3-1966

Effects of freezing on HCN potential of sorghum plants

David Wattenbarger

To the Graduate Council:

I am submitting herewith a thesis written by David Wattenbarger entitled "Effects of freezing on HCN potential of sorghum plants." 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:

John Reynolds, Edward E. C. Clebsch

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

December 29, 1965

To the Graduate Council:

I am submitting herewith a thesis written by David Wattenbarger entitled "Effects of Freezing on HCN Potential of Sorghum Plants." 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:

John H. Reynolds

Edward E. C. Clebsch

Accepted for the Council:

Hilton A. Smith
Dean of the Graduate School

EFFECTS OF FREEZING ON HCN POTENTIAL OF SORGHUM PLANTS

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

David Wattenbarger

March 1966

ACKNOWLEDGEMENTS

To the following persons, I wish to express my humble gratitude and appreciation:

Dr. Elmer Gray, my major professor, for his unselfish and sincere assistance, fellowship, inspiration and guidance throughout the course of my graduate study.

Dr. Edward E. C. Clebsch, my minor professor, for his cooperation and understanding in serving on my committee.

Dr. John Reynolds, for his helpful suggestions and critical reading of the manuscript.

Dr. Henry A. Fribourg for his help in the use of the IBM computer.

Mr. Alonzo J. Hester, Assistant in Agronomy, for his research assistance.

My wife, Jean, for her patience and encouragement during the course of the study.

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CHAPTER I

INTRODUCTION

Hydrocyanic acid is one of the most toxic and rapidly acting poisons found in nature (14). Dhurrin, a precursor of hydrocyanic acid is found in all species and varieties of the genus Sorghum (10).

Sorghums are tolerant to drought and have become an important summer crop in the United States (17). In 1963, there were 3,797,000 acres grown for forage and silage (29). In Tennessee in 1962, sorghums grown for forage and silage amounted to 23,000 acres (30). With the advent of the more productive sorghum x Sudangrass hybrids, this acreage is expected to increase (13).

Under certain conditions, the hydrocyanic acid content of sorghum plants may become high enough to be lethal to livestock (19). It is generally accepted that actively growing sorghum plants are unsafe for animal consumption after being frozen.

The objective of this study was to determine the effect of freezing on hydrocyanic acid potential of Sudangrass and sorghum x Sudangrass hybrids.

CHAPTER II

REVIEW OF LITERATURE

I. EARLY EXPLANATIONS OF SORGHUM POISONING

The fact that plants of the genus Sorghum are toxic to animals has been known since the 1800's. In India in 1877, great numbers of cattle died after eating sorghum. The season was especially dry and the crop was in a droughty condition. Sorghum poisoning was again heavy in 1887 and 1895 which were also drought years. The natives believed that a small insect, "bhaunri," attacked the sorghum plants in dry years and caused them to be poisonous. Another theory was that a gas which caused asphyxiation was given off by sorghum leaves while in the animal's paunch (28).

Other theories were: (1) poisonous fungi on the plants were eaten and caused death; (2) consumption of excessive amounts of sorghum plants caused suffocation; and (3) the accumulation of nitrates during long dry periods resulted in nitrate poisoning (10). Vinall (28) reported work in which potassium nitrate was found in withered sorghum plants and suggested that it caused sorghum poisoning.

II. DISCOVERY OF DHURRIN

The presence of hydrocyanic acid (HCN, prussic acid or hydrogen cyanide) in sorghum plants was discovered by Dunstan and Henry (10)

and Slade (26) in 1902. Slade (26), a chemist at the Nebraska Agricultural Experiment Station, tried to determine why sorghum plants were poisonous and isolated prussic acid from a fresh sorghum sample in August, 1902. Earlier that same year, Dunstan and Henry (10) in England analyzed sorghum plants from a pasture in Egypt on which several cattle had died. When the material was crushed and wet with water, a strong odor of prussic acid was detected. Chemical analysis revealed that the plant material contained prussic acid. A glucoside was found in the plant which was hydrolyzed by an enzyme to yield HCN. Dunstan and Henry (10) named the glucoside dhurrin from the vernacular name or sorghum in Egypt, "Dhurra Shirshabi."

III. TOXICITY OF HCN

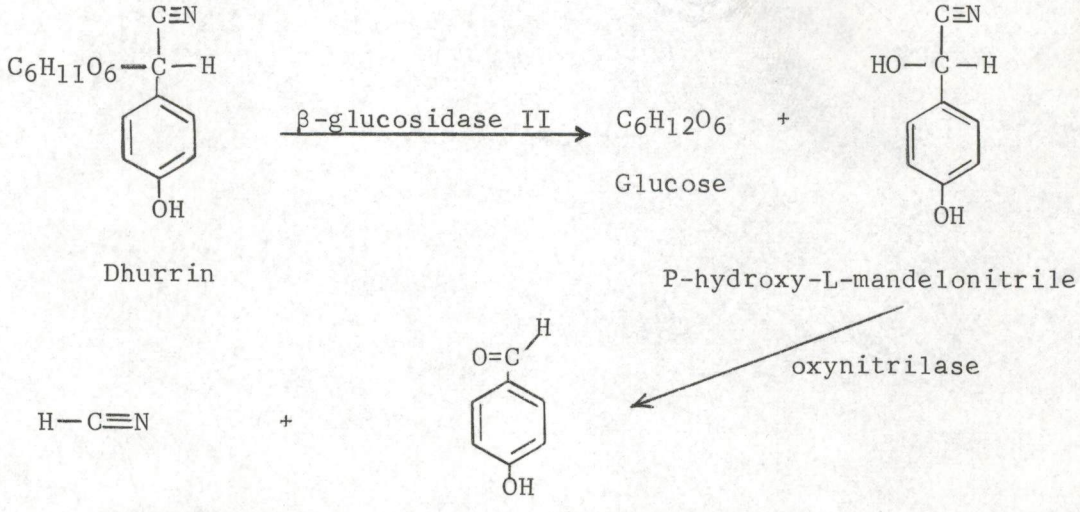
When HCN is liberated in the stomach, it is abosrbed by the blood stream and carried to the tissues where it inhibits the action of the oxygen-activating enzyme, cytochrome oxidase. As a result, the tissues cannot utilize the oxygen in their normal metabolic process. Due to this lack of utilization there is an accumulation of oxyhemoglobin on the venous side of the circulation. The accumulation of oxyhemoglobin causes the venous blood to be the same color as the arterial blood and is a symptom which is used in diagnosing HCN poisoning (15).

The amount of material required to kill an animal varies. Van der Walt (31) stated that poisoning in ruminants varied with quantity of the plant ingested, previous diet of the animal, pH of the stomach

contents, percentage of total HCN present in the free state in the plant, concentration of cyanide liberating enzyme and total HCN content of the plant. According to Couch (9) the minimum lethal dose for cattle is about 2.042 mg. per kg. of body weight.

IV. CHEMICAL BREAKDOWN OF DHURRIN TO HCN

The glucoside dhurrin (empirical formula $C_{14}C_{17}O_7N$) is the precursor of HCN. Dhurrin was the first glucoside to be found in nature. Dunstan and Henry (10) proposed a hydrolysis reaction in which an enzyme must be present for the reaction to occur. The enzyme performed the same function as emulsin found in sweet almonds; they concluded the enzymes were the same. Working with etiolated sorghum seedlings, Conn and Colette (7) found two enzymes involved in the breakdown of dhurrin. At a later date, Mao (18) at Wisconsin found that two enzymes were involved in the breakdown of dhurrin which supports the findings of Conn and Colette. He proposed the following enzymic decomposition:



Dhurrin

P-hydroxy-L-mandelonitrile

Hydrogen cyanide

P-hydroxybenzaldehyde

Coop and Blakley (8), as cited by Garner (14), reported that cyanide is rapidly released under the action of ruminal organisms, provided suitable conditions are obtained in the rumen.

