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Estimating hydrocyanic acid potential sorghum plants from leaf samples

Jim Allen Benson

To the Graduate Council:

I am submitting herewith a thesis written by Jim Allen Benson entitled "Estimating hydrocyanic acid potential sorghum plants from leaf samples." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agronomy.

Elmer Gray, Major Professor

We have read this thesis and recommend its acceptance:

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(Original signatures are on file with official student records.)

August 6, 1964

To the Graduate Council:

I am submitting herewith a thesis written by Jim Allen Benson entitled "Estimating Hydrocyanic Acid Potential of Sorghum Plants from Leaf Samples." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agronomy.

Elmer Gray
Major Professor

We have read this thesis and recommend its acceptance:

Henry H. Laude

Raymond W. Holton

Accepted for the Council:

Hilton A. Smith
Dean of the Graduate School

ESTIMATING HYDROCYANIC ACID POTENTIAL OF SORGHUM PLANTS FROM LEAF SAMPLES

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Jim Allen Benson

August 1964

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CHAPTER I

INTRODUCTION

All of the species and varieties of the genus Sorghum contain dhurrin, the precursor of hydrocyanic acid (HCN). Hydrocyanic acid is one of the most powerful poisons found in nature (9).

Sorghums have become an important summer crop in the United States. In 1954 there were 20,148,000 acres grown with the largest amount being grown in the dryer areas of Texas, Oklahoma, and Kansas (35). An estimated 30,000 acres were grown in Tennessee in 1961; with the advent of more productive hybrids between sorghum and Sudangrass the acreage probably will increase (18).

Sudangrass (Sorghum vulgare var. sudanense Hitchc.) is the most widely used summer annual pasture crop now grown in the United States (34). In comparison with sorghum (Sorghum vulgare Pers.) Sudangrass is relatively low in HCN content. However, under certain conditions, the HCN content of Sudangrass may be high enough to be fatal to cattle. Hybrids between sorghums and Sudangrass are likely to be higher in HCN than the Sudangrass parent (28).

The objectives of this study were: (1) to determine if a small portion of various leaves will give a representative sample of the hydrocyanic acid potential of the whole plant; (2) to determine the effect of three different cutting managements on hydrocyanic acid

potential; and (3) to determine the extent of variation in hydrocyanic acid potential among varieties.

CHAPTER II

REVIEW OF LITERATURE

I. EARLY EXPLANATIONS OF SORGHUM POISONING

The fact that plants of the genus Sorghum are toxic to animals was known in the 1800's. In Africa the plants were used as a poisonous protective hedge to prevent animals from eating crops such as peanuts. Wilted sorghum plants were used to scatter around other crops to keep the cattle away (14).

The toxic effect of sorghum plants was first thought to be caused by a poisonous fungus in the plants or by a small insect feeding on the plants. It was thought that the animal consumed the fungi or insects while eating the plants. Another belief was that the animals consumed the plants in excessive amounts causing suffocation. Another theory was that during long dry periods nitrates built up in the plant resulting in nitrate poisoning (14).

II. DISCOVERY OF DHURRIN

Early in 1902, H. B. Slade (36) tried to determine why sorghum plants were poisonous. He did not identify the toxic substance but believed it was an enzyme in the plant. Later the same year Dunstan and Henry (14), working in England, analyzed some material from Egypt which had caused several fatalities in a herd of grazing cattle. They detected a strong odor of hydrocyanic acid (HCN) when the material was

crushed and wet. A chemical analysis was run on the distillate of the liquid extract and HCN was found in the material. They also found that the plant contained a glucoside that was hydrolyzed by an enzyme to yield HCN. They named this glucoside dhurrin from the vernacular name of sorghum in Egypt "Dhurra Shirshabi". This work was reported June 27, 1902, in the Chemical News of London and confirmed Slade's theory. Before Slade had read this article he had detected HCN in sorghum plants that had killed some cattle. He determined the percentage of HCN and secured strong evidence in favor of the glucoside theory (3).

III. FACTORS AFFECTING HYDROCYANIC ACID CONTENT

Fertilization

The HCN potential of sorghum plants is affected by fertilization. Boyd et al. (4) found that the addition of nitrogen fertilizers to soils deficient in nitrogen increased the HCN content of Sudangrass and sorghum grown on these soils. Similar treatment of soils well supplied with nitrogen had little effect on the HCN content of plants produced. When adequate amounts of phosphate fertilizer were added to soils low in phosphorus, Sudangrass planted thereon grew rapidly, and in six weeks after planting only small amounts of HCN were found. On the other hand, high concentrations of HCN were found in plants of the same age grown on soils low in phosphorus.

Franzke et al. (16) found that the application of stall manure decreased the HCN content of sorghum plants. They also found that the HCN content was invariably lower in plants from plots receiving acid

phosphate than from corresponding ones not fertilized. Continued use of nitrogen in the cropping system has been found to be correlated with an increase in the amount of HCN content of sorghum plants grown upon the soil. Nelson (30) reported that the HCN content was always higher with increased amounts of nitrogen regardless of moisture level and stage of growth.

Patel and Wright (32) grew two strains of Sudangrass in nutrient solution in the greenhouse to determine the effect of nitrogen, phosphorus and potassium on the HCN content. Significant differences were found in plants that received different levels of nitrogen and phosphorus but the HCN content was not affected by various levels of potassium. High levels of nitrogen (364 ppm.), when associated with either low (15.5 ppm.) or optium (31 ppm.) levels of phosphorus, resulted in significant increases in HCN content. Willaman and West (39) found that on soils deficient in nitrogen, added nitrogen increased slightly the HCN content in sorghum; but with a plentiful supply of nutrients in the soil, added nitrogen did not affect the amount of HCN in the plants.

Stage of Growth

Stage of growth is another factor that affects the HCN content of sorghums. Acharya (1) in India found that the total quantity of HCN in the plant increased until the flowering stage. After the formation of grain, there was a decrease in the content of HCN so that the plants were not toxic. Contrary to Acharya's findings, Cassady (8) found that the HCN content of Sudangrass was highest in young plants and decreased

as the plants become older. Couch (12) stated that the quantity of potential HCN that can be formed in plants may vary considerably with the stage of growth. He also found that young second-growth and first-growth plants, including suckers, had a much higher rate index, speed in which the glucoside breaks down to form HCN, than leaves of well developed sorghum varieties and hence were more likely to cause poisoning. The rate index was high for young plants less than 15 inches tall and low for plants above 2 feet, regardless of the variety.

Franzke et al. (17) found that out of 24 comparisons between first and second growth, 17 had a higher amount of HCN for the first growth than for the second growth. Contrary to Franzke's findings, Hogg and Ahlgren (22) found the average HCN content of plants in the seedling stage to be 122 ppm. whereas that of the second growth was 224 ppm.

Moisture and Drought

It is generally thought that a long drought increases HCN content regardless of stage of growth (7, 11, 23). Franzke and Hume (16) found that plants grown on soil at 15, 25, and 35% moisture produced plants with an HCN content of approximately 1200, 500 and 250 ppm., respectively, on a dry weight basis.

Heinrichs and Anderson (20) reported the HCN content to be twice as high in plants grown under drought conditions as in plants grown under normal conditions. Hogg and Ahlgren (22) conducted a similar greenhouse experiment in which they subjected a variety of sorghum to

drought conditions. Plants were sampled at two-day intervals until the permanent wilting point was reached. At the beginning of the study the plants contained 159 ppm. of HCN. Twelve days later when the plants had reached the permanent wilting point, the concentration was 222 ppm.

Frost

The fact that sorghum is unsafe to pasture after a frost is generally accepted; however, it is not known exactly why a frost makes the sorghum dangerous to use as feed. Boyd et al. (4) found no increase in the HCN content when Sudangrass was frosted. They suggested that when favorable conditions for growth follows a killing frost, Sudangrass will send forth new shoots and leaves which are likely to be very high in HCN and, if pastured, may cause hydrocyanic acid poisoning. When this happens it is of course natural to infer that it is the frosted material that caused the poisoning rather than the new growth. Franzke et al. (17) reported that the HCN content of sorghum was higher in samples taken the evening before a heavy frost than in samples taken the day after the frost. Pickett (34) concluded that Sudangrass partially killed by frost may be dangerous to graze since the cattle will graze the young tender shoots that are much higher in HCN. Swanson (37) ran tests on frosted Sudangrass and found the HCN content to be much higher than in material that had begun to wilt.

Geographic Location

It is generally known that HCN poisoning is much less common in the Southern states than in states farther north. It may be that the

plant stores less glucoside or, it may be that the enzyme which exists in the plant and is instrumental in breaking down the glucoside and liberating the hydrocyanic acid is less active in the Southern states. The HCN may occur in a more unstable form in sorghums grown in the Northern states (38). Franzke et al. (17) found that some difference or differences in conditions of growth at two locations in South Dakota caused corresponding differences in the HCN content of 13 identical strains grown at both locations. Hogg and Ahlgren (22) tested 10 inbred lines in 6 different locations in the Midwest and Canada and found a difference of about 1200 ppm. in HCN content of the same line grown at different locations.

