A Study of Leguminosae Bacteria.

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A complete study of leguminosae bacteria would involve the consideration of many questions beyond the present ability of the writer while at the same time the briefest discussion of the points thus brought under consideration would prolong the extent of this paper beyond reasonable limits. It is not, therefore, my object to present a full discussion of the subject, but briefly to review some of the chief factors appertaining thereto. As an introduction to this study it will probably be well to speak of the economic importance of leguminosae bacteria.

It has long been known to agricultural investigators that the most essential elements of plant food are three in number, viz.; nitrogen, potassium, and phosphorus. Other elements are required, but in such small quantities and are almost everywhere present in such abundance that they may here be neglected. Potassium and phosphorus being products of the chemical disintegration of rock and soil are also relatively abundant, and, in addition are not easily soluble but combine readily with the bases in the soil and are not likely to become exhausted to such an extent as to cause serious apprehension on the part of the agriculturist. Not so, however, with nitrogen. A careful calculation by competent authority reveals the fact that while in the first eight inches of average soil in the United States there is, were it possible for all of the essential elements to be assimilated without waste, sufficient potential phosphoric acid for 129, and potash for 240 crops of maize of 50 bushels per acre each, there is nitrogen present sufficient for only 32 such crops. Nitrogen is the most essential element to the growth of living tissue, be it plant or animal, and in addition is the scarcest and the most liable to depreciation and loss. The question of the nitrogen supply is therefore of paramount importance not only to the agriculturist but to the world at

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large.

The nitrogen supply in the soil is almost exclusively in the organic matter. Any process which tends to decompose or destroy this organic matter, such as nitrification or other forms of oxidation, will also tend to reduce the total stock of nitrogen in the soil. Because of this fact the matter of restoring nitrogen to the soil becomes of very great importance. Of course a part of the nitrogen removed in crops may be returned to the soil in manure produced on the farm; and nitrogen may also be bought in the markets in such forms as sodium nitrate (containing 15 to 16 per cent of nitrogen), ammonium sulphate (containing 20 to 21 per cent of nitrogen), and dried blood (containing 12 to 15 per cent of nitrogen); but when we bear in mind that such commercial nitrogen costs about 15 cents a pound and that to produce 1 bushel of corn nearly 2 pounds of nitrogen are required, it will be seen at once that the purchase of commercial nitrogen cannot be considered practicable in general farming.

Nitrogen is removed from the soil not only in the crops grown, but also, and frequently in larger amounts per annum, in the drainage waters, and in some other ways, as by denitrification and by the blowing and washing of the surface soil. Professor Snyder, of the Minnesota Experiment Station, has shown that during a series of years the total loss of nitrogen from certain Minnesota soils in some cases amounts to several times the amounts actually used in the crops produced.

Considering all these facts and the additional fact that there are millions of pounds of atmospheric nitrogen resting upon every acre of land or water on the earth, the question naturally arises: Is there not some method whereby this great store of nitrogen may be made available for the use of farm crops? This identical question had agitated the minds of scientific investigators ever since it became the accepted opinion that nitrogen, unlike carbon dioxide, could not be assimilated directly from the air by growing plants.

From the earliest days of agriculture it has been recognized that all plants belonging to the Leguminosae Order had a decidedly beneficial effect on the soil. Pliny wrote, "The bean ranks first among the legumes. It fertilizes the ground in which it has been sown as well as any manure," and again, "The lupine enriches the soil of a field or vineyard as well as the very best manure. The vetch, too, enriches the soil and requires no attention in its culture." There are also in other ancient writings many references to the importance and necessity of including some leguminous crop in the regular rotation Naturally the explanations offered to account for this beneficial effect were various, perhaps the most universal belief being that the root system of these plants was much more extensive than that of grains and root crops and consequently brought up plant food from considerable depths, which not only served the legumes, but was likewise available for subsequent crops. Shaw in 1809, advanced the theory that the cultivation of leguminous crops might improve the soil by taking up nutriment from the air and depositing it in the soil through roots and stubble; but this was merely a conjecture without experimental basis. In 1854, Boussingault promulgated his classic experiments demonstrating the fact that plants could not assimilate free nitrogen gas. His work was verified by the joint labors of Gilbert, Lawes, and Pugh, and although Ville and others still maintained the fallacy of the investigations, it soon became as well established as any fact in plant physiology that the only efficient source of nitrogen supply was in the fixed forms supplied through the plant roots. It was not, however, until 1886 that Helriegel announced at a scientific meeting in Berlin that the source of nitrogen for these plants was undoubtedly the atmosphere, and two years later, together with Wilfarth, he demonstrated the fact that the growth of plants in soil free from nitrogen always occured after the development of nodules or swellings upon their roots. Later the results of the investigations of these two men were fully substantiated by many other investigators, and the explanation of the long unsolved problem was made possible.

