THE HYDROCYANIC ACID CONTENT OF SUDAN GRASS

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THESIS

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THE HYDROCYANIC ACID CONTENT
OF SUDAN GRASS

A Thesis Submitted to

the Faculty of

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By

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THESIS

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CONTENT

INTRODUCTION

It has long been known in countries where Sorghums are grown as forage that this crop frequently causes the death of cattle when they are allowed to eat it under certain conditions. The Sorghums have been studied extensively and considerable work has been done on Sudan grass. There is some confusion regarding the nomenclature of Sudan grass. It has been given various names by different authors. It is regarded by some botanists (1) as a separate species under the name *Sorghum sudanense*, Stapf., while others (2) consider it only a variety of Johnson grass and give it the name *S. halepense* var. *sudanense*. Hitchcock (3) in his recent publication gives it the name *S. vulgare* var. *sudanense* (Piper) Hitchc. There is also some confusion in the reports of the work done on Sudan grass due to the fact that most of it is reported merely as done on Sudan grass (4, 5, 6). Some Australian workers (2) contend that pure Sudan grass is non-poisonous but that the various Sorghum-Sudan hybrids are poisonous. This paper will consider in particular the poisonous properties of the commercial Sudan grass as grown at Michigan State College. The seed for this material was produced in Texas and obtained by the college from the Michigan Farm Bureau.

HISTORICAL

Prussic acid poisoning by the various sorghums has been studied extensively since the early part of this century. Poisoning by Sudan grass has been studied by comparatively few men over a much shorter time. In considering the history of this subject it is therefore desirable to consider essentially the history of Prussic acid poisoning by the Sorghums.

The first report of any great loss from Prussic acid poisoning was made by Pease (7) who reported that great numbers of cattle died from eating Sorghum in India in 1877. The year 1877 was one of extreme drought and was followed by the dry years of 1887 and 1895, which were also marked by the deaths of a considerable number of cattle from the same cause. Pease made a chemical study of the Sorghums in 1895 and came to the conclusion that the poison responsible was nitrate of potash present in some stems to the extent of 25% and especially abundant at the nodes. This observation was contradicted in 1900 by Hiltner (8) of Nebraska, who found that in most if not all cases there is not sufficient potassium nitrate present to produce the observed effects. He failed, however, to find the Prussic acid and came to the conclusion that the toxic effect which manifested itself at times was not due to a chemical poison inherent in the plant.

The fact that Prussic acid is present in the Sorghums was first reported in 1902 by Dunstan and Henry (9). In the same year Slade (10) suggested that a highly poisonous chemical compound might be produced by the action of an enzyme on a glycoside formed in a plant through a process of abnormal growth. Slade actually isolated Prussic acid from a sample of Sorghum late in 1902. Further work by Avery and Peters (11) conclusively proved that Prussic acid was the direct cause of Sorghum poisoning.

That Prussic acid poisoning is common in practically all Sorghum producing countries is evidenced by reports from Australia, Africa, Italy, China, Japan, Java, India, the West Indies, the Phillipines, Europe, and America. Actual accurate figures as to the number of deaths are not available, but Hiltner (8) states that 144 fatal cases were reported in a single year but unquestionably this represents only a small proportion.

Investigations by other workers have since established the presence of the Prussic acid principle in other plants belonging to the Sorghum family. Crawford (12) in 1906 reported it in Johnson grass. Francis (13) in 1915 reported it in Oklahoma-grown Sudan grass. The wide-spread occurrence of hydrocyanic acid in nature is illustrated by two papers by Rosenthaler (14) in which he lists 410 species, 148 genera, and 41 families as containing the poisonous principle.

The form in which the hydrocyanic acid occurs in the plant has been the subject of research by many workers. Slade (10) in 1901 suggested that the poisonous principle, then unknown, was produced by the action of an enzyme on a glycoside.

Avery (11) concluded from his work that it was evident that the hydrocyanic acid did not occur in a free state but rather in a combined form, probably as a glycoside as suggested by Slade. The conclusion of Avery was confirmed by Dunstan and Henry (9) who isolated dhurin, a glycoside readily hydrolized by the enzyme emulsin. The two English workers and the Americans, Slade and Avery (11), and Dowell (15) agree that the hydrocyanic acid occurs in the plant only in the form of a glycoside which is hydrolized during the early processes of digestion. Willaman (16), however, contends that the Prussic acid occurs not only as a glycoside but also uncombined or in a loosely combined form. Willaman has some support for this theory in the work of the Italians, Ravenna and Babini (6), who in 1909 reported the occurrence of free hydrocyanic acid in the leaves of cherry laurel, peach, and flax. The Italians, however, qualified their conclusions by indicating how readily a small amount of free hydrocyanic acid could be produced by autolysis during the progress of the experiment. Tuskunaga (17) in 1928 remarked that the amount of free hydrocyanic acid is greater in young plants while that of combined hydrocyanic acid becomes greater but decreases as the plant matures. Swanson (5) in 1921 concluded that the hydrocyanic acid is not present in the plant in the free state since it is not liberated by the action of acid on green material.