V. EFFECTS OF FROSTING ON HCN POTENTIAL

The literature contains reports of both increased and decreased HCN content after frost. Boyd et al. (3) did not find any increase in the HCN content of frosted material. Franzke et al. (12) reported a higher HCN content of sorghum the evening before a frost than in samples taken the morning after. Swanson (27) found that freezing of Sudangrass did not cause a decrease in the cyanide content present if the test was made before thawing and wilting. But when the test was made after thawing and wilting, the cyanide content dropped rapidly. Burns and Wedin (4) also found a decrease in HCN of sorghum after frost. According to the Merck Veterinary Manual (25), freezing does not ordinarily increase the glucoside content of HCN producing plants but it may tend to increase the quantity of free HCN in the plants. Other investigators have reported an increase in HCN content after frost. Manges (18) states that immediately after the first frost of the autumn a heavier concentration of prussic acid may develop than was normally present in the plants. Willaman (34) reported an increase in HCN yield from frosted material. Sorghum plants sampled before and after frost contained 5.50 and 7.16 mg., respectively, of HCN per 100 g. of dry plant material. He attributed this increase to both rupturing of cells

and disturbed enzyme equilibrium. Peters et al. (23) found a decrease in HCN after frosting. Three-inch tall sorghum plants sampled before and after frost contained 0.0133 and 0.0082 g. of HCN, respectively, per 100 g. of plant material. Willaman and West (32) suggested that the increase in hydrocyanic acid which sometimes occurs after a frost may be due to disturbed enzyme balance.

Boyd et al. (3) suggested that favorable conditions for growth after a frost may be conducive to new growth. This new growth is likely to be high in HCN content and, if pastured, likely to cause HCN poisoning. In a case such as this, a natural inference is that the frosted material caused the poisoning. Pickett (24) concluded that frosted and partially killed Sudangrass may be dangerous because cattle graze the younger more tender shoots which are higher in HCN.

VI. EFFECTS OF CHLOROFORM, ALCOHOL AND ETHER ON HCN CONTENT

Willaman (34) stated that chloroform, alcohol and ether apparently cause an increase yield of prussic acid. He proposed three possible explanations for the increased yield of HCN: (1) disturbed osmotic relations in the tissues bring about increased hydrolysis of dhurrin, (2) the presence of the chloroform, alcohol or ether stimulates the hydrolytic activities of the enzymes as well as their synthetic activities which result in an actual increase in production of dhurrin and hydrocyanic acid or (3) a combination of both (1) and (2).

Anderson (1) and Hogg (16) used chloroform in a picric-acid test for determining the HCN content of plant material.

VII. OTHER FACTORS AFFECTING HCN CONTENT OF SORGHUM PLANTS

Benson (2) studied varieties of the grain sorghum, forage sorghum and Sundangrass types and found them to be high, intermediate and low in HCN potential, respectively. Moodie and Ramsey (21) found that sorghum x Sudangrass hybrids contained three times as much HCN as the Sudangrass parent. Finnemore and Cox (11) reported that sorghum x Sudangrass hybrids contained more HCN than Sundangrass and less than sorghum.

Cassady (5) found that the HCN content of Sudangrass plants was highest in young plants and decreased as the plants became older. A much higher HCN content was found in young than in older sorghum plants by Couch (9). Martin et al. (20) reported that tillers were higher than main culms in HCN potential. Benson (2) stated that 20-inch tall sorghum plants had a higher concentration of HCN than 30-inch tall plants which, in turn, had a higher concentration than plants taller than 30 inches.

HCN content of sorghum plants is also affected by geographic location, soil fertility and soil moisture (2).

CHAPTER III

MATERIALS AND METHODS

I. LOCATION

The Sudangrass (Sorghum sudanense (Piper) Stapf.) and sorghum (Sorghum vulgare Pers.) x Sudangrass varieties used in this study were grown at Knoxville on a Linside sandy loam soil and at the Plant Science Farm on Sequatchie silt loam soil in 1964. In 1965 the varieties were grown only at Knoxville on an Etowah silt loam soil.

Daily minimum temperatures were recorded at a point which was about 0.25 and 0.10 mile from the 1964 and 1965 Knoxville plantings, respectively, and about 4.5 miles from the 1964 planting at the Plant Science Farm.

II. VARIETIES

The varieties used in this study were "GHS-1," "Greenleaf," "Hydan-37," "Piper," "Suhi-1" and "Tennessee Synthetic 1." Descriptions of the varieties are given in Table 1. These varieties were chosen because they represent a range of low to high HCN potential (2). All six of the varieties were studied in 1964, but only Greenleaf, Piper and Suhi-1 were studied in 1965.

TABLE 1. Descriptions of Sudangrass and sorghum x Sudangrass varieties.

Variety	Origin	Apparent characteristic
GHS-1	Tennessee Co-op	Sorghum x Sudangrass type
Greenleaf	Kansas State Univ.	Sudangrass type
Hydan-37	Frontier Seed Co.	Sorghum x Sudangrass type
Piper	Univ. of Wisconsin	Sudangrass type
Suhi-1	Univ. of Georgia	Sorghum x Sudangrass type
Tennessee Synthetic-1	Univ. of Tennessee	Sudangrass type

III. MANAGEMENT

Drilled plantings in rows spaced 36 inches apart were made during the first week of May in 1964 and 1965. Prior to planting, 10-10-10 fertilizer was incorporated into the soil at the rate of 600 lbs. per acre. An additional 60 lbs. of N per acre was applied by topdressing with 30 lbs. of N per acre after the second and third harvests. Plants were cut to a height of 8 inches each time they reached a height of 30 to 36 inches until time of expected frost. Time of the first cutting was varied for portions of the 1964 and 1965 Knoxville plantings to give different stages of growth of material throughout the season and at the time of frost. No harvests were made after the first autumn frost,

The first autumn frost at Knoxville in 1964 occurred on October 6. The six varieties which were grown at Knoxville were in two stages of growth. The younger plants were in the vegetative stage and about 20 inches high. The older plants were in the boot stage and about 30 inches high. The varieties which were grown at the Plant Science Farm were vegetative and about 15 inches high at the time of frost.

On October 1, 1965 the two stages of the three varieties at Knoxville were about 25 and 30 inches tall. Since the first frost in 1964 occurred October 5, no additional cuttings were made. However, the first frost of 1965 did not occur until October 25. At the time of frost the younger plants were in the early head stage and about 35 inches tall. The older plants were in the early seed stage and about 45 inches tall.

IV. CONTROLLED TEMPERATURE STUDIES

Seeds of Greenleaf, Piper and Suhi-1 were planted in 5-gallon buckets of soil. Dividers were used so that the three varieties could be grown in the same bucket. During the summer of 1965, these plants were subjected to controlled temperatures between -20 and 40°F. Leaves were split along the mid-rib and one-half was analyzed for HCN potential before freezing and the other half after freezing.

V. CHLOROFORM AND FREEZING STUDIES

Leaves of Greenleaf, Piper and Suhi-1 were used in an attempt to determine if the freezing of fresh material would cause a release of HCN from the plant. The freezing was accomplished by placing intact plants in a freezer at 0°F or by allowing plants to remain in the field during a 24°F frost. Frozen leaves were split along the mid-rib; one-half was subjected to the normal "picric-acid test" and the other half subjected to the same test but without the addition of chloroform.

VI. ESTIMATION OF HCN POTENTIAL

Plants were analyzed for HCN potential at weekly intervals from about October 1 until the first frost. HCN analyses were made daily for three or four days following a frost and then at weekly intervals until the next frost. On three occasions the frosted plants were analyzed twice during the day frost occurred. From 12 to 15 plants were taken at random from a row of each variety. Five of these plants

were used in HCN determinations and the remaining plants were used in determining dry matter percentages.

A modified version of the method used by Anderson et al. (1) at Wisconsin was used to estimate HCN potential. Each plant was chopped into one-quarter inch sections with a paper cutter. The chopped material from each plant was mixed thoroughly and two samples from each plant were weighed on a closed Torsion balance to the nearest hundredth gram. Sample weights ranged from 0.5 to 2.5 g. Each sample was placed in a 150 x 15 mm. test tube and chloroform was added with a medicine dropper until the cut plant tissue was thoroughly moistened (10-30 drops). A strip of filter paper saturated with sodium picrate solution was then suspended above the sample by a rubber stopper. The tubes were allowed to stand for approximately 24 hours at room temperature before the filter paper strips were removed and immersed in 25 ml. of distilled water in a colorimeter tube. The filter paper strips were allowed to soak in the water for approximately 30 minutes and were stirred gently two or three times to aid extraction of the colored material. After the filter paper was removed the tubes were placed in a Fisher Electrophotometer with a 525 mμ filter and the light absorbency was read. The readings were then substituted into the equation prepared from the standard curve to determine the HCN content of each sample.

