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General Summary Statements

Part I

The chemical method which was used in recovering HCN from sorghum plants is that of precipitating the substance with silver nitrate ($AgNO_3$) as approved by the Association of Official Agricultural Chemists.

Attempted use of the aspiration method invariably recovered lower amounts than the foregoing method. It was found impossible to recover any amount whatever of HCN from ensilage by the foregoing method.

Part II

The factors which control content of HCN in sorghum are heritable, and consequently subject to modification by selection and breeding. Such a demonstrated fact it is hoped might serve as a basis for production of varieties and strains of forage and grain sorghum utilizable in the northern Great Plains and elsewhere with little or no danger of cyanide poisoning.

Low-HCN appears to be partially dominant over high-HCN. The Mendelian ratio is not considered established as yet.

Part III

The amount of HCN occurring in strains of sorghum investigated is subject to decided modification by environment, weather, condition of soil, climate, or storage. The conditions of environment obviously apply themselves to various localities where sorghum may be produced.

The writers offer the tentative observation, as a result of these researches, that whatever conditions of environment may promote normal and regular growth in sorghum plants conducing minimum HCN content therein. Consequently, conditions which retard such normal development of sorghum plants, in greater or less degree, bring about corresponding increase in amounts of HCN in subsequent growth.

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A Study of Sorghum With Reference to The Content of HCN

By C. J. Franzke, Leo F. Puhr, and A. N. Hume[1]

Introduction

Farmers and others concerned with the production and feeding of forage crops long have known that certain crop plants may be poisonous to animals. It also has been found that the poisonous property of certain plants consists of or is associated with prussic acid or hydrocyanic acid (HCN). The researches reported in this bulletin are concerned with one principal forage crop, namely sorghum and its varieties. Other species which have been observed to contain HCN are as follows (Couch, 5)[2]:

Choke Cherry (wild), *Prunus virginiana;*
Black Cherry (wild), *Prunus serotina;*
Sorghum, *Sorghum vulgare;*
Johnson Grass, *Sorghum halepense;*
Flax, *Linum usitatissimum;*
Arrow Grass, *Triglochin maritima* and *T. palustis;*
Velvet Grass, *Holcus lanatus;*
Christmasberry, *Photinia salicifolia;* and
Sudan Grass, *S. vulgare,* var. *sudanense.*

Of indirect importance to the present problem is the fact that animal poisoning may occur from feeding forage plants that contain harmful substances other than HCN.

Upon one occasion, a report came to the Agronomy Department concerning the loss of 20 young cattle on a farm about 10 miles from Brookings. Members of the department and Dr. C. C. Lipp, staff veterinarian, visited the farm and found that the cattle had been feeding upon sudan grass which had been stored in a stack. Dr. Lipp observed professionally that the cattle had not shown the usual symptoms of HCN poisoning. An analysis of the Sudan forage revealed no HCN. It was, however, found to contain 8.47 percent of nitrates.

In another instance a sample of rough pigweed, **Amaranthus retroflexus L.,** was received by the department along with a report that this weed had apparently killed several cattle. It contained no HCN. However, it was found to contain 9 percent of nitrates.

The foregoing will serve to illustrate a rather broad application of the problem of forage poisoning. This bulletin, as indicated, deals with a comparatively narrow phase of the entire problem, namely forage poisoning from the use of sorghum containing HCN.

1. The writers gratefully acknowledge the helpful assistance of Dr. S. P. Swenson, Associate Professor of Agronomy, who informally provided much valuable information and helpfully reviewed the manuscript; also Edgar Joy, Farm Foreman at the Cottonwood Experiment Station, for the cooperation in securing samples for analysis.

2. Reference is made to Literature Cited, p. 51, by number in parenthesis.

Review of Literature

A rather extensive review of cyanogenesis in plants has been compiled by Leemann (12). Therefore only some of the literature having a bearing on the specific problems discussed in this bulletin will be reviewed.

The fact that sorghums may be toxic to animals has been recognized for a long time, perhaps since 1838 (Leemann, 12). Among the early reports of sorghum poisoning in the United States are those of Abbott (according to Leemann, 12), Hiltner (10), Peters, Slade, and Avery (16), Pammel (15), and Mohler (14).

A number of workers have published discussions on methods of determining HCN content in sorghums. Viehover and Johns (23) from work on cyanogentic plants concluded that it was necessary to estimate small quantities of hydrocyanic acid. The various titration methods as well as the silver gravimetric method had to be excluded because they were working with plant distillates which usually contain reducing compounds. Furthermore, the quantities to be determined were often too small to permit the use of the above methods. In the colormetric method, the deepest shade of Prussian blue was obtained from a cyanide only when the volume of the solution to be tested was sufficiently small.

Bishop (1) tested a number of methods for determining HCN and concluded that distillation led to untrustworthy results. He concluded that alcohol extraction was a sound method for determining cyanogentic glycosides in leaves. Swanson (21) stated that the maximum amount of hydrocyanic acid was obtained by macerating the material and digesting in water at room temperature for about six hours or over night. Practically all the hydrocyanic acid was found in the leaves while in well developed stems none was found. More hydrocyanic acid was found in younger plants than in those more matured, due mostly to stem development. If leaves only are used the differences between plants are small, except when the plants approach maturity. More was found in the summer than in the fall. Hydrocyanic acid does not exist as free hydrocyanic acid in the growing plants but it begins to be liberated as soon as the plant is macerated or undergoes wilting. Liberation of hydrocyanic acid is intimately associated with enzyme action but if this enzyme action is inhibited by the addition of hot water or acids, no hydrocyanic acid will be liberated. Hydrocyanic acid was obtained from wilted grass when hot water was added, because during the wilting process hydrocyanic acid was set free.

Several workers have found that Sudan grass contains an appreciably smaller amount of HCN than either the forage or grain sorghums (Swanson, 22; Couch 5; and Menaul and Dowell, 13). Williaman and West (25) suggested that variety might be an important factor in determining the amount of HCN in sorghum plants. Of particular interest is the work of Coleman and Robertson (3) who demonstrated that inbred lines of Sudan grass may differ in their ability to produce HCN. Furthermore, they found that high-HCN was associated with non-glossy leaves and purple-tipped seedling leaves. Rogers, Larson and Spracher (18) working on Sudan grass found no relationship between seed characteristics and HCN content.

The literature on environmental factors affecting HCN content is somewhat more extensive. Brunnich (2) listed a large number of "conflicting statements and theories" on the conditions under which sorghums

may become poisonous such as variations in maturity, rainfall, drought, frost, insect damage, molds or fungi, fertilizers, temperature, and humidity. A summary of the factors influencing HCN content in sorghums also has been presented by Couch (5).

Diurnal variation in the HCN content of sorghums has been reported by Ravenna (Leemann, 12) and Williaman and West (26). In each instance, the amount of HCN increased from morning to afternoon. Menaul and Dowell (13), on the other hand, concluded that there is more HCN in the plant in the morning than in the afternoon.

Williaman and West (26) concluded that climate is a more important factor than soil in the production of HCN. They stated that the apparent effect of humidity and temperature on the amount of cyanide in sorghum is probably due to the indirect effect on the rate of growth and that an adequate water supply is usually accompanied by low, and inadequate by high, hydrocyanic acid content. However, they also point out that soil may be an important factor as determined from adding nitrogen fertilizer to poor soils. Pinckney (17) demonstrated that the size, color, and prussic acid content of sorghum plants were markedly affected by adding different amounts of sodium nitrate to three Minnesota soils low in nitrogen. Brunnich (2) also found that the addition of sodium nitrate increased HCN.

Peters, Slade, and Avery (16) stated that growth arrested by drought presents a very favorable condition for the elaboration of the poison. Growth stunted by too much water, sterile soil, shade, or hardening of the soil, on the other hand, does not result in a higher HCN content. Francis (9) also concluded that drought-stunted plants are especially dangerous. Vinall (24) concluded from a critical survey of literature that the injury to growing sorghum plants by drought increases the prussic acid content, but that stunted growth from lack of plant food in the soil diminished it. Coleman and Robertson (3) concluded that soil and seasonal differences may influence the production of HCN in Sudan grass. Leemann (12) and Couch (5) have cited a number of other instances where climate and soil have been shown to influence the HCN content in sorghum plants.

The HCN content at different stages of growth also has been investigated by a number of workers. Peters, Slade, and Avery (16) found that young plants of vigorous growth contain more prussic acid than plants approaching maturity. They further concluded that second growth is not more dangerous than first growth but since the autumns in the west are often bright and dry, conditions may be favorable for the elaboration of larger amounts of HCN during the second growth. Second growth, according to Francis (9), is unsafe. Williaman and West (25) stated that prussic acid is concentrated in the stalks during the first three or four weeks but then decreases and disappears, persisting in the leaves in decreasing amount until maturity. A decrease in hydrocyanic acid with growth also was observed by Menual and Dowell (13).

Frost, according to Peters, Slade, and Avery (16), is without influence except as a forerunner of a period of bright dry weather. Francis (9) stated that frosted plants are unsafe. Vinall (24) also concluded that frost increases the prussic acid content of sorghums.

Changes in HCN content during the drying or curing process have been investigated by a few workers. Dowell (7) has found that approximately three-fourths of the acid is set free in the process of drying, confirming

the common belief that sorghum is safe for feeding after it has been dried. At the same time the results show that not all of the hydrocyanic acid disappears although the rapidity with which the sorghum is dried determines the percentage of the hydrocyanic acid retained. The fact that farmers quite frequently cut their sorghum during drought after it has been partially dried while yet standing may result in the retention of a large percentage of the hyrdocyanic acid in the fodder because of the prolongation of the drying period. According to Collison (4), well-cured sorghum usually can be eaten safely by cattle because of the effect of drying upon the poisoning properties of the plants.

Swanson (22) concluded that the rate of drying is important. The portion tested at once gave large amounts, that dried in the oven somewhat less, that dried in the sun still less, and that dried slowly in the shade none or only a trace. Making Sudan grass into silage did not diminish the amount of hydrocyanic acid obtained. He further stated that hydrocyanic acid was found in large amounts in Sudan grass used for pasture and no harm resulted to the cattle. Liberation of hydrocyanic acid from Sudan grass appeared to be associated with enzyme action. Tests made immediately on frosted Sudan grass gave large amounts of hydrocyanic acid, but it disappeared rapidly as soon as the plant began to wilt and when dry, the hydrocyanic acid had disappeared. While Sudan grass giving a strong test for hydrocyanic acid was not harmful to cattle, under other conditions it was harmful. Couch (5) concluded that dried cyanogenetic plants, such as those made into hay, commonly produce a very much less hydrocyanic acid than plants which are fresh. Well cured sorghum or Johnson grass, for instance, contain little or no hydrocyanic acid and may be eaten by livestock without danger of poisoning. It is thought that in some cases the poisonous acid is slowly given off during the process of drying and, being very volatile, passes into the air. In other cases some chemical alterations destroy the ability of plants to produce the acid. Some plants, however, retain a considerable proportion of the active acid even when dried. This applies especially to seeds.

Part I. Materials and Methods

The materials for the various experiments reported in this bulletin are listed and described in the separate discussions of the experimental results. Likewise, the methods of handling the material are discussed specifically for each experiment. This section of the bulletin therefore will be confined to a description of the following: (1) laboratory determinations of HCN and (2) methods of emasculation and pollination in making crosses.

Laboratory Determinations of HCN

It will be obvious to any reader that the great number of determinations of HCN reported in this bulletin must be the outcome of one or more laboratory methods of determination. It is thought well here to report the general method of determination utilized in the researches of this bulletin, with some additional studies made on laboratory methods. These are put down not so much as discussions of chemical theory but rather to set forth the way by which given results have been attained.

Usual Method. The usual method for HCN determination as employed in the researches of this bulletin was the following:

Sorghum leaves were stripped from the stalks and the midribs of the leaves were removed before cutting the leaf blades into smaller portions. The leaves were cut into pieces approximately one-half inch in length with a pair of grass shears. Fifty grams of stripped leaves were used for a bulk sample. The finely cut leaves were thoroughly mixed by hand before sampling for distillation. Samples were immediately weighed out for moisture and for HCN determinations in order to avoid losses of moisture and HCN as far as possible. The percent of moisture was determined by drying the samples in an electrically controlled oven at 105°C. under ordinary atmospheric pressure for approximately 20 hours.

Five or ten gram samples of the finely cut sorghum leaves were macerated in an iron mortar with a small quantity of pure silica sand moistened with a few cubic centimeters of distilled water. After maceration the samples were transferred to an 800 cc. Kjeldahl flask with approximately 300 cc. of distilled water to wash out the mortar. After transferring the macerated sorghum to the Kjeldahl flask, the flask was immediately corked tightly, using a large rubber stopper. The macerated sorghum in the tightly stoppered Kjeldahl flask was allowed to digest over night at room temperature. The HCN was distilled on the ordinary Kjeldahl distilling apparatus into a receiving flask containing 10 cc. of .05 normal silver nitrate ($AgNO_3$). One cubic centimeter of nitric acid (HNO_3) to keep the solution acid and 50 cc. of distilled water were then added. The receiving flasks were painted black in order to avoid reduction of silver nitrate solution by light.

The precipitate of silver cyanide was separated from the solution in the receiving flask by filtering through a Gooch crucible. The silver nitrate remaining in solutton was determned by titration with a .05 standard potassium thiocynate solution, using ferric ammonium sulphate as an indicator. The percent HCN was calculated from the titration on a moisture free basis and converted to parts per million (p.p.m.).

Recovery of HCN After Different Periods of Time for Digestion and After Digestion at Two Different Temperatures. An experiment was installed to determine whether or not the length of time allowed for digestion during the process of determination of HCN in green forage affects the amount of HCN recovered.

Three-gram samples of ground sorghum were placed (in duplicate) in Kjeldahl flasks. One set of Kjeldahl flasks were brought to a temperature of 45°C. and another to a temperature of 43°C. After being thus placed in the Kjeldahl flasks and brought to the required temperatures, varying periods of time were allowed to elapse before the several duplicate flasks were distilled. The following table is a summary of the results.

A comparison of the amounts shown in the table indicates that they are slightly higher at 43°C. in all cases where a comparison is possible. There is no indication that the time of digestion within the space of an hour has any considerable effect upon the amount of HCN recovered. It seems likely that HCN wherever present in green leaves of sorghum begins to be liberated at any moment when such leaves are macerated, and that the process of liberation becomes as complete through distillation at that instant as is possible during any later period of digestion.

TABLE 1. HCN in Sorghum Samples, Recovered After Given Length of Time of Digestion Before Distillation. (1933).

Sample No.	No. minutes digested	HCN content, p.p.m., in sample (duplicates) digested for given time at given temperature.	
		45°C.	43°C.
1	*None	590	590
2	*None		650
3	2	580	
4	5	580	620
5	10	590	630
6	15		640
7	20	610	640
8	25		620
9	30	580	610
10	35		650
11	40		640
12	45		640
13	50		630
14	55		640

* Cold water put in Kjeldahl flask along with samples placed on Kjeldahl distilling apparatus and distilled off immediately.

Application of Heat (103°C.) resulted in the Loss of HCN. A sample of leaves of sorghum was harvested and divided into two equal and similar parts. Duplicate samples from one part were cut finely and thoroughly macerated with white sand in a mortar, then placed directly into a Kjeldahl flask, and left to digest one hour at 43° C. before distillation. The samples of the other part were heated in an electric oven at 103°C. for 18 hours before macerating and digesting. Thereafter, the HCN was distilled into 10 cc. of .05N silver nitrate ($AgNO_3$) solution and titrated in the usual way.

This study of the effect of a comparatively high temperature, 103°C., as compared with a temperature of 43°C. was made in 1933. The outcome was briefly as follows:

HCN in sorghum receiving usual treatment, digested at 43°C. one hour before distillation	645 p.p.m.
HCN in sorghum heated in electric oven 18 hours at 103°C. before distillation	68 p.p.m.

It is evident that the application of heat (103°C.) to sorghum in some way prevented the recovery or resulted in the loss of HCN. Only a minimum amount was recovered from the heated sample by the standard method of determination. One possibility is that the application of heat previous to distillation inhibited the action of the enzyme apparently necessary to liberate HCN; another possibility is that HCN was lost during heating in the oven.

The Aspiration Method. It was believed that some of the questions about HCN in sorghum or sorghum silage or its escape therefrom might be easier to answer if more were known about the method of recovering it by aspiration. Such a method might be more analogous to ordinary atmospheric processes that occur in the course of handling sorghum or sorghum silage for feed.