By this time it was beginning to be more generally known that the Leguminosae were capable of growing in soil practically devoid of nitrogen, and consequently great difficulty was experienced in reconciling what certainly appeared to be two contradictory statements. So well established, however, was the work of Boussingault and others that no very great doubt was cast upon the question of how plants obtained their nitrogen, and an attempt was made to explain the difference through some inherent peculiarity of the legumes themselves.

While this conclusion is not strictly true it nevertheless has some elements of truth connected therewith, as we shall see later. More than two centuries ago, 1687, Malphigi had described what he called gall formations of the legumes, and in the early part of the 19th Century Karl Van Wulffen described the "tiny tubercles"which occur only on legumes, and recommended the cultivation of white lupines for the improvement of sandy soil. The first detailed description of the structure of the leguminosae tubercles was made by Woronin, in 1866, who at the same time propounded the theory for the first time that they were caused by vibrio-like organisims which he had discovered within the nodules. From this time up to the date of Helriegel's definite announcement there were numerous investigators who attempted to solve the problem, but without success.

While at first the observations of Helriegel and Wilfarth were by no means universally accepted by botanists, the numerous verifications of their work by Lawes and Gilbert, Atwater and Woods, Ward and many others soon left no other explanation plausible and it became practically an accepted fact that all legumes were beneficial to the soil because of the presence of peculiar swellings upon their roots which enabled the plants in some way to acquire nitrogen from the air. On the other hand the nature of the organism which produced the tubercle was not readily decided, and even up to the present time there is considerable discussion as to its character and systematic position.

This organism about which so many disputes have been waged by scientists, each of whom have apparently equal ability as observers, has been variously described. This is owing to the various forms taken on, according to the environment in which it is grown. In reality the organism is a very simple form of bacteria. When isolated and grown in pure cultures it has been found to be a short rod shaped organism with rounded ends. The characteristic u and y shapes found in the nodules are supposed to be due to the grouping together of two or more of the simple forms. According to Prozmowski's investigations the development and growth of the tubercles are as follows:- Bacterium pseudomonas radicicola lives normally in the earth and collect in numbers on the outside of the roots of the various legumes. Some of the organisms succeed in forcing their way into the root tissue, probably through the ends of the root hairs, sometimes remaining there for a time as pure bacteria but the plant plasm seems to exert an injurious influence on them, hence the development of a sheath or pouch around them. The bacteria which do not succeed in getting into this pouch degenerate into bacteroids which appear later in great numbers.

The bulk of the bacteria are enclosed within the pouch, where they continue to develop in great numbers and with much vigor. The pouches begin to grow into thread-like masses and these threads are often branched. These threads penetrate the root, looking very much like mycelium of a mold. The development of this mass of threads soon forms a swelling on the root, which develops into the tubercle. The cells of the plant continue to develop a corky-like layer around these bacteria which appears to be impervious to the bacteria thread and hence holds them within its limits. Further development goes on until it seems that the membrane enclosing the bacteria bursts, letting the organism into the cellular tissue of the root; at all events there appears the bacteroid tissue probably through the influence of the plant plasm. The bacteria continue to grow only as long as inclosed within the protecting membrane.

The principle structure of the tubercle is thus developed from the root tissue, while the inside, composed of bacteroids, is what gives the tubercle the flesh red color. Some of these central cells are so completely filled with the bacteroids that nothing else can be seen, while in others a nucleus can be seen. The bacteroids are afterwards absorbed by the plant, leaving the tubercle an empty pouch. This practically ends the life history of the tubercle.

