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The Composition of Soybean Plants at Various Growth Stages as Related to Their Rate of Decomposition and Use as Green Manure

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The Composition of Soybean Plants at Various Growth Stages as Related to Their Rate of Decomposition and Use as Green Manure

LLOYD M. TURK*

Abstract.—A study was made of the solubility of soybean plant material, at different stages of maturity, as it influences decomposition rate in the soil. The rate of decomposition of the soybean plant parts was studied by various methods, including the evolution of carbon dioxide, ammonia production, nitrate accumulation, and humus production. A study was made of the carbon and nitrogen changes of the soybean during the growth season and also the carbon and nitrogen changes which occurred in the soil during a definite decomposition period. The more soluble or easily decomposable constituents decreased as the plants became older, while the more insoluble material increased in amounts with maturity. There was a widening of the carbon-nitrogen ratio with advancing maturity. In general the rate of decomposition of the plant parts was in the following decreasing order: Tops, complete plants, and roots. The water-soluble fraction was found to be largely responsible for the initial decomposition behavior. A decrease in soil nitrates occurred following the incorporation of soybean root material into the soil, which may explain the detrimental effect frequently noticed on the wheat crop following soybeans, but the top and root material greatly increased the soil nitrates. There was a distinct narrowing of the carbon-nitrogen ratio in the soil during a thirty-day decomposition period. It was found that microbiological digestion and nitrogen "tie-up" depend mainly on the energy material supplied in comparison to the amount of nitrogen.

INTRODUCTION

The usefulness of a plant for green manure depends primarily on its chemical composition. Legumes make excellent green manure on account of their high nitrogen content, whereas straw may be detrimental to the crop following because of its low nitrogen content. Soybeans are often used as a green manure with success, for as a legume they are rich in nitrogen and if plowed under they return large amounts of nitrogen to the soil. It has been observed, however, that where the soybeans are removed from the land the wheat crop following often gives lower yields than when not preceded by this crop. The writer has observed in actual farm experience in Southwest Missouri that wheat yields are nearly always depressed following soybeans, sometimes as much as five bushels per acre, where the soybeans were removed from the land. This depressive effect is especially noted on the poorer soils where no nitrogenous fertilizer accompanies the wheat.

The composition of a green manure, as combined with that of the soil to make it a favorable microbiological medium for its decomposition and nitrogen release, is the final index of its value as a green manure.

*Submitted by the author in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the graduate school of the University of Missouri, 1931.

Since the speed and nature of the decomposition of plant residues varies with maturity^{59*} it is essential to learn more about the composition of the soybean as a possible explanation for the irregularities in its effects on succeeding crops.

The following study is an attempt to measure by chemical means the differences in organic composition of the soybean plant parts, which accompany increasing maturity, and their differences in decomposition behavior in the soil. From this study it is hoped that an explanation can be given for the depressing effect of soybeans on the crop which follows.

In the last few years the acreage of soybeans in Missouri has enormously increased. It has become a regular crop in a great many of the Missouri cropping systems. The progressive increase in soybean acreage invites a consideration of it as a green manure crop. The extensive use and general popularity of this crop prompted the work herein reported. Studies on the rate of decomposition of the soybean as related to the nitrogen of the soil seemed extremely significant both from the practical and scientific viewpoints.

REVIEW OF LITERATURE

Principles and Values of Green-Manuring Practices

Green-manuring is the practice of turning into the soil undecomposed green plant tissue. From time immemorial²⁶ the turning under of a green crop to better the condition of the soil has been a common agricultural practice. The effects claimed for green manuring are many fold²⁴. It is well known that the effectiveness of green manures may be increased by properly adjusting the green manure crop to the soil, the season, and the climate. Perhaps the most important function of a green manure crop is to increase the store of soil nitrogen and organic matter content. Hopkins quoted by Merkle³⁰ states that "it is the decay of organic matter and not the mere presence of it that gives life to the soils". The amount of organic matter found in any soil⁴² is the resultant of accumulation and of loss through decomposition, both of which factors are decidedly influenced by climatic conditions. Jenny¹⁹ points out that "it is possible to build up the nitrogen content of the soil by adding organic material in the North, because the low annual temperature would favor its preservation. In the South, however, it will be rather difficult, if not impossible, to increase permanently the nitrogen content by common green manuring practices, because the high temperature militates against nitrogen accumulation by favoring decomposition." It is next to impossible to assign a definite money

*Superscript numbers refer to citations of literature in the bibliography on pages 38 to 40 inclusive

value to organic matter because when incorporated with the soil it has several different effects, physical, chemical and biological, any one of which is of sufficient importance to justify its use. The value of organic matter must in the end be determined from the increase in crops produced.

Factors Influencing Rate of Nitrogen Liberation from Green Manures.

It has been demonstrated⁴⁶ that the rapidity and nature of decomposition of plant residues under aerobic conditions depend primarily upon the chemical composition of the particular plant materials. Rege³⁸ has suggested that two factors control the biological destruction of cellulose, namely, energy material as pentosans and an "inhibitory factor" as lignin. He believes that cellulose decomposition depends upon the pentosans-lignin ratio.

Jensen^{20,21}, Whiting⁶², Heck¹⁵ and others^{23,25} reported that the carbon-nitrogen ratio is a factor which exerts a profound influence on rate of nitrification. The ratio gives in general a fair idea of the end products which may be expected from a given material.

It is quite the general rule, as shown by Whiting⁶² and Starkey⁴⁴, for the material containing the higher nitrogen contents to be more rapid in decomposition. This is not true, however, of all materials. Some organic materials decompose easily, and others with much more difficulty irrespective of their nitrogenous contents. Waksman⁵⁹ shows that when the nitrogen content of the plant is about 1.7 per cent, it is just sufficient to cover the requirements of the microorganisms which are active in the decomposition of the plant material.

According to the experiments of Waksman and his co-workers^{59,54,51,46}, and Whiting^{62,63}, the water soluble substances are the first to be acted upon in the process of decomposition of plant residues in the soil and they determine to a large extent the initial rate of decomposition with most materials. The water-soluble nitrogen is especially an important factor.

In a study of a wide range of materials it was found by Fischer and Lieske¹², that in the decomposition of plant substances under natural conditions the process begins with the breakdown of the hydrolyzable constituents. The rapid nitrification of certain materials according to Whiting⁶² is traceable to the unstable nature of the protein and its derivatives contained in them.

A large number of investigators^{33,59,61,29,30,18,62,23,46,17,60} have found that the younger the crop when turned under the more rapidly it decomposes. With an increase in age of plants there is a decrease in the relative amounts of water-soluble substances, of the nitrogenous con

stituents, and of the minerals. There is an increase in the celluloses, hemicelluloses, and lignins.

There is almost complete agreement in the literature^{62,17,4,28,64} showing the green condition of plants and plant products to be faster in nitrate production than the dry condition. In this connection the work of Whiting and Schoonover⁶⁴ is outstanding.

According to Tenney and Waksman⁴⁶ and Anderson³ the addition of available inorganic nitrogenous salts hastens the decomposition of carbonaceous plant materials. A number of investigators^{1,2,6,9,40,14,5,31} in studying the influence of straw on the accumulation of nitrates in the soil have demonstrated a marked decrease in the nitrate content of the soil immediately following the incorporation of the material.

Gainey¹⁴ states that in Kansas, where more than 1,000,000 acres are annually grown to sorghums, and where a depression in yield of wheat following sorghum was found to be three bushels per acre compared with wheat following corn, such injury assumes considerable economic importance. Available evidence concerning the relative rates of decomposition of corn and kafir residues would indicate that there is present in kafir limited quantities of carbonaceous material somewhat more easily oxidizable by microorganisms than exist in corn. Such material being readily available as carbonaceous food tends to stimulate temporarily a more rapid development of microorganisms, probably resulting in a somewhat more rapid assimilation of soluble nitrogen.

Joshi²² found that roots and stems of cowpeas turned under decreases immediate yields of oats following. Especially, the roots gave a detrimental effect.

Wilson and Wilson⁶⁵ in studying the rate at which nitrate nitrogen disappeared and then reappeared in soil cultures, in the presence of timothy hay or clover hay, found that the decomposition of timothy residues in soil extended over a longer period than that of clover residues. Since the clover residues are more easily oxidized by the organisms, the number of organisms rises in a relatively short time to higher figures in the cultures with clover. Subsequently the counts fall with the utilization of the more readily oxidizable organic matter. There was a more rapid reappearance of nitrates in soil cultures containing clover hay.

These same investigators found⁶⁶ sorghum roots to cause a more rapid disappearance of nitrates than did corn roots. They suggested that the injurious after-effects of sorghum may be associated with the comparative ease with which its roots are oxidized in soil.

Conrad⁷ explains the sorghum injury to following crops on the assumption that the sorghum roots are relatively higher in sugars than other crops. Analyses of the roots of six varieties and of one variety of corn showed that the sorghum roots contained from about

65 per cent more sugars to over 15 times as much sugar as did the corn roots analyzed. There seems to be a direct correlation¹⁶ between the amount of cellulose decomposed and nitrogen assimilated.

The environmental conditions at which decomposition is carried out in the soil, especially aeration, moisture supply, soil reaction, temperature and fertility of soil, influence to a marked degree the rapidity of decomposition as shown by the work of Starkey^{43,44} Sievers and Holtz⁴², Neller³², Potter and Snyder^{36,37}, Waksman^{53,51,58}, Lyon and Wilson²⁷, and Gainey³. In general it may be said that increased temperature, aeration and moisture (up to certain limits) are all favorable for rapid decomposition of organic matter.

Methods of Measuring Rate of Organic Matter Decomposition.

It has been suggested by Stoklasa and Ernest according to Waksman⁵², p. 719 that the determination of carbon dioxide evolved by a soil under given conditions of moisture, temperature and time, can furnish a reliable and an accurate method for the determination of bacterial activities in the soil. Feher¹¹, Martin²⁸, Waksman⁵⁰, and Russell and Appleyard³⁹ have indicated that the liberation of carbon dioxide is a very good index for measuring the sum total of the decomposition processes.

The formation of ammonia (and nitrate) as an index of decomposition rate has been found by a number of investigators to give satisfactory results even though the method is quite indirect. Martin²⁸ points out several errors which are encountered in using this means as a measure of organic decay.

The rate of humus formation may indicate the rate of organic matter decomposition but the methods for determining humus are rather indefinite. However, it can be said that, in general, the more readily organic material is decomposed the more rapidly does humus accumulate.

The rate of decay has been measured²⁸ by studying the increased availability of plant nutrients as measured by the effect on subsequent plant growth.

A more detailed analysis of plant materials giving the proportions of the nitrogen-bearing compounds to those bearing no nitrogen will give²³ a better conception of the processes taking place in the soil when such materials are incorporated therewith. Very valuable information may be obtained by determining the disappearance or transformation of various chemical groups with the progress of decomposition.

NEED FOR SOYBEAN GREEN MANURE STUDIES

In the progress of agriculture there is noted a tendency toward a more complete and more intelligent utilization of crop residues and green manures. This tendency gives rise to many new questions dealing with the relative rates of decomposition of the different crops and the different parts of the same crop.

It has been observed by a number of farmers throughout the State that wheat following soybeans often gives lower yields than wheat following other crops. Soybeans, practically unknown in Missouri ten years ago, have increased from an acreage of less than 500 in 1920 to nearly 500,000 acres in 1930. Therefore, if the wheat crop following soybeans is cut in yield per acre even one or two bushels this becomes an extremely important problem. Soybeans are nearly always followed by wheat because of the ease in putting in the wheat crop; little preparation for the ground being necessary.

Thatcher¹⁷ reports that the yield of wheat following soybeans cut for hay decreases as the date of the harvest of the soybeans is deferred. He also found the percentage of nitrogen in the soybean roots to decrease with advancing maturity. Sears⁴¹ finds yields of wheat after soybeans cut for hay greater than after soybeans that are cut for seed. Wiancko, quoted by Deatricks⁸ states that "very early in our rotation experiments we found that it was necessary to have some readily available nitrogen in the fertilizer for wheat after soybeans in order to give it an equal start in the fall. Apparently the nitrogen in the soybean residue does not become available fast enough to do the wheat much good in the fall."

Since straw or other carbonaceous materials incorporated in the soil cause a subsequent depression in the available nitrogen content of the soil, and since the total nitrogen content of soybean roots at the time of harvest is less than one per cent, approaching that of straw, according to Erdman¹⁰, an additional source of available nitrogen will be required before it can be completely decomposed because the above figure for nitrogen falls far below 1.7 per cent demanded according to Waksman and Tenney⁵⁹ to cover the requirements of the microorganisms for active decomposition.

The fact that soybean roots were found by many analyses to be carbonaceous (low in nitrogen) led to the hypothesis that the depressive effect on wheat following soybeans may be due to the disappearing of the available nitrates as a result of the biological stimulation produced by the soybean roots and stubble left within the soil.

Due to the enormous increase in acreage of soybeans in Missouri within the last few years and the fact that soybeans are nearly always followed by wheat, this problem becomes one of very vital and practical significance.

