

TEXAS AGRICULTURAL EXPERIMENT STATIONS.

BULLETIN NO. 83

Botanical Section.—JANUARY, 1906.

NITRO-CULTURE.

BY

O. M. BALL,

MYCOLOGIST.



POSTOFFICE :

COLLEGE STATION, BRAZOS COUNTY, TEXAS.

TEXAS AGRICULTURAL EXPERIMENT STATIONS.

OFFICERS.

GOVERNING BOARD.

(BOARD OF DIRECTORS A. AND M. COLLEGE.)

M. SANSOM, President.....	Alvarado.
F. A. REICHARDT.....	Houston.
K. K. LEGGETT.....	Abilene.
GEO. T. JESTER.....	Corsicana.
W. J. CLAY.....	Austin.
A. HAIDUSEK.....	La Grange.
L. D. AMSLER.....	Hempstead.
A. J. BROWN.....	Dallas.

STATION OFFICERS.

H. H. HARRINGTON, M. S., President of the College.

JOHN A. CRAIG.....	Dean and Director.
M. FRANCES, D. V. M.....	Veterinarian.
F. S. JOHNSTON.....	Agriculturist.
E. J. KYLE, M. S.....	Horticulturist.
F. R. MARSHALL.....	Animal Husbandry.
EDWARD C. GREEN, B. S.....	Assistant Horticulturist.
G. S. FRAPS.....	Acting Chemist.
R. L. BENNETT.....	Cotton Specialist.
O. M. BALL.....	Botanist.
B. C. PITTUCK.....	Feed Inspector.
C. O. MOSER.....	Deputy Feed Inspector.
M. S. CHURCH.....	Deputy Feed Inspector.
JOHN C. BURNS.....	Deputy Feed Inspector.
J. G. HARRISON.....	Bookkeeper.
C. W. CRISLER.....	Clerk Feed Control.
MISS M. H. WATKINS.....	Stenographer.

STATE SUB-STATIONS.

J. K. ROBERTSON, Superintendent.....	Beeville, Bee County.
W. S. HOTCHKISS, Superintendent.....	Troupe, Smith County.

NOTE.—*The main station is located on the grounds of the Agricultural and Mechanical College, in Brazos County. The Post-office address is College Station. Reports and bulletins are sent free upon application to the Director.*

TABLE OF CONTENTS.

	PAGE.
Cross inoculation of alfalfa with germ from bur-clover.....	8
Record of pots in experiments on artificial inoculation	9
Inoculation with Nitro-culture.....	11
Inoculation with Nitro-culture for alfalfa.....	12
Cultures in soil from compost heap.....	12
Cultures in pure sand.....	13
Inoculation with Nitro-culture for bur-clover	13
Behavior of the check plants.....	13
Soil sterilized but seed not.....	13
Neither soil nor seed sterilized.....	14
Conclusion	14
Summary	15

[Blank Page in Original Bulletin]

NITRO-CULTURE AND INOCULATION.

BY O. M. BALL, MYCOLOGIST.

In view of the extravagant claims now being published regarding the wonderful benefits to be derived from artificial inoculation of the soil, or of the seeds of leguminous plants, with bacteria which fix the free nitrogen of the atmosphere, a careful investigation of the question was thought advisable. It is true that some of these extravagant expressions have lately been modified to a certain extent, but the numerous requests for information and advice which are being received by the Experiment Station of this State show that the public interest has been aroused to a high pitch by the sensational announcements of vast profits to accrue from artificial inoculation of leguminous crops. Many seedsmen and dealers are pushing the sale of a commodity which is guaranteed to produce these marvelous results at a price of from \$1.50 to \$2.00 per acre, when the actual cost of the stuff has been shown to be less than 10 cents, and is worthless, in most cases, at that. Moreover, the process of artificial inoculation, in essentials, is not a new one, but is merely a revival of an attempt first made by Nobbe, a German scientist, in 1898. Owing to questionable results, Nobbe's method quickly fell into disfavor. Nobbe distributed his inoculating material in a solution containing nitrogen; Carron, both as fluid and dry, and Moore sends his desiccated on cotton. It appears, however, that undue haste has been exercised by the last named investigator in announcing his results. Proper verification of results, before publication, has been lacking.

