SOIL FERTILITY AND AMINO ACID SYNTHESIS BY PLANTS.[1]

By WM. A. ALBRECHT, WM. G. BLUE *and* V. L. SHELDON,[2] *University of Missouri, U.S.A.*

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Soil science has the responsibility not only of testing the soil for ample supplies of all essential elements, but also of determining the nutritional services of the soil-borne ions as (a criteria) of the soil treatments it undertakes. Of the many synthetic plant products of nutritional value, protein presents itself for first consideration in that it (or its constituents) is essential for growth and reproduction. Previous studies indicate that the common Kjeldahl procedure for measuring total nitrogen is inadequate for determining protein in forages. (Sheldon, Blue and Albrecht, 1948). General diversity of amino acids in legume hays according to soil types and treatments, including the trace elements, promoted further study of the function of the soil-borne plant nutrients as they function in the biosynthesis of these vital substances. Blue, Sheldon and Albrecht (1948), and Tisdale *et al.* (1950) showed that the methionine and cystine contents of alfalfa were influenced by the supply of the sulfate ion. The extension of this work to a survey of wider ranges of soil conditions and the resulting concentrations of the amino acids in more forages should establish the basic nature of these findings. One may, however, anticipate that the supply of any element serving in a catalytic or structural capacity, either as an activator or in a prosthetic group of the enzymes involved in the biosynthetic conversion of the carbohydrates, may produce marked variation in the resulting amino acid array within the plant leaf. The studies here reported, were carried out on well-defined media as well as on natural soils in order to measure some amino acid output by plants in relation to some elements of soil fertility.

In addition to studying the influence of the soil-borne elements upon the amino acid composition of certain forages, it seemed important to establish the amino acid values for these commonly used in pasture systems. Alfalfa and soybeans, were grown in the greenhouse using both the sand-solution cultures, and the colloidal clay techniques. Korean lespedeza, barley, Sudan grass, oats, bluegrass, alfalfa, wheat, rye, sweet clover, ladino clover, and red clover were grown under field conditions with a wide assortment of soil types representing a wide range of soil fertility. The range in the fertility level of these soils was further increased by applying fertilizers.

Using a mixture of Gila and Putnam clays, soybeans were grown by Dr. Donald Brown of Fayetteville, Arkansas, to which Rio Grande irrigation water or a nutrient solution was added. The exchange complex of these cultures was previously reported (Brown and Albrecht, 1947). Soybeans were also grown by Hall C. Turley on dialized colloidal clay and silt mineral mixtures of Wyomingite, and glauconitic dolomite.

Alfalfa, lespedeza, barley, oats, rye, Sudan grass, sweet and ladino clovers were grown on soils of the Prairie Region of South-west Missouri. Alfalfa and barley samples were taken at random (*a*) where there was no soil treatment, (*b*) where trace elements or magnesium sulphate or (*c*) where a mixture of these was offered.

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[1] Contribution from Department of Soils, Missouri Agriculture Experiment Station, Columbia, Missouri.

[2] Professor of Soils; Research Assistant, now Assistant Biochemist, University of Florida; and Graduate Assistant, now Instructor in Soils, respectively.

Other soil treatments, such as, limestone, superphosphate, and rock phosphate were also used. A bluegrass pasture on the Putnam silt loam was treated with limestone, superphosphate, and these plus potash.

Nine of the ten amino acids, essential for the white rat were determined by using the microbiological method of Stokes and Gunness (1945, 1950). The total nitrogen was determined by the Kjeldahl-Gunning-Arnold method as modified by Murneek and Heinze (1937). Van Slyke determinations of nitrogen were made by means of the macro-type deaminization apparatus in the usual manner.

Sulphur and the Synthesis of Methionine.

Because much of the sulphur in the plant is thought to occur as a constituent of protein, the amount of sulphur offered to it might reflect itself in the resulting sulphur-containing amino acids, if the needs for the other elements have been met. The methionine assay of alfalfa and soybean hays suggested that the sulphur offered in the growth medium influenced the concentration of this amino acid in the forage as shown in Table 2. Maximum production of methionine occurred when the plants received 64 to 95 p.p.m. of sulphur in the solution.