The standard curve was developed using a mixture prepared by combining 50 ml. of a cyanide solution containing 0.241 g. KCN per

liter of water with 50 ml. of an alkaline picrate solution containing 25 g. Na_2Co_3 and 5 g. of picric acid per liter of water. Aliquots of this mixture were then dispensed into tubes in increasing amounts to give increasing concentrations of HCN. The tubes were then read for light absorbency on the Fisher Electrophotometer with a 525 mμ filter. A linear regression was calculated for colorimeter readings and concentrations to give "a" and "b" values to be used in the regression equation, $Y = a + bX$. Two alkaline picrate solutions were used in the study. The "a" values were 3.06 and 4.85, and the "b" values were 13.20 and 14.26 for the first and second solutions, respectively.

A program was developed for the IBM 7040 computer to convert plant sample data into p.p.m. of HCN of green or dry plant tissue.

CHAPTER IV

RESULTS

I. FIELD STUDIES

HNC potential of plants from different varieties and at different stages for both 1964 and 1965 are included in Tables 2-5. The data were not subjected to statistical analysis because it is questionable whether the assumptions of the Analysis of Variance were met by these data.

Suhi-1 was highest in HCN potential at all stages in both 1964 and 1965. Piper was lowest in HCN potential at all stages for both years. Greenleaf was intermediate and ranked above Tennessee Synthetic-1 and ranked below GHS-1 and Hydan-37 in 1964. GHS-1 was higher than Hydan-37 at both locations in 1964.

The effects of stages of growth on HCN potentials are in agreement with those reported by Benson (2). The shorter, younger plants had higher HCN potential than the taller, older plants. In 1964 all varieties in the 15-inch stage at the Plant Science Farm averaged 21 p.p.m. (green weight) and 82 p.p.m. (dry weight) (Table 2). At Knoxville in 1964 the 20-inch stage of all varieties averaged 16 p.p.m. (green weight) and 65 p.p.m. (dry weight) (Table 3); the 30-inch stage averaged 6 p.p.m. (green weight) and 27 p.p.m. (dry weight) (Table 4). For the three varieties which were grown at Knoxville in 1965, the 35-

TABLE 2. HCN potential (p.p.m.) of 15-inch plants of 6 varieties which were grown at Plant Science Farm in 1964

Date		Variety											
		GHS-1		Greenleaf		Hydan-37		Piper		Suhi-1		Tenn. Syn.-1	
		Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight
October	5	46[a]	290	13	66	28	152	T[b]	T	52	328	1	11
	6	61	492	15	67	32	214	1	6	46	282	2	10
	7	27	99	16	43	47	152	4	10	75	121	6	28
	8	71	177	8	25	55	165	1	1	58	252	4	14
	9	47	111	4	11	53	106	4	30	86	243	18	45
	12	61	184	4	14	49	231	T	T	55	280	1	2
	15	35	150	1	2	36	163	0	0	44	195	3	12
	19	26	121	7	29	17	77	1	2	33	145	2	7
	20	26	65	5	12	18	58	T	T	17	53	1	2
	21	23	80	7	17	38	129	T	1	38	109	2	4
	22	30	122	10	36	17	70	1	4	30	132	1	6
	23	49	196	31	105	46	217	18	56	57	250	7	38
	26	23	123	4	31	30	167	1	3	52	210	2	8
	27	22	99	5	17	35	184	1	3	46	204	3	14
	29	29	100	T	2	20	82	1	2	35	141	3	10
November	2	54	200	15	56	36	166	21	43	89	415	7	37
	5	24	35	7	11	28	45	8	15	75	126	2	3
	9	37	140	3	9	32	110	2	4	33	113	9	21
	12	32	63	8	11	26	40	0	0	35	140	4	5
	16	36	176	0	0	9	43	0	0	20	94	T	1
	19	30	182	9	26	43	265	3	10	50	505	2	16
	21	55	219	6	11	25	87	16	21	63	188	6	19
	22	27	111	11	39	33	153	6	23	48	172	5	14
	23	31	105	4	9	38	130	1	2	48	116	1	4
	24	31	92	T	1	27	94	0	0	45	127	2	7
	27	T	T	0	0	5	20	0	0	T	1	0	0
	30	0	0	0	0	0	0	0	0	0	0	0	0
	Average	35	138	7	24	30	123	3	9	45	183	4	13

All varieties averaged 21 p.p.m. green weight and 82 p.p.m. dry weight.

[a]Each value is an average of 2 samples from each of 5 plants.

[b]T represents a HCN potential of less than 1 p.p.m.

TABLE 3. HCN potential (p.p.m.) of 20-inch plants of 6 varieties which were grown at Knoxville in 1964

Date		Variety											
		GHS-1		Greenleaf		Hydan-37		Piper		Suhi-1		Tenn. Syn.-1	
		Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight
October	1	40[a]	374	26	150	23	190	6	41	43	360	14	8
	6	24	207	11	99	23	191	7	48	26	187	4	23
	7	24	181	5	18	19	100	1	4	26	156	2	8
	8	35	172	14	52	34	164	4	11	34	159	3	6
	9	27	109	6	18	28	72	T[b]	2	23	93	7	25
	12	51	330	5	25	61	493	1	2	29	232	2	6
	15	52	391	18	82	31	162	0	0	37	148	4	16
	19	13	86	3	16	10	63	4	2	22	124	1	9
	20	26	98	3	10	14	49	1	2	21	79	4	13
	21	29	154	3	10	4	17	T	T	13	46	0	0
	23	25	180	6	29	18	102	T	1	16	83	2	11
	24	42	322	15	95	18	137	10	55	26	170	8	43
	26	30	70	7	51	20	104	1	5	12	73	2	5
	27	18	124	3	12	6	40	0	0	6	37	T	1
	29	17	75	5	20	11	47	2	10	31	133	T	4
November	2	15	63	5	14	8	34	1	5	16	97	T	T
	5	18	51	5	9	9	26	10	12	13	30	3	5
	9	18	63	2	6	8	25	2	4	12	38	T	T
	12	14	47	2	2	7	31	1	4	18	36	2	6
	16	17	63	T	T	17	97	22	110	23	110	1	8
	19	26	152	4	27	45	362	3	21	24	159	1	7
	21	31	112	3	7	23	66	2	8	23	70	2	4
	22	21	85	2	6	11	55	4	14	25	117	1	2
	23	17	51	T	T	15	59	0	0	13	52	T	T
	24	18	61	2	6	4	20	T	1	26	90	T	T
	27	2	8	0	0	1	5	0	0	4	19	0	0
	30	0	0	0	0	0	0	0	0	0	0	0	0
	Average	38	135	6	28	17	100	3	13	28	107	2	8

All varieties averaged 16 p.p.m. green weight and 65 p.p.m. dry weight.

[a]Each value is an average of 2 samples from each of 5 plants.

[b]T represents a HCN potential of less than 1 p.p.m.

TABLE 4. HCN potential (p.p.m.) of 30-inch plants of 6 varieties which were grown at Knoxville in 1964

Date		Variety											
		GHS-1		Greenleaf		Hydan-37		Piper		Suhi-1		Tenn. Syn.-1	
		Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight
October	1	13[a]	91	4	22	7	46	4	22	33	200	2	14
	6	4	32	6	28	9	59	1	8	11	65	T[b]	3
	7	16	84	2	11	15	78	2	8	15	78	T	T
	8	21	124	6	27	12	77	4	13	14	80	6	3
	9	9	49	2	9	12	51	2	5	13	57	2	9
	12	12	85	2	9	19	103	1	4	11	66	2	14
	15	20	116	2	7	2	14	2	8	24	118	2	8
	19	7	40	T	T	4	25	1	3	10	48	T	2
	20	7	33	T	T	6	29	0	0	12	51	T	1
	21	5	23	T	1	9	43	0	0	9	41	T	T
	23	8	46	3	14	3	20	0	0	23	113	T	T
	24	27	161	5	29	13	74	6	23	35	189	5	29
	26	13	74	5	27	4	23	1	2	12	51	T	1
	27	13	77	1	4	5	32	0	0	5	24	T	T
	29	8	47	1	2	9	44	T	1	10	47	T	2
November	2	4	16	1	5	3	14	T	3	9	56	1	3
	5	7	29	2	5	4	21	T	T	14	44	T	T
	9	7	30	2	11	1	6	0	0	6	24	T	1
	12	8	32	1	4	2	5	T	1	14	62	1	2
	16	6	25	T	T	1	4	6	19	11	59	T	1
	19	4	22	T	1	4	17	T	1	15	71	T	2
	21	7	25	4	11	23	86	T	T	11	40	T	T
	22	5	20	2	8	9	37	0	0	12	48	1	5
	23	5	18	0	0	10	37	0	0	10	36	0	0
	24	T	T	0	0	5	19	0	0	7	23	0	0
	27	T	T	0	0	1	3	0	0	T	T	0	0
	30	0	0	0	0	0	0	0	0	0	0	0	0
	Average	9	48	2	8	7	36	1	4	13	63	1	4

All varieties averaged 6 p.p.m. green weight and 27 p.p.m. dry weight.

