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HAWAII AGRICULTURAL EXPERIMENT STATION,

E. V. WILCOX, Special Agent in Charge.

Bulletin No. 24.

THE ASSIMILATION OF NITROGEN BY RICE.

BY

W. P. KELLEY,

CHEMIST.

UNDER THE SUPERVISION OF

OFFICE OF EXPERIMENT STATIONS,

U. S. DEPARTMENT OF AGRICULTURE.

WASHINGTON:
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HAWAII AGRICULTURAL EXPERIMENT STATION, HONOLULU.

[Under the supervision of A. C. TRUE, Director of the Office of Experiment Stations, United States Department of Agriculture.]

WALTER H. EVANS, *Chief of Division of Insular Stations, Office of Experiment Stations.*

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LETTER OF TRANSMITTAL.

HONOLULU, HAWAII, *February 27, 1911*.

SIR: I have the honor to submit herewith and recommend for publication as Bulletin No. 24 of the Hawaii Agricultural Experiment Station, a paper on the Assimilation of Nitrogen by Rice, prepared by Mr. W. P. Kelley, chemist of the station. The formulas for fertilizers heretofore used for rice in Hawaii have been based on experiments in which insufficient attention was given to the comparative effects of the different forms of nitrogen. The experiments reported in this bulletin indicate quite conclusively that nitrates are unsuited as fertilizers for rice, while excellent results are secured by the use of nitrogen in the form of ammonia. The bulletin should, therefore, lead to changes in cultural practice which will bring about greater profits. From a scientific standpoint, also, the careful pot experiments in which nitrates are shown to be ineffective and harmful, and ammonia very effective, should be of considerable interest.

Respectfully,

E. V. WILCOX,
Special Agent in Charge.

DR. A. C. TRUE,
Director Office of Experiment Stations,
U. S. Department of Agriculture, Washington, D. C.

Publication recommended.
A. C. TRUE, *Director.*

Publication authorized.
JAMES WILSON, *Secretary of Agriculture.*

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THE ASSIMILATION OF NITROGEN BY RICE.

INTRODUCTION.

The absorption of plant food and the chemical changes that take place in the formation of organized living tissue from the inorganic substances of the soil and air are subjects that have long occupied the attention of scientists. During the past century certain phases of this question were investigated in no small measure, and among these perhaps none received more study and played a greater part in the development of agricultural science than that which pertains to the utilization of nitrogen in plant growth. The history of this question marks a progressive evolution, and connected with it are to be found the names of prominent chemists and biologists in many countries.

As a result of these researches, it has come to be generally accepted that practically all cultivated plants, with the possible exception of the legumes, absorb nitrogen in a combined form and preferably as nitrates. Without tracing the chronological development of these investigations, it is sufficient to say that to-day it passes current and is generally taught in agricultural texts that whatever be the form of combined nitrogen applied to the soil, bacterial changes result in its transformation into nitrates before it can be absorbed by plants. Nitrogenous fertilizers are said to be valuable in proportion to the rate at which they become nitrified. Thus it is common to refer to the nitrogen of hoof-and-horn meal as having less agricultural value than that of dried blood or ammonium sulphate, and even these latter substances are sometimes spoken of as slow acting, by which it is meant that nitrification must take place before the higher plants can utilize their nitrogen. The prevailing idea is traceable to the fact that nitrates are considered to be immediately available, whereas other forms including ammonium salts are not. This view receives support from the fact that all forms of nitrogen in the soil tend to become converted into nitrates under conditions favorable to plant growth, and in addition that the direct application of nitrate gives indications of its immediate absorption, while other forms do not usually act so readily.

While it is true that the application of nitrates usually results in greater economy and stimulates plant growth to a greater degree than ammonium salts, it does not necessarily follow from this that the former can be the more easily transformed into proteids, nor that nitrates occurring naturally in soils are really more easily assimilated than the naturally occurring ammonium compounds, for on the one hand it is not impossible that the basic ion combined with the nitrate exerts a stimulating effect, while on the other, the acid radicle of the ammonium salt may be injurious. This view appears the more tenable when it is considered that most cultivated soils have a decided tendency to become acid. Furthermore, nitrates are very soluble, and not being fixed to any great extent, become diffused throughout the soil, whereas ammonium salts are fixed and held more firmly and therefore, do not circulate as freely in the soil moisture. Consequently, it is reasonable to suppose that nitrates come in contact more freely with the active root hairs of plants.

In the process of nitrification ammonia represents one stage in the oxidation of organic nitrogen, and if conditions are not favorable for complete oxidation, the process may end at this point. In arable soils it is not infrequent to find rather large quantities of ammonia. Fraps,[1] for instance, found that the ammonia content of some Texas soils exceeded the nitrates, and that the application of certain organic nitrogenous fertilizers resulted in the accumulation in the soil of greater quantities of ammonia than of nitrates. This author refers to active nitrogen as including both nitrates and ammonia, and points out that in considering the available nitrogen of soils, greater attention should be given to ammonia.