Investigations have revealed the fact that the hydrocyanic acid content of the various plants is not a constant quantity. Work has been done therefore in an effort to determine the effect of soil fertility, soil and atmospheric moisture, stage of maturity, and injury such as frost and drought on Prussic acid content. Studies have also been made to determine the distribution of hydrocyanic acid in the different parts of the plant.

The effect of soil fertility has been studied by several workers. Willaman and West (18), in 1915, working on Sorghum found that when grown on poor, infertile soil, added nitrogen may slightly increase the amount of hydrocyanic acid in the plant. With fertile soil and abundant nitrogen this result may not be produced. Brunnich (19), in 1903, and Alway and Trumbull (20), in 1909, concluded that, since fertilized plants contained slightly more hydrocyanic acid than unfertilized plants, heavy nitrogenous soils and favorable climatic conditions would increase the amount of acid.

There is some confusion in the literature regarding the effect of moisture on the hydrocyanic acid content of Sorghums. Some of the early work done on Sorghums seems to show that healthy plants in the best growing condition contained the most hydrocyanic acid. Avery (11), in 1902, came to the conclusion that stunted plants contained more Prussic acid than the healthy plants. Brunnich (19), in 1903, Alway and Trumbull (20), in 1909, and Willaman and West (18), in

1915, all reported that those plants stunted by a lack of soil moisture contained less hydrocyanic acid than did the green healthy plants. Milligan (21), in 1909, came to the conclusion that atmospheric humidity had a greater effect on the Prussic acid content than did the soil moisture. Swanson (5), in 1920, concluded from his data that healthy green plants contained more hydrocyanic acid than those growing under dry conditions.

The effect of the stage of maturity has been the subject of a great deal of research and has led, as Vinall (6) says, to almost complete agreement among farmers and chemists concerning the effect of maturity on the hydrocyanic acid content of Sorghum plants. The percentage of hydrocyanic acid in the plants decreases steadily from the time they begin to grow until they reach maturity if the growth is normal. Vinall compiled in a table data from the work of Willaman, in Minnesota, Menaul and Dowell, in Oklahoma, and Schroeder, in Uruguay. This data illustrates very clearly the fact that the hydrocyanic acid percentage varies as stated above. Acharya (22) found, in 1933, however, that while the percentage decreases gradually, the total amount of hydrocyanic acid in the plant at first increases and then rapidly decreases toward maturity.

There is apparently a consensus of opinion as to the distribution of the hydrocyanic acid in Sorghum plants. Willaman and West (18), and Breakwell (23) each came to the conclusion that during the first three or four weeks the hydrocyanic acid is concentrated in the stalk and from then on it decreases there but persists in decreasing percentages in the leaves until maturity. Swanson (5) reported similar work from which he concluded that the Prussic acid was almost entirely in the leaves.

The effect of injury on the Prussic acid content of the Sorghums is of great interest and has been studied extensively. Data presented by Vinall (6) and Acharya (22) confirmed the contention that there is considerable increase in the Prussic acid content of the Sorghums due to injury or drought. Acharya also contends that too intense sunlight, even in the presence of sufficient moisture, will stimulate the production of Prussic acid in the Sorghums.

Acharya, in 1933, made a study of the variations in the Prussic acid content of Cholam during the day. From his data he concluded that the hydrocyanic acid content is least in the morning and increases to a maximum at about 2 p.m., after which there is a gradual fall until 6 p.m. followed by a rapid decrease in the night. He adds further that the variation in Prussic acid content shows a striking parallelism to the variation in photo-synthetic activity and of protein metabolism in the plant and lends support to the hypothesis that the formation of cyanogenic compounds is a normal part of the protein metabolism of the plant. Menaul and Dowell (4), in 1919, considered the subject and came to

the conclusion that the hydrocyanic acid content was higher in the morning than in the afternoon.

The question of the hydrocyanic acid content of the various Sorghum-Sudan hybrids is of interest since there is often considerable chance for the accidental production of such hybrids. The work of Moodie, Ramsay and Finnemore and Cox on this subject has been cited by Meadly (2). Moodie (25), in 1929, concluded that pure Sudan grass was non-poisonous and perfectly safe for feed at any stage of growth. He said further that the Sorghum-Sudan hybrids may contain more hydrocyanic acid than the Sorghum parent. Ramsay (26), in 1929, came to the conclusion that most hybrids were more toxic than the Sorghum parent and that Sudan grass did not contain enough Prussic acid to be dangerous. Finnemore and Cox (27), in 1931, however, came to the conclusion that the hybrids were lower in hydrocyanic acid content than the majority of the Sorghums.

There has apparently been a consensus of opinion that second growth is of a higher hydrocyanic acid content than the original growth. Menaul and Dowell (4), in 1919, presented data demonstrating this.

The effect of haying is of interest since if the Prussic acid is removed or driven off when the material is dried much more Sorghum fodder can be used. Vinall (6) and Swanson (5) agree that when Sudan grass is made into hay it is no longer dangerous.

