THE ROLE OF THE SOIL'S EXCESS NITROGEN IN THE BRUZONE OF THE RICE

By

R. VÁMOS

Institute for Plant Physiology of Szeged, (Hungary)
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The correlation between alkalinization, process of the formation of sodium carbonate therein and the circulation of nitrogen had been established by SZABÓ (12) almost hundred years ago.

Regarding this disease of the rice investigators as well as growers fully agree that its appearance is aided by the excess nitrogen of the soil, mainly in limeless alkaline soils and meadow clay. The correlation between the disease and the rich *N*-contents of the soil has been demonstrated by FRANK (2) and PRETTENHOFFER et al. (5), SOMORJAI and JÁRÁNYI (11) in our country. FÜLEKY and NAGYMIHÁLY (3, 4) have drawn identical conclusion. FRANK's (2) investigations show that the nitrogen available in the soil of sound (uninjured) rice-fields amounts to 2,0—4,0 mg, while that in the soil of injured rice is higher, on an average, ranges from 4,0 to 8,0 mg. Significant damage due to the disease occurs as a rule in fields of lush crop where the plants owing to the excess nitrogen contents are dark green.

As my examinations suggest that the appearence of the disease is to be attributed to the sulphides resulting from reductive processes connected with the alkalinization, i. e. to the effect of H_2S, the next step was to approach the problem of the correlation between the process leading to the bruzone and the unilateral effect of the nitrogen.

Experimental

The results of the examinations show the total and the possibly available nitrogen contents with reference to fields liable to some diseases and to those with ripened crop:

Ripened			Injured		
Soil sample	Available N mg/100 g	Total N %	Soil sample	Available N mg/100 g	Total N %
Kopáncs No 1	2,02	0,150	Kopáncs No 10	4,05	—
No 2	1,90	0,122	No 12	6,11	0,288
No 3	1,27	0,210	No 13	3,40	0,258
No 4	3,06	0,138	No 14	1,65	0,144
No 5	1,78	0,082	No 15	3,98	0,298
			Tiszasüly	—	0,311
				—	0,320

It is clearly seen that the total N-contents is considerably higher in the fields liable to the disease than in the sound ones.

Saturating the experimental soils with water and keeping them in the thermostat at 25° C for 3—4 weeks the amount of the available nitrogen is increasing. Using the so-called »ripening« method, the amount of the available *N* is frequently increased several times to that originally found in the soil.

The table below shows the available *N*-contents of some soils (original and after ripening): (1954—1955).

No	Soil sample from	State of plants	Available N-contens mg/100 g	
			in original soil	after ripening
1	Kopáncs (Ökröstó)	ripened	0,83	1,14
2	„	„	1,20	1,47
3	Kopáncs (Palé)	diseased	3,10	3,78
4	„	„	2,88	5,79
5	„	„	3,66	7,55
6	Karcag	ripened	3,07	4,08
7	Szeged	„	1,11	1,78

The increase of the organic *N*-content is especially marked where the water owing to the uneven surface can not be completely drained off. In such puddles or »plashes« the reductive processes, due to the organic matter of microbiological origin, start earlier than in the higher situated areas of the field. Soils from the deeper situated parts of some fields have also been examined regarding the total *N*. The quantity of the total *N* found in these places exceeds even the data mentioned above.

Soil sample from	Total N %
Vajhát	0,410
	0,399
Hódmezővásárhely	0,407
	0,512
Tiszasüly	0,301
	0,410

The decomposition of the organic nitrogen compounds is favourable for the formation of the anaerobic conditions. The ammonia is accumulated in the developing, airless environment. The accumulation of the ammonia during the flooding has been studied also by SIK (9, 10) and he stated that the

NH_3 in the fields liable to the disease *(Tiszasüly, Szarvas-Káka, Templomzug* etc.) ranges from 14,5 to 39,5 mg/100 g while in not-liable ones from 1,5 to 2,0 m/100 g. SIK found, further, that the quantity of the sulphide is parallel to the ammonia. So it seemed of importance to study the microbiological changes of the nitrogen compounds in order to continue successfully these investigations.

It is known that accumulation of ammonia occurs only when the carbon and nitrogen in the soil are in adequate proportion. According to examinations the bacteria need 2 g nitrogen to decompose 100 g orginic matter. As the C-contents in 100 g organic matter is 50 g the accumulation of the ammonia can only be expected when the $C:N$ ratio is less than 25.