Variety

Varietal difference probably has more effect on the amount of HCN in sorghum than the growing conditions (27, 40). It was found by Moodie and Ramsey (28) that sorghum-Sudangrass hybrids may contain three times as much HCN as the sorghum parent. On the other hand, Finnemore and Cox (15) working with 8 sorghum-Sudangrass hybrids, Sudangrass, and 9 varieties of sorghum found that Sudangrass had the least and feteria had the most HCN. The hybrids seem to yield less HCN, at least during the first month or two, than the majority of the sorghums.

Drying and Ensiling

There are considerable discrepancies among published reports as to the effects of drying on the HCN content of sorghum. Acharya (1) reported that drying sorghum in the shade decreased the HCN content by

about 10%. Drying in the sun resulted in a decrease of 30 to 40%. Heating the tissue to 100° C. and maintaining it at that temperature for some hours destroyed the HCN potential. Boyd et al. (4) found that plants that were high in HCN at the time of cutting did not lose appreciable quantities of HCN due to air drying or sun curing. Cassady (8) found that the amount of HCN decreased during the hay-curing process. Couch (11) stated that well-cured hay contained very little HCN. Dowell (13) found that approximately three-fourths of the HCN was set free in the process of drying.

Franzke et al. (17) reported that the HCN content of sorghum cured in the sun was not only lower than that of a comparable uncured sample but was lower than that of a sample cured in the shade. Swanson (37) found the rate of drying to be important in affecting the amount of HCN in a sample. Contrary to Acharya's work Swanson found that material tested at once contained more HCN than in oven dried material. The oven dried material likewise contained more HCN than material dried in the sun, and material dried in the sun contained more HCN than material slowly dried in the shade; the latter contained only a trace of HCN.

Briese and Cassady (5, 8) suggested that sorghum silage contained toxic amounts of HCN. They believed, however, that it was safe to feed since the HCN was in free form and dispersed immediately when exposed to the air.

IV. LOCATION OF HYDROCYANIC ACID CONTENT IN THE PLANT

There seems to be general agreement among workers that the HCN

is located mostly in the leaves, whereas the stems contain less and the roots contain even a smaller amount. Acharya (1) found that the HCN content of the leaves, stems, and roots was in a ratio of 9:3:2, respectively. Martin et al. (25) reported that the cyanogenetic compounds appeared to be synthesized in the leaves and were translocated to other parts of the plant. They determined the HCN content of various parts of sorghum plants using material grown in Texas, New Mexico, Colorado, and Virginia in 1936 and 1937. The HCN content of the leaves was 3 to 25 times that of the corresponding culms from plants that had reached the boot stage. The upper leaves contained more HCN than the lower leaves. The proximal half of the leaf was higher in HCN than the distal half. The HCN content of culm internodes decreased progressively downward, the lower internodes containing only small quantities. Axillary branches were much higher in HCN than the older, main culms and, in most cases, tillers were higher in HCN than the main culms. Willaman and West (38) concluded that during the first 3 or 4 weeks of the life of the plant the HCN is concentrated in the culm. The HCN content then decreased rapidly and disappeared, but apparently persisted in the leaves in decreasing percentages until maturity. The character of the growth of the plant affected the distribution of dhurrin between leaves and culms, there being a proportionally smaller amount in thick, heavy culms than in slender ones.

V. TOXICITY OF HYDROCYANIC ACID

Hydrocyanic acid is one of the most powerful poisons found in

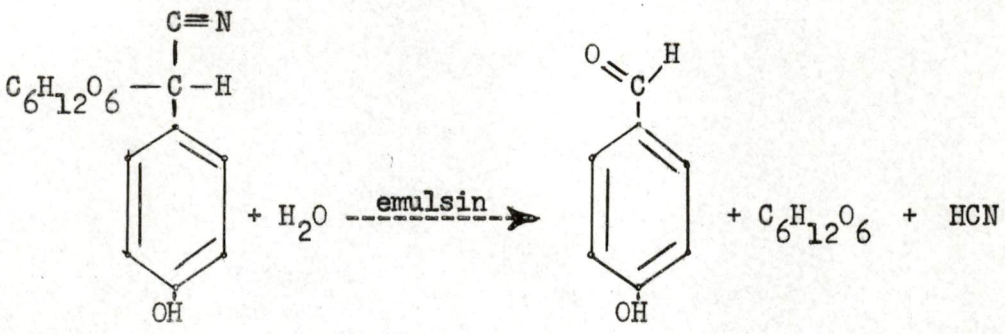
nature (9). When the acid is liberated in large enough quantities in the stomach, the HCN is absorbed and carried by the blood stream to all the body tissues where it inhibits the action of the oxygen-activating enzyme, cytochrome oxidase. As a result of this phenomenon, the tissues are unable to utilize the oxygen in their normal metabolic processes, and there is an accumulation of oxyhemoglobin on the venous side of the circulation. This causes the venous blood to be the same color as the arterial blood, a fact which can be used in diagnosing hydrocyanic acid poisoning (18).

The amount of material required to produce fatal results depends on the concentration of dhurrin in the plant, the rate at which HCN is released and the rate at which it is detoxified by the animal (27). According to Clawson, Bunyea and Couch (27) the minimum lethal dose of HCN for cattle is about 2.042 milligrams per kilogram of body weight. Since the material is detoxified as it is liberated, a 24-hour tolerance would range from about 15 to 50 milligrams per kilogram of body weight. Partially digested grain tends to make an animal more tolerant to HCN (3). Couch (11) stated that if a plant contains as little as 0.02% potential HCN, and if the animal consumes it rapidly, 5 pounds of the plant would be fatal. Boyd et al. (4) stated that it takes about one g. of HCN to kill a one thousand-pound cow, but an animal can detoxify the HCN at the rate of about 0.5 g. per hour.

VI. CHEMICAL BREAKDOWN OF DHURRIN TO HYDROCYANIC ACID

Dhurrin, the glucoside that is the precursor of HCN, has the

empirical formula C_{14} H_{17} O_7 N. At the time of its discovery (1902) it was the first dextrose glucoside to be found in nature. According to Dunstan and Henry (14) dhurrin upon hydrolysis reacts as follows:



Dhurrin

p-hydroxy-benzaldehyde

They found that an enzyme must be present before the reaction can take place. The enzyme performed the same functions as the enzyme emulsin, which occurs in sweet almonds, so they concluded provisionally that the two enzymes might be the same. Conn and Colette (10) working with etiolated sorghum seedlings, found that there was not one but two enzymes involved in the breakdown of dhurrin of HCN. The initial step in the process is catalyzed by a glucosidase which hydrolyzes the dhurrin to glucose and p-hydroxybenzaldehyde cyanohydrin. The sorghum plant contains a second enzyme, oxynitrilase, which catalyzes the decomposition of the cyanohydrin to hydrocyanic acid and p-hydroxybenzaldehyde. This latter enzyme has been purified many times from three-day old etiolated sorghum seedling plants and has been partially characterized.

Later work by Mao (24) of Wisconsin supports Conn and Colette's work. Mao found that dhurrin, in the presence of the enzyme β-glucosidase II, yielded glucose and p-hydroxy-L-mandelonitrile. The

p-hydroxy-L-mandelonitrile in the presence of the second enzyme, oxynitrilase, broke down to form p-hydroxybenzaldehyde and HCN. He found two types of β-glucosidases present in sorghum. The type I hydrolyzed salicyl alcohol glucoside and p-nitrophenyl β-glucoside. Type II β-glucosidase hydrolyzed p-hydroxy-L-mandelonitrile glucoside (dhurrin), p-hydroxy-D-mandelonitrile glucoside and dl-mandelonitrile glucoside. The oxynitrilase acted only on p-hydroxy-L-mandelonitrile (the aglycone of dhurrin) and not on the other two glucosides.

VII. SODIUM PICRATE METHOD FOR DETERMINING HYDROCYANIC ACID

The "picric-acid test" has been adapted so that it is now regularly used in determining the HCN content of a large number of individual plants. This test has the advantage of not only providing a measure of HCN content, but at the same time permitting normal development of the plants so that the usual studies on plant characteristics can be made. Furthermore, it permits the classification and selection of plants early in the growing season. Experiments have indicated that quantitative determinations obtained by means of this test are accurate for comparative purposes and may be safely used as a basis for selecting HCN free plants (31). The method consists of placing 0.15 g. of finely chopped green plant material in a test tube, adding 3 or 4 drops of chloroform, and suspending a strip of moist filter paper saturated with sodium picrate solution above the sample. The saturated filter paper is held in place with a cork stopper which also serves to seal the test tube. The tube with the contents is incubated at room temperature

(20° C.) for 12 to 24 hours. The sodium picrate present in the filter paper is reduced to a reddish compound in proportion to the amount of HCN evolved. The color produced is dissolved by placing the paper in a clean test tube containing 10 cc. of distilled water. The color of the water extract then is matched with the color standards (21).