The amount of Sorghum or Sudan fodder which must be eaten to produce fatal results varies greatly with the conditions. That this is true is evident from the fact that the hydrocyanic acid content varies greatly. It may be influenced in varying degrees by any or all of the various factors discussed above. The lethal dose of hydrocyanic acid for a cow has been placed at 0.5 to 0.6 grams. On the basis of this Vinall concludes from the data of several workers that 7.6 pounds of green Sorghum or 18.9 pounds of fresh Sudan grass would be necessary to produce fatal results. This is on the basis of one hundred per cent liberation of the hydrocyanic acid. Since a cow will eat 80 to 100 pounds of fodder a day it is evident that Sorghum or Sudan grass of .0164 per cent and .0066 per cent respectively, as given by Vinall, would be extremely dangerous.

The methods of determining hydrocyanic acid have been the subject of considerable investigation. Francis and Connell (28), in 1913, made a comparative study of six suggested methods:

1. The silver nitrate method in which silver cyanide is determined gravimetrically was found to be applicable only for amounts of 1 milligram or more of hydrocyanic acid.

2. The ferro ferricyanide method, in which the blue color developed is compared to a standard, was found to be applicable only to amounts of more than .5 of a milligram.

3. & 4. The ammonia alum and lead acetate methods were found to be applicable only when more than .1% of hydrocyanic acid

was present.

5. The mercurous nitrate method was purely a qualitative method.

6. The thiocyanate colorimetric method was found to compare favorably with the silver gravimetric method. By this method the hydrocyanic acid is distilled into potassium hydroxide. A portion of the distillate is treated with ammonium sulfide, evaporated to dryness, acidified with hydrocholoric acid, filtered and treated with ferric chloride; the resultant cherry red color is compared with a standard.

Smith (29) is reporting the work of several collaborating chemists made the following conclusions:

1. The prussian blue colorimetric method was the longest and most complicated of the three studied and offered the most chance for error. It was applicable to material containing volatile halides.

2. The alkali titration method was the simplest in that it consists in the titration of the distillate (in sodium hydroxide) with standard silver nitrate to the first turbidity. The end-point in the titration was rather difficult, especially in the presence of organic matter.

3. The acid titration method was found to be the most accurate. In this method the hydrocyanic acid is distilled into standard silver nitrate, the silver cyanide filtered off, and the excess silver nitrate is titrated with standard potassium thiocyanate. The end-point in this case was not

ideal but it was considerably better than that for the alkali method.

Acharya (22), in 1933, studied the three methods listed above by Smith and came to the conclusion that the alkali titration method was best suited for a routine analysis of a large number of samples. Acharya used this method in his work which will be quoted later.

EXPERIMENTAL

The experimental portion of this thesis consists of (1) a study of the suggested methods and a selection of one of them, (2) a study of the effect of the stage of growth on the hydrocyanic acid content, (3) a study of the diurnal variations in the hydrocyanic acid content, (4) a study of the hydrocyanic acid content of some known hybrids, (5) a study of the hydrocyanic acid content of second growth and hay.

Study of Methods

The study of the methods resolves itself into two parts, (1) a study of the methods of determining the hydrocyanic acid itself, (2) a study of the application of the method to plant material.

Three methods of determining hydrocyanic acid were considered:

1. The Alkali Titration Method.

In this method the hydrocyanic acid is distilled into about 2% sodium hydroxide. The distillate is then titrated with 0.02 N silver nitrate until the first permanent turbidity appears. This method was found to be satisfactory with pure solutions, but unsatisfactory when used with the distillate from plant material. The distillate became dark colored when titrated and this darkening made it impossible to determine the end point of the titration.

2. The Prussian Blue Method.

Under this method the distillate is received in sodium hydroxide and made up to a definite volume. An aliquot portion of the distillate is concentrated below 70° by means of a vacuum pump and a condensor to 1 cc. or less. Freshly prepared ferrous sulfate and a small amount of potassium fluoride are added and the flask exausted for 5-10 minutes. The mixture is acidified with 30% nitric acid and the blue color developed compared with a standard.

It was found that known amounts of potassium cyanide could be determined accurately as shown by Table I.

TABLE I

DETERMINATION OF HYDROCYANIC ACID BY PRUSSIAN BLUE METHOD

Trial	Mg. HCN added	Mg. determined		Per cent recovery
1	1.2506	1.2142		97.09
2	"	1.2856		102.79
3	"	1.1967		95.69
4	"	1.3266		106.08
		Average	=	100.412

It was noticed that there were some variations in the color and a considerable variation in the values determined for the various samples (see Table I). This method was not used because there seemed to be considerable chance for error and also because it is applicable only for amounts of .5 milligram or more of hydrocyanic acid. In many cases the amount of hydrocyanic acid in a reasonable sized sample is considerably below .5 milligram.

3. The Acid Titration Method.

By this method the hydrocyanic acid is distilled into standard silver nitrate. The distillate is filtered through a Gooch crucible and the excess silver nitrate titrated with standard potassium thiocyanate using ferric alum indicator.

