be that disease resistance in a like manner is sometimes secured easier by hybridizing than by the slower process of selection, that in the hybrid the disease-resistant force will be equal to the sum of the resistant forces of both parents, instead of intermediate between them.

BREEDING BACTERIA.

By Dr. GEO. T. MOORE, *U. S. Department of Agriculture.*

When Professor Hays asked me to attend the meeting of the American Breeders' Association and speak to you upon the growing of nitrogen-fixing bacteria for the inoculation of leguminous crops, I could not see how such a subject was adapted to your program. However, after the Assistant Secretary of Agriculture had talked to me for a little while, I began to realize that certainly a large part of the success of this work was due to the careful selection of the organisms used, and by the time he got through talking to me I was firmly convinced that any results recently obtained by the Department of Agriculture in the use of these bacteria were due entirely to that most important of sciences—plant breeding.

It is hardly necessary for me to discuss in detail the effect of leguminous crops upon soils or the necessity for the roots of these crops being provided with the proper nodules in order to obtain the greatest good from them. You all know how necessary nitrogen is in the soil, and any means calculated to increase the quantity of this most important plant food is, of course, of great practical value. From the earliest days of agriculture it has been recognized that all plants belonging to the Leguminosæ, that is, peas, beans, clover, alfalfa, etc., had a decidedly beneficial effect upon the soil, but it is only within recent years that we have been able to explain this phenomenon. Although it had been a matter of common observation that the roots of leguminous plants were usually provided with peculiar swellings or nodules, these were popularly supposed to be due to the bites of worms or insects, and that they were directly connected with the fertilizing power of the plant was not discovered for many years. Even after it was shown that leguminous plants devoid of these nodules were unable to benefit the soil, there was the widest difference of opinion as to why they appeared upon some plants and were absent from others. Now we know that the legume nodule is the direct effect of the presence of myriads of bacteria which have made their way into the roots through the root hairs, and as the result of the irritation the plant manufactures the nodule, each cell of which is full of bacteria.

Although it is true that thousands of acres of land in this country and abroad are well stocked with these nodule-forming bacteria, it is likewise a fact that there is equally as much land which does not naturally contain these beneficial organisms. Practical farmers have realized for years that some parts of a field would produce clover

or alfalfa successfully while others, which seemed to be in fully as good condition and were as carefully cultivated, failed to produce a good stand. Many times this condition could be remedied by the addition of lime or some careful cultural method, but it was often found that the roots of the plants which failed showed no nodules and it was evident that the nodule-forming bacteria did not exist in that spot. For this reason it became necessary to devise some means of artificially introducing into the soil the nitrogen-fixing bacteria. Naturally, the easiest and simplest way of accomplishing this was to transfer soil from a field which had produced nodule-bearing plants and by this means introduce the bacteria into the soils which were devoid of them. Transferring soil from one field to another on the same farm is a comparatively simple and inexpensive process, but when, as is often the case, thousands of pounds of soil have to be shipped hundreds of miles, the operation becomes more difficult. It is also a fact that certain diseases of plants, the spores of which remain in the earth, are widely disseminated by such means of attempting to introduce nodule-forming bacteria, and, in some cases where the disease causes great damage to leguminous crops it has become necessary to abandon altogether this method of inoculation. There is also great danger of introducing objectionable weeds and insect pests, so that if any better means of inoculating the soil could be devised, it would certainly be desirable. It should be understood, however, that many cases of inoculation by soil transfer in this country have been eminently successful, and although the percentage of failures is greater than is usually supposed, there is no question but that much good has been done by this means of inoculating the soil.

A number of years ago a German botanist conceived the idea of bringing about inoculation by the means of pure cultures. This was to be accomplished by isolating from the nodule the right organisms and then transferring them to tubes containing a medium which would enable them to multiply rapidly. There was no difficulty in obtaining these pure cultures, and within a short time the product was put upon the market under the name of "nitragin" and sold at the rate of \$1 per bottle, which was sufficient for one-half acre. Numerous experiments abroad with a few in this country soon demonstrated that, while in some instances nitragin produced an abundant formation of nodules, there was a vastly greater number of cases where no benefit was obtained.