Claims Advanced for Nitro-Culture.—Essentially, the claims advanced for Nitro-Culture are based on the following considerations:

1. The presence of nitrogen-fixing bacteria on the roots of legumes is requisite for the production of good crops, especially where the soil is lacking in nitrates.

2. The various legumes are affected by a species of bacterium which has so adapted itself to its host plant that each legume has its own variety of that species.

3. If soils are naturally deficient in nitrogen-fixing bacteria, these may be introduced by inoculation of the soil, or of the seed to be sown.

4. In order to obtain the best results, it is necessary to inoculate a given legume with its own variety of bacterium.

5. By cultivation of these bacteria under suitable conditions, for example, in solutions of food stuff in which no nitrogen is present, their "virility" or greed for nitrogen may be greatly increased.

6. After having been inoculated with these greedy bacteria, the crop of given legume will be vastly enlarged, because the bacteria

will fix a quantity of nitrates corresponding to their enhanced appetites, thus affording a greatly enlarged food supply for the legume.

In regard to these claims, the first is to some extent true. It is a matter of common observation that most legumes are more thrifty when their roots show a copious supply of the nodules produced by the activities of these bacteria. Their presence does not seem to be requisite, however, when the soil contains a plentiful supply of available nitrates, which fact has led to the statement that the bacteria will not thrive in such soil.

Secondly, in the light of recent experiments*, it is certain that in the case of the bacteria which produce nodules on the roots of sweet melilot, the same nodules will appear upon the roots of alfalfa, after "cross-inoculation." As will hereafter appear in this paper, the same is true of those bacteria infecting the roots of bur-clover. It will also appear that the bacteria sold by certain parties for the exclusive inoculation of alfalfa will sometimes infect the roots of bur-clover, and the bacteria designed for the exclusive use of bur-clover will in turn sometimes infect alfalfa.

As regards the third proposition, there are certain conditions under which it is conceivable that a given soil will be deficient in nitrogen-fixing bacteria; for instance, when a field has been cropped to excess with a growth which does not serve as host to these bacteria, such as wheat or corn. Under such circumstances, if the germs can not preserve their vitality for a year or two in the soil, they most inevitably disappear. These conditions are not apt to occur in a natural state of affairs, since, in most localities, one or other form of legume will be found abundant. On the barren sands of the uplands near the Brazos river, in land which has never been under cultivation, isolated legumes (*Lupinus subcarnosus*, *L. Texensis*, bur-clover, Japan clover and others) have been examined by the writer, and in every case show a plentiful supply of the nodules. The same fact was observed on the lately excavated embankment of the I. & G. N. Railroad, where the stiff and poor sub-soil of the "post-oak" country had been exposed to the gradual encroachment of vegetation, with scattered specimens (*L. subcarnosus*, *L. Texensis*, etc.). The writer has also noted, during four or five years, the gradual spread of Japan clover on the mountains of certain parts of Virginia. This plant has spread slowly over the Blue Ridge and the adjacent hills, on land never cultivated in any manner. From year to year, the plants appearing for the first time, even at elevations of 2500 feet, invariably show roots well supplied with nodules. The bacteria causing these nodules have either been obtained from other legumes, or the infection has been carried with Japan clover as it spread. Even isolated plants, on remote "knobs," have invariably been found to be supplied with tubercles. In another case, on land which had received a top dressing of stable manure, but which had not been

*Hopkins, Bulletin Illinois Experiment Station, 94, 1904. Lewis and Nicholson, Oklahoma Experiment Station, Bulletin No. 68, 1905, page 14 gives a table showing several other cases of cross-inoculation.

cultivated for many years, both alfalfa and bur-clover developed nodules, apparently to sufficiency. In fact, it would be difficult to find a soil in which a wild legume would show no nodules. Hence, the need for artificial inoculation would be a very rare occurrence.