The general shape of the tubercle varies more or less from nearly true spheres (found on soy beans and cow peas) to branching club shaped forms (found on alfalfa and the clovers). The size of the tubercles varies from semi-microscopic to that of common marbles. The size and number of tubercles formed depends upon several conditions among which may be noted; (1) Moisture. It has been shown by experiment that a moist condition of the soil is conducive to the most abundant formation of the tubercles. Since drought is in no way fatal to the bacteria it has been suggested that the reason for the non development of tubercles in a dry soil is that the bacteria are either unable to come in contact with the root hairs under such conditions or are unable to penetrate them. (2) Air. In artificial cultures it was conclusively shown that the organisms deprived of air soon perish, nitrogen especially being essential to their growth. (3) Light and heat. With artificial cultures it was found that except for the deleterious effect of strong sunlight there seemed to be no difference in organisms grown in the light or in the dark. The optimum temperature is between 23° to 25° C. (73° to 77° F.),and above 40C. (104 F.), there is usually no appreciable growth. It was not possible to produce death by any degree of cold although below 10°C. (50°F.) practically no multiplication took place. (4) The organism grows best in a slightly alkaline medium. (5) Nitrates. The presence of available nitrogen in the soil seems not only to retard the formation of the tubercles but to have an unfavorable effect on the bacteria themselves. A light sandy soil is a favorable medium for the development of large tubercles.

It having been definitely determined that the fixation of free nitrogen is dependent upon the presence of the leguminosae bacteria, the next problem is to secure the presence of the bacteria in the soil of fields to be devoted to the growing of leguminous crops. The old method of securing soil inoculation was to obtain soil from an old field known to be infected and scatter it about the new field. This is an absolutely certain way of securing the presence of the bacteria but the labor and expense attendant upon this method, to say nothing about the danger of spreading noxious weed seed and various plant diseases, practically precludes an extensive application of it. The successful isolation and cultivation of pure cultures of the bacteria naturally led to the idea of growing the organisms artifically on a large scale for inoculation purposes. To a German botanist, Professor Nobbe, of Tharandt, belongs the honor of first conceiving this idea and putting it to a practical test. To the cultures thus produced he gave the trade name of "nitragin." Several different kinds of nitragin were prepared from the nodules of as many different plants, and arrangements were made to have them placed on the market on a large scale. Experiments with nitragin in Germany met with varying success. In some instances its use seemed to produce an abundance of nodules while in others it produced no results. The chief difficulties seem to have been in securing cultures of the proper degree of virulence and in preventing deterioration because of being subjected to too much heat or varying degrees of moisture.

To meet these difficulties the United States Department of Agriculture made a thorough study of the nodule forming organism and has succeeded in perfecting a process whereby inoculation by pure cultures may be carried on on any scale. Their investigations too, have disproved the old theory that there are different species of bacteria for each legume. While it is undoubtedly true that the long adaptation of bacteria to the special reaction of a given plant will produce a physiological difference that will enable it to more readily penetrate the host upon which they have been accustomed to grow, there seems to be no question but that by a proper system of cultivation in the laboratory, a general culture may be secured capable ofproducing a limited number of nodules upon all the legumes that now possess these growths.

From this conclusion it is but a step, albeit a long one, that from the nitrogen fixing organism now adapted only to the legume, there may be developed by artificial culture a form adapted to growth on, and capable of securing free nitrogen from the air and combining it for the direct use of non leguminous plants. The economic value of

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such an organism would be immeasurably great. The whole system of crop rotation, if not the whole pratice of agriculture, would be revolutionized. It would not then be a mere question of making two blades of grass grow where but one grew before, for even the desert places could be made to blossom as a rose, the rich land would grow richer, and the soil now too poor for profitable cultivation would be made to bring forth abundantly.

It is yet too soon to predict that such an adaptation can ever be made but the possibility is apparent and the far reaching effect of the successful production of such an organism should be sufficient incentive to induce a life-long effort if necessary, in that direction to that end.