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Securing a Stand of Clover on the Southern Iowa Loess

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SECURING A STAND OF CLOVER ON THE SOUTHERN IOWA LOESS.

BY E. B. WATSON.

At the Sub-station at Leon, Decatur County, conducted by the Soil Section of the Iowa Experiment Station, clover was sown on the series of oat plots in the spring of 1905, some of the plots being treated with stable manure. It was noted during the season that the manured plots had the best stand, and much the best growth of clover. This is not a new discovery, but the cause seems to be a matter of speculation and conjecture. Clover is an important crop in Iowa, and is especially needed in that part of the State to supply lost humus and restore productiveness. Furthermore, it is often very difficult to get a good stand there especially on the points and clay hillsides.

In view of these facts it was decided to make a thorough study of the influences governing the germination and early growth of clover on the soil found there, including the action of manure on the clover.

The following is the report of the bacteriological work undertaken in connection with this study:

THE BACTERIOLOGICAL QUESTION.

A recent editorial in *Wallaces' Farmer* headed "What Barnyard Manure Does for the Soil" has the following statement: "The main value of manure does not lie in what is known as its fertilizing elements. What else does manure do to the soil? First, it inoculates the soil with bacteria, and soil that is full of bacteria is a soil in good physical condition—a productive soil." This very well expresses the general belief in regard to the bacteria brought by the manure. Bacterial life is very active and abundant in the manure, and it is but natural to suppose that these bacteria when added to the soil have a great deal to do with its added fertility. When this work was first started, it seemed altogether likely that an investigation of the causes of the benefit derived from barnyard manure, would lead into a bacteriological study.

In the first series run, pots treated with sterilized manure were run along with the regular manured pots. The manure was sterilized by heating in the autoclave for thirty minutes at 120°C.

This eliminated all bacteria, both the special clover bacteria and the numerous fermentations and decomposition bacteria.

Following are the green weights taken May 3d, one hundred and twenty-four days after planting:

Pot	Treatment	Wt. Crop g.	Average	No. of Plants	Wt. per Plant g.	Average
40	Horse Man.	38.9	.....	17	2.29	.....
41	Cattle Man.	53.3	.....	30	1.71	.....
42	Mixed I	45.4	47.	15	3.03	2.35
43	Mixed II	50.2	.....	21	2.39	.....
44	0	7.2	7.2	6	1.20	1.20
45	Horse M. Ster.	54.6	.....	15	3.64	.....
46	Cattle M. Ster.	51.7	45.	36	1.44	4.31
47	Mixed I. Ster.	34.2	.....	6	5.70	.....
48	Mixed II. Ster.	38.9	.....	6	6.48	.....

The stand varies so much that it is hard to make exact comparison, but surely the sterilized manure suffers nothing by the comparison, however we look at it. No. 40, horse manure, and No. 45, the same sterilized, have the same stand practically, but the sterilized pot has 40% heavier crop. The average of the four unsterilized pots is 47 grams per pot, and the sterilized pots 45 grams. Surely very close together. We see though that the unsterilized pots had much the heavier stand and when comparison is made on the weight per plant, the sterilized pots are very far ahead, 80% better in fact. However, where the stand was thinner the plants would have a much better chance to develop, so a comparison on this basis is not altogether fair. But taking everything into consideration, we feel safe in saying that sterilization has not lessened the benefit to be derived from the manure. In fact it really seems to have helped it.

In a later series this question was tested again. 71 was a check pot, 73 had cattle manure added and 74 had the same sterilized. They were planted February 24th. April 14th a study of the pots showed that manure had a decidedly beneficial effect, but no difference was noted between the sterilized and unsterilized pots.

There is a decided benefit from the use of stable manure and sterilizing it does not seem to decrease this effect.

