T H E S I S

A PRELIMINARY STUDY OF THE INHERITANCE

OF HCN ACCUMULATION IN SUDAN GRASS

(SORGHUM VULGARE VAR. SUDANENSE (PIPER) HITCHC.)

Submitted by

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Otto H. Coleman ENTITLED A PRELIMINARY STUDY OF THE INHERITANCE OF HCN ACCUMULATION IN SUDAN GRASS (SORGHUM VULGARE VAR. SUDANENSE (PIPER) HITCHC.) BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF Science

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TABLE OF CONTENTS

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INTRODUCTION

Sudan grass is an important forage crop under dryland conditions in eastern Colorado. Often cattle on sudan grass pasture die from poisoning that has been attributed to prussic acid (HCN) since an autopsy gave a positive test for HCN.

The lethal properties of these pastures are assumed to be due to three possible causes: (1) A mechanical mixture of sudan grass with the forage sorghums, (2) the presence of sudan x sorghum hybrids, and (3) sudan grass of extremely high concentrations of HCN.

The present study was designed to determine the feasibility of the third assumption. If only the first two assumptions are true and the third false, then the problem of maintaining a safe sudan grass pasture becomes relatively simple; but if the latter assumption is true, then the problem resolves itself into the selection and propagation of lines inherently low in HCN accumulation.

LITERATURE REVIEW

Leemann (1935) reported that either the cyanogenetic glucoside and its enzyme or the enzyme alone occurs in 48 genera and 88 species of grasses. Many workers have reported HCN as a hydrolysis component of the glucoside in the sorghum species. Willaman (1917) gave evidence that HCN may be in both the glucosidic and non-glucosidic form in Sorghum vulgare. Gortner (1929) reported that the glucoside dhurrin occurs in sorghum species. It is structurally para-hydroxymandelonitrile and hydrolyzes into glucose, p-hydroxybenzaldehyde, and HCN. This glucoside has been the object of much study because of its importance in the use of sorghums for forage. Leemann (1935) reported that amygdalin and dhurrin are the only glucosides that have been reported in grasses.

The lethal dose was studied by Peters, et al. (1903), who reported that a heifer died within 10 minutes after eating 1.5 pounds of sorghum. The material contained 0.016 percent HCN. He found the lethal dose for sheep to be about 1 mg. HCN per pound of body weight. Steyn (1934) reported the lethal dose for cattle and sheep to be about 2.2 mg. HCN per kg. (2.2046 pounds) of body weight.

Leemann compiled the following table concerning HCN in sorghums:

Highest Percent HCN Reported by Various Workers (Leemann, 1935)

Investigator	Plant Used	Percent HCN
Avery (1903)	Sorghum vulgare	0.0140
Willaman and West (1915)	Sorghum from Minnesota	0.1140
Dowell (1919)	Andropogan sorghum	0.0514
Swanson (1921)	Sudan grass	0.0150
Pinckney (1924)	Sorghum on caloma sand + 502 pounds Nitrate per acre	0.1360

Leemann (1935) cites Bruennich for the following list of factors that may cause the excessive toxicity of plants:

1. Immature plants.
2. Rapid growth after rainfall.
3. Stunted growth by frost or failure of rain.
4. Insect infection during a dry season.
5. Presence of poisonous fungus.
6. Seasonal rains.
7. Precipitation of KNO_3 in the stems.
8. Lack of rain, excess of humidity, damp cloudy weather, variable temperature, and high temperature.

Steyn (1934) cites Burt-Davey, who with Couch (1932), Willaman and West (1915), and Pinckney (1924) found that the addition of nitrates to poor soils gave a definite increase of HCN in grasses.

Narasimha Acharya (1933) reported that Sorghum vulgare had a high HCN content on dry soil and a low content on wet soil. However, the amount of available nitrogen may be low enough in the wet soils to cause a nitrogen deficiency, and thus lower the HCN content of the plant. Willaman and West (1916) also have reported an increase of HCN in sorghum under drought conditions.

Menaul and Dowell (1920) reported the effects of rain and drought on the production of HCN in sudan grass. A rain on June 28 increased the HCN from 0.0053 percent on June 23 to 0.0069 percent on June 30. Their results were corroborated by Swanson (1921) who reported an increase of from 7 mg. to 16 mg. HCN in a sample of sudan grass after a period of dry weather (followed by a rain). He reported similar results for kafir.

Cool temperatures seem to cause an increase of HCN in sorghums according to the results obtained by Willaman and West (1915).

Ravenna, as reported by Leemann (1935), found that the HCN in sorghum increased in the afternoon. Willaman and West (1916) found the maximum HCN content at midday. Acharya (1933) reported that in Sorghum vulgare the HCN content increased from early morning to 2:00 p.m., with a slight decrease until 6:00 p.m., followed by a rapid decrease at night. He believed that there was a correlation between photosynthesis and HCN production.

Miller (1924) found that total sugars increase in both sorghum and corn from 4:00 to 6:00 a.m., reach a maximum from 12 Noon to 5:00 p.m., and gradually decrease until daylight the next morning. If other grasses react relatively the same, then glucoside synthesis may be somewhat behind photosynthesis. This lag may be due to nitrogen metabolism.

Tottingham (1935) cited Vicchia's report that sorghum seedlings contained no HCN until the chlorophyll appeared. Willaman (1915, 1916, 1917) and other workers have found that the HCN was greatest in Sorghum vulgare when the seedlings were about 3 inches high and decreased rapidly until there were only traces at maturity.

Hamant (1935) found that the total N in sorghum grain, which contained an emulsin-like enzyme, was about 0.535 percent, of which 0.046 percent was NO_3-N. The NO_3 of the germinating plant increased at first and later decreased. This loss was accompanied by a corresponding increase in HCN in a glucoside, formed exclusively in the plant, which contained fructose and hydroxybenzaldehyde as components with HCN.

Willaman and West (1915, 1916, 1917) reported that disease, frost, bruising, and trampling increased the HCN in sorghum. Steyn (1934) cited Wehmer who found a high HCN content in Sorghum saccharatum (Pers.) when the plants were wilted. Swanson (1921) reported that frosted sudan grass had large amounts of HCN which disappeared rapidly.

Sudan-sorghum hybrids have been reported by Ramsay (1929) as being higher in HCN than either pure sudan grass or pure sorghum. Collison (1919) reported a difference in the amount of HCN found in the varieties of Sorghum vulgare. Amber sorghum had the lowest content and Dwarf Hegari the highest.