PLAN OF STUDY

It was believed that if the soybean roots do exert a depressive effect on the nitrates in the soil it could be demonstrated in the laboratory by following the nitrate accumulation curve in a soil in which soybean root material had been incorporated. If this could be demonstrated then one would be justified in concluding that the depressive effect on wheat following the soybeans can be explained, in part at least, on the basis of the carbonaceous material left in the soil in the form of stubble and roots.

The plan of this study included the following:

1. Solubility of soybean plant material and its influences decomposition rate in soil.
 - (a) Rapidly decomposable material—that which is soluble in water, (b) Slowly decomposable material—that which is insoluble in water but soluble in dilute alkali and acid, (c) Non-decomposable or extremely slowly decomposable material—the residue of the above treatments, or that which is soluble in ether and strong acids.
2. Variations in carbon and nitrogen content of soybeans as influenced by stage of maturity.
 - (a) Relation of total carbon to total nitrogen, (b) Relation of decomposable carbon to decomposable nitrogen.
3. Ratios of energy material to soluble nitrogen of the soybean plant material as influenced by maturity.
4. Decomposition rate of soybeans as influenced by stage of maturity.
 - (a) Differences in rate of carbon dioxide evolution, (b) humus accumulations from different soybean plant materials, (c) Changes in carbon and nitrogen during decomposition, (d) Ammonia and nitrate productions following the incorporation of soybean plant material in soil in the laboratory.

PREPARATION OF MATERIALS

The soybean plants were collected during the summer of 1929 from fertile, well inoculated Shelby loam soil that had been seeded with the Morse variety of soybeans on June 6. The beans were kept well cultivated throughout the summer. Beginning July 6, when the plants were about 15 inches high, a portion was harvested and at regular weekly intervals until October 5. The harvests were divided into three groups, i.e., whole plants, roots, and tops. They were allowed to air dry, after which they were ground and preserved for later study. About

three inches of the stems were included with the roots in order to duplicate field cutting by machine as nearly as possible.

At the fourth harvest July 27, some of the plants were beginning to bloom. The plants were in full bloom August 10, which was the date of the sixth harvest. On September 7 the pods were well developed, and by October 5 the plants had reached maturity. At maturity the soybeans were nearly waist high, stood up well, contained numerous pods, and were profusely inoculated.

The summer was rather dry. There was no rain of any significance during the month of August, which caused the dropping of many leaves. During the first week in September a rainfall of two inches stimulated the vegetative growth. The moisture content of the soil during the season is given in Figure 1.

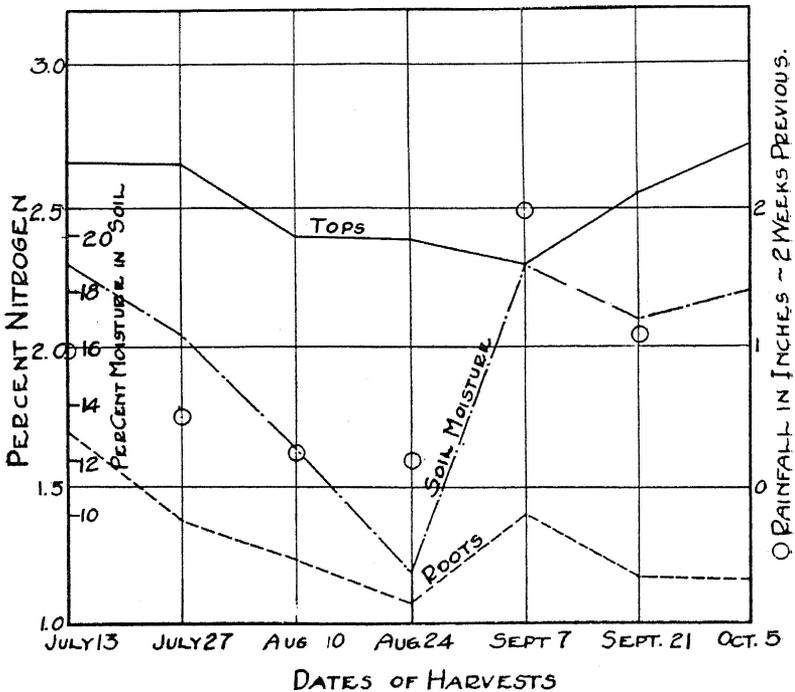


Fig. 1.—Nitrogen content of soybeans during the season as correlated with soil moisture.

EXPERIMENTAL WORK

Solubility of Soybean Plant Material at Various Growth Stages as it Influences Decomposition Rate in Soil

The following study is an attempt to measure by chemical means* the differences in organic composition of the tops and roots of the soybean plant, which accompany increasing maturity, in the belief that they may offer suggestions regarding the decomposition behavior of these plant parts in the soil.

The ease of digestion by chemical reagents was taken as a rough criterion of the readiness with which microbiological digestion, or decomposition, will take place within the soil. The study was based on the relative degree of solubility of the soybean plant material by various chemical treatments. The decomposition rate as related to chemical condition of such plant material, may be roughly divided into three divisions, as follows: (1) Rapidly decomposable material—that which is soluble in water, (2) Slowly decomposable material—that which is insoluble in water but soluble in dilute alkali and acid, (3) Non-decomposable material—the residue of the above treatments, or that which is soluble in ether and strong acids.

Analyses were made on the materials after they had been grouped according to the above treatments, or as they had arranged themselves according to the ease of chemical attack. Special attention was given to the nitrogen and carbohydrates present in each group, since they play such an important part in microbial activity.

All determinations were made on the oven-dry basis. Nitrogen determinations were made by the ordinary Kjeldahl method. The chemical treatments given the plant materials for partial digestions were those outlined by Waksman⁵⁶.

Analyses were made on soybean plant parts harvested every two weeks from July to October, as a means of determining the changes in organic composition with advancing maturity as such might indicate variation in the amounts of decomposable and non-decomposable substances.

TABLE 1.—PERCENTAGE OF NITROGEN IN THE ROOTS, TOPS, AND COMPLETE PLANT OF THE SOYBEAN AT DIFFERENT STAGES OF GROWTH DURING THE SUMMER 1929.

Material	Date of Harvest													
	July 6	July 13	July 20	July 27	Aug. 3	Aug. 10	Aug. 17	Aug. 24	Aug. 31	Sept. 7	Sept. 14	Sept. 21	Sept. 28	Oct. 5
Plants	2.81	2.68	2.52	2.37	2.24	2.19	2.22	2.21	2.13	2.28	2.26	2.24	2.26	2.39
Tops	3.03	3.01	2.68	2.66	2.65	2.40	2.43	2.37	2.22	2.29	2.39	2.54	2.76	2.72
Roots	2.11	1.69	1.43	1.37	1.49	1.23	1.14	1.06	1.31	1.39	1.28	1.15	.97	1.14

These data are assembled and correlated with the corresponding soil moisture content in Figure 1

*The data from the chemical measurements of the differences in organic composition of the soybean plant parts were gathered by Mr. W. H. Allison, and their interpretation in the following pages 11 to 18 is according to the report by W. A. Albrecht.

Table 1 shows the nitrogen content of the soybean tops and roots as influenced by the stage of maturity.

The total nitrogen in the tops fluctuated with the seasonal conditions, decreasing with the dry weather, dropping of leaves, and advancing maturity, and increasing again with the September rains which stimulated vegetative growth. This may have increased the nitrogen content. In the roots there was a continual decrease until late August and then a sudden rise in nitrogen content, followed by a decrease toward maturity. It is noticed that the nitrogen content of the roots is only about one per cent in the later growth stages. According to Waksman⁵⁹ in order to get nitrogen liberation the organic matter must contain 1.7 per cent total nitrogen. Therefore, we would expect available nitrates in the soil to be used by the organisms in the decomposition of soybean roots at the expense of the following crop.

TABLE 2.—COMPOSITION OF SOYBEANS (TOPS) ACCORDING TO CHEMICAL DIGESTION, AS RELATED TO MATURITY OF PLANTS. (EXPRESSED AS PERCENTAGE OF TOTAL WATER-FREE INITIAL SAMPLE).*

Fraction	July 13	July 27	Aug. 10	Aug. 24	Sept. 7	Sept. 21	Oct. 5
Ether Soluble	5.42	5.02	4.96	2.32	1.73	3.85	3.18
Cold Water Soluble	19.72	18.55	16.99	15.24	17.07	19.98	18.25
{ total substance	6.18	5.20	4.82	6.07	5.33	5.54	4.59
{ ash	.92	.90	.74	.91	.82	.64	.52
{ nitrogen	7.93	4.91	8.51	6.94	10.66	11.38	12.49
{ reducing sugar	5.14	6.94	5.88	2.72	2.55	3.13	4.79
Hot Water Soluble	1.09	1.70	1.33	.80	.68	.81	.76
{ total substance	3.24	.26	.26	.16	.14	.15	.18
{ ash	3.52	3.68	2.37	2.51	2.24	2.09	2.13
{ reducing sugar	33.58	32.76	33.70	35.49	36.68	36.61	44.70
Alkali Soluble	1.10	.95	1.02	.94	1.00	1.21	1.12
{ total substance	2.53	2.78	5.11	2.84	2.70	2.97	2.16
{ nitrogen	21.37	2.63	20.09	16.75	16.75	18.87	21.47
{ reducing sugar	2.47	2.64	3.13	3.19	3.59	4.43	4.67
Acid Soluble	.49	.52	.62	.64	.72	.88	.93
{ total substance	10.67	13.69	13.69	13.99	14.28	14.49	14.99
{ reducing sugar	9.74		10.66		10.99		11.94
Cellulose							
Pentosans							
Lignin (Ash and protein free)	11.88	12.91	13.76	15.79	18.91	22.51	23.03

*Data by W. H. Allison

The data of the analytical results for the digestive treatments, expressed in terms of percentage of sample, are assembled in Table 2 for the soybean tops, and in Table 3 for the roots. The ether soluble content in the roots decreases with maturity. In the tops there is also a decrease, with the lowest amount in late August and early September.

For both the cold and hot water fractions there are reported four sub-divisions, namely, the total substance soluble, the ash, the nitrogen, and the reducing sugars. The reducing sugar dissolved by hot water is that which was hydrolyzed by boiling in two per cent HCl. The cold

water solubility of both the tops and roots decreases with maturity, There is also a decrease for both the ash and the nitrogen. There is, however, a decided increase for the cold water soluble reducing sugar. This decrease in soluble nitrogen and increase in soluble sugar or carbohydrates, is significant with references to changes in the nature and rate of decomposition of the plant parts as they become more mature. It designates a widening carbon-nitrogen ratio, indicating that there would be a smaller amount of soluble nitrogen liberated from the decomposition of the mature material. This decrease in available nitrogen and increase in carbohydrates with maturity, especially in the roots, may leave such excessive amounts of readily decomposable carbohydrates and such deficient amounts of available nitrogen, that in the decomposition of the roots the soluble nitrogen from the soil is utilized and injures the succeeding crop.

TABLE 3. COMPOSITION OF SOYBEANS (ROOTS) ACCORDING TO CHEMICAL DIGESTION AS RELATED TO MATURITY OF PLANTS. (EXPRESSED AS PERCENTAGE OF TOTAL WATERFREE INITIAL SAMPLE).*

Fraction	July 13	July 27	Aug. 10	Aug. 24	Sept. 7	Sept. 21	Oct. 5
Ether Soluble	3.40	3.15	2.98	2.43	1.12	1.47	.22
Cold Water Soluble							
{ total substance	12.37	10.91	10.63	10.53	10.66	11.85	11.68
{ ash	4.34	2.41	3.68	2.91	2.96	2.90	1.79
{ nitrogen	.54	.31	.35	.32	.32	.32	.31
{ reducing sugar	3.35	3.82	3.64	4.53	8.57	8.71	8.62
Hot Water Soluble							
{ total substance	2.41	3.72	3.47	1.75	2.78	3.03	3.67
{ ash	.39	.79	1.11	.53	.64	.47	.45
{ nitrogen	.14	.13	.06	.13	.10	.13	.15
{ reducing sugar	1.57	2.87	2.54	1.35	1.95	2.19	2.54
Alkali Soluble							
{ total substance	27.18	24.69	28.36	30.92	32.09	31.80	33.40
{ nitrogen	.60	.51	.56	.52	.65	.58	.45
{ lignin (Protein free)	1.90	1.97	2.36	1.83	1.72	2.67	2.06
{ reducing sugar	12.26	14.36	15.84	17.06	23.91	26.38	29.30
Acid Soluble							
{ total substance	2.94	2.99	3.01	3.52	4.41	4.37	4.63
{ reducing sugar	.58	.59	.60	.70	.88	.91	.92
Cellulose	22.02	19.89	19.73	19.56	20.83	21.74	22.49
Pentosans	12.96		15.25		14.46		14.96
Lignin (ash and protein free)	9.12	18.32	17.39	17.41	17.38	17.77	22.99

*Data by W. H. Allison

The hot water soluble nitrogen in the roots is very low and fluctuates little through the season. The reducing sugar of this fraction from the roots increases but slightly, while that from the tops decreases slightly with maturity—the opposite of that for the cold water extraction.