Russell and Hutchinson[2] have recently shown that the increased fertility of soils following partial sterilization is due to accelerated ammonification, rather than the formation of nitrates, which again points to the importance of ammonia in plant nutrition.

Notwithstanding the prevalance of the idea that nitrates are necessary for plant growth, experiments are not wanting to show that other forms can function in normal plant nutrition. During the period of 10 years following 1884 Pitsch[3] conducted experiments with various plants under conditions that exclude nitrification, and found that ammonium salts were directly assimilated, but he also found that the yields were greater from the use of nitrates, especially during the early growth of the plants. Müntz[4] in 1889, and Griffiths[5] about the same time succeeded in growing beans, maize, barley, and hemp with ammonium salts as the only source of nitrogen. Similar experiments have been performed by a number of other investigators, but concordant results have not always been obtained. Recently Hutchinson and Miller[6] conducted a series of experiments with wheat

[1] Texas Sta. Bul. 106.
[2] Jour. Agr. Sci., 3 (1909), No. 2, pp. 111–114.
[3] Landw. Vers. Stat., 34 (1887), pp. 217–258; 42 (1893), pp. 1–95.
[4] Compt. Rend. Acad. Sci. [Paris], 109 (1889), pp. 646–648.
[5] Chem. News, 64 (1891), p. 147.
[6] Jour. Agr. Sci., 3 (1909), No. 2, pp. 179–194. These authors also give a very complete summary of the work done in this connection.

and peas in water and sand cultures under sterile conditions, and in practically every instance as satisfactory results were obtained from the use of ammonium sulphate as from nitrate of soda.

From the results of these and other experiments it may be concluded that some plants can utilize ammonium nitrogen equally as well as nitrate nitrogen, while others prefer nitrates, but it is uncertain that ammonium salts can ever produce better results than nitrates.

CONDITIONS IN RICE SOILS.

The process of nitrification is essentially one of oxidation. The bacteria affecting the chemical changes in this process require a free circulation of air in order to best accomplish these oxidations. In the absence of free circulating air nitrification proceeds at best at a very slow rate and probably ceases altogether. In water-logged or saturated soils natural aeration is reduced to a minimum. Rice is cultivated in just such soils. The land used in rice culture is usually puddled before planting in order to reduce the loss of irrigation water through natural drainage and seepage, and during the main growing period of the crop the soil is kept completely submerged. In other words, rice is grown in standing water and consequently in a saturated soil. It is reasonable, therefore, to suppose that under such conditions nitrification proceeds at a slow rate, if at all. In fact it is more likely that denitrification takes place, for in addition to being submerged, rice soils frequently contain large quantities of organic matter which is conducive to a reduction and probably to denitrification. Hence it is not without interest to inquire into the question of the absorption of nitrogen by rice.

NITROGEN EXPERIMENTS WITH RICE, HISTORICAL.

Kellner [1] in 1882 found that swamp rice in pots during its early development made better growth with ammonium salts than with nitrates, but during later growth nitrates proved the more effective. A combination of the two forms of nitrogen seemed best adapted to this culture. The total yield from the use of nitrates alone considerably exceeded that obtained from the use of ammonium nitrogen, whereas a combination of the two forms of nitrogen produced the greatest yield. Later the same author showed that the formation of nitrates in paddy soils of Japan [2] takes place very slowly, while ammonia is formed in rather large quantities.

In a bulletin on rice culture in Louisiana, Dr. Stubbs [3] in 1900 made the following statement: "All cultivated crops utilize nitrogen in the form of nitrates," and further on he says, "recent investigations at this station show that rice gets its nitrogen as nitrates."

[1] Landw. Vers. Stat., 30 (1884), pp. 18-41.
[2] Col. Agr., Tokyo Imp. Univ., 1 (1891), No. 9, pp. 1-25.
[3] Louisiana Sta. Bul. 61, pp. 396, 397.

In 1904 [1] the same author, in association with Dodson and Brown in discussing some sand cultures in which different forms of nitrogen were used, says: "The pot containing ammonia made quite vigorous growth, indicating the utilization of nitrogen in this form."

In 1905 Nagaoka [2] conducted a large number of pot experiments with wet-land rice by means of which he was able to compare the effects produced by the use of different nitrates, ammonium sulphate, etc. In some of these experiments he found that nitrates produced practically no effect, while in others a considerable increase was noticeable. Ammonium sulphate, however, produced noteworthy effects in every instance. This author also conducted similar experiments with dry-land and swamp rice, each being grown with and without irrigation, and again ammonium sulphate was superior to nitrates, although the effects of nitrate was relatively greater in the dry-land culture than under irrigation. For use in swamp-rice culture Nagaoka concluded that the value of ammonium sulphate and nitrates stand in the ratio of 100 to 40.

