

Graduating Thesis.
By.

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"The Crop Production of Bacteria."

The Importance of Nitrogen to Plant Life.

It is true that oxygen, carbon & hydrogen combine to form cellulose out of which the frame work of vegetation is built, and to form the starches, sugars & gums which make up by far the largest part of the dry weight of vegetable matter; but these appear to be only products which have resulted from the transformations which take place within the protoplasmic substance of which nitrogen sulphur & phosphorus appear to be the controlling or life-leading elements.

The amount of nitrogen required by different plants varies. One kind of grain will require more nitrogen than another. e.g. A crop of wheat which yields 30 bushels of grain to the acre, requires 48 pounds of nitrogen. Oats yielding 60 bushels of grain to the acre requires 73.3 pounds of nitrogen. Red clover yielding 2 tons per acre requires 102 pounds of nitrogen. With these figures, it is assumed that no nitrogen is given back to the soil, which, however, is not true regarding clover.

Distribution of Nitrogen in the Soil.

By referring to Ruess' book on "The Soil", page 108, it will be seen that the amount of nitrogen decreases as the depth increases. This decrease is evidently due largely to the variation in the distribution of roots in the soil. But there are other factors which tend to cause the destruction of nitric acid, setting free the nitrogen in a gaseous form.

Sources of Soil Nitrogen.

The greatest question of the source of soil nitrogen is one of the most important questions of practical agriculture. It was formerly thought that the nitrogen came from the air and was brought down by the rain, but it has been proven by observations made in various parts of Europe and in other places that this is not the case. The rain does not bring down sufficient nitrogen to produce the crops that grow from year to year. The following table gives the amount of nitrogen in rain per acre per year, as shown by experiments tried in three different places.

	Rothamsted, - 8 yrs pounds	Lincoln New Zealand. 3 yrs pounds	Barbados 8 yrs pounds
Nitrogen as ammonia	2.53	0.74	.93
" " nitric acid	.84	1.00	2.84
	3.37	1.74	3.77

These amounts, as shown, are only sufficient to contribute the necessary nitrogen for about two bushels of wheat per acre, when 48 pounds is counted sufficient for a crop.

Free Nitrogen-fixing Germs.

In 1887, Hellriegel published the results of his experiments, which satisfactorily prove that we have in nature great numbers of minute microscopic organisms living in the soil, locating themselves on the roots of plants, drawing nourishment from the plant but in turn giving to it compounds of nitrogen, which these germs are able to produce from the free nitrogen of the soil air. The organisms locate especially upon clovers, beans, peas and others of the Leguminous species. The germ of the pea will not act upon the roots of clover, or the bean germ on the lupine, etc. But each plant has its own particular kind of germ. The difference is easily noticed by the outward appearance.

The tubercles were first supposed to be normal parts of the plant on which they were found. The form of the tubercles was used as one of the characteristics of a variety of plants. Bivona took them to be fungi, and divided them into two classes, simple & lobed. Clos believed them to be normal structures with a physiological role and called them lenticels. Cornu treated them as root branches. Gasparini describes them as swollen, lateral roots and that they were organs for absorbing food. Trevisanus judged them as imperfect buds, which, in case the plant did not fruit, could themselves develop into perfect plants. Noble, Lachman and others thought they were places for the storage of reserve food material.

The period of serious investigation into their structure began in 1866 with Woronin, who described them as being composed of parenchymatous tissue. Following him came Eriksson, DeVries, Schindler, Kny and others. These are included in the middle period of investigation.

The recent period began about 1880, including Ward, Hellriegel, Beyerinck,

Villenium, Frank and Laurent who went farther in the investigations than those in the middle period.

We need not go farther into detail as to what each one's theory was and their explanations, but give reference to "The Botanical Gazette," Vol. 18, P. 357.

The following account of Hellriegel's experiments is given in the "Journal of the Royal Agricultural Society" Vol. II, Page. 664.

In 1883, he began his experiments with various families of plants set in pots of washed quartz sand. To all the pots nutritive solutions, but containing no nitrogen, were added. To one series nothing else was supplied; to a second a fixed quantity of nitrogen as sodium-nitrate was added; to a third twice as much was added; and to a fourth four times as much was added. The result was that in the case of the Gramineae, and some other plants, the growth was largely proportional to the amount of combined nitrogen supplied, whilst in that of the Leguminosae it was not so. In the case of these plants it was observed, that, in the series of pots to which no nitrogen was added, most of the plants were

apparently limited in their growth by the amount of nitrogen the seed supplied. On examination it was found, that, whilst no nodules were developed on the roots of the plants of limited growth, they were abundant on the roots of the luxuriantly grown plants, those to which nitrogen had been added. Hellriegel then tried experiments to determine whether, by the infection of the soil with appropriate organisms, the formation of root nodules and luxuriant growth could be induced, and whether by the exclusion of such infection the result could be prevented. To this end, he added to some of a series of experimental pots 25 cc. and sometimes 50 cc. of turbid watery extract from a fertile land, made by shaking a given quantity of it with five times its weight of distilled water, and then allowing the solid matter to subside. In some cases the extract they used was sterilized. In those in which it was not sterilized, there was almost always luxuriant growth, and abundant formation of root nodules; but with sterilization the result was entirely different. Thus proving that the soil could be infected and the root nodules produced.

