A STUDY OF HCN DETERMINATION IN SORGHUMS BY THE PICRIC ACID TEST

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INTRODUCTION

Plants poisonous to livestock have caused extensive losses to the livestock industry in many parts of the United States since the days of early settlements. They still constitute a major economic problem in many areas.

Farmers and other concerned with the production and feeding of forage crops have long known that some plants may be poisonous to animals under certain conditions. Of the several species which may cause such poisoning, the most important forage types are Sudangrass (Sorghum sudanense (Piper) (Stapf.), Johnson grass (Sorghum halepense (L) Pers.), and most all the varieties of Sorghums (Sorghum vulgare Pers.).

Sorghums contain a complex compound called gluoside which is nontoxic to livestock as long as the plant develops normally. However, if the plants are injured by drought, frost, mechanical means, or by some abnormal growth condition, this glucoside is broken down by an enzyme in the plant. One of the products of glucoside decomposition is HCN[1] which may be toxic if present in plant material eaten by ruminant animals.

The chemist can explain the cause of the poisoning, but present methods are inadequate when it comes to suggesting when the plants are safe and when they may be toxic. The various methods

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1. The term HCN will be used in this report to designate synomous terms prussic acid and hydrocyanic acid.

of measuring the HCN content of plants are very laborious and require several hours to reach a conclusion. The better methods require from 12 to 24 hours to determine the toxicity of plants, and this length of time is sufficient to allow for enzyme changes which will alter the content significantly. It would be highly desirable to have a quantitative test which could be applied in the field to determine the presence of prussic acid.

The sorghums are considered a very good feed but would occupy an even more important role in livestock feeding if their toxic properties could be eliminated. Much work has been done on this subject, but in spite of extensive study, the solution to the problem is still unsatisfactory. The development of a variety which is free of or very low in HCN would alleviate the danger of poisoning and establish sorghum as an even more valuable forage crop.

The major purposes of this preliminary study were to check the picric acid test as a rapid method of determination of HCN. Also, it was to see if this test could be used in selection of sorghum varieties low or free of HCN.

REVIEW OF LITERATURE

The literature concerning HCN in sorghums is very broad and volumetric. For this reason the materials presented here have been grouped in order to discuss each factor of HCN poisoning separately.

History of HCN Discovery

In countries where sorghum is an important crop, it frequently has caused death of livestock when they were allowed to eat the plant material in the green state. MacDonald (25)[2] reported in a general teaching outline that liberation of HCN by plants has been recognized for about 100 years. It was recognized by Hiltner (21) at the Nebraska station in 1900, that cattle were poisoned by eating green sorghum. However, this investigator made a chemical analysis of sorghum plants, from a field known to have killed cattle and found no HCN present.

It was not until 1902 that the presence of HCN in sorghum plants was discovered. Although Slade (35) suggested the possibility of such a poison in 1901, it was not actually isolated from a sample of fresh sorghum until August, 1902.

Peters, Slade, and Avery (31) continued the work of Slade and in 1903 published a rather complete summary of the results obtained at the Nebraska experiment station. The findings of this work left little doubt that HCN was the direct cause of sorghum poisoning.

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2. Figures in parenthesis refer to "Literature Cited", p. 41

It was not determined until several years later that the poisonous property existed in other plants belonging to the sorghum group. Crawford (12) found it in Johnson grass in 1906, and Francis (16) of the Oklahoma Agricultural Experiment Station made determinations of the HCN in Sudangrass in 1915. Menaul and Dowell (27), also of the Oklahoma station, reported a study of cyanogensis in Sudangrass in 1919.

Methods of Determinations

A number of workers have published discussions on methods of determining HCN content in sorghums. Viehover and Johns (39), from work on cyanogenetic plants using the Prussian blue method, concluded that it was necessary to detect small quantities of HCN. Bishop (2) tested a number of methods for determining HCN and concluded that distillation led to unreliable results but that alcohol extraction was a sound method for determining cyanogenetic glucosides in leaves. Francis and Connell (17) used 50 gm. samples of green plant material, Hogg and Ahlgren (23) 0.15 gm., and Swanson (37) 200 gm. when analyzing for HCN content.

A rapid method based on the picric acid test for determining HCN in green tissues was proposed by Pethybridge (32) in 1919. The use of chloroform in the test was suggested by Mirande (28), (according to Hogg and Ahlgren (23)), while Nowosad and MacVicar (30) later used toluene. The procedure followed in this method was to place a small amount of macerated plant material in a test tube, then add a few drops of toluene or chloroform, and suspend above the sample a strip of moist filter paper saturated with sodium picrate solution. The sodium picrate on the filter paper was reduced to a red colored compound by the HCN.

Nowosad and MacVicar (30) extracted the pigment from the test paper in 10 cc. of distilled water and compared it with standard colors produced by known concentration of HCN and concluded that the test was sufficiently accurate quantitatively for the selection of plants low in HCN.

Inheritance Studies

Hogg and Ahlgren (25) studied the inheritance of HCN in Sudangrass and tested 175 inbred lines for HCN content in 1938, 1939, and 1940. The results indicated that an inbred line high in HCN in any given year remains relatively high from year to year, and an inbred line low in HCN in any given year remains relatively low from year to year. By crossing plants with low HCN content, vigorous strains of Sugangrass uniformly low in HCN were developed. They concluded that the HCN content of Sudangrass is not controlled by a single pair of genes. The HCN content of F_1 plants was intermediate between that of the parents. The F_2 populations of plants from crosses between parents low and high in HCN resulted in a distribution extending to the limits of the parents with no tendency to be bimodal.

That HCN may be controlled by a genetic factor has been reported by Franzke et al. (19). They concluded from the frequency distribution of low and high parental strains and from their first and second generations that inheritance of HCN is Mendelian. Low HCN appeared to be partially dominant over high HCN with a ratio of low:high not yet established.

Coleman and Robertson (8) reported in progeny tests of Sudangrass that the differential ability to produce HCN may be inherited in inbred lines. In the lines studied, they found that high HCN production appeared to be more closely associated with non-glossy leaves than with glossy leaves. Similarly, high HCN content seemed to be associated with purple-tipped seedling leaves, but to a lesser degree.

Form Found In Plants

Peters, Slade, Avery (31), and Dowell (14) stated that the poison was usually not present in appreciable amounts as free HCN but in the form of complex compounds called glucosides. These must be broken down and the free HCN liberated before poisoning occurs. However, the glucosides are readily broken down by an enzyme usually present in the plant. The cyanogenetic glucoside in sorghums is called dhurrin, which, on hydrolosis in the presence of the enzyme, emulsin, yields glucose, parahydroxybenzaldehyde, and HCN.