The apparatus for determining HCN by the aspiration method was set up as follows: Three aspirator flasks were hooked up in a series. Air was pulled through the aspirator flasks by a water vacuum pump. The air entered at the top of No. 1 flask, passed downward to the bottom of the flask, being released in a concentrated sodium hydroxide solution, purified, rising to the top opening of flask No. 1, passed into No. 2 flask downward

to the bottom, released passing upward through the macerated sorghum leaves, digesting in water at room temperatures.

The purified air from the No. 1 flask passed to the No. 2 flask and released into the digesting sorghum where the HCN was being liberated. The pure air in No. 2 flask carrying the HCN was pulled through the opening of the top of No. 2 flask to the bottom of No. 3 flask, released in a standard silver nitrate solution. No. 3 flask was covered with black paper to prevent light rays from disintegrating the silver nitrate solution. The HCN was thus washed out in the standard silver nitrate solution before passing out the top opening of No. 3 flask. The air was pulled through slowly and continuously during the digestion process. After the aspirator had run over night at room temperature the standard silver nitrate solution in No. 3 flask was filtered and titrated the usual way.

In order to test the efficiency of the aspiration method a check was first made as follows: Ten cubic centemeters of a standard solution of HCN containing .0112 gms. of HCN was placed in water in the aspirator apparatus described in the foregoing paragraph and allowed to aspirate 5 hours. The amount of HCN recovered thus was found to be .01066 gms. An exact duplicate of the foregoing experiment was repeated on September 22, 1938, with the exception that the aspiration process was allowed to continue 15 hours. In this duplicate experiment, the amount recovered was likewise .01066 gms. It is obvious that the process of aspiration was effective in recovering all of a known quantity of HCN as such. Obviously such a check on the method itself constitutes helpful information in considering the following data which may be called a brief comparative study of the effectiveness of the aspiration method as compared with the usual method in recovering HCN from sorghum and sorghum silage.

Relative Amounts of HCN Recovered From Several Sorghum Strains at Successive Stages of Growth (1) With the Aspiration Method and (2) the Regular Distillation Method. Table 2 gives a summary of comparisons of the amounts of HCN in several strains of sorghum recovered first by the method of aspiration and second by the method of distillation.

TABLE 2. Comparative Amounts of HCN Recovered with Aspiration and with Usual Distillation Method from Sorghum Harvested at Several Stages. (1935 and 1936).

Strain No.	Year	First or Second Growth	Stage at Harvest and Ensiling	Date of HCN Determination on Silage	HCN Determined p.p.m. Aspir.	HCN Determined p.p.m. Distil.	Percent HCN recovered by aspiration, using usual distillation method as base.
15-30-S	1935	First	Fertilized	8-14	0	1900	0
20-30-S	1935	First	Fertilized	8-14	590	2500	23.6
Ave.					290	2200	11.8
19-30-S	1936	First	Fertilized	8-3	2640	4000	66.0
19-30-S	1936	First	Early Milk	8-11	2310	2660	86.8
18-30-S	1936	First	Early Milk	8-12	840	1260	66.7
20-30-S	1936	First	Matured	8-24	260	1030	25.2
Ave.					1510	2240	61.2
*15-30-S	1936	First	Fertilized	8-3	750	2980	25.2
19-30-S	1936	Second	Shooting	8-13	4260	4410	96.6
18-30-S	1936	Second	Shooting	8-14	2720	3950	68.9
15-30-S	1936	Second	Shooting	8-17	4340	4440	97.7
1-30-S	1936	Second	Shooting	8-18	2450	3830	64.0
Ave.					3440	4160	81.8
Ave. All					1910	2990	56.4

* Strain No. 15-30-S harvested August 3, 1936, was cured six weeks in the shade—analyses made September 14, on the cured forage.

A comparison of the amounts recovered by the two methods will reveal that the method of aspiration in every instance recovered a lower percentage of HCN than the regular method with distillation. It has already been demonstrated and pointed out that the aspiration method in and of itself was effective in recovering a known quantity of HCN from a water solution.

The inference therefore is that the sorghum itself gave up its amount of HCN less completely with aspiration than the solution of HCN. Moreover, a comparison of the amounts of HCN recovered by the two methods might indicate that the different strains and different conditions (stages) of sorghum at time of harvest have influence upon the effectiveness of aspiration as a method for recovering HCN.

Aspiration Method Recovered no HCN From Sorghum Silage. It occured to the writers that the method of aspiration might be serviceable for making of determinations of HCN in sorghum.

On August 30, 1934, sorghum was harvested then cut fine and placed in Mason jars for preservation in a way to stimulate commercial ensiling of sorghum. On October 17, and February 1, 1935, 50-gram samples of this sorghum silage were placed in the aspirator apparatus and allowed to aspirate over night to determine the resulting liberation of HCN. No HCN was liberated from the silage either time it was aspirated. The HCN content in the silage samples as determined by distillation were .580 and .390 p.p.m. respectively.

Further, in order to learn whether the presence of sorghum in and of itself might by any means inhibit the recovery of HCN by aspiration, a known quantity of HCN solution was added to the aforementioned material previously aspirated October 17. The amount added was 10 cc. of a standard solution containing .00972 grams of HCN, placed directly into the watery suspension of the silage. On October 18 the material was then re-aspirated for six hours, with the result that there was recovered .009045 grams of HCN. Obviously the method of aspiration which had recovered no HCN out of the sorghum silage itself did recover practically all of the amount added later.

Methods of Emasculation and Pollination

The Hot Water Method of Emasculating Sorghum Plants. In order to secure hybrids or crosses of selfed strains of sorghum it is necessary to first remove the stamens from flowers of plants to be crossed or otherwise destroy the pollen before self-fertilization can occur.

All of the emasculations for the crosses reported in this bulletin were made by the bulk emasculation method described by Stephen and Quinby (20), and modified by Franzke to meet local conditions.

The equipment used was as follows:

A hole was cut in the bottom of a one-gallon can from which the top had been removed, sufficiently large for a No. 1 can with the top and bottom removed to be soldered over the hole. The two cans thus soldered together formed an improvised funnel, with a holding capacity of one gallon of water and a fairly wide outlet, the latter by the No. 1 can. An 18-inch section of a small inner tube of an automobile tire was stretched over the outlet of this improvised funnel, in such manner as to make a rubber exten-

sion of the outlet. The funnel with the rubber extension downward was mounted on an adjustable tripod made of 1 x 2 soft pine.

A campstove with a large kettle was used for heating the water. The temperature of water desired for emasculation (48°C.) was secured by pouring boiling water gradually into another receptacle containing cold water.

When the emasculator was in use, the rubber tube extension was drawn carefully over the sorghum panicle down and around the stem and adjusted so that the sorghum panicle to be emasculated would be one or two inches above the bottom on the inside of the gallon can. After thus adjusting the emasculator, the inner tube was tied water-tight around the sorghum stem. The panicle was kept in water for ten minutes before being released. After a panicle was thus emasculated it was bagged with a glassine bag for later pollination.

Method of Collecting and Applying Pollen to Emasculated Flowers. The pollen used for application to the pistils of the emasculated flowers was secured from the panicles of other plants which had been previously selected. The panicles of any plant which was to furnish pollen was also covered with a glassine bag securely, shortly before pollination occurred and the glassine bag closely secured at the bottom in order to retain the pollen dust. After a sufficient amount of pollen had been discharged from the flowers on the panicle, the latter was bent over or removed from the plant and shaken slightly in order to collect the pollen in the bottom of the glassine bag. The latter was removed from the panicle and the pollen poured out into an ordinary glass Petri dish with a cover. The pollen was then carried to the emasculated plant where it was to be applied and dusted upon the emasculated flowers with a camel's hair brush.

Part II. Experimental Results: The Inheritance of HCN Content

Numerous Sorts and Varieties of Sorghum Were Found to Contain Varying Amounts of HCN. At the beginning of these researches many inquiries existed as to whether all kinds of sorghum would contain HCN. It was necessary at first to secure and analyze a large number of samples of sorghum of many kinds from a great number of locations to attempt to answer the foregoing inquiries. Not all of the results of such analyses can be given in this bulletin but the results from analyses of 194 selfed strains made in 1933 and 1934 are reported in Table 3.

TABLE 3. HCN Content in Varieties of Sorghum, all Produced at Brookings with Seed From Various Sources.

Variety	Year	Source of Seed	Stage of Growth	No. of Analyses	Average HCN p.p.m.
Dakota Amber	1933	Commercial	Shooting	82	660
Dakota Amber #6586	1933	Redfield, S. D.	Shooting	5	500
Sooner Milo C.I. 917	1933	Hayes, Kan.	Shooting	5	450
Brown Kaoliang Sp. #62428	1933	Redfield, S. D.	Shooting	7	730
Kaoliang	1933	Highmore, S. D.	Shooting	7	740
Dakota Amber	1934	Commercial	Dough	78	1080
Kaoliang	1934	Highmore, S. D.	Dough	7	280
Sudan grass	1934	Cottonwood, S. D.	Dough	1	140
Ambur Sudan Grass (cross)	1934	Brookings, S. D.	Dough	2	390

It is evident that varying amounts of HCN occurred in every separate kind of sorghum analyzed, Sudan grass possessing the smallest amount. The writers have observed no strain of sorghum which contains no HCN.

Strains Within Varieties Were Observed to Vary in HCN. In 1933 and 1934 a total of 194 strains of grain and forage sorghums were examined for content of HCN. Of the 194 lines, 160 were Dakota Amber selections. In Table 4 are listed the Dakota Amber selections along with their respective amounts of HCN.

TABLE 4. HCN Content in Green Forage of Pure-line Strains of Dakota Amber, Selected in 1930 from an Open-pollinated Commercial Dakota Amber at Brookings. (1933 and 1934).

HCN Content p.p.m.	No. of strains per class	HCN Content p.p.m.	No. of strains per class
242	4	210	4
343	6	440	8
450	16	670	10
554	14	900	14
658	11	1130	12
762	10	1360	15
886	5	1590	8
970	10	1820	4
1074	4	2050	1
1178	2	2280	2
Ave. 660		1080	
Total	82		78

Strains within varieties apparently show variation similar to that between varieties. The 82 S_3 lines of Dakota Amber examined in 1933 at the shooting stage had an average of 660 p.p.m. in HCN. The 78 S_4 lines of Dakota Amber examined in 1934 at the late dough stage had an average of 1,080 p.p.m. in HCN. The strains examined in 1934 show a greater spread than the lines examined in 1933 but the distributions of lines in classes of HCN content are similar. This consistent distribution of classes as shown in Table 4 emphasizes the idea that the HCN content of sorghum plants arranged in a series may vary in a regular curve from high to low in any given season. Such is the basis for selection. It was thought possible that continued selection might establish pure-line HCN strains and that the HCN content might be further studied from a genetic standpoint.

In Table 5 are listed three-year seasonal (1934, 1935, and 1936) averages of HCN content in green forage for four high-HCN amber sorghums and four low-HCN amber sorghums.

TABLE 5. Average Seasonal HCN Content of Four High-HCN Pure-line Dakota Ambers and Four Low-HCN Pure-line Dakota Ambers for the Years 1934, 1935, and 1936 (Brookings).

High HCN					Low HCN				
Strain Number	1934 HCN p.p.m.	1935 HCN p.p.m.	1936 HCN p.p.m.	3 yr. Ave. HCN p.p.m.	Strain Number	1934 HCN p.p.m.	1935 HCN p.p.m.	1936 HCN p.p.m.	3 yr. Ave. HCN p.p.m.
1-30-S	1720	2040	2230	1997	3-30-S	840	1200	1110	1050
15-30-S	1940	2240	2590	2257	13-30-S	780	1220	1410	1137
18-30-S	1520	1890	2910	2107	30-30-S	560	1070	1390	1007
19-30-S	2400	2330	2990	2573	39-30-S	160	470	600	410
Ave.	1895	2125	2680	2234		585	990	1128	901

The four high strains had an average of two and one-half times more HCN than the four low Ambers. The yearly average hydrocyanic acid content for both the four high strains and the four low strains shows a consistent seasonal increase in HCN content from 1934 to 1936. The average

for the four high strains shows an increase of 785 p.p.m., and the average for the four low strains shows an increase of 543 p.p.m., over the same period.

If it is to be assumed that these differences in HCN content between lines are to serve as a basis for selection in sorghum breeding, it is necessary to know that the differences are significant. The analyses of variance given by Fisher (8) was therefore applied to the data obtained on 12 strains of sorghum and one strain of Sudan grass, analyzed at seven different growth stages in each of three different years, 1934 to 1936 inclusive. The analysis is given in Table 6. The F values for comparing variances were calculated as given by Snedecor (19).

TABLE 6. Analysis of Variance of the HCN Content in the Green Forage of 12 Lines of Sorghum and One Line of Sudan Grass at Seven Different Growth Stages in Three Different Years.

Variation Due to	Degrees of Freedom	Sum of Squares	Mean Squares	F	F[1]
Lines	12	1.2806	.106725	18.24†	64.29†
Years	2	.2749	.137450	23.50†	82.80†
Lines x years (Error 1)	24	.1404	.005850		3.52†
Stages	6	.5753	.095883		57.76†
Lines x stages	72	.1448	.003450		2.08†
Stages x years	12	.2443	.020350		12.26†
Error (2)	144	.2393	.001660		
Total	272	2.8996			

† Highly significant
F is the ratio of variance for lines and years to the variance for lines x years.
F[1] is the ratio of the corresponding variance to the variance for Error 2.

The differences in HCN content between lines are highly significant as are the differences between stages and years. The highly significant interactions indicate that the lines do not react similarly in different stages or years and that the differences between stages are not similar in different years. Nevertheless, this analysis bears out the contention that it is possible to isolate pure-line strains of sorghum which differ consistently in their HCN content.

HCN in Low Strains Also Lower in Early Growth Stages. Figure 1 shows graphically the three-year average HCN content and specifies also the HCN content found in green forage at given growth stages for the four high strains and the four low strains listed in Table 5.

The three-year average for the four high strains was 2,740 p.p.m. of HCN at the eight-leaf stage and the four low strains had 1,450 p.p.m. of HCN at the same stage. The high strains had 1,090 p.p.m. more HCN than the low strains.

The four high strains increased in their HCN content up to and including the shooting stage. From the shooting stage their HCN content shows a decrease up to and including the mature stage, this decrease being especially marked from the early dough stage to the late dough stage. The four low strains in this particular comparison show a gradual decrease from the eight-leaf stage up to and including the mature stage. From the shooting stage to fertilization the difference in HCN content between the high and low strains was the greatest, while at maturity the difference was the smallest.

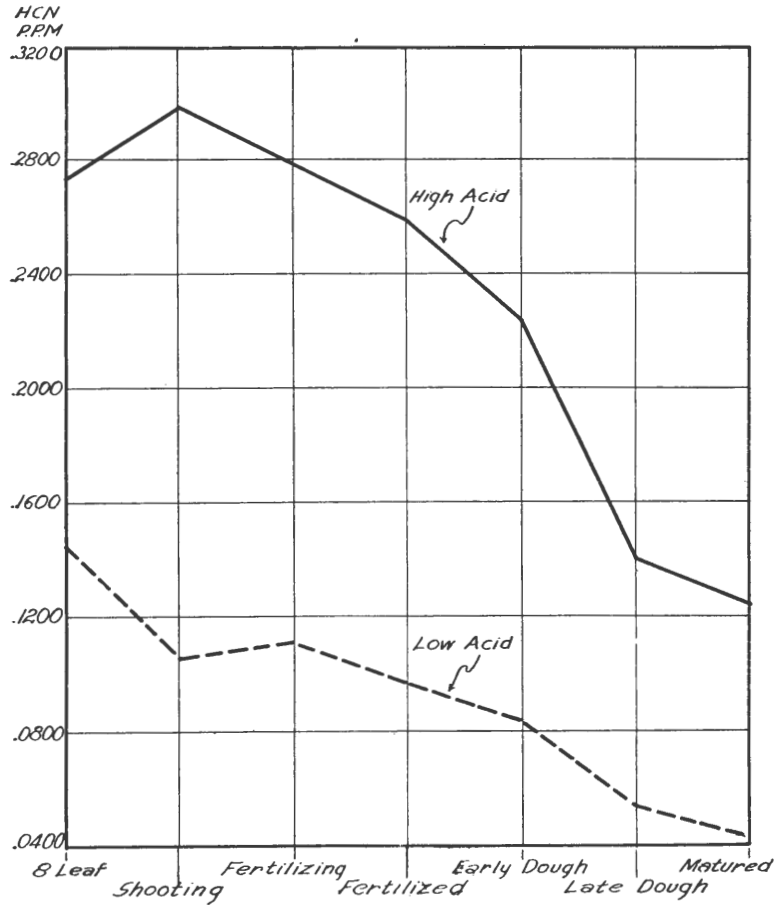

Fig. 1. HCN in green forage at given growth stages expressed as averages for four high- and four low-HCN Dakota Ambers at Brookings. (Three-year average, 1934-1936.)