The claim put forward in the fourth of the foregoing considerations has been directly contradicted by results obtained by the writer, working in the laboratory, as will appear below. In fact, it will appear, that in no case of artificial inoculation was the supply of nodules on the roots of the plants inoculated so copious as where the plants grew in the same soils but which had not been previously sterilized.

As regards the claims put forward in the fifth statement, the writer has seen no adequate statement of the methods by which the alleged increased "virility" has been obtained. Though great caution should always be exercised in reasoning by analogy, it may be urged against such doctrine that other like organisms do not show an increased appetite for their natural food after having been deprived of this food for a time. An aerobic bacterium (one that requires oxygen) may be, at another time, an anaerobe (requiring no oxygen), but no one has ever observed an increase in the "virility" of such anaerobes after having been reared again in the presence of oxygen; at least, to the writer's knowledge, no one has reported such observation. There are other so-called facultative bacteria, organisms which have the power of living with or without certain articles of food. There has been no recorded observation, in the knowledge of the writer, of an increase in "virility" of such organisms after having been changed to a medium containing a normal food substance which was lacking in a previous culture. Against this doctrine, the facts observed in the laboratory have even greater weight. In no case of artificial inoculation with "Nitro-culture" or other like preparations was there an observable increase in thriftiness in the inoculated plant, whether in good soils or in pure sand or water cultures in which all traces of nitrogen had been removed except that of the atmosphere.

Experiments to Test These Claims.—In amplification of these objections to the claims which have been made for the "Nitro-culture" and other substances of like nature, a detailed statement will follow of a series of experiments which were made in the laboratory of the Department of Botany and Mycology during the winter and spring of 1904-5, and the fall of 1905. Circumstances prevented immediate publication of these results. Further detailed and quantitative work has been planned for the field.

The work here described was wholly by means of pot cultures and falls into two groups. In the first series, the object was to determine whether nodules will appear on the roots of alfalfa when this plant is grown in soils where none had ever before been cultivated, but which produced a fine growth of bur-clover (*Medicago denticulata*), and further, whether the nodules already present on the roots of bur-clover could be carried over to alfalfa, when grown in soil that had previously been sterilized; in short, whether artificial inoculation of alfalfa with the germs from bur-clover is

feasible. In the second series, the question was to determine the value, if any, of the so-called Nitro-culture and other like "cultures."

The method of procedure was very similar in the two series. A variety of soils was used in the first series, as will appear in the following notes of pot cultures. Soil of the best quality, obtained from the compost heap of the College Horticultural Department and pure crushed quartz sand, were used in the second series. The pots were of white clay, of the the ordinary six-inch pattern.

CROSS-INOCULATION OF ALFALFA WITH GERM FROM BUR-CLOVER.

Soils of several varieties were obtained from the College lands, from points where no alfalfa had ever been cultivated. Some samples were from the parade ground directly in front of the buildings and others from the cultivated fields. The seed were selected from samples sent by various commercial houses in the State. These seeds were carefully washed through many changes of water, but chiefly by placing them under the tap from the 700-foot well, and allowing them to remain for several hours. They were then placed in a 2 per cent solution of corrosive sublimate and vigorously stirred for from several minutes to a half hour. In this connection, it may be of interest to state that the seed does not absorb the sublimate, since it was a matter of frequent observation that the young sprouts would appear from the seed accidentally left in the solution, and would grow to a length of half an inch before being killed by the poison. The careful washings and treatment with sublimate were designed to get rid of any bacteria which might adhere to the outer coats of the seed, if such were possible, and was simply a precautionary measure, the utility of which is doubtful. However, we were never troubled with the appearance of injurious fungi, "damping off," or other disagreeable contingencies of this kind.