It was found as is shown by Table II that known amounts of potassium cyanide could be determined accurately.

TABLE II

DETERMINATION OF HYDROCYANIC ACID BY ACID TITRATION METHOD

Trial	Mg. HCN added	Mg. determined	Per cent recovery
1	2.000	2.0033	100.11
2	"	1.9175	95.88
3	"	1.9383	96.92
4	"	1.9682	98.41
		Average =	97.84

Since the Prussian blue method was not applicable to amounts of less than .5 milligram the acid titration method was tried with plant material. The material was produced in the Botany green house by the courtesy of Mr. Beeskow.

Two methods of preparing the sample for distillation were considered:

1. The Method of Acharya (22).

By this method the sample was cut up and ground in an iron mortar and extracted with water until the volume of the extract was 1000 cc. The pulp was then placed in a flask and covered with water and both were allowed to stand 24 hours. Then the pulp and 500 cc. of the extract were distilled and the hydrocyanic acid content determined. The second half of the extract was allowed to stand another 24 hours and then distilled. If this portion was found to give a higher value that value was used and the total hydrocyanic acid content of the sample calculated.

This method was checked and discarded since as Table III shows there was no hydrocyanic acid left in the pulp and there was practically no difference between the two portions of the extract.

TABLE III

THE DETERMINATION OF HCN BY THE METHOD OF ACHARYA (22)

Sample	Milligrams of HCN		
	Pulp	24 hour filtrate	48 hour filtrate
1	-	.864	.864
2	-	.567	.562
3	-	.637	.648
4	-	.648	.621

From the results obtained it was decided that this method of preparation was not necessary.

2. <u>The Method of the Association of Official Agricultural Chemists (30).</u>

In this method it was suggested that the sample be ground to pass a 20-mesh sieve, placed in a Kjeldahl flask with 100 cc. of water and macerated two hours at room temperature. Another 100 cc. of water was then added and the mixture was steam distilled into acidified silver nitrate.

Since there was such a discrepancy between the maceration periods suggested by the two methods a series of analyses at various intervals of time was made. For this work dried material from one of the Dairy Department pasture fields was used. The results are given in Table IV.

TABLE IV

THE DETERMINATION OF HYDROCYANIC ACID AFTER VARYING PERIODS OF MACERATION.

Sample	Time of Maceration	Mg. HCN Determined
1	0 hrs.	.615
2	2	.648
3	4	.637
4	6	.669
5	8	.675
6	12	.648
7	16	.632
8	20	.583
9	24	.702

This data indicated that the hydralysis was probably complete after a two-hour maceration period.

In order to make a thorough check of the methods selected a series of determinations was made in which potassium cyanide was added to the plant material and then determined. The results obtained are presented in Table V.

TABLE V

RECOVERY OF ADDED HYDROCYANIC ACID (AS KCN)

Trial	Mg. HCN added	Mg. in check sample	Total in test sample	Mg. recovered	Per Cent recovered
1	.3852	.756	1.1772	.4212	109.34
2	"	.8586	1.2096	.3510	91.12
3	"	.5616	.9072	.3456	89.72
4	.7704	.8154	1.5552	.7398	96.04
5	.7704	.6264	1.4256	.7992	103.74
				Average =	97.99

This data was considered sufficient to warrant the use of the acid titration and the method of the Association of Official Agricultural Chemists in the rest of the work reported here. The method of the Association of Official Agricultural Chemists was used with some slight variations. The sample was cut in small pieces and ground in an iron mortar. A one liter round bottomed flask was used instead of a Kjeldahl and 300 cc. instead of 200 cc. of water was added to the sample.

Effect of Stage of Growth

Material for the study of this subject was taken from the southeast portion of the first dairy pasture field.

A complete series of analyses was made from the time the material was 3-5 inches tall until it was 50 inches tall and headed out. All samples were taken at 10 a.m. The plants were cut close to the ground and taken immediately to the laboratory where they were cut up with scissors and mixed. From this material samples to be distilled and for dry weight were weighed out. Ten plants were also measured and weighed and the average taken. The samples for dry weight were dried to a constant weight at 100 degrees centigrade in a vacuum oven. The data obtained is presented in Table VI, and Fig. 1. The number of milligrams per 100 plants was calculated from the average weight per plant and the total number of milligrams in the sample used.