Swanson (1921) found that the HCN in sudan grass did not materially diminish in silage. He also reported that the plants tested immediately after harvest contained large amounts of HCN; those dried in an oven less; those dried in the sun less; and those dried in the shade, slowly, had only traces. In his study of Sorghum verticuliflorum, Stapf., Leemann (1935) placed six tubes in a desiccator (Con. H_2SO_4-dehydrant) with Guignard paper as an indicator. After 2 days, he obtained a slight test for HCN. He heated the dry hay to 52° C. in solutions of HCl, NH_4OH, and distilled water (leaves of grass partly immersed) and obtained the following results:

1. Dilute HCl gave strong positive reaction for HCN.
2. Dilute NH_4OH gave strong positive reaction for HCN.
3. Distilled H_2O gave strong positive reaction for HCN.

Hay tested 5 months later yielded no HCN.

MATERIALS AND METHODS

Inbred Lines

In the spring of 1935, 91 selections of sudan grass were obtained from Mr. G. W. Deming, Assistant Agronomist, Division of Sugar Plant Investigations, United States Department of Agriculture, Rocky Ford, Colorado. He made self-fertilized selections from a uniform field of sudan grass during the season of 1933 and selfed several plants from each inbred selection in 1934. The seed of 1 plant per inbred line was obtained for 36 selections, 2 plants for 20 selections, and 3 plants for 5 selections. Two of these lines did not set enough seed to plant in the field in 1936.

In the fall of 1934, W. W. Austin made head selections from a pure seed plat of sudan grass located on the Agronomy Farm at Fort Collins, Colorado. Twenty-three of these selections had lavender seeds and were included in the study.

The total number of inbred lines of sudan grass grown in 1935 was 114.

Nineteen additional selfed plants of sudan grass were obtained from Mr. Deming in the spring of 1935. These consisted of 7 original (1933) selections, 2 of 4 plants each, 1 of 2 plants, 1 of 6 plants, and 3 of 1 plant. These 19 plants were included in the 1936 study, making a total of 131 inbred lines of sudan grass.

The lines of sudan grass are quite different in plant types. Most of them are between 5 and 7 feet tall but No. 36 is very short. Plate 1 pictures No. 36 (second from the left) in comparison to some of the taller lines. This line is extremely fine in comparison to the others. Plate 2 shows one of the very weak culmed lines. Most of the lines are dark seeded while a few are brown. Some have a thick waxy cuticle and are classed as glossy.

Comparison of Chemical Analytical Methods

The Prussian Blue method, as described by Viehoever and Johns (1915), was used. The plant material to be distilled was treated with 50 cc. of commercial concentrated H_2SO_4, many of the

Plate 1. Inbred Lines of Sudan Grass, Summer of 1936.

Plate 2. Inbred Lines of Sudan Grass, Summer of 1936.

distillates being badly colored with organic matter. In the final step of the procedure no color was obtained in the samples tested. In some there seemed to be a faint blue but even after warming slightly no readable color appeared. Johnson's (1916) sulfocyanate method, however, gave positive results for all samples.

To test further the value of the two methods of analysis and also to determine the possible use of H_2SO_4 in distillation, three 50-gram samples of Black Amber sorghum were treated with 50 cc. concentrated H_2SO_4, distilled and tested by the sulfocyanate method. Each of these distillates was colored with organic matter. Three other 50-gram samples of Black Amber sorghum from the composite were distilled over without the concentrated H_2SO_4 and each distillate was made up to 250 cc. in a standard measuring flask. These distillates were only slightly colored with organic matter. Each sample was divided into halves, then one half was analyzed by the Prussian Blue method and the other by the sulfocyanate method. The mean for the former was 26.064 p.p.m. HCN and for the latter was 26.667 p.p.m. of HCN. The samples treated with H_2SO_4 averaged 25.005 p.p.m., while those distilled over without H_2SO_4 averaged 28.326 p.p.m.

It was very difficult to adjust the pH of the mixture when the Prussian Blue method was used. Thus, even with known amounts of KCN with which to check the colorimeter, the color would not always be the best for colorimetric analysis since the mass of the blue molecule is very large, and unless the pH is adjusted within a very narrow range, precipitation will take place.

This difficulty was not found with the sulfocyanate method, since the mixture is nearly neutral in reaction and no strong base or acid is added. The reaction between $FeCl_3$ and KSCN takes place instantly, forming a deep red color $\{Fe(SCN)_3 3H_2O\}$.

A simple laboratory test was made with pure chemicals to determine the relative sensitivity of the Prussian Blue and sulfocyanate methods of estimating HCN in solution.

A stock solution of 10 mg. KCN was made up to 20 cc. from which different concentrations were made as reported in Table 1; 14.9 mg. KSCN was treated in a similar manner. The actual CN reported as HCN in solution varied from 216 to 1 p.p.m. A concentration of 26 p.p.m. HCN was the color standard for the sulfocyanate method.

The degree of agreement of the estimated concentrations of HCN in solutions with the theoretical amount was determined by the use of the X^2 test (Fisher, 1934). Since the Prussian Blue method gave a X^2 of 0.4490 for 9 groups with $p > 0.99$, the latter method is evidently more accurate for the determination of small amounts of HCN.

Fig. 1 indicates the narrow range in which the Prussian Blue method is effective. The sulfocyanate method, however, gives a very close estimate of the theoretical HCN in solution throughout the entire range of concentrations used.

Table 1. Comparison of the Sensitivities of the Prussian Blue and Sulfocyanate Tests for Small Quantities of HCN

10 mg. KCN equivalent to 4.151 mg. HCN (Prussian Blue Test)
14.9 mg. KSCN equivalent to 4.143 mg. HCN (Sulfocyanate Test)

Prussian Blue		Sulfocyanate		
Estimated HCN in solution	Theoretical HCN in solution	Estimated HCN in solution	Corrected estimation HCN in solution	Theoretical HCN in solution
p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
281	206	216	215	207
128	103	106	105	104
52	52	53	52	52
23	26	26	25	26
2	13	13	12	13
0	6	6	5	6
0	3	3	2	3
0	2	2	1	2
0	1	1	Trace	1
X^2 55.0276		0.4490	1.9342	
p Very small		>0.99	>0.98	

A correction was made for the small amount of color due to the addition of a slight excess of $FeCl_3$. This corrected value gave a X^2 value of 1.9342 with $p > 0.98$ as compared with the X^2 of 0.4490 with $p > 0.99$ for the uncorrected estimate. It is apparent that the correction is not necessary.