In 1906 Fraps [3] published a bulletin dealing with rice soils in Texas, in which it is shown that nitrification takes places very slowly in submerged rice soils, whereas ammonium formation proceeds more rapidly. He also states that rice probably absorbs a considerable portion of its nitrogen from ammonium compounds, since during the main growing period the soil contains much larger quantities of ammoniacal nitrogen than of nitrates.

In 1907 Daikuhara and Imaseki [4] found from pot experiments that for irrigated rice ammonium sulphate was much more effective than either nitrate of soda or a combination of the two forms. The value of the nitrate was also found to be greatly reduced in the presence of organic matter. When the rice was grown as a dry-land crop, however, nitrate of soda and ammonium sulphate proved to be of about equal value.

Experiments already reported from this station [5] also indicate that ammonium sulphate is a more effective carrier of nitrogen in rice culture than nitrate of soda, although concordant results have not always been obtained. From the foregoing it is apparent, therefore, that the question of the form in which nitrogen is absorbed by rice is far from being settled. It is important that we have more definite knowledge concerning this question, for such knowledge will not only enable the farmer to better select his fertilizer, but also may suggest the advisability of a modification of the system now employed in rice culture, especially in Hawaii and on the mainland. Among the Chinese rice growers in Hawaii it is quite common to use cane fer-

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[1] Louisiana Sta. Bul. 77, pp. 392, 393.

[2] Bul. Col. Agr., Tokyo Imp. Univ., 6 (1904), pp. 285–334.

[3] Texas Sta. Bul. 82.

[4] Bul. Imp. Cent. Agr. Expt. Sta. Japan, 1 (1907), No. 2, pp. 7–36.

[5] Hawaii Sta. Rpts. 1907 and 1908.

tilizers, such as is applied on the cane lands near by, and which often contain nitrogen in two or more forms. Furthermore, the scientific phase of this question is of no little importance. Consequently an investigation of the question is not without interest.

FIELD TRIALS.

Accordingly this station for the past two years has conducted fertilizer experiments with rice. Field experiments under practical conditions have been made at the rice trial grounds on a uniform piece of land, which proved to be well adapted to experiments of this nature. The soil is deficient in nitrogen without at the same time requiring other fertilizers. In fact, the application of various nonnitrogenous substances has in practically every instance failed to show any influence on the crop; while marked influences have consistently followed the application of certain nitrogen fertilizers. The plats used in these experiments were separated by low dikes constructed of puddled soil and rendered practically impervious to the passage of water, in order to prevent interchange of fertilizing substances. The soil was prepared just as is done in field practice. In Hawaii rice seedlings from 20 to 30 days old are transplanted by hand from seed beds to the field. The soil after being thoroughly prepared is flooded and completely submerged before transplanting the seedlings, and the irrigation is usually continued throughout the main growing period. Two crops of rice are grown on the same land each year.

To one plat ammonium sulphate was applied, to another nitrate of soda, a third plat received ammonium sulphate at intervals of ten days in six equal applications, and finally one plat was treated with nitrate of soda which was also applied in six equal applications at intervals of ten days. Each of these plats received the same amount of nitrogen, viz, 70 pounds per acre. Applications in other experiments near by indicated this to be about the maximum amount that could be profitably used on this soil.

The following table will give the yield in pounds per acre of straw and paddy obtained from three successive harvests:

Yields from field experiments.

Nitrogen applied.	Fall crop, 1909.			Spring crop, 1910.			Fall crop, 1910.		
	Straw.	Paddy.	Total.	Straw.	Paddy.	Total.	Straw.	Paddy.	Total.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Ammonium sulphate (applied before planting)	3,168	4,603	7,771	3,316	3,564	6,880	2,920	4,010	6,930
Nitrate of soda (applied before planting)	1,881	2,475	4,356	2,029	2,128	4,157	2,227	3,312	5,539
Ammonium sulphate (applied in six applications)	2,475	3,465	5,940	2,772	3,078	5,850	2,722	3,762	6,484
Nitrate of soda (applied in six applications)	2,277	2,623	4,900	1,633	2,079	3,712	1,831	2,427	4,258
Check				1,930	2,178	4,108	2,145	2,762	4,907

From these data it is apparent that the application of nitrate of soda produced only slight effects on the yields either when applied in one application before transplanting or when applied at intervals during the growth of the crop. Ammonium sulphate, on the other hand, brought about considerable increase in yield, although the single application proved the more beneficial.