Thus, it is emphasized by Fig. 1, that the low strains are consistently lower in their HCN content, throughout the period of growth than the high strains in comparison.

Strain Highest in HCN Has Six Times as Much as Lowest Strain. Latter Comparable to Sudan Grass. In Figure 2 are presented the three year seasonal average HCN contents in the green forage at given growth stages for the highest high strain, 19-30-S, the lowest low strain, 39-30-S, and a strain of Sudan grass, 176-S.

Strain 19-30-S increases 780 p.p.m. in its HCN content from eight-leaf stage to the fertilizing stage and decreases 2,080 p.p.m. from the

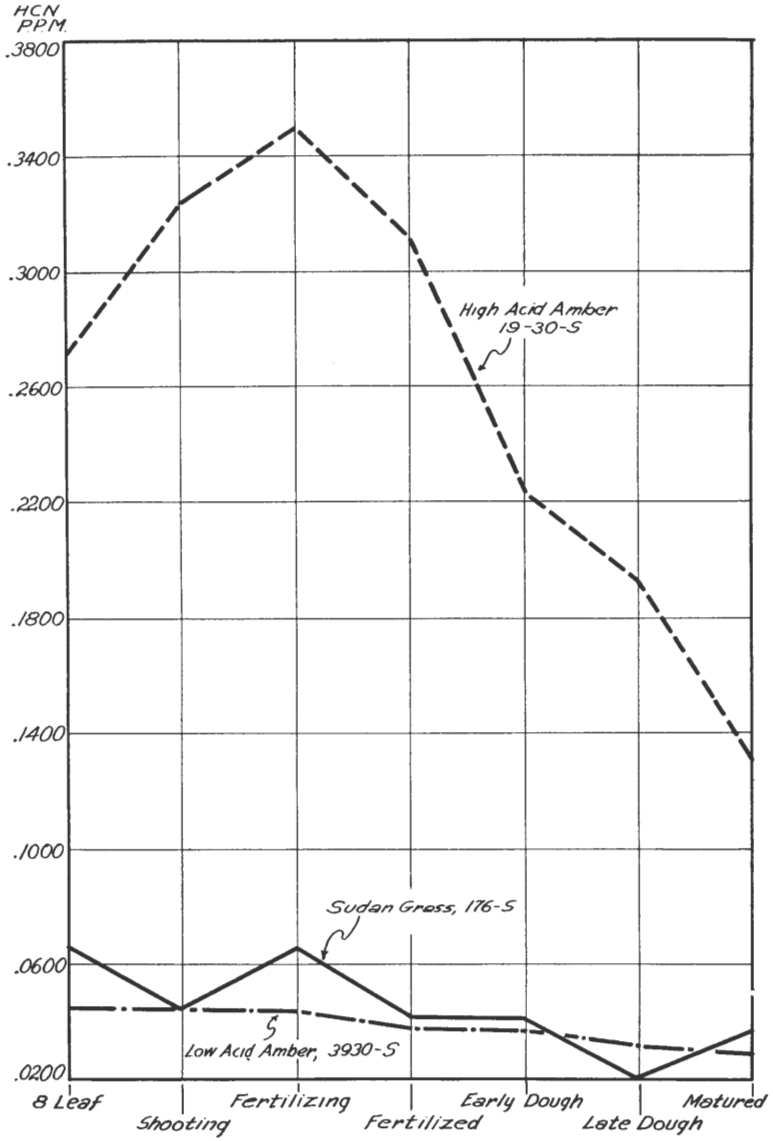

Fig. 2. HCN in green forage at given growth stages for high-HCN strain, 19-30-S, low-HCN strain 39-30-S (Dakota Ambers) and Sudan grass strain, 176-S, at Brookings. (Three-year average, 1934-1936.)

fertilizing stage to maturity. Strain 39-30-S shows a gradual drop of 500 p.p.m. in HCN from the eight-leaf stage to maturity. The Sudan grass strain 176-S shows only a small amount of fluctuation throughout the growing season. Sudan grass tillers considerably, making it relatively difficult to secure a uniform sample for a given growth stage.

The high strain, 19-30-S, Fig. 2, had 2,570 p.p.m. of HCN for the three-year seasonal average and the low strain, 39-30-S had 410 p.p.m. of HCN. **Strain 19-30-S therefore had 6.27 times more HCN than strain 39-30-S.** Sudan grass 176-S had 420 p.p.m. of HCN for the three year seasonal average. Strain 39-30-S and Sudan grass 176-S were close together in their HCN content and followed a similar trend throughout the season.

It was temporarily assumed by the writers at the beginning of these investigations that other strains of sorghum might be found which would be characterized by an HCN content consistently as low as that of Sudan. The results which have been presented substantiate such a hypothesis, not only indicating again that sorghum strains may be selected in general on a basis of HCN content but making the possibility appear that the low content of HCN in Sudan, which makes it so comparatively safe to utilize as a forage crop, is not confined to it alone. It is hopeful to note that such **low HCN content** exists in at least one strain of sorghum of another variety, and **may be found to characterize strains of all or many varieties of sorghum.**

Variability of the HCN Content in a Single Sorghum Strain. In the fall of 1934 at the time of threshing sorghum strains for the 1935 planting, nine panicles from the selfed seed lot of strain 39-30-S (low-HCN Amber) were threshed and bagged individually. The seed from these selected panicles was labeled 39-30-S-a to 39-30-S-i inclusive. The remaining selfed panicles of the strain 39-30-S were bulk threshed. The lots of seed from the bulk and individually selected panicles were planted in one-rod cultivated rows June 1, 1935. On August 12, samples from the fertilized stage were taken from each individual row and the HCN determined. Table 7 lists the HCN content for the bulk lot of strain 39-30-S and the nine individual selections.

TABLE 7. HCN Content in Plants from (1) Bulk Seed, and (2) Seed of Nine Selected Heads, From Strain 39-30-S, Brookings, 1935.

Strain No.	Bulk	a	b	c	d	e	f	g	h	i
HCN p.p.m.	400	320	320	390	270	300	340	440	390	370

The variation in the HCN content is not great. Strain 39-30-S-d had the lowest (270) p.p.m. of HCN and strain 39-30-S-g the highest (440) p.p.m., showing thus an extreme difference of 170 p.p.m. The average HCN content for the nine selections was 349 p.p.m. and for the bulk lot, 400 p.p.m.

Strain 39-30-S as indicated by the foregoing is apparently consistent in its low content of HCN. **The data of Table 7 therefore furnish some additional evidence to the effect that low-HCN may be a gentic character of a selfed strain of sorghum.**

The Inheritance of HCN Content as Determined From Crosses Between High and Low-HCN Strains. In the summer of 1934, crosses were made in both directions between the high-HCN strain, 19-30-S, and the low-HCN strain, 39-30-S. A cross also was made between 19-30-S and a low-HCN

strain of Milo, 109-32-S. These crosses were intended to combine the characters of parent strains with high content of HCN on one hand, and low content of HCN on the other. F_1 seed from these crosses was planted June 1, 1935, in two-rod rows for cultivation. Unfortunately, a heavy rain occurred immediately after planting which destroyed a large number of the rows. However, an amount of F_2 seed was secured from some plants in remaining rows which seed was planted in turn in 1936. These were planted in three-rod cultivated rows, each row having 60 to 70 individual plants. All rows were planted in duplicate on the same day. The rows were so arranged that the parent strains were planted with eight rows of the F_2 intervening. It also was possible to plant a number of rows from F_1 seed of 19-30-S (high-HCN) x 39-30-S (low-HCN).

Beginning July 15, when the F_2 plants in the nursery were at about the nine-leaf stage, samples were taken daily for the determination of content of HCN. A total of 72 samples was taken every day, 18 samples four times daily. The 18 samples were taken as follows: Two from the low-HCN parent strain, two from the high-HCN parent strain, and 14 from individual F_2 plants. The foregoing was varied slightly on occasional days when the number of samples from F_2 plants was reduced to make room in the laboratory for an equal number of samples from the F_1 plants, where it was possible to prepare and analyze only 72 samples per day. The four daily periods of sampling at which the 18 samples were taken were as follows: 8:00 a.m., 10:00 a.m., 1:15 p.m., and 3:00 p.m. Results from the determinations are given in Table 8.

TABLE 8. Frequency Distributions of Plants According to HCN Content Including the Low-HCN Parent, High-HCN Parent, F_1 and F_2. (1936).

Parents and Progeny	p.p.m. HCN in green forage											
	370	780	1190	1600	2010	2420	2830	3240	3650	4060	N	Ave. HCN p.p.m.
9-leaf Stage												
39-30-S (low-HCN)		4	2	1							7	1020
19-30-S (high-HCN)							2	3	1	1	7	3360
High x Low F_1		3	0	2							5	1110
High x Low F_2		1	16	16	21	12	2				68	1790
Shooting stage												
39-30-S	4	29	3								36	830
19-30-S						5	15	7	5		32	3010
High x Low F_1		8	1	1							10	820
High x Low F_2		8	29	39	24	21	9	2		1	133	1740
Heading stage												
39-30-S		6	4								10	890
19-30-S						1	7	6	3		17	3120
High x Low F_1	2	7	4	3	1						17	1040
High x Low F_2	2	32	65	61	41	18	1	2	1		223	1510
Fertilizing stage												
39-30-S		3	11								14	710
19-30-S							1	5	1	2	9	3350
High x Low F_2	2	31	43	49	17	11	1				154	1400
Fertilized Stage												
39-30-S	6	4									10	580
19-30-S					1	1	5	3			10	2860
High x Low F_2	2	24	32	20	7	1	1				87	1220
All stages												
39-30-S	10	46	20	1							77	800
19-30-S					1	7	30	24	10	3	75	3090
High x Low F_1	2	18	5	6	1						32	980
High x Low F_2	7	111	185	190	101	53	12	4	1	1	665	1370

The plants were grouped into 10 separate classes for HCN content in the green forage. These classes range from 370 to 4,060 p.p.m., the class center values, and the difference between the classes was 410 p.p.m.

The two parental strains allot themselves into widely separate classes, based on the content of HCN at all stages. These classes never overlapped, in short the two strains were consistently different.

It is fair to assert that all plants of F_1 fall into classes of low-HCN. **Such a fact indicates that low-HCN is dominant in some degree over high-HCN in the strains and plants examined.** The classes into which plants of F_2 group themselves with respect to HCN content, **indicate genetic segregation, the same being true for all stages of growth at which samples were taken.**

The foregoing facts may be considered **strongly indicative of the Mendelian character of inheritance for HCN content in the strains of sorghum examined.**

The data in Table 8 are further substantiated by the data presented in Table 9 which is made up from crosses made in the reciprocal of those in Table 8.

TABLE 9. Frequency Distributions of Plants According to HCN Content, Including the Low-HCN Parent, High-HCN Parent, and F_2. (Latter are reciprocals of those in Table 8.) (1936).

Parents and Progeny	p.p.m. HCN in green forage											
	370	780	1190	1600	2010	2420	2830	3240	3650	4060	N	Ave. HCN p.p.m.
Heading stage												
39-30-S (low-HCN)		6									6	760
19-30-S (high-HCN)							3	3			6	3060
Low x High F_2				2	2	4	1				9	2230
Fertilized stage												
39-30-S	4	6									10	640
19-30-S					2	3	5	2		2	14	2900
Low x High F_2				3	2	1					6	1830
Milk stage												
39-30-S	12										12	370
19-30-S					6	2	4				12	2320
Low x High F_2	1	9	25	32	14	1					82	1440
Early dough stage												
39-30-S	6										6	280
19-30-S					2	1	2	1			6	2560
Low x High F_2		2	7	11	6						26	1560
All stages												
39-30-S	22	12									34	500
19-30-S					10	6	14	6		2	38	2690
Low x High F_2	1	11	32	48	24	6	1				123	1550

The most important deduction from Table 9 is the fact that it bears out the previous deductions from Table 8. The S_6 plants of the two separate parental strains are found to fall in classes with respect to HCN content that are distinct and that do not overlap at any of the stages of growth. Furthermore, the numbers of F_2 plants which fall into the several classes **indicate segregation** so far as HCN content is concerned. It is indicated further, that the large number of plants in F_2 tend to fall into classes with

TABLE 10. Frequency Distribution of Plants According to HCN Content, Including the High-HCN Parent, 19-30-S, Low-HCN Milo, 109-32-S, and the F_2. (1936).

Parents and Progeny	p.p.m. HCN in green forage											
	370	780	1190	1600	2010	2420	2830	3240	3650	4060	N	Ave. HCN p.p.m.
Fertilized stage												
109-32-S		2	6	2							10	1180
19-30-S						6	4	2			12	2310
High x Low F_2	3	21	33	30	25	15	6				133	1570
Milk stage												
109-32-S	8	1	19	8							36	1140
19-30-S					9	17	7	5			38	2520
High x Low F_2	27	97	88	37	19	5					273	1100
Early dough stage												
109-32-S	1	12	11								24	970
19-30-S				8	5	7	2	2			24	2170
High x Low F_2	16	53	57	33	21	6	1	1			188	1210
All stages												
109-32-S	9	15	36	10							70	1090
19-30-S				8	4	30	13	9			74	2370
High x Low F_2	46	171	178	100	65	26	7	1			594	1250

low-HCN content which conforms to that put down for Table 8 that **low-HCN is partially dominant over high-HCN content.**

The data in Table 10 were obtained from a cross between high-HCN Amber, 19-30-S, and low-HCN, Milo, 109-32-S, grown in F_2 at Brookings in 1936.

It may be noted from the heading of Table 10 that the parental strains were of two distinct varieties, namely Milo and Amber. It may be further explained in detail that one reason for choosing the strain of Milo for the low-HCN parent, was the fact observed that wherever the kernel, head, or stalk characters of Milo appeared, there likewise appeared low-HCN. **These characters appeared to be correlated with the low-HCN character although further work will be necessary to establish this fact.** It was found practically possible, therefore, to identify whatever hybrid plants observed to carry the Milo kernel, head and stalk character as also having the character of low HCN. The hybrid plants segregating for a combination of Milo and Amber kernel head or stalk characters were intermediate in their HCN content. This also was true where they segregated for Amber kernel, head or stalk characters.

Examination of Table 10 makes it further evident that the several plants characterized either by low-HCN or high-HCN fall into separate and distinct classes for each of the separate stages of growth put down in the table; likewise, the classes in F_2 with respect to HCN content display a segregation with apparently a greater number of the population possessing the character of low-HCN which in turn may indicate a degree of dominance for the latter character.

The foregoing in general are indications of the Mendelian character of inheritance for HCN content in the sorghum strains concerned.

Table 11 summarizes the classes into which all of the growth stages of the parental strains were classified whether of one variety or another with respect to low-HCN or high-HCN; likewise it summarizes all plants of F_2 progenies of whatever parentage for the year 1936 according to class of low-HCN or high-HCN.

TABLE 11. Classification of All Plant Groups for Low-HCN and High-HCN Made in 1936, Including Parental Strains, and F_2 Progeny. (Summarized from Tables 8, 9, and 10.) (1936).

Parent and Progeny	Group of HCN in green forage, all growth stages (p.p.m.)											
	370	780	1190	1600	2010	2420	2830	3240	3650	4060	N	Ave. HCN p.p.m.
Low parents	41	73	56	11							181	855
High parents				8	15	43	57	39	10	5	187	2723
F_2 progeny	54	293	395	338	190	85	20	5	1	1	1382	1184

It is possible to observe from such a summary table that the plants from the parent strains allot themselves within the HCN groups with very little overlapping. It is further possible to observe that the 1,382 F_2 progeny plants allot themselves so that the greater number occur under the low-HCN groups. Such a summary of all the plant groups analyzed for HCN in 1936 substantiates the indication that the character of low HCN is dominant over high HCN.

In the season of 1936, as already noted in Tables 8, 9, 10, the F_2 plants were found to segregate with relation to the HCN content, and to classify themselves into groups which could be called: (1) Low; (2) Intermediate, and (3) High-HCN.

It was possible to select five plants from each of the foregoing groups in 1936 and subject flowers which grew on tillers of these plants to the process of selfing, in order to secure seed from them for planting an F_3 progeny test in 1937.

The seeds from each one of the five selfed plants of each separate group were planted in separate rows for the study of 1937. Representative progeny plants from each of these groups were analyzed for HCN. The plants are classified in the lower part of the following Table 12. Also in Table 12 will be found the classification of the F_2 plants, out of which the seed for these F_3 plants was selected, also the F_1 plants of the previous generation. Likewise in the upper part of the table the original parental strains are classified. It may be recalled here that the sorghum plants which furnished data for the following tables were produced under uniform nursery conditions in 1937.

