

BULLETIN No. 282.

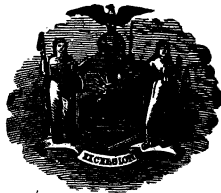
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QUALITY OF COMMERCIAL CULTURES FOR LEGUMES
IN 1906.

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PUBLISHED BY THE STATION.

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SUMMARY.

Cultures of legume bacteria dried upon cotton according to Moore's method have been tested by sixteen Agricultural Experiment Stations in 1904-5 and all have found such cultures to be of little or no practical value.

Metal containers have been recently put forth as a means of protecting such cultures and it was claimed that cultures packed in this way would remain active for long periods.

A careful examination of fourteen such cultures showed that the claims made for the metal container were not borne out in practice.

The results from the examinations of twenty commercial cultures indicate that the goods upon the market for 1906 were little if any better than those offered in 1905.

In neither year was there any evidence that the purchaser had had more than the remotest chance of receiving the worth of his money from the use of such cultures.

INTRODUCTION.

During the years 1904-5 great interest was manifested throughout the country in the artificial inoculation of legumes with their appropriate bacteria in order to stimulate the fixation of nitrogen from the air. This interest was due to the sensational manner in which this subject was brought to the public notice at a time when farmers were beginning to realize the importance of legumes in soil enrichment and to know what substantial results had been obtained by the use of naturally inoculated soils. That this interest was due to the manner of presentation rather than to the newness of the subject matter is seen from the following facts: Practically identical culture media¹ had been employed in growing these germs for many years, the idea that the activity of the cultures could be increased by controlling their environment has long been held, especially by Hiltner,² and the method of shipment on absorbent material has been considerably used with yeast cultures. The main value of this presentation lay in the fact that it succeeded in bringing these scientific facts to the attention of the agricultural public.

Coincident with this public interest in the subject of artificial inoculation there appeared commercial companies which offered cultures for this purpose. Inquiries concerning the value of these commercial cultures began to pour in at the various Agricultural Experiment Stations and in order to obtain data upon which to answer these inquiries the Experiment Stations made tests of these cultures.

¹The cultivation of legume bacteria in nitrogen-free media has long been a common practice. Laurent (*Recherches sur les nodosités radicales des Légumineuses. Ann. Inst. Pasteur*, 5: 105. 1891) used the following medium: Distilled water 1000 c.c., potassium phosphate 1 gram, magnesium sulphate, 1 gram, and saccharose.

²*Cent. Bakt.* [etc.], II, 10: 660. 1902.

INVESTIGATIONS.

RESULTS FROM PREVIOUS EXAMINATIONS.

The tests by the various Agricultural Experiment Stations were made under a wide range of conditions and in practically all possible ways. They included extensive examinations in the laboratory as well as trials by pot experiments and field tests.

In the tests by sixteen Stations³, the results from which are now available, there was a striking similarity in one particular; they unite in saying that they have failed to find evidence that these cultures are of any value to the agriculture of their particular regions.

Bulletin 270 of this Station gives the results of bacteriological examinations in 1905 of eighteen packages of inoculated cotton cultures for legumes, put up by the National Nitro-culture Co., of West Chester, Pa. Duplicate portions from six of these packages were examined at the Agricultural Experiment Stations in Delaware, Michigan, New York and New Jersey and by Parke Davis & Co., at Detroit, Mich. These eighteen packages were all found to be worthless for practical purposes. It was further shown experimentally that the failure of these commercial cultures was inherent in the manner of their preparation.

Great stress is laid by the commercial companies upon the re-

³Okla. Agr. Exp. Station Bul. 68. 1905.
New York Agr. Exp. Station Bull. 270. 1905.
W. Va. Agr. Exp. Station Bul. 105. 1906.
Ga. Agr. Exp. Station Bul. 71. 1905.
Me. Agr. Exp. Station Bul. 128. 1906.
Cornell Univ. Agr. Exp. Station Bul. 237. 1906.
Ky. Agr. Exp. Station Bul. 125. 1906.
Ontario Agr. Coll. Bull. 148. 1906.
Pa. Agr. Exp. Station Bull. 78. 1906.
Ann. Rept. Wis. Exp. Station 22: 242. 1905.
Ann. Rept Mass. Exp. Station 18: 77. 1906.

Unpublished results were kindly furnished by Agricultural Experiment Stations of North Carolina and Virginia.