CHAPTER III

MATERIALS AND METHODS

The plants in this study were obtained from two different tests. One was an HCN determination test which was set up for this particular study. The other was a variety test in which part of the material was cut as green-chop; the other part was cut as silage. The variety test material was sampled to give a larger comparison among varieties.

I. HCN DETERMINATION TEST

Two varieties of Sudangrass, Piper and Suhi-1, were used in this experiment. The Piper is low in HCN potential and the Suhi-1 is relatively high in HCN potential.

The two varieties were planted June 10, 1963, in drilled rows 36 inches apart and approximately 100 feet long. They were planted on a Lindside sandy loam soil. One row of each variety was subjected to each of three cutting managements. The first cutting management consisted of allowing the material to reach 20 inches in height before it was cut back to 3 inches. This cutting management will be referred to as the 20-3 management. For the second cutting management, the material was allowed to reach 30 inches in height and was cut back to 8 inches. This cutting management will be referred to as the 30-8 management. In the third treatment, the plants were allowed to reach the early bloom stage and were cut back to four inches. This cutting management will be

referred to as the E.B.-4 management. After each cutting, nitrogen fertilizer was applied at the rate of 30 pounds of N per acre.

II. VARIETY TEST

The varieties sampled from the green-chop and silage regrowth tests are presented in tables 1 and 2. These varieties were planted May 6, 1963, in drilled rows 36 inches apart and 22 feet long. They were planted on Huntington and Sequatchie silt loam soils. The material sampled was taken from 9 to 11 feet of the row. The plants in the green-chop yield test had been cut from 3 to 7 times, depending on the variety. The plants in the silage test were regrowth material, after the first growth had been harvested at the dough stage for silage. All of the plants were approximately 30 inches tall at the time of sampling and were cut to 8 inches.

III. SAMPLING TECHNIQUE

When the material reached the desired height, 30 to 35 plants were selected at random throughout the row. The material was taken to the laboratory immediately after cutting and samples were taken from 15 plants. When more than one variety was sampled during the day the material was put in an ice chest above a small quantity of ice.

The plants were divided into the individual portions to be analyzed. These included the whorl, and the first, third and fifth leaves. The remaining portion of the plant was chopped up and mixed in a small container. A 2 to 5 g. random sample was taken from this

Table 1.--Varieties from the green-chop test sampled for HCN potential.

Variety Name	Origin	Apparent Characteristics
Greenleaf	University of Kansas	Sudangrass type
Tennessee Synthetic 1	University of Tennessee	Sudangrass type
Piper	University of Wisconsin	Sudangrass type
Trudan 1	Northrup King	Sudangrass type
Su-1	University of Nebraska	Sudangrass type
H-6160	Asgrow	Sudan x forage sorghum type
Sordan	Northrup King	Sudan x forage sorghum type
Hydan 37	Frontier	Sudan x forage sorghum type
Sudax	DeKalb	Sudan x forage sorghum type
Sweet Sioux	Paymaster	Sudan x forage sorghum type
Mor Su	Rudy Patrick	Sudan x forage sorghum type
Suhi-1	University of Georgia	Sudan x forage sorghum type

Table 2.--Varieties from the silage regrowth test sampled for HCN potential.

Variety Name	Origin	Apparent Characteristics
Su-1	Rudy Patrick	Sudan x forage sorghum type
Yieldmaker	Taylor-Evans	Sudan x forage sorghum type
Milkmaker	Taylor-Evans	Sudan x forage sorghum type
Dairy D	Asgrow	Sudan x forage sorghum type
Sudax	DeKalb	Sudan x forage sorghum type
4D-F	Rudy Patrick	Sudan x forage sorghum type
Aztec	Paymaster	Sudan x grain sorghum type
N. K. 330	Northrup King	Sudan x grain sorghum type
S-214	Frontier	Sudan x grain sorghum type
Su-Chow 1	Pfister	Sudan x grain sorghum type
Su-Chow 2	Pfister	Sudan x grain sorghum type
1071 F	Advance	Grain sorghum type

material to represent the whole plant. The whorl was considered as the first leaf above the uppermost leaf with a collar. The first leaf was considered as the uppermost leaf with a collar and the third and fifth leaves were the third and fifth leaves from the top, respectively. Approximately 0.5 g. was taken from the distal portion of each leaf to be analyzed. Samples similar to those taken for the HCN analysis were taken from 15 other plants for dry matter determination.

IV. CHEMICAL DETERMINATIONS OF HCN

The method used in the laboratory was a modified version of the method used by Anderson et al. (2) at Wisconsin. The material was weighed on a closed, torsion balance to the nearest hundredth g., and then cut into small pieces and placed in a test tube. Chloroform was added to the material in a ratio of approximately 8 drops to each 0.5 g. of plant tissue. A strip of filter paper saturated with sodium picrate solution was suspended in the tube with a rubber stopper which also served to seal the tube. The tubes were then set aside at room temperature for 16 to 24 hours.

After the material had incubated for the designated time the strips of filter paper were removed and placed in colorimeter tubes. Twenty-five ml. of distilled water was added to each tube and the paper was allowed to soak for approximately 20 minutes. When the color of the filter paper was extremely dark it was dissolved into 50 or 100 ml. of distilled water. During the period the paper was soaking the containers were gently swirled to aid in the dispersion of the colored

material from the filter paper. After the filter paper had been removed the tubes were placed in a Fisher Electrophotometer with a 525 millimicron filter and the percentage light absorbence was read. The readings then were inserted in the equation prepared from the standard curve to determine the actual content of HCN in each sample.

The standard curve was developed by dissolving 0.241 g. of potassium cyanide into one l. of distilled water. The solution was then dispensed into tubes in increasing amounts to give increasing concentrations of HCN. These tubes then were read for percentage light absorbence on the colorimeter. A linear regression was run on the colorimeter readings and the concentration to give an "a" and "b" value which could be used in the equation, $Y = a + bX$. For the two batches of sodium picrate solution used during the experiment, the "a" values were 2.57 and 3.06 and the corresponding "b" values were 10.86 and 13.20.

V. MATHEMATICAL CALCULATIONS

A program was developed for the IBM 1620 computer to take the raw data from two input cards and produce the output data using the equation $Y = a + bX$. Card one of the input data contained the green sample weights, the colorimeter readings and the dilution factors. Card two of the input contained the wet and the dry weights of comparable material. From the information given on these two cards, output was obtained which consisted of the HCN concentration of each sample, in parts per million, on both green and dry weight bases. The dry weight percentage also was calculated.

Another program was developed to take the output mentioned above and average any number of plants desired and give the number of observations making up the average. This was used to obtain the average values for the 15 plants of each of the cuttings.

A third program was developed to compute the various simple correlations of interest. This program was used to obtain the r values which are presented in the results of this thesis. Copies of these programs are available from the University of Tennessee Agronomy Department.

CHAPTER IV

RESULTS AND DISCUSSION

The HCN potential of Suhi-1 and Piper on both green and dry weight bases are presented in tables 3, 4, 5 and 6. In the results and discussion, the samples are referred to as L_0 for the whorl, L_1 for the first leaf, L_3 for the third leaf, L_5 for the fifth leaf, and WP for the whole plant. The values for the whole plant were obtained by taking a 2 to 5 g. random sample from the material remaining after the L_0, L_1, L_3 and L_5 had been removed.

I. COMPARISONS OF THE HCN POTENTIAL OF LEAVES AND WHOLE PLANTS

The correlation coefficients for the HCN potential in the leaves versus that in whole plants of all cuttings and all managements of Piper were significant for most of the cuttings (Table 7). The comparisons L_0 x WP and L_1 x WP have significant r values for all of the cuttings of the 20-3 management. The r values for the comparisons L_1 x WP and L_3 x WP were significant for more cuttings than the comparisons L_0 x WP and L_5 x WP for the 30-8 management. The r values were significant for all comparisons of all cuttings for the E.B.-4 management.

There were fewer cuttings with significant correlation coefficients for Suhi-1 than there were for Piper (Table 8). The comparisons L_0 x WP and L_1 x WP had significant r values for three cuttings of the 20-3 management. The comparisons L_0 x WP, L_1 x WP, and L_3 x WP had

Table 3.--HCN potential (ppm.) on a green weight basis of different leaves and whole plants of Piper subjected to different cutting managements. Averages of 15 plants.

Cutting Date	L_0	L_1	L_3	L_5	WP
		20-3 management			
July 9	8	34	48	43	28
July 25	2	9	20		12
Aug. 7	24	23	33		25
Aug. 20	20	14	30		15
Sept. 5	4	5	1		12
Sept. 30	5	6			12
		30-8 management			
July 12	1	6	10	20	14
July 23	0	1	11		11
Aug. 5	9	15			21
Aug. 23	6	4	3		11
Sept. 13	2	3			7
		E.B.-4 management			
July 29		4	9	16	3
Aug. 29		3	16		2

Table 4.--HCN potential (ppm.) on a dry weight basis of different leaves and whole plants of Piper subjected to different cutting managements. Averages of 15 plants.