Then, if 50 grams of plant material are used and HCN is present in the amount of 1 p.p.m., the extract made up to 20 cc. will have an HCN concentration of 2.5 p.p.m., which is well within the limits of quantitative estimation by the sulfocyanate method. The spread between the theoretical value and that found by the

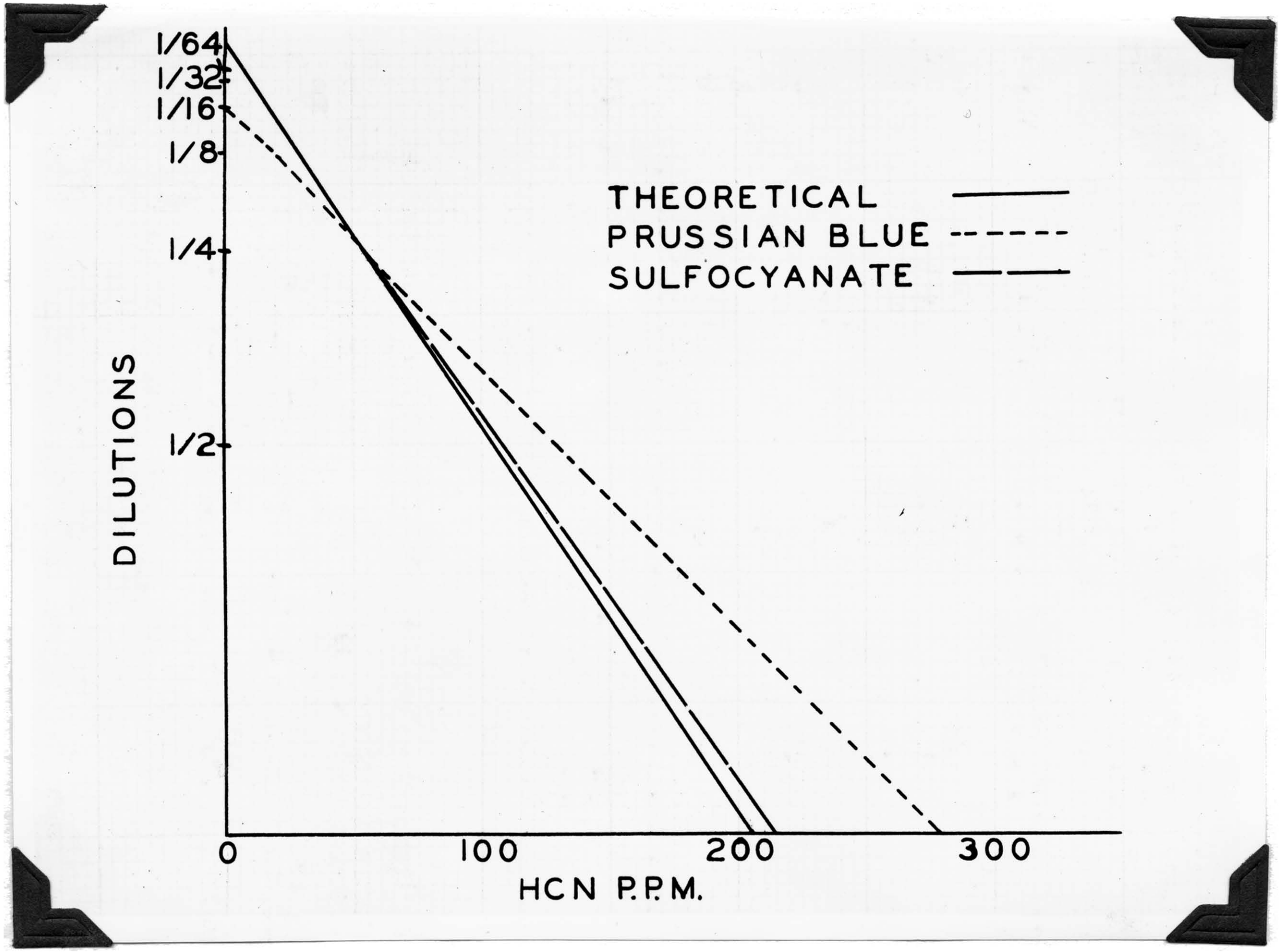


Fig. 1. Comparison of the Sensitivities of the Prussian Blue and Sulfocyanate Tests for Small Quantities of HCN.

sulfocyanate method increases from 0 p.p.m. at 1 p.p.m. concentration to 9 p.p.m. at 207 p.p.m.

Smith (1934) made a comparison of the old alkaline titration method of determining HCN in plant material with the acid titration method and the Prussian Blue method. He found that the alkaline method was the most variable since the end point was hard to determine and, consequently, he advised dropping this method from the official list. The variability of the 3 methods was as follows: Alkaline, 120-1033 p.p.m.; acid, 97-143 p.p.m.; and Prussian Blue, 80-259 p.p.m. Later, Green and Williams (1936) reported a modified alkaline method, which used a AgI precipitate as the end point. This was much more reliable. They also found that the Prussian Blue method was unreliable because some of the work must be done under reduced pressure. The adjustments of pH were very difficult, and the blue was not as intense as the red sulfocyanate. They recommend their modified alkaline method and a photoelectric method developed by Bartholomew and Raby (1935). The latter seemed to be more consistent and the results were very similar to the sulfocyanate method. A statistical analysis of the data presented by Green and Williams indicated that for a comparison of the results of different workers, the sulfocyanate method was less variable than the modified alkaline method, since the mean for the former was 136.78 with a coefficient of variability of 8.50 percent, while the latter was 179.84 with a coefficient of variability of 12.39 percent. It is apparent, then, that the sulfocyanate method is a better measure for comparing relative amounts of potential HCN in plant material than the modified alkaline method.

Laboratory Study of the Sulfocyanate Method

A laboratory check on the sulfocyanate method was made in the summer of 1936. In this study, pure chemicals and green plant material were analyzed. A series of 8 amounts of KCN were dissolved in water, made up to about 250 cc., (5 cc. dilute H_2SO_4 added) and distilled. Two consecutive 100 cc. distillations were made and kept separate. The mean KCN estimated for the first 100 cc. distillation was 11.67 mg., and for the second was 0.15 mg. or 1.29 percent of the first. Eight samples of sudan grass and 4 samples of Black Amber sorghum were tested in the same manner as described above. The mean of the first 100 cc. distillate from sudan grass was 38.75 p.p.m. HCN. The mean of the second was 1.38 p.p.m. or 3.55 percent of the first. For the sorghum, the first 100 cc. gave a mean of 61.30 p.p.m. HCN, and the second 15.90 p.p.m. HCN or 25.94 percent. An explanation for the behavior of the sorghum is not possible at the present time. In general, the first 100-125 cc. distillate carried most of the HCN available from the plants of sudan grass. It is significant that one of the sudan grass samples had well over 100 p.p.m. HCN. Thus, the amount of HCN present had no apparent effect on the amount carried in the second 100 cc. of distillate.

From the study with pure chemicals, it was found that not all of the KCN in the original sample was carried over in the final analysis. For example, the mean KCN used was 26.13 mg. and the recovered 11.67 mg. The actual was 2.24 times as large as the estimated.