Rice soils in common with many other soils throughout the islands are extremely porous and naturally drain away large quantities of water. As previously mentioned, it is common among the rice growers to puddle the soil in order to reduce drainage and seepage, but in spite of every precaution considerable water passes through the soil. If the inflow be shut off the submerging water will pass below the surface in a few hours, thus indicating that rather large volumes of water drain away in this manner. Ammonium sulphate, however, is fixed by the soil to a much greater extent than nitrate of soda and the latter would consequently suffer greater loss by leaching than the former. It may be that in case of the application before planting the nitrate was largely leached below the zone of root penetration before the seedlings began active growth. Even in the case of the repeated applications it is possible that serious loss was brought about in this way. It is improbable, however, that the inefficiency of the nitrate applications was brought about entirely in this manner, for the yields from the single application were greater two times out of three than the yields from the repeated applications. It is also of interest to note that the yields from the single application of ammonium sulphate were in every instance greater than from the repeated applications.

POT EXPERIMENTS.

In addition to losses through leaching, other factors demand consideration. It is important to know not only whether nitrates are leached out of reach of the roots, but also whether denitrification may not play a prominent part. As previously pointed out, the conditions in rice soils are such as naturally suggest denitrification and consequently loss of nitrogen. The reduction of nitrogen compounds in soils is brought about by bacteria, of which there are a number of species. One class of these converts nitrates into nitrites, another reduces nitrites to free nitrogen, while still another class has the power of converting inorganic forms into insoluble albuminoids. The conditions under which each of these classes of bacteria is most active are the absence of free oxygen and the presence of an abundant source of energy, such as carbohydrates. In submerged rice soils each of these conditions is fulfilled in a large measure.

With a view of throwing more definite light on these questions, a considerable quantity of soil from the rice field was taken to the laboratory for use in pot experiments. In the field the irrigation water is cut off and the soil allowed to dry for a week or 10 days before harvest. Immediately after harvest the land is plowed and left fallow until the time of preparation for another crop. In this way the soil dries out and becomes quite thoroughly aerated. The soil used in the pot experiments was taken from the field after having been aerated for two months. By analysis it was found to contain 0.189 per cent total nitrogen, with 55 parts per million of nitrates, 10.8 parts per million of ammonia, and a trace of nitrites.

The pots employed in this work were of a Japanese design and made of glazed porcelain provided with an aperture at the bottom by means of which drainage can be effected if desired. In these experiments the apertures were closed so as to prevent leaching. Each pot was filled by placing a layer of gravel in the bottom, on top of which were placed about 2 inches of coarse volcanic sand and then 4 kilograms of well-prepared soil. Each pot received the same quantity of a basic fertilizer composed of sulphate of potash and superphosphate. The soil was neutral and in addition was not deficient in lime.

The nitrogenous fertilizers used include ammonium sulphate, sodium nitrate, calcium nitrate, magnesium nitrate, and soy-bean cake, applied in series of three pots each, and in quantities so as to provide 0.6 gram of nitrogen per pot. The entire amounts of these substances, except in Series VI and VII, were thoroughly mixed with the dry soil, after which each pot was irrigated with nitrogen-free water in sufficient quantities to provide a column of about 2 inches above the surface of the soil. Five uniform seedlings of a Japanese variety were transplanted to each pot on the following day, April 15. On each succeeding day water was again added to replace that lost by evaporation and transpiration. The pots in Series III received a second application on May 28, thus providing this series with double the amount of nitrogen supplied to any other. The nitrogen in Series VI and VII was divided into four equal parts and applied at intervals of 10 days, the first application being made before planting.

At intervals during the growth of the rice, nitrates, nitrites, and ammonia were determined in samples of the soil and water from each pot. These tests showed that nitrites were formed in all the pots within from 5 to 10 days after having been irrigated, and were developed in considerably greatest amounts in the pots to which nitrate had been added. At the end of 20 days only a faint reaction for nitrite was obtained in any case, except where additional application of nitrate had been made. In no instance did nitrites accumulate to an extent greater than two parts per million of the irrigating water. A slight increase in nitrites followed the application of ammonium sulphate and a still greater amount was developed where soy-bean cake was used, but uniformly the greatest accumulation of nitrites was found in the pots to which nitrates had been applied. In Series VI and VII nitrites usually disappeared within from 5 to 10 days following the nitrogen applications. The amounts of nitrate and ammonia present in the pots are shown in the following table:

Parts per million of nitric acid and ammonia in pots.

Series.	Nitrogen applied.	Apr. 28.		May 6.		May 16.	
		NO_3.	NH_3.	NO_3.	NH_3.	NO_3.	NH_3.
I	Calcium nitrate	11.3	18.0	3.6	18.5	(1)	(1)
II	Magnesium nitrate	9.0	19.2	7.2	18.0	7.0	18.0
III	Sodium nitrate	9.0	16.8	5.9	20.4	7.2	18.0
IV	Ammonium sulphate	9.0	76.8	7.2	85.0	7.2	58.9
V	Soy-bean cake	18.0	64.8	5.9	59.3	14.4	63.4
VI	Sodium nitrate (in four applications)	72.0	(1)	18.0	20.4	36.0	18.2
VII	Ammonium sulphate (in four applications)	18.2	(1)	9.0	36.2	9.0	45.3
VIII	Check	18.2	14.4	5.9	18.2	9.0	13.6

[1] Not determined.