Cutting Date	I_0	L_1	L_3	L_5	WP
		20-3 management			
July 9	28	120	217	215	244
July 25	9	36	49		108
Aug. 7	117	105			213
Aug. 20	56	42			105
Sept. 5	20	19	4		97
Sept. 30	17	21			64
		30-8 management			
July 12	6	32	41	99	90
July 23	3	6	33		86
Aug. 5		57			164
Aug. 23	19	13			73
Sept. 13	10	11			44
		E.B.-4 management			
July 29		10	32	45	15
Aug. 29		8	3		6

Table 5.--HCN potential (ppm.) on a green weight basis of different leaves and whole plants of Suhi-1 subjected to different cutting managements. Averages of 15 plants.

Cutting Date	L_0	L_1	L_3	L_5	WP
		20-3 management			
July 11	110	135	114	89	76
July 23	182	160	163		88
Aug. 7	184	162	150		98
Aug. 23	161	114	103		70
Sept. 10	185	187	144		84
		30-8 management			
July 15	84	56	45	22	59
July 24	132	153	137		84
Aug. 5	104	89	79		77
Aug. 20	148	134	124		95
Sept. 5	170	167	72		69
Sept. 30	183	127	49		69
		E.B.-4 management			
July 31		107	62	53	32
Sept. 10		147	76	5	19

Table 6.--HCN potential (ppm.) on a dry weight basis of different leaves and whole plants of Suhi-1 subjected to different cutting managements. Averages of 15 plants.

Cutting Date	L_0	L_1	L_3	L_5	WP
		20-3 management			
July 11	588	431	456	355	549
July 23	1152	550	816		761
Aug. 7	735	596	720		658
Aug. 23	742	511	308		531
Sept. 10	831	748	578		637
		30-8 management			
July 15	352	266	193	112	419
July 24	396	763	695		744
Aug. 5	⊕	406	343		601
Aug. 20	330	601	495		740
Sept. 5	705	575	252		551
Sept. 30	660	396	158		358
		E.B.-4 management			
July 31		430	222	174	135
Sept. 10		442	229	16	76

⊕Dry matter sample lost.

Table 7.--Simple correlation coefficients for the HCN potential between leaves and whole plants of Piper subjected to different cutting managements.

Cutting Date	L_0 x WP	n[⊕]	L_1 x WP	n	L_3 x WP	n	L_5 x WP	n
			20-3 management					
July 9	.61*	15	.84**	15	.62*	15	.76**	15
July 25	.67**	15	.68**	15	.31	12		
Aug. 7	.73**	15	.65**	15	.60	6		
Aug. 20	.82**	15	.62*	15	.61	8		
Sept. 5	.51*	15	.52*	15	.25	12		
Sept. 30	.59*	15	.62*	15				
			30-8 management					
July 12	.71**	15	.66**	15	.78**	15	.34	15
July 23	.51*	15	.60*	15	.57*	13		
Aug. 5	.55*	15	.70**	15	.59*	9		
Aug. 23	.47	15	.52*	15	.92**	10		
Sept. 13	.43	15	.29	15	.21	14		
			E.B.-4 management					
July 29			.94**	15	.94**	15	.95**	15
Aug. 29			.96**	15	.66**	15		

[⊕]n = number of observations.

* and ** indicate significance at the .05 and .01 level of probability, respectively.

Table 8.--Simple correlation coefficients for the HCN potential between leaves and whole plants of Suhi-1 subjected to different cutting managements.

Cutting Date	L_0 x WP	n[⊕]	L_1 x WP	n	L_3 x WP	n	L_5 x WP	n
			20-3 management					
July 11	.07	15	.35	15	.13	15	.10	15
July 23	.64**	15	.63*	15	.01	13		
Aug. 7	.65**	13	.51*	13	.74**	8		
Aug. 23	.63*	15	.34	15	.07	9		
Sept. 10	.34	15	.72**	15	.22	15		
			30-8 management					
July 15	.16	15	.10	15	.14	15	.42	15
July 24	.54*	15	.29	15	.29	10		
Aug. 5	.79**	15	.85**	13	.71**	15		
Aug. 20	.25	15	.84**	15	.63*	12		
Sept. 5	.68**	15	.38	15	.75**	15		
Sept. 30	.66**	15	.52*	15	.38	15		
			E.B.-4 management					
July 31			.58*	15	.65**	15	.61*	15
Sept. 10			.39	15	.67**	15	.34	15

[⊕]n = number of observations.

* and ** indicate significance at the .05 and .01 level of probability, respectively.

significant r values for an approximately equal number of cuttings for the 30-8 management. The comparison L_3 x WP was the only comparison to have a significant r value for both cuttings of the E.B.-4 management.

All cuttings of each management were grouped together to give one correlation coefficient for each comparison of each management (Table 9). The r values for all comparisons of the 20-3 management of Piper were highly significant. All comparisons for the 30-8 management were highly significant with the exception of the last comparison, L_5 x WP, which was not significant. All comparisons were highly significant for the E.B.-4 management.

The comparisons L_0 x WP and L_1 x WP were highly significant for the 20-3 management of Suhi-1 but the r values for the other two comparisons were not significant. The comparisons for the 30-8 management were all highly significant with the exception of the comparison L_5 x WP which was not significant. The comparisons L_3 x WP and L_5 x WP were the only comparisons to have significant r values for the E.B.-4 management.

The ratios of HCN potential in the leaves to the whole plant were very inconsistent for Piper (Table 10). In some cuttings the leaves had a higher HCN potential than the whole plant and in other cases, the reverse occurred.

The ratios were much more consistent for Suhi-1 than for Piper. With the exception of four instances, the leaves had a higher HCN potential than the whole plant (Table 11). In the 20-3 management the leaves were higher in HCN potential than the whole plant by a larger ratio than they were for the other managements.

Table 9.--Simple correlation coefficients for the HCN potential of leaves and whole plants of all cuttings of Piper and Suhi-1.

Variety	Management	L_0 x WP	n[⊕]	L_1 x WP	n	L_3 x WP	n	L_5 x WP	n
Piper	20-3	.60**	90	.76**	90	.61**	59	.76**	15
Piper	30-8	.56**	75	.68**	76	.64**	51	.34	15
Piper	E.B.-4			.94**	30	.85**	30	.95**	15
Suhi-1	20-3	.51**	73	.56**	73	.23	60	.10	15
Suhi-1	30-8	.39**	90	.52**	88	.57**	82	.42	15
Suhi-1	E.B.-4			.33	30	.53**	30	.56**	30

[⊕]n = number of observations.

** indicates significance at the .01 level of probability.

Table 10.--Leaf to whole plant ratios of HCN potential for Piper subjected to different cutting managements. Averages of 15 plants.

Cutting Date	L_0: WP	L_1: WP	L_3: WP	L_5: WP
		20-3 management		
July 9	1: 3.50	1: 0.82	1: 0.58	1: 0.65
July 25	1: 6.00	1: 1.33	1: 0.60	
Aug. 7	1: 1.04	1: 1.08	1: 0.75	
Aug. 20	1: 0.75	1: 1.07	1: 0.50	
Sept. 5	1: 3.00	1: 2.40	1: 12.00	
Sept. 30	1: 2.40	1: 2.00	1: 12.00	
		30-8 management		
July 12	1: 14.00	1: 2.33	1: 1.40	1: 0.70
July 23		1: 11.00	1: 1.00	
Aug. 5	1: 2.33	1: 1.40		
Aug. 23	1: 1.83	1: 2.75	1: 3.66	
Sept. 13	1: 3.50	1: 2.33		
		E.B.-4 management		
July 29		1: 0.90	1: 0.30	1: 0.12
Aug. 29		1: 0.15	1: 0.13	

Table 11.--Leaf to whole plant ratios of HCN potential for Suhi-1 subjected to different cutting managements. Averages of 15 plants.

Cutting Date	L_0: WP	L_1: WP	L_3: WP	L_5: WP
		20-3 management		
July 11	1: 0.69	1: 0.56	1: 0.66	1: 0.85
July 23	1: 0.48	1: 0.55	1: 0.53	
Aug. 7	1: 0.53	1: 0.60	1: 0.65	
Aug. 23	1: 0.43	1: 0.61	1: 0.67	
Sept. 10	1: 0.45	1: 0.44	1: 0.58	
		30-8 management		
July 15	1: 0.70	1: 1.05	1: 1.31	1: 2.68
July 24	1: 0.63	1: 0.64	1: 0.61	
Aug. 5	1: 0.74	1: 0.86	1: 0.97	
Aug. 20	1: 0.64	1: 0.70	1: 0.76	
Sept. 5	1: 0.40	1: 0.41	1: 0.95	
Sept. 30	1: 0.37	1: 0.64	1: 1.40	
		E.B.-4 management		
July 31		1: 0.29	1: 0.51	1: 0.60
Sept. 10		1: 0.12	1: 0.25	1: 3.80

The ratios of the HCN potential in the leaves compared to the whole plant were inconsistent when comparisons were made among the managements of both varieties (Table 12). The whole plants of Piper were higher than the leaves in HCN potential in most cases. Suhi-1 leaves were always higher in HCN potential than the whole plant.

