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LEGUME INOCULANT TESTS IN 1931

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BULLETIN No. 602

LEGUME INOCULANT TESTS IN 1931

A. W. HOFER AND H. J. CONN

ABSTRACT

An investigation of legume inoculants on sale in New York State has been made by plate and greenhouse studies. All except one of the products examined were found to be satisfactory. A number of cultures on sale in the State were found to be old. Consequently, farmers are advised to pay careful attention to the expiration dates given on the labels when legume cultures are secured, whether purchased or accepted gratuitously with the seed.

INTRODUCTION

During 1930, the New York Legislature passed a law covering the inspection of legume inoculants. The need for this law has arisen from the fact that in the case of this product, as with many others, there is keen competition between manufacturers coupled with a strong tendency toward overemphasis of trivial advantages which one culture may have over another. When this is done by a number of companies, confusion is caused in the minds of the users of the products, so that it is quite difficult to make an intelligent selection of a culture. Losses due to the use of a poor or worthless inoculant are much greater than the loss of the small amount of money paid for the culture, as crop yields may be greatly reduced by a lack of inoculation of the legume crop. If the plants are grown for soil enrichment and if there are no root nodule bacteria for this particular crop in the soil or on the seed, there will be little or no atmospheric nitrogen fixed by the legume crop grown, and as a result, succeeding crops will suffer.

THE LAW

A statement of the main portion of the law[1] follows:

Definition. Soil or plant inoculants shall include any carrier or culture of a specific micro-organism or mixture of micro-organisms represented to improve the soil or the growth, quality, or yield of plants, and shall also include any seed or fertilizer represented to be inoculated with such a culture.

[1]Chapter 318, Laws of New York for 1930.

License. No person shall sell, offer or expose for sale in this state any soil or plant inoculant unless licensed as provided in this section. Application for a license upon a form prescribed by the commissioner shall be made annually. The application shall include a statement as to whether the inoculant is represented as effective for inoculating legumes or for some other purpose, and if represented as effective for the inoculation of legumes, for which legume or legumes it is so represented. With the application, the applicant shall present a representative sample of the soil or plant inoculant described in the application. The commissioner, if satisfied that the inoculant may be depended upon to produce an effective inoculation for the purpose represented, shall issue to such applicant a license for the sale of such inoculant, expiring on December thirty-first next following. The applicant shall pay annually, at the time of presenting the application, to the commissioner for remittance to the state treasury, a license fee of ten dollars for each brand of inoculants as defined in the rules and regulations adopted by the commissioner as provided in this article.

Label requirements. Each soil or plant inoculant, when sold or offered or exposed for sale within this state, shall be clearly and plainly labeled to show whether the inoculant is represented as effective for inoculating legumes or for some other purpose, and if represented as effective for the inoculation of legumes, for which legume or legumes it is so represented; and the date to which the inoculant is represented to produce effective inoculation.

Misrepresentations prohibited. No person shall sell or offer for sale or advertise for sale any soil or plant inoculant if the package containing it, or the label or tag attached thereto, or any advertising relative to it, shall bear any statement or device regarding such soil or plant inoculant which is false or misleading in any particular.

Inspection and examination. The commissioner shall, as frequently as he deems necessary, transmit to the New York State Agricultural Experiment Station for analysis, examination and testing, samples taken from the different brands of inoculants which are or may be sold or offered or exposed for sale in this state. The director of said experiment station shall cause such samples to be analyzed, examined or tested, and shall report the results thereof to the commissioner and in such report shall state whether the label or any advertising relating to the inoculant shall be found to be false or misleading. The commissioner shall, from time to time, publish in bulletins the findings and other pertinent matter in relation to such inoculants.

Since the law was enacted, manufacturers of inoculants have been required to submit samples of this product to the State Department of Agriculture and Markets in order that the labels may be examined

before the material goes on the market. By this plan, false and misleading statements are to be kept from appearing on the labels, as products bearing such statements cannot now be legally sold in this State. In the tests made in 1931, a number of the cultures sent in for label control were tested, together with samples purchased by an Inspector of the State Department of Agriculture and Markets in various places in the State.

METHODS

For thoro inspection, legume cultures should be tested both in the laboratory and in the greenhouse. Adequate greenhouse equipment for tests of these cultures is now being made available at this Station, so that careful control of legume inoculants will soon be possible.

