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Leguminous root tubercles :results of experiments

William Rufus Dodson

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SECOND SERIES,
No. 46.

BULLETIN

OF THE

AGRICULTURAL EXPERIMENT STATION

WM. C. STUBBS, PH. D., Director and State Chemist.

LEGUMINOUS ROOT TUBERCLES.

RESULTS OF EXPERIMENTS,

— BY —

W. R. DODSON, A. B., S. B., BOTANIST AND MYCOLOGIST.

ISSUED BY THE BUREAU OF AGRICULTURE AND IMMIGRATION,
J. G. LEE, COMMISSIONER.

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LOUISIANA STATE UNIVERSITY AND A. & M. COLLEGE.

BUREAU OF AGRICULTURE.

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LOUISIANA STATE UNIVERSITY AND A. AND M. COLLEGE,
OFFICE OF EXPERIMENT STATIONS,
Baton Rouge, La.

Major J. G. Lee, Commissioner of Agriculture and Immigration, Baton Rouge, La.:

DEAR SIR—The leguminous plants have long been known to be restorative in their character when used for improvement of soils in a systematic rotation of crops. Some years since it was discovered that the chief virtue of these plants in abstracting and appropriating nitrogen from the air, was due to the tubercles which occur upon their roots. With the view of throwing light upon this subject, and especially of studying these leguminous plants which are in common use for soil restoration in this State, the following experiments were inaugurated, and have been successfully conducted by Prof. W. R. Dodson, Mycologist of the Station. For the purpose of diffusing information upon this subject among our farmers and planters, I ask that you publish this report as Bulletin No. 46;

Respectfully submitted,

WM. C. STUBBS,

Director.

Experiments on Leguminous Root Tubercles.

PREFACE TO EXPERIMENTS.

The farmers and planters of the Southern States have long recognized the necessity of cultivating a soil-renovating crop in the course of rotation.

The cow pea, Dolichos sinensis, has been most universally used for this purpose. Whatever theories may have been advanced in time past to account for it, it has been known for a great many years that this plant has a wonderful influence in restoring fertility to worn land, and increasing the productiveness of land already fertile to a fair degree. Other sections of our country and the countries of other lands have had their soil renovating crops in the form of clovers, vetches, lupines, or other leguminous plants. The beneficial results were recognized and taken advantage of by progressive farmers, but the means by which the plant accomplished such ends remained a mystery until within the last few years. In fact it has not yet been revealed in full, but the essential points in the process have been discovered. They get nitrogen from parasitic micro-organisms that live upon their roots; these organisms taking the nitrogen from the atmosphere which is not available directly as food material for plants.

Any one who will take the trouble to carefully remove the soil from the roots of a vigorously growing cow pea, peanut, garden pea, or other plant of the same family, will find upon the roots numerous warty formations, or tubercles, varying in size from the head of a pin to the third of an inch or more in diameter. The shape of the tubercle varies quite a good deal in different genera of host plants, but the well developed tubercles of any individual species will be so nearly constant in its general characters, that one accustomed to examining them can tell from the tubercle the genus of the host plant in a great many cases,

The accompanying cut from a photograph of tubercles on twenty species of plants will give a fair idea of the relative typical sizes and shapes, these specimens being selected as representative ones at the blooming period of the plant. In most cases where the tubercles are very numerous, they are small, and when they are large there are but few on the roots, and sometimes they are few and small. Generally a vigorous and healthy looking plant will be well supplied with tubercles. In the case of the peanut the tubercles are always abundant and quite large, considering the multitude found on every root. When thin slices of these tubercles are examined with a good, high power microscope, they are found to be filled with myriads of organisms resembling bacteria. It has been established beyond question that these organisms are directly responsible for the tubercles. Growing parasitically upon the roots, they cause an irritation of the tissue which results in this abnormal development, very much after the manner in which tubercles are produced in animal tissues in cases of tuberculosis. Several investigators have shown that when plants are cultivated in pots under conditions where contact with these organisms is prevented, the accumulation of nitrogen in the tissues of the plant is only equal to what is lost by the soil in which they grew; but if these bacteroid organisms gain access to the roots and tubercles are formed, there is an increase in the nitrogen of the plant that can be explained only by the assumption that the free nitrogen of the atmosphere has been utilized.