TABLE VI

EFFECT OF STAGE OF GROWTH

Material	Age in days	Height in inches	Wt./plant in grams	% HCN fresh wt.	%HCN dry wt.	Mg. HCN/ 100 plants
Sudan Grass	24	3-5	.12	.01302	.0609	1.563
"	26	3.5-5.5	.125	.01493	.0779	1.866
"	28	4-6	1.45	.01449	.0783	2.1
"	29	5.5	.15	.0126	.0835	1.89
"	32	8	.26	.01381	.0850	3.603
"	33	8.3	.42	.01099	.0763	4.575
"	36	12	.7	.00859	.0542	6.02
"	39	16	1.4	.00603	.0427	8.45

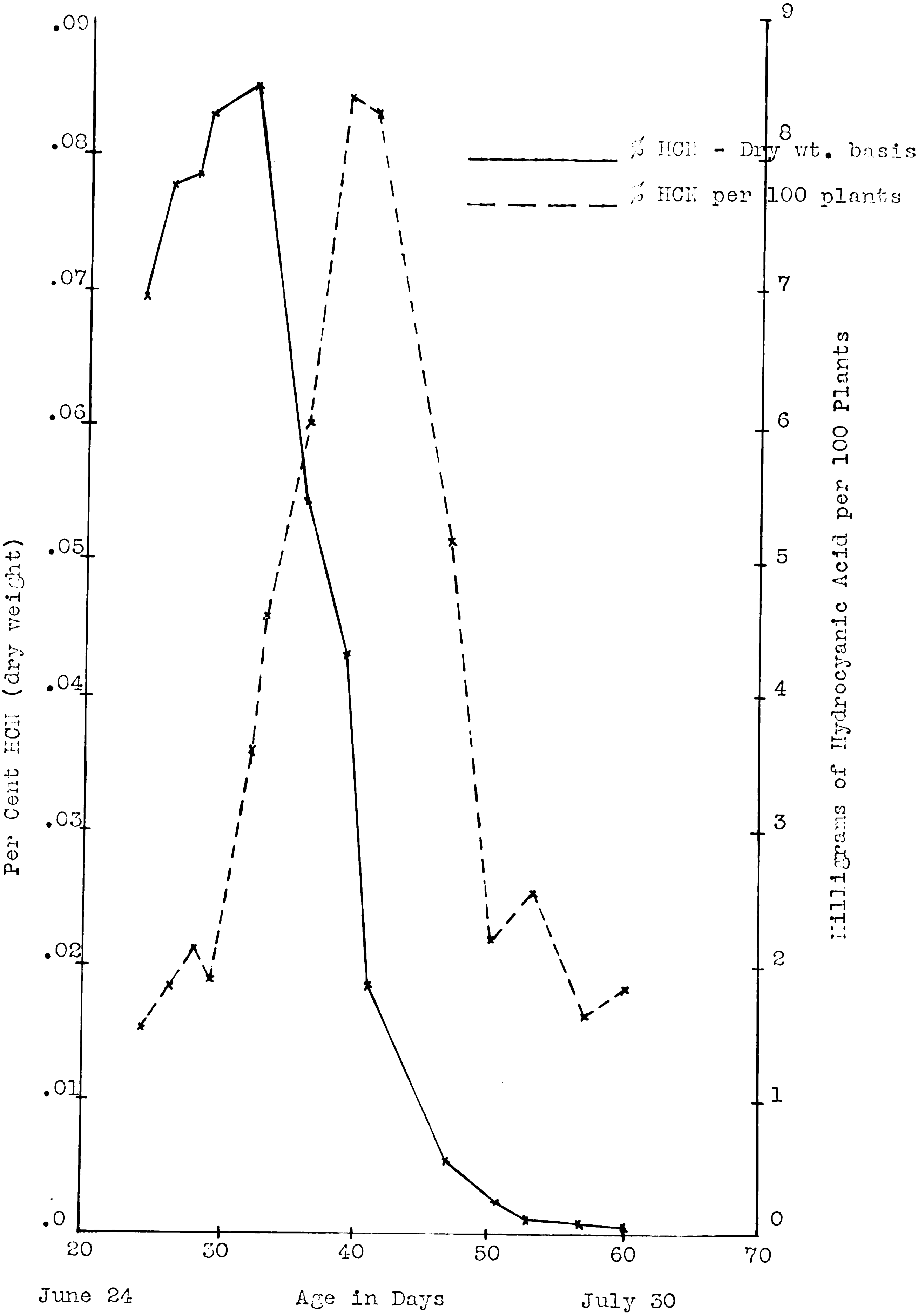

Fig. 1 - EFFECT OF STAGE OF GROWTH

TABLE VI (Continued)

Material	Age in days	Height in inches	Wt./plant in grams	% HCN fresh cut	% HCN dry cut	Mg. HCN/ 100 plants
Sudan Grass	41	24	3.3	.00252	.0130	8.31
"	47	31.7	6	.00085	.00526	5.13
"	50	31	4.6	.00048	.00237	2.2
"	53	36.7	7.3	.000342	.00205	2.5
"	57	47	13.4	.000121	.00069	1.64
"	60	50	16	.000112	.00059	1.8

Diurnal Variations

In order to obtain information on the diurnal variations in the hydrocyanic acid content of Sudan grass six series of analyses were made. The determinations were made at two-hour intervals between 6 a.m. and 6 p.m. The procedure used was as described under Effect of Stage of Growth. The data obtained is presented in Table VII and Fig. 2. The data for July 5, 10 and 22 represents original growth from the southeast portion of dairy field No. 1. Material from dairy field No. 3 was used July 17 and 26. Second growth material from field No. 1 was used August 12.