TABLE 12. Frequency Distribution of Plants According to HCN Content, Including the High-HCN Parent, 19-30-S, the Low-HCN Parent, 39-30-S, and the F_1, F_2 and Three Selected F_3 Progenies. (1937).

Parents and Progeny	p.p.m. HCN in green forage											
	370	780	1190	1600	2010	2420	2830	3240	3650	4060	N	Ave. HCN p.p.m.
39-30-S (Low-HCN)	43	47	12	2							104	680
19-30-S (High-HCN)						10	20	37	29	8	104	3360
High x Low F_1	19	20	45	68	46	29	6	2			235	1580
High x Low F_2	4	49	105	106	60	20	13	2	1		360	1530
High x Low F_3 Low*	40	147	55	3							245	820
High x Low F_3 Intermediate†	1	29	103	23	11	3					305	1620
High x Low F_3 High§		1	14	22	55	112	74	27	4		309	2400

* Ave. of the 5 F_2 low plants selected 990 p.p.m. of HCN.
† Ave. of the 5 F_2 intermediate plants selected 1960 p.p.m. of HCN.
§ Ave. of the 5 F_2 high plants selected 3040 p.p.m. of HCN.

Examination of Table 12 makes it evident that: (1) The HCN content in parent strains, are in classes (a) low, and (b) high, distinct from one another. (2) The groups of F_1 place themselves toward the left-hand columns of the table, which mark the lower HCN contents **indicating a degree of dominance for characters determining low HCN over those determining high HCN.** (3) The distribution of plants in the horizontal line marked F_2 is grouped in the low-HCN classes. (4) The groups of F_3 plants in the three lowest horizontal lines, which were produced from three corresponding classes of seed out of F_2 in 1936, conform only approximately to the F_2 segregations. The inheritance studies summarized in Table 12 apply to numbers of plants produced in 1937. They corroborate the studies made and previously summarized for 1936.

There remains slight room for questioning that HCN content in sorghums is controlled by heritable factors. Further, low HCN appears to be partially dominant over high-HCN. It is impossible to arrive at a definite Mendelian ratio but the results indicate that only one or two main factors and possibly several minor modifying factors are involved in the inheritance of HCN content.

Part III. HCN Content, Influenced by Conditions Other Than Inheritance

Location of HCN in Plant Parts, viz. Leaves and Stems. The data in the foregoing section used as a basis for inheritance studies, were all arrived at from analyses of leaves of sorghum plants. Obviously, the location of HCN in the several parts of the sorghum plants is a matter of theoretical and practical importance.

In two separate seasons, separate analyses for HCN were made from leaves and stems of plants. In 1933 two sets of these analyses were made from plants of a commercial variety and in 1936, six sets of such comparative analyses of leaves and stems were made from S_6 lines. The results are tabulated for comparison in Table 13.

TABLE 13. Comparative Amounts of HCN in Leaves and Stems of Sorghum Plants. (1933 and 1936).

Strain	Year	Type of Growth	Date	HCN in leaves p.p.m.	HCN in stems p.p.m.	Percent in stems utilizing leaves as 100 percent
Commercial	1933	First	8-28	1080	60	5.56
Commercial	1933	First	8-28	810	145	17.90
Ave.	1933			945	103	10.90
1-30-S	1936	Second	8-24	2340	290	12.39
15-30-S	1936	Second	8-24	2520	390	15.48
23-30-S	1936	Second	8-24	3280	430	13.11
18-30-S	1936	Second	8-24	3360	330	9.82
20-30-S	1936	Second	8-24	3410	640	18.77
19-30-S	1936	Second	8-24	3510	230	6.55
Ave.				3070	385	12.87
Ave. All				2539	314	12.37

It is apparent that the relative amount of HCN in leaves and stems is not constant, but it is further evident that the percentages of HCN in leaves are invariably higher than the percentage in the stems of the same plants. The outstanding fact of the table is that the average HCN content in the stems of plants analyzed is only 12.37 percent of that in the leaves.

HCN Content in Two Strains at Different Periods of Day. Obviously, if it were possible to establish a definite period within the time of day when the HCN content would be low or negligible, such information would be of practical importance as well as scientific interest. In 1936, samples were taken from two separate S_6 strains of sorghum on dates extending from July 17 to July 24. Likewise one sample on each date was taken at 8:00 a.m. and another at 1:30 p.m. for both strains. The principal object was to determine whether the HCN content in any or all of the samples taken at 8:00 a.m. would be greatly different from that in samples taken at 1:30 p.m.

Table 14 summarizes the HCN content found in the two strains on the different dates, likewise at the two periods of day when the samples were taken.

TABLE 14. Comparative Amounts of HCN in Sorghum Samples Secured at 8:00 a.m. and 1:30 p.m. on Four Different Dates. (1936).

Strain No.	Date	Stage of Growth	HCN content in sample taken at given hour of day. p.p.m. 8:00 a.m.	1:30 p.m.	Difference
19-30-S (High-HCN)	7-17	9-leaf	3980	2870	−1110
19-30-S	7-21	Shooting	2720	2680	− 40
19-30-S	7-22	Shooting	2590	3090	+ 500
19-30-S	7-24	Shooting	3450	3350	− 100
Ave.			3185	2997	− 188
39-30-S (Low-HCN)	7-17	9-leaf	870	1010	+ 140
39-30-S	7-21	Shooting	940	970	+ 30
39-30-S	7-22	Shooting	740	880	+ 140
39-30-S	7-24	Shooting	890	910	+ 20
Ave.			860	943	+ 83
Ave. All			2023	1970	− 53

It may be observed that samples of high-HCN sorghum secured at 8 a.m. were slightly higher in four instances out of five than corresponding samples taken at 1:30 p.m. The third comparison in the given group makes a somewhat higher HCN content for the samples taken at 1:30 p.m. The average difference for said group is so low that evidently the components which make it up are not significant. In the lower part of the table which deals with the HCN content in a low-HCN strain, the amounts are consistently, although very little, larger for the samples taken at 1:30 p.m. than at 8:00 a.m.

It appears certain from the foregoing table that differences, if any, in amounts of HCN occurring in the strains of sorghums at two different periods in the day, are small and perhaps insignificant.

HCN in Several Strains Grown in Two Locations. It has been asserted in the earlier part of this bulletin that the content of HCN in sorghum is evidently controlled by heritable factors. It is none the less necessary to know whether differences in environmental conditions that may occur between situations more or less widely removed from one another may still modify the HCN content. In 1934 12 S_4 lines of Dakota Amber and one bulk selection of Sudan grass were planted in the nurseries at both the Brookings Experiment station and Cottonwood sub-station. The methods of planting and the culture were similar at the two places. Obviously the conditions of soil and climate were dissimilar. The immediate purpose in planting the strains at the two places was to find out if possible whether corresponding differences in HCN content would develop rather than to define the difference in environment.

On August 20, when it occurred that the strains were in the early

dough stage at both Brookings and Cottonwood, samples were harvested for analysis. At Brookings, the samples were analyzed immediately after being taken from the plants which presumably allowed minimum loss of HCN from the samples. The samples at Cottonwood were taken from the plants and placed immediately in Mason jars and sealed in the usual manner. The jars were placed on the afternoon train so that they arrived at Brookings at 10:20 a.m. the next day. They were removed from shipment at the earliest possible moment and analyzed for HCN. The authors are aware that the treatments given the samples from Brookings and those from Cottonwood were not identical but it was as nearly so as possible under the circumstances.

In Table 15 are given the comparative parts per million of HCN in samples of the same strains produced at Brookings and Cottonwood.

TABLE 15. HCN in Identical Strains of Sorghum Grown at Brookings and Cottonwood (1934).

Strain	HCN content p.p.m. Brookings	Cottonwood	Difference
	p.p.m.	p.p.m.	p.p.m.
20-30-S	2140	4070	1930
15-30-S	2080	6430	4350
1-30-S	2040	4430	2390
7-30-S	1990	4590	2660
18-30-S	1910	6330	4420
23-30-S	1830	5040	3210
5-30-S	1510	5790	4280
24-30-S	1160	2770	1610
13-30-S	720	4780	4060
3-30-S	580	2350	1770
30-30-S	350	3520	3170
39-30-S	250	2040	1790
*Sudan Grass	100	1770	1670
Ave.	1282	4146	2864

* Sudan was from seed grown 10 years at Cottonwood.

It is evident that the amount of HCN in every one of the 13 comparisons is higher for the Cottonwood samples. As an average, it is 3.2 times as high as at Brookings. This wide consistent difference is the outstanding indication of the table. Evidently some difference or differences in conditions of growth at two locations caused corresponding differences in HCN content of the 13 identical strains.

In the second column of the table the strains are listed in order of their content of HCN at Brookings. The order for the samples from Cottonwood is at variance, indicating that all strains do not react similarly at the two locations. Careful examination, however, may reveal that the trend is the same as that at Brookings.

HCN in a Single Strain, Placed in 11 Separate Localities in Comparison With Commercial Strains. The foregoing section which summarizes tests of several strains grown at Brookings and Cottonwood substations, demonstrates that although HCN content in sorghum is heritable, it may be nevertheless modified by conditions where grown. In 1938, it was possible to introduce seed from selfed strain 39-30-S into sorghum trials at different locations in several counties. At given stages of growth, samples were taken from plants of this strain and from comparative plants of commercial strains in the same trials.

The samples when taken were immediately wrapped in oiled paper and forwarded to Brookings where they were analyzed for HCN. The results of these analyses are tabulated in Table 16.

TABLE 16. HCN in Samples of Selfed Strain Grown in Several Locations in Direct Comparison with Known Commercial Strains. (1938).

County where trial field was located	Commercial Variety			Selfed Strain 39-30-S Dakota Amber	
	Name	Stage	HCN p.p.m.	Stage	HCN p.p.m.
Lyman	Dakota amber	Headed	5700	Headed	2370
Kingsbury	Dakota amber	Headed	4550	Headed	3000
Kingsbury	Sudan grass	Heading	3170	Headed	2990
Kingsbury	Dakota amber	Headed	5080	Headed	2730
Brown	Altamont	Fertilizing	810	Dough	960
Brown	Altamont	Fertilizing	1900	Fertilizing	1880
Pennington	Altamont	Hard dough	3280	Hard dough	2880
Clark	Red amber	Medium dough	4470	Medium dough	2390
Perkins	Dakota amber	Early dough	5380	Fertilizing	2740
Pennington	Atlas	1 ft.	6140	1½ ft.	3570
Brookings	Dakota amber	Fertilized	2500	Fertilized	390
Average			3907		2345

A comparison of amounts of HCN indicates that the content in the selfed strain of low-HCN sorghum (39-30-S) was lower in all locations, with one exception, than the content in the samples of the commercial strain taken from the same trial field. Such a fact is in line consistently with the fact that low-HCN content is controlled by inheritance.

Likewise in the last column there is some degree of variation in p.p.m. of HCN in the selfed strain itself as between four locations all taken at the same stage, with the greater difference between samples taken from separate counties, corroborating the belief that HCN content though controlled by heritable factors is modified by environmental conditions.

HCN Content on Differing Soil Types. In the previous two sections of this bulletin it was indicated that HCN content of sorghum though controlled by heritable factors, was influenced at differences in growing conditions at locations more or less widely separated.

It remains to be found what the factor or factors might be that cause such differences. It was recognized that differences in soil at the two locations might be an explanation in whole or part. Pursuant of that idea, greenhouse cultures were arranged for producing sorghum on two separate kinds or types of soil where conditions were otherwise identical.

Orman clay was shipped from Cottonwood sub-station Farm, and Barnes loam was secured from an area near Brookings. The exact description of these two soil types is not included here. The low-HCN strain, 39-30-S, and the high-HCN strain, 19-30-S, were used in this comparison. At given stages of growth comparative samples were taken from the pots having (1) Orman clay and (2) Barnes loam which had been planted on April 9 and held at a moisture content of 25 percent.

The results of analyses of these comparative samples are tabulated in Table 17.

TABLE 17. HCN Content of Sorghum from (1) Orman Clay and (2) Barnes Loam. (1937).

Type of Soil	Kind of Sorghum	Ave. Weekly Growth Inches	Stage at time of first sampling	HCN p.p.m.	Ave. Weekly Growth Inches	Stage at time of second sampling	HCN p.p.m.
Orman	Low-HCN	1.00	8-leaf	1760*	1.40	Shooting	730†
Barnes	Low-HCN	1.58	Shooting	570*	1.73	Heading	90†
Orman	High-HCN	1.22	8-leaf	4310*	1.55	Shooting	2670†
Barnes	High-HCN	1.86	Shooting	950*	2.03	Heading	930†

* Harvested June 19.
† Harvested June 25

It is noted from the third and sixth columns of the table that the rate of growth of sorghum plants, on Barnes loam, was decidedly more rapid than that of those on Orman clay. The plants on Barnes loam had arrived at the shooting stage June 19, those on Orman clay required until June 25 to arrive at the same stage of growth. Whatever the technical differences in the soils of two types on which the plants were produced, they grew more rapidly on the Barnes loam.

The content of low-HCN plants produced on Barnes loam at the shooting stage was 570 p.p.m., whereas samples taken six days later from plants at a similar stage of growth on the Orman clay analyzed 730 p.p.m. of HCN. Likewise high-HCN plants at the shooting stage, from Barnes loam were found to contain 950 p.p.m. of HCN whereas those at a similar stage of growth from Orman clay six days later, contained 2,670 p.p.m. of HCN.

Obviously the slow growing plants of both low-HCN and high-HCN strains on Orman clay developed a higher content of HCN (at shooting stage) than the more rapid growing plants on Barnes loam. It is reasonable to assume that the two types are representative of large areas, Barnes east of the Missouri river and Orman west. Type descriptions are available in reports of the United States Bureau of Chemistry and Soils. It is of interest here to recall that the soil types are widely different.

HCN Content Modified by Soil Fertility. A soil fertility experiment is conducted with the use of a series of treated plots on the East Farm, Brookings, Hutton (11). These differential fertility treatments involve applications of the following materials, individually and in combination—stall manure, acid phosphate, fine ground phosphate, limestone.

After the small grain harvest in 1937, four separate pure S_6 lines of Dakota Amber were planted on the several treated plots. This was done with the clearing a small area on the edge of each plot in the series and hoeing off the grain stubble, thus making a clean seed bed for the sorghum. Then a short row of each of the four strains was planted with the sorghum rows four feet apart. The sorghum was planted on July 17. Because of drought, it was necessary to supply water in equal amounts to the plants in the different plots by hand sprinkling.

During the growth of the plants it was noted that the color of the leaves seemed uniform though there may have been some variation in the width of leaves, and slight irregular variations in the number of leaves per plant.

The plants on the several plots were sampled September 7 and their HCN contents determined. The summary of soil treatments regularly applied in the course of the fertility experiment already cited, the amounts of HCN in the four pure lines, and the height of plants measuring the growth on the several plots are given in Table 18.

An examination of Table 18 may make it appear that the average **parts per million of HCN for the plants of all the selfed strains on plots where stall manure is applied was invariably lower than on the corresponding check plots receiving no treatment.** The HCN content was roundly twice as high on the check plots as on the manured plots. The unmanured plots had an average for the four lines of 3,943 p.p.m. of HCN and the manured plots had an average for the same four lines 1,983 p.p.m. of HCN.

It is hardly tenable to say that this difference was reflected in the manner of growth of the plants, though it was observed that the leaves

TABLE 18. HCN Content (p.p.m.) in Plants of Four Selfed Strains Grown on the Regular Fertility Plots at Brookings. (1937).

	Plot treatment per acre in course of regular fertility experiment							
Strain	None	Manure 10 T.	Acid Phos. 300 lbs.	Fine ground Phos. 600 lbs.	Lime 2000 lbs.	Manure 10 T. Lime 2000 lbs.	Manure 10 T. Lime 2000 lbs. Acid Phos. 300 lbs.	Manure 10 T. Lime 2000 lbs. Rock Phos. 600 lbs.
1-30-S	3000	960	830	680	1100	3100	2130	2960
15-30-S	5300	2220	5020	3330	5520	4880	4780	5880
18-30-S	5440	3530	3310	3520	3960	4320	4220	4100
39-30-S	2030	1220	1440	1170	930	1320	1810	1380
Ave.	3943	1983	2650	2175	2878	3405	3235	3580
Height	10.1	10.3	10.0	12.3	12.3	10.8	11.3	13.5

were narrower on the manured plots than on the others. The difference in average height of plants was evidently slight, though it was in line with a little more growth on the manured plots.