The table shows the ratio of the organic matter and of the total nitrogen from soils of injured fields and where the rice ripened:

Soil sample from	Organic matter %	Carbon %	Total N %	C : N ratio
		Soils with injured rice		
Kopáncs	3,41	1,7	0,20	8,7
	3,1	1,55	0,165	9,4
	3,85	1,92	0,253	7,6
	3,46	1,73	0,209	8,3
Tiszasüly	3,58	1,78	0,208	8,55
	3,05	1,52	0,214	7,10
		Soils with ripened rice		
Vajhát	2,83	1,44	0,14	10,3
Baktó	2,7	1,35	0,13	10,4
	2,83	1,41	0,14	10,0
Hódmezővásárhely	2,49	1,24	0,086	14,4
Sik's data	2,30	1,15	0,048	23,9

The average ratio of $C:N$ of the fields included in this table has been calculated from the data available in the literature. Results are:

	Injured fields			Ripened		
	C %	N %	C : N	C %	N %	C : N
C : N ratio	1,70	0,208	8,17	1,31	0,108	12,1

As it is seen the $C:N$ ratio is less in the fields liable to the disease than in the soil of fields with ripened crop. The difference, however, is not always significant and so it may be stated that often slight differences, associated with other factors, may suffice to intensity the demaging effect of the disease. In fact the signs of the disease are shown, in the overwhelming majority of the cases, on the harvested plants, even on those classified »resistant kind«. The growers, however, consider only such fields injured with bruzone where a more or less poor crop is to be expected.

The course of the disease, influenced by several factors, throws some light upon how the crop, due to slight differences, can greatly differ in different parts of two adjacent fields cultivated at the same time under identical circumstances and sown the same seeds; in one field there is no yield at all, while in the other quite an average crop can be harvested (in the state owned farm at *Kopáncs*, 1955).

Particular study has been made to determine the proteolytic bacterium flora in the soils of rice fields. The agar plate method was used pouring broth on the plates. After inoculation with adequate dilutions and incubation, colonies of mainly ammonifying bacteria developed on the plates: especially *Proteus vulgaris, Bac. mycoides, Bac. subtilis* etc. In soils less liable to the bruzone, colonies of other than ammonifying bacteria develop; the latter, however, prevail, especially the *Proteus vulgaris*. The anaerobic bacteria were isolated in BURRY-tubes. In this way the *Proteus vulgaris* of the facultative anaerobic species and of the obligatory species the *Clostridium sporogenes* appeared most frequently. The latter decomposes also the carbohydrates and decomposing the proteins produces considerable hydrogen sulphide.

Nitrate-reduction. The formation of the anaerobic conditions will arrest not alone the nitrification but it will aid also the reduction of the nitrates. 80—85% of the determined and undetermined bacteria, isolated only in pure culture, can reduce the nitrates. As the majority of the bacteria possesses the reducing capacity practically it means that almost 100% of the ammonifying bacteria present can reduce the nitrates.

Examinations made on the dry material of the plant proved both the excess nitrogen in the soils and the accumulation of the ammonia.

Investigations resulted that the total nitrogen, in terms of percentage of the dry material (leaf-stem-panicle) has always been higher in the plants injured than in the uninjured ones. This is shown in the table below. The results are the means of triple parallel examinations.

The bruzone disease of the rice may be affected by the excess nitrogen in many ways. Physiologically e. g. it lengthens the vegetation time and by delaying the ripening the of plant, still in development, may be unfavourably affected by the fall of temperature in August. Moreover the ammonia-N is the source of nitrogen for the bacteria reducing the sulphate.

According to recent investigations anaerobic proteolytic bacteria themselves are able to reduce the sulphates. Hence the strong proteolysis aids directy as well as indirectly the reduction of the sulphate. Besides the nitrogen, i. e. accumulating ammonia has an other role that is suggestive of utilizing the ammonia known is Solvay's soda-manufacture.

The ammonia produced by the ammonification is water-soluble in any quantity and forms ammonium-hydrocarbonate with the carbonic acid deriving from decomposition of organic matter and from the atmosphere.

$$NH_4OH + H_2CO_3 = NH_4HCO_3 + H_2O$$

In my opinion one of the effects of the ammonia is that by binding the carbonic acid prevents it from being released from the water. The ammonium-

Ripened plants	Total N in % of dry material
1. Baktó	0,72
2. Vajhát	0,68
	0,89
	0,91
3. Kopáncs	0,77
4. Fülöpszállás	0,94
	0,99
	0,90
Diseased plants	
1. Besenyszög	1,33
	1,11
2. Kopáncs	1,284
	1,28
	1,21
	1,17
3. Vajhát	1,48
	1,64

hydrovarbonate thus produced, together with the successively produced sulphides, may react.

$$NaSH + NH_4HCO_3 = NaHCO_3 + NH_4SH$$

The water-soluble CO_2 as a stronger acid may release the hydrogen sulphide from the ammoniumhydrosulphide and so a part of the hydrogen sulphide may get into the air.

After a complete drainage of the rice fields most of the substances solved disappear. No significant accumulation of salts occurs.