[a] Each value is an average of 2 samples from each of 5 plants.

[b] T represents a HCN potential of less than 1 p.p.m.

inch stage averaged 9 p.p.m. (green weight) and 48 p.p.m. (dry weight); the 45-inch stage averaged 7 p.p.m. (green weight) and 28 p.p.m. (dry weight) (Table 5).

Daily minimum temperatures, which were taken only at one location, are presented in Table 6. Apparently temperatures were lower in the field locations than at the recording location because frost occurred sometimes at temperature readings of 37 to 38°F.

Daily minimum temperatures and average HCN potential, obtained by averaging all varieties and all stages sampled on a given day, were plotted for the different locations (Figures 1-6). In the graphs (Figures 1 and 2) for 1964 Plant Science Farm, each point represents an average of 60 HCN determinations (six varieties, five plants, and two samples). In Figures 3 and 4 each point is an average of 120 HCN determinations (six varieties, two stages, five plants and two samples). In Figures 5 and 6 each point is an average of 60 HCN determinations (three varieties, two stages, five plants and two samples).

During the studies of 1964 and 1965 there were 5 and 4 frost periods, respectively. A frost period was defined as a time in which frost occurred one or more successive days. Frost periods were separated by one or more days on which no frost occurred.

1964 Study

The first frost period occurred from October 6 through 12 with recorded temperatures ranging from 32 to 38°F. For the 15-inch plants at the Plant Science Farm, HCN potential (green weight) increased

TABLE 5. HCN potential (p.p.m.) of 25-inch and 30-inch plants of 3 varieties which were grown at Knoxville in 1965

Date		Variety											
		25-inch plants						30-inch plants					
		Greenleaf		Piper		Suhi-1		Greenleaf		Piper		Suhi-1	
		Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight	Green weight	Dry weight
October	6	12[a]	86	1	6	34	315	12	72	2	12	33	264
	12	3	27	5	25	17	85	6	22	0	0	51	221
	21	5	30	0	0	14	91	3	20	0	0	30	60
	25	4	21	1	4	22	116	1	5	T[b]	T	30	110
	25[c]	1	6	1	8	33	202	3	10	2	6	30	102
	26	3	15	1	5	29	145	T	T	T	1	18	67
	26[c]	7	36	1	5	30	186	1	7	T	T	2	7
	27	3	15	1	5	21	120	T	T	0	0	17	42
	28	4	22	0	0	38	196	T	2	1	2	13	47
	29	2	10	T	T	23	106	T	T	T	T	25	77
November	3	1	4	1	4	33	137	1	6	0	0	12	46
	8	2	12	1	4	25	95	T	3	0	0	20	55
	16	T	4	T	T	35	160	T	T	0	0	13	36
	18	T	1	0	0	16	64	0	0	0	0	7	17
	18[c]	1	4	0	0	14	61	0	0	0	0	7	22
	19	0	0	0	0	4	8	0	0	0	0	7	16
Average		3	18	1	4	24	123	2	9	T	1	20	74
		All varieties averaged 9 p.p.m. green weight and 48 p.p.m. dry weight.						All varieties averaged 7 p.p.m. green weight and 28 p.p.m. dry weight.					

[a]Each value is an average of 2 samples from each of 5 plants.

[b]T represents a HCN potential of less than 1 p.p.m.

[c]Two samples were taken on this date.

TABLE 6. Minimum daily temperatures (°F) at Knoxville, Tennessee for October and November, 1964 and 1965

Date	1964	1965	Date	1964	1965
October 1	60	56	November 1	47	34[a]
2	58	66	2	46	30[a]
3	60	60	3	44	32[a]
4	56	60	4	40	46
5	48	47	5	40	44
6	34[a]	44	6	39	44
7	32[a]	58	7	40	42
8	34[a]	54	8	56	44
9	38[a]	42	9	45	42
10	34[a]	42	10	40	52
11	32[a]	44	11	40	50
12	34[a]	46	12	42	46
13	42	40	13	56	52
14	44	47	14	40	38
15	54	46	15	38[a]	34[a]
16	50	51	16	50	50
17	50	54	17	60	40
18	48	54	18	56	24[a]
19	50	56	19	60	40
20	30[a]	60	20	32[a]	30[a]
21	30[a]	62	21	28[a]	36
22	48	53	22	16[a]	44
23	37[a]	48	23	22[a]	36
24	33[a]	42	24	28[a]	34[a]
25	37[a]	30[a]	25	40	42
26	37[a]	34[a]	26	40	52
27	40	39	27	30[a]	48
28	45	39	28	38	34[a]
29	50	38	29	26[a]	24[a]
30	50	30	30	26[a]	20[a]
31	46	34[a]			

[a]Days on which frost was observed on the plants.

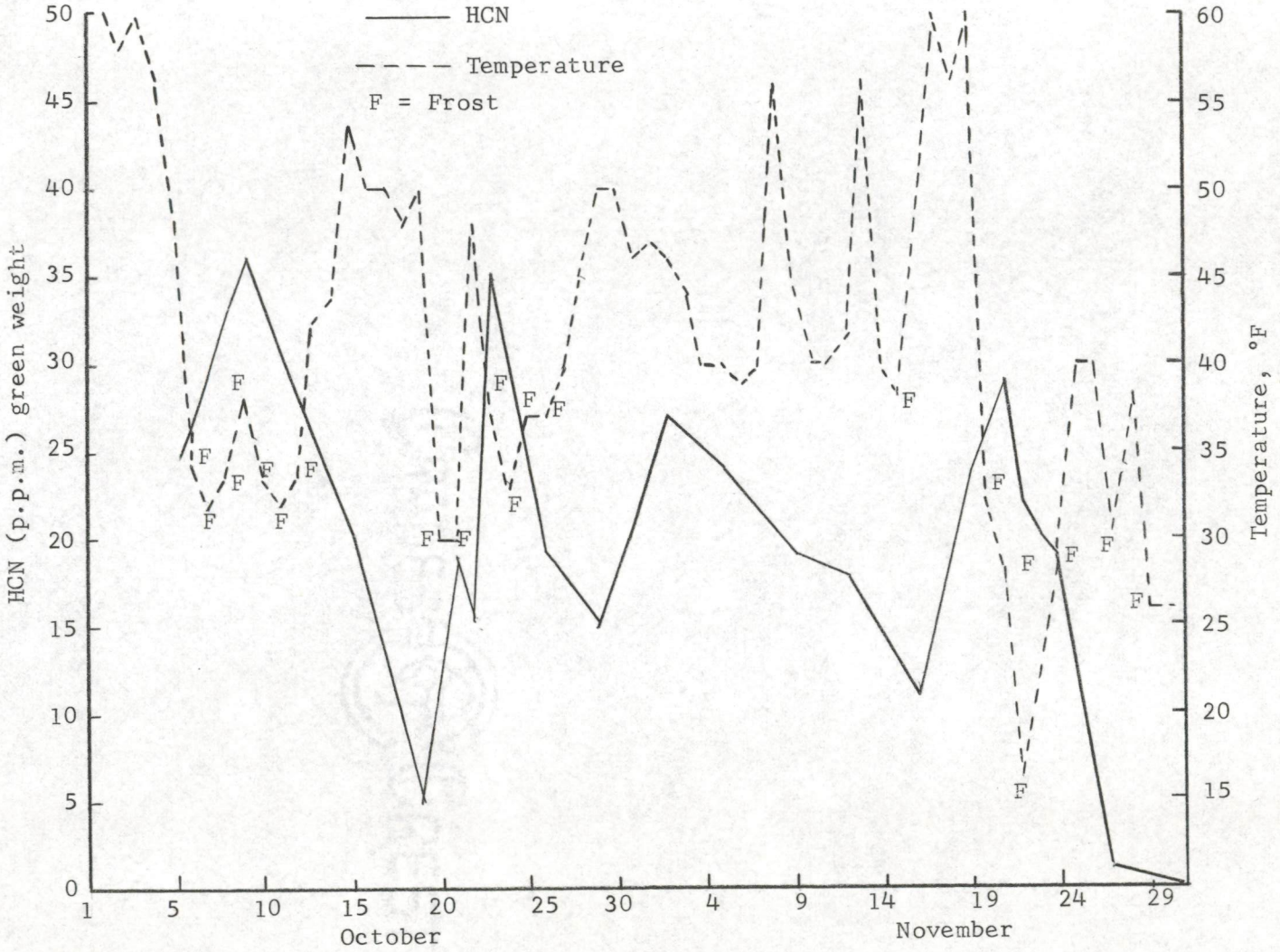


FIG. 1. Temperature versus HCN in p.p.m. of green weight, Plant Science Farm, 1964.

