is confined to members of the same species. To get a pure cultivation of a particular species, one removes with a platinum needle a small part from a colony, and inoculates a fresh gelatine tube. In general outline, then, this is the method, but in very exact work other points must be attended to, *e.g.* certain bacteria only grow in absence of oxygen (anaerobic), and such must be cultivated accordingly. Questions of temperature, and differences in nourishing media to suit the varying bacterial tastes must also be considered.

BACTERIA OF THE SOIL.

In the soil are a very large number of different species, some of which have been investigated and proved harmful or useful, but many more, as far as research has gone, have not yet been proved to have any significance as regards man himself or man's cultural operations. I propose to choose out of the mass three sorts, all of much importance to agriculturists, *viz.*:—

1. The Putrefactive.
2. The Nitrifying.
3. The Nodule forming Bacteria of Leguminosæ.

There are a number of bacteria which act on nitrogenous organic compounds in the soil and cause putrefaction, resolving the albuminous organic matter into simpler principles. We place as manure on our land complex organic substances, which by the aid of the putrefactive germs are rendered simpler, and brought into conditions such that they can be assimilated by the plant. When as a result of the action of the putrefactive bacteria on albumen, ammonia, say, is produced, this is seized and

acted on by other organisms, the so-called nitrifying bacteria; the nitrous organism converting the ammonia into nitrous acid, this nitrous acid by the agency of the nitric organism passing into nitric acid, from whose union with a base come the nitrates so important as the form in which plants obtain that nitrogen, without which their growth and development is impossible. There is then a sort of circle of changes by which the nitrates taken up by the plant once more find themselves as nitrates in the soil, on the completion of the circuit in which bacteria play so important a part.

“Death,” as Liebig says, “the source of a new generation”; or, as Klein quotes, “from earth to earth.”

Morphology of the Nitrifying Organisms.—Globular to begin with, but later, oval and somewhat elongated. The nitrous organism is larger than the nitric, and measures $\frac{1}{25000}$ inch.

Conditions which favour Nitrification with Annotations.—

1. *The presence of the organisms.*—These organisms vary in number greatly in different soils. The more highly cultivated soils will contain them in greatest quantity. Where they are few in number what has been termed “microbe-seeding” may be wisely practised, *i.e.* soils which, owing to their deficiency in these organisms, can be looked on as so far sterilised, could have added to them soil which from the conditions was known to be rich in nitrifying germs. If the new environment were favourable the

introduced bacteria would rapidly multiply and spread. This is a favourable place to notice one of the values of stable manure. "Dung," as Hilltner has expressed it, "is the heaven of the agricultural soil," meaning that in adding it one gets, over and above other well-known advantages, a supply of vigorous nitrifying organisms.

2. *Access of Oxygen.*—This is why the bacteria of nitrification occur in greatest number towards the surface, and decrease with the depth. A new meaning is hereby given to tillage and good cultivation, as these ensure a good circulation of the necessary air.

3. *Light acts injuriously.*

4. *A certain moisture* is necessary, but marshy ground will lack the germs.

5. *A suitable temperature.*—In this case, say, between 90° and 100° F.

6. *A base with which the nitric acid, on its appearance, can combine.*—It is an interesting fact that as a result of the activities of soil organisms, their own further development may be inhibited. Thus, yeast is killed by excess of alcohol produced by itself, and so, too, with bacteria. Their own products may forbid their further growth. In the world of medicine, by inoculation of the matter prejudicial to the life of certain bacteria, we can put an end to them. The presence of a base then prevents the accumulation of nitric acid. A favourite base is lime, and among the numerous advantages of lime in a soil, I desire at present to single out its favouring so largely the development of the nitrifying bacteria.

7. *The other necessary plant foods.*

And now there is a dark side to this story. Research has proved the presence in soils of denitrifying organisms, which may bring the work of the useful organisms to nought. Their work is a reducing one, the nitrates being decomposed with an ultimate giving off of free nitrogen. Instead of being a gain, these denitrifying bacteria occasion a loss. Generally it may be stated that the conditions under which these harmful organisms flourish are the opposite to these we have mentioned as favourable to the nitrifying germs.