Dunstan and Henry (15) believed that sorghum poisoning was caused by the dhurrin and emulsin coming together in the early processes of digestion when the enzyme, by the addition of water to the glucoside, breaks the latter down and liberates the poisonous HCN. Slade and Avery (35, 31) arrived at practically the same conclusion while working independently and without knowledge of the findings of Dunstan and Henry in England.

Findings reported in later literature (25, 29, 22, 24) and those in Nebraska, Oklahoma, and England agreed that the acid existed

in sorghums combined only in the form of a glucoside. Willaman (41) of the Minnesota station stated, however, that HCN was found in sorghum both in the glucoside and a non-glucosidic form.

That the non-poisonous form exists as a glucoside called dhurrin in sorghums is the most accepted theory. The sorghum becomes poisonous only after an enzyme, emulsin, acts on the glucoside, liberating HCN as one of the products of decomposition.

Drought

The belief by farmers and ranchers that sorghums injured by drought and other adverse conditions are higher in toxic properties than those which have made normal growth has been shown to be true by several investigations. Willaman and West (42) found that the HCN content of plants grown under inadequate moisture conditions was higher than that of plants grown under optimum moisture conditions. Frankzke et al. (19) presented data which showed that plants grown on soil with a low moisture content contained more than twice as much HCN as plants grown at a high moisture level. Hogg and Ahlgren (23) found that plants grown under drought conditions produced in the greenhouse showed a steady increase in HCN as the moisture content of the soil decreased. Peters, Slade, and Avery (31) stated that growth arrested by drought presented a favorable condition for the elaboration of the poison. Francis (16) also concluded that drought-stunted plants are especially dangerous. Vinall (40) surveyed the literature concerning HCN in sorghum and concluded that when sorghum was stunted by drought the HCN content increased.

Swanson (37) found, however, that HCN was more abundant in rapidly growing plants than in plants stunted by drought. Boyd (3) (according to Hogg and Ahlgren (23)) also concluded that drought did not cause an increase in the amount of HCN present although the plants remained for longer period in the stage where they contained a high level of HCN.

Frost

According to Peters, Slade, and Avery (31) frost does not influence the content of HCN. Franzke et al. (19) also found that frost was not a direct cause of increase of HCN in sorghum. Francis (16) stated that frosted plants are unsafe. Vinall (40) also concluded that frost increased the HCN content. Heller (29) stated that young succulent plants were likely to be dangerous after a frost. Swanson (37) found when frosted Sudangrass contained a high content of HCN that the amount decreased as the plants began to wilt and continued to decline until none was present when the plants were dry.

Maturity

Regarding the effect of maturity or age on the HCN of sorghum plants, there is almost complete agreement among chemists as well as farmers that the percentage of HCN decreases from the time the plant begins growth until it ripens its seeds. Willaman and West (43, 42) presented data which was conclusive proof that HCN in sorghums decreased as the plants reached maturity. Peters, Slade, and Avery (31) found that young vigorous growing plants

contained more HCN than plants at maturity. A decrease in HCN in plants as growth progresses also was observed by Menual and Dowell (27). Hogg and Ahlgren (23) found that the HCN content of the plant tissue decreased as it became older. Swanson (38), Boyd et al. (4), and Franzke et al. (19) have also reported that young plants contain more HCN than do older plants. Martin et al. (26) found the upper (younger) leaves contained more HCN than the lower (older) leaves. Also, the proximal (younger) half of the leaf was higher than the distal (older) leaf. The HCN content of the stalk decreased progressively downward with the lower (older) internode containing only a small quantity.

It is a common belief that the second growth sorghum is more toxic than the first growth. Conflicting data have been published by several workers. Menual and Dowell (27), Willaman and West (43), Boyd et al. (4), Swanson (38), and Martin et al. (26) have all shown the second growth to contain more HCN on the unit weight basis than does older plants. Hogg and Ahlgren (23) found that plants low in HCN in the seedling stage were even lower in the second growth stage, whereas the HCN content of plants which were relatively high doubled from the seedling to the second growth stage. Franzke et al. (19) found 15 percent less HCN in second growth of sorghum than in the first growth.

Soil Fertility

The effect of soil fertility on the HCN content of Sudangrass has been reported by many investigators. Willaman and West (43) found that heavy nitrogen fertilization had no effect on the HCN content of Sudangrass under field conditions, except when the plants showed signs of nitrogen starvation. Boyd et al. (4) found that Sudangrass which was short and dark green was more dangerous than plants which were pale or yellowish green in color. High available nitrogen and low available phosphorus tend to increase the poison content of the plant, but if there is a low amount of nitrogen and a high amount of phosphorus in the soil, the level of HCN is reduced. Alway and Trumbul (1) found that yellow stunted plants contained less HCN than vigorous green growing plants in the same field. Pinckney (33) stated that in general the percent of HCN in green plants was in proportion to the nitrate used, the dark green plants being highest and the yellowish green plants being lowest. Brunnich (5) also found that the addition of sodium nitrate increased HCN. Franzke et al. (19) concluded that applications of stale manure and phosphate reduced the HCN content of sorghum, but that lime and nitrogen increased it. Manure, phosphate, and lime, however, did not produce as high HCN content in plants as did lime alone. It can be concluded that plants grown on poor soil contain less than those on good soil, and fertilization with nitrates markedly increases the percentage of the acid.

Location in Plant

It has been reported by some investigators that the amount of HCN found in the leaves is greater than that found in the stem. Franzke et al. (19) found that the leaves contained roughly eight times as much HCN as the stems of sorghum. Vinall (40) stated that the leaves of sorghum plants contained greater amounts of HCN than the stems. Martin et al. (26) found that sorghum leaves contained three to 25 times the amount of HCN as the stalks when the plants were in the boot stage. Swanson (37, 38) showed that the leaves of Sudangrass have a higher HCN content than the stalk. Menual et al. (27) also found more HCN in the leaves than in the remainder of the plant.

Willaman and West (43, 42) presented data conflicting to that mentioned by the above investigators. They found that before the plants were 47 days old the amount of HCN in the stalks of Feterita was higher than the amount in the leaves; after that, they were lower. After 67 days, the stalks contained almost no HCN. Orange sorgo stalks were higher than the leaves for 25 days; after that, the content of HCN in the stalk began to decrease. These workers also found more HCN in the stalks of Early Amber and Southern Cane when the plants were young, but as the plants became older, the leaves contained more HCN than the stalks.