Correlation between the pH-value and nitrogen content

Investigating the pH-value and the nitrogen content in the soils of the rice-fields a correlation could be noted. Namely, at draining the pH-value in the water of the nitrogen-fertilized fields ranged from 8,5 to 8,8 while the pH-value of the controls, i. e. in the water of not-fertilized fields never rose above 7, 6. (1955.).

The pH-value of the flood-water regulates the ratio of the dissociation of the hydrogen sulphide. The higher pH-value renders possible the absorption of the -*SH*-ions resulting the appearance of the bruzone on the nodes, leaf and panicle. The intake of hydrosulphide produces sterility in the blooming plants (14).

Experiments in fields

In order to demonstrate that the excess nitrogen contributes to the appearance of the disease the following experiments have been made.

It is commonly known that the nitrogen fertilizer favours the appearance of the disease, especially in cool weather. This statement can be explained on the basis of the observations mentioned above. Using ammonium nitrate the ammonium is bound by the colloids in the clay, the nitrate is reduced and the nitrogen is lost into the air. In case of ammon-sulphate the sulphate is reduced whereas the ammonium is bound. In both instances the quantity of the ammonium is increased. The product of the reduction of the ammonsulphate is at the same time an immediate causative factor of the disease and so it was not surprising that the disease appeared first and most intensively in all my experiments where an excess ammonsulphate was used to bring about the disease artificially.

1. Experiments carried out in small plots (100—200 sq. fathoms) produced the disease using ammonium sulphate 80 kg/per acre in 3 consecutive instances. The disease appeared on 2. Aug. 1955 in *Tiszasüly* as well as in *Kopáncs*. In the rice-fields of the state farm at *Tiszasüly* (about 8000 acres), however, no disease could be observed at this time. In the state farm at *Kopáncs* the disease appeared regularly at the same time year by year.

2. The disease could be repeatedly produced in small plots using ammonium nitrate 80 kg/acre in liable soils. Its appearence was defihitely observed on 6. Aug.

3. Succesful attempts were made to bring about the disease locally in uninjured field without its appearance elsewhere. In this experiment pieces of 50 dkg of ammonium-nitrate had been placed in certain spots of the field on 4. Aug. the clorotical leaves became dark green within a range of 2 m. of the nitrate and in these spots the disease appeared, though to a somewhat milder extent, especially on the leaves. The seeds in the upper part of the ear became brownish, however, without damaging the crop.

4. Efforts to produce the disease in the same way but with 25 dkg pieces were also successful. Here is commonly experienced that the disease usually appears where previously fertilizers were stored or dunghill stood.

PRETTENHOFFER (7, 8) obtained identical results carrying out his experiments in rice-fields in 1954 and 1955 in order to study the effect of the two nitrogen fertilizers, i. e. of ammonium sulphate and of the calcium nitrate in soils liable to the brown disease. The quantity of the fertilizers were increased. According to the results of the two year experiments (one in 1954, eight in 1955) the ammonium sulphate produced the disease and the occurrence was proportionate to the quantity increased. At the same time, however, only a slight effect could be observed with the calcium-nitrate. On the basis of his experiments he suggests to avoid the ammonium sulphate fertilizer in soils liable to the bruzone.

In 1956 there was an unusually hot Summer in Hungary. Neither low temperature nor cool periods interferred the normal biological processes of the rice. Under such circumstances the rice could overcome the toxic effect of the sulphides (methyl sulphide and hydrogen sulphide) formed in the soil. In this year the abundant nitrogen fertilisation (80 kg ammonium sulphate per hectar) produced no disease, on the contrary well over an average yield has been harvested in these fields.

The low temperature is a generaly known stimulatory factor of the bruzone. As Hungary is one of the countries situated most northernly where rice is produced, ist is obvious that the disease appear more powerfully here than in South.

The examinations and experiments described above naturally do not mean the definite determination of the role played by the nitrogen. The results of further investigations may give possibilities to learn more about these extremely important processes in soil biology.

Summary

In the growers' opinion, supported by experiments made in laboratories, the total-N is high in the soils of rice fields liable to the bruzone disease.

The reasons, why the excess nitrogen of the soil is instrumental in the process leading to the disease, so far stated, are:

1. The excess nitrogen prolongs the developmental cycle of the plants, enhances the energy of the vegetative processes, the formation of the seeds is delayed and the plant my be exposed to the injurious sequels due to the low temperature in August.

2. NH_3-nitrogén the result of proteolysis, is the N-source of the sulphate reducing bacteria and the H_2S by binding the oxigen gives favourable conditions to the increase of these bacteria.

3. The proteolytic bacteria at the same time may reduce the nitrates inhibitory to the bacteria reducing the sulphate.

4. The powerful proteolysis contributes to the formation of the anaerobic conditions, as well as to that of the reductive processes.

5. Certain proteolytic bacteria possess also the capacity to reduce the sulphates.

6. The rise of pH-value in the water of fields rich in nitrogen renders possible the intake and the absorption of the hydrosulphide-ions.

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