THE BACTERIA OF LEGUMINOSÆ.

Of the substances which are taken in by the roots of plants, the most important in plant metabolism are the nitrogenous compounds. Generally it may be stated that while plants can and do take in oxygen as a free gas for their respiration, they are quite unable to make use of free nitrogen. The nitrogen so necessary in the plant economy can only be absorbed in the combined state, say, as nitrates.

For long this was held to be true of all orders of plants, until it was noticed that while in soils deficient in nitrogen most plants came to nought, yet members of the order Leguminosæ (peas, and clovers, and vetches) grew quite well, and left the land after their growth richer in nitrogen than before. When it came to be admitted that leguminous plants must and did make use of the free uncombined nitrogen, men cast about for an explanation of their advantage as regards nitrogen, and several theories were advanced before it came to be admitted that in the nodule formation was to be found the real solution of the problem.

These warts, or swellings, or tubercles—as they are variously called—were shown to be little houses inside which lowly plant forms lived in partnership with the pea, or bean, or clover, deriving support from the plant, and repaying this support by fixing the free nitrogen that surrounded the roots, and forming it into compounds that were absorbed by the leguminous plant.

These swellings had been written of long before their real origin was discovered. As far back as 1687 Malpighi wrote of these as galls, and, coming nearer our own time, they were looked upon by some as a sign of a diseased condition, and by others as resulting from nematode attack. Now we know that these nodules only appear on the roots in case of infection with a bacterium, the *Bacillus radiclecola* of Beyerinck (*Rhizobium leguminosarum*, Frank). This bacillus is widely spread, and can be obtained in quantity, for examination, if perfectly fresh leguminous roots are laid in water and left to steep for a few hours in a warm room. Amidst the bacteria, which

will have rendered the water cloudy, will be found many of the nodule-making forms. The bacteria penetrate a root hair or a non-cuticularised epidermal cell (the young parts are the seat of infection, the older parts of the root not allowing an entrance), where they multiply at the expense of the cell contents. The irritation set up in the cortex cells, in which the bacteria ultimately find themselves, gives rise to repeated cell division, and the wart is formed. Finally, these bacteria take on a forked or Y-shaped appearance, when they are known as bacteroids. The full significance of these bacteroids will appear later, sufficient here to say that their contents are finally absorbed by the host plant. A certain number of bacteria remain behind, which, on the breaking down of the nodules, are set free again in the soil, where they may proceed to new infection. Frank had previously noticed that nodules did not appear on leguminous plants grown in sterilised soil, but the proof that the Leguminosæ are not able to make use of the free nitrogen in absence of nodules, but only when nodules are present in their roots, was given by Hellriegel and Wilfarth, after a series of very accurate recorded experiments. Their experiments showed that peas and lupins grown in soil sterilised, but with all the necessary plant foods added save nitrogen, died off without any nodule formation as soon as the original store of nitrogen present in the seeds had been exhausted. Peas and lupins, on the other hand, sown under the same conditions, save that a little soil was added from a field where peas and lupins had been growing the year before (in which soil it was reasonable to expect the infecting bacteria to be present), showed a large formation of nodules and a most excellent growth.