Nitrogen is one of the essential food elements of plants in which most soils are deficient and of which they are most easily exhausted. Though growing plants are surrounded by the greatest abundance of nitrogen in the air, they are unable to use it as food material until it is brought into combination with other substances by these parasitic organisms, or chemical and electric agencies. The amount of nitrogen gained by the latter process is small. In some way which we do not yet fully understand, this combination is accomplished through the root parasites, and it is then available as food material. The nitrogen thus acquired being distributed throughout the roots as well

as the stem, a goodly portion of the nitrogen is left in the soil when the crop is harvested. The tubercles themselves are very rich in nitrogen, as will be seen by reference to the analyses made by the chemists of the Station, Mr. Blouin and Mr. Clark. It may be suggested, since "pulled hay" includes many roots and adherent tubercles that are not eaten by stock, that this is not the best way to harvest a crop of leguminous plants. The agricultural possibilities opened up by the discoveries already referred to, are far reaching, and give promise of much practical application. It is possible that in the future the farmer will give as much attention to a bacteriological understanding of his soil as he now does to its physical and chemical properties. To furnish conditions favorable to the development of a world of plants so infinitely little as to be totally beyond his natural vision, will be the most potent factor in fertilization. Soils now thought to be incapable of growing certain crops, may be made to produce them abundantly, and that, too, with little expense and trouble. In several instances in this State, soil infertile to alfalfa has been made to yield a splendid growth in two or three years either by sprinkling over it small bits of soil from a field where this plant was growing luxuriantly, or by taking the roots of the healthy alfalfa and mascerating them in water and pouring it over the land. This process is thought to simply convey the organism necessary to develop the tubercles, and when once they are started to growing in the soil and on the roots of the alfalfa, it is only a matter of a very few years till they will be well established there, and the alfalfa will flourish.

The question naturally arises, can this process be perfected so as to extend to all soils of this climate, and may not other crops be affected by the character of the microscopic flora in the soil to an extent that will warrant thorough investigation. Can the continued failure of a particular crop in a given locality be explained by the absence of certain living micro organisms, and can these organisms be artificially applied; what conditions of soil and cultivation are most favorable for their development and the influence of one form upon another; these are some of he questions that call for our consideration.

Some experiments that presented the possibility of practical results were begun in February, 1896, two series of which an account here follows:

EXPERIMENTS, SERIES 1.

The object of these experiments was to determine the influence of deep and shallow planting on the tubercle formation of the roots. The plants selected for the experiments were cow peas, peanuts and garden beans. On account of the difficulty of getting a stand in deep planting, the observations were practically limited to cow peas. Seeds were planted at a depth of one, two, three, four, five and six inches. After eight weeks of growth the soil was thoroughly softened by soaking and the roots were preserved as far as possible by washing the dirt away from them with a stream from the hose. The root system was studied as the soil was washed away from it and the amount of roots compared after the harvesting. After making allowance for factors not under our control in the experiment there was a striking indication that the shallow planting showed more surface root formation and the greatest abundance of tubercles. Planting at two and three inches of depth seemed to give the maximum of roots that spread near the surface, and the greatest number of tubercles. Below three inches the greater the depth the less extensive the root system and less vigorous the plant. There was but little difference in the first three plantings.

Roots often come very close to the surface of the soil and run parallel with it for a considerable distance. Roots of the cow pea have been traced a distance of six feet without at any time being more than three or four inches below the surface. While the tubercles are generally most abundant near the base of the stem, they are found throughout the surface root system. Roots penetrating directly downwrrd are generally free from tubercles at a depth of ten or twelve inches, or soon after the root gets into the compact clay.

The observations made in these plantings were supplemented by an examination of all the leguminous plants that could be found in the vicinity of Baton Rouge.