In the laboratory tests, a count is made of all bacteria that will grow upon the food materials supplied to them. This is carried out by weighing a definite amount of the carrier, consisting of sand, soil, peat, humus, or charcoal on sterile paper which is then poured into a known amount of sterile water. In the case of an agar culture, the entire content of the container is washed into 100 cc of water. Then 1 cc will contain 1/100 of the total number of bacteria in the original container. If 10 cc of this mixture are removed and put into a flask or bottle with 90 cc of sterile water, the latter will be 10 times as dilute as the former, and each cc of this dilution will carry 1/1000 of the total number of bacteria present in the original sample. This process of dilution is carried on, always with careful mixing, until 1 cc of the last sample contains 1/100,000,000 of the bacteria found in the original. Then, 1-cc portions are taken out of the dilution bottles and each portion deposited in a sterile, glass-covered culture plate. (See Fig. 1.) When this operation is completed, a small amount of warm, sterile agar is poured into the plate. This agar carries food materials and like gelatin, has the ability to solidify when cooled. When the agar has hardened, the plates are set away to incubate.

Some days later, depending upon the legume plant which the culture is supposed to inoculate, the plates are examined, and it is seen that small, gummy, white colonies of bacteria are growing upon the plate. Each of these colonies represents a spot where an isolated bacterium or a cluster of the original bacteria fell. On the plates from the 1/100 dilution, the colonies will perhaps be so numerous that they cannot be counted, while in the 1/100,000,000 dilution, there may be no colonies. When possible, a dilution is selected which

gives between 30 and 300 colonies on a single petri plate, and the number of colonies is then counted. (See Fig. 2.) For instance, with culture D, in Table 1, the plates containing the most favorable number of bacteria for counting were made from the 1/10,000,000 dilution. When counted, it was found that these plates averaged 148

FIG. 1.—METHOD OF ESTIMATING NUMBERS OF BACTERIA.

The laboratory worker is placing a small fraction of the commercial culture into a culture plate to see how many "colonies" develop. The principle involved is the same as tho a farmer, who wanted to count a lot of seeds that were too small to see, had sprinkled them over a plot of weed-free soil and later counted the number of seedlings that had appeared. The laboratory worker uses a sterile culture plate filled with sterile culture media instead of a plot of weed-free soil; otherwise the procedure is about that which the farmer would use.

colonies each. If 148 colonies developed on each plate containing 1/10,000,000 of the original sample, naturally there must have been at least 10,000,000 times that number of bacteria present or at least 1,480,000,000 bacteria in the original container. This culture was intended for inoculation of 30 pounds of seed, so that the count of bacteria in this case is expressed as at least 49,000,000 per pound of seed.

In the case of cultures other than agar, where a portion of the material is weighed out for dilution, a further correction is necessary, as the count gives only the number of bacteria in the material used in the dilutions. After the correction is made and the total number

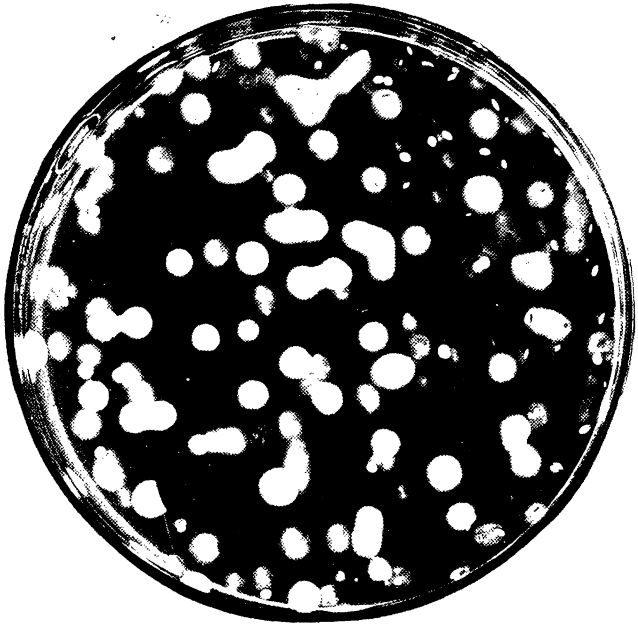

FIG. 2.—A CULTURE PLATE, LIKE THE ONE BEING INOCULATED IN FIG. 1, AFTER SEVERAL DAYS' INCUBATION.