Many workers, British and foreign, have since verified these results, but Professor Nobbe and Dr. Hilltner, of Tharand, have taken a step in advance by being the first to induce nodule formation by inoculation with pure cultures. Here is the method of making such pure cultures: Take a fresh nodule and wash it carefully. After drying it in blotting paper, drop it for a moment into corrosive sublimate, to kill any bacteria on the surface. Next wash it with absolute alcohol. Having

sterilised a scalpel by heating in a flame, cut the nodule. Dip a platinum needle into the cut surface, and streak the gelatine already prepared. It is well to have a large surface of gelatine, as the *Bacillus radicumicola* is an aerobic form. It is important to remember that this bacillus will not grow on the ordinary gelatine, but there must be incorporated with the gelatine-nourishing medium a decoction of leguminous shoots or leaves. Having obtained in this way, after a few days, a pure growth of the bacillus, in order to inoculate plants growing in pots some of the bacilli are transferred to water, a little of which may be sprinkled over the soil in which the plants are growing; or, better still, by means of a glass tube the mixture is introduced to the deeper layers, near the roots. The experiments of Nobbe and Hilltner aimed at winning information and clearness in three directions, viz.: (1) What are the true factors and occurrences by which a nodule-beset leguminous plant is able to make use of the free nitrogen? (2) What is the action and efficacy of the nodules in soils holding different quantities of nitrogen? (3) Are the bacteria from the warts of the different leguminous species one and the same species of bacterium, or has each leguminous group or species its own distinct nodule-former?

As to Question 1.—Some hold that the nitrogen assimilation is by means of the green leaves, others that the bacteria are the chief agents in taking up the free nitrogen. Nobbe and Hilltner believe strongly that the power is vested in the bacteroids, which, arranged in the form of a network, expose a large surface to the air, to which the bacteroids come into relation as regards nitrogen in the same way as the gills of a fish to oxygen. The conclusions from their experiments so far are: (a) Nodules whose bacteria have not followed on to the bacteroid stage (as happens in certain circumstances) are of no value to the plant, the bacteria in them being out-and-out parasites, and performing no nitrogen assimilation. (b) The stronger in life the bacteria are, the less is the tendency to become bacteroids; the stronger the nodule-possessing plants, the easier is the changing from bacteria to bacteroids. (c) With bacteroid formation comes nitrogen assimilation.

Question 2.—Hellriegel experimented with sterilised soil, and grew his plants with the conditions—no nitrogen and non-inoculated, and no nitrogen but inoculated. Of greater complexity are the experiments of Nobbe and Hilltner, who added to some of the sterilised soils (left sterilised or else reinoculated) varying proportions of nitrogen, and noted results. Their answer to Question 2 is, that the effect of the nodule reaches its full worth when the soluble nitrogen of the soil is almost used up by the growing plants. The more a soil contains of bound assimilable nitrogen so much the later will the differences stand out externally between inoculated and non-inoculated plants. Quickly-growing leguminous species, like peas and vetches, which sooner exhaust the supply of combined nitrogen, will show the effects of inoculation earlier than clovers or lathyrus, which, growing at first more slowly, make more trifling demands on the soil nitrogen.

Question 3.—Do the bacteria from the nodules of different leguminous plants belong to one and the same species? Beyerinck is of opinion that the bacteria cultivated from the different species of Leguminosæ are not identical, though certainly very similar. He distinguishes two groups: (1) Those whose large colonies are more hyaline and give rise to bacteroids, forked or roundish. Such are bean, vetch, broom, medicago, melilotus, clover, pea, lathyrus. (2) Those with opaque, dull white colonies giving bacteroids seldom branched, but more like bacteria, *e.g.* ornithopus, lotus, lupinus, robinia, caragana. Nobbe and Hilltner believe that though the bacteria springing from the different leguminous species can scarcely be distinguished from one another externally, yet in their effect and behaviour and attitude to the plants they show noteworthy differences. For example, bacteria won from nodules on the pea, infect most powerfully other pea plants, and act most beneficially on them; infect and act less favourably to plants not peas, but closely related to them, but produce no impression on leguminous species like robinia or clover, whose affinity with the pea is not close. *Vice versa*, bacteria from the nodules of the red clover infect other red clover plants, but are quite ineffectual on peas.