The fraction removed by sodium hydroxide increases with maturity for both the tops and roots. The percentage of alkali soluble nitrogen and lignin is almost constant. The reducing sugar in this fraction of the tops remains low throughout the season, while that in the roots increases decidedly. Again we find the nitrogen in the roots remaining fairly constant while the carbohydrate content increases with maturity, agreeing with the results of Waksman and Tenney⁵⁷. This widening

of the carbon-nitrogen ratio lessens the readiness of nitrogen liberation when this difficultly soluble material is decomposed in the soil.

It is observed that a relatively small amount, only two to five per cent of the residue after alkali treatment, was soluble in sulfuric acid. The total soluble substance and the reducing sugar increased with maturity for both tops and roots. Also in this fraction, the carbohydrate increased as the plants became older.

Cellulose, determined by the method of Waksman,⁵⁶ increased in the tops with maturity, going from over 10 per cent to 15 per cent. The roots' cellulose content remains nearly constant at about 20 per cent, reaching the maximum of 22.5 per cent on October 5. Since the decomposition of cellulose requires the presence of certain minerals, especially of available nitrogen, it is interesting to note the ratio of cellulose to total nitrogen through the growth period as shown in Figure 2.

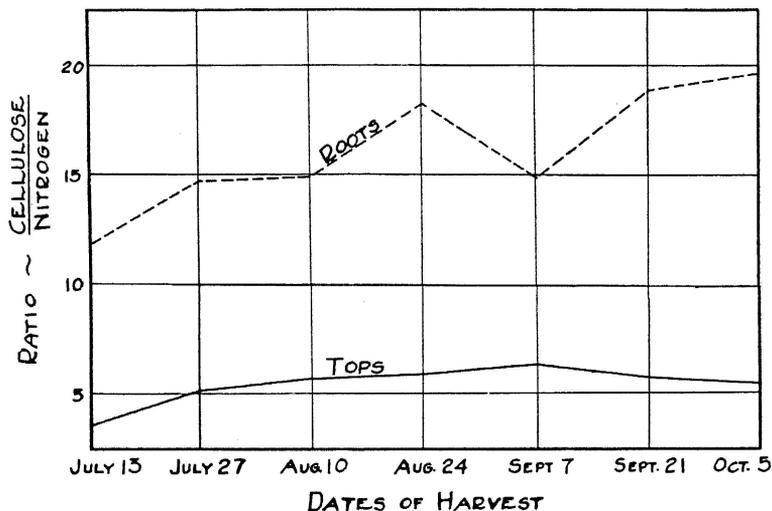


Fig. 2.—Cellulose-nitrogen ratio of soybeans during the season

This ratio was about 5:1 in the tops at the beginning, showing an increase and then a decrease with advancing maturity. The roots show a much wider ratio of 11:1 at the outset, becoming as wide as 19:1 in the late harvest. Waksman points out that a ratio of 50:1 of cellulose to available nitrogen facilitates cellulose decomposition. If this is the case then the results cited above suggest that if the total amount of nitrogen in the soybean plant material is all readily available it is sufficient for the decomposition of the cellulose. However, a large portion of the nitrogen is in very stable compounds and the ease of decomposition can not be predicted by this ratio.

Rege³⁸ has suggested that two factors control the biological destruction of cellulose, namely, energy material as pentosans and an "inhibitory factor" as lignin. The pentosans of the soybean plants were determined by the usual phloroglucide method on the harvests of four week intervals. This constituent increased gradually with maturity. The lignin determination showed that this organic substance almost doubled itself, going from 12 to more than 23 per cent in the tops, while in the roots it was 9 per cent at the first harvest, and increased to almost 18 for the rest of the dates except the last one, when it mounted to 23 per cent. Since lignin is not digested by 72 per cent H₂SO₄ and is considered decomposable in the soil only very slowly, the more mature soybean plant when plowed into the soil adds about one-fourth of its organic matter in a slowly decomposable form.

It is believed by Rege³⁸ that cellulose decomposition depends on the pentosan-lignin ratio. This, in his opinion, should be greater than 1.0:1.0 for rapid decomposition while a ratio falling below this as far as .5:1.0 signifies slow decomposition. These ratios for the soybean plant roots at all times and for the tops on all later dates fall below the figures 1.0:1.0 and would—if Rege's deductions are significant—indicate slow decomposition of their cellulose fraction in the absence of other sources of energy.

Since the nutrient elements within the lignin are poorly available on account of its lack of rapid decomposition, a determination of the lignin nitrogen was made to learn how much of this element is locked up in the inert form. This remains fairly constant throughout the growth period as shown in Table 4.

TABLE 4. COMPOSITION OF LIGNIN OF SOYBEANS WITH INCREASING MATURITY (EXPRESSED AS PERCENTAGE BASED ON LIGNIN SAMPLE)*

		July 13	July 27	Aug. 10	Aug. 24	Sept. 7	Sept. 21	Oct. 5
Ash	Tops	3.33	.62	5.01	1.49	.64	1.52	1.19
	Roots	6.71	3.67	4.39	4.53	4.87	8.32	10.63
Carbon ^a	Tops	7.48		8.67		11.91		14.50
	Roots	5.74		10.95		10.95		14.48
Nitrogen	Tops	.49	.61	.41	.45	.44	.49	.48
	Roots	.32	.42	.40	.28	.29	.44	.38
Protein ^b	Tops	3.10	3.80	2.60	2.82	2.75	3.09	2.99
	Roots	2.01	2.66	2.54	1.77	1.85	2.76	2.36
Carbon ^c	Tops	39.40	40.92 ^d	42.43	42.99 ^d	43.54	44.59 ^d	45.64
	Roots	40.16	41.65 ^d	43.14	43.42 ^d	43.71	44.15 ^d	44.58

a—Lignin x .63

b—Nitrogen x 6.25

c—Based on original water free sample of plant material.

d—By interpolation from preceding and succeeding dates.

*Data by W. H. Allison

According to Waksman and Tenney,⁵⁷ lignin contains 63 per cent carbon. Using this figure as a basis for calculation, it was found that

this undecomposable or lignin carbon increases from 7.5 in the young plant to 14.5 per cent at maturity. With the content of nitrogen in the lignin remaining constant while the carbon increases, the nitrogen-carbon ratio of the lignin becomes correspondingly wider.

Since the carbon and nitrogen in the lignin material are generally considered inert it seemed desirable to subtract the percentages of these two elements from the respective total amounts and to designate the resultant values as "decomposable carbon and nitrogen". The relationship of these values is expressed in Figure 3.

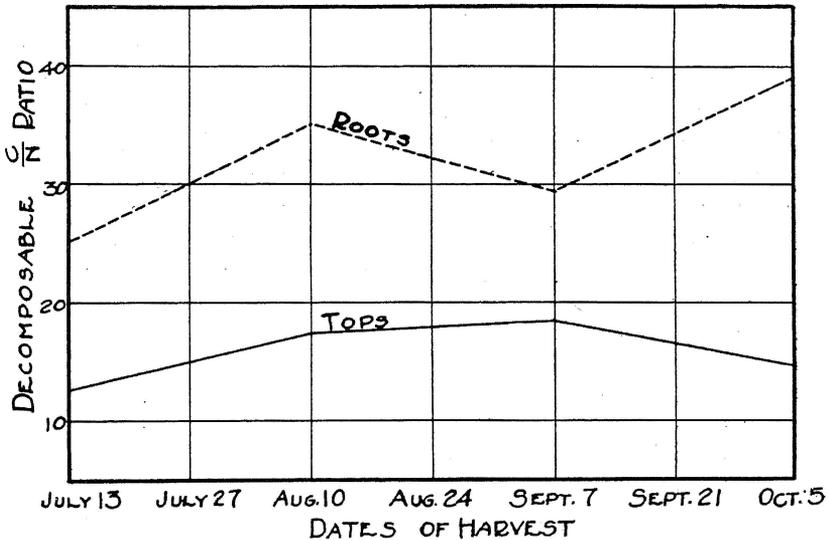


Fig. 3.—Ratios of carbon to nitrogen as considered "decomposable" for soybeans during the growth season.

The ratios for the tops became widest for the first harvest in September following the extreme drought, and then became narrower again with the fall rains. The reverse fluctuation occurred in the roots. Since the narrower carbon-nitrogen ratio favors rapid decomposition, these soybean roots with their wide ratio would require an additional source of available nitrogen for rapid and complete decomposition. This nitrogen must necessarily come from the soil and, as a result, the crop following soybeans may suffer because the decaying soybean roots are competing with it for the soil's supply of available nitrogen.

Microbiological digestion depends mainly on energy material which may be supplied in reducing sugars, cellulose, or pentosans. For the purpose of calculation we shall consider the growth material, or nitrogen, as being supplied mainly by the water soluble and alkali soluble nitrogen. Now let us note the changes in amounts of energy

material and nitrogen so supplied by the tops and roots as the plant changes with maturity. The sum of the sugars, pentosans and cellulose is taken as energy material. These data are assembled in Table 5 and Figure 4. The outstanding fact revealed by these calculations is the change in the ratio of energy material to the nitrogen in the tops and especially in the roots. In the tops this value increased from 23.6 to 34.8 during the season, while in the roots these values go from 39.6 to 85.7. For the decomposition of cellulose alone, Waksman⁵² cites the need of one part of available nitrogen for each 50 to 60 parts of cellulose. In the more mature soybean roots this ratio is wider than this even though partially stable nitrogen is included in the calculations.

Certainly this wide ratio suggests that the soybean roots supply far too great an amount of energy material in proportion to their nitrogen or growth material for rapid decomposition, and suggest that if such mature roots were turned under they would draw on the soil's supply of soluble nitrogen and endanger the succeeding crop.

TABLE 5.—READILY SOLUBLE NITROGEN AND ENERGY MATERIALS OF SOYBEANS AS RELATED TO MATURITY OF PLANTS. (EXPRESSED AS PERCENTAGE OF TOTAL WATER-FREE SAMPLE)*

	July 13	July 27	Aug. 10	Aug. 24	Sept. 7	Sept. 21	Oct. 5
TOPS							
Nitrogen	Cold water soluble	.92	.90	.74	.91	.83	.64
	Hot water soluble	.24	.27	.27	.16	.14	.15
	Alkali soluble	1.11	.95	1.02	.94	1.01	1.22
	Total readily soluble	2.27	2.12	2.03	2.01	1.98	2.01
	Balance—inert	.74	.54	.38	.35	.32	.54
Reducing sugars	33.33	31.76	31.61	26.85	30.39	33.34	37.04
Cellulose	10.67	13.69	13.69	13.99	14.28	14.49	14.99
Pentosans	9.74	10.20 ^a	10.66	10.83 ^a	10.99	11.47 ^a	11.94
Total energy materials	53.74	55.65	55.96	51.67	55.65	59.20	63.98
Ratio	$\frac{\text{Energy}}{\text{Soluble nitrogen}}$	23.6	26.2	27.5	25.6	28.1	29.4
ROOTS							
Nitrogen	Cold water soluble	.54	.31	.36	.32	.32	.32
	Hot water soluble	.15	.13	.07	.13	.10	.13
	Alkali soluble	.60	.52	.56	.52	.66	.59
	Total readily soluble	1.29	.96	.99	.97	1.08	1.04
	Balance—inert	.41	.42	.25	.10	.32	.11
Reducing sugars	17.77	21.67	22.64	23.66	35.32	38.20	41.40
Cellulose	20.02	19.89	19.73	19.56	20.83	21.74	22.49
Pentosans	12.97	13.11 ^a	13.25	13.86 ^a	14.47	14.71 ^a	14.96
Total energy materials	50.76	54.65	55.62	57.08	70.62	74.65	78.85
Ratio	$\frac{\text{Energy}}{\text{Soluble nitrogen}}$	39.6	56.5	55.7	58.8	65.3	71.5

*Data by W. H. Allison

^a — Interpolated from preceding and succeeding dates.