In all, about forty pots were used in this series, of which several were used as checks. The soil in the others was sterilized by heating for six to eight hours in an Arnold's sterilizer at a steam heat of 206 to 208 degrees F. They were then left in the sterilizer over night and the heat again applied for a like period. This resulted, in all but one case, in a complete sterilization of the soil. A sufficient quantity of the seed, treated as above outlined, was now sown in each pot. After germination, the pots were carefully examined at intervals to ascertain if nodules were present. This was done by means of thoroughly sterilized section lifters. One or two seedlings would be lifted out of the soil and examined. With one exception, the young plants when four inches high and five weeks old showed no nodules. The sterilization was then considered complete.* If no nodules were found, the re-

*It had previously been observed on many young bur-clover plants which make their appearance above the ground at College during the month of November, that nodules are very evident when the seedling is two weeks old and from one to two inches high.

maining plants were then inoculated and set aside, together with the check pots, in an isolated section room, and were watered with distilled water. A careful record of each pot was kept. A condensed statement of this record follows:

Record of Pots in Experiments on Artificial Inoculation.—Pot I. Seeded February 9, 1905, in sterilized soil. On March 11th the plants were about four inches high, and their roots showed no nodules. The pot was then inoculated with a "solution" from carefully selected and thoroughly washed nodules from the roots of bur-clover found growing on the grounds of the Agricultural Building. These nodules were crushed in sterilized water, in sterilized glass beakers, and the "solution" was poured around the roots of the young alfalfa plants. Six weeks later, on April 28th, the plants were thrifty and flourishing, and their roots were plentifully supplied with nodules. The check pot showed no tubercules, although there was no observable difference in the appearance of the plants.

Pot II. The soil in this pot was sterilized by heating in porcelain crucibles for several hours, by which means the organic matter was thoroughly burned out and all forms of life destroyed. Seeded February 9th, and inoculated March 11th with the same "solution" used in No. I. April 28th plants, as was to be expected, from the burning of all organic matter in the soils, were not so thrifty as in No. I, but rootlets showed a fair supply of nodules, though by no means so many as in No. I.

Pot III. Sterilized as before and seeded February 2d. On March 16th no nodules were present; whereupon the plants were moistened with water, in which the roots of bur-clover had been allowed to lie in the dark for forty-eight hours. April 29th the rootlets showed abundant nodules. The plants were no better than their checks, grown in sterilized soil and which showed no nodules.

Pot IV. Sterilized, and seeded February 1st. By March 16th no nodules had appeared on the roots, and water which had been allowed to filter through soil containing bur-clover roots was poured around the young plants. On April 25th there was a very fine growth of nodules on the roots.

Pot V. Unsterilized soil, of very poor quality and containing but little organic matter; taken from a spot where bur-clover was growing, but very sparsely. This soil became very compact in the pots, and was with great difficulty kept from packing. Seeded February 8th with sterilized seed and allowed to grow until May 15th. At this time the plants were about one-eighth as large as those grown in sterilized rich soil. Nevertheless, the rootlets showed a very large number of nodules.

Pot VI. Seeded February 5th, in soil taken from the compost bin of the Horticultural Department, and which was very rich, both in humus and in nitrates. The alfalfa seed sown in this pot were sterilized with extra precaution. On March 16th the rootlets showed very fine nodules. Thereafter, the plants thrived well, but made no better growth than the check alongside in the same soil, but which had been previously sterilized by steam heat.

Pot VII. Soil taken from parade ground, near flagpole, and was dense and unpromising. There are no legumes at this point. Seeded February 15th, and examined May 23d, but no nodules made their appearance. The plants were of about the same size and appearance as those described in No. V.

Pots VIII-IX. Soil from compost bin, sterilized as above and sown with alfalfa seed which had been allowed to lie for a few minutes in "soil solution" used in No. 1. Four weeks later the plants in these two pots showed a large supply of nodules on the rootlets. When two months old the difference between these plants and their checks, of same age, growing in sterilized soil, of the same kind without inoculation, and never showing nodules, was so slight as to be negligible.

Pots X-XI. Soil from compost bin, sterilized as above, sown with alfalfa seed which had received treatment from a solution of crushed nodules from rootlets of young bur-clover plants. These plants all showed the presence of nodules when examined three weeks later. If there was any difference between these plants and their checks, growing in sterilized soil of the same kind, and never showing nodules, such was not observable.