An attempt was made to determine the stage during analysis at which the HCN was lost. About 100 cc. of distilled water were added to 75 mg. KCN, yellow $(NH_4)_2S$ was added and the solution was placed in a 250 cc. Erlenmeyer flask and evaporated on the hot plate. Glass tube connections were made in relay with a flask holding a dilute solution of H_2SO_4 and then on to a flask of dilute KOH. The contents of each flask were analyzed for HCN. Of the 75 mg., 23 were recovered from the first flask, 0.48 from the second, and 0.40 from the third. The actual was 3.14 times as great as that estimated by analysis.

Four extractions of each of 6 solutions of pure chemicals were made with acetone, the mean of the first being 17.23, the second 0.35, the third 0.11, and the fourth 0.09. Three extractions were usually made. The mean of the fourth extraction was 0.59 percent of the sum of the means of the first 3 extractions.

To check the analysis further, three 2 mg. solutions of KSCN were made up in acetone. The first was evaporated to dryness on the hot plate, the second was heated 5 minutes after drying, and the third was heated 15 minutes after drying. The results were about the same, being 2.27, 2.17, and 2.34 mg. of KSCN, respectively.

Pure chemicals were used to test which of the 4 following forms of sulfur was the best: (1) $(NH_4)_2S$, concentrated solution with some free sulfur but no extra sulfur added; (2) a concentrated solution of K_2S, (3) a concentrated solution of K_2S plus an excess of flowers of sulfur, and (4) a concentrated solution of $(NH_4)_2S$ plus an excess of flowers of sulfur. The latter was divided into two tests: (a) evaporation to dryness on the hot plate, medium heat, and (b) evaporation to dryness on a water bath. Four different amounts of KCN were used, 1, 10, 20, and 50 mg. Only one sample of each amount was used in testing (1) and (2); two samples of each amount were used in testing (3) and (4). Table 2 summarizes the results of this study.

When either $(NH_4)_2S$ or K_2S was used without adding sulfur, most of the KCN was lost, but even then the samples held their original positions according to KCN concentration. But when either K_2S or $(NH_4)_2S$ was used plus an excess of sulfur, from one half to two thirds of the KCN was recovered. The use of the hot plate had little or no effect on the recovery of KCN as KSCN. Whether this loss in percentage is constant for all amounts of KCN present or whether it increases with the amount cannot be determined from these meager data. Up to 20 mg. KCN, the percent loss seems to be about the same, but for the 50 gm. sample the loss was nearly one half. That difference, however, may or may not be a true picture of the usual circumstance.

The study, to date, would indicate that the major loss

Table 2. The Effect of Different Forms of Sulfur and Different Methods of Evaporation on the Amount of KCN Recovered from Known Quantities

	1 mg. KCN	10 mg. KCN	20 mg. KCN	50 mg. KCN
1	0.7204	0.8271	1.0075	4.6708
2	0.8133	1.3863	8.5394	--
3	0.7184	7.1162	11.8603	21.3486
4 a	0.6517	4.9779	13.3952	28.5919
b	0.6929	7.2389	15.6324	26.6858
Ave. of 3 and 4	0.6876	6.4444	13.6293	25.5421
Percent of theoretical	68.76	64.44	68.15	51.08

takes place during the transformation of KCN to KSCN.

The estimated HCN p.p.m. for the summer of 1935 was very low. Table 3 indicates that laboratory technic may have had some influence on the results, since the average for July 29, in 1935, was the highest average of the 3 dates, while it was lowest in 1936.

Table 3. Amount of HCN in Sudan Grass for Each Date Sampled in 1935 and 1936

Date Sampled		No. Lines		HCN	
1935	1936	1935	1936	1935	1936
				p.p.m.	p.p.m.
7/22	7/21	28	51	1.6	33.03
7/25	7/25	35	50	1.6	34.46
7/29	7/29	42	30	2.4	31.69

In the summer of 1935, the Prussian Blue method of analysis was used but, as has already been pointed out, this method failed to give any positive results. Consequently, the distilled samples remained in the laboratory several days before the sulfocyanate method could be perfected and used. A laboratory check on the effect of leaving the solution of KCN, in an alkaline medium, standing for several days was made. When 1, 10, 30, 60, and 100 mg. KCN were used and the solution left standing 25 days, the amount of KCN estimated was 0.0422, 0.0523, 0.0471, 0.0687, and 0.0516 mg., respectively. There can be little doubt that the July analyses of 1935 are of little value in estimating the HCN in the plant material.

Sampling Technic

Field Samples, 1935-1936.--Tillers from plants in the late boot stage were cut off just above the ground in July, 1935 and 1936. Several plants in each row were sampled and the resulting tillers bulked into one composite sample.

The normal green leaves on the under side of frosted plants were taken for samples and bulked from each inbred line in October, 1935. The leaves included the first 6 from the soil surface. The sorghum leaves were in the same general location on the culm, but were partially damaged by frost.

All the field samples were analyzed in duplicate in the laboratory.

Drought Samples, 1936.--At least 4 plants from each plat were taken for a sample when the plants in the dry plats were at the temporary wilting stage. Two samples were taken from each plat, and each sample was analyzed in duplicate in the laboratory. Table 4 gives the results of the statistical interpretation (Tippett, 1931) of these analyses. The variance due to sampling in the plat was much greater than the variance due to laboratory

Table 4. Sampling Technic, 1936
Drought Study for HCN Accumulation

Variance due to	D.F.	Sums of Squares	Mean Squares	F
Blocks	1	126.28	126.28	
Selections	3	38,782.44	12,927.80	4.35*
Treatments	1	1,080.47	1,080.47	
Tr. x Sel.	3	768.12	256.37	
Error	7	20,032.35	2,970.09	
Total Plats	15	60,790.66		
Samples within plats	16	7,168.48	448.03	6.72**
Analysis within samples	32	2,132.28	66.63	
TOTAL	63	70,091.42		

* 5 percent point
**1 percent point

analysis. The F value (Snedecor, 1934) was 6.72 with $p < 0.01$, i.e., the chances of the laboratory variance being as great as that of field sampling, or greater, are less than 1 in 100 trials. The practice of taking the tillers from at least 8 or 10 plants, however, should aid in obtaining a valid estimate of the composition of that particular inbred line.

Laboratory Technic

Each sample was placed in a 1-liter open-mouthed bottle, sealed with a rubber stopper. The samples were taken into the laboratory, ground in an ordinary hand-power rotary food grinder, and weighed immediately. Where possible, 50-gram duplicate samples were analyzed for HCN.

The method of analysis was described by Johnson (1916) as a modified Francis and Connell procedure, and was further altered to meet the requirements of this study.

Each sample was washed into a 250 cc. Erlenmeyer flask which was filled to within one inch of the top with distilled water and allowed to stand over night.