Notes were taken at this time and again May 24th. Final green weights were obtained June 1st. The following table gives all these data. The pots are compared with each other by rating the check pot 100 and giving the relative standing of the others:

No.	Treatment	Relative Growth May 3d.	Relative Growth May 24th	June 1st—Green Weight		
				Weight	No. Plants	Relative Weight
71	0	100	100	17.15	13	100
73	Manure	300	240	43.3	13	252
74	Sterilized Manure	275	240	40.4	13	240

These figures show that the sterilized pot was practically as good as the unsterilized, the slight difference being within the limits of error.

These two tests, or rather five tests, for there were five pair of pots tried, settle beyond dispute the question of the influence of the bacteria brought by the manure. The bacteria in the manure have nothing to do with the growth of the clover, for the clover grows just as well without them. This also settles the question of inoculation with the especial clover nodule bacteria. It has been stated many times that manure was one of the ways of inoculating alfalfa or clover. This shows that such inoculation is not necessary. Further than that, care was taken to ascertain if the clover was inoculated on the field plots. It was found that all the clover plants on the untreated plots were inoculated. Also the poor, stunted plants in pot 37, a check pot, were pulled up March 14th and were found to be well inoculated with tubercles on their roots. This soil was undoubtedly inoculated with the clover nodule bacteria, and failure to produce good stand and growth had nothing to do with this bacteria.

But what about the other bacteria that the manure contains? The following in an extract from a short article by Joseph E. Wing, that prince among agricultural writers, in the *Breeder's Gazette* of June 13, 1906: "If I could use horse manure made by horses feeding on alfalfa hay, I would snap my fingers at purveyors of commercial cultures. And I guess that any sort of manure whatsoever when incorporated with the soil will put in the yeast that will start the ferment that we call nitrification and that bacteria will be found there (how they will get there we can only guess) and that alfalfa will thrive if only the land has lime enough in it to make it sweet, and is not wet."

Here is also an extract from an article by Dr. Chas. D. Wood,[1] Director of the Experiment Station at Orono, Maine, on "Nitrogen in Relation to Soil Fertility": "The application of farm manure

1. Agriculture of Mass. 1905, page 185.

is advantageous from two wholly different reasons. They carry considerable quantities of plant food, particularly nitrogen. This is the chemical side which has been in the past perhaps unduly emphasized. There is another equal and in some cases greater advantage derived from the application of farm manures, because of the large amount of nitrifying bacteria which they carry to the soil."

This opinion expressed by the scientist agrees with that expressed by the practical man, and they both voice the general idea in regard to the function of the bacteria in the manure. But here we find a mistake has been made. There may be numerous bacteria in the manure but they have nothing to do with the growth of the clover. When they are taken away the clover grows just as well.

But this by no means settles the bacteriological question. A count of the number of bacteria was made on March 17, 1906, or 77 days after the loess pots were treated and planted, and 73 days after the till pot was planted. The media used for the first count was gelatine of rather poor quality and much liquefaction took place and the count was uncertain. A few days later another count was made using agar, and this proved entirely satisfactory. The results of both counts are given in the following table:

Pot	Soil	Treatment	Gelatine Media		Agar Media	
			Bac. per g.	Molds per g.	Bac. per g.	Molds per g.
44	Loess	Check	100,000	15,000	1,000,000	32,000
41	Loess	Manure	2,400,000	3,000,000	4,000,000	240,000
46	Loess	Man. Ster.	7,500,000	150,000	14,000,000	300,000
65	Till	Check	1,300,000	200,000	700,000	70,000

As was expected, the second count gave larger numbers but they should be depended upon rather than the first figures. It is seen that there are a great many more bacteria in the soil that had been treated with the manure than in the untreated soil. This means that the manure made a good medium for growth of the bacteria. It is also true that clover grows a great deal better on the manured soil, and the question now is, what connection, if any, do these two facts have with each other.