Effect of Curing

Changes in the HCN content during drying or curing processes have been investigated by a few workers. Dowell (13) found that approximately three-fourths of the acid was set free in the process

of drying. Couch (10) concluded that dried cyanogenetic plants commonly contained less HCN than plants that were fresh. Churchill (6) stated that sorghum was rendered safe for feeding by curing. Collison (9) reported that well-cured sorghums usually could be eaten safely by cattle because drying reduced the poisonous properties of the plant. These investigators have confirmed the common belief that sorghum is safe for feeding after it has been dried. However, other results have shown that not all the HCN disappears upon curing although the more rapidly the sorghum is dried the higher the percentage of acid retained. Boyd (4) found that Sudangrass cut and put up for hay without much exposure may be poisonous. Heller (22) stated that plants cut and subjected to hot dry conditions, which caused quick drying, lost less than 40 percent of the poison. Swanson (37) found that Sudangrass dried in the shade contained less HCN than that dried in the sun. However, Frankze et al. (19) found that plants cured in the sun were invariably lower in HCN than those cured in the shade. The rate at which the sorghum is cured seems to be the major factor determining whether or not hay is toxic. When allowed to dry slowly, the plants contain little or no HCN and should be safe to feed.

It is generally recognized that injuries to livestock through HCN probably never occur when sorghum is preserved as ensilage. However, work by Swanson (37) showed that making Sudangrass into silage did not necessarily diminish the amount of HCN. Franzke et al. (19) found in three different years that sorghum contained a lower amount of HCN preserved as ensilage than it did at the time of being ensiled.

Geographical Variations

The fact that HCN poisoning is much less common in the Gulf States than in the states farther north is generally accepted among those who work with forage plants. Vinall (40) reported that very few complaints regarding HCN poisoning are received from points in the United States south of 35° north latitude. Just why the sorghum plant and its related species should be so much more dangerous in Kansas, Nebraska, and Eastern Colorado than in Eastern Texas, Louisiana, Mississippi, Alabama, Georgia, South Carolina, and Florida has never been satisfactorily explained. The crops are widely grown in both regions, and acute dry periods are likewise common to both areas. Vinall gave three possible explanations for the fewer cases of sorghum poisoning in the Southeastern states which are as follows: (1) the quantity of glucoside stored by the plant may be less, (2) the enzyme which breaks down the glucoside into HCN may be less active, or (3) the HCN may occur in a more unstable form in sorghums grown in the North and West. However, more work is needed to prove or disapprove Vinall's theories.

Couch (10) also stated that livestock losses from HCN poisoning in sorghums were much less in the Southern States than in the states farther north. The reason for the difference is probably climatic, but little is known about the exact cause of the formation of the poisonous acid in this case. Hogg and Ahlgren (23), using 10 inbred lines of Sudangrass at eight different locations, concluded that HCN content of Sudangrass is influenced by variations in soil and climate. Couch et al. (29) found that no cor-

relation existed between HCN content and temperature, rainfall, or altitude. Willaman and West (42) of the Minnesota Station made an attempt to study the effect of climate on the HCN content of sorghum and concluded that varietal differences were a larger factor than climate in determining the amount of HCN in a sorghum plant.

Varietal Differences

Varietal differences in sorghum are probably of more importance in determining the amount of HCN than are conditions of growth. Brunnich (5) reported two to 2.5 times as much HCN in "Imphee" (Sumac) as in Early Amber sorgo. Francis (16) found less HCN in normal mature kafir leaves than in second growth Sudangrass. Willaman and West (43) published figures indicating that Feterita produced considerably more HCN than Orange sorgo. Later, they (42) reported figues showing that, except for plants 33 days old, Early Amber sorgo yielded slightly less HCN than "Southern Cane" under Minnesota conditions. Swanson (38) found the following quantities of HCN in the varieties tested: Kafir 36 mg. per 100 g. of green tissue, sorgo 21, and Sudangrass 16. Menaul and Dowell (27) reported that Sudangrass contained about one-third as much HCN as grain sorghums. Couch et al. (11) found Feterita, Hegari, Chiltex, and Sumac sorgo to be high in HCN; Milo, Darso, Atlas sorgo and Kansas Orange sorgo intermediate; and Leoti sorgo, "African Millet" sorgo, and a selected strain of Dakota Amber sorgo relatively low in HCN. Varieties of kafir showed wide variations, some being high and others low in HCN.

Coleman and Robertson (8) demonstrated that inbred lines of Sudangrass may differ in their ability to produce HCN. Collison (9) of the Florida Station published an extensive comparison of the HCN content of different varieties. The material was collected from plants 12 to 24 inches high. Amber sorgo was low at zero percent. Other varieties showed HCN increasingly higher in the following order: Orange sorgo 0.0008 percent, Dwarf milo 0.0016, Pink kafir 0.0016, Sunrise kafir 0.0018, Darso 0.0022, Dwarf kafir 0.0024, Shallu 0.0026, Brown kaoliang 0.0031, Feterita 0.0032, Blackhull kafir 0.0033, and Dwarf hegari 0.0037. These percentages were all calculated on the basis of fresh material.

Diurnal Effect

Diurnal variation in the HCN content of sorghum has been reported by several workers. Willaman and West (42) found that the amount of HCN increased from morning to afternoon. Stakelenburg (36) concluded that HCN increased during the day and decreased at night. Rimington (34) suggested there might be an increase in the HCN content from early morning to noon. Menual and Dowell (6) found that the HCN content of Sudangrass was somewhat higher in the morning than in the afternoon. Boyd (3) obtained 30 percent more HCN at 1:00 p.m. than at 9:00 a.m. or 7:00 p.m. Franzke et al. (19) found slightly less HCN in the plant at 1:30 p.m. than at 8:00 a.m. Later he (34) observed the greatest diurnal drop in HCN occured at the close of the day between 6:00 and 8:00 p.m., when photosynthetic activity was low. The most rapid diurnal increase in HCN occurred between 8:00 and 10:00 p.m. Hogg and Ahlgren (23) observed no variation in HCN attributable to the diurnal factor.

Animals Affected

Horses and Swine are not very susceptible to HCN poisoning because their stomachs are made strongly acid by their content of HCl. According to MacDonald (25) this HCl reacts upon HCN and produces formic acid. Swanson (38) reported feeding green Sudangrass to a horse without any toxic effect.

Ruminants are more affected by cyanogenetic glucosides than other animals because they have a large flora of micro-organisms and much emulsin in the rumen. These serve to release HCN from the glucoside.

Speed and Symptons of Poisoning

Several investigators have reported that death may occur within 15 minutes after an animal starts eating the poisonous plants. Boyd et al. (4) reported that cattle grazing on Sudangrass which had a high HCN content usually stop eating after about 15 minutes due to the action of the poison. Heller (22) stated that sometimes cattle will die in 15 minutes after eating poisonous sorghum plants while in other cases as much as five hours were required for the poisonous material to have a lethal effect. Peter et al. (31) found that when a cow was allowed to graze on poisonous sorghum she stopped eating in 10 minutes and was dead 15 minutes later.