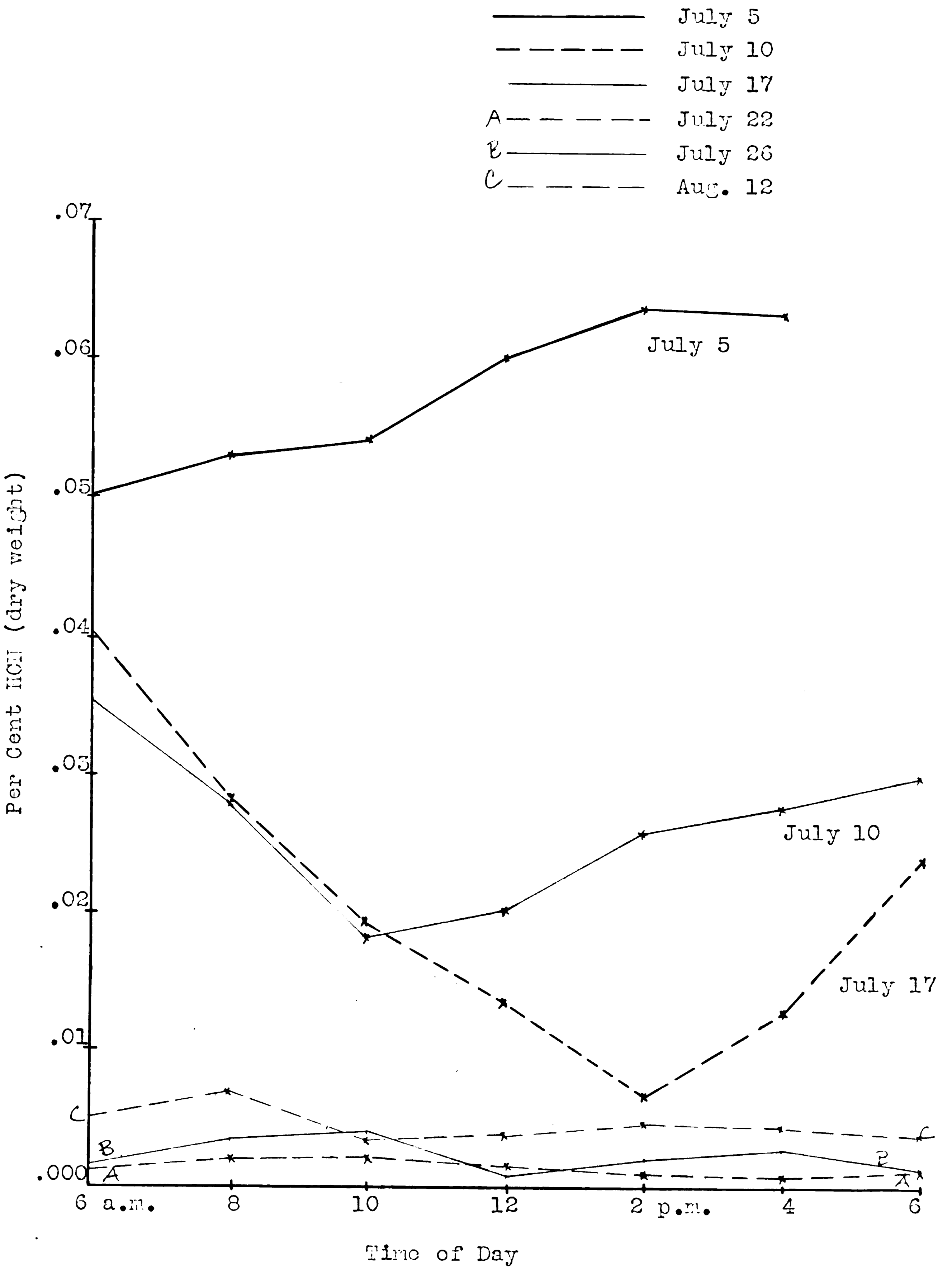

Fig. 2 - DIURNAL VARIATIONS

TABLE VII

DIURNAL VARIATIONS

Time of Day		Per cent HCN (dry weight) 6 a.m.	8 a.m.	10 a.m.	12 N.	2 p.m.	4 p.m.	6 p.m.
Date	Age in days							
July 5	36	.0507	.0529	.0542	.0603	.0636	.0633	-
" 10	41	.0353	.0278	.0180	.0200	.0256	.0272	.0295
" 17	37	.0408	.0280	.0188	.0132	.0066	.0123	.0232
" 22	53	.0012	.0019	.00205	.00149	..0010	.00075	.00105
" 26	46	.00176	.00346	.00375	.00098	.00194	.00278	.00122
Aug.12	2nd. growth 15 days old	.00508	.00528	.00278	.00386	.00457	.00444	.00387

In an effort to obtain an explanation of the observed variations the weather data for the days involved was obtained from the United States Weather Bureau at East Lansing. Table VIII presents the hourly temperature in degrees Farenheit and the sunshine in percentage of the total possible. The value 1 represents 100 per cent sunshine.