The sterile manure as soon as it touched the soil was inoculated. The untreated soil was low in bacterial life, not because it lacked inoculation, for all kinds of bacteria were there, but because the

conditions for growth were not favorable. As soon as the manure was added they multiplied because there were food and favorable conditions. Scientific researchers have demonstrated that bacteria have a great deal to do with preparing food for the higher plants. They produce decay of the organic matter in the soil. The carbon after numerous steps finally is changed to carbon dioxid, the protein nitrogen finally takes the form of ammonia, nitric acid or free nitrogen. The higher plants obtain their nitrogen from nitrates, their carbon from carbon dioxid and even the mineral elements owe their availability largely to bacterial action.

"The[1] greater part of the bases are taken up as nitrates and phosphates, and also as salts of the organic acids. Phosphoric acid exists in the soil in the form of insoluble basic phosphates, which under the action of organic acids are converted into neutral or acid salts which are soluble. Hence the production of organic acids by bacterial fermentation renders phosphoric acid available to plant roots. Carbonates unite with silica to form zeolites and these in turn are slowly decomposed by organic acids and their contained bases again liberated as organic salts. Thus by the combined production of carbonic, nitric and the various organic acids, through the action of bacterial life, we have all the necessary agencies at hand for the dissolution of the mineral elements of plant growth."

F. Dafert[2] and Kornauth, Vienna, Austria, report experiments with oats inoculated with alinit, *Bacillus megatherium*, *B. subtilis*, etc. The yield averaged 62.5 g. for the check which received a complete fertilizer, 66.2 g. where inoculated with alinit, 66.5 g. where inoculated with *B. megatherium* and 76 g. where inoculated with *B. subtilis*.

We see that bacteria may play a prominent part in soil fertility, and the question is how much did they do in the case of the clover?

It may be that the manure by supplying food for the bacteria, or correcting the reaction of the soil, has produced a growth of bacteria which is favorable to the growth of the clover. There are undoubtedly more bacteria there in the soil. It may be that these have worked upon the plant food in the soil or the manure, and by this action made it more available for the clover. In the long run, bacteria undoubtedly do have much to do in the preparation of plant food and it would seem that in the case cited above that *Bacillus subtilis* helped the growth of the oats about 20%. If they are responsible for the better growth of the clover, it would seem that the clover would suffer if they were removed.

1 F. D. Chester, Penn. Dept. Agriculture. Bul. 98.
2 Experiment Station Record 16, p. 851.

The following experiment was planned to secure data on this point. Soil from plot 113 of the Leon station was used:

Pot 161 and 162 check.
Pot 163 and 164 manured.
Pot 165 and 166 sterilized.
Pot 167–168 manured and sterilized.

Manure leachings were used for treatment. 3 inch flower pots were used because four of them would go under the available bell jars. 165-166-167-168 after being filled were wrapped in paper and put in the autoclave and kept for 45 minutes at 10 pounds pressure. 4 Petri dishes were also sterilized. These were for the pots to set in. The inside of the bell jar was washed out with a saturated solution of mercuric chloride and a glass plate was also washed off with this disinfectant. Red clover seed was put in a small E flask and washed with a saturated solution of mercuric chloride and then well rinsed with sterile water three times. About fifteen of these sterile seeds were planted in each pot by means of a flamed forceps. The surface was scratched and the seeds covered about one-fourth inch deep. The pots were watered with sterile water and the four sterilized pots covered with a large bell jar, and the four unsterilized pots covered with another bell jar, so that conditions would be parallel. The planting was done October 23d. By November 7th it was seen that sterilization was not complete, for the four pots which were supposed to be sterile had mold on the surface and the plants were looking sickly. The clover on the unsterilized pots was looking fairly well, but growing very slender. By November 27th the clover on the sterilized pots was all dead, evidently killed by the mold. On the unsterilized pots the clover had made a fair growth. A count of the bacteria and mold on the sterilized pots was made at this time by inoculating some agar plates by the usual method. The idea was simply to see if the soil was sterile, and not to know just how many bacteria or molds were present.