The correlation coefficients were significant for many of the comparisons between leaves and whole plants. However, the inconsistency of the ratios indicate that these leaves cannot be sampled to provide a representative estimate of the HCN potential of the whole plant. The leaf to whole plant ratios give an indication as to the manner in which they are related.

The HCN potential of Piper was so low that a difference of 8 to 10 ppm. between a leaf and whole plant could result in the leaf being 2 to 4 or more times higher or lower in HCN potential than the whole plant. The ratios of HCN potential of leaves versus whole plants were more consistent for Suhi-1 than they were for Piper. The HCN potential of Suhi-1 was higher than that of Piper and a larger difference in HCN potential of the leaves and whole plants did not cause a large change in the ratio.

All simple correlations were done using both green and dry weights. Since there were no differences in the r values obtained, all the r values and ratios are presented on a green weight basis.

The HCN potential of the various leaves of the plants was different in the two varieties, Piper and Suhi-1. In Piper, the HCN potential of the whorl was lower than the first leaf in 8 out of 11 cuttings

Table 12.--Leaf to whole plant ratios of HCN potential in Piper and Suhi-1 subjected to different cutting managements. Averages of all cuttings.

Variety	Manage-ment	L_0: WP	L_1: WP	L_3: WP	L_5: WP
Piper	20-3	1: 1.60	1: 1.00	1: 0.57	
Piper	30-8	1: 4.70	1: 2.80	1: 2.10	
Piper	E.B.-4		1: 0.71	1: 4.00	1: 2.00
Suhi-1	20-3	1: 0.42	1: 0.45	1: 0.51	
Suhi-1	30-8	1: 0.55	1: 0.62	1: 0.94	
Suhi-1	E.B.-4		1: 0.19	1: 0.36	1: 0.86

(Table 3, page 23). The HCN potential of the first leaf was lower than the third leaf in 8 out of 13 cuttings and in 3 of the 13 cuttings the third leaf was missing so no comparison could be made. The first cuttings were the only cuttings to have a fifth leaf. The HCN potential of the fifth leaf was higher than that of the third leaf for two of these cuttings and was almost as high for the other. These results do not agree with the work reported by Martin et al. (25).

The results for Suhi-1 were almost opposite to those obtained with Piper. The HCN potential of the whorl was higher than that of the first leaf in 8 out of 11 cuttings (Table 5, page 25). The HCN potential of the first leaf was higher than that of the third leaf in 12 out of 13 cuttings. The first cuttings of the 20-3 and 30-8 managements and both cuttings of the E.B.-4 management were the only cuttings to have a fifth leaf. In each case the HCN potential of the third leaf was higher than that of the fifth leaf. These results agree with the work reported by Martin et al. (25).

The HCN potential of Piper leaves followed the same trend as that in the whole plant throughout the season. In the 20-3 management the third leaf had a higher HCN potential than any of the other leaves or the whole plant except for the fifth cutting (Fig. 1). In the 30-8 management the whole plant had a higher HCN potential than the leaves for every cutting (Fig. 2). The lines joining the observation points on the graphs are for convenience only and do not indicate HCN potential for any period other than the date on which the material was analyzed.

The HCN potential of the leaves followed the trend of the whole

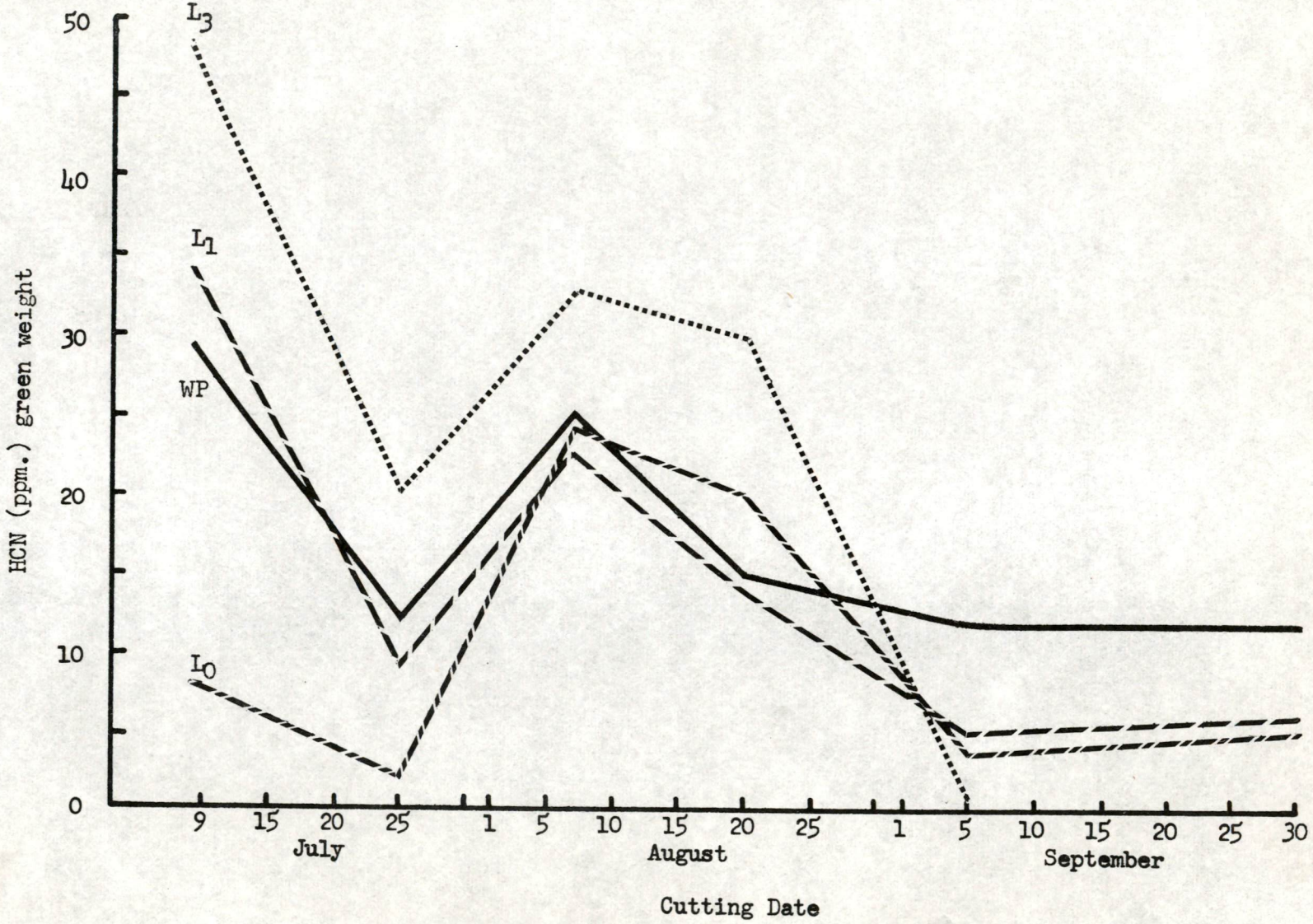


Fig. 1.--HCN potential of leaves and whole plant of Piper subjected to the 20-3 management.

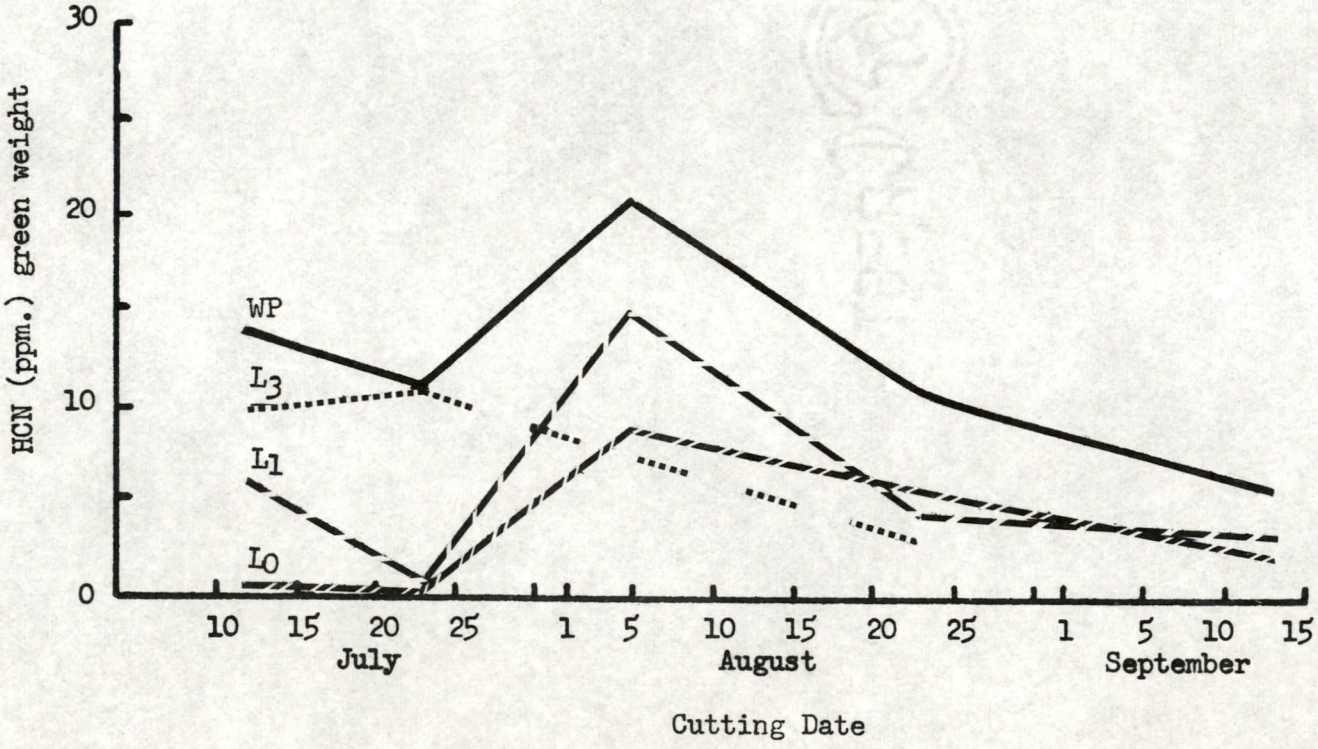


Fig. 2.--HCN potential of leaves and whole plant of Piper subjected to the 30-8 management.