TABLE VIII

WEATHER ON DAYS DIURNAL VARIATION WAS STUDIED

Date	Time of Day	6	8	10	12	2	4	6
July 5	Temp. °F	70	73	84	87	88	87	87
	Sunshine	.92	1.0	1.0	1.0	1.0	1.0	1.0
July 10	Temp. °F	67	72	81	84	84	85	84
	Sunshine	.87	1.0	1.0	1.0	1.0	1.0	1.0
July 17	Temp. °F	61	69	81	84	82	82	81
	Sunshine	.77	1.0	1.0	1.0	1.0	1.0	1.0

TABLE VIII (Continued)

Date	Time of Day	6	8	10	12	2	4	6
July 22	Temp. °F	70	74	83	84	85	83	82
	Sunshine	0	.2	1.0	.4	1.0	.1	.9
July 26	Temp. °F	63	67	79	82	83	84	83
	Sunshine	.63	1.0	1.0	1.0	1.0	1.0	1.0
Aug. 12	Temp. °F	72	77	87	88	87	84	79
	Sunshine	1.0	1.0	1.0	1.0	1.0	1.0	1.0

The Hydrocyanic Acid Content of Some Hybrids.

A study of some Sorghum-Sudan hybrids was made in an effort to determine the relative amounts of hydrocyanic acid in known hybrids and the available Sudan grass and Early Amber Sorghum. The material used and its source are presented in Table IX.

TABLE IX

HYBRID MATERIAL AND ITS SOURCE

Material	Source
Early Amber Sorghum	C. R. Megee, Michigan State College
Sudan grass	" " " " "
Sorghum x Sudan (tall)	J. C. Ireland, Oklahoma A & M "
" " (short)	" " " " "
Orange Sorgo x Sudan(F_2)	R. E. Karper, Texas Agr. Exp. Sta.
Leati F.C. 6610 x " "	J. C. Stephens, U.S. Dept. Agr. Bu. Plant Indus.

All samples were taken at 1 p.m. and treated as previously described. This material was grown in the experimental plots of the Farm Crops Department. The data obtained is presented in Table X.

TABLE X

THE HYDROCYANIC ACID CONTENT OF SOME HYBRIDS

Material	Age in days	Height in inches	% HCN fresh cut	% HCN dry cut
Early Amber Sorghum	29	12.4	.0421	.223
" " "	47	42	.0103	.0757
" " "	58	63	.0036	.0261
Sudan x Sorgo (tall)	29	11	.0353	.2031
" " "	43	27	.0159	.1144
" " "	58	59	.0036	.0257
Sudan x Sorgo (short)	-	-	-	-
" " "	43	25.5	.0176	.1267
" " "	57	57	.0037	.0264
Sudan x Orange Sorgo	29	12.5	.0267	.183
" " "	44	38	.0095	.0896
" " "	61	51	.0039	.0191
Sudan x Leoti	30	15.7	.0273	.142
" "	44	35	.0038	.0224
" "	61	59	.0008	.0048
Sudan Grass	30	11	.0183	.1302
" "	47	39.6	.0068	.0405
" "	57	55	.0023	.0117

The Hydrocyanic Acid Content of Second Growth & Hay

In order to obtain information in this subject three analyses were made on material from the experimental plots of the Department of Farm Crops. A sample of the original growth was taken July 9, when the plot was being cut for hay. Eleven days later the second growth and the hay were analysed. The procedure was the same as described before. The results are presented in Table XI.

TABLE XI

HYDROCYANIC AICD CONTENT OF SECOND GROWTH & HAY

Material	Age in Days	Height, inches	%HCN (dry wt.)
Original growth	54	36	.0196
Second "	11	18.7	.0560
Hay	11	36	.00118

DISCUSSION

Methods for Determining Hydrocyanic Acid

The alkali titration method proved to be unsatisfactory. It was possible to determine known amounts of potassium cyanide in a pure solution, but the distillate from plant material became dark colored when titrated. This darkening Smith (29) says was caused by the presence of organic matter and made the titration practically impossible.

The Prussian blue colorimetric method proved to be fairly accurate in pure solutions as indicated in Table I. There were, however, variations in the colors which made them difficult to match. This method is long and complicated and offers considerable chance for error as indicated by the variations in Table I. These results confirm the opinion of Smith (29).

The acid titration method was found to be simpler than the Prussian blue method and less subject to error as indicated by the small variations in Table II. The end point, however, is not as clear as might be desired. These results again confirm the findings of Smith.

Method for Preparing the Sample

When the method of Acharya (22) for preparing the sample was compared with the simpler one suggested by the Associations of Official Agricultural Chemists (30) the former was found to be unnecessary as shown by Table III. Table IV shows that the two-hour maceration period suggested by the second method is sufficient.