In one case, after the usual sterilization, the young plants were found to be infected, and in still another this infection was found four weeks after the young plants had been inspected and found to contain no nodules; at least none were present on the rootlets of those taken up. These were the only irregular occurrences in the whole series of forty pots, and may be explained as the result of incomplete sterilization or of accidental inoculation. Unfortunately, the department possesses no green house where conditions may be rigidly controlled and all visitors excluded. It is possible that infection may have been caused by some visitor with good intentions but poor judgment.

The experiments above outlined were repeated quantitatively, and the work was carried on by two different parties, working separately, and whose results were not compared until the end of the series. From a survey of the results, it will appear that the bacterium which affects the roots of bur-clover will produce nodules on those of alfalfa with equal readiness. Further, in good soil, as in No. VI, it is not necessary for the welfare of the plant that nodules be present. This, however, has long been known to be true. In poor, stiff soil, the presence of nodules did not appear to materially affect the growth of the plant, there being no noticeable difference in the plants in pots in Nos. V and VI, in which the soil was of very similar character.

It may possibly be urged against the results that the time of experimentation was not sufficiently long to permit the plants to derive any benefit from the presence of the nodules, which had been formed on the rootlets in plants in Nos. I, III and VI. To this it may be replied that the plants were large and well grown, being in most cases from one and one-half to two feet high and about two and one-half month old, when the work was brought to a close by the ending of the session of the College. During this

time, it seemed that some difference between the infected and non-infected plants would have appeared, if such were ever to occur.

INOCULATION WITH "NITRO-CULTURE."

To determine the value of the claims put forward for "Nitro-culture," and, further, whether "Culture," intended for alfalfa would affect bur-clover and vice versa, one package of the "Culture" for alfalfa and one for bur-clover were obtained from the National Nitro-Culture Co., West Chester, Pa., and one for alfalfa from the Texas Seed & Floral Co., Dallas, Texas. The latter was obviously the product of the former concern, since the packages and instructions in all were alike. All were marked "Acre Package." The solutions were made precisely and carefully according to directions found in the packages; not all of the materials being used in the first preparation. The remainder was carefully enclosed in the original papers and laid aside for future use. After about twenty-four hours, a milky color appeared in the bottles, according to schedule. This cloudy appearance increased during the following day and was so decided that yeasts were suspected, and a microscopic inspection of a drop showed the presence of a yeast; but this examination was carried no farther.* A second and third solution was prepared from the remainder of the materials of the packages at intervals of three days. Each behaved as the first, and hence was considered to be good, since there was no observable difference in the solutions. The soil used in the experiments was taken from the compost heap of the Horticultural Department, and was carefully sterilized, as above described, by steam heat, in Arnold's sterilizers.

In one series, alfalfa seed was inoculated with the solutions and sowed in pots carefully marked, so that there would be no chance of confusing the bur-clover with the alfalfa cultures. In the other series, young alfalfa plants about four weeks old were watered with these solutions, after having been scrutinized in the manner above explained, to determine if nodules were already present. These cultures were likewise carefully kept apart. A number of pots of the same soil were kept alongside of the above as check pots. One-half of these had been sterilized previous to seeding; the other was of native, unsterilized soil, fresh from the compost heap. As far as was possible, the seed in any one series was sown at the same time, in order to keep the plants of the same age and development.

To resume, these experiments were intended to determine:

1. If the solution made from the materials sold as "Nitro-culture" will in reality produce nodules on the roots of legumes in soil which had previously been thoroughly sterilized.
2. Whether "Nitro-culture" for alfalfa will inoculate bur-

*This observation of the presence of yeasts in the cultures so prepared has been fully verified by Harding & Prucha, New York Experiment Station, Bulletin No. 270, November, 1905.

clover, and whether the culture for bur-clover will in turn affect alfalfa.

3. To compare the results of inoculation, if such occurred, with conditions found in plants growing naturally in the same soils and affected with tubercle-producing bacteria.

INOCULATION WITH NITRO-CULTURE FOR ALFALFA.