The HCN content was invariably lower from plots receiving acid phosphate than from corresponding ones given no treatment. The same was true concerning plants on plots treated with fine ground phosphate.

The HCN content on the plot treated with lime in addition to manure, was higher for all strains than where manure was applied alone.

Without attempting here to draw analogies too closely, it is suggested that the HCN content of sorghum plants in this experiment was relatively lower on land treated with those fertilizers known to promote the growth of crops in general. Moreover, the fact that HCN content of plants was higher where stall manure plots were treated with lime accords with the observed fact that lime seems to retard the maturity of crop plants on the soils here involved.

HCN Content in Plants From Complete Fertility Tests. A complete fertility test also is conducted on the East Agronomy Farm, Brookings, Hutton (11). Sorghum plants were produced from seeding on the several plots of this fertility test after harvest in the season of 1937. The plants were produced in the same manner as those described in the previous section. The plants grew sufficiently so that it was possible to take samples and analyze them for HCN on September 7.

The kind of fertility regularly applied on the several plots and the amounts of HCN found in the samples from the various strains are given in Table 19.

TABLE 19. HCN Content (p.p.m.) in Plants of Four Selfed Strains Grown on Complete Fertility Plots at Brookings. (1937).

	Plot treatment per acre in course of regular fertility experiment							
Strain	None	Nitrogen 350 lbs.	Acid Phos. 200 lbs.	Potassium 200 lbs.	Nit. 350 lbs. Phos. 200 lbs.	Nit. 350 lbs. Pot. 200 lbs.	Phos. 200 lbs. Pot. 200 lbs.	Nitrogen 350 lbs. Phos. 200 lbs. Pot. 200 lbs.
1-30-S	3610	3860	4130	2720	4660	3380	2770	4430
15-30-S	5200	6220	5870	3810	6740	5890	3660	5580
18-30-S	4900		4110	3960	5690	4970	4310	5400
39-30-S	2230	2980	2030	1960	2090	2930	1380	2320
Ave.	3985	4353	4035	3113	4795	4293	3030	4433
Height	13.9	10.7	12.5	16.0	14.8	11.6	15.5	13.6

The average amount of HCN is invariably higher on plots where nitrogen is regularly applied than where nothing is applied. It is likewise true that the HCN content is higher in all cases where nitrogen is applied with acid phosphate than where acid phosphate is applied alone. Moreover, the corresponding amounts of HCN are higher where nitrogen is applied in addition to both phosphorous and potassium than where the latter are applied together without nitrogen. Evidently the application of nitrogen in addition to potassium in the regular cropping system has increased the HCN content in sorghum plants in the experiment.

From the foregoing, it would appear that for some reason, the regular use of nitrogen in the cropping system is correlated with an increase in the amount of HCN content of sorghum grown upon the soil. The average height of plants at the time of sampling which may be taken to indicate bigger growth is greater on the check-plots receiving no nitrogen than on those where nitrogen alone was applied. Likewise the average height of plants where nitrogen was applied along with both phosphorous and potassium was less than where only phosphorus and potassium were applied together.

The height of plants grown where nitrogen is applied in addition to potassium is less than where potassium is applied alone. Apparently the only instance where an average increase in height of plants is associated with the application of nitrogen is that where nitrogen is applied in addition to phosphorous as compared with the application of phosphorous alone.

The HCN content of plants on potassium plots was invariably lower than where nothing was applied and likewise lower than where either nitrogen or acid phosphate were applied either singly or in combination. The HCN content of plants on potassium plots was also invariably lower than that on the comparative plots where all three elements, nitrogen, phosphorous and potassium were applied. The application of potassium in addition to phosphorous invariably caused reduction of HCN in sorghum, as compared with the application of phosphorous alone. For whatever reason, it appears that the application of potassium alone or in combination in this experiment is associated with lowering of the HCN content in sorghum plants grown upon the soil.

The average height of sorghum plants at times of sampling was highest on the plot where potassium alone was applied, likewise the plants were higher where potassium was applied with nitrogen alone and higher where applied with phosphorous than with phosphorous alone.

HCN in Sorghum as Affected by Nitrogen and Phosphorous in Greenhouse Cultures. Additional information concerning the effect of fertilizers containing nitrogen and phosphorous and their combinations was secured in the summer, 1938, with the use of greenhouse cultures. Twelve 2-gallon stone jars were prepared March 21, each with 4000 grams of Barnes soil. A series of six pots was planted with a low-HCN strain and six pots with a high-HCN strain, in all cases eight plants per pot. Sufficient water was added to the soil of each of the pots to bring it up to 25 percent moisture, which percentage was retained throughout the experiment.

Differential fertility treatments were applied to the several pots of soil in the series, each fertility application being made in duplicate. One of these duplicates was planted with low-HCN 39-30-S and the other with high-HCN 19-30-S.

During the season two crops of sorghum were produced and harvested from the two series of pots. The first crop being harvested at the time of sampling, May 9. They were then replanted without addition of more fertility, thus any effect of the nitrogen and phosphorous treatments on the second crop must have been residual. The summary of the foregoing experiment is put down in Table 20.

TABLE 20. HCN Content in Two Separate Strains of Sorghum from Greenhouse Cultures, as Affected by Application of Nitrogen, or Prosphorus, and Combinations. (1938).

Treatment	Amt. per Acre lbs.	Low HCN Strain Ave. Weekly Growth in Inches 1st. crop	2nd crop	HCN p.p.m. 1st. cr.	2nd. crop	High HCN Strain Ave. Weekly Growth in Inches 1st crop	2nd. cr.	HCN p.p.m. 1st. cr.	2nd. cr.
None		1.375	3.50	1220	90	1.375	3.50	2940	1140
Sodium Nitrate	200	1.417	2.75	1320	260	1.667	3.31	2970	570
Sodium Nitrate	300	1.417	3.00	940	270	1.292	3.19	3280	1130
Sodium Nitrate	500	1.917	3.00	600	320	1.833	3.00	3100	1330
Acid Phos.	200	2.083	3.00	530	60	1.750	3.06	2630	940
Sodium Nitrate	300)								
Ac. Phos.	200)	2.083	3.06	1130	220	1.667	3.06	3270	860

The relation between the application of phosphorous and the amount of HCN makes it evident that the latter **was always lower from pots where acid phosphate was applied alone** than in the check pots where no fertilizer was applied. The same was true with both the low-HCN and high-HCN strains, and with first- and second-crop plants.

It is generally true that where nitrogen was added to phosphorus the amount of HCN from the corresponding plants was slightly greater than where phosphorous was applied alone. The apparent exception came in the case of the second crop of high-HCN where the difference was hardly significant.

The foregoing might be taken as a tentative indication that the addition of nitrogen was the cause of increasing HCN in sorghum, under the controlled conditions. In this connection it is true that the addition of nitrogen alone caused only slight general increase in HCN in sorghum as compared with that from similar check or untreated plots. However, the addition of nitrogen in some amount was always found to increase HCN content as compared with that in plants from pots with no treatment.

It may be stated here that the foregoing statements relative to HCN content in sorghum plants as affected by application of nitrogen under controlled conditions are in general agreement with tests carried on in the field and described in foregoing section. The comparative results from the two tests where phosphorous is involved also agree substantially.

HCN Content Influenced by Application of Stall Manure, Limestone, Potassium, or Combinations in Sorghums From Greenhous Pot Cultures. A further experiment was carried out in the greenhouse to secure further information as to the effect of application of stall manure, limestone, potassium, alone or in combination, upon the amount of HCN in sorghum.

Thirty-six two-gallon stone jars were filled, each with 4,800 grams of Barnes soil from a West Agronomy farm plot, that had been in continuous winter rye and untreated with any fertilizer so far as known, certainly not since 1908.

These were arranged in two groups of 18 pots each, in order to plant

each of the groups with a separate strain of sorghum. Likewise nine pairs of duplicates (total 18) in each of these sets were treated with fertilizer as given in Table 21.

TABLE 21. HCN Content in One Strain of Low-HCN Amber and One Strain of High-HCN Amber from Greenhouse Pot Cultures Treated With: 1, nothing; 2, manure; 3, lime; 4, potassium; 5, combination. (1938).

Treatment	Height of Plants July 11		Average Weekly Growth, Inches		Average HCN p.p.m.	
	39-30-S Low HCN	19-30-S High HCN	Low HCN	High HCN	Low HCN	High HCN
None	14½	14½	1.30	1.25	740	3260
Manure, 10 T.	17½	18½	1.65	1.65	80	890
Lime, 2 T.	17	17	1.55	1.50	90	1670
Lime, 6 T.	17	18½	1.55	1.65	140	1870
Lime, 10 T.	17½	17½	1.60	1.55	170	2320
Potassium 400 lbs.	17½	18½	1.60	1.65	210	1440
Manure, 10 T. Lime, 6 T.	18½	18	1.70	1.60	100	720
Manure, 10 T. Potassium 400 lbs.	19	20	1.75	1.80	120	870
Manure, 10 T. Lime, 6 T. Potassium 400 lbs.	18	18	1.65	1.60	100	1560

An examination of the data will substantiate the other observations in this bulletin that the application of stall manure in and of itself brings about reduction in the amount of HCN in sorghum plants produced on soil where such application is made. The amount indicated is lower for both low and high-HCN strains where stall manure was applied than where nothing was applied. Moreover, the content of HCN in plants from pots where manure was applied in combination with potassium was lower in both instances than where potassium was applied singly.

The application of limestone which was applied in the two separate pots in increasing amounts was found associated with correspondingly increased amounts of HCN in the plants harvested therefrom. Such a fact in itself would substantiate the observation made previously that the application of lime tends to inhibit the rapidity of growth of sorghum plants which accordingly may cause an increase in the HCN content.

HCN in Plants From Several Crop Systems (With or Without Green Manure). Some additional observations bearing on the extent to which variation in soil conditions might affect HCN content in sorghum were made by growing four separate selfed strains as catch crops on the soil of several rotation experiments after the regular crops had been harvested in 1937. The manner of making them was same as that described in two sections previously discussed.

The several crop rotations which served as a basis for making these differential trials were located on the West Agronomy Farm, Brookings. They are as follows: The kind of crop in the regular rotation which occupied the land in 1937 and which consequently immediately preceded the sorghum in this experiment is the crop in larger print.

Rotation 1: Corn, oats, **winter wheat,** red clover (1st. crop hay, 2nd. crop seed)

*Rotation 1: Same as above rotation 1, excepting an application of 10 tons of stall manure once in the rotation.

Rotation 27: Corn, **wheat,** barley, oats, clover (1st. crop hay, 2nd. crop seed)

Rotation 19: Continuous **wheat** since 1897.

Rotation 16: Corn and **wheat.**

Rotation 3: Corn, **wheat,** sweet clover (1st. crop hay, 2nd. crop green manure)

**Rotation 3: Same as above rotation 3, except an application of 10 tons of stall manure once in the rotation.

The sorghum plots were seeded July 17 and supplied with water by hand, samples being taken for HCN determinations on September 7. Table 22 summarizes the HCN content found in plants from the several rotations.

TABLE 22. HCN in Sorghum Plants Produced on Soil of Several Crop Rotations. (1937).

Strain	HCN in plants from given Rotations, p.p.m.						
	1	*1	27	19	16	3	†3
1-30-S	3000	960	3610	1190	450	1840	1530
15-30-S	5300	2220	5200	4550	2280	3570	3510
18-30-S	5440	3530	4900	3100	990	4380	2850
39-30-S	2030	1220	2230	1070	310	750	300
Ave.	3943	1983	3985	2478	1008	2635	2042
Height at time of sampling	10.13	10.25	13.9	10.1	11.4	24.1	16.5

It is evident that some differences have been brought about in HCN content of sorghum plants produced on soil of several crop systems. The lowest HCN content occurs in plants produced on Rotation 16, with only one insignificant exception in the case of Strain 39-30-S which is slightly lower in Rotation 3†. In comparison, plants from Rotation 3 (where a legume is introduced) are higher in HCN than in 16 where there is no legume. The same is true when a comparison is made between plants from Rotation 3 and Rotation 1, where likewise a second crop of clover is plowed under as green manure.

Thus it is the case in both rotations of this series where green manure is included, that the amounts of HCN in plants produced after harvest upon the soil is higher than in plants from the soil of a two year rotation (16) without green manure. In Rotation 3 where the sweet clover was plowed under for green manure, the average HCN content for the four lines is 2,635 p.p.m. In that portion of Rotation 3† where 10 tons of stall manure is added along with the green manure, the average HCN content for the four lines is 2,042 p.p.m. Where stall manure was applied in Rotation 3† along with the green manure, the HCN content is lower than where the stall manure was left off and green manure turned under. Without attempting to draw close analogy here, the immediate effect of green manure is exactly opposite from that of stall manure as pointed out in Table 18.

One tentative suggestion as to this apparent effect of green manure in increasing HCN content of sorghum grown subsequently, is that the growth of green manure in itself may have reduced soil moisture available for the rapid subsequent growth of sorghum. Such moisture reduction might in turn inhibit the rapid sorghum growth.

In this specific connection the average HCN content of sorghum plants of all four strains produced on soil of Rotations 1 and 27, where legumes were harvested for hay and seed was invariably higher than in the case of Rotation 3 where similar legumes were turned under somewhat earlier for green manure.

It is an observation of the writers that whatever condition inhibits the

growth of sorghum or renders it irregular, makes a corresponding increase in content of HCN.

HCN Content in Two Strains of Sorghum Not Greatly Affected by Moisture From Ordinary Rainfall. It has been assumed in some instances by growers of forage and others, that natural moisture supplied to the crop by rain, and consequent vigorous growth ofttimes following periods of comparative drought, would be accompanied by increases in HCN content of sorghum plants. Some information has been assembled bearing upon the above assumption.

In 1936, conditions in the month of June were favorable for the growth of sorghum. However, from the middle of July until August 13, temperatures were high, precipitation low, and vegetative growth was retarded. During the day leaves of sorghum were often rolled. On August 13, there came a shower of 1.01 inches of rain, obviously enough to cause sorghum to resume normal growth. Table 23 may serve as a summary of conditions, and of the amounts of HCN in one low and one high-HCN strain of sorghum before and following the aforementioned shower of August 13.

TABLE 23. HCN in Two Strains of Sorghum one day Previous to Rainfall and at Periods Extending One to Six Days Thereafter. (1936).

Stage of Growth of Sorghum	Date of Taking Sample	No. of days before or after shower on August 13.	Average HCN p.p.m. Low HCN (39-30-S)	Average HCN p.p.m. High HCN (19-30-S)
Heading	7-26	1 day before	910	3350
Heading	7-27	Rained (0.2")	800	3090
Heading	7-28	1 day after	730	2820
Heading	7-29	2 days after	510	2760
Milk	8-12	1 day before	510	2630
Milk	8-13	Rained (1.01")	420	2300
Milk	8-14	1 day after	420	2130
Milk	8-18	6 days after	460	1710

Examination of the two separate series of the HCN content in the last and next to last columns of the table will make it evident that said amounts of HCN in both instances decreased gradually from the date when the earliest sample was taken down to and including the date when an appreciable shower of rain occurred, namely August 13. Further examination of the amounts of HCN on the subsequent dates thereafter will indicate likewise that the rain which occurred August 13 could have had no great effect in the direction of increasing the HCN content in either strain of sorghum.

The general trend of HCN put down in Table 23 is in accord with the outcome described in Figures 1-2, pp. 16-17 that there is normally a gradual diminution in HCN content of sorghum from early to late stages of growth and that ordinary rainfall will not influence the content enough to obviate such a general trend.

HCN Content in Irrigated and Non-Irrigated Sorghum. In the foregoing sections of this bulletin it appeared that the amount of HCN in sorghum produced on different soils was evidently caused to vary by the soil differences whether the soils were in different localities or under controlled conditions in the greenhouse. In Table 24 are presented comparative amounts of HCN from different strains of sorghum produced in the same season on irrigated and non-irrigated land in Butte county, S. D.

TABLE 24. HCN Content in Comparative Samples from Irrigated and Non-irrigated Sorghums. (1937).

Date of Receiving Samples at Brookings	HCN in given samples, p.p.m. Low-HCN Amber		Commercial Amber	
	Irrigated	Non-irrigated	Irrigated	Non-irrigated
Aug. 22	2120	3400	6300	
Aug. 25	2530	3530	5540	9310

It may be noted that two sets of samples of sorghum were sent in from Butte county by growers in that locality on two separate dates. Each of the two sets of samples contained two kinds of sorghums, namely a low acid strain (39-30-S) and a variety of commercial amber. Both lots of low-HCN amber were produced on irrigated and non-irrigated land and one of the lots of commercial amber was also produced on irrigated and non-irrigated land.