Experimental Details.

The plants produced in the greenhouse were seeded in two-gallon pots containing white quartz sand. This had previously been leached with concentrated hydrochloric acid and washed repeatedly until the test for chlorides was negative. The nutrient solution (given in Table 1) was applied except where one element at a time was first omitted and then added as increments to give the desired treatment. During the growth of the plant an attempt was made to keep light, water, humidity, temperature and other environmental factors at optimum levels. The nutrients were added each week during the early stages of growth and more frequently as the plants approached maturity. Before a new supply of nutrients was added, the pots were leached with distilled water in order to keep the nutrient supply at the appropriate level. When the plants were in full bloom the above ground portions were harvested and dried in a forced-draft oven at 60° to 65° C. Using the above technique, soybeans were grown on varying levels of magnesium, manganese, boron, sulphur, and iron. Alfalfa was grown on divergent levels of manganese, boron, sulphur, iron and potassium.

Table 1.

Composition of Nutrient Solution used in Quartz Sand Cultures.

Element.	Amount in Full Nutrient (p.p.m.)
K	547·0
Ca	400·0
Mg	96·0
N	25·0
P	124·0
S	128·0
Fe	34·0
Mn	1·1
B	1·1

TABLE 2.

Methionine Concentration in Alfalfa and Soybean Hays according to the amounts of Sulphur offered in the Nutrient Solution.

Sulphur offered (p.p.m.)	Alfalfa (mg./gm.)	Soybeans (mg./gm.)
0	1·96	2·67
16	4·50	3·08
32	5·01	3·56
64	5·37	..
96	4·61	6·41
128	2·70	5·90

In a field study of a transitional soil in the South-west Prairie region of Missouri, applications of magnesium sulphate to the soil were found to increase the methionine content of the alfalfa. When barnyard manure was added to the soil along with the sulphur, even greater increases were evident, as indicated in Table 3.

TABLE 3.

Methionine Concentration (mg./gm.) in Alfalfa Hay according to Sulphur applied to the soil.

Treatment.	Methionine at different dates.		
	6/24/48	7/31/48	4/11/49
None	3·10	3·21	2·91
100 lbs./a. $MgSO_4$	3·41	3·64	5·87*

* 10 tons per acre of barnyard manure applied.

In an effort to substantiate further these general findings, flowers of sulphur, at the rate of 200 pounds per acre, were applied to an adjoining field where the phosphate requirement had previously been met by the addition of 1,000 pounds of rock phosphate per acre. This sulphur treatment almost doubled the methionine concentration in the Sudan grass while the total nitrogen increased only slightly, as shown in Table 4. Further application of sulphur in the form of magnesium sulphate depressed the methionine yield per unit weight and the nitrogen content.

TABLE 4.

Methionine Concentration in Sudan Grass According to Sulphur Applied to the Soil.

Treatment.	Total N (%)	Methionine (mg./gm.)
1. No sulphur	2·20	2·04
2. 200 lbs./a. sulphur	2·44	4·06
3. Same as 2 plus 100 lbs./a. $MgSO_4$ ..	1·96	1·53

SOIL FERTILITY AND THE SYNTHESIS OF TRYPTOPHANE.

Indol-3-acetic acid has frequently been associated with the boron supply from the soil. There was the suggestion, in the data in Table 5, of a possible relation between this element and the concentration of the indol amino acid, or tryptophane, in both alfalfa and soybean hays. Even though the enzymatic function of boron remains quite obscure, these data substantiate the conclusions of Briggs, namely, that an interruption of the immediate synthetic processes just prior to protein formation occurs when boron is in low supply.

TABLE 5.

*Tryptophane Concentrations in Alfalfa and Soybean Hays according to the amounts of Boron offered in Nutrient Solution.**

Soybean Hay.		Alfalfa Hay.	
Boron offered (p.p.m.)	Tryptophane (mg./gm.)	Boron offered (p.p.m.)	Tryptophane (mg./gm.)
0	1·38	0	1·27
0·27	1·89	0·22	1·36
1·08	3·10	0·44	2·17
..	..	1·08	2·55

* Composition of Nutrient Solution given in Table 1.