The next day each sample was washed with 50 cc. distilled water into a Kjeldahl flask and about 100 cc. were distilled over into a 125 cc. beaker containing 5 cc. of 4 percent KOH solution. To the distillate were added about 2 cc. of saturated yellow $(NH_4)_2S$ solution and the treated solution was evaporated to dryness on an electric hot plate (low heat) at a temperature just below the boiling point of water. To the residue, which was cooled to room temperature, were added 10 cc. of acetone. The insoluble matter was rubbed with a small glass pestle to insure thorough extraction of KSCN. This extraction was repeated twice with 5 cc. of acetone, and the combined acetone extracts were evaporated to dryness on the same hot plate and cooled to room temperature.

The residue was taken up with about 10 cc. of distilled water and about 0.5 cc. of 2 percent $FeCl_3$ solution were added. The red color appeared immediately. More $FeCl_3$ was added, one drop at a time, until no color change was noted, then 0.5 cc. more were used. The solution was made up to 20 cc.

The color was matched by a standard made up of one mg. of KSCN, taken up in 10 cc. of distilled water, treated with one cc. of $FeCl_3$ and made up to 20 cc. The Kelt colorimeter was used. This colorimeter is accurate to dilutions of 1/45 of the standard.

Design of Experiments

In 1935 the inbred lines were laid out in the order of their filing number. One row of each line was planted. In 1936 a random distribution was used for the field trials. Lack of seed necessitated the use of only one row for each inbred line per year.

A preliminary study on the effect of drought on the accumulation of HCN in sudan grass and Black Amber sorghum was made. One series of 9 plats, each 13' x 10', was covered with canvas similar to that reported by Robertson, et al. (1934). Three feet on each side of the series were planted to 2 rows (1 foot apart) of bulk sudan grass. The end plats and each alternating plat in the series were planted to bulk sudan grass. The remaining 4 plats were planted with 2 rows each of 3 inbred lines of sudan grass and 1 line of Black Amber sorghum, as indicated in the planting plan, Fig. 2. The plats were planted on May 28, 1936 and all plats were given a 1-inch irrigation on June

Fig. 2.

<u>Planting Plan for Drought Study</u>

1936

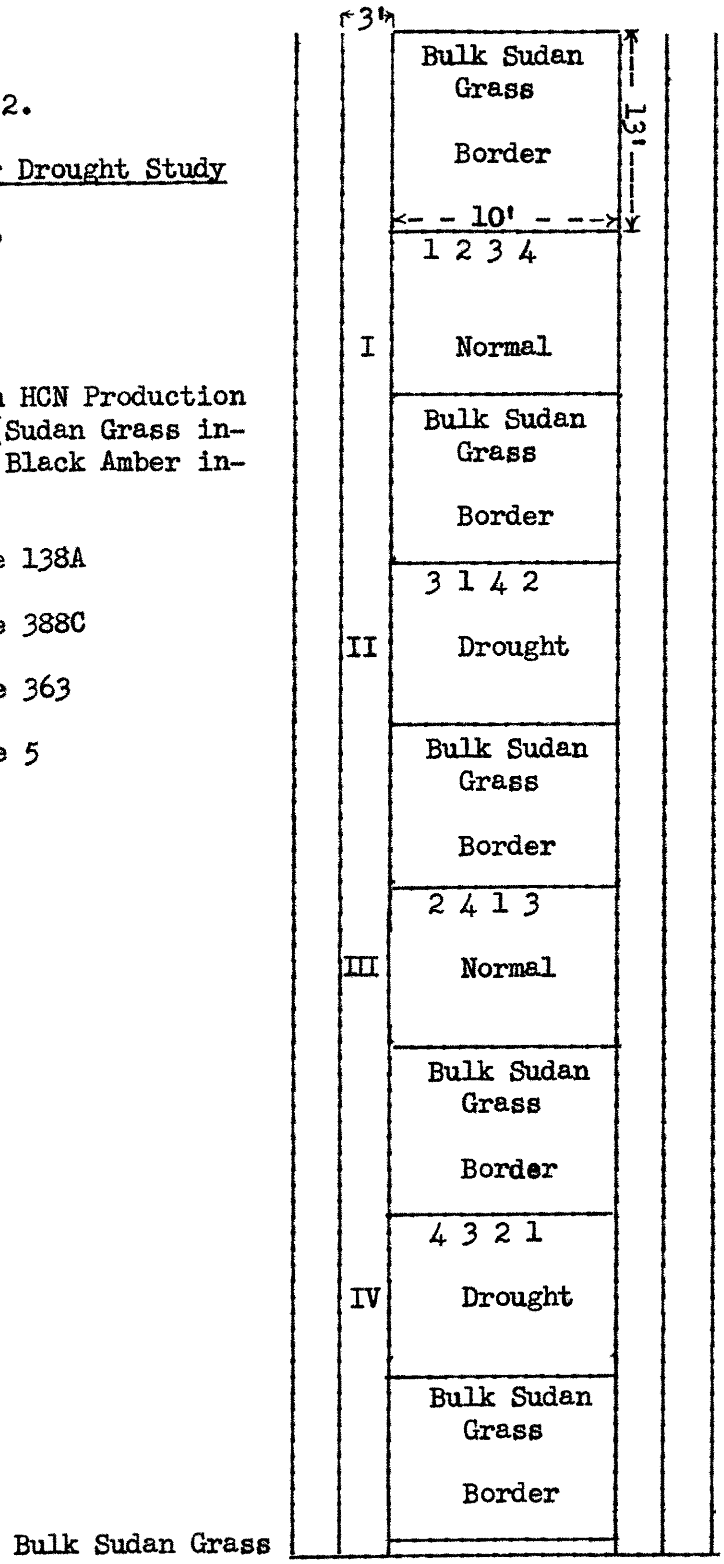
Effect of Drought on HCN Production in Sorghum vulgare (Sudan Grass inbred selections and Black Amber inbred selections)

1 = Sudan Grass Line 138A

2 = Sudan Grass Line 388C

3 = Sudan Grass Line 363

4 = Black Amber Line 5



1. Plats I and III were given a 4-inch irrigation on July 20. The whole series was sampled and plant height and leaf width measurements made on August 1.

EXPERIMENTAL RESULTS

HCN in 1935:1936

A comparison between the July analyses of 1935 and 1936 cannot be made since the July analyses of 1935 are of little or no value in determining the relative amounts of HCN in the various inbred lines of sudan grass.

The October samples of 1935, however, were analyzed by the improved method and the results should be comparable even though the plants were frosted at the time of sampling. The 14 lines, when compared for the 2 years (Table 5) gave a correlation coefficient of +0.2603, $p > 0.1$ (Fisher, 1934). This low correlation coefficient was evidently due to 2 of the selfed lines. Line 363 contained 91.1 p.p.m. HCN in October, 1935, and 25.3 p.p.m. in July, 1936; Line 389 had 20.9 p.p.m. HCN in October, 1935 and 61.7 p.p.m. in July, 1936. Whether or not these two selections react differently when frosted is open to question, but when they were left out of the comparisons the correlation coefficient was +0.8254, $p < 0.01$.