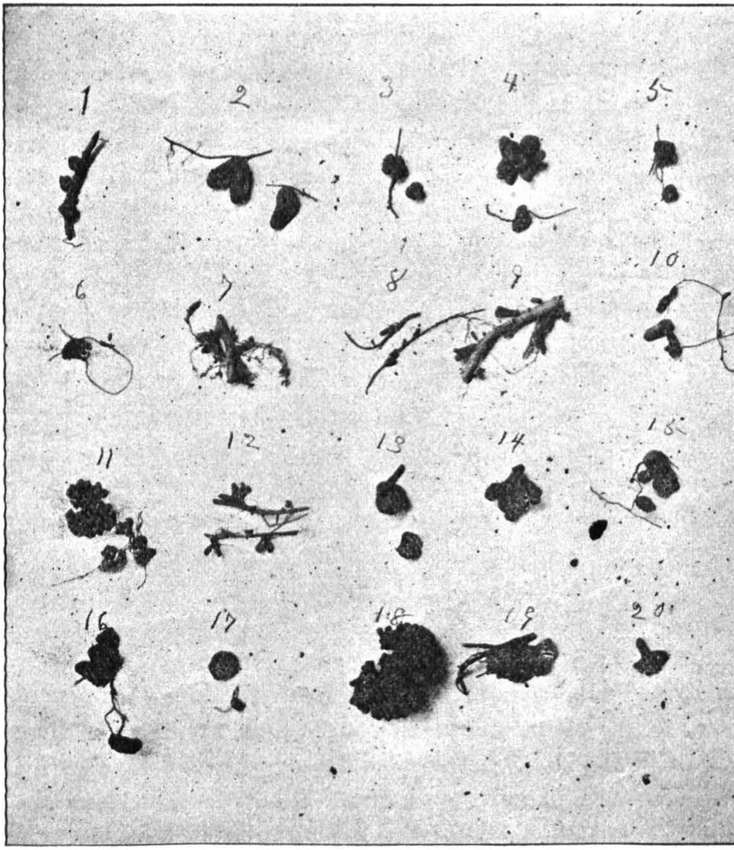
The observations gathered indicate that the nature of the soil has much to do with the depth to which the root tubercles are well developed. A striking illustration of this may be seen by digging a bunch of white clover that has grown in a soil that has been well cultivated and comparing the roots with those of a plant of the same kind grown in the compact soil of the headland. In the former case the tubercles will be found plentiful to a depth of four inches or more, while on the roots of the plants from the headland they will be confined to roots much closer the surface. A number of plants have two distinct sets of roots, especially is this noticeable in the cow pea. One set forages the surface soil, while the other goes directly downward.

Tubercles are formed at a greater depth in sandy soil than in a clayey soil, and deeper where deep cultivation has been practiced than where shallow cultivation has prevailed.

SECOND SET OF EXPERIMENTS.

By these experiments it was sought to obtain an approximate idea of the depth to which the nitrifying organisms penetrate and find conditions favorable to their development. Eighteen six inch flower pots were thoroughly sterilized, and six of them filled with clay from three feet below the surface, six with clay from a depth of two feet, and six from one foot below the surface. A portion of iliz ed sand was mixed to prevent excessive compactness, and the pots planted with the following seeds: Crimson clover, Lima bean, yellow trefoil, new era cow pea, peanuts, and white lupines. The pots were watered with prepared nutritive solution, but all grew very poorly. It was difficult to keep the necessary amount of moisture and prevent caking of the soil. The plants lived for about two months when all but the cow pea and peanut were beginning to die. The roots were examined and a few small tubercles were found on the cow peas of some of the pots of clay from a foot below the surface, others had none. No difference in the vigor of the plant with the tubercles was noticeable.

The experiment was repeated with some modifications. The pots were filled with sterilized sand and to six of these was added about an ounce of clay from a depth of three feet. The



The root tubercles figured in cut were taken from the following plants :

No. 1, Cow pea, Dolichos sinensis; No. 2, Dolichos sesquipedalis; No. 3, Dolichos mingo; No. 4, Dolichos cultratis from Hindoostan; No. 5, Dolichos forneosa; No. 6, Crimson clover, Trifolium incarnatum ; No. 7, White clover, Tritolium repens ; No. 8, Red clover, Tritolium pratense; No. 9, Sweet clover, Melilotus alba ; No 10, Alfalfa, Medicago sativa ; No. 11, Black medic, Medicago lupulina ; No. 12, Spotted medic. Medicago maculata ; No. 13, Soja bean, Soy hispida ; No. 14, white lupine, Lupinus albus ; No. 15, Canavalia ensiformis ; No. 16, Canavalia gladiata ; No. 17, St. Helena wild pea, Phaseolus helvolus, var.; No. 18, Hairy vetch, Vicia vilosa ; No. 19, Common vetch, Vicia sativa ; No. 20, Wild vetch, Vicia Caroliniana.

clay was stirred up with sterilized water and mixed well with the sand. The pots were then planted with the same seed as before, omitting the clover and trefoil. They were kept watered with nutritive fluid, and most of them lived until August, when they were taken up for examination. Excluding one or two instances, where contamination had possibly taken place, no tubercles were formed on the roots of any of the plants.