Also see Report on field and pot culture experiments at Woburn (Eng.) Experiment Station. 1904.

sults obtained by farmers from the use of these cultures. Of the large number of farmers (approximately a hundred) whose experience has come to us during the past two seasons, but six have believed that they had obtained any result whatever from the use of such cultures. One had tried the inoculation on garden peas. When the matter was reported in November the crop had disappeared, but the statements of both the man and his neighbors left little room for doubt that good results had been obtained. A second farmer had tested inoculation on cow peas, and while he maintained that there was a marked increase in the nodule formation on the inoculated area, there was no discoverable difference in the crop obtained from the two portions. A third was enthusiastic concerning results obtained with alfalfa, but as no plat had been sown without inoculation it was hard to see upon what he based his conclusions. Two other fields of alfalfa where the owners believed that they had obtained good results from the use of commercial cultures were examined without finding anything upon which to base such conclusions. Another alfalfa field has been reported but has not yet been examined. It would thus appear that the negative results obtained from these cultures in the laboratory at the Experiment Station are in close accord with what is being actually obtained in practice upon the farms.

EFFECT OF THESE RESULTS UPON TRADE CONDITIONS.

It is encouraging to note that while the trade in these commercial cultures was brisk during the season of 1905 the adverse reports which began to appear at the end of that season from the Experiment Stations, the agricultural press and from the farmers themselves, have very markedly reduced the use of these cultures.

MODIFICATION OF THE ORIGINAL METHOD.

Kellerman & Beckwith⁴ have shown that when cultures of legume bacteria are placed on cotton, dried promptly and kept absolutely dry, they retain their vitality for a considerable time.

⁴Kellerman, K. F., & Beckwith, T. D. Effect of drying upon legume bacteria. *Science*, N. S., **23**: 471-472. 1906.

The National Nitro-culture Co., took advantage of this fact and put upon the market cultures on cotton inclosed in metal containers. The company claimed thereby to obviate all the objections which had been raised against their cultures as put out during 1905. These metal containers were collapsible tubes similar to those in which bicycle cement is commonly sold, except that they had no small opening and were closed by rolling up and compressing the large end. That such a closure does not completely cut off the air is plain to anyone who has purchased a tube of bicycle cement which has been long in stock.

Since it was this company whose preparations had been found worthless last year, common justice demanded that their product be again tested and if found to be as much improved as claimed by them the fact should be given as wide circulation as had the condemnation of their product of the preceding season.

EXAMINATION OF CULTURES IN 1906.

Duplicate acre packages for three legumes were purchased from each of three seedsmen, or eighteen packages in all. Mr. C. K. Scoon, a local farmer, very kindly made the purchase for us.

The cultures had all been put up by the National Nitro-culture Co., and since they bore the date 1906 were surely not old cultures which had been long in the hands of the seedsmen. Twelve packages, six for alfalfa and six for crimson clover, were contained in the metal tubes, while the six cultures for vetch were wrapped in parchment paper and tin foil, as was the case with the packages last season. This could hardly have been an accident since it was equally true of the cultures received from each of the three seedsmen.

Method of making the examinations.—The chemicals used in all cases were those accompanying the cultures. They were dissolved in the appropriate amount of rain water and the solutions sterilized in order to reduce the chance of outside contaminations and enable us to determine just what germs were on the cotton. In making a test of these commercial cultures the packages were carefully opened and the inoculated cotton divided into three equal portions with sterile instruments and under con-

ditions which exposed the material to the least possible opportunity for contamination. In each case one of these portions was placed in a flask containing 100 c. c. of the sterile nutrient solution above described. The remainder of the cotton was returned to the original container and sealed as before.

The flasks were held at 25° C. (77° F.). At the end of twenty-four hours the proper amount of sterile ammonium phosphate was added. The formation of turbidity was noted and the contents examined by means of hanging drop and stained cover glass preparations. Peptone-free agar plates were inoculated from each flask at the end of two, and again at the end of five days. Any colonies resembling the legume bacteria which appeared upon the plates were given further study.

In the case of each examination of these cultures two series of control flasks were also used. One series received the same treatment as the test flasks with the exception that in place of the cotton these flasks received a portion of a pure culture of legume bacteria. In the other series the flasks received the same chemicals and exposure to contamination but did not receive either cotton or intentional inoculation. The object of the first series was to show that the conditions in the flasks were such that the legume bacteria could make successful growth, while the second series was intended to measure the probability of the flasks becoming accidentally contaminated during the process of manipulation. The flasks inoculated with legume bacteria uniformly produced both an abundant and a pure growth of the germ with which they had been seeded. This shows that the conditions were good for the development of any legume bacteria which might be present upon the cotton. The uninoculated flasks remained sterile, indicating that there was slight probability that any of the flasks became accidentally contaminated during the process of the test.

Results of the examinations.—A test of each of the eighteen cultures was begun on November 10 and a second test, using a second portion from each commercial package, was started November 15, 1906. While each of these tests was carried through entirely separate the data are here combined into a single table, in order that the results may be more easily compared.