In an effort to determine the magnitude of the synthesis of tryptophane by the plants when the elements commonly supplied by the soil are in low amounts, magnesium, boron, manganese, sulphur, iron, calcium and phosphorus were withheld from the growth medium. The results of these modifications of the plant environment, recorded in Table 6, show the pronounced variation in this essential amino acid according to the corresponding variation of these elements of soil fertility.

TABLE 6.

Tryptophane Concentrations in Alfalfa, Soybean, and Redtop Hays according to the Inorganic Nutrients in the Substrates.

Plant.	Treatment.	Tryptophane (mg./gm.)
Soybeans†	Mg withheld	1·80
Soybeans†	B withheld	1·89
Soybeans†	Full Nutrient	3·10
Soybeans†	Mn withheld	1·93
Soybeans†	B ,,	0·57
Soybeans†	Fe ,,	1·76
Soybeans†	Full Nutrient	2·10
Soybeans*	Wyomingite	0·47—0·50
Soybeans*	Glauconitic Dolomite	0·66—0·90
Soybeans*	Ex Mg on clay	1·06—1·09
Alfalfa†	Mn withheld	1·47
Alfalfa†	B ,,	1·15
Alfalfa†	S ,,	2·64
Alfalfa†	Fe ,,	1·74
Alfalfa†	Full Nutrient	2·80
Redtop*	High P, High Ca	2·65
Redtop*	High P, Med. Ca	2·21
Redtop*	High P, Low Ca	2·09
Redtop*	Med. P, High Ca	2·38
Redtop*	Med. P, Med. Ca	1·88
Redtop*	Med. P, Low Ca	1·38

† Grown on Nutrient Solution given in Table 1.
* Grown on Colloidal Clay.

In studies of some magnesium minerals from which this element could be weathered at varying rates by the acid colloidal clay, the tryptophane content was lowered according to the magnesium made available from that reaction. The concentration of this amino acid in the case where Wyomingite was offered was only one-half the value of that where the magnesium was readily exchangeable on the clay. Magnesium from the dolomite was more available and expressed itself through increased synthesis of tryptophane by the plant. These data carry the suggestion that either less carbohydrate was built by photo-synthesis or less was respired to yield a critical linkage in the tryptophane molecule. The enzymatic formation of the indol ring could require magnesium as the activating cation at some stage of its synthesis.

In a general fertility study of the calcium-rich Gila clay, with its high exchange capacity and free carbonates, marked increases in propein synthesis would not be expected when additional calcium, magnesium, potassium and sodium are offered to the plant. Yet, more arginine, threonine and valine were produced when the Rio Grande irrigation water containing these elements was applied.

TABLE 7.

Tryptophane, Valine and Basic Amino Acids in Soybean Hays according to Addition of Nitrogen and Phosphorus to Natural Gila Clay. (mg./gm.).

Medium.	Arginine.	Histidine.	Lysine.	Valine.	Threonine.	Sum of Basic Amino Acids.*
Gila Clay ..	5·7	3·7	11·8	8·4	4·3	21·2
Gila Clay plus irrigation water ..	6·5	4·1	11·3	9·1	4·9	21·9
Gila Clay plus solution containing N and P	8·4	4·7	12·2	10·9	7·8	24·3

* The sum of the histidine, arginine and lysine.

That even more of those amino acids were built where a nutrient solution containing nitrogen and phosphorus was offered the soybean plants is indicated by the evidence in Table 7. Since nitrogen is a constituent of these molecules, and since phosphorus is required to convert the simple carbohydrates into their organic acid precursors, an increase in any or all of the amino acids could be expected. There were general increases in arginine, threonine, and valine. In view of the leadership rôle commonly assigned to the basic amino acids, namely, arginine, histidine, and lysine, in the theories of protein structure, one might expect these compounds to be synthesized in a constant ratio in any given plant species. Only arginine showed a striking increase.