Cultures in Soil from Compost Heap.—In the first series, eighteen pots were used. Twelve of these were sterilized, and were sown with alfalfa seed which had been thoroughly washed and allowed to lie for a short time in corrosive sublimate solution. The seed planted in six of these pots was treated with the solution of "Nitro-culture" for alfalfa, according to directions found in the packages; the second set of six received no "culture," while the third set served as controls and were filled with the same soil, but unsterilized, and the seed were also unsterilized. From ten to twelve seeds were sown in each pot.

After two weeks, when the young plants were about two inches high, two or three were carefully removed with sterilized section lifters and their rootlets examined for tubercles. Not a single plant of those inoculated showed tubercles, and none were found on those in the sterilized uninoculated pots. Every pot of natural soil showed tubercles on the rootlets of the alfalfa plants. This result was so surprising and so unexpected that the remaining plants were allowed to grow for two weeks longer, after which they were again examined, with the same results. There were no tubercles to be found, either in inoculated or in the sterilized pots, while the check pots continued to develop a good crop.

Since there was a possibility that some error in preparation of the "culture" had occurred, a fresh solution was prepared with the utmost care, exactly according to the directions given. A portion of the package previously used was made up, reliance being placed upon the statements found in the sheet of directions, that "The package accompanying this direction will keep for one year from date, if it is not wet or overheated"; which, to say nothing of the grammar, is certainly ambiguous. A new set of twelve pots was prepared, as before, by careful sterilization. Six were sowed with alfalfa seed inoculated with the new solution, and six were sowed with sterilized but not inoculated seed, and a third set of six were filled with natural soil, sown with unsterilized seed. At the same time the six which had previously been inoculated, and had failed to catch, were watered copiously with this new solution, to obtain, if possible, a "setting" of the tubercles. In both cases, absolute failure to obtain nodules resulted, though tubercles were uniformly formed in the unsterilized soils. Later, forty-eight pots were thus inoculated, and in this number we succeeded in inoculating nine pots, at least tubercles were found on the rootlets of plants in that many pots. In this series, twelve pots were used as checks, being filled with unsterilized soil. In none of the inoculated pots were the tubercles abundant, the plants showing few and scattered nod-

ules, which did not seem to thrive, nor did the plant once affected seem to be able to develop the tubercles in increasing numbers. All of the check pots developed abundant nodules.

Cultures in Pure Sand.—Six pots were filled with pure crushed quartz sand, which had first been washed by tap water for several hours before sterilization. The tap water was from a cistern under the roof of the Agricultural Building, and was pure rain water, falling on and collected from a slate roof. This water was used also in making up the Nitro-culture solutions. These pots were sown with inoculated seed, and were watered with a von der Krone's nutrient solution, in which the nitrate of potash had been omitted. These plants throve remarkably well, considering the lack of nitrates, but never at any time showed tubercles, though they received frequent waterings with the "Nitro-culture" after they had reached a height of several inches and had developed no tubercles from the first inoculation.

One alfalfa plant, grown in a water culture of v. d. Krone's solution without nitrates, developed, six days after receiving "Nitro-culture," three tubercles on the rootlets. This plant was kept under observation for more than four months. It grew fairly well, but never developed more than the three nodules which first appeared.

INOCULATION WITH NITRO-CULTURE FOR BUR-CLOVER.

A parallel series of thirty-six pots filled with compost was prepared at the same time as the foregoing and placed in another room. Eighteen of these received alfalfa seed inoculated with Nitro-culture for bur-clover, prepared and sent out by the firms above mentioned. Of the remaining eighteen, nine were sterilized and planted with sterilized seed without previous inoculation, and nine were not sterilized and were planted with unsterilized seed. Four of the inoculated pots developed tubercles, but as in the case of alfalfa culture, the number of nodules appearing was very small, being only three or four on each plant. The check series of nine pots all showed an abundant supply of nodules.

BEHAVIOR OF THE CHECK PLANTS.