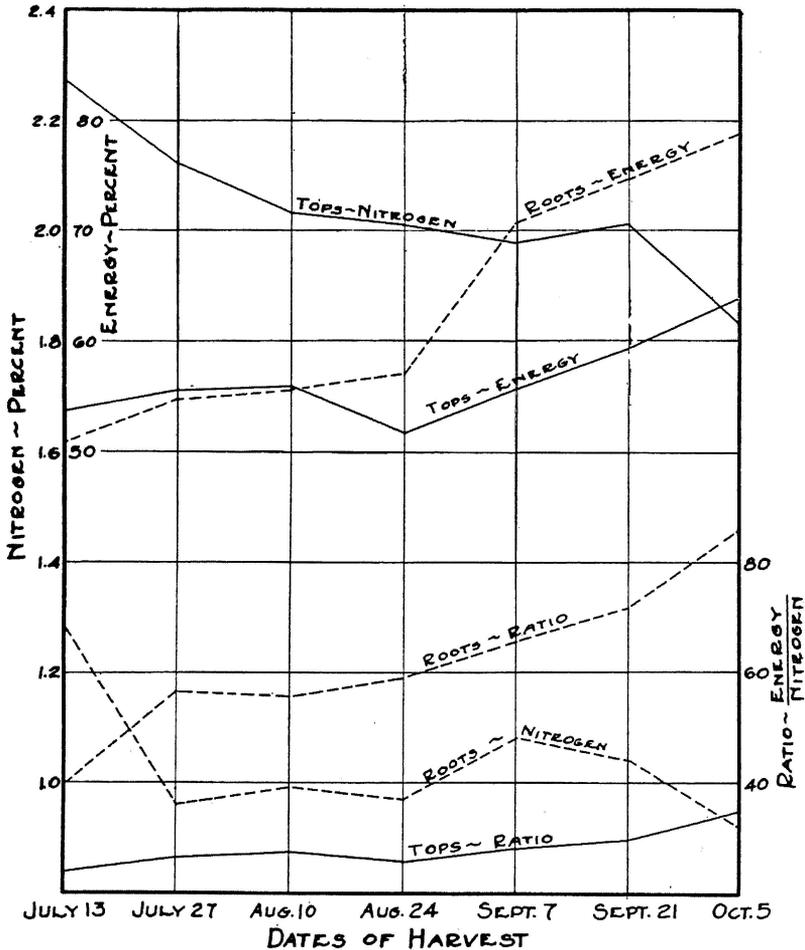


Fig. 4.—Changes in soluble nitrogen and energy materials in soybeans during the growth season.

Decomposition Rate of Soybeans as Influenced by Stage of Maturity

As a result of the chemical composition studies of the soybean plant and from the work of other investigators, one would naturally expect the soybean tops to decompose much more readily than the roots, and the more mature harvests of each plant part to decompose more slowly than the corresponding more immature harvest. One of the important characteristics of a good green manure crop is that it should decompose readily. A number of methods were used to determine the relative rates of decomposition of the soybean plant parts in the soil.

Evolution of Carbon Dioxide Following Incorporation of Soybean Plant Material in Soil.—There are many objections against employing methods of measuring the rate of decomposition by the evolution of carbon dioxide. Yet the advantage of such methods seems to compare well with methods based upon any other principle according to Gainey¹⁴, when dealing with materials such as corn and kafir stubble. In this experiment we are primarily interested in the relative rates of decomposition.

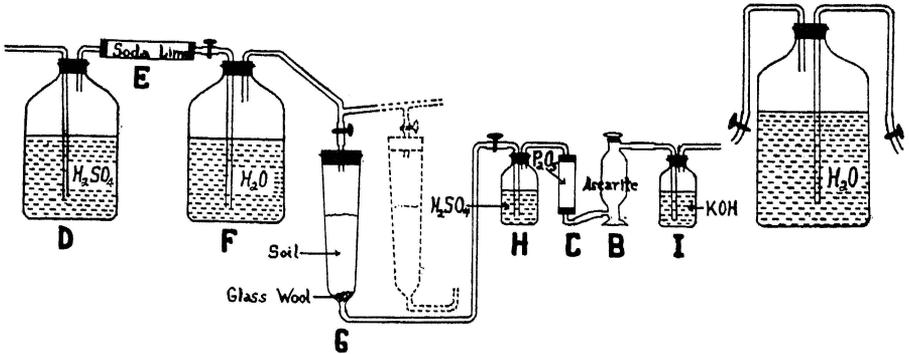


Fig. 5.—Apparatus used in carbon dioxide evolution studies.

Figure 5 shows a diagrammatic sketch of a portion of the apparatus used in the carbon dioxide evolution studies. By the use of this apparatus one is able to run twelve samples in duplicate. The samples mixed with the soil were placed in the bottom of the containers "G" in which a bit of glass wool was placed to prevent the soil from stopping the outlet leading to "H". After all the samples were placed in the containers and the apparatus arranged as shown in the diagram, the amount of carbon dioxide evolved could be easily measured by aspirating each container separately and weighing the carbon dioxide. At the start of the experiment air was drawn through the apparatus to free it of all carbon dioxide. The amount of carbon dioxide produced was determined every two days by drawing two liters of air through each sample and collecting the carbon dioxide in an Ascarite absorption bulb and weighing on the analytical balance. "A" was filled with water and the apparatus so arranged that when water was siphoned from "A" and the proper connections made, air was drawn through the apparatus entering "D" which contained sulfuric acid, taking out the water passed through "E" which contained soda lime removing the carbon dioxide of the air, through "F" which contained distilled water, moistening the air so it would

not dry out the soil, and down through "G" through the soil. The air removed the carbon dioxide produced in the soil and was carried through sulfuric acid and phosphorus pentoxide into "C" which removed the water and through "B" (Ascarite bulb) which removed the carbon dioxide. By siphoning two liters of water out of "A" two liters of air passed through each soil sample, taking them one at a time. Two liters of air passing through the sample was sufficient to change the soil air fifty times. The volume of "G" was sufficiently large to prevent the carbon dioxide concentration from becoming a disturbing factor in the biological activity in the soil. As soon as the carbon dioxide had been removed from the soil and collected in "B" the amount was determined by weighing on the analytical balance. This apparatus has been found to be very convenient and reliable in determining differences in decomposition rates of various substances. It was possible to make the twenty-four determinations in one and a half hours.

For this work soil was obtained from a farm in southwest Missouri on which the depressive effect of soybeans on wheat following had been especially noted. The soil is of the type known as Gerald silt loam⁴⁵. The carbon-nitrogen ratio of this soil is 12.4:1. The soil is low in nitrogen, low in fertility, acid, and has poor air and water drainage.

A preliminary experiment was run with the apparatus using 100 grams of soil plus 1 gram of sweet clover tops in one case, 100 grams of soil plus 1 gram of glucose in another, and 100 grams of soil with no additional material. The amount of carbon dioxide evolved was determined by the method outlined above. Table 6 gives the daily production of carbon dioxide.

TABLE 6.—DAILY EVOLUTION OF CARBON DIOXIDE IN MILLIGRAMS.

<i>Days</i>	<i>Dextrose</i>	<i>Sweet Clover</i>	<i>Checks</i>
1	46.4	93.4	5.7
2	67.5	89.3	1.1
3	101.7	73.0	2.9
4	162.4	54.0	2.8
5	157.3	39.7	2.0
6	114.4	32.9	2.7
7	70.5	30.7	1.9
8	49.8	26.9	2.1
9	40.1	21.4	3.2
10	35.2	20.4	2.5
11	28.5	17.1	3.1
12	23.9	15.8	2.2
13	22.3	16.0	3.5
14	18.7	14.5	2.8

The values are the averages for duplicate determinations given in milligrams. The accuracy of the method is indicated by the checks. The values are shown graphically in Figure 6.

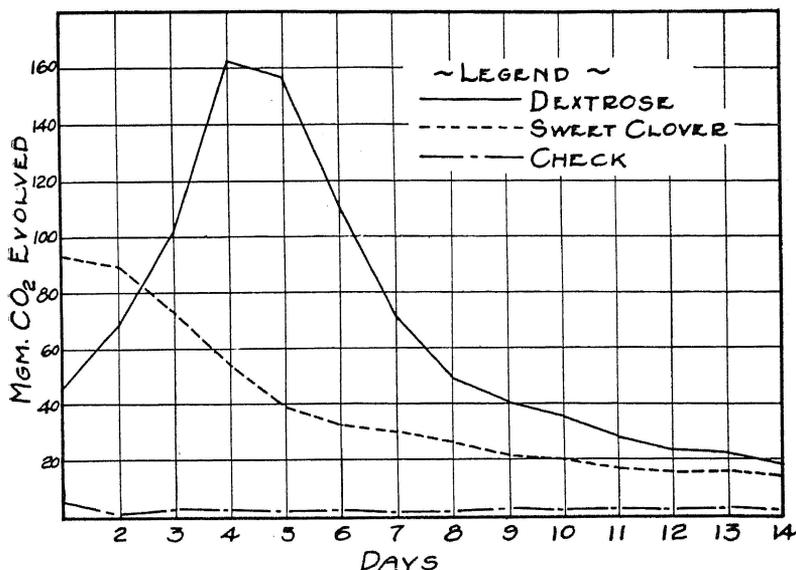


Fig. 6.—Evolution of carbon dioxide from decaying organic materials in soil.

This preliminary experiment demonstrated several things, namely, (1) The apparatus described is satisfactory for carbon dioxide studies of this sort as indicated by duplicate determinations; (2) It is accurate and simple to operate; (3) Substances higher in nitrogen are not always oxidized most rapidly; (4) The rate of carbon dioxide evolution from dextrose and sweet clover tops is almost equal and constant at the end of 14 days; and (5) The soil being low in organic matter (2.55%) the amount of carbon dioxide evolved is very slight in the checks.

Because of the very minute quantities of carbon dioxide evolved from this soil without the addition of organic matter, checks were eliminated from subsequent carbon dioxide evolution studies.

After having successfully completed the preliminary work, carbon dioxide evolution studies were made of soybean plants, soybean tops, and soybean roots for four different harvest, i. e., July 13, August 10, September 7, and October 5. Fresh soil equivalent to 100 grams oven-dry soil was used; it has a moisture content of 20 per cent which was optimum. Two per cent organic matter was added in each case. Samples were run in duplicate and the amount of carbon dioxide produced was measured every two days.

Table 7 and Figures 7, 8, 9, and 10 show the average yield of carbon dioxide at successive two day intervals of the various soybean plant

materials. The values are averages of duplicate determinations given in milligrams.

TABLE 7.—CARBON DIOXIDE EVOLVED (MGMS.) AT TWO-DAY INTERVALS FROM SOYBEAN PLANT MATERIAL, ROOTS, AND TOPS INCORPORATED IN SOIL

Date of Harvest		2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
July 13	Roots	93	130	124	95	73	66	40	38	41	35	27	22	28	20	17
	Tops	172	200	184	113	73	54	45	32	30	28	17	15	20	13	16
	Plants	166	204	169	127	86	68	48	37	33	31	14	17	21	15	15
Aug. 10	Roots	111	120	113	86	61	48	38	33	33	31	28	20	26	19	19
	Tops	170	181	130	91	60	46	35	27	26	24	13	14	17	12	12
	Plants	156	194	123	107	75	64	52	38	37	32	24	18	24	17	15
Sept 7	Roots	102	102	96	80	63	49	41	38	36	33	22	21	25	18	18
	Tops	197	189	139	104	70	60	45	34	35	30	21	16	23	18	15
	Plants	179	178	137	104	82	66	62	39	36	35	25	21	24	19	16
Oct. 5	Roots	105	87	79	68	51	45	41	35	29	30	21	20	26	20	17
	Tops	187	174	168	144	107	84	64	38	48	41	25	18	27	21	19
	Plants	181	151	134	104	79	66	52	38	38	33	22	22	17	18	19

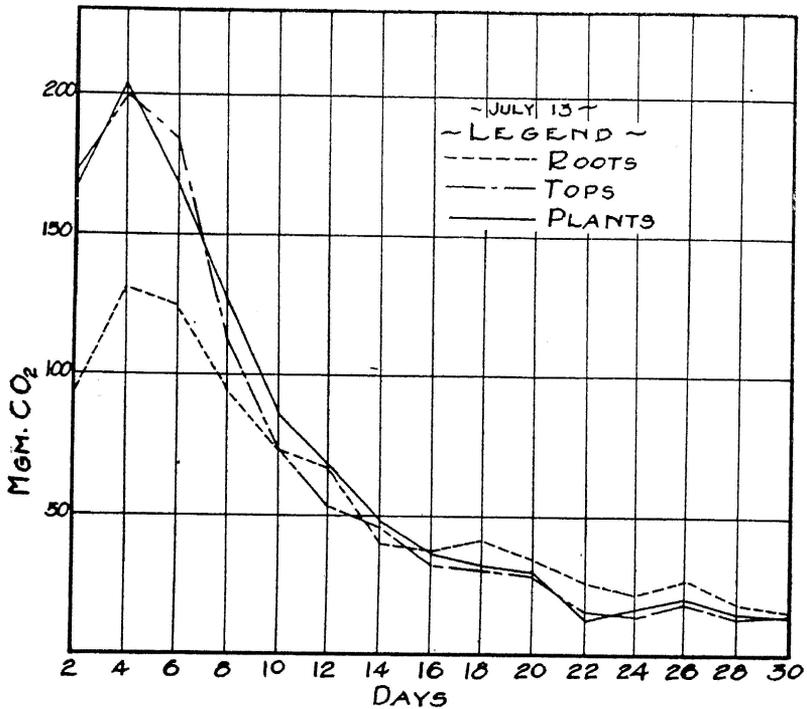


Fig. 7.—Evolution of carbon dioxide from soybean plant material harvested July 13.