In 1936 the HCN p.p.m. varied from a low of 7.9 p.p.m. HCN for Line 371 to 98.0 p.p.m. for Line 376. Ten lines of sudan

Table 5. HCN Content of 14 Inbred Lines of Sudan Grass Grown in 1935 and 1936

Line No.	Date Sampled: October 1935 HCN (p.p.m.)	Date Sampled: July 1936 HCN (p.p.m.)
138A	17.0	24.9
140A	67.5	41.2
150B	12.1	14.6
153B	21.5	33.3
160	20.8	23.6
162	50.0	31.2
165A	61.7	31.7
166A	71.0	32.7
206B	20.2	18.3
303C	81.3	45.7
363	91.1	25.3
389	20.9	61.7
404	18.4	26.7
405	34.1	30.9
Mean	41.97	31.56
Correlation coefficient for all lines	$+0.2603,\ p > 0.1$	
Correlation coefficient excluding lines 363 and 389	$+0.8254,\ p < 0.01$	

grass were higher than Black Amber Lines 2 and 5, but none were as high as Black Amber Line 1.

Progeny Test

Two or more S_3 (selfed three generations) progeny of each of 24 original selfed selections were planted in 1936 and tested for HCN. Table 6 gives the results of this test. Some of the original selections were apparently quite homozygous for HCN accumulation, while others appear to be segregating for high and

Table 6. The Effect of Three Years of Selfing on the HCN Accumulation in Sudan Grass Lines

Selection No.- 1933	1936 Progeny	1936 HCN(p.p.m.)	Selection No.- 1933	1936 Progeny	1936 HCN(p.p.m.)
137	A	42.7	185	A	14.4
	B	52.1		B	16.7
138	A	24.9	206	A	42.7
	B	25.6		B	18.3
	3[a]	11.7		1-1[b]	43.3
	5	21.1		1-2	28.3
				4	26.5
140	A	41.2		5	22.8
	B	24.9			
			214	A	27.3
147	A	35.8		B	25.3
	B	33.3		C	22.2
150	A	29.0	228		19.1
	B	14.6		1	25.1
	9	30.0		2	18.5
				3	25.1
152	A	56.5		4	26.0
	B	15.9			
	3	20.7	280	A	51.3
				B	38.3
153	A	36.6		C	46.3
	B	33.3			
	1	33.3	296	A	33.7
	2	66.7		B	32.4
	7	75.8			
	8-1[b]	48.3	303	A	41.2
	8-2	47.0		B	39.7
	8-3	55.6		C	45.7
155	A	25.4	322	A	20.3
	B	34.0		B	27.8
165	A	31.7	329	A	29.0
	B	54.6		B	66.7
166	A	32.7	341	A	33.7
	B	18.2		B	50.5
				C	47.6
				6	61.7
167	A	32.4			
	B	10.4			

Table 6 (continued).

Selection No.- 1933	1936 Progeny	1936 HCN(p.p.m.)	Selection No.- 1933	1936 Progeny	1936 HCN(p.p.m.)
386	A	13.7	388	A	61.3
	B	24.9		B	74.1
387	A	40.7	Black Amber[c]	1	141.8
	B	40.7		2	58.5
				5	54.6

[a] The numbered lines in 1936 came from Deming in the spring of 1936.

[b] The first number indicates 1934 plant and the second number the 1935 plant.

[c] Each line selected and selfed first in 1935.

low HCN accumulation. Both reselections of Line 185 were low in HCN accumulation in 1936; A had 14.4 p.p.m., and B had 16.7 p.p.m. Lines 138, 214, 228, 322, and 386 were relatively uniform and moderately low in the accumulation of HCN, while Lines 137, 147, 155, 280, 296, 303, and 387 were relatively high and uniform. The highest uniform line was 388. The uniformity of these lines indicates a possible differential inheritance of the ability of sudan grass to accumulate HCN. Contrariwise, there were some lines that did not show uniformity in HCN accumulation; among these are 152, 153, 167, 206, and 329. The mean of some of these lines was high and of others low, but the extremes were too far apart to be explained by error in laboratory analysis. Line 152 ranged from 15.9 p.p.m. HCN for B to 56.5 p.p.m. for A. Lines 153, 167, 206, and 309 also had a wide range in HCN accumulation. Line 206 seems to be segregating for high and low amounts of HCN accumulation since the two lines selfed in 1935 from 206-1 had widely different HCN contents, i.e., 206-1-1 yielded 43.3 p.p.m. HCN and 206-1-2 yielded 28.3 p.p.m. HCN. Line 153, however, gave indications of becoming uniform for HCN accumulation. The progeny of this line selected from the 1934 plant 153-8 had similar values for HCN.

The behavior of the three inbred lines of Black Amber sorghum is significant because the HCN in these lines varied from 54.6 p.p.m. to 141.8 p.p.m.

Relation of HCN to Agronomic Characters (Phenotypes)

A study was made of the relation between certain plant characters and HCN accumulation in the various lines in 1936. The summarized data for this investigation are reported in Table 7.

Table 7. The Relation Between Plant Characters and HCN Accumulation in Sudan Grass Lines, 1936

Character	No. Lines	Mean HCN (p.p.m.)
All lines	131	33.27
Glossy leaves	74	30.40
Non-glossy leaves	57	36.99
Coarse stemmed	53	34.30
Fine stemmed	78	32.56
Small seeds	16	29.31
Medium seeds	54	34.29
Large seeds	61	33.57

There was, clearly, no relation between HCN accumulation and glossy leaves, texture of stems, or size of seeds.

Where possible, 20 plant height measurements (in inches) were made for each inbred line. The width of the second leaf from the top of each of 20 plants per line, where possible, was measured to the nearest 1/32 inch. The mean of these 20 measurements was used as the measure of height and leaf width, respectively, for each line. The correlation coefficient (Table 8) for HCN and plant height was -0.0580, $p > 0.1$, and for HCN and leaf width was +0.0322, $p > 0.1$. There is no apparent relation in the lines tested between HCN and plant height or leaf width.

Since some of the lines were much more variable in plant height or leaf width than others, correlation coefficients were calculated for HCN, plant height, and leaf width, using only those lines which were fairly uniform for plant height or leaf width, respectively. The correlation coefficient for plant height was -0.0967, $p > 0.1$, and for leaf width was -0.1963, $p > 0.1$. The lines fairly uniform for both plant height and leaf width had correlation coefficients for HCN and plant height and leaf width, respectively, of -0.1889, $p > 0.1$ and -0.2812, $p > 0.1$. The variability of height or leaf width had little or no effect on the relation between HCN and plant height or leaf width.