The symptoms of HCN poisoning have been studied extensively by Peters et al. (31) as well as by several others. They reported the following symptoms of an animal poisoned by HCN: dropping to its knees; stupor, difficult breathing, and frequent convulsions which result from the action of the poison on the brain centers that control respiration. When the animal is lying down, its head

is usually turned toward the abdomen as in the case of a horse having colic; the muscles, expecially of the nose and head, twitch; the pupils of the eyes are dilated and give off a watery discharge; the tongue is partially paralyzed and saliva runs from the mouth; the limbs and ears are cold; the pulse not perceptible; and the mucous membrane of the rectum protrudes with involuntary discharges of urine and feces. In the last stages, the limbs are paralyzed and the animal becomes unconscious. Couch (10) reported that death was caused by respiratory paralysis and that the heart continued to beat for some time after breathing had stopped.

Lethal Dose

Investigations indicate that the lethal amount of cyanide to cattle is somewhat variable. Boyd et al. (4) found that animals in a low state of vigor and very hungry are more apt to eat a lethal dose than well fed animals. He found that when certain recommendations are followed no cases of poisoning have occurred. The recommendations as listed by the author are as follows:

Mg. HCN per 100 grams dry tissue	Relative degree of toxicity
0-25	Very low (Safe to pasture)
25-50	Low (Safe to pasture)
50-75	Medium (Doubtful to pasture)
75-100	High (Dangerous to pasture)
100	Very high (Very dangerous to pasture)

Couch (10) stated that enough HCN to make an animal sick was usually a sufficient amount to be fatal. In one experiment, he found that a fatal dose of poison for a cow was somewhat in excess

of six grains of pure acid. A 100 pound sheep was fatally poisoned when fed about 1 2/3 grains. If a plant contains as little as 0.02 percent potential HCN, and the animal consumes it all rapidly, five pounds of the plant would be fatal for a horse or cow, and 1.25 pounds would kill a sheep if no factors entered in to prevent the development of the acid. MacDonald (25) stated that it required about one gm. of HCN to kill a 1000 pound cow, but Heller (22) reported four-tenths of a gram would be toxic. Peters et al. (31) reported that 0.5 to 0.6 gram or 0.02 ounce would probably be fatal to a mature animal in most cases. Vinall (40), in reviewing the literature on HCN poisoning, concluded that an animal would have to eat 7.6 pounds of sorghum or 18.9 pounds of Sudangrass to ingest the 0.02 ounce considered as a lethal dose.

Antidotes

Numerous remedies for this poison have been proposed but an effective safeguard against the poison is difficult to devise because of its rapid lethal effect. Glucose, dextrose, and other forms of sugar are known to act as antidotes to the poison. Peters et al. (31) and Dowell (14) presented experimental evidence that glucose and dextrose retard the liberation of HCN. They suggest that one way of reducing the danger of HCN poisoning is to feed cattle or sheep a starchy food, such as corn or grain sorghum, before allowing them to graze on the sorghum plants. The starch in the grain yields glucose in the digestive tract and thus aids in preventing lethal effects. Haring (20) recommended an ever-ready

antidote for HCN that was used in cyanide plants of gold mines.

Its preparation and administration is as follows:

(a) Solution 1---dissolve one ounce of sodium carbonate (washing soda) in a pint of soft water and put in a long-necked quart capacity bottle.
(b) Solution 2---dissolve 1/2 ounce of iron sulfate (copperas) in a pint of water.
(c) Keep in a dark place and when needed, mix solution 2 with solution 1 and give to animal A quart is sufficient for one cow and 1/2 quart for a sheep.

Francis (16) recommended a strong solution of glucose (corn syrup or molasses); a dose of quick acting purgative, such as a mixture of Epson salts and linseed oil; and the inhalation of ammonia. Clawson (7) has shown that if the affected animal can be reached in time, a combination of sodium thiosulfate and sodium nitrate given intravenously by a veterinarian is an effective remedy against doses of cyanide up to three minimal lethal doses. In order for most of these remedies to be practical, they should be given soon after the animal showed symptoms of poisoning because of the rapid action of the poison.

MATERIALS AND METHODS

A study of HCN determination in sorghum was conducted at the Oklahoma Experiment Station near Perkins in 1952. The varieties and selections tested represent a wide range of plant material consisting of standard varieties, selections, crosses, and introductions. The sorghums used were planted in three series of 30 rows each with the rows numbered from north to south. The first 30 rows made up the West Series; rows 31 through 60 made up the Middle Series; and the East Series consisted of rows 61 through 90 with a row of Sugar Drip used as a border row on both the north and south side of each series.

From previous work there had been some indication that Kavirondo was a genetically low type in HCN content. For this reason selections from a cross of Early Dwarf kafir and Kavirondo were used extensively in this experiment. The first 47 rows were F_3 progenies of this cross. The row number corresponded consecutively to the selection number. Five selections from an F_2 generation of a cross of Male Sterile (Dwarf milo No. 51 x Kaferita C. I. No. 811) x (Early Dwarf kafir x Kavirondo) were planted in rows 48 through 52 with the selections numbered one to five consecutively with the rows. A cross of a Male Sterile Dwarf kafir and (Early Dwarf kafir x Kavirondo) which was in the F_2 generation was planted in row 53. Rows 54 and 55 were planted to two F_5 selections of Early Dwarf outcross (918 x 71-27-2-1, and -6). Early Dwarf kafir (918 x 71-27-2) was planted in row 56. Rows 57 through 59 were selections one, two, and three of the cross Shallu (85) x Dwarf White feterita x Early

Tillering x Early Hybrid milo-2. These selections were in the F_5 generation. Early Tillering x Early Hybrid milo-1-2 was planted in row 60.

Row 61 was a cross of Shallu (85) x Dwarf White feterita x Early Tillering x Early Hybrid milo-1. Rows 62 through 76 were planted to consecutive selections one through 15 of the cross Highland x Early Tillering x Early Hybrid milo-1-2. Dwarf milo (51) x Kafir x Darso outcross x Early Tillering x Early Hybrid milo-2 was planted in row 77. Rows 61 through 77 were in the F_5 generation. Dwarf Freed (971) x Sudan-3-7-7, Early Tillering x Sudan-20-5-3, White-Seeded Sudan, Tift Sudan, Sweet Sudan, Grain-of-the-Plains, Kavirondo, Sorghum verticilliflorum, and Rancher were planted in rows 78 through 86 in the order listed above. The East Series was completed by planting Sugar Drip in rows 87 through 90. Each border row of Sugar Drip was assigned a number and tested for HCN content at the same time as the other material.