Nobbe and Hilltner, however, emphasise that the cultures from different Leguminosæ have not to be looked on as different bacterial species, but only as adaptable forms, forms adapted to the varying leguminous species by their living on different plants. To make this clear, they have coined a new phrase—neutral bacteria, viz. those living in the soil with no special biological adaptability to any special leguminous species, but able to get in a weak degree into symbiosis (partnership) with all genera of Leguminosæ. These neutral bacteria, having penetrated the roots of a special leguminous plant, form nodules. They multiply, and their descendants have been so influenced by the host plant that, having ceased to be neutral like the original parent forms, they effect their full strength only on the same leguminous species, and lose their power to set up infection in any other. Here will be found the explanation of strange Leguminosæ forming nodules on their roots, which nodules go on increasing from year to year in number and efficacy. Leguminous plants then will only, with certainty, show nodule formation if the soil contain either the germs accustomed to the planted species or else neutral germs.

I would now call your attention to some experiments relating to Question 2:—

I. AN EXPERIMENT WITH PEA (*Pisum sativum*).

In this experiment 6 pots were taken and filled with a mixture of 4 parts quartz sand and 1 part garden earth, the whole being sterilised, *i.e.* all germ life was killed by heating. In each pot 5 pea plants were sown in the beginning of June.

Pot 1 was inoculated with pure cultures of Phaseolus bacteria.				
" 2	"	"	Pea	"
" 3	"	"	Clover	"
" 4	"	"	Robinia	"
" 5	"	"	Lupin	"
" 6 was left uninoculated.				

At first all the pots showed an equally good growth, but as time went on and the soluble nitrogen of the soil had been largely consumed, the effects of inoculation began to show themselves. The plants in the pot inoculated with pea bacteria, and, later, those in the pot inoculated with phaseolus bacteria were seen to be green, and were richly

flowering and fruit-bearing up till the autumn. The plants in the other four pots were quite withered by the end of August. On 20th October the plants were cut, and the above-ground parts analysed. For comparison, here are the results in the cases of the non-inoculated, inoculated with phaseolus, and inoculated with pea—

	Not Inoculated.	Inoculated with Phaseolus.	Inoculated with Pea.
Dry substance in gr. .	3·878	28·821	95·452
Nitrogen in the green substance in gr. .	0·055	0·852	2·791
Nitrogen per cent. of the dry substance .	1·43	2·96	2·92

That is, the plants inoculated with pea bacteria, compared with the non-inoculated, showed twenty-four times more of dry substance and fifty times as much nitrogen content.

At the time of cutting, the plants had for each experimental pot—

	Flowers still.	Fruits.	Seed.
1. Inoculated with Phaseolus bacteria	18	41 (20 still unripe)	55
2. Inoculated with Pea bacteria	36	134 (77 still unripe)	161
3. Not inoculated	0	2	3

II. EXPERIMENT WITH *VICIA VILLOSA*.

This experiment was conducted under the same conditions as the last, only the pots contained *Vicia villosa* plants instead of peas.

The most noteworthy thing about this experiment was that the best results were given with the vetch plants in the pot inoculated with pea bacteria (vetches and peas are nearly related), and the next best results in the case of inoculated with phaseolus bacteria. The plants inoculated

with clover, robinia, or lupin, soon began to show nitrogen hunger, shedding their leaves. The non-inoculated plants were ultimately quite leafless.

	Uninoculated.	Inoculated with Phaseolus Bacteria.	Inoculated with Pea Bacteria.
Dry substance in gr.	4.292	63.929	93.693
Nitrogen in gr.	0.070	2.310	3.444
Nitrogen in per cent. of dry substance	1.84	3.61	3.68

EXPERIMENT III.

contains additional interest. The plants experimented with this time were red clover, five in each pot, and all the conditions of the other experiments the same.

In this experiment the inoculations with pea bacteria, with robinia bacteria, and with lupin bacteria showed in the first two no effect whatever, and with the lupin only a very limited effect.