A study of this table reveals some interesting facts. It should be kept in mind that the soil before having been irrigated contained 55 parts of nitrate per million and 10.8 parts of ammonia. Thirteen days thereafter nitrates were present to the extent of less than one-third of the former amount, while the ammonia content had become practically doubled. At the end of 10 days later the nitrate content had become reduced to a low minimum, whereas the ammonia was being maintained. It is surprising to note that the nitrates disappeared so soon after having been applied. In no instance was there a maintenance of the nitrate content, except in the case of the repeated applications. Many tests not recorded in the table, showed that nitrates disappeared in this soil within five days from the time of application. It is of interest in this connection that the application of ammonium sulphate or soy-bean cake resulted in considerably increasing the ammonia in the soil, and that a slight increase in ammonia followed the application of nitrates.

In order better to compare the growth of the rice in these pots, measurements of the height of the plants at different periods of growth were made. These measurements are recorded in the following table:

Average height in inches of rice in pots at different intervals.

Series.	Nitrogen applied.	May 11.	May 28.	May 31.	June 6.	July 16.
I	Calcium nitrate	18.5	31	32.5	33.5	41
II	Magnesium nitrate	19.0	30	30.5	32.5	41
III	Sodium nitrate [1]	19.0	30	31.5	33.5	44
IV	Ammonium sulphate	24.0	36	39.0	42.0	48
V	Soy-bean cake	21.0	33	35.0	36.0	46
VI	Sodium nitrate (in four applications)	19.0	31	32.0	36.0	46
VII	Ammonium sulphate (in four applications)	21.0	34	37.0	39.0	38
VIII	Check	20.0	30	31.0	32.0	40

[1] This series received an additional application May 28.

The effects of the ammonium sulphate were noticeable within one week from the time of transplanting and became more and more apparent throughout the main growing period. Soy-bean cake produced results intermediate between the unfertilized and the ammonium sulphate series. The increase in height, however, does not fairly represent the full effects of the treatment, for the rice that was fertilized with ammonium sulphate stooled, or tillered, to a much greater extent than when it was fertilized with nitrates or unfertilized. The average number of the fruiting stems produced from the use of ammonium sulphate was six for each seedling transplanted, while the unfertilized and nitrate series each had an average of three. Nitrates appeared to exert no influence on the rice, neither in the size and number of the fruiting stems nor in its color or appearance, until the crop had passed through a considerable period of its growth. Near the time of heading nitrate seemed to exert a stimulating effect and was most pronounced in Series III and VI, to which additional applications were made.

The rice was cut on July 17 and after having become thoroughly air dried was weighed and subjected to analysis for nitrogen. The results are recorded in the following table:

Average yields and nitrogen absorbed per pot.

Series.	Nitrogen applied.	Straw.	Paddy.	Total.	Nitrogen in—		
					Straw.	Paddy.	Total.
		Grams.	*Grams.*	*Grams.*	*Per ct.*	*Per ct.*	*Grams.*
I	Calcium nitrate	35	26	61	0.361	0.862	0.3504
II	Magnesium nitrate	28	22	50	.398	(1)	....
III	Sodium nitrate	37	39	76	.428	.947	.5276
IV	Ammonium sulphate	72	46	118	.388	.960	.7309
V	Soy-bean cake	56	41	97	.420	.960	.6288
VI	Sodium nitrate (in four applications)	38	33	71	.388	(1)	....
VII	Ammonium sulphate (in four applications)	61	44	105	.404	(1)	....
VIII	Check	27	20	47	.408	(1)	....

[1] Samples destroyed by mice before being analyzed.

The above table shows that markedly increased growth was produced from the use of ammonium sulphate or soy-bean cake, while only a slight increase followed the use of calcium and magnesium nitrates. Series III, it should be remembered, received an additional application of sodium nitrate on May 28, which seemed to bring about some increase in growth; likewise the repeated applications in Series VI also produced noticeable effects. The percentage of nitrogen in the straw and grain was in no instance greatly affected by the fertilizer, and from the amount of nitrogen absorbed by the total plant it is apparent that greater assimilation of nitrogen followed the use of ammonium sulphate or soy-bean cake than the use of nitrates.