When this line of investigation was taken up in the Department of Agriculture, the demand for some artificial means of inoculating soil was already sufficiently great to make it desirable, if possible, to increase the bacteria very rapidly and to insure a sufficient quantity of them so that they could be distributed to various parts of the country where it seemed that they were needed. For this reason the means used for propagating them was similar to that of the German botanist, that is, clover, or bean, or other plants from which the bacteria originally came were stewed and to this decoction was added sugar, peptone, and other substances ordinarily used

in cultivating bacteria. The result was a medium very rich in nitrogen and the growth obtained was all that could be desired. At this stage the problem seemed an exceedingly simple one and it was thought that in a short time it would be possible to send out bacteria by the keg, barrel or any quantity desired. Unfortunately, however, when these organisms were tested in the greenhouse and in the field, it was found that they did not produce nodules. There could be no question about their having been derived from the true nodule-forming organism, but it looked as though they had by some means or other lost the power of infecting the plants upon which it was so desirable to have them produce root nodules. After a little experimenting, it became evident that the manner of growing these bacteria was not one designed to make them efficient nitrogen fixers. When you remember that these organisms reproduce themselves in from thirty to forty-five minutes, you can readily see that if they are at all susceptible to their environment, the tremendous number of generations obtained at the end of a few days enables any slight difference in their efficiency to be transmitted in a most astonishing way, and that, although one started with an organism capable of producing nodules and fixing nitrogen, unless the conditions were exactly right, this function might be entirely lost. Experiments soon proved that this loss of power was the result of growing upon a rich nitrogenous medium. It was as though the bacteria soon discovered that it was easier to get nitrogen out of the substance upon which they were growing than it was to fix it from the air, for it requires a tremendous energy to combine free nitrogen. Consequently, they simply degenerated and became lazy, like a number of other organisms do under similar circumstances, and, so long as they were allowed to grow under such luxurious conditions, they refused to perform the beneficial function for which Nature intended them. As soon as this fact was thoroughly demonstrated, an attempt was made to find some medium which would sustain the life of the bacteria and yet not enable them to degenerate and lose their beneficial power. By transferring the bacteria to a medium made up of chemically pure salts which contained no nitrogen and solidifying with silica jelly, it was possible to obtain colonies which had never had access to nitrogen in a fixed form. These colonies could then be transferred to nutrient solutions devoid of nitrogen, and in this way the beneficial function of combining nitrogen from the air was so augmented that chemical tests proved these organisms to be from seven to ten times as efficient as when they were originally isolated from the nodule. Of course, large numbers of the original bacteria were not able to stand this sudden transition from a comparatively rich nitrogenous medium to one absolutely devoid of this element, but a few million bacteria more or less make practically no difference. If one organism persisted, it was a matter of a very short time when this could be multiplied to any desired quantity. Thus by a process of selection, it was possible to obtain a culture which, so far as the direct fixation of nitrogen was concerned, certainly was very much more efficient than the ordinary wild nodule-forming organisms.

There is one other important element, however, which must be maintained or increased if these organisms are to produce the best results, that is, their ability to penetrate the root hairs, for at present at least it does not seem that these bacteria are able to obtain sufficient energy outside of the plant to fix nitrogen in any perceptible quantity. It is probable that they need large amounts of carbohydrates to enable them to carry on their important function, and these, of course, are readily obtained from the roots of the plant. Consequently, if they are not able to enter the plant, they are of little or no benefit to the soil. The penetrative function of these bacteria is generally restricted to a very small motile form which is seldom found within the root nodule after it has developed to any size. Growing nodule-forming organisms upon nitrogenous jelly or agar tends to fix the large rod-shaped form which cannot penetrate the root hairs. In fact, it is almost impossible after these bacteria have been grown under what would seem to be the most favorable conditions for a considerable length of time, to cause them to break up into the small root-hair penetrating form. On the other hand, bacteria cultivated upon nitrogen-free media, particularly when kept in liquids, readily break up into this small form and thus are able to produce large numbers of nodules filled with bacteria which are at what may be considered their highest state of efficiency.

In order to test the results of these selection methods several acres of different legumes were planted upon the experiment farm near Washington, part of which was inoculated with bacteria grown upon rich nitrogenous media and part with organisms taken from the same nodule but transferred immediately to silica jelly agar and thereafter kept growing in solutions absolutely devoid of any fixed nitrogen. The results were very striking indeed. In the case of soy beans, some thousands of plants grown in rows and inoculated with bacteria cultivated after the method of Nobbe upon decoctions of soy beans with peptone added failed to show a single nodule, whereas other plants in rows within 4 feet of these and inoculated with bacteria taken from the same nodule but grown upon nitrogen free media, showed an average of from 10 to 25 nodules per plant. Of course, the contrast above ground was equally striking.

It will be impossible for me to read you the great number of favorable reports we have had from practical farmers throughout the country who have successfully used these selected bacteria. Of course, it was expected that soils which were devoid of any of these organisms would be improved by inoculation, but it was not supposed that, where the bacteria already existed and had been producing a fair number of nodules upon the standing crop, the addition of the cultivated form would be of any distinct benefit. Reports demonstrate, however, that in some instances the inoculation with the cultivated forms shows as much difference upon the field as in those places where bacteria had not previously existed, and it seems probable that, by following the method so well understood by plant breeders, eventually we may be able to develop an organism which

will be available for a number of conditions which at the present time it is not supposed possible to benefit.