TABLE I.—RESULTS OF THE EXAMINATION OF NITRO-CULTURES.

No. of package.	Legume.	Series. No.	Became turbid.	Remarks.
I	Alfalfa	1	Not in 7 days	Sterile.
		2	Not in 7 days	Sterile.
II	do	1	3 days	Bacterial contamination, 30 per ct. <i>Ps. radicola.</i>
		2	6 days	Bacterial contamination, 20 per ct. <i>Ps. radicola.</i>
III	do	1	Not in 7 days	Sterile.
		2	5 days	Yeast.
IV	do	1	Not in 7 days	Sterile.
		2	7 days	Yeast and molds.
V	do	1	7 days	Yeast and molds.
		2	7 days	Molds and pink yeast.
VI	do	1	Not in 7 days	Sterile.
		2	7 days	Yeast and foreign bacteria.
VII	Crimson clover	1	4 days	Yeast.
		2	3 days	Yeast and molds.
VIII	do	1	7 days	Yeast.
		2	4 days	Yeast.
IX	do	1	7 days	Pink yeast.
		2	5 days	Pink yeast.
X	do	1	3 days	Foreign bacteria.
		2	6 days	Foreign bacteria and molds.
XI	do	1	7 days	Pink yeast and foreign bacteria.
		2	4 days	Yeast and molds.
XII	do	1	7 days	Molds.
		2	5 days	Molds.
XIII	Vetch	1	5 days	Foreign bacteria and 5 per ct. <i>Ps. radicola.</i>
		2	1 days	Foreign bacteria
XIV	do	1	2 days	40 per ct. <i>Ps. radicola</i> , foreign bacteria.
		2	4 days	50 per ct. <i>Ps. radicola</i> , foreign bacteria.
XV	do	1	2 days	Foreign bacteria.
		2	2 days	Foreign bacteria.
XVI	do	1	4 days	Yeast and molds, 5 per ct. <i>Ps. radicola.</i>
		2	7 days	Molds and yeast.
XVII	do	1	6 days	Molds and foreign bacteria.
		2	4 days	Molds and yeast.
XVIII	do	1	5 days	Molds.
		2	2 days	Foreign bacteria, molds and yeast.

It will be seen that no legume bacteria could be found in fourteen of the eighteen cultures examined. In two of the four commercial cultures where legume bacteria were found they were present in very small numbers, and had they been exposed to the mixed growth which occurs when the cultures are developed upon the farm, it is very doubtful whether any result would have been obtained from the use of these cultures. In the two commercial cultures where the legume bacteria developed sufficiently to represent from 20 per ct. to 50 per ct. of the germs present, they would probably have developed in some numbers under ordinary conditions. That such cultures do occasionally develop in this way seems probable from the few apparently well authenticated cases where good results have followed the use of commercial cultures.

The finding of even this small number of the desired bacteria marks a decided advance over the conditions found last season, but at best makes only a very poor showing for the cultures.

Yeast and molds invariably developed in the flasks. In some cases the turbidity appearing in three or four days was due to these organisms. The bacterial contaminations consisted of several different organisms.

Packages Nos. 1 to 12 were in metal containers while packages Nos. 13 to 18 were wrapped in parchment paper and tin foil. Our data fail to show that the metal containers exerted any favorable influence upon the legume bacteria. The only apparent difference between the cultures inclosed in the metal containers and those wrapped in parchment paper and tin foil was that the latter were more heavily contaminated.

CULTURES FROM OTHER SOURCES.

During the season we examined two other packages of inoculated cotton cultures put up by this same firm and inclosed in metal containers.

In May, 1906, Prof. J. L. Stone developed a culture on cotton for alfalfa, following the directions carefully. The solutions became turbid at the proper time and everything seemed normal. The seed was inoculated on the second day, and plates inoculated with the fluids at that time showed only an occasional colony of

the legume bacteria. There was an abundant growth of miscellaneous forms. Professor Stone reports that the culture was without discoverable effect upon the alfalfa.

In August, 1906, a package of Nitro-culture in metal container was sent in by Mr. W. A. Runyon, Westtown, Orange Co., with a request that its quality be determined. A portion of the cotton cultures was placed in the proper sterile solutions and allowed to develop. Examinations with the microscope and by means of plates failed to detect the presence of the desired legume bacteria.

CONCLUSIONS.

It would seem from these results that the strong claims made by the culture company for the metal containers are not at all in accord with the facts.

It should be clearly understood that this publication concerns itself only with the commercial cultures which up to this time have been exclusively those dried upon cotton in accord with the method of Dr. Moore. These cultures have proved essentially a complete failure in tests made in practically all parts of the country and it is hard to understand how any firm can feel justified in continuing to offer such cultures for sale.