The origin of the material is as follows:

Early Dwarf kafir (918 x 71-27-2)--a selection from a cross of Wheatland milo C. I. No. 918 and standard Blackhull kafir C. I. No. 71 made at the Oklahoma Experiment Station.[3]

Kavirondo--a forage type sorghum from Kenya, East Africa, and reported safe for grazing under African conditions. It was one parent of 47 F_3 selections tested.

Male Sterile (Dwarf milo No. 51 x Kaferita C. I. No. 811)--a male sterile selected at the Oklahoma Experiment Station.

Male Sterile Dwarf kafir--a male sterile line of Dwarf kafir developed at the Oklahoma Experiment Station.

Early Dwarf kafir outcross (918 x 71-27-2-1)--and outcross from the Early Dwarf kafir parent used in the Kavirondo cross.

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3. C. I. No. refers to the Cereal Investigation Number.

Shallu (85) x Dwarf White feterita--a selection made at Stillwater, Oklahoma, in a study of Shallu C. I. No. 85 crosses.

Early Tillering x Early Hybrid milo--an early grassy-type sorghum derived from a cross made at Woodward, Oklahoma.

Highland--an early sorghum developed at the Akron Station, Colorado, in 1920 from Dawn kafir.

Dwarf milo (51) x Kafir x Darso outcross--a selection from a field cross of Darso with a Dwarf milo Kafir cross which was made at the Oklahoma Experiment Station.

Dwarf Freed (971) x Sudan-3-7-7--a selection from a cross of Dwarf Freed C. I. No. 971 and Common Sudan made at the Woodward, Oklahoma, Station.

Early Tillering (755)--a grassy-like selection from a cross of White feterita C. I. No. 755 and Common Sudan made at Woodward, Oklahoma.

White Seeded Sudan--a white seed selection from a cross between White feterita and Common Sudan at Woodward, Oklahoma.

Tift Sudan--derived by crossing Leoti and Common Sudan at the Tifton, Georgia, Station by Dr. Glenn Burton.

Sweet Sudan--a variety of Sweet Sudan introduced from Texas.

Grain-of-the-Plains--a prolific tillering grain-type sorghum from Kansas.

Sorghum verticilliflorum--a wild grass sorghum from Kenya Colony, East Africa, thought to be one parent of Kavirondo.

Rancher--a low HCN selection from Black Amber sorgo made by C. J. Franzke at the South Dakota Station.

Sugar Drip--a pure variety of sweet sorghum used as the check variety and in the series as guard rows.

The plantings were made May 22 on Norge, very fine sandy loam, terrace soil. A hill-drop, two-row planter which had been modified for hand dropping was used. The seed were hand-dropped in single rows 40 feet long and 42 inches apart. One hundred seed of each variety were planted in the 40 foot row. Only three plants emerged in rows 15 and 45, and none in row 18. A relatively good stand was obtained in other rows. Because of the small number of seed planted, no thinning was necessary. Cultivation was frequent enough to control weeds.

The method used was essentially that of Nowosad and MacVicar (30). The procedure was as follows: Filter paper was cut into strips six mm. long and one mm. wide and saturated in a picrate solution which was prepared by dissolving 25 g. of Na_2CO_3 and five g. of picric acid in 1000 ml. of distilled water. These prepared strips were stored away from light until ready for use. The plant material tested was taken each time from the first fully developed leaf so that uniform sampling was practiced throughout the test. A sample consisted of 20 pieces of the leaf blade, each 1/4 inch in diameter, taken by the use of a standard hand operated paper punch. Each sample was placed in a 21 x 70 mm. size shell vial and three or four drops of chloroform were added. A strip of filter paper which had been previously treated with picrate solution was moistened with distilled water and suspended above the mixture. Then the filter paper was thumbtacked to the cork which also served to seal the vial. The materials used in taking samples are shown in Fig. 1 and 2.

The vials with their contents were then stored at room temperature for 12 to 18 hours. The sodium picrate present on the filter paper was reduced to a reddish compound in amounts proportional to the amount of HCN evolved. The results were recorded in numbers from zero to six; a zero value was recorded when no reddish color was shown. The values increased as the intensity of red increased, with a value of six given when the filter paper was a deep red. All samples tested were classified entirely by visual observations without the use of a color chart or standards for comparison. The assigned values from zero to six were relative figures with no number specifying any definite concentration of HCN.

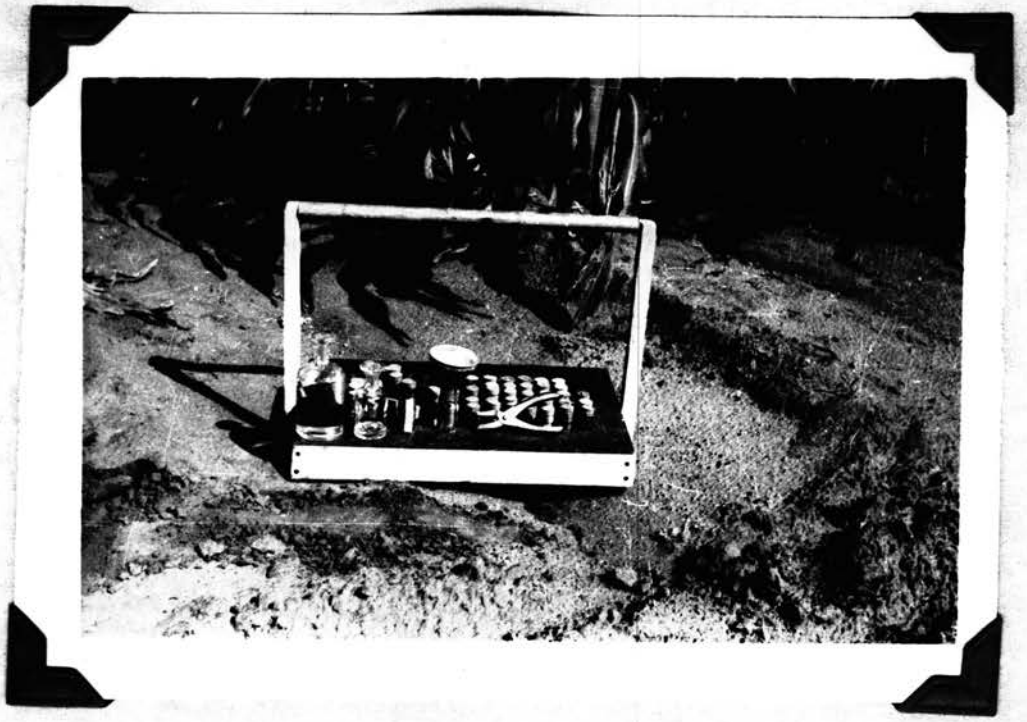

Fig. 1 - The materials used in taking samples in the field.