By again referring to the table (p. 12), it will be seen that the application of nitrates did not permanently increase the amount of nitrate in the soil. Usually no increase was detected later than five days after the time of making the application, but on the other hand, an increase in the ammonia content of the soil followed the application of nitrate. From these facts it seems reasonable to suppose that at least a part of the influence brought by the application of nitrates may be traceable to the fact that they gave rise to an increase in ammonia. This seems the more reasonable when we consider that nitrates were not developed from the use of ammonium sulphate, but a permanent increase in the recoverable ammonia was brought about in this way, and at the same time increased growth was produced. In addition, we find by comparing the height of the rice in Series IV and VII on the one hand and the ammonia present on the other that growth was proportional to the amount of ammonia present.[1]

EXPERIMENTS IN ERLENMEYER FLASKS.

With the view of determining whether the reduction in the amount of nitrates present in the original soil and the disappearance of nitrates added to the pots were due to denitrification or to the nitrates having been absorbed by the rice, additional experiments were made in Erlenmeyer flasks. On April 19, 100 grams of soil with 100 cubic centimeters of distilled water were added to each flask. Ammonium sulphate, nitrate of soda, and soy-bean cake were then added in a series of three flasks each. After thoroughly mixing the flasks were placed in a dark room and tested from time to time for nitrites, nitrates, and ammonia. In each flask nitrites were developed to a slight extent and were found present to the greatest extent in the flasks to which nitrate of soda was added. In no instance did the nitrite exceed more than two parts per million of the original soil. The following table shows the nitrates and ammonia in these flasks at three different times during the first month:

Parts per million of nitric acid and ammonia in flasks.

Nitrogen applied.	Apr. 28.	May 5.		May 19.	
	NO_3	NO_3	NH_3	NO_3	NH_3
Sodium nitrate	675.0	540.0	23.8	270.0	17.0
Ammonium sulphate	11.0	10.8	102.0	8.1	91.8
Soy-bean cake	6.7	17.9	81.6	5.6	56.0
Check	11.0	8.1	27.2	5.4	27.2

[1] Previous experiments have shown that rice absorbs a large part of its nitrogen during early growth. Consequently greater yields may be expected where an abundant source of available nitrogen is present. See Hawaii Sta. Bul. 21.

These data show that the disappearance of nitrate is due to denitrification and indicates that such change probably accounts for the loss of nitrates in the previous pot experiments. The flasks to which nitrate was added rapidly lost nitrate, so that within one month over one-half of that added had either become converted into insoluble albuminoids or lost as free nitrogen.

From the foregoing experiments it is evident that nitrate is not a suitable form of nitrogen for paddy soils. The nitrates that accumulate during the period between crops of rice while the soil is dry and aerated do not to any considerable extent function in the nutrition of rice because they are soon converted into lower forms and probably lost as gaseous nitrogen, while the amount of ammonia in the soil considerably increases after being irrigated and can be permanently increased by the application of ammonium sulphate or organic nitrogen. With such increase, increased growth of rice follows. On the other hand, only a small increase in growth was produced by supplying the crop with nitrates, not even under conditions that prevented the loss of nitrogen through drainage. In practice organic or ammonia forms of nitrogen should be employed.

EXPERIMENTS WITH SAND CULTURES.

From the scientific standpoint it is of interest to carry these experiments a step further. In the previous experiments both nitrates and ammonia were available to the growing plant. While it is true that nitrates exist in Hawaiian rice soils to no such extent as ammonia, it is not certain from the foregoing experiments that the small amount of nitrates present did not function advantageously to the rice, for nitrate was found in the rice plants at different periods in their growth and was most abundant in the plants fertilized with nitrates. The question in this connection arises, Can ammonia nitrogen fully supply all the nitrogen requirements of rice, and how does this plant behave with nitrates as its only source of nitrogen? The following experiments are of interest in this connection:

Thoroughly leached silica sand free from nitrogen was sterilized by baking over a free flame for several hours and then used in pot experiments. In addition to a general nutritive solution,[1] different forms of nitrogen were applied to different pots. The seedlings were transplanted and treated in every way as the pot experiments already described. Two series were grown, a dry-land and a wet-land series. While sterile solutions and sand were used at the beginning, the facilities at hand did not make it possible to grow the rice under sterile conditions throughout. Consequently it is not impossible that bacterial changes brought about some transformation in the nitrogen

[1] The cultural solutions were prepared according to MacDougal. Text-book of Plant Physiology, New York and London, 1901, pp. 225.

applied. With a view of having a check on such changes it was planned to determine the nitrates, nitrites, and ammonia in these cultures at intervals during the growth, but stress of other work prevented the making of any of these except the determination of nitrites. From some preliminary experiments it is found to be imperative to know the content of nitrites in sand cultures of this sort. The sand used in these experiments was found to contain large quantities of denitrifying bacteria and several methods of sterilization were employed before a thoroughly satisfactory one was found. Unless all the denitrifying bacteria were killed it was found that either nitrates or ammonia became rapidly converted into nitrites, and that in the presence of excessive amounts of nitrites the rice turned yellow, made poor growth, and usually died. Numerous experiments proved that in a concentration of five or more parts of nitrite per million rice becomes seriously injured, and if such concentration is maintained for any considerable length of time the plant will die.