The second set of six pots was treated as the first, except that clay from a depth of two feet was used. The same results given above apply to these pots.

The third set of six pots was treated as the others, except clay from one foot below the surface was used. Trefoil and clover were omitted from the planting. Results from this set indicate that the organisms capable of producing tubercles on the above four plants are found at a depth of one foot, but that they are not very abundant, as in no case was the infection as general as from a surface inoculation.

EXPERIMENTS ON TRANSFERRING TO DIFFERENT HOST.

Since there has been but little work done in this country towards settling the question as to whether there is one plastic form of organism that can adapt itself to all leguminous plants and produce tubercles on the roots, or that each species has its own peculiar parasite or parasites, the following experiments were undertaken in transferring the organism in the tubercles of one species, to the roots of a different species.

Before giving the results of the experiments, the general precautions of sterilization and inoculation may be briefly stated once for all. The sand and pots used were thoroughly sterilized by steam heat. The plants were nourished with prepared sterilized nutritive media.

The material for inoculation was secured by taking the tubercles from the roots, washing thoroughly with water, using a brush to remove all traces of dirt, then immersing in a solution of bichloride of mercury, 1 part to 1000, where they were again carefully brushed with a fine stiff brush and allowed to stand in the solution for twenty minutes. They were then trans

ferred to a second solution of bichloride of mercury where theouter surface of the tubercles was scraped away, hands, knife and tubercle being all the while immersed in the fluid. The remaining portion of the tubercles were thoroughly washed in sterilized water, then mascerated with sterilized water and the solution added to the pots.

In selecting tubercles those of smooth surface were chosen, care being taken that none with cracked epidermis should be used. With these precautions it was thought the possibility of transferring a form not normally in the tubercle was reduced to a minimum. It was thought better to use forms directly from the tubercle than to use cultures from the laboratory.

By arranging felt paper and cotton batting at the top and bottom of the pots to serve as filters to organisms from without, the roots were almost as well protected as a culture in the laboratory. Under these precautions the following pots were planted:

Six pots were left exposed to the air with no attempt to protect them for a period of one week; they were then planted with cow peas, peanuts, lupines and red clover. The surface of the pots was left uncovered and were supplied with nutrition under the same conditions as all the other. With the exception of the clover all the plants made a fair growth, but when the roots were harvested for examination the sparing formation of tubercles on them indicated that there had been no general infection of the soil with the forms that produce the tubercles on these plants. From the long exposure and the imperfect protection it would seem that dissemination through the air does not take place to a very great extent, if at all.

Three pots, Nos. 7, 8, 9, were filled with sterilized sand and planted with white lupines, Lupinus albus. No. 7, was inoculated with cow pea (Clay) tubercles. No. 9, with tubercles of white clover. When the plants were about nine inches in height they were examined for tubercles; but none were found.

No. 7 was then replanted with Black Eye Cow Pea, and after two months' growth numerous tubercles were found on the roots. This indicates that the forms from the Clay pea were still living in that pot and were capable of developing on the

roots of the Black Eye Cow Pea, but were not able to grow on the roots of the lupine.

No. 9 was replanted with Peanuts, *Arachis hypogœa*, and after two months' growth examined for tubercles, but none were found.

No. 8 formed no tubercles. This was a control to show that the sand was sterilized. Pot 9 indicates that the forms from the white clover are not able to develop on the lupine or the peanut.

Pots Nos. 10, 11, 12, were filled with sand as above, and planted with New Era cow pea. Nos. 10 and 12 were inoculated with white clover tubercles. After six weeks' growth they were examined, and no tubercles were formed save a few at the very bottom of two of the pots, and this was thought to have been because of imperfect protection from small particles of soil getting into the saucers.