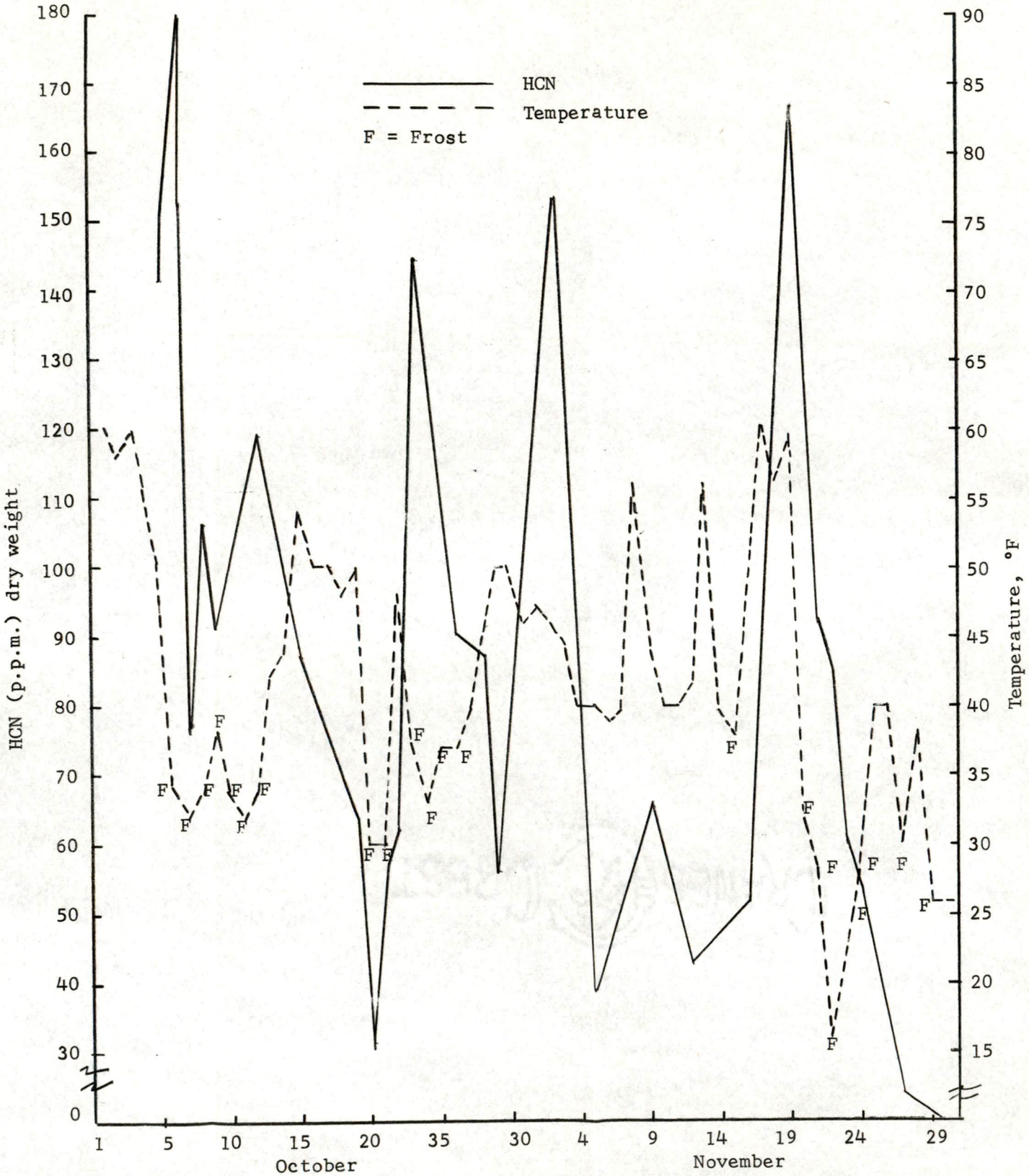


FIG. 2. Temperature versus HCN in p.p.m. of dry weight, Plant Science Farm, 1964.

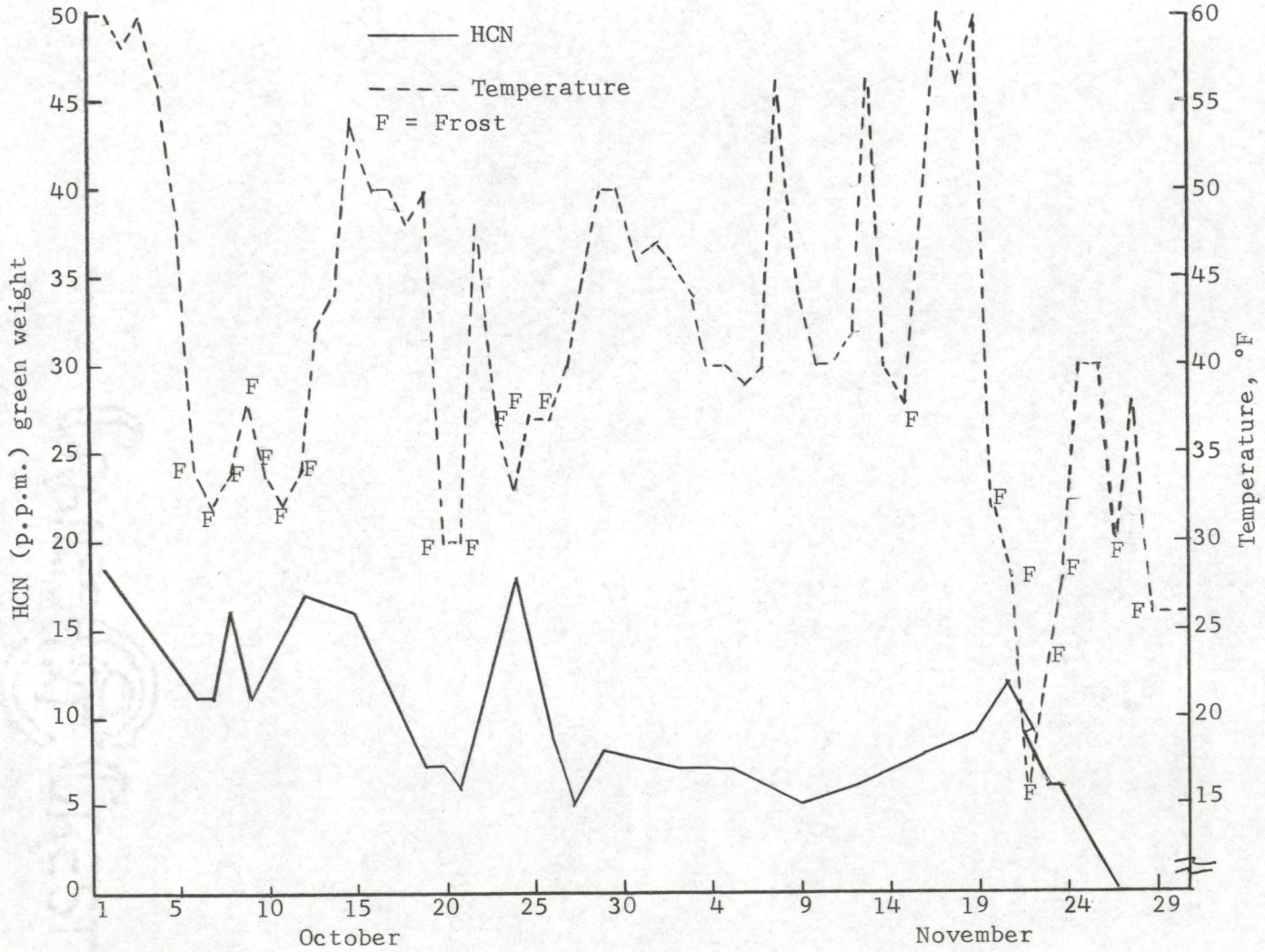


FIG. 3. Temperature versus HCN in p.p.m. of green weight, Knoxville, 1964.