In all instances where direct comparison is possible, it appears that the HCN content from irrigated land is decidedly lower than the HCN content from non-irrigated.

It is only possible here to assume that the rate of growth was greater for the irrigated sorghum. It was observed that the samples of sorghum from non-irrigated land in all cases were of a relatively scanty appearance as compared with others from irrigated land. It is likewise possible that adequate moisture may have caused the vigorous growth and likewise inhibited the development of maximum HCN content directly or indirectly.

HCN in Sorghum, Affected by Variation in Moisture Content of Soil. It has been indicated in foregoing sections of this bulletin that difference in soil type may bring about change in HCN content of sorghum plants—likewise differences in fertilizer application, including stall manure, green manure, nitrogen, potassium, phosphorous, and lime. It was thought that variation in moisture content of soil might likewise induce variation in the HCN content of sorghums.

On March 21, 1938, 20 3-gallon jars were each filled with 9,000 grams of air-dry soil. Ten of these jars were planted with low-HCN amber 39-30-S and 10 with high-HCN amber 19-30-S. Each of the series of 10 jars was divided into five duplicates which were arranged in series with increasing percentages of soil moisture. The jars and contents were weighed three times per week and water added to bring the five duplicated in each set to their several moisture standards.

On May 6, the sorghum had grown in the several pots to a 9-leaf stage, at which time samples were taken for analysis for HCN. The summary of this experiment is put down in Table 25.

It appears from the foregoing that the average weekly growth of both strains of sorghum was caused to increase by increases in the amount of

TABLE 25. HCN Content in Two Strains of Sorghum Grown in Greenhouse Cultures With Varying Moisture Contents. (1938).

Moisture Percent	Low HCN (39-30-S) Ave. Weekly Growth Inches	HCN p.p.m.	High HCN (19-30-S) Ave. Weekly Growth Inches	HCN p.p.m.
15	1.417	880	1.417	3920
20	1.500	750	2.458	2120
25	2.583	510	2.417	2270
30	3.125	590	2.917	1570
35	3.167	380	3.125	1410

moisture supplied to the soil in which they grew. It appears likewise that the amount of HCN in the samples from the several pots decreased with negligible exception with the same regularity as the weekly growth in inches increased along with the regular increase of water added to the soil. Thus, this exeriment emhasizes the deduction from former sections of this bulletin that the growth environment of sorghum plants furnished by the soil, influences the HCN content. Apparently the change in water content of soil could influence growth even more regularly than any fertilizer application thus providing a more definite correlation between increased rate of growth and lower HCN content in sorghum.

HCN Content of Sorghum Affected by Atmospheric Conditions. It has been suggested in previous sections that modifications of soil in which sorghum may grow have direct influence upon the HCN content therein. It was considered that atmospheric environment, including temperature (as influenced by sunshine and wind) might likewise have influence.

The Agronomy greenhouse has two compartments called east and west, separated by a partition. For the present experiments, the roof panes of the east compartment were heavily whitewashed in order to cut down direct sunlight as much as possible by such means. The roof panes of the west greenhouse were left clear.

The jars described in the foregoing section of this bulletin, after completion of soil moisture experiment summarized in Table 25, were replanted with the same strains of low and high-HCN. The plants growing upon them had arrived at the four-leaf stage, May 28.

On that date, the odd-numbered duplicate jars of each pair having a given percentage of moisture were moved into the west compartment, the other duplicates were left in the east compartment. The ventilators in the west compartment were opened only a little, those in the east compartment were opened widely to accentuate difference in temperature as much as possible by that means. At various times during this experiment the temperature was 30° higher in the east than in west compartment.

All the jars were weighed three times per week and enough water added to bring them to the required percentage of moisture. In addition, a 14-inch electric fan was stationed in the west compartment to maintain circulation of air across the growing sorghum plants. During a seven-day period just previous to sampling for HCN the amount of water applied to the pots daily was recorded.

The plants in the west greenhouse on bright days showed the effects of heat, the leaves rolling during the hottest part of such days, whereas the leaves of plants in the duplicate jars in the east greenhouse gave no such indications.

Examination of Table 26 yields the information that the HCN content in this experiment with both strains of sorghum employed and in both compartments of the greenhouse decreases regularly with the increase of water content in the soil of the several pots. That is in general agreement with the outcome of the previous section where variations aside from soil moisture were disregarded. In short, an increase of moisture content in soil is accompanied by a decrease in the HCN content of plants.

With negligible exception the amount of HCN in plants from the east compartment was lower than in the west compartment. (It may be recalled that observed temperature was lower in the east compartment, due to re-

TABLE 26. HCN Content of Sorghum Plants of Two Strains, in Greenhouse with Induced Differences in Atmospheric Condition. (1938).

Moisture Percent	Condition under which grown: WEST—Sunshine, heat, breeze, slight ventilation — Low HCN 39-30-S			WEST—Sunshine, heat, breeze, slight ventilation — High HCN 19-30-S			EAST—Reduced sun, ventilation, lower temperature — Low HCN 39-30-S			EAST—Reduced sun, ventilation, lower temperature — High HCN 19-30-S		
	Ave. weekly growth inches	Ave. no. cc. water added daily	HCN p.p.m.	Ave. weekly growth inches	Ave. no. cc. water added daily	HCN p.p.m.	Ave. weekly growth inches	Ave. no. cc. water added daily	HCN p.p.m.	Ave. weekly growth inches	Ave. no. cc. water added daily	HCN p.p.m.
15	2.12	313	700	2.31	256	2950	2.88	150	320	3.12	159	2190
20	2.87	401	600	3.87	321	2500	3.44	178	230	3.06	151	1520
25	3.44	469	470	4.19	439	1790	4.01	179	150	4.00	227	1010
30	4.37	568	240	4.94	595	940	4.00	201	150	4.06	250	830
35	4.69	566	110	5.00	580	920	4.06	267	150	4.06	319	770
Ave.	3.50	463	424	4.06	438	1820	3.68	195	200	3.66	221	1264

stricted sunlight, and better ventilation was more complete via open ventilator. The plants were not exposed to direct draught from a fan as in the west greenhouse).

The average water requirement for each and all plants was higher in the west compartment than in the corresponding duplicates in the east compartment. In short, whereas an increased use of water under a given set of conditions, tended to decrease the amount of HCN, the higher water requirement (caused by change in atmosphere) was accompanied by a rise in content of HCN. It is not attempted at this moment to formulate the ultimate cause of such rise. It was an apparent fact that the conditions of growth in the west greenhouse were disturbed—perhaps in spite of an ample moisture supply.

HCN in Sorghum at Successive Stages of Maturity. It was observed early in these researches that sorghum in the earlier succulent stages of growth had a relatively high content of HCN whereas plants harvested at more mature stages were likely to have a lower HCN content. In 1933, analyses of 47 strains at the shooting stage produced an average of 8,970 p.p.m. whereas the same strains in the late dough stage were found to contain an average of 2,490 p.p.m. of HCN.

This problem was investigated further in three successive seasons at Brookings, by sampling and analyzing 12 pure lines of Dakota Amber and one line of Sudan grass, at seven stages of growth. The summary of these analyses is shown graphically in Fig. 3.

One outstanding indication of Fig. 3 is that the maximum HCN content was found at the stages of active growth. In 1935 and 1936, the maximum HCN content occurred as early or earlier than the shooting stage, while in 1934 it occurred at the fertilizing stage. In all three years there was a gradual reduction from the maximum HCN content toward the stage of maturity, with one exception in 1936 when a shower occurred on August 13, causing considerable renewed growth which might have delayed the maturity of the plants. Many side tillers were produced by the plants though these were discarded in taking samples for HCN determination.

The average HCN content for the three years 1934-36 indicates that the maximum HCN content occurs early, at the eight-leaf stage, diminishes slowly until the sorghum has reached the stage of becoming fertilized, after which it diminishes more rapidly to a minimum at complete maturity.

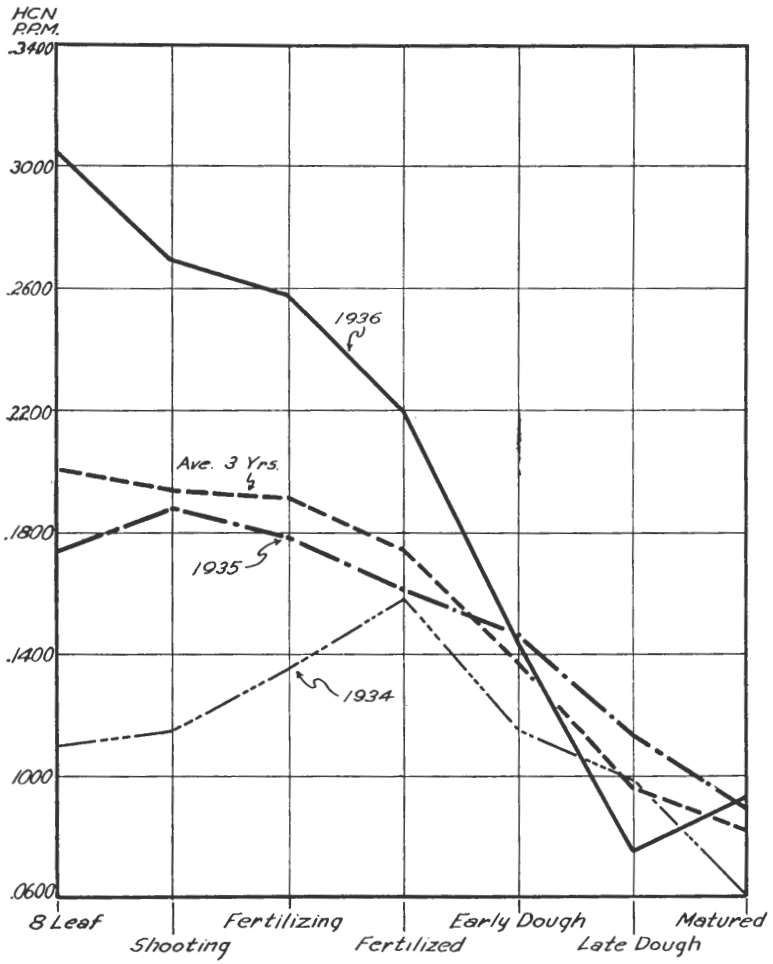

Fig. 3. HCN content in green forage of 12 pure lines of Dakota Amber and one strain of Sudan grass at seven growth stages at Brookings. (Three-year average, 1934-1936.)

HCN in Second Growth as Compared With First Growth in Sorghum. In a previous section it was shown that the leaves contained the higher HCN content as compared with the stalk. In the same general manner it was possible to compare the HCN content in second growth and first growth, using the same plants of 10 different strains out of four varieties in the three seasons of 1933, 1935, and 1936. Such a comparison is of interest because of the somewhat prevalent impression that second-growth sorghum is more poisonous to livestock than first-growth. Table 27 furnishes a summary of the comparative amounts of HCN from the first and second growth.

TABLE 27. Comparative HCN Contents of First Growth and Second Growth of the Same Sorghum Plants, at Comparative Stages. (1933, 1935 and 1936).

Year	Variety	Strain	Stage	HCN on Date Given: First growth Date	First growth HCN p.p.m.	Second growth Date	Second growth HCN p.p.m.
1933	Kaoliang	162-32-S	Shooting	7-19	870	10-13	580
1933	Milo	155-32-S	Shooting	7-20	620	10-13	270
1933	Dakota Amber	4-30-S	Shooting	7-21	880	10-13	340
	Ave.				790		397
1935	Dakota Amber	15-30-S	Shooting	7-24	2720	9-9	2390
1935	Dakota Amber	19-30-S	Shooting	7-24	2740	9-9	2070
1935	Dakota Amber	20-30-S	Shooting	7-24	2330	9-9	2080
1935	Dakota Amber	39-30-S	Shooting	7-24	620	9-9	460
1935	Sudan grass	176-S	Shooting	7-24	260	9-9	190
	Ave				1734		1438
1935	Dakota Amber	15-30-S	Heading	7-31	3070	9-16	1960
1935	Dakota Amber	19-30-S	Heading	7-31	2820	9-16	1650
1935	Dakota Amber	20-30-S	Heading	7-31	2950	9-16	1630
1935	Dakota Amber	39-30-S	Heading	7-31	510	9-16	510
1935	Sudan grass	176-S	Heading	7-31	450	9-16	110
	Ave.				1960		1172
1935	Dakota Amber	15-30-S	Fertilization	8-7	2820	9-23	3050
1935	Dakota Amber	19-30-S	Fertilization	8-7	2590	9-23	2690
1935	Dakota Amber	20-30-S	Fertilization	8-7	2440	9-23	2030
1935	Dakota Amber	39-30-S	Fertilization	8-7	420	9-23	420
1935	Sudan grass	176-S	Fertilization	8-7	1010	9-23	560
	Ave.				1856		1760
1936	Dakota Amber	19-30-S	Shooting	7-20	4530	8-13	4410
1936	Dakota Amber	18-30-S	Shooting	7-20	3930	8-14	3950
1936	Dakota Amber	15-30-S	Shooting	7-20	4060	8-17	4340
1936	Dakota Amber	1-30-S	Shooting	7-20	3000	8-18	3830
1936	Dakota Amber	39-30-S	Shooting	7-20	700	8-19	380
1936	Dakota Amber	176-S	Shooting	7-20	700	8-19	480
	Ave.				2820		2898
	3 year average				1958		1683

A comparison of the HCN contents in the first and second growths reveals that out of 24 comparisons, 17 have a higher amount of HCN for the first growth than for the second. In only five instances was the HCN higher for second growth and in two instances it was equal.

As an average the amount of HCN was 1,958 p.p.m. for the first growth and 1,683 p.p.m. for the second growth on corresponding plants.

HCN in Sorghum After Successive Days of Air-Drying. Obviously it is important to know what effect if any the drying or curing of sorghum may have upon the content of HCN.

TABLE 28. HCN in Sorghum on Successive Days, During a Period of Air-drying at Room Temperature. (1933).

No. days drying	HCN in samples from sheaves of given weight, p.p.m. 1500 gms. (3.3 lbs.)	1000 gms. (2.2 lbs.)
None	1080	1170
1	1130	1100
2	810	1000
3	890	750
4	850	650
6		840
7	1080	690
8	760	980
9	1050	710
10	1100	
13	520	
14	680	
15	640	
16	780	
17	740	
18	610	

In 1933, 24 sheaves of commercial Dakota amber sorghum weighing 1,500 grams (3.3 lbs.) each were dried at room temperature and sampled every day for a period of 18 days. Another group of sheaves of the same commercial Dakota amber, each weighing 1,000 grams were similarly dried over a period of nine days, with samples taken and analyzed daily for HCN. The amounts secured from these analyses are summarized in Table 28.

Apparently there was a gradual loss of HCN in the sorghums during the process of drying. The losses were somewhat irregular; perhaps due to the nature of the samples, taken as they were from commercial sorghum and not a uniform strain. The uniformity of reduction in amount of HCN in the second series of samples where the sheaves were smaller was apparently greater than that of the first series where larger sheaves were used.

Loss of HCN During Drying Process of Pure Lines of Three Varieties. Additional analyses of sorghum before and after drying gave more definite information concerning the loss of HCN during drying.

In 1934, samples were taken from leaves of two pure line strains of Dakota Amber, one of Kaoliang and one of Sudan grass. These samples consisted of portions of leaves cut fine with a pair of grass shears and weighed out to 15 grams. One series of analyses of these samples were made in duplicates immediately, and another at the close of the four-day period (eight days in case of one fertilized sample). The summary of these analyses is given in Table 29.

TABLE 29. Loss of HCN From Samples of Sorghum Varieties and Strains After 4 Days Air-drying at Room Temperature. (1934).

Variety	Date of Sampling and 1st. analysis	Stage	HCN in undried sample p.p.m.	HCN in sample dried p.p.m.	Percent HCN lost during curing
Dakota Amber	7-10	Shooting	532	324	39.1
Dakota Amber	7-11	Shooting	432	379	13.3
Kaoliang	7-11	Shooting	430	350	18.6
Sudan Grass	7-11	Shooting	170	130	23.5
*Dakota Amber	8-29	Fertilizer	830	500	39.8
Average			479	337	26.9

* Cured 8 days.

The last column makes it evident that both strains of Amber sorghum, and one strain of Kaoliang and one of Sudan **lost appreciable but different amounts of HCN through the drying of the cut samples** over a period of four days. The sample of the one strain of Dakota Amber taken at the fertilization stage (dried eight days) lost over one-third of its HCN.