The data was as follows:

Pot	Dilution	Bacteria	Molds
165.	1-10.	Many	
165.	1-100.	Many	
166.	1-10.	.....	Many
166.	1-100.	.....	Few
167.	1-10.	.....	Many
167.	1-100.	Few.	Many
168.	1-10.	.....	Many
168.	1-100.	Few.	Many

This shows that none of the pots were sterile but that the molds were more numerous than the bacteria. There was apparently only one species of bacteria but three kinds of mold.

These pots were sterilized over again leaving them a longer time in the autoclave. The seeds were sterilized by leaving them 2½ hours in the corrosive sublimate solution. Planted November 27th. This time the clover on the unsterilized pots was attacked and killed by mold while the plants on the sterilized pots grew slender and sickly and finally died. Thus nothing was learned from these two experiments except that clover was a difficult crop to grow under these conditions.

As clover was found to be so unsuitable, it was decided to try wheat, as the probabilities are that the relation between wheat and the bacteria is very nearly the same as that between clover and bacteria.

The same soil was used. The series was as follows:

Pot 401 and 402 checks.
Pot 403 and 404 leachings of manure.
Pot 405 and 406 sterilized.
Pot 407 and 408 leachings added and sterilized.

The wheat for the seeding was sterilized with corrosive sublimate for two hours, and then washed several times with sterile water. The pots with the soil in them were sterilized in the autoclave for one hour at 10 to 15 pounds pressure. 4 inch pots were used and one was put under each bell jar. 5 seeds were planted in each pot one-fourth inch deep on January 11, 1907. None of the wheat sprouted, evidently being killed by the corrosive sublimate.

The series was planted again February 4th. The soil was re-sterilized, and the seeds kept only twenty minutes in the saturated solution of corrosive sublimate. Only one seed in this series grew.

February 16th the wheat series was replanted for the third time. The soil was not resterilized. The seed was sterilized by immersion for ten minutes in 5% formalin and then rinsed in sterile water. This time the seed grew, or enough of them grew so that a comparison could be made. All pots both sterilized and unsterilized were covered with bell jars so that conditions would be identical. All plants grew well up till the first of March, when 406, the sterilized check began to fail. In a few days 405, its duplicate began to show signs of dying. By March 6th, 406 was turned decidedly yellow and the tips of the leaves were dried up.

On March 8th a sample was taken from each pot to determine the number of bacteria in each. Each sample was secured by taking a small amount on the end of a flamed spatula from three different places just below the surface on each pot. This was placed in sterile Petri dishes, thoroughly mixed and about one-fourth gram taken from each and placed in sterile test tubes for dilution. Two agar plates were poured from each with dilutions of 100 and 1,000 respectively. No effort was made to get exact numbers, but the notes made were as follows:

401. Many bacteria, many species, colonies all sizes, one mold.
402. Many bacteria, many species, colonies all sizes, one mold.
403. Ten times as many bacteria as 401, colonies the same, twenty molds.
404. Twenty times as many bacteria as 401, colonies the same, twenty molds.
405. Many bacteria, colonies mostly very small. Few large ones.
406. One-tenth as many bacteria as 401, colonies mostly very small.
407. Bacteria very numerous, colonies all quite small.
408. Bacteria very numerous, colonies all quite small.

It is seen that none of the pots were sterile, but it is plainly seen that the character of the flora has been changed by the sterilization. It would seem that one species which has the characteristic of forming very small colonies on agar plates survived the sterilization in all four pots and that in addition, pots 405 and 406 became later inoculated with other bacteria. There were no molds on the sterilized pots. On March 9th the poorest one of each of the duplicates was harvested and the data is here given:

Pot	No. of Plants	Green Wt. g.	Wt. per Plant	Relative Weight
401	2	.240	.120	100
404	3	1.265	.422	351
406	3	.445	.148	123
408	3	1.275	.425	354

It is here seen that sterilization helped the growth of the wheat on the untreated soils, and did not hinder it any on the manured soil. The plants on all the pots were thrifty, except 406. The plants on it were nearly dead.