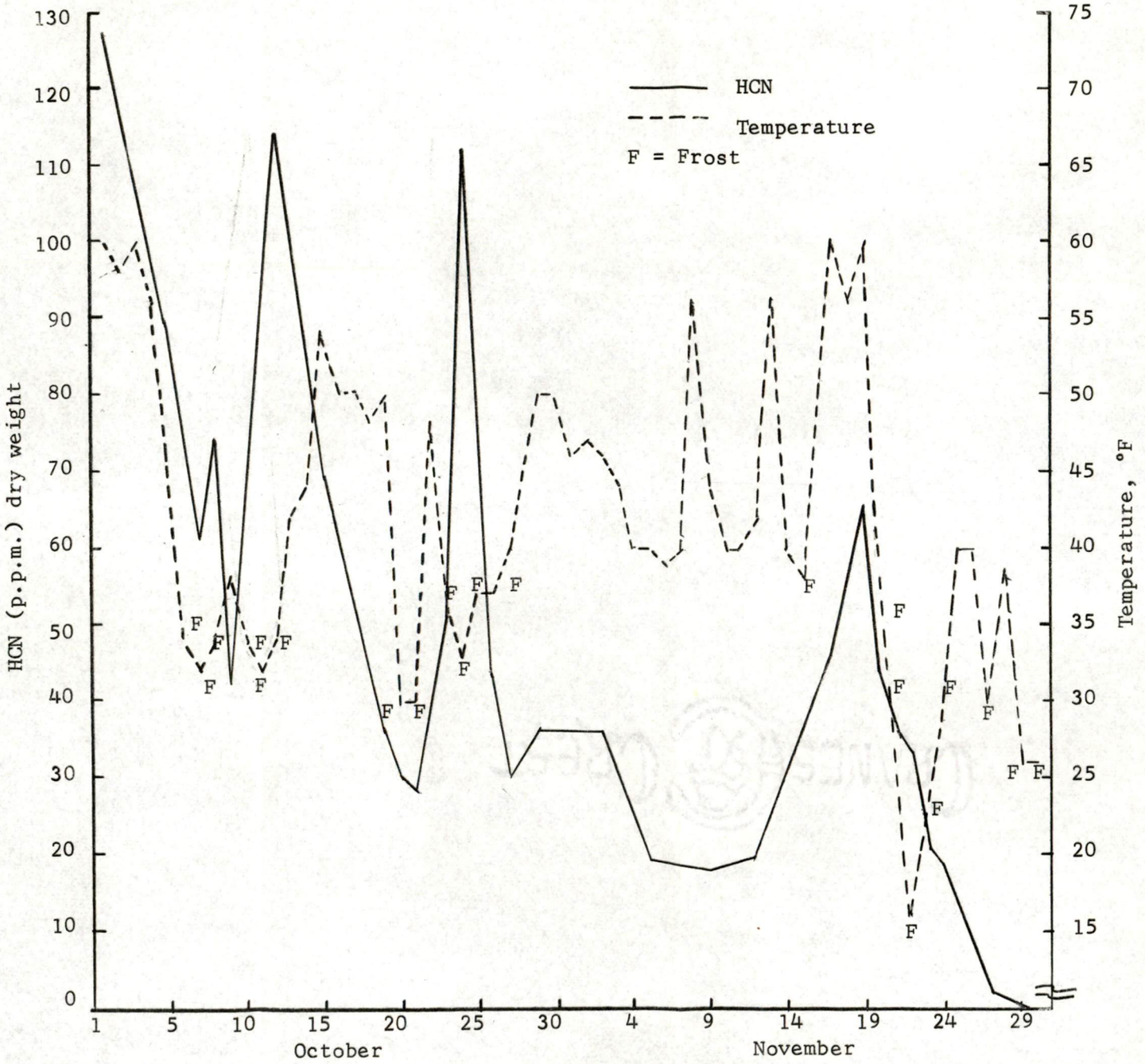


FIG. 4. Temperature versus HCN in p.p.m. of dry weight, Knoxville, 1964.

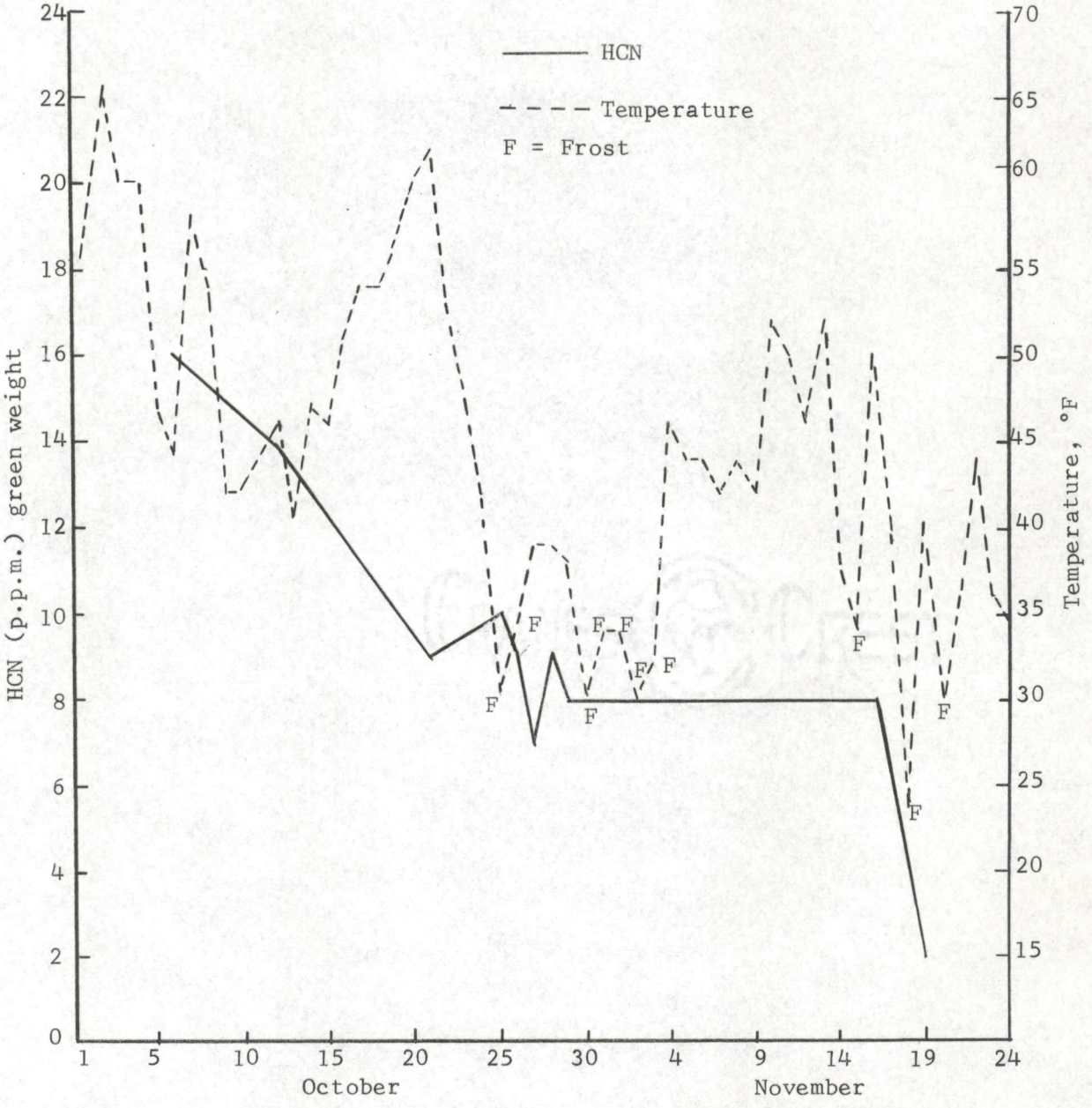


FIG. 5. Temperature versus HCN in p.p.m. of green weight, Knoxville, 1965.