Comparing the pea inoculation with the clover inoculation—

	Inoculated with Pea Bacteria.	Inoculated with Clover Bacteria.
Dry substance in gr.	6.23	70.99
Nitrogen in green substance in gr.	0.108	2.136
Nitrogen in per cent. of dry substance	1.74	3.01

As to flowers and fruit. On cutting in October, the five clover plants inoculated with clover bacteria had had 54 flower-heads, while the pot with clover plants inoculated with pea had only 8 flower-heads.

EXPERIMENT IV.

will have an interest for foresters. Robinia plants were inoculated, some with pea bacteria and some with robinia bacteria. The robinias inoculated with robinia bacteria greatly excelled the robinias inoculated with pea bacteria.

So far for laboratory experiments with sterilised soil. There has not yet been time to obtain many results from experiments made with pure culture inoculation on a field scale, and yet here, too, there is at least an earnest of success.

Three plots out in the open, each 120 yards square, were planted with *ornithopus*. The sowing was in May.

Plot 1 was inoculated with pure culture of *ornithopus* bacteria.

„ 2 „ „ raw earth from another *ornithopus* field.

„ 3 was left uninoculated.

When these plants were revised in August, the non-inoculated plot averaged 2 small nodules per root.

Those inoculated with raw soil plot averaged 3 medium-sized nodules per root.

Those inoculated with pure *ornithopus* culture averaged 9 mostly large nodules per root.

Many experiments on a field scale have been started on the Continent, and, doubtless, during the year many more will be undertaken in Britain. Certainly a good case has been made out in the experiments in the experimental station at Tharand for the value of soil inoculation, and now field experiments under many conditions must give the final answer as to whether or not this special branch of soil inoculation is likely to prove advantageous.

As to Methods.—Where a soil is deficient in *Bacillus radicicola* there are two methods of infection—

1. The bringing to such a field a quantity of soil from another field in which the species of the plant to be sown has already grown well. This soil is then worked in. Dr. Salfeld, of Hanover, holds the honour of having employed this method on a large scale with gratifying results, getting good leguminous crops where such had refused to grow before. Difficulties in the way of using this method of inoculation on a large scale will occur, *e.g.* uncertainty, questions of cost and transit, the possible introduction of harmful forms, and so on.

2. There remains the second method, viz. Nobbe and Hilltner's method of inoculation with pure cultures. Such cultures can be made by oneself if the necessary bacteriological training is possessed, but if not, bottles containing the cultures can be bought from Lucius & Bruning, Hoechst a Main, at 2s. 6d. each. So far, cultivations of the following plants can be procured:—

Trifolium pratense or *repens* or *hybridum*, *T. incarnatum*, *Vicia villosa* and *sativa*, *Pisum sativum*, *Vicia faba*, *Medicago sativa*, *M. lupulina*, *Lupinus luteus*, *L. angustifolius*, *Ornithopus sativus*, *Onobrychis sativa*, *Lathyrus sylvestris*. Each bottle contains enough to supply half an acre.

In the inoculation with pure cultures there are two variations—

(a) *The inoculation with infected soil.* Take one of the above-mentioned bottles. Gently warm the bottle by placing it in lukewarm water (great care must be taken not to overheat—keeping in one's pocket for a few minutes is enough), and the gelatine with the culture is liquified. Pour this (thoroughly cleaning the bottle out) into a vessel containing sufficient water to moisten half a hundred weight of soil. Mix this liquid well with the earth. Then add some dry soil till a condition is reached when it can be conveniently spread. Work this into the field to a depth of 3 to 4 inches. Sow as soon as possible.

(b) *The inoculation of the seed directly.* As before, take a bottle and pour the liquified contents into $1\frac{1}{2}$ pint of water (this will do for small seeds like clover, but for larger seeds use 2 or 3 quarts). Pour the mixture over the seeds, and mix thoroughly, making sure that each seed gets moistened. Then add some soil from the field to be sown, so that the seeds don't stick together in clumps, and when dry enough sow.

In both *a* and *b* I have given the quantities necessary to inoculate half an acre.