GENERAL VARIATIONS IN NITROGEN FRACTIONS WITHIN SPECIES.

Considerable controversy has existed as to whether the amino acid contents of a plant, i.e., the free amino acids plus those hydrolyzed from the proteins, may be modified by alterations of the plant environment and particularly whether or not these concentrations vary proportionally with the total nitrogen. Maximal and minimal values of the nitrogen fractions and their ratios for different species have been assembled in Table 8. Those acids having simpler structures gave ratios corresponding to those of the percentages of nitrogen. In the amino acids histidine, tryptophane, arginine and methionine, the ratios were considerably different from

TABLE 8.

Maximal and Minimal Concentrations of the Amino Acids arranged by Plant Species with the ratios of the former to the latter values.

Plant species.	Maximum or Minimum.	% N.	Valine.	Leucine.	Isoleucine.	Threonine.	Tryptophane	Histidine.	Lysine.	Arginine.	Methionine.
Alfalfa	Maximal	5·53	27·8	18·7	39·4	13·5	3·91	9·9	25·8	13·8	6·51
	Minimal	1·93	9·4	6·5	11·0	4·8	0·84	2·3	7·0	2·2	trace
	Max./Min.	2·86	2·96	2·88	3·58	2·82	4·65	4·3	3·69	6·28	..
Soy-beans	Maximal	5·04	20·3	26·6	39·1	16·0	3·35	11·3	39·5	10·4	5·90
	Minimal	1·11	8·1	9·2	10·9	3·5	0·32	3·5	10·2	2·4	1·83
	Max./Min.	4·54	2·51	2·89	3·59	4·57	10·48	3·23	3·88	4·33	3·22
Korean Lespe-deza	Maximal	3·60	20·5	19·3	28·2	11·8	3·19	8·6	28·8	9·4	3·31
	Minimal	1·98	10·2	8·3	16·7	5·3	0·79	2·1	9·9	1·6	0·90
	Max./Min.	1·82	2·07	2·33	1·69	2·23	4·04	4·1	2·91	5·88	3·68
Sudan grass	Maximal	2·78	11·9	11·9	..	7·5	2·42	3·1	..	3·7	4·06
	Minimal	1·71	8·6	8·6	..	5·5	0·88	2·3	..	1·9	1·53
	Max./Min.	1·62	1·43	1·39	..	1·36	2·75	1·35	..	1·95	2·64
Barley	Maximal	5·96	18·9	20·3	14·4	12·1	3·58	10·2	22·0	5·1	5·40
	Minimal	4·24	12·2	12·3	8·9	6·8	1·50	5·5	9·8	2·7	2·64
	Max./Min.	1·41	1·55	1·65	1·62	1·78	2·39	1·85	2·24	1·89	2·05
Blue-grass	Maximal	4·05	19·8	19·6	36·2	16·4	2·41	8·1	28·3	7·6	4·69
	Minimal	1·36	4·7	5·6	12·3	3·6	0·76	2·8	6·2	3·4	1·26
	Max./Min.	2·98	4·22	3·50	2·94	4·56	3·17	2·89	4·57	2·23	3·72

the ratio of the total nitrogen. In view of their more complex linkages, their synthesis by modification of the sugar chain should be more involved.

Legumes are generally considered to contain small quantities of methionine, yet large quantities were found in some of the legume forages. The ratios of the maximal to minimal concentrations of methionine were, in general, larger for the legumes than for the non-legumes. This points directly to the fallacy of referring to a species of plant as being low in some particular constituent unless plants grown under a wide variety of soil conditions are examined.

DISCUSSION.