Fig. 2 - The addition of chloroform to green material in the vial.

After some data had been collected on the material by the picric acid test, it was deemed desirable to check the results by a quantitative test. The method used was that mentioned by Franzke et al. (19). Two low, one intermediate, and three high varieties were selected to be tested. The picric acid test was run at the same time on the material which was to be tested in the laboratory.

As soon as the samples were taken in the field, they were placed in paper bags and then in a cardboard box containing dry ice. The material was immediately taken to the laboratory where the leaves after the removal of the midribs were cut into smaller portions with a pair of scissors. The finely cut leaves were thoroughly mixed by hand before sampling for distillation. Samples were immediately weighed for moisture and 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 on an electrically controlled plate.

Five or 10 gram samples of the finely cut sorghum leaves were macerated in a mortar with a small quantity of pure silica sand moistened with a few ml. of distilled water. After maceration, the samples were transferred to a Kjeldahl flask using distilled water to wash out the mortar. The flask was corked tightly with a large rubber stopper. The macerated sorghum in the tightly stoppered Kjeldahl flask was allowed to digest over night at room temperature. Ten ml. of 0.05 N silver nitrate ($AgNO_3$), one ml. of nitric acid (HCO_3) and 50 cc. of distilled water were added to a receiving flask. The HCN was distilled on the ordinary Kjeldahl distilling apparatus into the receiving flask.

The precipitate of silver cyanide was separated from the solution in the receiving flask by filtration. The silver nitrate remaining in solution was determined by titration with a 0.05 standard potassium thiocynate solution, using ferric alum as an indicator. The percemt of HCN was calculated from the titration to a moisture-free basis and also converted to parts per million (p.p.m.) by an appropriate factor.

The efficiency of the picric acid test was checked throughout the growing season by comparing samples of corn with those of sorghum, 20 punches of plant material with 10 punches, macerated samples with non-macerated samples, and water with chloroform.

CLIMATIC DATA

A relatively wet spring preceding the growing season at Perkins in 1952 made optimum soil conditions for seed germination and growth. After planting was completed on May 22, a light rain fell with a heavier rain on May 23 (Table 1). June was an abnormally dry month with only 1.62 inches of rainfall being recorded. The sorghums were making no growth and showed some signs of burning until 4.11 inches of rain fell in July. A below average rainfall of 2.92 inches was recorded in August with only .48 inches received in September. Because of the very dry June, conditions for plant growth were at a record low.

The mean temperature for the month of June was 76.9°F. in comparison to 81.3°F. for a 22 year average (1931-1952 incl.). The temperature mean for the other months followed along the same trend as the long time temperature mean (Table 2). The maximum temperature of the growing season was recorded as 104°F. in August and the minimum temperature was 26°F in April.

Table 1.--Daily moisture precipation at Perkins, Oklahoma, January 1, 1952, to September 30, 1952.

1952	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.
1			.35		.17	.28	.10		
2	.20								
3.	.37	.07	.37	.09			.06	.08	
4			.02			.93		.57	
5									
6								.19	
7							1.30		
8								1.11	
9			1.07	.24	.01				
10			.68						
11		.26		.27					
12				Tr.*					
13							.13		
14			Tr.		Tr.				
15		Tr.					.05		
16									
17			.71		1.51		2.24		.48
18				.02			.06	.03	
19				.22				.30	
20			Tr.	.57					
21	.23	.03		.16					Tr.
22				.23	.25	.40			
23					1.04			.48	
24		.55	Tr.	Tr.					
25		.37							
26									
27					.03			.16	
28									
29			.06						
30				.03					
31					.01				
Totals	.80	1.28	3.27	1.83	3.02	1.61	4.11	2.92	.48

* Trace of moisture.

Table 2.--Summary of precipitation data by months at the Perkins Agronomy Farm. Also, precipitation and temperature data for Stillwater, Oklahoma, 1952.

Year	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Seasonal Apr.-Sept.	Annual
Precipitation - Perkins Agronomy Farm														
1952	.80	1.28	3.27	1.83	3.02	1.61	4.11	2.92	.48	.00	2.04	.97	13.97	22.33
Sum 1931-51	28.59	28.91	36.92	72.55	97.00	94.11	57.15	69.45	79.30	50.25	42.95	30.01	469.56	592.77
Av. (21 yrs.)	1.36		1.75	3.45	4.62	4.48	2.72	3.31	3.78	2.39	2.05	1.43	22.36	28.23
Precipitation - Stillwater, Oklahoma. U. S. Weather Bureau														
1952	.77	1.09	3.01	2.22	2.52	2.45	3.50	4.93	.68	.00	2.23	1.07	16.30	24.47
Dep. from Normal	-.36	-.18	.82	-1.59	-2.25	-1.64	.47	1.70	-2.84	-2.98	.00	-.38	-6.15	-9.23
Normal	1.13	1.27	2.19	3.81	4.77	4.09	3.03	3.23	3.52	2.98	2.23	1.45	22.45	33.70
Temperature - Stillwater, Oklahoma. U. S Weather Bureau														
1952														
Max.	78°	72°	76°	85°	90°	100°	100°	104°	100°	96°	84°	68°	104°	104°
Min.	12°	23°	16°	26°	36°	59°	56°	66°	45°	24°	15°	16°	26°	12°
Mean	43.7	45.6	46.4	55.9	67.9	81.3	80.7	83.2	73.6	58.2	47.9	38.2	73.8	60.2
New Mean	36.9	40.0	50.2	59.8	67.9	76.9	81.4	81.3	74.5	61.8	49.2	38.8	73.5	59.8

RESULTS AND CONCLUSIONS

The first samples were taken on July 2, 1952, from the West Series. At that time the plants were not making any noticeable growth because of the extremely dry weather preceding that date. The plants were tagged as to the date sampled. The samples received relative values ranging from one to five (Table 3).

When the Middle series and rows 79 through 86 in the East series were checked on July 3, White-Seeded Sudangrass showed no HCN content but several rows of other sorghums received number five rating. (Table 4).

The complete East series was checked on July 4, when several rows showed no HCN content (Table 5). All three series received two more complete checks at intervals, and rows 79 through 86 were sampled each time, regardless of the series being checked.

The last row check was taken on July 12, at which point enough data had been collected to determine those rows which showed a trend toward high HCN content and undesirable agronomic characters. All the selections and rows except 12, 15, 26, 35, 38, 45, 51, 60, 61, 79, 80, 84, 85, and 86 were eliminated after this date.