Each living bacterium (or clump of bacteria) has now grown into a "colony" and appears as a white spot on the culture medium. Each colony corresponds to one of the seedlings which would show the farmer where one of his invisible seeds had been planted. The laboratory worker can count these colonies and thus learn approximately the number of living bacteria in the fraction of the original culture which was placed in the culture plate. From this he can very simply estimate the number of bacteria in the original culture and the number that would be applied per pound of seed, if the culture were used according to the manufacturer's directions.

of bacteria in the container determined, this is divided, as in the case of the agar cultures, by the number of pounds of seed the culture will inoculate. In the case of cultures manufactured without previous sterilization of the material that carries the bacteria there is further

difficulty due to the problem of deciding which of the bacteria on the plate are probably legume bacteria. To overcome this difficulty, a dye (crystal violet) is used in the agar to prevent the growth of the greater part of these types of organisms that do not cause nodules on legumes. When this dye is used, however, the count obtained is not always as accurate as when dyes are not used.

Because of this fact and because there is no way of identifying the legume organisms with certainty except by plant inoculation, the laboratory method of testing is not as satisfactory as greenhouse tests. In pure culture legume inoculants, however, where no bacteria are found, or where only a few are found, the conclusion can be safely drawn that the culture will be practically worthless for inoculation purposes.

Laboratory tests constituted the greater part of the tests made during 1931 when the only greenhouse work done was in bottles of sterile agar in which inoculated seeds were grown. During 1932, it is expected that plants can be grown in the new greenhouse in pots of sterile sand. The greenhouse procedure is simpler than that of the laboratory since a definite amount of seed is inoculated with the amount of inoculum recommended by the manufacturer. If the culture is supposed to inoculate 30 pounds of seed and if 1/100 pound is used in the test, 1/3000 (1/30 and then 1/100) part of the inoculant material in the container is used. The manufacturer's directions are followed as nearly as possible in making the inoculations, and samples of the inoculated seeds are then planted. If the plants form nodules, the culture is said to be satisfactory. This year no cultures have been reported as unsatisfactory because all tests had to be made in bottles, and occasionally even a good culture will not form nodules under such conditions.

RESULTS OF 1931 TESTS

Results of the work, so far as it has been possible to undertake it, have brought out the interesting fact that there are worthless cultures available to farmers who buy inoculants or who receive inoculants given free with the seed. Even tho it was not possible for the inspector to buy them, often cultures were found in the dealer's stock which were quite old, and presumably were either being given or sold to farmers for inoculation purposes. Newer cultures made by the same company (Culture F) were found to be of very low value, and the older cultures were undoubtedly worthless.

From this it is plain that the expiration date on the label should be noticed before a legume culture is purchased. It must also be remembered that when an old culture is obtained free with a bag of seed, it is equally worthless, and the expiration date is just as important as when the culture is purchased directly. Bacteria are living organisms (plants), and they may all die after being held for one or two years in a container. Dead bacteria are worthless for inoculation purposes, and the only way the farmer can protect himself against this danger is by using care to select a container whose label shows plainly that the culture is good at least until the date on which the material is to be used. If there is nothing on the label to indicate the age of the culture, or if the date to which the culture is good is already past, the culture should not be purchased.

TABLE 1.—RESULTS OF TESTS OF LEGUME CULTURES PURCHASED BY A STATE INSPECTOR, 1931.

BRAND	CROP	BACTERIAL COUNTS*	GREENHOUSE OBSERVATIONS
Culture A	Soybean	76,000,000	———
Culture A	Alfalfa	32,000,000	Satisfactory
Culture A	Clover	31,000,000	Satisfactory
Culture A	Pea	6,700,000	———
Culture A	Bean, pea, sweet pea	None found	———
Culture B	Clover	42,000,000	Satisfactory
Culture B	Alfalfa	27,000,000	Satisfactory
		202,000,000	Satisfactory
Culture B	Pea	50,000,000	———
		8,000,000	———
Culture C	Clover	None found	———
Culture D	Alfalfa	49,000,000	Satisfactory
Culture E	Clover	3,900,000	———
Culture F	Clover	Contaminated	———
Culture F	Alfalfa	None found	———
Culture F	Bean, pea, lima bean	73,000	———
Culture F	Pea	20,000,000	———
Culture G	Soybean	25,000,000	———
		35,000,000	———
Culture G	Bean	125,000,000	———
Culture G	Clover	21,000,000	Satisfactory
Culture G	Alfalfa	67,000,000	Satisfactory

*Count of apparent root nodule bacteria per pound of seed.