In order, therefore, to know definitely the amount of nitrites present, each culture solution was tested daily, and only such as were found to contain small amounts or no nitrites are recorded. Chemically pure compounds were used in making the culture solutions. In the wet-land series the cultures to which nitrate of soda was added failed to bring about any growth of rice whatever. Repeated trials were made and in every instance the seedling stood for some days, turned yellow, and died. In the trials with other nitrates the seedlings also became yellow, but finally overcame this and developed into fairly normal plants. In each of these instances, however, nitrates seemed to be unable to properly nourish the young seedlings. It was about a month after transplanting before the rice that was fertilized with nitrates made effective growth. In the cultures containing ammonium nitrogen, however, except in the case of ammonium nitrate, the seedlings made vigorous growth from the first. The rice in these was of a deep green color and in every way normal in appearance. The use of ammonium nitrate, however, was not attended with such favorable growth. With this compound the seedlings turned yellow and appeared to be stunted at first, but fairly normal development was made later and, as will be seen from the table, greater yields were obtained than from the use of nitrates alone.

The dry-land series acted in most ways similar to that of the wet-land series, except that nitrates seemed to bring about normal growth earlier than in the wet-land series. The average of the air-dried weights and nitrogen absorbed per pot under the different treatments will be given in the following table:

Yields and nitrogen absorbed in sand culture.

WET-LAND SERIES.

Nitrogen applied.	Straw.	Paddy.	Total.	Nitrogen in—		
				Straw.	Paddy.	Total.
	Grams.	*Grams.*	*Grams.*	*Per ct.*	*Per ct.*	*Grams.*
Calcium nitrate	7.3	3.7	11.0	0.320	0.826	0.0539
Magnesium nitrate	13.3	6.0	19.3	.372	.800	.0975
Ammonium nitrate	15.5	9.0	24.5	.344	.854	.1302
Ammonium phosphate	38.0	16.0	54.0	.329	.980	.2814
Ammonium sulphate [1]	64.0	32.0	96.0	.388	1.028	.5772

DRY-LAND SERIES.

Sodium nitrate	5.6	1.6	7.2	.644	1.276	.0559
Magnesium nitrate	6.3	2.7	9.0	.574	1.248	.0708
Ammonium nitrate	10.0	3.0	13.0	.718	1.416	.1142
Ammonium sulphate	13.0	4.0	17.0	.824	1.366	.1617

[1] The greater growth obtained from the use of ammonium sulphate than from ammonium phosphate is believed to be due mainly to the fact that the ammonium sulphate series was grown in pots of about twice the capacity of those used in the other series. In each instance the cultural solutions were of equal concentration.

DISCUSSION.

The question of why nitrates are not as effective in the nutrition of rice as ammonia has been the subject of some speculation. In 1898, Suzuki [1] concluded from his experiments on the utilization of nitrogen by phanerogams that the presence of certain quantities of sugar is necessary for the transformation of nitrates into asparagin, which he holds is a necessary step in the formation of proteids. On the basis of this investigation Nagaoka [2] concluded that the failure of nitrates in submerged rice culture may in part be due to the accumulation of insufficient quantities of sugar in the leaves. Later Daikuhara and Imaseki [3] pointed out that dry-land rice which responded to the use of nitrate contained no larger quantities of sugar than were found in wet-land rice, and therefore the failure of nitrates could not be attributed in this instance to the lack of sugars in the plant. These investigators attribute the unfavorable action of nitrates in paddy soils to the loss of nitrogen by denitrification, the formation of poisonous nitrites and loss of nitrates through leaching.

In the foregoing experiments it has been shown that serious loss of available nitrogen may follow the use of nitrates in rice soils and also that poisonous nitrites are formed. When the accumulation of nitrites reaches a concentration of about five parts per million the rice turns yellow and may be seriously injured. On the other hand, when denitrification is prevented and no nitrites are formed, young rice seedlings are still unable properly to utilize nitrates. The appearance of the plants indicates a physiological disturbance. They turn

[1] Bul. Col. Agr., Tokyo Imp. Univ., 3 (1898), pp. 488–507. [2] Loc. cit. [3] Loc. cit.

yellow and remain standing for several weeks where nitrates furnish the only source of nitrogen. The percentage of sugar in the leaves, as pointed out above, does not offer sufficient explanation of this phenomenon, for the leaves of young rice seedlings contain rather large percentages of sugar; in fact, similar quantities to that found in the leaves of most cereals.