plant over the season in both the 20-3 and 30-8 managements of Suhi-1 (Figs. 3 and 4). In all managements the whole plant was lower in HCN potential than any of the leaves.

The trends for the HCN potential from cutting to cutting were different between the two varieties. The trends for the 20-3 and 30-8 managements of Piper (Fig. 5) agree with the trends reported by Peters (33) of Nebraska and Burger et al. (7) of Illinois. The HCN potential decreased from the first to the second cutting and increased from the second to the third cutting. This also agrees with the work reported by Franzke et al. (17). The trends for Suhi-1 (Fig. 6) were different from those of Piper. The HCN potential increased from the first to second cutting for the 20-3 and 30-8 managements. The HCN potential continued to increase for the 20-3 management but decreased for the 30-8 management. These results do not agree with the work reported by Burger et al. (7) and Franzke et al. (17); however they do agree with the work reported by Hogg and Ahlgren (22).

II. COMPARISONS AMONG THE THREE MANAGEMENTS

There was a difference among the different managements of both varieties. The 20-3 management of Piper was higher in HCN potential than the 30-8 management at any given period (Fig. 5). The 30-8 management was higher than the E.B.-4 management.

The 20-3 management for Suhi-1 was higher in HCN potential than the 30-8 management for all cuttings except the fourth (Fig. 6). The 30-8 management was considerably higher than the E.B.-4 management.

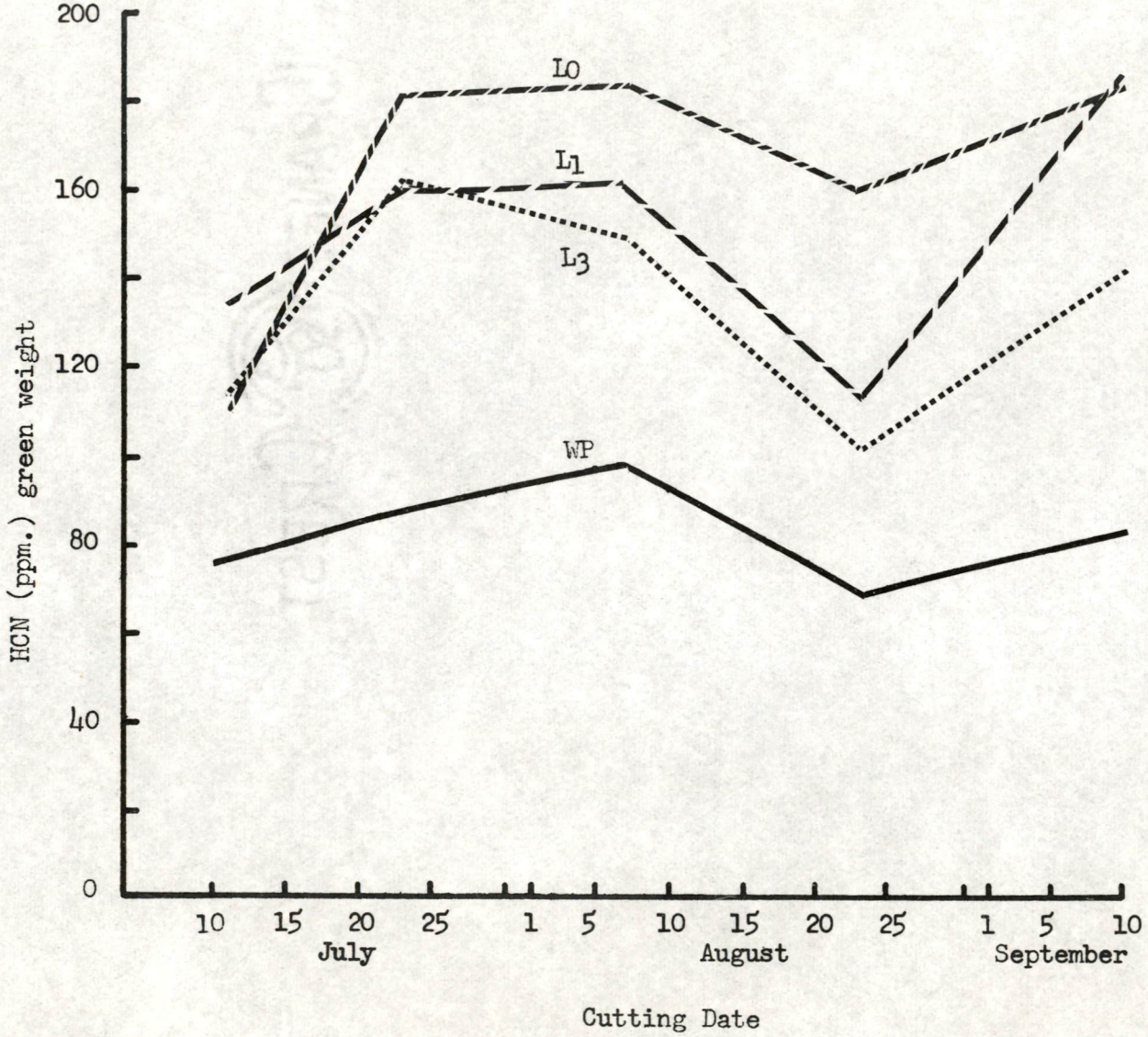


Fig. 3.--HCN potential of leaves and whole plants of Suhi-1 Subjected to the 20-3 management.