Effect of Stage of Growth

The variations observed are not as a whole in agreement with other reports on the subject of seasonal variations. Vinall (6) states that there is almost complete agreement among farmers and chemists that the percentage of hydrocyanic acid decreases steadily from the time the plant starts to grow until it reaches maturity if the growth is normal. The data presented in Table VI and Fig. 1, however, indicates that the percentage of hydrocyanic acid at first increases and then gradually decreases toward maturity. There is, however, considerable difference in the material used. In the table given by Vinall, on the subject, most of the work was started on material 30-44 days old and 8-26 inches high. The weather conditions are not given. The material used in this work was 24 days old at the start and only 3-5 inches tall. The seed was planted May 30 and during the first month growing conditions were very poor. The normal rainfall for June is 3.51 inches while the rainfall for June of this year was 4.95 inches. The normal sunshine for the same month is 72% while this year June received only 54%. This excess rain and defficient sunshine may be a factor causing the discrepancy, or the fact that younger material was studied may be the explanation.

When the data obtained is calculated on the basis of milligrams of hydrocyanic acid per 100 plants as shown in column 6 of Table VI and in Fig. 1, it is shown that the total amount of hydrocyanic acid at first increases and then gradually decreases toward maturity. This is in complete agreement with the conclusions of Acharya (22) who is the only worker to report on this subject to date.

Diurnal Variations

The diurnal variations as given in Table VII and Fig. 2 show that with one exception the hydrocyanic acid content of Sudan grass tends to decrease during the morning and rise again in the afternoon. This is in exact disagreement with the conclusions and data of Acharya (22). (The exception mentioned was for very young material and unfortunately could not be repeated. This one case is in agreement with the work of Acharya.) It is, however, in agreement with the work of Menaul and Dowell (4). The work of Menaul and Dowell, however, is very incomplete in that the data is only for morning and afternoon of one date and the time of day is not mentioned.

No hard and fast conclusions should be drawn from this work on diurnal variations until it has been repeated for several years. The hydrocyanic acid content is small and the variations are proportionally small and there is considerable chance for error due to the fact that it is impossible to obtain exactly uniform samples.

Hybrids

The hydrocyanic acid content of the hybrids studied as given in Table X varied between that of Early Amber Sorghum and the commerical Sudan grass as grown here. This is not in agreement with the work, cited by Headly (2), of Moodie (25) or Ramsay (26), but confirms the conclusions of Finnemore and Cox (27). There is, of course, no reason to expect exact agreement, since the hybrids used are very likely not the same.

The Australian workers Moodie (25) and Ramsay (26) each contend that pure Sudan grass is not dangerous at any stage of growth. The data presented in Table VI indicates that the commerical Sudan grass as grown here this year would be dangerous at least until it was 16 inches high, at which time 18.3 pounds of green material contained about .5 ~~milli~~-gram of hydrocyanic acid. This amount according to Vinall (6) would be sufficient to produce fatal results if it were all liberated. This difference of opinion as to the toxicity of Sudan grass here and in Australia may be explained by a fact mentioned by Vinall. He says that there is much less Prussic acid poisoning reported in the southern than in the northern portion of the United States. All of Australia lies north of 40 degress south latitude while East Lansing lies between 42-43 degrees north latitude. The reason for this climatic variation would be a study of much interest.

Second Growth and Hay

The composition of the hay and resulting second growth as presented in Table XI shows that the hydrocyanic acid content of hay is considerably less than that of the original growth. This is in agreement with Swanson (5) and Vinall (6).

Table 11 also shows that the hydrocyanic acid content of the second growth at the stage tested was higher than that of the original growth. When, however, the hydrocyanic acid content of the second growth is compared to that of a sample of original growth of comparable size, Table XII, there is seen to be little difference. This contradicts the prevalent opinion that second growth is more toxic than the original. This may be explained by the fact that the second growth has not been compared with original growth of the same age.

TABLE XII

A COMPARISON OF SECOND AND ORIGINAL GROWTH

Sample and Source	Height in inches	Per cent HCN (dry wt.)
Second growth (Table XI)	18.7	.0560
Original " (" VI)	16	.0427

No conclusions should be made on this subject until a series of analyses has been made on both the original and second growth, taking each to the same stage of growth. It appears from the available data that the second growth may be little if any more dangerous than the original growth at the same stage of growth.

CONCLUSIONS

1. The alkali titration method was found to be the most satisfactory.

2. The percentage of hydrocyanic acid in the commercial Sudan grass at first rises and then gradually decreases toward maturity.

3. The commercial Sudan grass as grown at Michigan State College this year contains sufficient hydrocyanic acid to be dangerous at least until it is 16 inches tall.

4. The total amount of hydrocyanic acid per plant at first increases and then falls off toward maturity.

5. The hydrocyanic acid content varies during the day, being in general highest in the morning and evening and lowest in the middle part of the day.

6. The hybrids tested contained more hydrocyanic acid than Sudan grass and less than the Early Amber Sorghum.

7. The second growth does not necessarily contain more hydrocyanic acid than the original growth at the same stage of growth.

8. Haying causes a very decided decrease in the hydrocyanic acid content.

9. Due to the fact that this work covers only one season's work the above conclusions should not be taken as final until the work has been repeated during the course of several years.

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