The conversion of nitrates into proteids is essentially a reduction process. Nitrates as such do not occur to any considerable extent in plants and that which is present in most instances may be looked upon as being unassimilated. Likewise, proteids, derived from whatever source, do not contain nitrogen as an immediate derivative of nitrate, but are more properly looked upon as being made up of the ammonia derivatives. From this point of view it seems that ammonia ought to be more easily assimilated by plants than nitrates, and in fact there are certain evidences that give support to this view. It has been observed by investigators that while ammonia as such does not occur in plants to any considerable extent, their nitrogen content may be considerably greater if ammonium salts are used as fertilizers. Russell and Hutchinson [1] recently found this to be true in their experiments, and the analytical results in previous pages indicate the same.

It seems reasonable, therefore, that while the solubility and free circulation of nitrate in the soil moisture may bring it into more intimate contact with the absorbing surfaces of plants (thus making possible its greater absorption than is ordinarily effected by ammonia), the actual assimilation of nitrogen may be easily accomplished from the ammonia form. In the instance of paddy rice the experiments already described indicate this point. The taking up of nitrates through osmosis can be effected so far as known equally as readily as ammonium salts, but in some way rice appears not to have sufficient power of converting nitrates into proteids, but is abundantly able to transform ammonium salts into these substances.

It has been known for some time that extract from living plants [2] have the power of reducing nitrates into nitrites. This change has been attributed to enzymotic action, and while it may not be necessary in the process of catabolism for nitrates to be changed into nitrites, it is not unlikely that the whole series of changes which nitrogen undergoes in the construction of proteids is in a large measure brought about by enzymotic agents. Natural selection, breeding, and environment are known to have a great influence on plants. May it not be that rice, which having grown for centuries under conditions that largely exclude the formation of nitrates, has in a large measure lost the power of reducing nitrates? Further experiments will be conducted to determine the correctness of this view.

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[1] Loc. cit. Similar results were also obtained by Hutchinson and Miller. Loc. cit.
[2] See Kastle and Elvove. Amer. Chem. Jour., 31 (1904), pp. 606-641.

SUMMARY.

Experiments reported in this bulletin show that in field trials the application of ammonium sulphate produced considerable increases in the yields of straw and grain of rice, while nitrate of soda was ineffective. The application of ammonium sulphate before planting gave greater yields than were produced by applications at intervals during the growth of the crop. Nitrate of soda produced little or no effect, however applied.

In pot experiments with the use of soil the application of nitrates was without effect until near the heading period of the rice. Only small increases in yields were brought about by nitrates when applied before planting the rice. A somewhat greater yield resulted from the use of nitrates applied at intervals in the growth of the crop. Soy-bean cake as an organic form of nitrogen produced considerable increase in the growth, while ammonium sulphate was the most effective of all forms applied. Ammonium sulphate not only increased the height of the rice, but also brought about greater tillering than nitrates, thus insuring a large number of fruiting stems in a given area.

Denitrification was found to take place in paddy soils, thus resulting in the formation of nitrites and possibly bringing about loss of nitrogen as the free gas. Ammonia developed to a considerable extent during the time of irrigation, while the nitrates originally in the soil soon became reduced to a low minimum.

The application of nitrates gave rise to a slight increase in the ammonia of the soil. The addition of soy-bean cake resulted in considerably increased ammonia formation, and still greater quantities of recoverable ammonia occurred where ammonium sulphate was applied. Measureable increases in the ammonia content of the soil were found at the end of one month's growth of the rice, and at this time the ammonia content was still considerably greater in the pots to which ammonium sulphate or organic nitrogen had been applied. Differences of the same order but of unequal magnitude were found in the soil from the field plat experiments two months after the applications were made.

Experiments in flasks prove that denitrification is sufficiently active to account for the loss of nitrates found in pot experiments, and that ammonification takes place to a considerable extent in submerged rice soil.

Sand cultures resulted in showing that, if rice has access to nitrates as the only source of combined nitrogen, unhealthy and stunted growth follows, while on the other hand ammonium salts brought about vigorous growth and in every way normal-appearing plants.

In practice the use of ammonium salts or organic nitrogen and not nitrates are recommended for rice culture.

The more nitrates there are in rice soils the greater will be the quantity of nitrites formed, and if nitrites accumulate to any considerable extent injury to rice will result.

The failure of rice to properly assimilate nitrates can not be adequately explained on the basis of insufficient sugar content in the leaves, but may be due to a lack of nitrate-reducing enzyms, which, having long ceased to function through nonuse, are no longer developed in sufficient quantities to enable the rice plant to fully satisfy its nitrogen requirements.

Credit is due and thanks are here extended to Mr. F. G. Krauss for cooperation in this work and to Mr. William T. McGeorge for the determination of the nitrogen in the paddy and straw.

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