In the physiology of an organism, we have regularly recognized the necessity of considering the inorganic environment in attempts to explain performances by it. The measurement of the delivery by the soil of the inorganic elements constituting the ash of the plant material, as the soil's only contribution to the quality of the crop, has been an unfortunate criterion of the importance of the soil factor of environment. This little appreciation of the soil has persisted even when the ecological array of plant species suggests that plants must be of diverse organic quality as they evolve on the various and diverse soils. Efforts to evaluate the protein status of a crop by the common total nitrogen techniques fail to present the true picture of forage protein. Since the food value of the forage depends upon the complete list of the amino acids and not on the total nitrogen *per se*, we need to adopt organic assays as newer criteria by which to measure the service of the soil in plant production for good nutrition.

That the quality of forage protein can be altered by any factor of environment is of utmost significance. Whether the protoplasmic protein or merely the free amino acids in the plant sap is modified is of little significance from a standpoint of nutritional values.

Plants stand in the cycle of nutrition as enzymatic factories wherein sufficient quantities of the essential amino acids are made according to the soil. Diagnostic techniques using any biological assay of soil fertility must ultimately measure molecules both organic and inorganic, as the reactants in biosynthesis. The *status quo* of the complete biotic pyramid could thus be viewed as merely the union of colloidal particles having enzymatic specificity to yield the numerous protoplasmic components according to the fertility of the soil on which the entire pyramid rests.

To sum up, we now have suggestive evidence from the relative effects of phosphorus, sulphur, nitrogen, boron, magnesium, iron, and manganese on the amino acid array in the organic composition of alfalfa, soybeans, bluegrass, lespedeza, redtop and Sudan grass hays, that the soil serves in the control of the nutritional quality of forages. This is substantiated particularly by considerations of the elements sulphur and boron in the formation of methionine and tryptophane respectively. A single species of plant may vary widely with respect to its amino acid content. These conclusions are in no way contradictory to the general experience of plant physiologists who have worked with the processes of biosynthesis. Indeed, the fairly close relation between our experiments and the findings of the enzymologists is entirely consistent with the present view.

SUMMARY.

A microbiological assay for nine of the ten amino acids required by the white rat in alfalfa (*Medicago sativa*), soybeans (*Soja max*), redtop (*Agrostic stolonifers major*), and Sudan grass (*Sorghum vulgare* var Sudanese) showed that these organic substances of particular food values varied widely according to the inorganic composition of the substrate upon which the plants were grown. The synthesis of methionine was inhibited when sulphur was withheld from the solution or was in low supply in the soil. Application of flowers of sulphur doubled the methionine concentration in the Sudan grass.

The formation tryptophane was found to be proportional to the available boron when this anion was the limiting element in the culture solution. Tryptophane synthesis was decreased when magnesium, boron, manganese and iron were withheld from nutrient solutions offered alfalfa and soybeans.

LITERATURE CITED.

Blue, W. G., Sheldon, V. L., and Albrecht, W. A. (1948). Microbiological Assays for their Amino Acids According to Soil Types and Treatments including Trace Elements. *Proc. Soil Sci. Soc. Amer.*, **13**, 318–322.

Brown, D. A., and Albrecht, Wm. A. (1947). Plant Nutrition and Hydrogen Ion; VI. Calcium Carbonate, a Disturbing Fertility Factor in Soils. *Proc. Soil Sci. Soc. Amer.*, **12**, 342–347.

Murneek, A. E., and Heinze, P. H. (1937). Speed and Accuracy in the Determination of Total Nitrogen. *Mo. Coll. Agri. Expt. Sta. Bull.*, **261**,

Sheldon, V. L., Blue, W. G., and Albrecht, W. A. (1948). Diversity of Amino Acids in Legumes According to the Soil Fertility. *Sci.*, **108**, 426–428.

Stokes, J. L., and Gunness, M. J. (1950). Microbiological Methods for the Determination of the Amino Acids. *J. Biol. Chem.*, **160**, 35–49.

——— ———(1945). Microbiological Methods for the Determination of the Amino Acids. *Ibid.*, **157**, 651–659.

Tisdale, S. L., Davis, R. L., Kingsley, A. F., and Mertz, E. T. (1950). Methionine and Cystine Content of Two Strains of Alfalfa as Influenced by Different Concentrations of the Sulphate Ion. *Amer. J. Agron.*, **42**, 221–225.

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