Soil Sterilized, But Seed Not.—Twenty-eight pots, in all, were used as checks, in which the soil was sterilized, and the seed not. Of this number, only two showed the presence of tubercles, the remainder being at all times free from nodules. These two cases may have resulted from imperfect sterilization in the first place, and this imperfect sterilization may reasonably account for some of the cases observed of infection after inoculation, though certainly not in the case of the water culture where the nodules were observed to form within a few days after the rootlets of the plant had been treated with the solution.

Neither Soil nor Seed Sterilized.—Each of the twenty-four pots containing unsterilized soil gave an abundant catch of tubercles, but the general appearance of these plants was no better at the close of the experiments than those growing in the same unsterilized soil.

CONCLUSIONS.

The results obtained by this method of investigation were so uniform that there could be no room for doubt but that something was wrong with the substances sold as Nitro-culture. So directly at variance with the claims put forward by the Department of Agriculture and by numerous writers, presumably inspired from the same source, were the results of this investigation, that in May, 1905, the writer addressed a summary of these to Dr. Geo. T. Moore. The statement of results was purposely made in a guarded manner, since the matter was of such far-reaching importance that it was thought necessary to make fuller and more extended experiments in order to verify and extend the work already done. The claims then put forward were essentially as follows:

1. In no case was the supply of nodules found on rootlets after inoculation as abundant as in plants growing normally in the same soil unsterilized.

2. It is not necessary to make a "culture" before cross-inoculation can be affected.

Later experiments have not only verified the exactness of these results but have demonstrated that only in a small percentage of cases is there any inoculation produced at all by the cultures used. Since the first series of these experiments was ended in April, 1905, bulletins from several experiment stations have appeared dealing with the problem by other methods of procedure. All have substantiated the results here put forward to the extent of showing that the so-called cultures are worthless for practical purposes. Notes of warning had been given from time to time, as, for instance, those of Director A. M. Soule and Dr. Meade Ferguson, Virginia Agricultural Experiment Station, Bulletin 154, April, 1905; of Professor Bailey, in Country Calendar, June, 1905; also of Lawrence T. Clark, in Bulletin 231, of the Agricultural College of Michigan, June, 1905, who deprecates especially the injudicious enthusiasm aroused by the questionable methods of heralding inoculation as a "panacea for all crop failures." The most authoritative statements of the value of the "cultures" are as follows:

New York Experiment Station, November, 1905, appearing January, 1906. H. A. Harding and M. J. Prucha. We quote:

"These packages of treated cotton have had a wide sale at a high price—two dollars for a package sufficient to treat an acre—while the cost of production was less than ten cents.

"These examinations make it very evident that the packages were worthless for practical purposes."

Oklahoma Agricultural Experiment Station, Bulletin 68, December, 1905:

"The pot cultures proved that the tubercle-forming germ of the alfalfa plant was present, but the results as compared with those from pots inoculated with cultures isolated in the laboratory show that the germ was very scarce in the 'Nitro-culture.'

"In the plates poured from the culture, the alfalfa germ could not be found, as the plates showed almost a pure culture of an entirely different organism. This germ, when used in pure cultures on alfalfa, gave negative results."

The latter finding sustains the work of Messrs. Harding and Prucha, and that of the other examinations quoted by them. In the case of the investigations made at the Oklahoma Station, it is possible, though scarcely probable, that in the cultivation of pure cultures, by plates, conditions arose which were fatal to the germ. It is also possible, in the work of this laboratory, that incomplete sterilization may account for the presence of the tubercles on the rootlets, and that they were not due to the inoculation by the "Nitro-culture" at all.

SUMMARY.

Summarizing the results obtained in this laboratory, we find:

1. Alfalfa will become infected with the tubercle-forming bacterium:

When grown in soil which is producing a crop of bur-clover. After being watered with a "solution" of such soil.

By making an infusion of the nodules on the roots of bur-clover.

2. Inoculation with the so-called "Nitro-culture" is of more than doubtful value since only a small proportion of the plants treated developed tubercles, and these were in no observable degree benefited by their presence.

3. In no case of artificial inoculation were the number and vigor of the tubercles so great as in that occurring by natural means.