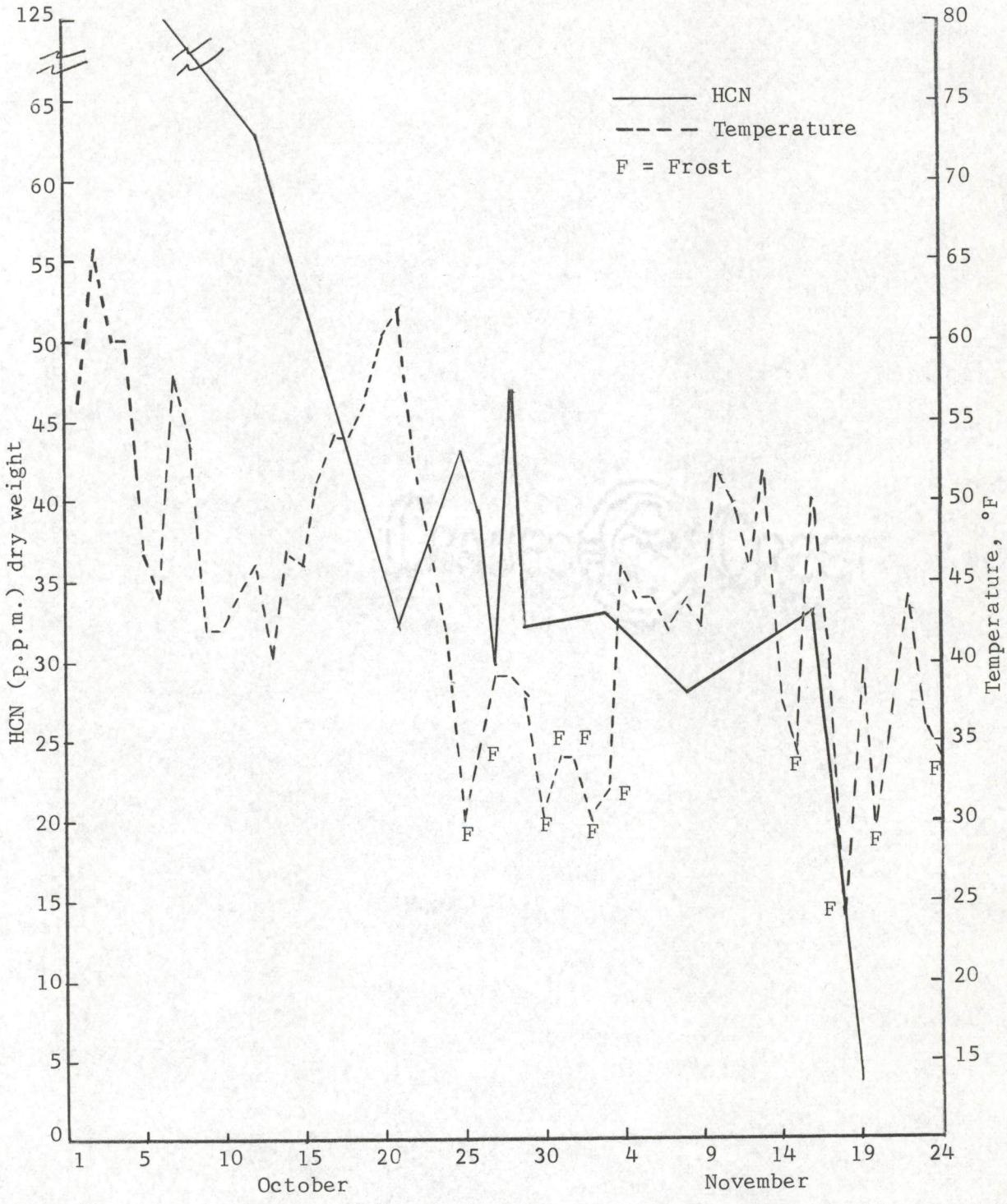


FIG. 6. Temperature versus HCN in p.p.m. of dry weight, Knoxville, 1965.

slightly each day until the fourth day after the first frost (Figure 1). After this increase, the HCN decreased steadily to a level lower than that prior to frost. Average HCN estimates for the 20- and 30-inch plants at Knoxville were more variable (Figure 3) than those for the 15-inch plants. During the first four days of frost the HCN estimates were variable; however, estimates made on the seventh and tenth days were approximately equal and about 5 p.p.m. above the estimate on the first day of frost. The HCN potential then decreased to a level lower than that prior to frost as was also observed for the 15-inch plants.

The results using dry weights were similar to those for green weights for the first frost period at the Plant Science Farm and at Knoxville with one major exception. For the 15-inch plants there was a pronounced decrease in HCN between the first and second frost days. The dry matter for the first frost day and the day prior to frost averaged 16.5 per cent, whereas, dry matter for the second day of frost averaged 32.0 per cent. Apparently the plants lost moisture after the first frost resulting in the higher dry matter percentage. Although a slight increase was observed for HCN (green weight), the increase in dry matter percentage on the second frost day resulted in an observed decrease in HCN (dry weight).

The second frost period consisted of two 30°F days which occurred October 20 and 21. HCN (green weight) increased and reached a high the fourth and fifth days after the first frost for Plant Science Farm and

Knoxville, respectively (Figures 1 and 3). Following this high HCN decreased and reached a low in about six days. Dry weight results (Figures 2 and 4) were similar to green weight results for the second frost period.

The third frost period, October 23 through 26, followed the second by one day in which no frost occurred. At the Plant Science Farm the HCN (green weight) was low seven days after the first frost of this period but had increased by the eleventh day (Figure 1). There was only a slight increase in HCN of the plants at Knoxville (Figure 3). At the Plant Science Farm HCN potential decreased for 15 days, but at Knoxville the decrease was less pronounced and continued for 12 days. For HCN dry weight, the increase at the Plant Science Farm was pronounced (Figure 2), but the increase was slight at Knoxville (Figure 4). The decrease in HCN (dry weight) was similar to the decrease in HCN (green weight).

The fourth frost period consisted of one day, November 15, in which the recorded temperature was 38°F. In both the Plant Science Farm and Knoxville readings an increase followed this date by seven days (Figures 1 and 3). The decrease which followed continued into the fifth frost period. The increase of HCN (dry weight) in this period was pronounced for plants at both locations. The decrease of HCN (dry weight) (Figures 2 and 4) was similar to that of HCN (green weight).

The fifth frost period occurred November 20 through 24 and recorded temperatures ranged from 16° to 32°F. During this time HCN (green weight) was decreasing following the high of the fourth frost

period. The decrease continued until no HCN was detected in the samples. Similar results were obtained for HCN (dry weight).

1965 Study

Four frost periods occurred during the 1965 study. The first frost period did not occur until October 25 and 26. The daily minimum recorded temperatures were 30° and 34°F. At this time HCN (green weight) was about 10 p.p.m. Average HCN content had decreased from about 16 p.p.m. at the time of the first sampling on October 6. Plants in the younger stage had advanced from the boot to the heading stage. Plants in the older stage had advanced from heading to seed stage. The decrease accompanying advanced maturity is in agreement with results reported by Benson (2), Cassady (5), Couch (9) and Morton (20).

There was little if any change in HCN (green weight) following the first frost period (Figure 5). The pattern of variation in HCN (dry weight) (Figure 6) was similar to that for green weight except there was more spread between the points.

The second frost period which occurred October 30 through November 3 had a recorded temperature range from 30° to 34°F. This frost period apparently had little or no effect on HCN when expressed on either green or dry weight basis.

The third frost period consisted of one day, November 15, with a minimum recorded temperature of 34°F. After one day with a 50°F minimum recorded temperature, the fourth frost period, a 24°F day, occurred. The day following the third frost period, HCN (green weight) and HCN

(dry weight) began to decrease and continued through the fourth frost period. The readings approached zero with subsequent death of the plants.

On three days of the 1965 study, samples were taken twice daily. No consistent differences were observed between samples taken in the morning and in the afternoon (Table 5).

II. CONTROLLED TEMPERATURE STUDIES

Results of the controlled temperature studies are given in Table 7. The HCN potential of leaf halves was not affected by temperatures of 30°, 38° and 40°F when the chloroform was applied immediately, 18 or 36 hrs. after the end of the 6-hour treatment. However, a similar treatment of 6 hrs. at 30°F in which chloroform was applied 60 hrs. after treatment resulted in a decrease of HCN potential. Temperatures of -20°F for 0.2 hr. with addition of chloroform 0.5 hr. after treatment also resulted in a lower HCN potential. No HCN was detected in the leaf halves when chloroform was applied 15 hrs. after exposure to -20°F for 0.2 hr.