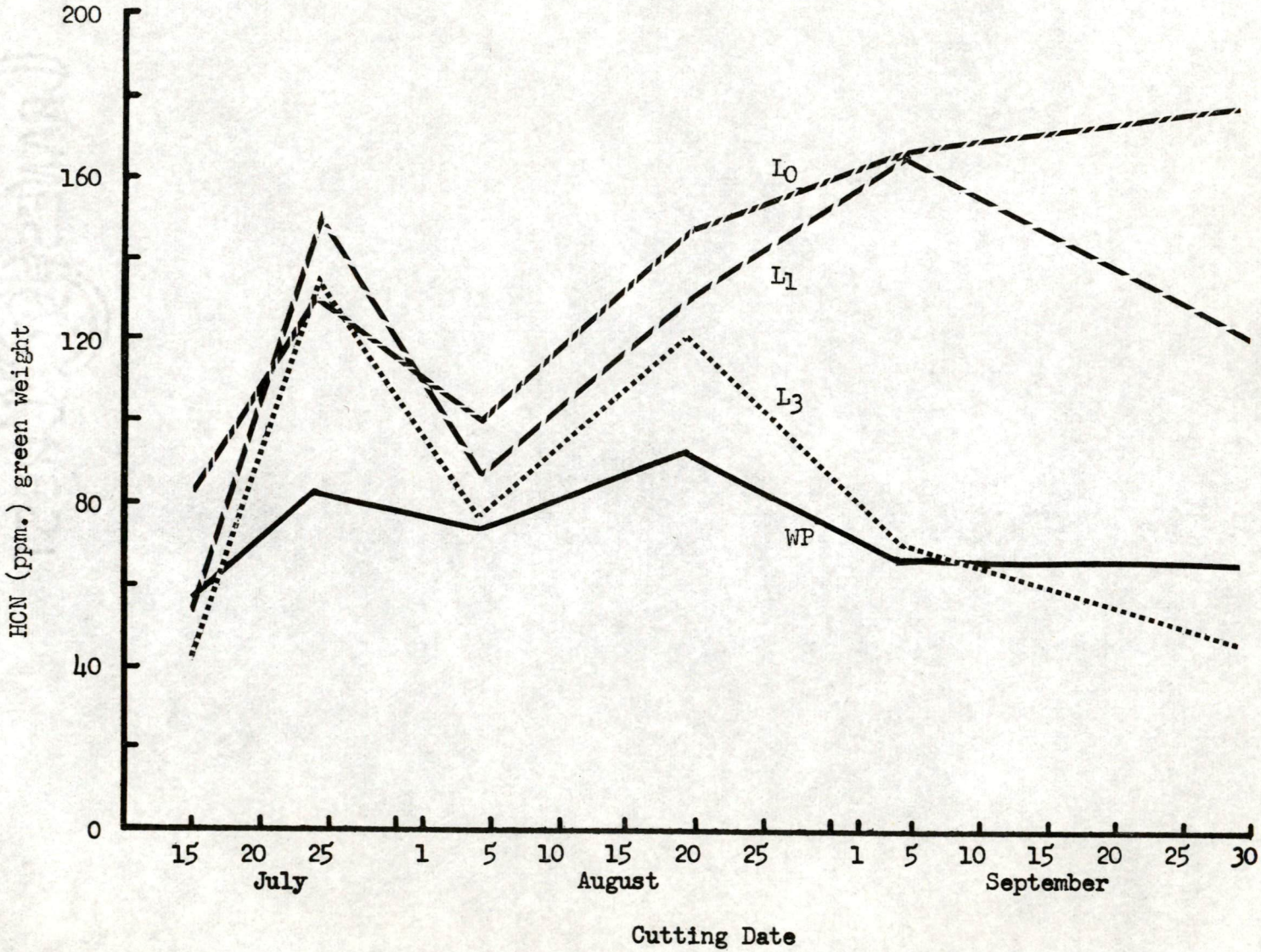


Fig. 4.--HCN potential of leaves and whole plant of Suhi-1 subjected to the 30-8 management.

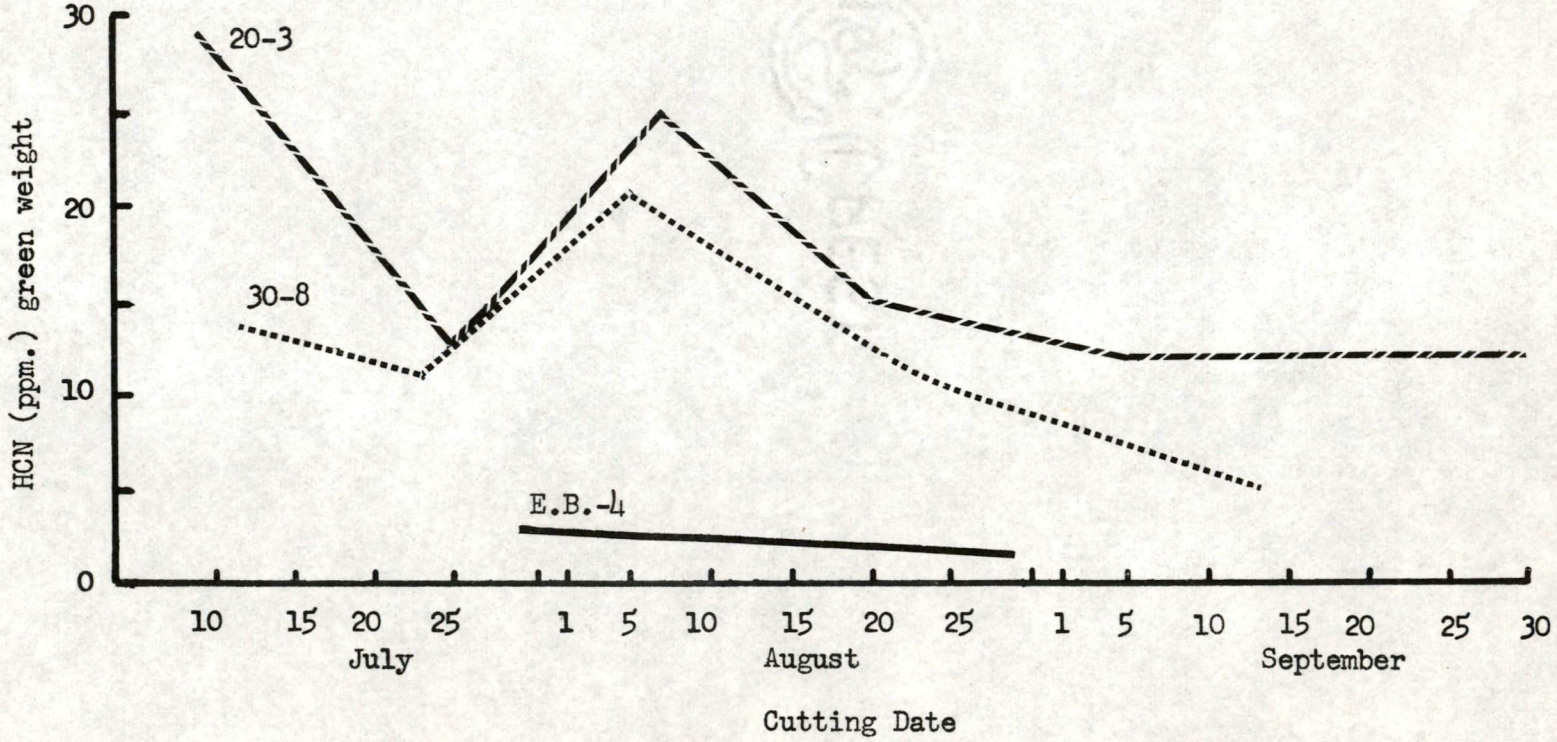


Fig. 5.--HCN potential of whole plants of Piper subjected to 3 different managements.

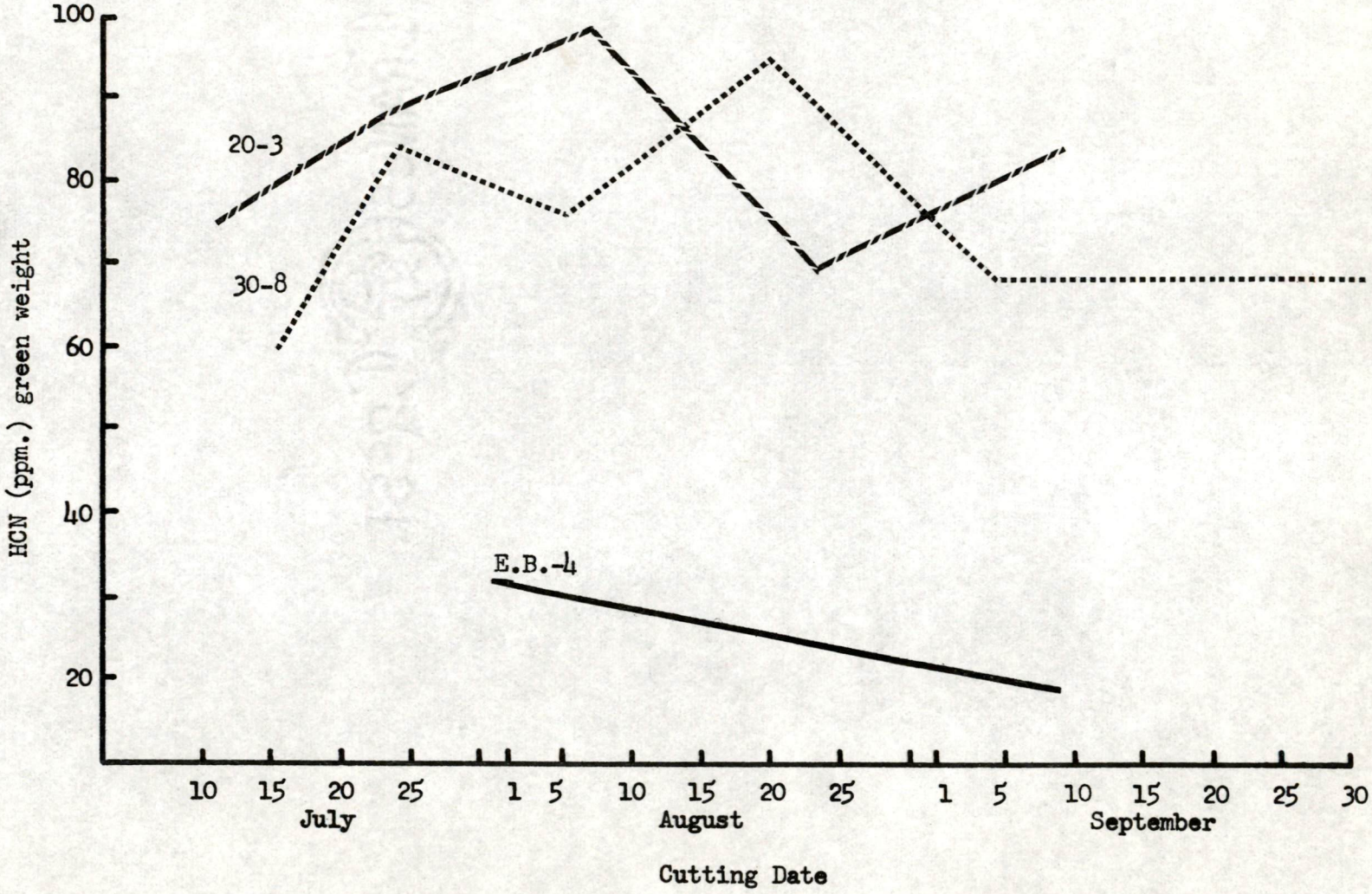


Fig. 6.--HCN potential of whole plants of Suhi-1 subjected to three different managements.