When several plants within selected rows were sampled on July 21, selections number 15 and 45 and row 79 were found to be intermediate in HCN content and were not tested any more. White-Seeded Sudan, row 80, and Rancher, row 86, showed consistently low HCN from the July 3 up to the July 21 testing. Kavirondo, row 84, showed a definite trend toward high HCN content but was not omitted because of desirable breeding characters.

Table 3. Cyanide content of plants in rows of the West Series with 0 indicating no cyanide and 6 indicating the highest concentration of cyanide.

Row	Date (month and day) 7-2	7-4	7-5	7-9	7-21	7-29	8-6
Sugar Drip 1	1		2	3	1		
1	1	3	4	4			
2	4	2	4	5			
3	5		2	3			
4	5		5	3			
5	3		5	3			
6	4		6	2			
7	3		2	1			
8	2		2	3			
9	5		4	5			
10	3		2	3		4	
11	2		3	1		5	
12	4		5	5		1	4
13	3		5	4	4	4	4
14	4		4	5	2		
15	5		4	4	2		
16	5		5	4	4		
17	5		3	4			
18							
19	5		6	4			
20	5		4	1			
21	3		5	2			
22	4		2	1			
23	4		5	5			
24	2		4	0			
25	6		4	4		5	
26	5		6	4		3	
27	5		6	4		1	
28	1		3	2			
29	5		4	4			
30	4		1	3			
Sugar Drip 2	4		2	4	0	0	

Table 4.--Cyanide content of plants in rows of the Middle Series with 0 indicating no cyanide and 6 indicating the highest concentration of cyanide.

Row	Date (month and day)								
	7-3	7-7	7-8	7-10	7-11	7-21	7-23	7-29	8-6
Sugar Drip 3	1	1		0			1		
31	4	5		4			1		
32	4	4		4			3		
33	4	4		4			5	3	
34	4	4		5			4	3	
35	5	6		5			4	3	
36	4	5		2			2	4	
37	5	5		3			1		
38	2	1		5			3		
39	3	3		4			2		
40	5	2		4			2		
41	3	4		0			0		
42							1		
43							0		
44						1	4		
45	4	6		1		2	1		
46						2	1		
47	5	3		2		5	1		
48	5	5		3			0		
49	3	3		4			3		
50	4	4		3			0		
51	5	6		5				5	
52	3	2		2				4	
53	2	2		2				4	
54	4	4		5				0	
55	5	3		2					
56	5		5		4				
57	1		0		2			4	
58	2		0		1			4	
59	2		0		2			1	1
60	4		6		5			0	2
Sugar Drip 4	1	2		4				5	

Table 5.--Cyanide content of plants in rows of the East Series with 0 indicating no cyanide and 6 indicating the highest concentration of cyanide.

	Date (month and day)															
	7-2	7-3	7-4	7-5	7-7	7-8	7-9	7-10	7-11	7-12	7-21	7-22	7-23	8-6	8-15	8-25
Sugar Drip 5		3	1	2	1	1	3	0	3	1		2	4	3		
61			2			1			1	3		0		0		
62			0			0			3	3		0		0		
63			1			5			2	3		0				
64			4			2			3	0		0				
65			0			4			4	2		0				
66			5			1			4	2		1				
67			2			4			1	2		0				
68			3			0			2	2		0				
69			5			3			0	1		1				
70			4			0			2	3		0				
71			4			2			4	2						
72			3			4				0	3					
73			1			2			1	0	5					
74			2			5				1	3					
75			2			4			2	3	1					
76			4			2			3	4	2					
77			5			5			1	2	2					
78			4			1			3	4	3					
79		4	4	3	3	3	3	3	3	0	1			0	0	0
80		0	2-	0	1	0	1	1	1	0	0			0	0	0
81		3	1	0	3	0	4	2	3	4	0					5
82		2	0	1	1	3	2	3	3	3						5
83		1	5	0	1	0	1	1	0	1	4		2	5	5	4
84	4	3	0	1	5	3	5	3	5	4	1		4	5	4	4
85		3	3	0	6	3	5	4	4	5	1		5		5	5
86	1	1	0	1	2	1	0	0	0	1	3		5		1	4
Sugar Drip 6		2	1	1	1	1	2	2	3	1	1					3

All 10 samples taken on July 22, from row 61, a selection which possessed the desirable character of tan plant color, showed a low HCN content.

Selection number 38 and rows 84 and 85 were checked on July 23. The results obtained from the 20 plants tested from selection number 38 were very erratic, and this row was discarded. The samples from Kavirondo, row 84, and Sorghum verticilliflorum, row 85, showed the same trend toward high content of HCN as observed in the earlier samples.

Of the samples taken on July 29, from selections 12, 26, and 35 and rows 51 and 60, all but selection 12, a Kavirondo appearing plant, and row 60, and early, tillering sorghum, were discarded because of their continued exhibition of high HCN content.

Of the six varieties subjected to a quantitative test in the laboratory on August 6, and compared to the readings obtained from the picric acid test (Table 6), the same general trend was noted between the two tests. White-Seeded Sudan had the lowest value assigned from the picric acid test and zero p.p.m. of HCN from the quantitative test, and row 60 received the highest rating from both tests. The picric acid test showed to be effective in the selection of lines low in HCN but did not give consistent results in high HCN selections.

When White-Seeded Sudan, Kavirondo, and Sorghum verticilliflorum were tested again on July 15 and 25, the same relative results were obtained as previously recorded.

The White-Seeded Sudan (Fig. 3) proved to be constantly low and almost always free of HCN. Row 61 (Fig. 4), an early dwarf, tillering, tan colored selection, and row 86, Rancher (Fig. 5), were the next lowest throughout the test. Sugar Drip (Fig. 5) was an intermediate variety in HCN content. Rows 84, Kavirondo, and 85, Sorghum verticilliflorum (Fig. 6) both proved to be relatively high throughout the test.

When corn was sampled to check the accuracy of the picric acid test, no red color was shown on the filter paper.

Table 6.--Comparative results obtained from a quantitative analysis and the picric acid test.

		Quantitative test			Picric acid test
Row No.	Number leaves tested	Percent dry weight	HCN p.p.m. green weight	HCN p.p.m. dry weight	Relative color value
80	30	36.30	---	0	0
61	38	35.33	183	517	1
Sugar Drip	23	32.68	372	1137	3
12	24	32.68	522	1547	4
84	18	30.51	599	1964	5
60	40	35.24	1298	3684	5

Fig. 3. - Row 80, White-Seeded Sudan, which was consistently low and usually free from HCN.