No relation between HCN accumulation and time of maturity was found. The early, medium, and late selections had means of HCN, respectively, 33.03, 34.46, and 31.69 p.p.m. obtained from

Table 8. The Relation of Plant Height and Leaf Width to HCN Accumulation in Inbred Lines of Sudan Grass in 1936

Relationships Studied	No. Lines Used	Correlation Coefficient	p Value
	All Inbred Lines		
Plant Height and HCN Accumulation	131	-0.0580	>0.1
Leaf Width and HCN Accumulation	131	+0.0322	>0.1
	Inbred Lines Uniform for Plant Height and Leaf Width, Respectively		
Plant Height and HCN Accumulation	75	-0.0967	>0.1
Leaf Width and HCN Accumulation	32	-0.1963	>0.1
	Inbred Lines Uniform for both Plant Height and Leaf Width		
Plant Height and HCN Accumulation	21	-0.1889	>0.1
Leaf Width and HCN Accumulation	21	-0.2812	>0.1

51, 50, and 30 lines.

Relation of HCN to Insect Damage

An estimate of grasshopper and aphid damage was made for each line. The correlation coefficient for HCN versus grasshopper damage was +0.0839, $p > 0.1$, and for aphid damage was +0.0421,

$p > 0.1$. These data indicate that there was no relation between HCN and damage due to grasshoppers and aphids.

Drought Study

The effect of drought on HCN accumulation, leaf width, plant height, and percentage of dry matter of sudan grass and Black Amber sorghum is recorded in Table 9. The HCN content of Black Amber was shown to be statistically greater than the sudan grass lines, except 388C.

Table 9. The Effect of Drought on HCN Accumulation, Leaf Width, Plant Height, and Percentage of Dry Matter of Three Sudan Grass Inbred Lines and One Black Amber Sorghum Inbred Line in 1936

Selection No.	Average HCN Oven-dry Basis	Average HCN Green Plant Basis	Average Plant Height	Average Leaf Width	Average Dry Matter
	(p.p.m.)	(p.p.m.)	(inches)	(1/32")	(Pct.)
138A	143.9	30.8	50.91	20.56	21.58
388C	274.3	60.7	48.59	24.46	22.07
363	147.0	31.4	45.25	22.10	21.69
Black Amber #5	449.6	91.1	45.35	63.21	20.17
2 x S.E. of Diff.	171.2	37.6	4.16	4.04	0.13
Normal Moisture	244.6	49.5	50.71	34.24	20.11
Drought Conditions	262.6	57.8	44.29	30.93	22.70
2 x S.E. of Diff.	121.1	26.6	2.94	2.86	0.09

The average plant height (inches) of sudan grass Line 138A was more than twice the standard error of a difference greater than any of the other lines, including Black Amber. The leaf width (1/32 inch) of Black Amber was statistically greater than any of the sudan grass lines. All sudan grass lines had a greater dry matter percentage than Black Amber. Lines 138A and 363 had about the same percentage of dry matter, while 388C had the highest percentage of dry matter.

A comparison of the plants grown with an adequate supply of water with those having a reduced supply of water indicates that in this study drought had very little effect on HCN accumulation, whether reported on oven-dry or green plant basis, but reduced the plant height, reduced the width of leaves, and increased the percentage of dry matter.

Table 10 records a comparison of plant height, leaf width, and HCN p.p.m. of the sudan grass and Black Amber in the field and the normal plants in the small plats. The average plant height in the plats under canvas was 68.0 percent of the field. Black Amber had the greatest reduction or was only 49.0 percent of the field height. The leaf width averaged 84.6 percent of the field. Black Amber and sudan grass Line 363 had the larger reductions. In general, the HCN p.p.m. was higher under the canvas than in the field with a mean of 118.2 percent of the field test. Black Amber again showed the greatest change with the mean 150.4 percent of the field test.

The correlation coefficient for plant height and HCN p.p.m., green plant basis, was $+0.0799$, $p > 0.1$ when Black Amber was included, but was $+0.0527$, $p > 0.1$ when only the sudan grass plats were used. The width of leaves gave a correlation coefficient of

Table 10. A Comparison of HCN and Plant Growth in the Drought Study and in the Field, 1936

Selection No.	Plant Height (Inches)			Leaf Width (1/32 in.)			HCN (p.p.m.)		
	Plats	Field	Pct. of Field	Plats	Field	Pct. of Field	Plats	Field	Pct. of Field
138A	53.10	65.70	80.8	21.03	23.95	87.8	29.8	24.9	119.7
388C	51.08	68.45[a]	74.6	25.05	24.88[a]	100.7	55.7	67.7[a]	82.3
363	50.09	74.00	67.7	22.74	31.95	71.2	30.5	25.3	120.6
B. A. #5	48.56	99.10	49.0	68.14	86.40	78.9	82.1	54.6	150.4
Average	50.71	76.81	68.0	34.24	41.80	84.6	49.5	43.1	118.2

[a] Mean of 388A and 388B in field, 1936.

$+0.7758$, $p < 0.01$ with HCN p.p.m. on the oven-dry basis and $+0.7324$, $p < 0.01$ on the green plant basis when Black Amber was included, but when Black Amber was excluded $r = +0.5309$, $p = 0.1 - 0.05$ on the oven-dry basis and $r = +0.4908$, $p > 0.1$ on the green plant basis. Thus, the lines used in the drought study followed the same general trends as the entire group in the field test.

DISCUSSION

Many methods have been used for analyzing plant material for HCN but none of them have been entirely satisfactory. The sulfocyanate method has been demonstrated to be a valid relative test. Further investigation of this and other methods, however, must be made before an exact quantitative test for HCN in plant material is developed. When such a method is perfected, the study of cyanogenesis in plants will be facilitated.

The nature of the inheritance of the ability to accumulate HCN in plants is not known, but the fact that this ability is inherited has been demonstrated by many workers. It is common knowledge that some genera of grasses, such as Poa, produce little or no potential HCN while others, such as Sorghum, do produce lethal amounts. For some time, the fact that certain forage sorghums contain more potential HCN than sudan grass has been known. However, many workers have assumed that so-called pure sudan grass would contain HCN below lethal amounts.

Ahlgrene, Aamodt, and Wright (1936) made eight selections from inbred lines of sudan grass that to all appearances were what they called pure sudan grass. They believe that at least one of these selections should be safe for feeding under all conditions. They planned to test these lines for HCN during the summer of 1936.

The assumption that pure sudan grass has very low concentrations of HCN may not be true. Sudan grass is a native of Africa and was introduced into the United States in nearly its native condition. It, like many of our native grasses, is not entirely self-pollinated and, consequently, the population as a whole is quite heterogeneous. Then, if selections were made from the pure sudan grass of Africa and kept pure by selfing, there would probably result many different plant types. Since a certain amount of crossing has taken place between the sorghums and sudan grass, even greater variations would be expected by selection and selfing.