Numerous laboratory experiments have seemed to demonstrate that it is impossible for the nodule-forming bacteria to penetrate the roots of legumes after the plants are of any size. For this reason it has always been considered that it was useless to attempt to add the nitrogen-fixing bacteria to a growing crop. However, experience with these cultivated forms seems to indicate that under some circumstances the use of inoculating material upon a standing crop of any age will be beneficial.

In order to test the value of these cultivated bacteria which have been improved by selective methods, it was decided to distribute sufficient material for inoculating an acre or more of ground to each of a large number of practical farmers throughout the country. This distribution was begun about a year ago and we have already received some 3000 reports giving the results of the experiments. It would be very interesting if I could read you the experience of the men who have used these bacteria, but, as some of the reports have already been published and it would take altogether too much time to even give you an abstract of them, I can only say that the results have far exceeded our expectations, the percentage of success upon all crops under all conditions and in every part of the country being over 75 per cent. This means that the farmer was able to see a decided difference in the legumes which had been treated with the bacteria distributed by the Department as compared with those grown under ordinary circumstances. Sometimes the percentages of increase were as much as 500 per cent or more, and in the majority of cases the difference in favor of the inoculated crop was 100 per cent. When it is remembered that all of these experiments were carried on by men who had no other knowledge of the bacteria than that given them in the printed directions accompanying the cultures, that they were not selected in any way, and that oftentimes the region in which they attempted to grow the specific crop was not by any means best adapted for it, I think you will agree with me that the results have been very satisfactory.

Thus far the attempt has naturally been to secure an organism best adapted for general conditions, and since most of the legumes in this country are grown upon comparatively poor soil, these bacteria show the best results when used under such conditions. Whether or not it may be desirable to breed or select a special strain of bacteria for different kinds of soils remains to be seen. It appears that it would be possible to secure organisms adapted to alkali or acid soils, but whether bacteria of high efficiency can ever be produced which will have the desired effect in rich nitrogenous soils is a question. As a matter of fact there is probably very little demand in this country for such an organism, as in general soils of this character are devoted to wheat or corn or cotton, and it is only the wornout or comparatively poor soil which is used for growing leguminous crops. The possibilities of selecting certain strains of highly efficient, beneficial bacteria are just beginning to be realized in this country. While the

introduction of solid media by Koch has, of course, been of inestimable value in enabling us to secure pure cultures of the bacteria, it seems as though we have lost a great deal of valuable information by neglecting some of the older and less convenient methods of growing these organisms. It has been shown that certain bacteria which have the power of fermenting calcium citrate in a liquid medium will almost immediately lose this ability if transferred to a gelatinous medium. The bacteria pathogenic to rats lose their virulence if cultivated in contact with the air, and many other instances of the great rapidity with which these micro-organisms may modify their seemingly fixed functions might be given. I think enough has been said, however, to illustrate the fact that we really know very little about these most interesting and essential plants. That we shall be able to accomplish as much by selection with certain groups of bacteria as has already been done with the higher plants may seem too much to expect, but I believe it is certainly possible to improve greatly many processes in nature as well as in various industries dependent upon the bacteria by applying the knowledge and information gained from plant breeding in other lines.

BREEDING PROBLEMS.

By W. M. HAYS, *U. S. Department of Agriculture.*

The farmers of America produce annually nearly \$5,000,000,000 worth of products. Most of this vast production, say \$4,000,000,000, is handled by living forms of plant and animal life, which may be bred by man to greater efficiency. Many species can be so bred as to increase their production 25 per cent, and some only 10 per cent, without altering the other conditions of production. These other conditions of production can also be improved so as to increase production in nearly like amount. Broadly speaking, production can be increased 10 per cent by breeding better plants and better animals, and another 10 per cent by improving the methods of farming. In either case 10 per cent of \$4,000,000,000 is \$400,000,000, but the increase by better breeding can be had at a much less cost than the increase from better farming.

Betterments from breeding reproduce themselves and with no very great expense may be perpetuated and made continuously effective. Many of the betterments in the methods of farming, as the purchase of fertilizers and better cultivation, require considerable expenditure of money and labor every year. Burbank's potato, Gideon's Wealthy apple, Wilson's strawberry, Minn. No. 169 wheat, Cruikshank's Shorthorns, or the Jersey blood from the sire "Noble," go forward adding blessings year by year as a result of the work of the original breeder.

It is not argued that the wise purchase of commercial fertilizers does not pay good profits, nor that really superior cultivation and