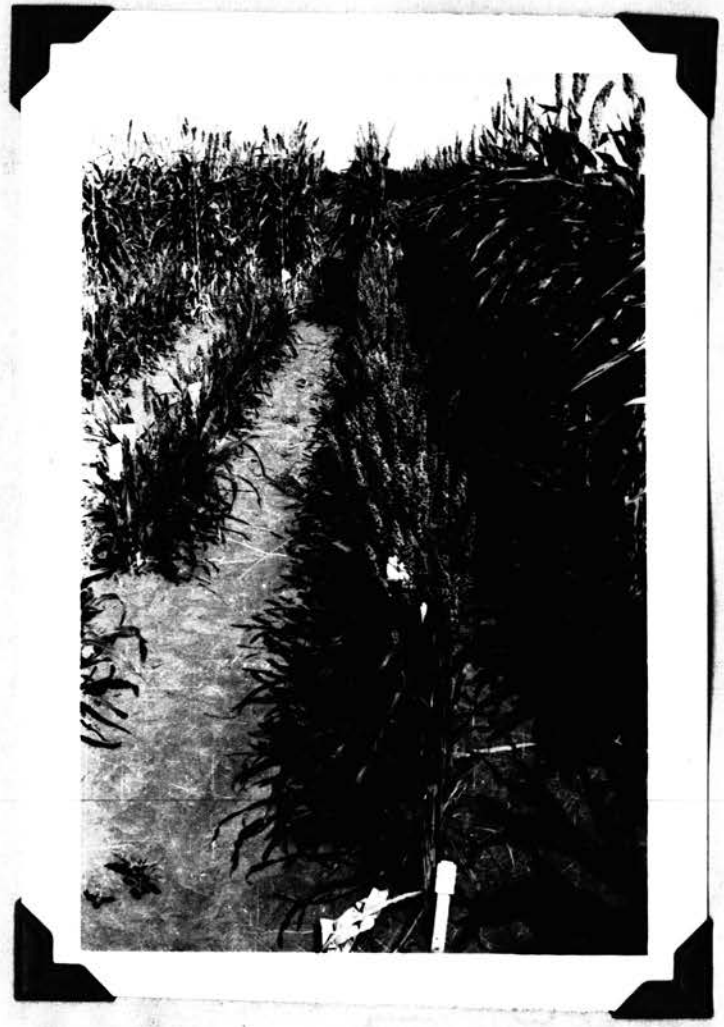

Fig. 4. - Row 61, an early, tillering sorghum, which was relatively low in HCN throughout the tests.

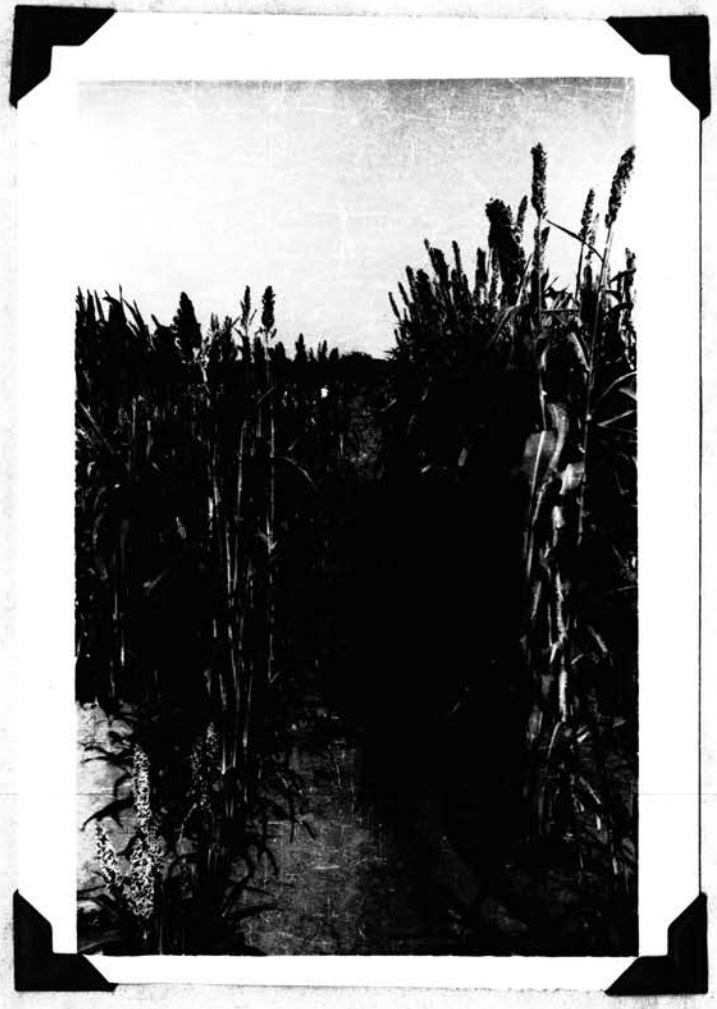

Fig. 5. - Left-Row 86, Rancher, a low HCN variety from South Dakota. Right-Sugar Drip used as border row.

Fig. 6. - Left-Row, Kavirondo, high in HCN content. Right-Row 85, Sorghum verticilliflorum, a wild type sorghum high in HCN content.

SUMMARY

This project was conducted in 1952 at the Oklahoma Agricultural Experiment Station at Perkins, Oklahoma, in an attempt to find a sorghum variety which is very low or free of HCN and to check the efficiency of the picric acid test in determining the presence of HCN in sorghum.

The bulk of the material tested consisted of crosses involving Kavirondo; because reports from Africe, its place of origin, indicated that this variety was a low HCN type.

The following limited conclusions, which may be modified by future results, were drawn:

1. The picric acid test gave the same general results when compared to a quantitative test run in the laboratory.

2. White-Seeded Sudan was very low or free from HCN.

3. Row 61, a tan, early, tillering selection, and Rancher were relatively low in HCN content. These two selections have future possibilities as breeding material.

4. Kavirondo and Sorghum verticilliflorum were constantly high although they had been expected to be relatively low.

5. Corn showed no HCN content.

6. Erratic results were obtained from comparisons of 20 punches of leaf with 10 punches and from macerated with non-macerated plant material. No indication of the desirable number of punches resulted nor was a definite preference of macerated over non-macerated plant material shown.

7. Water can not be substituted for chloroform in the picric acid test.

8. Color standards of known concentration with which to compare the colored filter paper would greatly increase the value of the picric acid test. The numbers from zero to six, which were assigned the samples, could then be associated with a definite HCN concentration.

9. The picric acid test was sufficiently accurate only for the selection of plants very low in HCN.

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