Huskins and Smith (1932, 1934) have reported that S. vulgare sudanensis and S. sorghum have 2n = 20 chromosomes, while S. halepense (Johnson grass) has 2n = 40. They also report that usually 10 bivalents are found in the somatic tissue of the 2n = 20 species of sorghum, but that S. sudanensis had 6 bivalents and 2 quadravalents in at least one case. Fertile progeny from crosses between S. sudanensis and S. halepense would be expected to be very rare.

The lines studied in this investigation were selected from what appeared to be a relatively pure field of sudan grass so that no true sorghum or broomcorn types would be expected to appear. This has been true although some lines tended toward the broomcorn or Johnson grass types.

The results of this study indicate that there is no relation between the plant characteristics studied and the accumulation of HCN. It follows, therefore, that the selection of plants in the field, based on plant characteristics, may not necessarily have any effect on their HCN accumulating ability. The evident uniformity of several of these original lines, as demonstrated three years later by their progeny behavior, may indicate a possible differential inheritance of HCN accumulation. These lines are apparently quite homozygous for HCN accumulation. On the other hand, many of these original lines appeared to be quite heterozygous for HCN as indicated by their progeny behavior. This would be expected even though a single pair of genes were involved, and more so if many genes were active in effecting HCN accumulation.

Whether or not HCN accumulation is inherited in sudan grass cannot be stated at the present time, but the indications are strong enough to warrant further studies on this problem.

SUMMARY

1. The progeny test indicates that the differential ability to accumulate HCN may be inherited in sudan grass lines.

2. No relation was indicated between HCN production and such plant characteristics as glossy or non-glossy leaves, coarse or fine-stemmed, small, large, or medium sized seeds, short or tall plants, wide or narrow leaves, early or late dates of heading.

3. HCN production does not seem to be correlated with grasshopper or aphid feeding preferences.

4. Sudan grass and Black Amber sorghum grown under the restricted water supply were not consistently higher in HCN p.p.m. than that grown under sufficient water supply.

5. In general, the amount of HCN in the plants grown under canvas was slightly higher than in those grown in the field.

6. All plants grown under canvas were reduced in plant height and leaf width but Black Amber plants had the greatest reduction.

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Abstract of Thesis

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ABSTRACT

Sudan grass is an important forage crop under dryland conditions in eastern Colorado. Often cattle on sudan grass pasture die from poisoning that has been attributed to prussic acid (HCN) since an autopsy gave a positive test for HCN.

The lethal properties of these pastures are assumed to be due to three possible causes: (1) A mechanical mixture of sudan grass with the forage sorghums, (2) the presence of sudan x sorghum hybrids, and (3) sudan grass of extremely high concentrations of HCN.

The present study was designed to determine the feasibility of the third assumption. If only the first two assumptions are true and the third false, then the problem of maintaining a safe sudan grass pasture becomes relatively simple; but if the latter assumption is true, then the problem resolves itself into the selection and propagation of lines inherently low in HCN accumulation.

The primary source of HCN in the plant is one of several glucosides of which only dhurrin has been reported in the genus Sorghum.

In general, the lethal dose of HCN is considered to be about 1 mg. HCN per pound of body weight.

The amount of HCN in the plant is dependent upon many factors. The plant species determines, to a large extent, the amount of HCN in the plant. The stage of growth of the plant seems to have some influence on the HCN content, thus the sorghums contain large amounts of HCN when they are young but contain much less at maturity. The addition of nitrates to soils tends to increase the amount of HCN found in sorghum. Some workers report that a limited water supply increases the HCN content of the plant while others maintain that the highest amount of HCN is found in the plant during any period of vigorous growth. Among the other factors which have been reported to increase HCN are cool temperatures, disease, frost, bruising, and trampling. Usually, the dried forage of sorghums of high HCN content has much less HCN than the green plants.

In 1935, 91 inbred lines that had been self-fertilized for two years, and 23 head selections, were grown. Fourteen of the former were used for specific study. In 1936, these 91 lines, excepting two that did not set seed in 1935, were planted along with 19 more inbred lines that had been selfed three years.

All of the lines were analyzed for HCN in 1936. The effect of drought on HCN accumulation was studied with three inbred lines of sudan grass and one inbred line of Black Amber sorghum grown in 16 small plats that were covered with canvas during any period of rainfall.

Since the Prussian Blue method of analyzing HCN in plant material failed to give any positive results in the summer of 1935, the sulfocyanate method was finally adopted for the analytical work. The latter method was not only more sensitive but gave better comparative results in estimating HCN in solution.

Some studies were made of the sulfocyanate method and this method was shown to give less variability in laboratory results than any other method investigated. The method, however, gives only relative estimations of HCN.

Field samples for analysis were composed of the tillers from several plants in each row of inbred lines of sudan grass or sorghum at the time that the plants were in the late boot stage. The samples were analyzed in duplicate in the laboratory.

The drought samples were composed of four plants each and two samples were taken from each plat. These samples were also analyzed in duplicate in the laboratory. By the use of statistics, the sampling error in the field was shown to be much greater than the error due to laboratory analysis.

The 14 inbred lines of sudan grass that were tested after a frost in October, 1935, and samples from their progeny (inbred) taken in July, 1936, had a correlation coefficient of +0.2603, $p > 0.1$ for HCN accumulation. Two of the lines disagreed and when these were not included the correlation coefficient was +0.8254, $p < 0.01$.

The HCN p.p.m. for the various inbred lines of sudan grass in 1936 ranged from a low of 7.9 to 98.0. There were ten lines of sudan grass higher than two of the lines of Black Amber sorghum but none were as high as the highest sorghum line.

Two or more S_3 (selfed three generations) progeny of each of several original selections of sudan grass were tested for HCN accumulation. Six lines were relatively uniform and moderately low in HCN accumulation; seven lines were relatively high and uniform, and five seemed to be segregating for high and low HCN accumulation. The Black Amber sorghum inbred lines varied from 54.6 p.p.m. to 141.8 p.p.m. HCN.

The progeny test indicates that the differential ability to accumulate HCN may be inherited in sudan grass lines.

No relation was indicated between HCN production and such plant characteristics as glossy or non-glossy leaves, coarse or fine-stemmed, small, large, or medium sized seeds, short or tall plants, wide or narrow leaves, and early or late dates of heading.

The production of HCN does not seem to be correlated with grasshopper or aphid feeding preferences.

Sudan grass and Black Amber sorghum grown under the restricted water supply were not consistently higher in HCN p.p.m. than that grown under a sufficient water supply.

In general, the amount of HCN in the plants grown under canvas was slightly higher than in those grown in the field.