The percentage loss may be less important than the total amount remaining in the samples after drying. The actual amount remaining in the sample taken at the fertilization stage was greater than that remaining in any other.

Average Loss of HCN From Sorghum, Harvested at Seven Successive Stages, During the Process of Curing in the Shade. In 1935 12 S_4 lines of Dakota Amber and one of Sudan were harvested at seven growth stages. Each portion harvested was divided into two parts; one part was sampled by stripping off leaves immediately and analyzing them for HCN; the other was tied into a small bundle and hung to dry in a large dark store room where there was ample circulation of air. The two portions of the same pure lines therefore in all instances furnished a comparison between

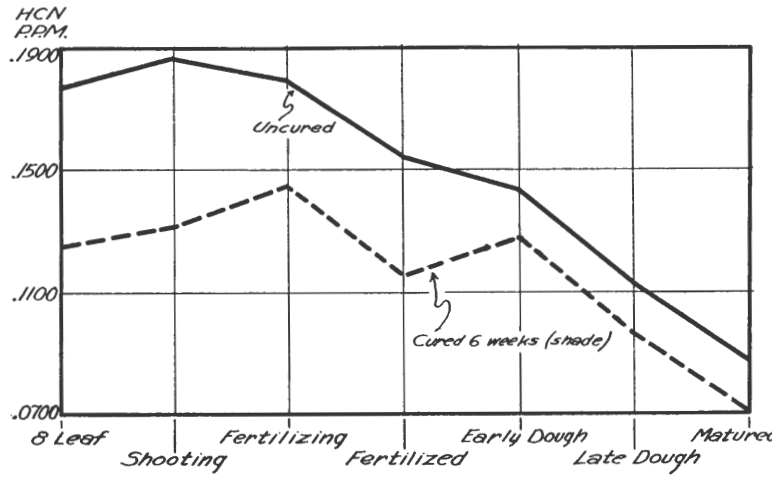

Fig. 4. HCN content in green and cured forage of 12 pure lines of Dakota Amber and 1 pure line of Sudan grass at seven stages. Brookings, 1935.

the HCN content in sorghum at the time of harvest, and the same after six weeks of curing in the shade. The summary of the average amounts of HCN in the 13 strains, cured and uncured, are shown graphically in Fig. 4.

The average HCN in all of the strains diminished generally from the earliest stage of growth to the stage of complete maturity, similar to what has been shown for the green forage. Slight exceptions in this respect scarcely disturb the general trend. The diminution of HCN occurred regardless of whether the material was analyzed immediately after being harvested or after curing six weeks in the shade. The average amounts in the cured portion of every sample was lower than that in the comparable portion analyzed immediately after it was harvested. The reduction in HCN due to the process of curing appeared to be generally less in the more mature stages, as might be expected due to the fact that the total amount of HCN is relatively reduced at such stages.

Loss of HCN Greater From Curing in Sun Over Curing in Shade. In the foregoing section, it became apparent that the drying or curing process, in the shade, caused a reduction of HCN. That being ascertained, it becomes important to know whether the loss might be modified by the condition of curing.

In 1936, strains of sorghum were harvested at seven successive stages, extending from the eight-leaf stage to maturity. The amount harvested was divided into three equal portions. Leaves of one portion were stripped immediately, samples taken and analyzed. A second portion was hung up in a dark room for curing in the shade after the manner of that described in the previous section. The third portion was hung in a similar manner, but placed in the Agronomy greenhouse, where it was exposed under glass to direct sun during daylight hours, where the temperature also became unusually high.

The storage period for the portion thus placed in the sun to cure and likewise for that placed in the shade for comparison was six weeks. Analy-

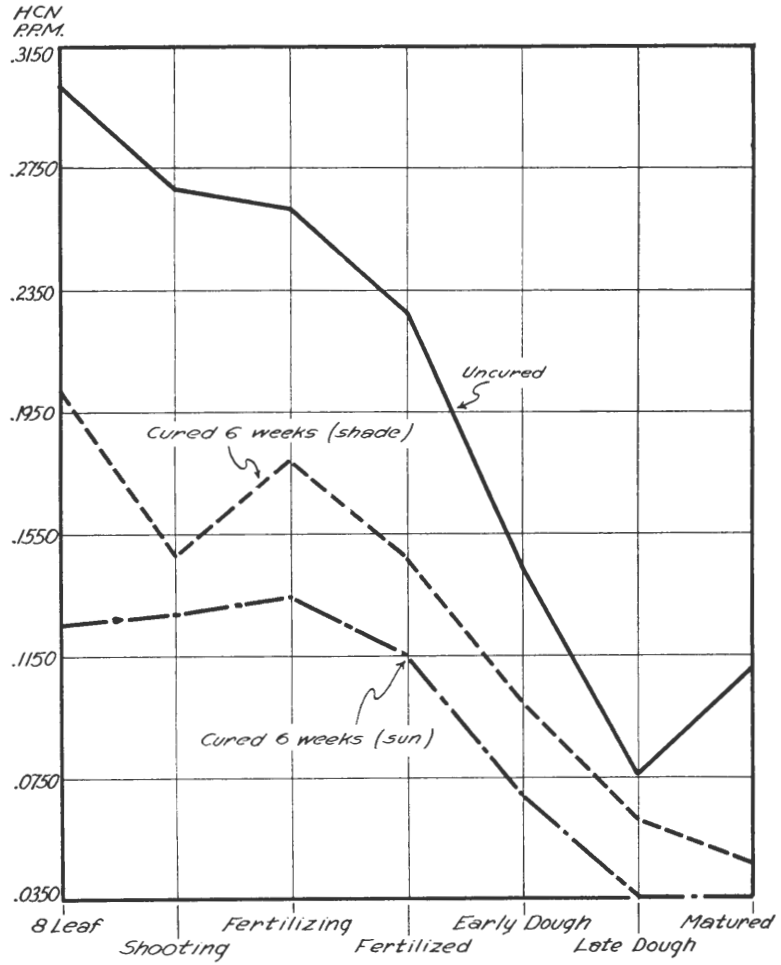

Fig. 5. HCN content in forage expressed as average of 12 pure lines of Dakota Amber and one pure line of Sudan grass at seven stages: Uncured and cured 6 weeks in the shade and cured 6 weeks in the sun at Brookings. (1937).

ses then were made for HCN in the leaves of the portions cured by the two methods.

The average results of the analyses are shown graphically in Fig. 5.

The specific point to be noted is the fact that the HCN content in the portions cured in the sun is not only invariably lower than that of comparable sample uncured, but likewise lower than the one cured in the shade.

Again the average amounts of HCN indicate that the reduction of HCN is associated with advancing maturity—with minor exceptions. Likewise it

is evident that the process of curing tends always toward a reduction of HCN at whatever stage of growth harvesting may take place.

Loss of HCN Varies Between Different Strains of Sorghum. It has been indicated in previous sections that all kinds of sorghum lost some HCN

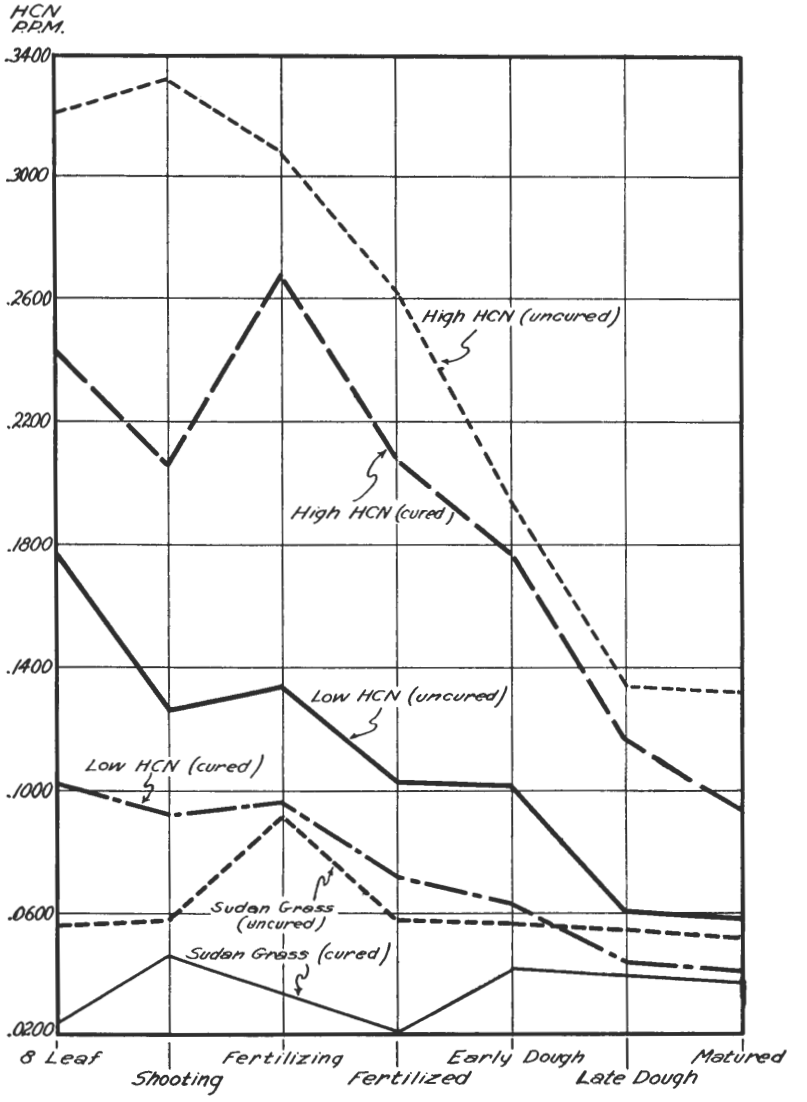

Fig. 6. HCN content in green forage and cured forage (6 weeks in the shade) for four high-HCN pure lines of Dakota Amber, four low-HCN pure lines of Dakota Amber and one pure line of Sudan grass at seven stages at Brookings. (Two-year average 1935 and 1936.)

during the process of drying or curing—moreover, the manner of curing, whether in sun or shade influenced the total loss. It seems probable that strains differing in HCN content may differ in the rapidity with which they lose HCN during the curing process. For example, it has been found in these investigations that high-HCN strain, 19-30-S, lost 29.9 percent of its total HCN during the process of curing six weeks in the shade, whereas strain 15-30-S, likewise a high-HCN strain, lost an average of only 7.9 percent.

Figure 6 shows graphically the average amounts of HCN for two seasons (1935 and 1936) of four high-HCN strains and likewise of four low-HCN strains and one strain of sudan before and after the curing process. The analyses for HCN were made at seven successive stages of growth between the early 8-leaf stage and complete maturity.

The lines in Fig. 6 indicate that the loss of HCN during curing is decidedly more rapid in the four high-HCN strains than in either the four low-HCN strains or the strain of Sudan. It appears that different strains of sorghum release widely differing amounts of HCN at the several stages during the process of curing. The release of HCN during the early stages of the curing process was more rapid for the high-HCN strains than for the four low-HCN strains and the one Sudan grass. From the fertilizing stage to maturity the loss in HCN for the four high strains was considerably less than at any other period.

Loss of HCN During Field Curing. In the previous section it was pointed out that different pure lines of sorghum may lose varying amounts of HCN during the curing process. The case in point involved controlled or partially controlled conditions.

In 1934 samples from the several pure lines were analyzed for HCN not only immediately after harvest, but also after a two weeks period and again after six weeks. In 1936, the samples were analyzed immediately after harvest and later after a period of curing six weeks.

A summary of losses of HCN brought about during the periods of storage is put down in Table 30.

It is of interest to know the extent of reduction in HCN content which several strains of sorghum may undergo during the process of field curing in shocks. In two seasons, 1934 and 1936, 12 pure lines of Dakota Amber and one of Sudan were harvested at maturity on a given date. Portions of

TABLE 30. Reduction in p.p.m. of HCN From Several Pure Lines in 1934 and 1936 Attendant Upon Field-curing in Shocks.

Strain Number	HCN in Matured 8-27 Uncured	HCN in Cured 2 wks.	HCN in Cured 6 wks.	HCN in Matured 8-24 Uncured	HCN in Cured 6 wks.	2 Year Average HCN Content Uncured	Cured 6 wks.	Percentage loss in HCN
1-30-S	990	310	370	1130	190	1060	280	73.6
15-30-S	1050	740	280	1630	720	1340	500	62.7
18-30-S	480	450	60	1450	190	965	125	87.1
19-30-S	1810	1180	0	880	560	1345	280	79.2
20-30-S	1210	400	250	1030	280	1120	265	76.3
23-30-S	640	180	0	910	330	775	165	78.8
2-30-S	910	400	10	960	120	935	65	93.1
3-30-S	180	180	70	580	160	380	115	69.8
13-30-S	310	110	0	280	20	295	10	96.7
30-30-S	180	70	60	870	230	525	145	72.4
34-30-S	60	80	0	1460	280	760	140	81.6
39-30-S	0	0	0	580	70	290	35	87.9
176-S(Sudan)	140	70	0	640	190	390	95	74.7
Ave.	612	321	85	954	257	783	171	79.5

these pure lines harvested were made into sheaves which in turn were shocked in a long shock. The long shock was supported by a wire on steel posts. The object of making such a long shock was to insure that all sheaves representing the several kinds of sorghum would be exposed under as nearly similar conditions during the curing process which obviously might not be the case with a shock of another shape.

Examination of the foregoing table shows that the average percentage loss of HCN for all strains in 1934 and 1936 over a six weeks period of curing in shock was 79.5. It would thus be indicated that the total reduction in HCN content during field curing may be as high as losses brought about by other processes of drying.

It also is apparent that the total amount of HCN remaining in the several pure lines varies considerably, a fact which is in general agreement with what was pointed out in the preceding section, namely that various strains of sorghum may differ in the degree and rapidity of losing HCN during drying or curing. The lowest percentage loss in HCN shown in the last column was 62.7 for strain 15-30-S which is in accord with what was pointed out in the previous section to the effect that this strain lost only the small percentage of 7.9. The seasonal difference also is of interest in that the amount of HCN remaining in the cured sheaves of the several pure strains after six weeks curing was appreciably lower (85 p.p.m.) in 1934 and in 1936 (257 p.p.m.).

Position in Shock Affects Loss of HCN During Field Curing. The foregoing section indicated that the loss of HCN during the process of curing in a shock in the field was as great or greater as an average than losses during other systems of drying. An experiment next was arranged to test whether the foregoing would be applicable to the conditions of storing sorghum in the ordinary form of shocks in the field.

On August 25, 1937, 43 two-rod rows of high-HCN sorghum, 19-30-S, were harvested at maturity. One-half of the harvested material was shocked immediately, and the other half was left lying in sheaves on the ground over a period of three days before shocking. Each of the two shocks (one having all sheaves put up immediately after harvest, and the other having sheaves left on the ground to dry before being set up into shock) were started with having four sheaves set upright and held in position by an upright metal post. The four sheaves in each instance were bound to the post with twine. Thus the four sheaves in each instance formed the middle of a shock. A second tier of sheaves was set around the first four sheaves and a third tier was set around the second. Each tier was securely tied in place. Each shock was thus made up of an outside set of sheaves exposed to the elements, a central set of four on the inside of the shock protected by all the sheaves outside, and another set of sheaves midway between the two.

A composite random sample was taken for determination of HCN immediately after harvest of each set of sheaves going into the makeup of the two shocks. A sample was also taken and analyzed of the sheaves after three days drying on the ground before shocking. Then after six weeks storing in the shocks, composite samples were taken and analyzed from: 1, the outside tier of sheaves; 2, the middle tier of sheaves; and 3, the inside tier in the middle of each of the two shocks. The summary of these analyses is shown in Table 31.

TABLE 31. HCN in Sheaves of 43 Rows of the High-HCN Strain (19-30-S) of Sorghum as Affected by Differing Positions in Shock or by Three Days Drying in Sheaf. (1937).

Drying or Curing	HCN in composite random sample of leaves in given group. p.p.m.	
	Shocked immediately	Dried 3 days on ground
Immediately after harvest	2220	2090
Three days on ground in sheaf before shocking		430
Outside tier of sheaves after 6 weeks	60	100
Second tier of sheaves after 6 weeks	350	
Center sheaves of shock after 6 weeks	160	80

The amount of HCN in the sorghum of the two separate shocks was practically the same immediately after harvest.

The HCN content in sheaves from the outside tier of the shock put up immediately after harvest was lower at the close of six weeks curing than that of either of the inside tiers in the same shock. This is in accordance with the previous conclusion that curing in direct sunlight reduces the content of HCN more completely than curing in shade. Obviously the inner tiers of sheaves in any shock would be shaded by the outer tier which itself would be exposed to sun.