HINTS AS TO EXPERIMENTS.

To all intending to make experiment I would recommend that in each experiment, for purposes of comparison, three plots be dealt with. Let plot 1 remain uninoculated; let plot 2 be inoculated with infected soil; let plot 3 have the seed inoculated directly. In the case of experiment on these lines, for safety let the non-inoculated plot be sown before the inoculated, and, to prevent after infection, let great care be taken to have no traffic between the inoculated and the non-inoculated plots.

Experimenters will also note—

1. That the cultures are very sensitive to light, and heat, and drought, all of which destroy the efficacy of the bacteria. Therefore do not allow the inoculated soil or seed to become too dry, and do not allow the seeds to remain exposed on the surface.

2. The results of inoculation must not be looked for too early. Remember that the efficacy of the nodules only comes into full activity when the soil nitrogen has been used up. The more of this in the soil the later will the effects of inoculation show themselves.

3. In order that a proper judgment be passed as to the effect of inoculation in parallel experiment, a mere naked eye overlook is not sufficient and not trustworthy. There must follow a careful weighing. Dr. Hilltner told me of a case where Dr. Salfeld made an experiment with ornithopus, the pure cultures for which were supplied from Tharand. When, towards the end of the experiment, an inspection was made, there seemed, as far as the eye could judge, to be no difference between the inoculated field and the non-inoculated, and, as the plants in the latter also showed nodules, everyone thought this experiment had proved a failure. Just eight days after the inspection, the two crops having been cut in the interval, Dr. Salfeld wrote to Professor Nobbe to the effect that the inoculated plot had borne stronger plants than the non-inoculated, and that a careful weighing had given a twenty-five per cent. advantage in green substance to the inoculated over the non-inoculated.

4. It must not be forgotten that the condition of the soil has a most important influence on the results of inoculation. The—apart from nitrogen—necessary plant foods must be present in sufficient quantity. The nodules collect only nitrogen, so that the plants must find in the soil all the other foods. A chain has only the strength of its weakest link, and “a field is as poor as its most deficient fertilising principle.” Apart from nitrogen, if there be only one of the other necessary foods not present in sufficient amount, then the capacity of the nodules will fail from the time when this substance begins to disappear.

5. The most profitable results of inoculation are likely to be in soils where nodule bacteria are naturally absent, and the least profitable where Leguminosæ have been greatly cultivated. Between these two there are all grades.

I cannot close this article without placing on record my indebtedness to Professor Nobbe and Dr. Hilltner, who received me at Tharand with great courtesy, and gave me every opportunity of becoming acquainted with the methods and experiments.

EXCURSION OF THE SCOTTISH ALPINE BOTANICAL CLUB TO CLOVA, IN JULY 1896. By WILLIAM CRAIG, M.D., F.R.S.E., F.R.C.S.E., Secretary of the Club.

(Read 11th March 1897.)

The Annual Excursion of the Club in 1896 was to Clova, a place exceedingly rich in botanical treasures, and one which is, and has been for generations, a great favourite with botanists. When the Club first visited Clova in 1872, the whole district was pastoral, and perfect freedom was permitted to all botanists. It is now, however, like many other of our highland glens, converted into a deer forest, and consequently there is sometimes a difficulty of access to these glens. The Club, however, were highly favoured in obtaining permission for a week in July to botanise the district. Permission was obtained from the proprietrix, Mrs. Macpherson of Glen Doll, through her agent, Mr. W. Gibson, W.S., Edinburgh.

The members of the Club left Edinburgh on Monday, 27th July 1896, with the 9.40 A.M. train for Kirriemuir. They travelled by the Caledonian Railway, the officials of which Company sent a private saloon carriage to Kirriemuir for the special use of the members. Kirriemuir was reached about 12.45 P.M. After lunch in one of the hotels the members had a very pleasant drive to Clova, and stayed at the Ogilvy Arms Hotel. Nine members of the Club attended this excursion, including our president, Mr. W.