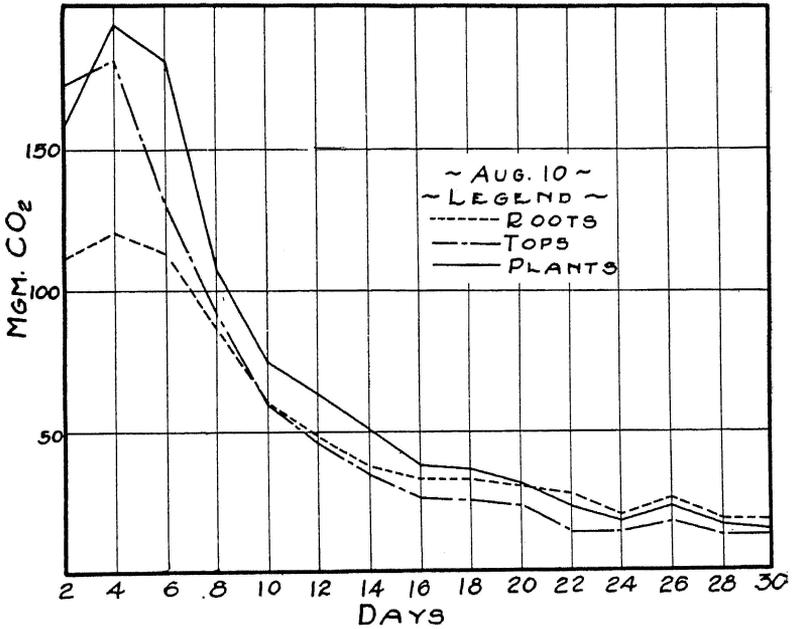


Fig. 8.—Evolution of carbon dioxide from soybean plant material harvested August 10.

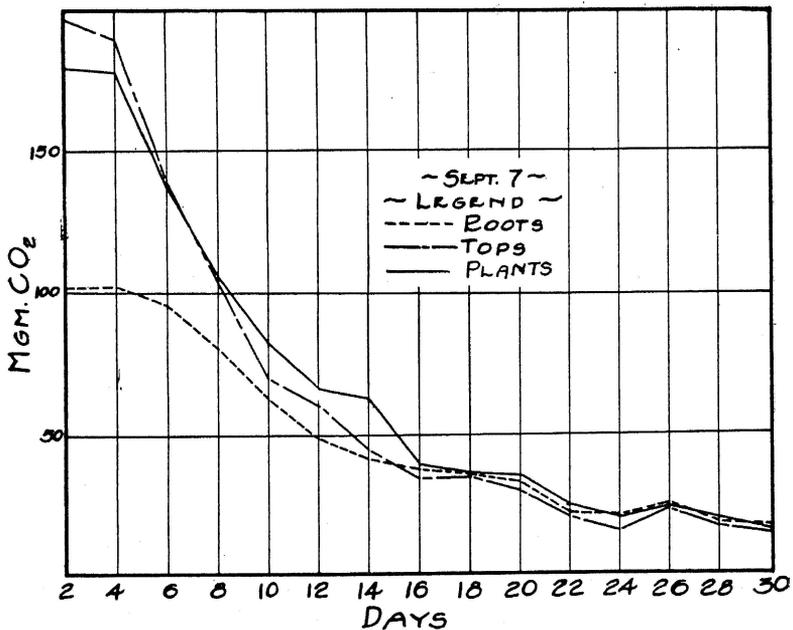


Fig. 9.—Evolution of carbon dioxide from soybean plant material harvested September 7.

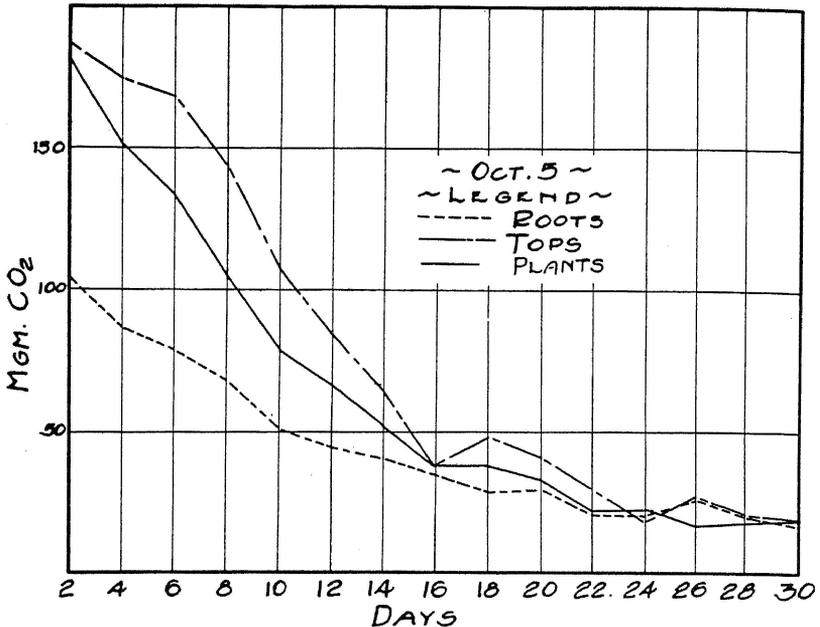


Fig. 10.—Evolution of carbon dioxide from soybean plant material harvested October 5.

It is observed by studying the curves of the carbon dioxide liberated with time, as measured in two day intervals, that there is considerable difference in the curves between the early and extremely late soybean harvests. This difference is especially noticeable during the first ten days of the experiment. For the July and August harvests the curves all give a very noticeable rise for the first four days and then a gradual decrease, but for the September and October harvests the curves decrease from the start. This difference in the curves is very striking and is worthy of further consideration. From the results of other work previously referred to it may be due to the water-soluble portion of the organic material. If it is due to this fraction then we would expect the curves to behave differently if the carbon dioxide evolution was determined with the same materials except with the water-soluble material removed. This experiment was performed and is discussed later.

In general, throughout the course of the curves the later harvests are below the earlier ones and the "root curves" are much below those of the plants and tops in any one particular harvest. These differences may be due either to differences in amount and nature of the water-soluble fraction, total nitrogen, carbon-nitrogen ratio, or to any one or a combination of factors already discussed as influencing the rate of decom-

position. From the results of the chemical analysis of the soybean plant material one would expect the earlier harvest to decompose more readily than the later harvests for the following reasons: (1) The percentage of total nitrogen decreased with the age of the plant; (2) The water-soluble constituents decreased as the harvest was deferred; (3) The alkali soluble material, considered slowly decomposable, increased markedly toward plant maturity; (4) There was a widening of the carbon-nitrogen ratio with increasing maturity; and (5) The older the plant the narrower the pentosan-lignin ratio which might indicate slower decomposition.

Table 8 gives the amount of carbon added in the roots and tops of the preceding experiment for the four harvests and the percentage of total added carbon oxidized for each two-day interval.

TABLE 8.—PERCENTAGE OF TOTAL ADDED CARBON OXIDIZED AND EVOLVED AS CARBON DIOXIDE FROM THE SOIL.

Date	July 13		Aug. 10		Sept. 7		Oct. 5	
	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops
Mgms. C added as carbon dioxide	2,839	2,805	2,943	2,879	2,967	2,885	3,036	3,065
Days	% of total	%	%	%	%	%	%	%
2	3.28	6.11	3.76	5.92	3.44	6.81	3.45	6.09
4	7.87	13.25	7.78	12.20	6.89	13.37	6.31	11.45
6	11.11	19.80	11.70	16.70	10.11	18.19	8.91	17.26
8	15.59	23.82	14.63	19.84	12.80	21.79	11.15	21.95
10	18.15	26.43	16.70	21.94	14.92	24.21	12.82	25.76
12	20.48	28.35	18.33	23.55	16.57	26.30	14.31	28.18
14	21.87	29.96	19.62	24.77	17.96	27.86	15.66	30.29
16	23.20	31.09	20.75	25.80	19.23	29.01	16.83	31.53
18	24.66	32.17	21.88	26.60	20.43	30.22	17.78	33.09
20	25.89	33.18	22.92	27.43	21.53	31.27	18.77	33.09
22	26.82	33.79	23.80	27.89	22.26	31.98	19.47	35.24
24	27.61	34.31	24.54	28.36	22.97	32.55	20.10	35.84
26	28.58	35.01	25.43	28.94	23.82	33.34	20.00	36.72
28	29.21	35.49	26.07	29.36	24.44	33.96	21.64	37.40
30	29.88	36.06	26.72	29.78	25.05	34.47	22.20	38.01

It is noticed in each case at the end of 30 days a larger percentage of the total carbon of the tops was oxidized in comparison to that of the roots. As the difference in the carbon-nitrogen ratio between these two plant parts increased, the difference in the percentage total carbon oxidized at the end of 30 days becomes greater. Figure 11 shows the percentage of the total carbon of the soybean roots oxidized at two day intervals. In these curves the influence of maturity on the rate of decomposition is very strikingly brought out. As the stage of maturity advances the rate of decomposition is decreased as is indicated by a slower rate of carbon dioxide evolution based on the percentage of total carbon added. The results of the plant analyses adequately explain differences observed in the rate of decomposition. These soil materials remaining from the carbon dioxide production studies were preserved for a study

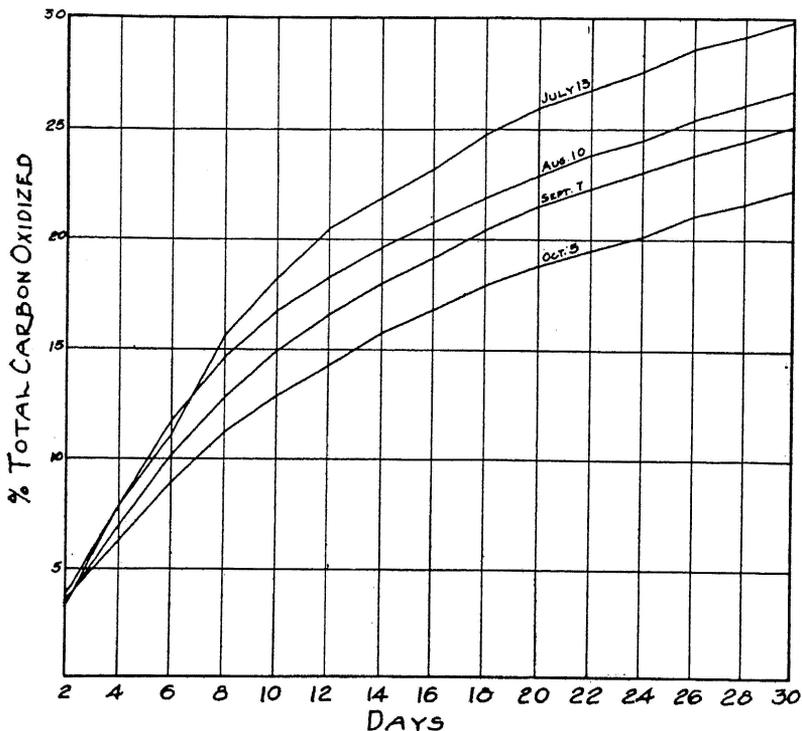


Fig. 11.—Percentage of total added carbon in the form of soybean roots that was oxidized and evolved as carbon dioxide.

of the nitrogen and carbon changes that occurred and for humus accumulation studies.

In order to determine what effect the water-soluble material has on the rate of carbon dioxide evolution, samples of the same material as used before were extracted with cold water and the carbon dioxide produced by the residue in soil was determined. The experiment was run exactly as before except that the water-soluble material had been removed from the organic matter added. In this experiment only the roots and tops for the July and October harvests were considered. The cold water soluble material was removed by soaking the material in 200 cc. distilled water for 24 hours, filtering, and washing the residue thoroughly with cold distilled water. Two samples of each harvest were thus treated.

Table 9 gives the percentage total nitrogen remaining in the cold water extracted residues and the percentage of the original total nitrogen that is water soluble. These values were calculated from the results given in Tables 1, 2, and 3.

TABLE 9.—PERCENTAGE OF TOTAL NITROGEN THAT IS WATER SOLUBLE.

Date of Harvest	Nitrogen in residues	Water-soluble nitrogen
July 13 Roots	1.15	32.0
Tops	2.09	30.5
Oct. 5 Roots	.83	27.2
Tops	2.20	19.1

In the early harvest over 30 per cent of the total nitrogen is cold water soluble, and the percentage of cold water soluble nitrogen decreases with advancing maturity.

If the percentage water soluble nitrogen is the determining factor in the initial rapid decomposition rate, then one should have found the root material of July 13, after water soluble extraction, to decompose more readily than the tops of the same harvest, because the former is higher in percentage of cold water soluble nitrogen. But this was not the case and the only explanation offered is that no one factor controls the rate of decomposition but rather a number of factors, probably the predominating one being within the cold water soluble fraction.