This indicates that forms from the white clover tubercles are not able to grow on the roots of the New Era cow pea. Three plants from pot 10 were replanted as follows: One was replaced in pot No. 10, where it matured seed without forming any tubercles. One was planted in a pot of sterilized sand and covered to a depth of three inches above the highest root and a layer of garden soil was placed on top of the sand. The pot was watered so the water would run through the soil before getting to the sand. The plant matured seed without forming any tubercles.

A third was replanted in some rich garden soil and in a few weeks was bountifully supplied with tubercles on the new roots, though they never developed to a considerable size.

Nos. 16, 17, 18 were filled with sterilized sand and planted with Hairy vetch, *Vicia vilosa*, and Clay cow pea. No. 17 was inoculated with tubercles of white lupines. The vetch grew for a time but died as summer advanced. No tubercles were formed on the roots of either of the plants, indicating that the forms from the white lupine were not able to develop on the vetch or the cow pea. No. 18 was inoculated with tubercles of the peanut. The same may be said as to growth of the plants as was said of the above. Results indicate that the forms from the tubercles of the peanut are not able to develop on the roots of the

vetch or the cow pea. No. 16 served as a control and upon the roots there were no tubercles found.

Nos. 19, 20 and 21 were planted with the Virginia peanut, *Arachis hypogœa.* No. 19 served as a control and developed no tubercles, but after the first pod was formed it was replanted in garden soil and tubercles were developed on the roots.

No. 20 was inoculated with cow pea tubercles, but failed to show any tubercles formed on its roots.

No. 21 was inoculated with the tubercles of white clover with the same results as given above.

Nos. 22, 23, 24 were sown with alfalfa, *Medicago sativa.* No. 22 was mixed with tubercles of the peanut. No. 23 was mixed with tubercles of the cow pea, and No. 24 was mixed with both kinds of tubercles. The plants died about midsummer without forming any tubercles.

Nos. 25, 26, 27 were sown with peanuts. No. 25 was inoculated with tubercles from the white clover, cow pea, Canavalia ensiformis and Hairy vetch at the time of planting. No. 26 was treated with the same mixture two weeks after planting, and No. 27 three weeks after planting. With the exception of a few tubercles near the surface of one of the pots and the bottom of another where a particle of foreign matter may have been introduced, there were no tubercles formed on the roots.

Nos. 29, 30, 31 and 32 were planted with Saddle Back cow pea. No. 29 was inoculated at the time of planting with a masceration of tubercles from Burr clover, *Medicago denticulate,* pea nut, Hairy vetch, *Canavalia ensiformis.* No. 30 was inoculated with the same mixture one week after planting, and No. 31 two weeks after planting. No. 32 three weeks after planting. Excluding one or two instances, where contamination had possibly taken place, there were no tubercles.

Nos. 33 and 34 were planted with Black Eye cow pea and inoculated with tubercles of a different variety of cow pea, and tubercles were formed in both cases.

Nos. 35, 36 and 37 were sown with New Era cow pea and watered with a masceration of all the tubercles that could be found except from the genus Dolichos. No general infection

took place and it was thought that the few tubercles that were developed were due to unintentional introduction of a few small portions of garden earth.

The same line of work was extended in soil cultures but the results were very unsatisfactory and are not yet ready for presentation.

The above results would indicate that each plant, or at most each genus of plants, will support but one kind of parasitic organism capable of developing the root tubercles on its roots. For instance, in order that tubercles may be developed on alfalfa a particular organism must be present in the soil, and any quantity of cow peas or other leguminous plants will not furnish that organism. The cow pea likewise has its peculiar parasite, and so on with others. Yet dozens of leguminous plants may be grown side by side in the same soil, and each develop its own tubercles. Several species have been imported and are growing at this station, forming root tubercles, and yet the plant is not found in this country, and has never before been grown in this soil. The organism capable of producing the tubercle must have been in the soil before the seeds were planted, as there was no inoculation from soil where the plant had been previously grown. These organisms are therefore not dependent upon any particular plant for their existence, but the plant may be dependent upon them for its fullest development. Their absence from some soils and presence in others cannot be altogether satisfactorily explained. The effect of climatic conditions upon their continued development has not been studied, but I doubt not in some cases it is an influence in determining the geographical limitations of leguminous plants.