Another fact brought out by these tests is that there is a tremendous variation in the number of bacteria supplied by individual cultures, even when produced by the same company. In Table 1, cultures purchased by the inspector are listed, and the results of tests

TABLE 2.—TESTS OF LEGUME CULTURES SUBMITTED BY THE MANUFACTURER, 1931.

BRAND	CROP	BACTERIAL COUNTS*	GREENHOUSE OBSERVATIONS
Culture A	Pea	116,000	———
		None found	
Culture A	Soybean	147,000	———
		1,400	———
Culture A	Bean	22,000	———
		2,600,000	
Culture A	Clover	125,000,000	———
		3,000,000	Satisfactory
Culture A	Alfalfa	11,000,000	Satisfactory
Culture A	Cowpea	26,000,000	———
		5,000,000	———
Culture B	Clover	940,000,000	———
		18,000,000	Satisfactory
Culture B	Alfalfa	2,500	———
Culture B	Pea	145,000	———
Culture D	Bean	5,000,000	———
Culture D	Alfalfa	118,000,000	———
Culture D	Soybean	681,000,000	———
Culture D	Clover	720,000	———
Culture D	Pea	2,000,000	———
Culture F	Alfalfa	370,000	———
Culture H	Soybean	41,000,000	———
		4,500,000	
Culture H	Alfalfa	96,000,000	Satisfactory
		61,000,000	Satisfactory
Culture H	Pea	76,000,000	———
		932,000	
Culture H	Pea, bean, lima bean	620,000,000	———
Culture H	Bean	180,000,000	———
Culture H	Clover	792,000,000	Satisfactory
		Not recorded	Satisfactory
Culture H	Cowpea	250,000,000	———
		9,500,000	

*Count of apparent root nodule bacteria per pound of seed.

of these cultures are given. In Table 2, the results are given of the tests of the cultures sent to the Dairy and Food Bureau of the Department of Agriculture and Markets for label examination when application for registration was made. The tests showed that the

cultures sent to Albany were much more satisfactory than the cultures (often older) purchased on the open market. The names of the manufacturers are omitted in these tables, since it was not possible to make conclusive tests of the value of the products this year.

The results are summarized in Table 3. Since it is obviously unfair to express an average which is distorted by the occurrence among the inoculants tested of one or two extremely high-count cultures, another average was found, after the omission of one or two of the cultures with the highest counts. The second average probably more closely represents the number of bacteria that may be expected to be present in any culture purchased on the open market.

TABLE 3.—SUMMARY OF 1931 RESULTS.

BRAND	AVERAGE COUNT PER POUND OF SEED (IN MILLIONS)		PERCENTAGE OF SAMPLES ABOVE FIVE MILLION
	All samples	Excluding samples with unusually high counts	
Culture A	20	13	50
Culture B	143	21	78
Culture C	—	—	—
Culture D	143	35	67
Culture E	—	—	—
Culture F	4	0	20
Culture G	55	37	100
Culture H	194	80	82

A determination was also made of the percentage of the cultures of a given company which gave a count above a certain minimum. Assuming that only 1 of the 25 or 30 legume bacteria that grow upon plates will form nodules after plant inoculation and considering the fact that there are about 200,000 alfalfa seeds in a pound, this would mean that 5,000,000 bacteria would be necessary for good inoculation of a pound of alfalfa seed. When the percentage of cultures with a count above 5,000,000 manufactured by each company is noted, it is seen that there is great variation in the value of the different products.

Culture F was poor, as most of the samples of this product gave very low counts. The summary of results in Table 3 shows that the farmer's chance of buying a satisfactory sample of this culture is small. Since it is not advisable this year to publish the names of the companies that produce inferior cultures as judged by the tests made

and since there is not sufficient evidence to justify the refusal of registration to any company, the only thing that could be done was to warn these companies that their products would be subject to stringent tests in 1932. It is expected that action can be taken in the future to bar undesirable cultures from sale.

DISCUSSION

In conclusion, it is well to emphasize again the importance of care on the part of the purchaser of legume cultures in regard to the dates on the labels. *Purchase only cultures whose dates of expiration have not been reached. Do not purchase undated cultures.* Also, remember that cultures vary greatly in the number of living bacteria present and in their value for inoculation. Information as to the value of different brands of cultures should be available for publication in 1932.