The process of permitting sheaves to lie on the ground three days before shocking apparently reduced their content of HCN appreciably. Apparently the sheaves thus dried on the ground before shocking were more evenly reduced in HCN content both on the outside and inside of shock after such drying than those in the shock put up immediately after harvest.

These results may have a bearing upon the method which might be used in handling sorghum after harvesting for fodder.

Analyses of Variance for Comparing HCN in Different Lines of Sorghum, at Successive Stages of Maturity and in Uncured Versus Cured Forage. It has been pointed out in this bulletin that different lines of sorghum and different stages of growth may be characterized by differing amounts of HCN. Furthermore the time and method of curing, and seasonal variation may have an influence upon the content of HCN.

TABLE 32. Analysis of Variance for Comparing the Percent of HCN in the Green Forage With the Same Forage Cured Six Weeks in the Shade, Using 12 Lines of Sorghum and one Line of Sudan Grass, Harvested at Seven Growth Stages, in Two Different Years at Brookings. (1935 and 1936).

Variation Due to	Degrees of Freedom	Sums of Squares	Mean Squares	F	F^1	F^2
Lines	12	1.384079	.115340	13.36†	48.69†	82.39†
Years	1	.066987	.066987	7.76†	28.28†	47.85†
Lines x years (Error 1)	12	.103596	.008633		3.64*	6.17†
Uncured vs. cured (U.C.)	1	.216438	.216438		91.36†	154.60†
Lines x U.C.	12	.055775	.004648		1.96	3.32†
Years x U.C.	1	.031090	.031090		13.12†	22.21†
Lines x years x U.C. (Error 2)	12	.028426	.002369			1.69
Stages	6	.841592	.140262			100.19†
Lines x stages	72	.221796	.003061			2.20†
Years x stages	6	.182151	.030359			21.69†
Treatments x stages	6	.047453	.007909			5.65†
Lines x years x stages	72	.111762	.001552			1.11
Lines x U.C. x stages	72	.031411	.000436			.31
Years x U.C. x stages	6	.010280	.001713			1.22
Error (3)	72	.100793	.001400			
Total	363	3.433634				

† Highly significant.
F is the ratio of variance for lines and years to the variance for error 1.
F^1 is the ratio of the corresponding variance to the variance for error 2.
F^2 is the ratio of the corresponding variance to the variance for error 3.

A statistical analysis has been computed to substantiate the foregoing findings. An analysis of variance for comparing 13 lines of sorghum (12 amber sorghum and one Sudan), seven stages of growth, uncured and cured (in shade for six weeks) forage, and two seasons (1935 and 1936) is presented in Table 32.

The F values obtained show that there were highly significant differences in HCN content between lines, years, uncured versus cured forage, and stages. The general conclusion to be drawn from the significant interactions is that such differences between lines, years, treatments of forage, and stages did not remain constant but changed significantly under varying conditions.

These results merely furnish additional evidence to substantiate the conclusions reached in previous sections of this bulletin.

Analysis of Variance for Comparing Different Methods of Curing in 13 Lines at Various Stages. It has already been stated that losses in HCN from curing in the sun are consistently greater than losses from curing in the shade.

In Table 33 is given an analysis of variance for comparing HCN in the 13 lines of sorghum, (1) as green forage, (2) as cured six weeks in shade, and (3) as cured six weeks in the sun, at seven stages of growth.

TABLE 33. Analysis of Variance for Comparing the Percent of HCN in the Green Forage With the Same Forage Cured Six Weeks in the Shade and Sun, Using 12 Lines of Sorghum and One Line of Sudan Harvested at Seven Growth Stages at Brookings. (1936).

Variation Due to	Degrees of Freedom	Sum of Squares	Mean Square	F	F^1
Lines	12	1.015801	.084630	15.34†	77.01†
Treatment of forage	2	.484867	.242434	43.85†	220.60†
Lines x Treatment (Error 1)	24	.132892	.005516		5.02†
Stages	6	.980759	.163460		148.74†
Lines x stages	72	.294962	.004097		3.78†
Treatment x stages	12	.103814	.008651		7.87†
Error (2)	144	.158302	.001099		
Total	272	3.170897			

† Highly significant
F is the ratio of variance for lines and uncured and cured forage to the variance for error 1.
F^1 is the ratio of the corresponding variance to the variance for error 2.

In Table 33 highly significant differences are found between lines, treatments of forage, and stages. The highly significant interactions for lines x stages, lines x treatment and stages x treatment lead to the conclusion that all lines do not react similarly at all stages or under all treatments. Likewise, differences between treatments vary significantly in different lines and stages, and differences between stages do not remain constant in different lines and treatments.

TABLE 34. Analysis of Variance for Comparing the Percent of HCN in Mature Sorghum, (1) curing in shade, (2) curing in sun, (3) curing in field, (4) uncured forage of thirteen lines. (1936).

Variation Due to	Degree of Freedom	Sums of Squares	Mean Squares	F
Lines	12	.027290	.002274	0.55
Treatment of forage	3	.033269	.011089	2.69†
Error	36	.014849	.004125	
Total	51	.075408		

† Highly significant.

Analysis of Variance for Comparing HCN in Mature Sorghum Between Three Different Methods of Curing as Compared to Uncured Forage in 13 Lines. Table 34 sets forth the analysis of variance implied in the foregoing heading. The forage was cured for six weeks in each method of curing.

There is no significant variation in HCN content between the 13 lines, perhaps because of a high interaction variance of lines x treatment which in this case is used as the error variance. There is, however, significant variation in the effect of curing these lines by the different methods.

HCN in Sorghum Not Increased Through Freezing. The fact that livestock losses have occasionally occurred with the pasturing or feeding of sorghum subsequent to frost or freezing temperatures has given rise to the belief that the freezing process in itself causes an increase in HCN in the forage. Some information has been arrived at relative to changes in HCN as related to freezing.

A sample of green leaves from Dakota Amber was collected on August 30, 1934. The total sample was divided into two parts: one part was cut fine, weighed out in duplicate and allowed to digest over night. The other portion was placed in a refrigerator over night and retained at a temperature below freezing. After such freezing duplicate samples were taken and allowed to air-dry for a period of 36 hours.

The analysis for HCN proved that the sample of the green forage which was weighed out and digested over night and immediately analyzed for HCN contained 560 p.p.m. The comparative sample that was harvested at the same time, then frozen, and air-dried for thirty-six hours, contained 440 p.p.m.

It is obvious from this comparison that the process of freezing with additional curing thereafter for 36 hours caused no increase in HCN; on the contrary there was a loss of 21.4 percent.

A more extensive experiment was carried out later which gives results bearing more directly upon effects of freezing alone.

Second growth sorghum was growing in a nursery row, from the high-HCN strain 19-30-S on September 15, 1937. This second growth had come on after the harvest of the first crop which occurred July 16. The second growth had reached the heading stage on September 15, and on that evening a light frost was predicted for this area.

September 15, at 5 p.m. a check sample of the second growth green forage was secured, and brought in for immediate analysis. Events proved that this analysis was made from material taken from the field just before a heavy frost. It was observed on the morning of September 16 that a heavy white frost had occurred, the frost being the first of the season.

The plan which was carried out included the taking of samples of the second growth sorghum previously described, beginning at 4 a.m. and at every subsequent hour of September 16, up to and including 8 a.m. Thereafter, samples were taken every two hours up to and including 6 p.m. The weather conditions for the day September 16 included sunshine, with a gentle breeze from the northwest.

The summary of observations taken during the time of sampling and the amount of HCN found at the successive periods is shown in Table 35.

It may be seen from Table 35 that the content of HCN in the sample taken the evening of September 15 before frost is higher than that of any sample taken on the next day following the heavy frost. This would nearly

TABLE 35. HCN in Second Growth of High-HCN Amber (1) Previous to Frost, and (2) at Nine Successive Periods on Day Following. (1937).

Time of Sampling	Appearance of frost on plants at time of sampling	Condition of plants	Odor of HCN from harvested plants	HCN in samples p.p.m.
5 p.m.	Before frost	Normal	None	4550
4 a.m.	Light coverage of white frost	Leaves frozen medium hard	Considerable	3970
5 a.m.	Heavy coverage of white frost	Leaves frozen hard	Considerable	4010
6 a.m.	Heavy coverage of white frost	Leaves and stems frozen hard	Considerable	3070
7 a.m.	¾ white frost gone Plants wet	Leaves limp. Dark watery green	Little	3390
8 a.m.	Plants wet, stems slightly frozen	Plants and leaves limp. Light green	Very little	1960
10 a.m.	Plants dry	Leaves drooped, drying. Light green	Slight	1410
12 noon	Plants dry	Leaves drooped slightly, rolled, dry, whitish green	None	1210
2 p.m.	Plants dry	Leaves upright, rolled, dry, whitish green	None	1500
4 p.m.	Plants dry	Leaves upright, rolled, dry, very whitish green	None	1260
*6 p.m.	Plants dry	Leaves upright, rolled, dry, brittle, very whitish	None	1590
*Nov. 2	(Cured Forage)			760

* Cured in shade 48 days, taken from sampling at 6 p.m.

establish it as a fact that **mere freezing, in and of itself** in this instance **was no direct cause of increase in HCN content of sorghum.**

It also is of interest to note that there was a general reduction in the content of HCN, which however is not very pronounced until 8 a.m. by which time the plants and leaves had thawed out and become wilted.

HCN must have escaped from the plants during the time they were thawing, assuming that strong odor of the HCN was an indication. Evidently, freezing results in a more rapid and complete liberation of HCN than is normally found in sorghum plants. This may explain some of the cases of sorghum poisoning after heavy frosts, especially if the animals have access to the sorghum immediately after freezing or when fed in a frozen condition.

It is apparent, however, that not all the HCN escaped from the plants within the day following process of freezing during their curing process. It may be observed that a portion of the sample harvested at 6 p.m. was cured in the shade through a period of 48 days. This portion evidently lost an additional amount of HCN subsequent to being frozen and analyzed immediately, indicating that although sorghum may lose HCN while thawing or after it has thawed, it may not lose its entire content within a 24 hour period.

HCN in Sorghum Preserved as Silage. It is a generally recognized fact that injuries to livestock through HCN probably never occur when sorghum is preserved as ensilage.

Comparative tests were made in three separate seasons (1933, 1934, and 1937) with Dakota Amber sorghum to determine facts about the amount of loss, if any, of HCN during the process of curing in a silo. In these experiments, the sorghum was immediately stripped and either ground or chopped fine with a shears in a manner to imitate harvesting and cutting of silage under the usual conditions. After being thus ground or cut into small pieces, a portion was analyzed immediately and the remainder was put into Mason jars and firmly tamped. After that the lids were placed on

the jars, however without being sealed. Thus, the Mason jars served as miniature silos, which appeared to provide storage comparable to the usual storage in commercial silos. The data which were obtained are presented in Table 36.

TABLE 36. HCN in Sorghum at Time Harvested and Later at Successive Dates When Preserved as Silage. (1933, 1934 and 1937).

	HCN (p.p.m.) at given date										
	At harvest				After being ensiled						per-
Year	July 31	Aug. 30	Sept. 5	Sept. 14	Oct. 9	Oct. 17	Nov. 2	Dec. 18	Feb. 1	Feb. 21	cent loss
1933			735			710					3.4
1934		590				580			390	500	16.9
1937	3860			1370	720		460	450			88.4

It is evident from the foregoing table that the dates of harvesting sorghum for silage in the three separate seasons of this experiment and the coinsequent degree of maturity of the forage at the time of harvest are different. Likewise, the dates of taking samples for analysis and the periods of time involved are different. Furthermore, the strain of sorghum utilized in the several years was not the same, although it was Dakota Amber in all instances.

The sorghum placed in storage as ensilage lost a proportion of its original content of HCN in all three separate seasons although the amounts lost varied widely. Careful examination of the amounts thus lost in relation to the date of harvest and probably maturity of the forage at time of being placed into the Mason jars for ensilage indicates that the losses may depend upon the degree of maturity at harvest which in turn probably regulated the total HCN content in the forage at the time it was put into the jars.

HCN Not Correlated With Total Sugar Content in Sorghums. One matter of scientific and practical importance would be the possible relation between total sugar in strains of sorghum and HCN found therein. Accordingly some experiments have been made with a view to securing information to this question.

In 1936 a number of pure lines of Dakota Amber, also some crosses of the same, and certain Milo x Dakota-Amber crosses were sampled and analyzed for both HCN and total sugar. The portion of each plant analyzed for sugar content was the upper stalk of the upper node immediately below the panicle. The leaves and leaf sheaths were stripped from this portion of the stalk before the sample was taken. Analyses for total sugar were made by method of the Association of Official Agricultural Chemists, 1925.

Table 37 shows the amounts of HCN and the corresponding percentages of sugar in six high-HCN strains, five low-HCN strains, and a strain of Sudan.

Careful examination of the amounts of HCN and total sugar thus placed opposite one another for comparison fail to reveal any general correlation between the two. The average amount of HCN in the six strains of high-HCN Amber is 3,160, and for the five low-HCN strains, 1,150 p.p.m. The average amount in the high-HCN strains is 2.8 times the average amount in the five low-HCN strains. The average percentage of total sugar in the low-HCN and high-HCN strains show no such difference. In fact, the average percentage of sugar in the high-HCN strains is 38.4 and

TABLE 37. Comparative Amounts of HCN and the Percentage of Total Sugar in 11 Pure-line Strains of Amber Sorghum and One Strain of Sudan. (1936).

Strain No.	Date	p.p.m. of HCN	Percent of Sugar
High-HCN strains:			
1-30-S	8-6	2720	35.0
15-30-S	8-6	2470	33.6
18-30-S	8-6	3260	42.2
19-30-S	8-6	4000	42.0
20-30-S	8-6	3680	38.5
23-30-S	8-6	2820	39.0
Ave. (High-HCN)		3160	38.4
Low-HCN strains:			
2-30-S	8-7	1400	33.4
3-30-S	8-7	1000	39.6
13-30-S	8-7	1580	37.0
30-30-S	8-7	1340	39.6
39-30-S	8-7	430	41.8
Ave. (Low-HCN)		1150	38.3
Ave. all		2740	38.3
176-S (Sudan)	8-7	680	9.4

the comparative percentage of total sugar in the low-HCN strains is 38.3, essentially the same in both cases. There is apparently no correlation between the content of HCN and of total sugar in the several strains of sorghum so far as it is apparent from the foregoing experiment.

Experiments also were conducted on two sets of hybrids produced with pure line parents. The first hybrid was a cross of high-HCN Amber, 19-30-S low-HCN Milo, 109-30-S, and the second, with the same high-HCN amber and a low-HCN amber, 39-30-S. In one cross the low-HCN sorghum strain was used as the male parent.

Samples were taken from the several parental strains and their F_2 progeny after the manner mentioned in previous sections, with the use of material from the upper node of the several plants for sugar determination. The outcome of the amounts of HCN and percentages of sugar are summarized in Table 38.

TABLE 38. HCN Content and Percentages of Total Sugar in Parental Strains and in Groups of Their F_2 Progeny. (1936).

Strain No.	Type	No. tested	Stage	HCN p.p.m.	Ave. percent Sugar
19-30-S	High-HCN Dakota Amber	3	Dough	2240	26.7
109-32-S	Low-HCN Milo	4	Dough	1160	30.0
High x Low	Low-HCN F_2 progeny	11	Dough	780	37.0
High x Low	Medium-HCN F_2 progeny	2	Dough	1460	41.1
High x Low	High-HCN F_2 progeny	6	Dougs	2510	33.0
Ave.	F_2 progeny			1710	39.7
Ave.	Parents			1620	28.5
19-30-S	High-HCN Dakota Amber	5	Milk	2350	30.5
39-30-S	Low-HCN Dakota Amber	6	Milk	380	31.4
Low x High	Low-HCN F_2 progeny	6	Milk	770	37.5
Low x High	High-HCN F_2 progeny	5	Milk	2140	36.6
Ave.	F_2 progeny			1320	37.6
Ave.	Parents			1230	30.7

The amounts of HCN and total sugar content reveal no correlation between the amounts of the two substances. In the upper half of the table it may be observed that the amounts of HCN in the highest parental strain was roundly twice that in the low-HCN Milo, whereas the percentage of sugar in the latter is higher than the former. Likewise, in the three groups of F_2 progeny, where they were classified into low, medium, and high-

HCN, the corresponding percentages of sugar fail to correspond. Likewise in the lower half of the table the lack of any correlation between amounts of HCN and percentages of total sugar is apparent. In short, the foregoing experiments indicate that there is no correlation between amounts of HCN and percentage of total sugar in the strains of sorghum studied.

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