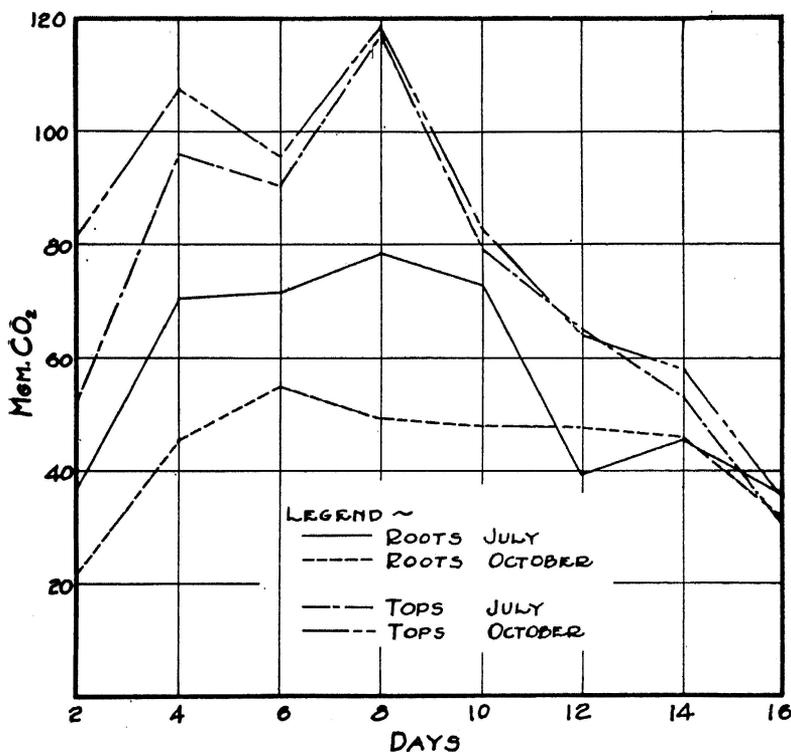


Fig. 12.—Evolution of carbon dioxide from the cold water extracted residues of the soybean tops and roots of the July and October harvests.

If the cold water soluble fraction is largely responsible for the early rapid evolution of carbon dioxide then one would expect the cold water extracted residue to decompose much more slowly, particularly at first, and such was the case in these trials.

The curves showing the carbon dioxide evolved during the successive two-day intervals for the cold water residue are shown in Figure 12. A comparison of the curves for the original material and of the cold water extracted residue is made in Figures 13 and 14.

The curves indicate a direct correlation between the percentage of nitrogen of the residue and the rate of carbon dioxide production. The tops for both July and October harvests gave a carbon dioxide production curve much higher than the corresponding "root curves"; again showing a correlation with the total nitrogen content. Far more interesting is the comparison of these curves with the "carbon dioxide production curves" of the corresponding material before removing the water soluble material.

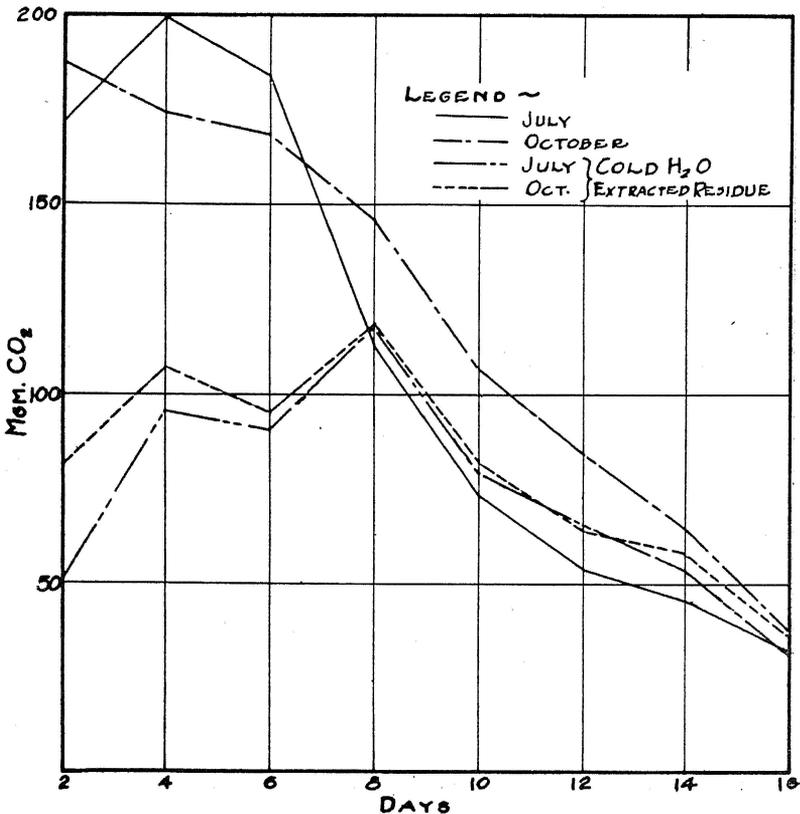


Fig. 13.—A comparison of the carbon dioxide evolution from the original soybean top material, and the corresponding cold water extracted residue for the July and October harvests.

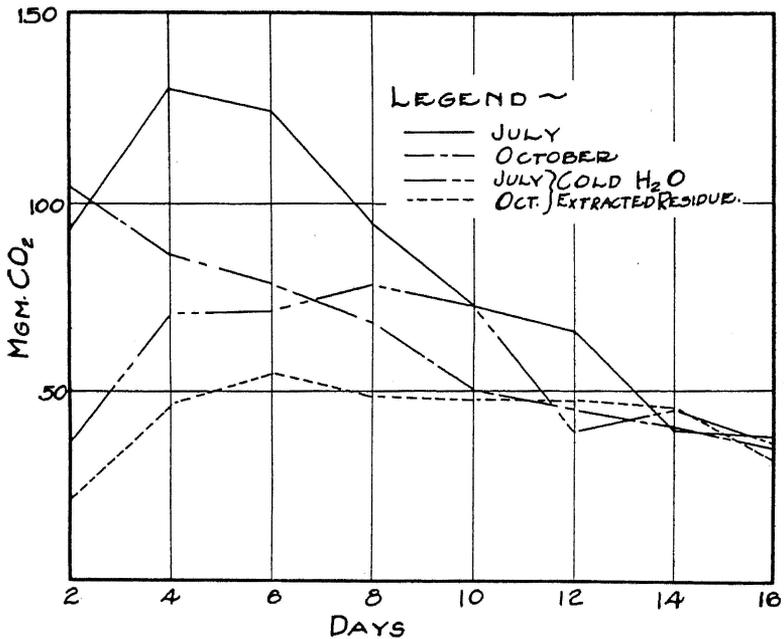


Fig. 14.—A comparison of the carbon dioxide evolution from the original soybean root material, and the corresponding cold water extracted residue for the July and October harvests.

It is seen that the immediate production of carbon dioxide of the material from which the water-soluble material was removed is only about one-half as great as of the original material. In other words, the water-soluble material is largely responsible for the immediate and rapid decomposition or the corresponding rapid production of carbon dioxide.

Humus Accumulation.—The more easily the organic material decomposes, the more rapidly humus accumulates. Therefore, we would expect to find a greater accumulation of humus in the soil, at the end of a given time under similar conditions, where the younger and more immature material was added. A humus analysis was made of the soil and residue remaining from the carbon dioxide production studies in order that this analysis might indicate, in another way, the relative rates of decomposition of the different plant materials incorporated into the soil. The procedure followed was that as outlined by Waksman⁵⁵. Twenty-five grams of oven-dry soil were used in each case. A summary of the analysis of the Alpha fraction* of humus is presented in Table 10.

It must be remembered that only 2 per cent of organic material was added to the soil in each case and that it was allowed to decompose

*Total removed by 2½% solution of sodium hydroxide.

in the laboratory only thirty days. Therefore, we would not expect any great differences in amount of humus accumulated in that short a time. Moreover, the methods for determining humus are more or less indefinite and makes this method very difficult for determining the rate of organic matter decomposition.

TABLE 10.—HUMUS ANALYSIS OF ALPHA FRACTION OF SOIL REMAINING FROM THE CARBON DIOXIDE PRODUCTION STUDIES.

Date of Harvest of Material Added to Soil		Weight of Alpha fraction	% Alpha humus	% N	% ash
July	Roots	.2028	.811	2.70	1.18
	Tops	.2085	.834	3.33	1.43
Aug.	Roots	.1816	.726	3.07	.32
	Tops	.1808	.723	3.40	.46
Sept.	Roots	.1884	.754	3.29	1.68
Oct.	Roots	.1859	.744	3.10	1.40
	Tops	.1859	.744	3.68	1.35
Check		.1524	.610	3.57	1.43

The quantities of humic materials extracted from soils⁴⁸ vary with the chemical nature of the alkaline solutions, their concentration, the temperature and the time of reaction. These determinations were made under identical conditions so that the results may be considered comparable. However, it is observed that where the material of the earlier harvests was incorporated in the soil there is a larger percentage of humus than in the case where the mature material was used. This, of course, indicates a more rapid rate of organic matter decomposition. The soil to which no organic matter was added stands much below the others in percentage of humus that it contains.

The percentage of nitrogen in the Alpha fraction of the humus is practically the same in all cases with an average of about 3.25 which agrees with the results of other investigators⁵⁵. This part of the soil organic matter which is soluble in dilute alkali solutions comprises the so-called "humic acids" and is characterized by a definite nitrogen content.

Changes in Carbon and Nitrogen During Decomposition.—Soils in general have a definite carbon-nitrogen ratio, which is approximately 10:1. In other words, for every ten parts of carbon in the soil there is, on the average, one part of nitrogen. Most of the organic materials that are added to the soil have a much wider ratio than this and during their decomposition there is a narrowing of the ratio of this plant material. The carbon loss during organic matter decomposition is generally more rapid than the nitrogen loss and it is for this reason, of course, that we get a narrowing of the carbon-nitrogen ratio of added organic materials.

Therefore, we would expect to find a distinct narrowing of the carbon-nitrogen ratios in the soils used in the carbon-dioxide production studies which have been discussed.

Table 11 gives a summary of the data dealing with these carbon and nitrogen changes. In each case there was a distinct change from the relatively wide ratio to a much narrower one. The soil to which the soybean top material had been added resulted in a narrower ratio in each case than did the corresponding root material. These results are to be expected since the top material contains a much higher percentage of readily decomposable material which favors a more rapid rate of decomposition.

TABLE 11.—CHANGES IN THE NITROGEN AND CARBON DURING DECOMPOSITION OF SOIL ORGANIC MATTER AND THE RELATIVE AMOUNTS OF THESE TWO ELEMENTS REMOVED BY DILUTE ALKALI TREATMENT.

		% C after humus ext.	% C lost on humus ext.	% N after humus ext.	% N lost on humus ext.	c/n ratio, beginning of de- comp.	c/n ratio at end of decomp.	c/n ratio after ext.	% dif. in c/n be- fore and after ext.
July	Roots	.56	68.0	.030	76.8	14.9:1	13.5:1	18.6:1	37.8
	Tops	.39	76.6	.034	80.4	12.6:1	9.6:1	11.0:1	14.6
Aug.	Roots	.59	66.5	.032	77.9	15.6:1	12.1:1	18.6:1	53.7
	Tops	.40	76.6	.033	79.2	13.9:1	10.7:1	12.0:1	12.1
Sept.	Roots	.55	68.0	.030	78.2	16.0:1	12.5:1	18.3:1	48.8
Oct.	Roots	.57	68.1	.031	77.8	16.6:1	12.8:1	18.5:1	44.5
	Tops	.47	71.8	.033	79.5	13.2:1	9.8:1	11.2:1	14.3
Check		.42	68.6	.031	75.6	12.4:1	10.6:1	13.5:1	27.3

Carbon and nitrogen determinations were made on this soil material after the so-called humus was removed by dilute alkali. These data are assembled in Table 11. The results are very striking in that the percentage of nitrogen left in the extracted soil is practically the same in all cases (.030 to .034), but great differences are noted in the percentage of carbon remaining (0.39 to 0.57). A much larger amount of carbon was found in the extracted soil to which the soybean root material had been added. Calculated on a percentage basis there was in each case a higher percentage of the total added carbon removed in the extraction from the soil to which soybean top material had been added than where the root material had been added. The percentage of total nitrogen removed by the alkali extraction was also greatest where the top material had been added, but the percentage differences were greater in the carbon removal. The ratios of the soil to which root material had been added was about 13:1 before extraction and about 18:1 after extraction. In the cases where the top material was added the ratios were widened from about 10:1 to 11.5:1. These ratio changes calculated on a percentage difference basis are extremely significant. The average percentage difference in the carbon-nitrogen ratio of the soil before and after extraction in the case

of the root material addition was 45.5 in contrast to only 13.7 in the case of the top material additions. This difference could be due either to a more complete removal of carbon in the latter case or a more complete removal of nitrogen in the former. But, as pointed out above, the percentage differences in removal were greater in each case for the carbon than for the nitrogen. Therefore, one must conclude that the differences found in the resulting carbon-nitrogen ratios of the extracted soil are due to a more complete removal of the carbon where the soybean top materials were added.