The value of work upon this subject to the farmer will be determined by a number of circumstances. The cow pea, which is most extensively used in the Southern States as a soil renovator, seldom fails to find the necessary organism to develop the desired tubercles, and upon such soil no inoculation would be necessary. Although this plant from several considerations possibly deserves to rank first, under the present methods of agriculture, it is not impossible that with proper inoculation its

superior may be discovered. At present the growth of alfalfa in this State is almost limited to the bottoms of the Mississippi and Red rivers, but, as already referred to, its profitable growth may be very much extended by soil inoculation. There is also a possibility that the condition of the soil may be such as to prevent the development of the infecting organisms after they are introduced, and some preliminary preparation may be necessary before making the inoculation. Supposing, however, that the conditions are favorable, the infecting organisms are scattered upon the soil, the young roots come in contact with them and the organism effects an entrance into the tissues of the young root, the tubercle begins to develop, the bacteria multiply in the tissues of the tubercle, until a period of dissolution begins to take place, and the protein matter of the parasite is absorbed by the host plant and the tissues of the tubercle become disintegrated till only the thick epidermis remains as an almost empty shell. Numerous living bacteria remain in these fragmentary portions, and the infectious character of the soil is increased. Where only one organism may have started the tubercle, thousands of them are left in the skeleton.

While the tubercles present some peculiarity of shape or the appearance of the surface, the organisms within can scarcely be said to present any characteristics that would enable one to distinguish them under the microscope. This fact at first led to the belief that there was but one form, and it was given the name *Rhizobium mutabile*; others called it *Bacilus radicicola*. When the subject is well studied, we will probably have as many varieties as we have species of leguminous plants.

There is no room to doubt the successful results of yot experiments, but can the same process of inoculation be practiced with ease and profit by the farmer? Few tests have been made on an entensive scale, but the reports from these experiments are promises of success. Most of the reports have been from an application of a portion of soil from a field where there was a healthy growth of the same plant of which inoculation was desired; for instance, if it was desired to inoculate alfalfa, soil must be taken from a field where alfalfa is growing luxuriantly

However, these organisms can be grown in the laboratory upon specially prepared media, and successful inoculations made from these artificial cultures. Nobbe and Hiltner, of Germany, have prepared pure cultures from a number of plants in such a way as to place them upon the market. They have named this preparation "Nitragin," and it is now being prepared and tested on a commercial scale as a substitute for nitrogen fertilizer. It is put up in bottles, the contents of which is sprinkled upon the soil, or mixed with the seed before sowing. It is nothing more than a great mass of a particular bacterial form, separated from all others, with sufficient nutritive media to sustain them until they are placed in the soil. The plant sown must be accompanied with "Nitragin" made from the tubercles of that plant. What influence these organisms may have upon the soil in the absence of the given host plant is but little, if at all, understood. We do know that they live and flourish in some soils, and if they appropriate free nitrogen when growing parasitically, and do not when not parasitic, by what means are we to explain the difference, and will it be possible to supply artificially the necessary environments to sustain their continuous development in the soil in the presence of all crops, and at the same time bring free nitrogen into such a combination that will be available as plant food?

Some experiments along these lines have been begun and will be continued, and it is hoped that something of value to the farmer will be developed that may be put into practice in the field on a profitable basis.

The following shows the per cent. of nitrogen in the dried tubercles of a few leguminous plants. The analyses were made by Mr. R. E Blouin and Mr. J. D. Clark, Chemists of the Experiment Stations. The tubercles ranged in size from the largest to the smallest, so as to make a fair average:

1.	Medicago lupulina, Black medic	5.28	per cent.	nitrogen.
2.	Vicia vilosa, Hairy vetch	7.87	"	"
3.	Vicia sativa, Common Smooth vetch	8.40	"	"
4.	Trifolium repens, White clover	8 55	"	"
5.	Dolichos sinensis, Cow pea	5.02	"	"
6.	Arachis hypogæa, peanut	5.78	"	"
7.	Dolichos forneosa	8.97	"	"
3.	Dolichos sesquipedalia	6.54	"	"
9.	Desmodium tortuosum,	6.09	"	"