More extensive experiments have been carried out since, in 1888-89-90-91, proving the fixation of nitrogen germs.

The question yet remains, What is the practical importance of this newly recognized source of nitrogen to the Leguminosae?

The discovery of these germs has placed in the hands of every farmer, a means whereby he can at any time cause to be drawn from the atmosphere the free nitrogen and have it fixed in any field he wishes to enrich. In

Germany, a man named Schultz has for some years farmed a considerable area of gravelly soil by growing leguminous crops - various clovers, lupines, serradella, etc, by means of kaimit and phosphate manures. He finds that thereby the land is very much enriched for future cereal and other crops. And from our own experience we know that leguminous crops enrich the ground.

There are three distinct genera of these organisms; those which form ammonia from organic nitrogenous compounds, such as albumen; those which transform ammonia into nitrous acid, and those which transform nitrous into nitric acid.

Each genus is necessary in the complete transformation of proteid matter into nitric acid, in which form alone nitrogen is chiefly available for plant food.

Formation of Ammonia.

The Bacteria which are especially active in the formation of Ammonia are found constantly in surface soils, and in the air and rain waters. By the activity of these organisms quantities of ammonium carbonate are produced by the decomposition of albuminous bodies. A temperature of from 50° to 100° F. is found most favorable to the production of fermentation. As the temperature approaches freezing point the activity diminishes and finally ceases altogether, but their vitality is not destroyed. Above a temperature of 110° F. the activity of the ferment is greatly diminished, and finally ceases at a still higher temperature. A temperature near the boiling point of water continued for some time destroys their vitality altogether.

Production of Nitrous Acid.

The conversion of ammonia into nitrous acid is the next step. With a

moderate store of ammonia, the oxidation into nitrous acid takes place, as a rule, without loss of nitrogen. When however there is an excess of ammonia much nitrogen may be lost. Thus nitrogen fertilizers should be applied in moderate quantities. The nitrous ferment is by far the largest and most vigorous of the nitrifying organisms. They are multiplied by spores which develop rapidly into full size.

Production of Nitric Acid

The last steps of the process of nitrification consists in oxidizing nitrous into nitric acid. The organisms are much smaller than the nitrous organisms, but of the same general shape. It must not be supposed that these steps in the preparation of food are in entire distinctness.

They are almost synchronous.

Conditions Favoring Nitrification.

Position — The vitality of the organisms, as a rule, is greatly reduced, as it occurs below the surface at greater depth. The land should be in position for good drainage

2d good cultivation.

Temperature — The soil should be warm, from 85° to 90° F. Diminished temperature lessens the activity of the organisms, thus lessening plant food.

Light — Exposed to bright light, the activity of the organisms diminish to an almost zero point. Thus a warm night is much better for the development of soil organisms than a warm day.

Air — It may be inferred that proper aeration is necessary for the development of functional activity of the germs. This is secured by proper cultivation and drainage.

Lime — Since the final action of nitrification is the production of nitric acid, it is highly important that the soil contain some substance capable of combining with it and preventing its accumulation in a free state. The activity of these ferments is diminished by the presence of an acid, and increased by a slightly alkaline substance. The lime is usually slacked lime.

All of these above mentioned favoring conditions will prove of no advantage in a soil which is practically sterilized. But if a few organisms can be supplied, a sterilized soil, after a time, by natural growth and distribution of nitrifying organisms, becomes fully impregnated with the nitrifying germs. As a rule, the richer the soil or the more highly fertilized it has been and the more fully cultivated, the greater will be the number of organisms which it contains and the higher the degree of their vitality. The organisms may be placed in soil where they are wanted, in several ways. A very common method consists in scattering a certain amount of soil, taken from a field where the crop to be raised has been successfully grown, over the surface of the new land before plowing it. This method has been practiced successfully on various occasions. Another method is to abstract with water some of the soil, which from previous observation is known to contain the

desired root bacterium germs, and sprinkle the watery extract over the soil before plowing. One of the latest developments in this direction is the appearance in the general market of a patented germ fertilizer for leguminous crops. They are manufactured or grown on a commercial scale, and sold in bottles. A firm in Germany gives the following directions for use.

Every bottle contains sufficient for the inoculation of $2\frac{1}{2}$ woods.

If the contents of the bottle have already become liquid they are used for the direct inoculation of the seed. If solid the contents can be easily liquified by warming gently for a few minutes. Exposure to a temperature greater than the heat of the body, which is amply sufficient to melt it, or to direct sunlight must under all circumstances be strictly avoided.

The liquid contents are poured into a vessel containing from one to two pints of clear water, and then shaken

or stirred until the fertilizer is equally distributed thruout the vessel. The inoculated water is then poured over the seed and worked with the hands until every seed is moistened. The seed is then mixed with dry sand so as to be sown. If possible avoid sowing in the glaring sunlight. Attention is especially called to the fact that the 'germ fertilizers' should only be used for the species of leguminosae marked on the bottle.

The principle firms in this business are in Germany, altho there are a few stations experimenting on it in this country. Hatch Experiment Station is one of them.

The discovery of these germs has caused a great advance in agriculture, but still "the half has not been told."