What are the explanations and of what significance are these findings? Earlier in the paper data were presented showing that the change in the ratio of energy material to the nitrogen in the soybean tops ranged from 23.6 to 34.8 during the season while in the roots the range was from 39.6 to 85.7. These wide ratios for the roots suggested that the soybean roots supply too much energy material in proportion to their nitrogen for rapid decomposition. This is without question a possible explanation for the great differences found in the amount of carbon remaining in the alkali extracted soils cited above. In other words, there was a less complete decomposition of the root material and an accumulation of more resistant material, perhaps lignin-like material. However, the plant analysis showed little differences in the total lignin content of the materials added, yet, because of the differences in the nitrogen and energy material of the two groups of substances, more complete decomposition occurred in the case of the top material. Waksman and Gerretsen⁴⁹ and M. Phillips and others⁵⁴ have recently pointed out that lignin under proper environmental conditions is decomposed at a reasonably rapid rate. This is a possible explanation for the greater accumulation of the material more resistant to decomposition where soybean roots were incorporated into the soil. These findings emphasize the importance of an adequate supply of available nitrogen in the soil when material of a carbonaceous nature is added and a rapid rate of decomposition with corresponding liberation of nitrogen is desired.

Ammonia and Nitrate Production.—Ammonia and nitrate production in soil following the incorporation of soybean plant material was studied in the laboratory. Four soybean harvests were used, i. e., July 13, August 10, September 7, and October 5. The entire plants, tops and roots were studied separately for each harvest. Fresh soil equivalent to 100 grams oven-dry soil was mixed with 2 per cent of the soybean material in each case, made up to optimum moisture content, and placed in jelly tumblers. Sixteen tumblers for each plant part of each harvest were set up including 16 checks. Ammonia and nitrate determinations were made on duplicate samples at the start and thereafter at two week intervals. Ammonia determinations were discontinued at

the end of twelve weeks, but the nitrate determinations were continued until the end of the eighteenth week. This experiment was performed for the purpose of demonstrating, if possible, the cause for the detrimental effect on wheat following soybeans. The depressing effect of soybeans on the crop which follows is readily possible in consequence of the large percentage of carbonaceous matter and small percentage of readily usable nitrogen which the mature soybean crop, especially the roots, supplies to the soil microorganisms. The chemical composition studies showed that the mature plant material, particularly the soybean roots, which is high in carbonaceous matter, may jeopardize the soils

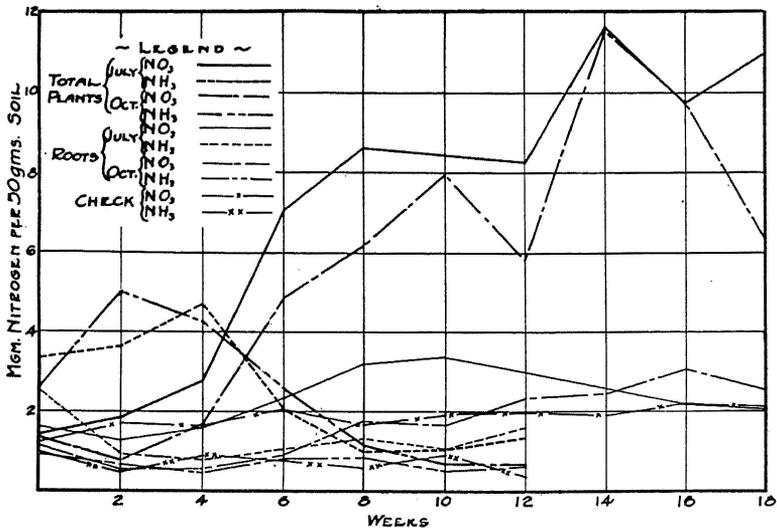


Fig. 15.—Ammonia and nitrate accumulation in soil from the total plants and roots for the September and October harvests.

supply of available nitrogen for other crops. If the soybean roots are responsible for the depressive effect associated with their decomposition by reducing the nitrates in the soil, it was believed that it could be demonstrated in the laboratory.

Figure 15 summarizes the "ammonia and nitrate production curves," following the incorporation of the total soybean plants and the roots for the September and October harvests and the checks. The curves for the soybean tops and for the other harvests have been omitted. By studying these curves it is very evident that the total plant material is very effective in increasing the nitrate content of the soil, whereas, the root material gives a very marked decrease in nitrate content. The ammonia accumulation is much greater and more rapid following the incorporation

of the total plant material than of that following the roots, and consequently greater nitrate production.

It is observed that the "ammonia production curves" and "nitrate accumulation curves" fall in line with the corresponding ones for carbon dioxide evolution, in that the earlier harvested material gave greater and more rapid response to decomposition. The most significant part of the results is the reduction in the nitrates below the checks following

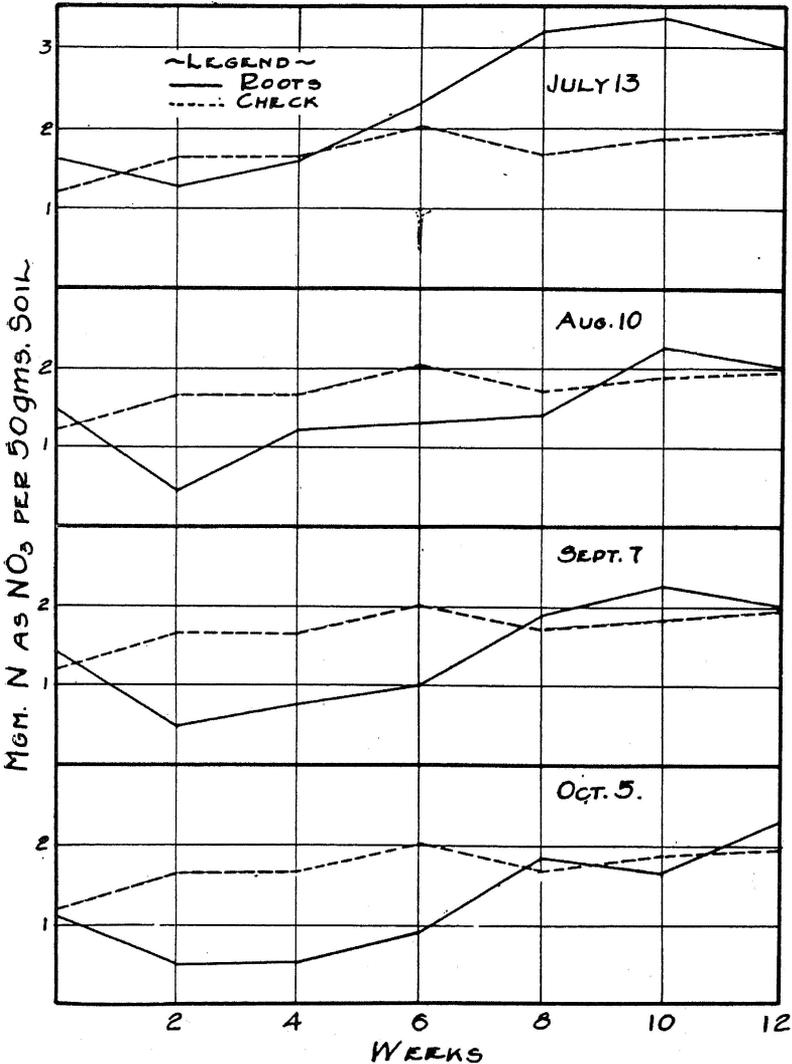


Fig. 16.—Nitrate accumulation when soybean root material is incorporated in the soil at different growth stages.

the incorporation of the root material. The "root curve" runs considerably below the check for the first eight weeks. This is no doubt due to the carbonaceous nature of the root material stimulating biological activity and the bacteria using up the available nitrates in their body synthesis. This decrease in nitrates appears to be the cause for the depressive effect on the crop following.

The "nitrate production curves" for the root material of the four different harvests in comparison with the checks are assembled in Figure 16. It is at once observed that as the stage of maturity advances the depressive effect of the roots on the soil nitrates increases. This is no doubt due, for the most part at least, to the decrease in percentage of readily available nitrogen and an increase in carbohydrates or energy material as the plants become older. In general it was found for all the materials studied, that as maturity advanced the ammonia accumulation and nitrate production decreased. A large production of nitrates followed the incorporation of total soybean plants in the soil but a very marked decrease in nitrates followed the incorporation of root material. This point is of great practical importance to the farmer who follows soybeans with wheat in his cropping system. During the decomposition of the soybean roots, stubble, and other carbonaceous residue in soil a deficiency of available nitrates is apt to occur which may greatly decrease his yield of wheat. This would suggest that an available nitrogenous fertilizer should be applied to the wheat under such circumstances. In farm practice this has been found to alleviate the depressing effect on the crop following the growth of soybeans, indicating an available nitrate deficiency in the soil.

DISCUSSION

Within recent years the growing of soybeans in Missouri has increased considerably, and they are generally considered a good legume crop to put into the rotation. The numerous reports from farmers in the state, that the yields of wheat are at times decreased below normal when the wheat follows soybeans, have led to experimentation under controlled conditions in the laboratory.

A chemical study of the organic constituents of soybeans with advancing maturity of the plants suggests that the effectiveness of this crop as a green manure to liberate nitrogen in the soil will vary widely with different stages of growth. It is known that the amount of nitrate nitrogen available in the soil at the time of planting the wheat is an important factor for its success. Deatrick⁸ points out that the relatively high nitrogen content of soybean hay indicates a high nitrogen absorption from the soil and that possibly nitrogen assimilation by the soybean

plants progresses until the nitrate nitrogen content of the soil is very low, and a consequent effect is that of lowering the yield of wheat that follows.

Evidence is presented in this paper to show that the depressive effect of soybeans on wheat following is due, in part at least, to the reduction in nitrates as a result of the biological stimulation by the carbonaceous root material. As the stage of maturity of the roots advances, a greater amount of nitrates is removed. This nitrate disappearance under field conditions, as has often been pointed out, may be caused by plant absorption through late season growth or by a microbiological assimilation, in consequence of the decay in root residue of highly carbonaceous nature, or by a combined effect of these two.

The depressing effect of soybeans on the crop which follows is readily possible in consequence of the large percentage of carbonaceous matter and small percentage of readily usable nitrogen which the mature soybean crop, especially the roots, supply to the soil microorganisms. This study shows that the percentage of more readily soluble nitrogen decreases as the plants become older while the carbonaceous matter usable by microorganisms increase so rapidly that the more mature crop is a bacterial ration with such a deficiency of soluble nitrogen that its decomposition may decrease the soil's supply of available nitrogen for other crops. There is need for a more complete knowledge of the chemical changes of the plant's organic complexes as it becomes more mature, and for a fuller knowledge of the decay of these constituents within the soil, in order that the chemical studies of green manures may more wisely guide their use of maximum effective nitrogen liberation in the soil.

SUMMARY AND CONCLUSIONS

Decreased yields of wheat immediately following soybeans led to the assumption that microbiological assimilation was responsible for nitrate disappearance.

A study was made of the variations in the amount of decomposable and undecomposable material in the soybean plants at different stages of maturity. Soybean tissue was successively treated with ether, cold water, hot water, dilute alkali, and dilute acid and determinations were made of the organic constituents in the various extracts and residue, as well as in the original material. A study was made of the carbon and nitrogen changes of the soybean during the growth season. These various analyses were made for the purpose of explaining or indicating a solution for the problem in question.

The rate of decomposition of the soybean plant and its parts at different growth stages was studied by various methods, including the

evolution of carbon dioxide, ammonia production, nitrate accumulation, and humus production.

The cold water-soluble fraction of the soybean material was studied with special reference to its total nitrogen content and its influence on the rate of decomposition.

A study was also made of the carbon and nitrogen changes which occurred in the soil during a definite decomposition period. The relative amounts of each of these constituents removed in the humus extract were determined.

The work reported herein permits the following conclusions:

1. The percentage of total nitrogen decreased with the age of the soybean plant. The roots were below 1.7 per cent nitrogen except in the earliest part of the season.

2. The water soluble constituents decreased in both roots and tops as the plants became older.

3. The alkali soluble material, considered slowly decomposable, increased markedly toward plant maturity and the nitrogen content of this portion increased, especially in the alkali soluble lignin.

4. The carbonaceous material, including the reducing sugars, the cellulose, and pentosans, all decomposable by soil microorganisms in the presence of soluble nitrogen, increased with maturity of the plant.

5. There was a widening of the carbon-nitrogen ratio with advancing maturity.

6. The percentage of total lignin increased enormously during the season's growth.

7. The pentosan-lignin ratio was a narrow one which might indicate slow decomposition, especially of the soybean roots.

8. In general the rate of decomposition of the different plant parts decreased with advancing maturity as indicated by the evolution of carbon dioxide.

9. The rate of decomposition of the plant parts was in the following order: tops, complete plants and roots.

10. The cold water-soluble fraction is largely responsible for the immediate and rapid evolution of the carbon dioxide.

11. There was a distinct narrowing of the carbon-nitrogen ratio in the soil during the thirty day decomposition period.

12. A more complete removal of carbon from soil by alkali treatment was noted where the soybean top materials were added than in the case of the root material indicating a more rapid decomposition of the former into the "humus" form.

