THE NODULES OF LEGUMINOUS PLANTS.

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For many centuries practical agriculturists have realised that legumes, such as peas, beans and vetches, when grown under suitable conditions, enrich the soil for the succeeding crop; but it is only within modern times that a satisfactory explanation of that increased soil fertility was forthcoming.

The experiments of Lawes, Gilbert and Pugh in 1857 had led to the irresistible conclusion that plants in general obtained their nitrogen requirements from the soil and not from the atmosphere. This conclusion agreed with the results of field experiments except where leguminous plants were concerned. The very troublesome fact remained that leguminous crops require no nitrogenous manure, yet they contain large quantities of nitrogen, and the soil in which they are grown is enriched. The problem of where this nitrogen comes from remained unsolved for many years.

Hellriegel and Wilfarth in 1888 were able to show from experiments in sand culture that the growth of a non-leguminous plant, such as the oat, was directly proportional to the amount of nitrate supplied, and that at the end of the experiment the amount of nitrogen contained in the oat crop and sand was less than that originally supplied. The growth of a leguminous plant, such as the pea, under similar conditions was erratic. In some cases the peas behaved exactly as the oat, but in others the peas grew well even in sand containing no nitrogen, and in these cases there was always an increase of nitrogen at the end of the experiment. They concluded that some factor, which came into the experiment by chance, enabled the peas to obtain their nitrogen from the air.

Berthelot in 1885 had shown that certain micro-organisms in the soil were capable of assimilating gaseous nitrogen from the air; Lachmann in 1858, and Woronin in 1866, had shown that the nodules of leguminous plants contained bacteria. By connecting these two facts, Hellriegel and Wilfarth came to the conclusion that the bacteria in the nodules obtained gaseous nitrogen from the air and then handed on some of the resulting nitrogenous compounds to the plant. In other words the nodule bacteria constituted the factor which entered the experiments by chance and caused the conflicting results.

That this hypothesis was well founded was amply demonstrated. They found that legumes grew quite normally in soils lacking nitrogen, provided that these soils had not been previously sterilised. In sterilised soils pea plants behaved like oats, and obtained their nitrogen from the soil. But if to the sterilised soil was added an extract of arable soil on which peas had previously been grown, the peas grew well even when the soil contained no nitrogen, and the resulting crop was rich in nitrogen. If, however, the extract was previously boiled, its addition produced no effect. They also showed that an extract which worked well for peas might be without effect on lupins. Further reference will be made to this finding.

The legumes which happened to grow satisfactorily in unsterilised soil and those from sterilised soil watered with soil extract had on their roots small tubercles or nodules, whereas in sterilised soil no nodules were formed. The presence of such tubercles had long been known on the roots of leguminous plants and various explanations had been offered of them. Frank in 1879 had shown that the formation of nodules could be prevented by sterilisation of the soil in which legumes were grown, and Marshall Ward not only proved that nodule formation is due to outside infection but that such infection could be brought about by placing pieces of old nodules in contact with the roots of growing legumes. It remained however for Beijerinck in 1888 to isolate the specific organism which produces the nodules, and to this he gave the name *Bacterium radicicola*.

Later researches have confirmed all of Hellriegel and Wilfarth's results. Laboratory experiments have shown conclusively that where sufficient precautions are taken to sterilise the soil and to prevent chance infection of the plant by *B. radicicola* no nodules result on the roots of legumes grown in it, and that, in the absence of root nodules, leguminous plants are dependent, like non-leguminous plants, on the nitrogen compounds in the soil for their supply of nitrogen. It is the bacterium within the nodule which has the power of assimilating gaseous nitrogen, and not the leguminous plant independent of the bacterium.

The question naturally arises whether the nodule bacterium fixes nitrogen in the absence of the leguminous host plant. To this question contradictory answers have been given and the problem cannot be regarded as completely settled. Various explanations have been offered for the differences in the results obtained. The purity of cultures employed has in some cases been doubtful, and other results are vitiated by faulty methods of analysis. Recent investigations point strongly to the fact that legume nodule bacteria do not fix atmospheric nitrogen when grown under ordinary laboratory conditions independent of the host plant. It would be foolish to say that these bacteria never fix nitrogen under any conditions because obviously no one can test all possible variations. There, however, appears little justification for the assumption that the nodule bacteria can fix even small quantities of nitrogen when grown independently of the host.

Thus a leguminous plant and its nodule-forming organism are individually unable to assimilate gaseous nitrogen; when living together nitrogen fixation undoubtedly takes place in sufficient quantities to be of practical importance in increasing the nitrogen supply of cultivated soils and in reclaiming barren sand and clay soils.

Reference has already been made to Hellriegel and Wilfarth's experiments which indicated that the bacteria having a beneficial effect on peas might have no effect on lupins or serradella. This observation indicated that there was not one kind of nodule bacteria but two or more. Carefully designed cross inoculation experiments carried out by a number of workers have led to the conclusion that if classified with regard to the leguminous plants on which they are found, the nodule-forming organisms can be divided into the following 12 groups:—

(1) Clover group. Includes only species of the genus Trifolium.