The cause of the trouble with the wheat on pots 405 and 406, the untreated sterilized pots, was given consideration. It was thought that it might be due to lack of nitrates in these pots; as nitrification could not go on in a sterile soil, possibly the nitrates were all used up and the plants were consequently dying. To test this hypothesis the following experiment was planned: The pots from which the plants had just been cut, were elevated on a ring stand, distilled water was applied to the surface until it percolated through, this dropped on to a filter and from this was caught in a graduate. This filtrate was tested for nitrates with the brown ring test. The percolates from all the pots showed the presence of nitrates very decidedly. The percolate from 406 was tested the second time and each time it gave a distinct test for nitrate. Pot 408 gave a very strong test for nitrates. So it is evident that the plants in pot 406 were not dying for lack of nitrates.

March 16th the remaining pots were harvested and the data is given below. The plants on 403 and 407 were very thrifty, those on 402 were one-third dead and those on 405 were one-fourth dead, and the whole plant looking yellow and sickly.

Pot	Treatment	No. Plants	Green Wt. g.	Wt. per Plant g.	Relative Weight
402	0	2	.552	.276	100
403	Man.	4	2.988	.747	270
405	Ster.	5	1.600	.320	116
407	Man. Ster.	4	3.150	.787	285

It is seen here also that sterilization has helped the growth of the wheat, whether the soil is manured or not. The fact that sterilization was not complete, but only partial, destroys much of the force of this argument, but still it is felt that there was some sterilization and that some of the species of bacteria were eliminated and that this data has some value.

It was decided to test the reaction of the soil to see if the species which formed the small colonies, made the soil acid. To do this, soil extract was obtained as before, 100 cc. portions were placed in a beaker, brought to a boil to expel CO_2 and titrated with N-50 acid and alkali solutions, lacmoid being used as an indicator. None

of the pots were found acid, but all were slightly alkaline, as normal soils should be. So the cause of the dying of the plants on pots 405 and 406 is unexplained. It was not due to acidity of the soil and it seems that it could not be due to lack of plant food, for there were free nitrates present and the corresponding pots unsterilized showed no lack of plant food.

The following investigation[1] on the effect of soil sterilization on the development of plants bears directly on the point under discussion. It was made by C. Schulze of Germany. He made pot experiments with field, meadow and garden soil. The crops grown were oats, mustard, peas, buckwheat and grasses. One pot of each series was untreated, one was sterilized for one hour at 125°C. before fertilizing, one sterilized the same after adding the fertilizers, and a fourth was sterilized at 100°C. for 18 hours. During sterilization, there was found to be a formation of more or less injurious decomposition products and also a release of otherwise non-available nitrogen. The addition of lime counteracted the injurious effects of the decomposition products. In general sterilization seemed to retard growth for a time, but later the plants became more vigorous in the sterilized pots, often exceeding in total growth those in the untreated pots. In most cases there was an increase in the total plant product which was attributed to sterilization.

This agrees exactly with my results and it seems safe to conclude that the bacteria in the soil did not aid in the better growth of the clover. They may have, and in fact seem to have a great deal to do with elaboration of plant food and the probabilities are that a soil kept sterile, would soon become unproductive, but it also seems true that the loss of the bacteria would not be felt the first few months or during the production of the first crop. If that is the case, the greater number of the bacteria in the manured pots, 41 and 46, noted above over the check No. 44, was due to the same cause as the better clover, but the larger number of bacteria were not responsible for the larger clover. That is the question we were working on. If this may be considered settled, then the conclusion is that bacteria in no way were responsible for the beneficial action of the manure on the growth of the clover, for in the first place it was shown that sterile manure was as beneficial as the unsterilized, and in the second place it was shown that when the whole pot was sterilized, the crop did not suffer but was even a little better.

1. Abst. Experiment Station Record 18, p 542.