13. Ammonia and nitrate accumulation following the incorporation of the plant materials in soil were less with the successive or more mature growth stages.

14. A positive accumulation of nitrates occurred following the incorporation of the total soybean plants and the tops in soil.

15. A negative accumulation of nitrates or their reduction occurred following the incorporation of soybean root material into the soil. The later the growth stage, the more pronounced was the depressive effect.

16. Possible practical applications for the foregoing results emphasize the need for careful attention to the growth stage and time at which soybeans or their root remnants are turned under if they are to be used to the fullest advantage as green manure.

- (a) When soybeans are used for green manure if a rapid accumulation of nitrates is desired turn them under in the more immature stages of growth.
- (b) An increase in nitrates following the growth of soybeans should not be expected because of the carbonaceous nature of the root material which stimulates biological activity increasing the number of organisms which consume the nitrate.
- (c) The less mature the soybeans are when cut and removed, the less will be the depressive effect on the soil nitrates.
- (d) Soybeans cut for seed will give a greater decrease in soil nitrates under wheat than when wheat follows soybeans cut for hay.
- (e) A liberal application of a nitrogenous fertilizer to wheat following soybeans eliminates any depressive effect that may be noted.
- (f) The present study of the composition of the soybean plant in relation to the accumulation of nitrates in the soil will be of value in the better adjustment of this leguminous plant to the many varied cultural practices in which it is used.

BIBLIOGRAPHY

1. Albrecht, W. A. *Nitrate Accumulation in Soil as Influenced by Tillage and Straw Mulch.* Jour. Amer. Soc. Agron. 18, 1926, 841-853.
2. Allison, F. E. *Nitrate Assimilation by Soil Microorganisms in Relation to Available Energy Supply.* Soil Sci. 24, 1927, 79-91.
3. Anderson, J. A. *The Influence of Available Nitrogen on the Fermentation of Cellulose in the Soil.* Soil Sci. 21, 1926, 115-126.
4. Bal, D. V. *Studies on the Decomposition of Some Common Green Manuring Plants at Different Stages of Growth in the Black Cotton Soil of the Central Provinces.* Agri. Jour. India, 17, 1922, 133-155.
5. Blair, A. W., and Prince, A. L. *Influence of Dry Organic Matter on Crop Yields.* Soil Sci. 25, 1928, 281-286.
6. Collison, R. E., and Conn, H. J. *The Effect of Straw on Plant Growth.* New York Agr. Exp. Sta. Tech. Bul. 114, 1925.
7. Conrad, J. P. *Some Causes of the Injurious After Effects of Sorghums and Suggested Remedies.* Jour. Amer. Soc. Agron. 19, 1927, 1091-1111.
8. Deatrick, E. P. *Reduction of Soil Nitrates During the Growth of Soybeans.* Jour. Amer. Soc. Agron. 20, 1928, 947-958.

9. Doryland, C. J. T. *The Influence of Energy Material upon the Relation of Soil Microorganisms to Soluble Plant Food*. North Dakota Agr. Exp. Sta. Bul. 116, 1916.
10. Erdman, L. W. *The Percentage of Nitrogen in Different Parts of Soybean Plants at Different Stages of Growth*. Jour. Amer. Soc. Agron. 21, 1929, 361.
11. Feher, D. *Untersuchungen über den Zeitlichen Verlauf der Bodenatmung und der Mikrobentätigkeit des Walbodens*. Biochem. Z. 206 (1929) 416-435.
12. Fisher, F., and Lieske, R. *Experiments on the Behavior of Lignin in the Natural Decomposition of Plants*. Experiment Sta. Rec. 61, 1929, 805.
13. Gaaney, P. L. *Parallel Formation of Carbon Dioxide, Ammonia and Nitrate in Soil*. Soil Sci. 7, 1919, 293-311.
14. Gaaney, P. L. *Relative Rates of Decomposition of Corn and Kafir Stubble*. Jour. Amer. Soc. Agron. 21, 1929, 323.
15. Heck, A. F. *A Study of the Nature of Nitrogenous Compounds in Fungous Tissue and their Decomposition in the Soil*. Soil Sci. 27, 1929, 1-50.
16. Heukelejian, H. *Decomposition of Cellulose by the Various Groups of Microorganisms of the Soil*. Abs. Bact. 8, 1924, 9.
17. Hill, H. H. *Decomposition of Organic Matter in Soil*. Jour. Agri. Res. 33, 1926, 77-99.
18. Hutchinson, C. M., and Milligan S. *Green Manuring Experiment*. Agri. Res. Inst., Pusa, Bul. 40, 1914.
19. Jenny, H. *Relation of Climatic Factors to the Amount of Nitrogen in Soils*. Jour. Amer. Soc. Agron. 20, 1928, 900-912.
20. Jensen, H. L. *On the Influence of the Carbon-Nitrogen Ratios of Organic Material on the Mineralization of Nitrogen*. Jour. Agr. Sci. 19, 1929, 71-82.
21. Jensen, H. L. *The Microbiology of Farmyard Manure Decomposition in Soil*. Jour. Agri. Sci. 21, 1931, 38-81.
22. Joshi, N. V. *Comparative Manurial Value of the Whole Plants and the Different Parts of Green Manures*. Agri. Res. Inst., Pusa, Bul. 141, 1922.
23. Leukel, W. A., Barnette, R. M., and Hester, J. B. *Composition and Nitrification Studies on *Crotalaria Striata**. Soil Sci. 28, 1929, 347-371.
24. Lipman, J. G., and Blair, A. W. *Microbiological Aspects of Green Manuring*. Proc. First Intern. Cong. of Soil Sci. 3, 1929, 312-316.
25. Lyon, T. L., Bizzell, J. A., and Wilson, B. D. *Depressive Influence of Certain Higher Plants on the Accumulation of Nitrates in Soil*. Amer. Soc. Agron. Jour. 15, 1923, 457-467.
26. Lyon, T. L., and Buckman, H. O. *The Nature and Properties of Soils*. Macmillan Company, New York, 1929, p. 365.
27. Lyon, T. L., and Wilson, B. D. *Some Relations of Green Manures to the Nitrogen of a Soil*. Cornell Univ. Agr. Exp. Sta. Mem. 115, 1928.
28. Martin, T. L. *Decomposition of Green Manures at Different Stages of Growth*. Cornell Univ. Bul. 406, 1921.
29. Maynard, L. A. *The Decomposition of Sweet Clover as a Green Manure Under Greenhouse Conditions*. Cornell Agr. Exp. Sta. Bul. 394, 1917, 119-149.
30. Merkle, F. G. *The Decomposition of Organic Matter in Soils*. Jour. Amer. Soc. Agron. 10, 1918, 281-302.
31. Murray, T. J. *The Effect of Straw on the Biological Soil Processes*. Soil Sci. 12, 1921, 233-259.
32. Neller, J. R. *The Oxidizing Power of Soil from Limed and Unlimed Plots and its Relation to Other Factors*. Soil Sci. 10, 1920, 29-39.
33. *Decomposition of Plant Residues*. New Jersey Sta. Report 1928, p. 67-68.
34. Phillips, M., Weihe, H. D., and Smith, N. R. *The Decomposition of Lignified Materials by Soil Micro-organisms*. Soil Sci. 30, 1930, 383-390.
35. Pieters, A. J., and McKee, R. *Green Manuring and its Application to Agricultural Practices*. Jour. Amer. Soc. Agron. 21, 1929, 985-993.
36. Potter, R. S., and Snyder, R. S. *Decomposition of Green and Stable Manures in Soil*. Jour. Agr. Res. 11, 1917, 677-698.
37. Potter, R. S., and Snyder, R. S. *Carbon Dioxide Production in Soils and Carbon and Nitrogen Changes in Soils Variously Treated*. Iowa Agr. Exp. Sta. Res. Bul. 39, 1916.
38. Rege, R. D. *Biochemical Decomposition of Cellulose Materials with Special Reference to the Action of Fungi*. Ann. of Applied Biology, 14, 1927, 1-44.
39. Russell, E. J., and Appleyard, A. *The Influence of Soil Conditions on the Decomposition of Organic Matter in the Soil*. Jour. Agr. Sci. 8, 285-417.

40. Scott, H. *The Influence of Wheat Straw on the Accumulation of Nitrates in the Soil.* Jour. Amer. Soc. Agron. 13, 1921, 233-258.
41. Sears, O. H. *Wheat not Always Unsatisfactory after Soybeans.* Ill. Agr. Exp. Sta. Rpt. 33, 1927.
42. Sievers, F. J., and Holtz, H. F. *The Significance of Nitrogen in Soil Organic Matter Relationships.* Wash. Agr. Exp. Sta. Bul. 206, 1926.
43. Starkey, R. L. *Evolution of Carbon Dioxide as an Index of Decomposition of Organic Matter in the Soil.* Abs. Bact. 8, 1924, 9.
44. Starkey, R. L. *Some Observations on the Decomposition of Organic Matter in Soils.* Soil Sci. 17, 1924, 293-314.
45. Sweet, A. T., and Jordan, H. V. *Soil Survey Lawrence County, Missouri.* No. 34 Series 1923, p. 1137.
46. Tenney, F. G., and Waksman, S. A. *The Nature and Rapidity of Decomposition of the Various Organic Complexes in Different Plant Materials, under Aerobic Conditions.* Soil Sci. 28, 1929, 55-84.
47. Thatcher, L. E. *The Soybean in Ohio.* Ohio Agri. Exp. Sta. Bul. 384, 1925.
48. Vincent, M. M., Hervaux and Gaudin. *Recherches Les Matieres Organiques. Des Sols.* Annales De La Science Agronomique. 47, 1930, 654-671.
49. Waksman, S. A., and Gerretsen, F. C. *Influence of Temperature and Moisture upon the Nature and Extent of Decomposition of Plant Residues by Microorganisms.* Ecol. 12, 1931, 33-60.
50. Waksman, S. A. *Chemical and Microbiological Principles Underlying the Decomposition of Green Manures in the Soil.* Jour. Amer. Soc. Agron. 21, 1929, 1-19.
51. Waksman, S. A. *Chemical Nature of Soil Organic Matter, Methods of Analysis, and the Role of Microorganisms in its Formation and Decomposition.* Trans. Soc. 2nd Com. Inter. Soc. Soil Sci. Vol. A, 1929, pp. 172-197.
52. Waksman, S. A. *Principles of Soil Microbiology.* Baltimore 1927, p. 702, 727.
53. Waksman, S. A. *Microbiological Analysis of Soil as an Index of Soil Fertility.* Soil Sci. 17, 1924, 141-161.
54. Waksman, S. A., and Diehm, R. A. *Chemical and Microbiological Principles Underlying the Transformation of Organic Matter in Stable Manure in Soil.* Jour. Amer. Soc. Agron. 21, 1929, 795-809.
55. Waksman, S. A. *Method of Determining Humus in Soil.* Soil Sci. 22, 1926, 221-232.
56. Waksman, S. A. and Tenney, F. G., *Methods of Quantitative Analysis of Plant Materials.* Soil Sci. 24, 1927, 275-283.
57. Waksman, S. A. and Tenney, F. G. *Influence of Age of Plant upon Rapidity and Nature of Decomposition.* Soil Sci. 24, 1927, 317-333.
58. Waksman, S. A., and Starkey, R. L. *Microbiological Analysis of Soil as an Index of Soil Fertility.* Soil Sci. 17, 1924, 141-161.
59. Waksman, S. A., and Tenney, F. G. *Influence of Age of Plant upon Rapidity and Nature of its Decomposition.* Soil Sci. 24, 1927, 317-334.
60. Waksman, S. A., and Tenney, F. G. *The Influence of Nature of Plant upon the Rapidity of its Decomposition.* Soil Sci. 26, 1928, 155-171.
61. White, T. H. *Crimson Clover as a Green Manure for Tomatoes.* Maryland Bul. 199, 1916, 100-106.
62. Whiting, A. L. *Some Important Factors Controlling the Rate of Nitrification of Organic Materials.* Jour. Amer. Soc. Agron. 18, 1926, 854-876.
63. Whiting, A. L., and Richmond, T. E. *The Relative Rates of Nitrification of Different Parts of Sweet Clover Plants.* Soil Sci. 24, 1927, 31-37.
64. Whiting, A. L., and Schoonover, W. R. *The Comparative Rate of Decomposition of Green and Cured Clover Tops in Soil.* Soil Sci. 9, 1920, 137-149.
65. Wilson, B. D., and Wilson, J. K. *An Explanation of the Relative Effects of Timothy and Clover Residues in the Soil on Nitrate Depression.* Cornell Univ. Agr. Exp. Sta. Mem. 95, 1925.
66. Wilson, B. D., and Wilson, J. K. *Relation of Sorghum Roots to Certain Biological Processes.* Jour. Amer. Soc. Agron. 20, 1928, 747-754.