(2) Alfalfa or lucerne group. Includes members of the genera Medicago, Melilotus and Trigonella.

(3) Cowpea group. A large one including several genera amongst which are, Vigna, Dolichos, Desmodium, Acacia, Arachis, Pueraria and Cassia.

(4) Vetch group. Includes species of Vicia, Lens, Pisum and Lathyrus.

(5) Soybean group, (*Glycine hispidus*).

(6) Bean group. Contains members of the genus Phaseolus but not *Phaseolus lunatus* (lima bean).

(7) Lupin group. Includes Seradella (*Ornithopus sativus*) in addition to the lupins.

(8) Hog peanut group, (*Amphicarpa monoica*).

(9) Lead plant group, (*Amorpha canescens*).

(10) Trailing wild bean group, (*Strophostyles helvola*).

(11) Common Locust, (*Robinia pseudo-acacia*).

(12) Wood's Clover, (*Dalea alopecuroides*).

The organisms belonging to any one group are incapable, as a rule, of producing nodules on the roots of plants belonging to any other group though they will form nodules on the roots of other members of the same group. Thus the nodule-forming organism which is found on the roots of red clover (*Trifolium pratense*) will cause nodules on the roots of other plants belonging to the genus Trifolium. It is specific to that genus. On the other hand the organism which invades the roots of the cowpea (*Vigna sinensis*) will also invade plants belonging to other, and apparently not closely related, genera such as Acacia, Desmodium and Cassia, but will not induce nodules on clover or other plants not belonging to Group III above.

These facts are of practical importance because they indicate that the optimum benefit cannot be derived from leguminous plants unless the necessary specific organism is present in sufficient numbers to form the nodules, even when other conditions are favourable for satisfactory growth. The benefit derived from introducing soil from land on which a particular leguminous plant has been grown, to new land where the crop is being grown for the first time was probably realised before the reason for the practice was understood. Soil on which a particular legume, or another from the same group, has been successfully grown will contain the proper organisms for that legume; the legume becomes naturally inoculated. But where a legume from another group is being introduced the requisite organism may have to be provided artificially. For instance, red clover has been grown so long in parts of the United States that its nodule bacteria are present everywhere, but when soy bean was introduced, the crop had everywhere to be artificially inoculated, as the soy bean organism appears not to have been originally present in the United States. Similarly where lucerne has been grown in England, the best results have been obtained where the crop has been artificially inoculated with the proper organism.

Although it had long been recognised that all legumes are not inoculated by the same organism, very little difference in physiological and cultural characteristics was noticed between the cultures from different plants. After more careful study it became apparent that all strains were not exactly alike. Differences in size, morphology, flagellation and other characters were observed, and experienced workers have been able to place the various strains in groups corresponding with the cross-inoculation groups already referred to. It was also assumed that all cultures inoculating any one legume were alike, but later work drew attention to the fact that there were differences between the cultures of any one cross inoculation group. It was shown that differences in nitrogen fixing power were correlated with such differences in cultural characteristics. Different strains from soy bean and from lucerne on trial have been shown to be not equally efficient in fixing atmospheric nitrogen. It follows, therefore, that where artificial inoculation is practised, a strain of organism having a high nitrogen fixing ability is necessary.

The maintenance and restoration of humus and nitrogen in Ceylon tea soils is a matter of great economic importance. These soils owing to climatic conditions are subjected to a rapid destruction of humus, consequently the necessity for incorporating organic residues into the soil is deservedly receiving increasing attention. Economic conditions render it necessary that the organic matter must take the form of vegetable residues, i.e., green manures, and not of animal manures which have always held first place in European agriculture. Because legumes, when grown under favourable conditions, enrich the soil as a result of the fixation of atmospheric nitrogen, they have consequently been the first choice for green manures. It is here emphasised that the fixation of atmospheric nitrogen is carried on by the bacteria within the root nodules. In the absence of such nodules the nitrogen requirements of the legume are obtained directly from the soil; in such cases the legume is no more efficient in soil improvement than are many non-leguminous plants. Very little is known of the nodule-forming organisms present in Ceylon soils and in the nodules of indigenous leguminous weeds and crop plants. Many of the green manure plants grown on tea plantations are imported species, but in no case has the requisite nodule-forming bacteria been specially imported with the seed. Those species which have succeeded have no doubt found the requisite organism in the Ceylon soil, and it seems possible that the failure of other species to flourish has possibly resulted from the absence of the necessary organism. It is not suggested that the absence of the requisite bacterium is always the cause of failure, because it must be recognised that a legume may fail owing to adverse climatic or soil conditions, drought, unfavourable soil reaction, poor seed, animal depredations and other causes. But if a legume is to have a fair chance of success it is not only important that the specific bacteria be present but that they be present in sufficient numbers.