These results agree with the reports of Cassady (8) and Couch (11). Their findings showed the HCN potential to be highest in young material and to decrease as the plants matured. The results do not agree with the work of Acharya (1). He stated that the HCN potential was highest in the plants at the flowering stage.

Mays and Washko (26) in Pennsylvania reported that Sudangrass was grazed most uniformly and with less waste when the plants are from 18 to 24 inches tall. HCN poisoning could be a problem when grazing plants at this stage of growth. Broyles and Fribourg (6) in Tennessee stated that the E.B.-4 cutting management yielded the most dry matter. Plants at this stage of growth would not present a problem of HCN poisoning; however the quality of the forage would be greatly reduced due to the larger percentage of stems (18).

III. COMPARISONS OF VARIETIES

The HCN potential of plants differed among varieties. The Suhi-1 plants that were allowed to reach the early bloom stage, where the HCN potential was lowest, were higher in HCN potential than Piper at 20 inches. The young material of Suhi-1 was much higher in HCN potential than comparable material of Piper (Table 3, page 23, and Table 5, page 25).

There was a larger range in HCN potential of varieties in the green-chop test than there was in the varieties of the silage regrowth test (Table 13). The average value for 15 plants of Tennessee Synthetic 1 was 19 ppm. and the average value for plants of Frontier Hidan was 114

Table 13.--HCN potential (ppm.) on a green weight basis of leaves and whole plants of the varieties in the silage regrowth and green-chop trials. Averages of 15 plants.

Variety	Growth* Characteristics	L_0	L_1	L_3	L_5	WP
	Green-chop (30-8)					
Greenleaf	S	114	169	92		66
Tennessee Synthetic 1	S	4	11	17		19
Piper	S	35	45	35		33
Trudan 1	S	58	74	43		49
Su-1	S	116	150	151		54
H-6160	S x F	242	283	200		89
Sordan	S x F	217	301	202		110
Hydan 37	S x F	233	347	297		114
Sudax	S x F	276	382	240		102
Sweet Sioux	S x F	102	85	66		62
Mor Su	S x F	270	281	150		100
Suhi-1	S x F	187	191	134		111
	Silage regrowth					
R. P. Su-1	S x F	24	15	18		16
Yieldmaker	S x F	34	31	56	40	24
Milkmaker	S x F	32	61	43		27
Dairy D	S x F	33	59	74		23
Sudax	S x F	7	17	2		12
40-F	S x F	87	155	174		38
Aztec	S x G	84	118	178		34
N. K. 330	S x G	44	42	65	85	23
S-214	S x G	30	38	55	37	23
Su-Chow 1	S x G	103	139	176		56
Su-Chow 2	S x G	47	56	88		26
1071 F	G	8	16	61		12

*S - Sudangrass type.
S x F - Sudangrass x forage sorghum type.
S x G - Sudangrass x grain sorghum type.
G - Grain sorghum type.

ppm. on a green weight basis.

In the silage regrowth test the differences were not so great but the values were all much lower than they were in the green-chop test. The range was from 12 ppm. in Advance 1071 F to 56 ppm. in Pfister Su-Chow 1. One possible reason for the difference in HCN potential of the varieties in the green-chop test and the varieties in the silage regrowth test is the difference in cutting practice. The material in the green-chop test had been cut from 5 to 7 times whereas the material in the silage regrowth test was the first regrowth after the initial growth had been cut for silage at the early bloom stage. Sudax was the only variety sampled from both tests. In the green-chop test the HCN potential was 102 ppm.; in the silage regrowth test the HCN potential for Sudax was 12 ppm.

The r values for the four comparisons of all plants from the silage regrowth and green-chop tests and the plants from the 30-8 management in the HCN determination test were .80, .77, .63, and .07 for the L_0 x WP, L_1 x WP, L_3 x WP, and L_5 x WP, respectively. The r values for the first three comparisons were highly significant.

The ratios of the HCN potential of the leaves to the whole plant were consistent for those varieties high in HCN potential (Table 14), but were inconsistent for the varieties low in HCN potential. The leaves had a higher HCN potential than the whole plant for some varieties while for other varieties the whole plant had a higher HCN potential than the leaves.

The highly significant correlation coefficients for the whorl,

Table 14.--Leaf to whole plant ratios of HCN potential for the varieties in the silage regrowth and green-chop trials.

Variety	L_0: WP	L_1: WP	L_3: WP	L_5: WP
Greenleaf	1: 0.57	1: 0.39	1: 0.71	
Tennessee Synthetic 1	1: 4.75	1: 1.72	1: 1.11	
Piper	1: 0.94	1: 0.75	1: 0.94	
Trudan 1	1: 0.84	1: 0.66	1: 1.13	
Su-1	1: 0.46	1: 0.36	1: 0.35	
H-6160	1: 0.36	1: 0.31	1: 0.44	
Sordan	1: 0.50	1: 0.36	1: 0.54	
Hydan 37	1: 0.48	1: 0.33	1: 0.38	
Sudax	1: 0.37	1: 0.26	1: 0.42	
Sweet Sioux	1: 0.60	1: 0.73	1: 0.94	
Mor Su	1: 0.37	1: 0.35	1: 0.66	
Suhi-1	1: 0.59	1: 0.58	1: 0.83	
R. P. Su-1	1: 0.66	1: 1.06	1: 0.88	
Yieldmaker	1: 0.70	1: 0.77	1: 0.42	1: 0.60
Milkmaker	1: 0.84	1: 0.44	1: 0.63	
Dairy D	1: 0.69	1: 0.39	1: 0.31	
Sudax	1: 1.85	1: 0.76	1: 6.50	
40-F	1: 0.43	1: 0.24	1: 0.22	
Aztec	1: 0.40	1: 0.29	1: 0.19	
N. K. 330	1: 0.52	1: 0.55	1: 0.35	1: 0.27
S-214	1: 0.76	1: 0.60	1: 0.41	1: 0.62
Su-Chow 1	1: 0.54	1: 0.40	1: 0.32	
Su-Chow 2	1: 0.53	1: 0.46	1: 0.29	
1071 F	1: 1.50	1: 0.75	1: 0.19	

the first and third leaves indicate that either of these leaves would be an adequate leaf to sample to rank the varieties for HCN potential. Although they could be used to rank the varieties they could not be used to estimate the HCN potential of the whole plant.

The HCN potential of the plants in the green-chop and silage regrowth tests seem to be related to their growth characteristics. The varieties having the highest HCN potential have growth characteristics approaching that of grain sorghum, whereas plants with the lowest HCN potential approach the true Sudangrasses in growth characteristics. The plants with growth characteristics of the forage sorghums were intermediate between Sudangrass and grain sorghum types in HCN potential.

CHAPTER V

SUMMARY AND CONCLUSIONS

Two varieties of Sudangrass, Piper and Suhi-1, were subjected to three different cutting managements in order to determine the effect of variety and cutting management on HCN potential. Samples were taken from the whorl (L_0), the first (L_1), third (L_3), and fifth (L_5) leaves from the top of these two varieties to determine which leaf would give a representative estimate of the HCN potential of the whole plant (WP). In another experiment 21 varieties were sampled to provide a wider comparison of HCN potential of different varieties.

The HCN potential of Piper ranged from 2 ppm. up to 28 ppm. for the whole plant. The HCN potential of Suhi-1 was higher than that of Piper and ranged from 19 ppm. up to 98 ppm. for the whole plant. Some of the leaves of Suhi-1 were much higher than the whole plant and went as high as 185 ppm.

The correlation coefficients of the comparisons L_0 x WP, L_1 x WP, and L_3 x WP were significant for most of the cuttings and could be used to rank varieties for HCN potential. However, the inconsistency of ratios between the HCN potential of the leaves versus the whole plant indicated that none of these leaves would be suitable representative samples for estimating the HCN potential of the whole plant.

There were differences resulting from the three managements. There was a difference of approximately 20 ppm. among the managements of

Piper and a difference of approximately 60 ppm. among the managements of Suhi-1. The plants that were allowed to grow to 20 inches in height and were cut back to 3 inches were higher in HCN potential than the plants that were allowed to grow to 30 inches in height and were cut back to 8 inches. One exception was the fourth cutting of Suhi-1 where the latter management was higher in HCN potential. The plants that were allowed to grow to the early bloom stage and were cut back to 4 inches were lower in HCN potential than plants subjected to the other managements for both varieties.

There were differences in HCN potential among the varieties sampled and their HCN potential was associated with their growth characteristics. Plants with grain sorghum type of growth characteristics were associated with high HCN potential. Varieties with a Sudangrass type of growth characteristics were lowest in HCN potential. Plants with forage sorghum type of characteristics were intermediate between the plants with grain sorghum types and those plants with Sudangrass types of growth characteristics. There was a difference in HCN potential of the varieties in the green-chop test and the silage regrowth test.

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