The temperatures above freezing caused no apparent injury to the plants and resulted in no change in HCN potential. A temperature of 30°F caused slight injury to the material, however no change in HCN was noted within 18 hrs. after treatment, which is in agreement with field results where increases were usually observed about 4 to 7 days after frost. The decrease in HCN for plants subjected to 30°F and treated

TABLE 7. Effects of controlled temperatures on HCN potential of sorghum leaves[a]

Variety	Number of plants sampled	Temperature °F[b]	Duration of treatment (hr.)	Time of chloroform application[c]	HCN potential (p.p.m.) Before treatment	HCN potential (p.p.m.) After treatment
GHS-1	10	40	6.0	18	42	44
Hydan-37	10	40	6.0	18	66	88
Suhi-1	10	40	6.0	18	77	74
Greenleaf	3	38	6.0	18	57	62
Piper	3	38	6.0	18	6	4
Suhi-1	3	38	6.0	18	118	135
Greenleaf	3	38	6.0	36	39	37
Piper	3	38	6.0	36	14	20
Suhi-1	3	38	6.0	36	102	91
Greenleaf	3	30	6.0	0	24	17
Piper	3	30	6.0	0	2	1
Suhi-1	3	30	6.0	0	79	93
Greenleaf	6	30	6.0	18	50	40
Piper	6	30	6.0	18	9	8
Suhi-1	6	30	6.0	18	83	79
Greenleaf	3	30	3.0	60	90	33
Piper	3	30	3.0	60	23	3
Suhi-1	3	30	3.0	60	109	48
Greenleaf	7	-20	0.2	0.5	108	50
Piper	7	-20	0.2	0.5	40	8
Suhi-1	7	-20	0.2	0.5	169	54
Greenleaf	3	-20	0.2	15	71	0

TABLE 7 (continued)

Variety	Number of plants sampled	Temperature °F[b]	Duration of treatment (hr.)	Time of chloroform application[c]	HCN potential (p.p.m.) Before treatment	HCN potential (p.p.m.) After treatment
Piper	3	-20	0.2	15	27	0
Suhi-1	3	-20	0.2	15	237	0

[a]Leaves were split along the mid-rib; one-half was analyzed prior to treatment and the other half was left attached until after treatment.

[b]The reported temperatures were obtained by the following methods: 40°--ice chest, 38° and 30°--cold storage room, and -20°--quick-freeze room.

[c]Number of hours after end of treatment at which chloroform was added.

with chloroform 60 hrs. later was not in agreement with field results where HCN content was increasing by this time. Exposure of plants to -20°F resulted in a rapid decrease in the HCN potential of the plants such as was observed after a severe freeze under field conditions.

III. CHLOROFORM AND FREEZING STUDIES

In the "picric-acid test" used in this study, chloroform was added to the chopped plant material. In this portion of the study, the effect of omitting chloroform on fresh and frozen plant material was investigated (Table 8). No HCN was measured from fresh samples when chloroform was omitted from the procedure. However, measured HCN of frozen samples not treated with chloroform was about 75 per cent of comparable frozen leaf halves treated with chloroform.

TABLE 8. Release of HCN (p.p.m.) from fresh and frozen samples with and without chloroform treatment[a]

Variety	Number of plants sampled	Fresh samples		Frozen samples	
		With chloroform	Without chloroform	With chloroform	Without chloroform
Greenleaf	6	8	0		
Piper	6	0	0		
Suhi-1	6	24	0		
Greenleaf	11			28[b]	21
Piper	11			0	0
Suhi-1	11			94[b]	70
Suhi-1	8			66[c]	50

[a]Leaves were split along the mid-rib; one-half received chloroform, the other half did not.

[b]Leaves were from plants placed in a freezer at 0°F for 15 minutes.

[c]Leaves were from plants exposed to 24°F in the field.

CHAPTER V

DISCUSSION

The effect of freezing on HCN potential of sorghum plants varied with the stage of growth of the plants and the severity of the freeze. Results found in this study as well as results reported in the literature indicate that freezing may cause either an increase or a decrease in HCN potential.

Manges (18) reported a heavier concentration of HCN following the first frost of the autumn. Willaman (34) also reported an increased yield in HCN of frosted material. In this study, a pronounced increase in HCN potential was observed for 15-inch plants (Figures 1 and 2) within a week after the first two or three periods of light frost of the season. Similar but less pronounced increases were observed for the 20- and 30-inch plants (Figures 3 and 4). HCN potential of mature plants showed little or no increase following light frost (Figures 5 and 6).

A light freeze causes formation of ice crystals in the intercellular spaces and usually does not result in death of the cells. The increase in HCN potential which was observed a few days after light freezes may have resulted from increased chemical activity accompanying higher temperatures. This is based on the assumption that the speed of the reaction as proposed by Mao (19) is decreased by the low temperatures and results in a buildup of intermediary products.

Severe or killing frosts resulted in a decrease of HCN potential. This phenomenon was observed at all locations for both years (Figures 1-6). Similar results were found for all plants subjected to -20°F (Table 7). Within 0.5 hr. the HCN potential had decreased by over one-half. Fifteen hours after the -20°F treatment, no HCN was detected in the material. Following a treatment of 0°F, HCN was found to be released without the application of chloroform. This was also true for plants exposed to 24°F in the field (Table 8). Peters et al. (23) and Burns and Wedin (4) reported results which agree with these findings.

In the event of a severe freeze, ice crystals form in the cells causing mechanical injury and disorganization of the protoplasm. Metabolic activity ceases with death of the cells. Upon thawing the HCN apparently escapes from the cells.

Either a light or severe freeze could cause otherwise non-toxic sorghum plants to become toxic to cattle. In the event of a light frost on actively growing plants the HCN would increase and the material would be most dangerous approximately one week after the time of frost. A severe freeze of actively growing sorghum plants, which normally have a high HCN potential, would cause plants to be unsafe for consumption by cattle immediately after thawing because of the rapid HCN release. HCN potential of mature sorghum plants is at a low level and does not tend to increase following a light frost. Therefore, such material should not be toxic following frost.

CHAPTER VI

SUMMARY

The effect of freezing on HCN potential of sorghum plants was studied using Sudangrass (Sorghum sudanese (Piper) Stapf.) and sorghum (Sorghum vulgare Pers.) x Sudangrass hybrids. The varieties which were studied in 1964 included: GHS-1, Greenleaf, Hydan-37, Piper, Suhi-1 and Tennessee Synthetic-1. Plants were in three stages of growth--15, 20 and 30 inches tall--when the first autumn frost occurred. In 1965, Greenleaf, Piper and Suhi-1 were studied. At the time of the first autumn frost the two stages of plant growth were about 35 and 45 inches tall. During the summer of 1965, plants of Greenleaf, Piper and Suhi-1 were grown in buckets and subjected to controlled temperatures.

A light frost on young actively growing plants resulted in an increase in HCN potential which reached a maximum about one week after the first frost of a frost period. No increase was observed following a light frost on plants in the heading or early seed stages.

A severe or killing freeze resulted in a decrease of HCN potential which seemed to occur immediately after thawing and continued until no HCN could be detected.

It was proposed that either a light or severe freeze could cause plants of high HCN potential to become more unsafe for consumption by livestock. Following a light frost, plants would be most dangerous about a week after frost, whereas, after a severe frost, plants would

be most dangerous immediately. The mature plants were low in HCN potential and would not be expected to be dangerous after either a light or severe frost.

Variety differences in HCN potential were observed. Piper and Tennessee Synthetic-1 were low, Greenleaf was intermediate and Hydan-37, GHS-1 and Suhi-1 were high. Stage of growth affected HCN potential. HCN decreased as the plants became more mature.

Similar results were obtained when HCN was expressed on green or dry weight basis except in young succulent plants in which freezing resulted in a loss of moisture and an increase in dry matter percentage.

LITERATURE CITED

LITERATURE CITED

1. Anderson, H., W. E. Tottingham, H. L. Ahlgren, D. C. Smith, M. J. Wright, R. M. Patel, C. J. Patel, and J. D. Blocher. 1960. Precise estimation of hydrocyanic acid in Sudangrass and sorghum. Univ. Wisconsin (Mimeographed).

2. Benson, J. A. 1964. Estimating hydrocyanic acid potential of sorghum plants from leaf samples. Master's Thesis, University of Tennessee.

3. Boyd, F. T., O. S. Aamodt, G. Bohstedt, and E. Grough. 1940. Sudangrass management for control of cyanide poisoning. J. Amer. Soc. Agron. 30:569-582.

4. Burns, J. C., and W. F. Wedin. 1964. J. Amer. Soc. Agron. 56: 457-460.

5. Cassady, A. J. 1952. Sudangrass, tepary bean, safflower and sweetclover investigations. Kansas Agr. Exp. Sta. Cir. 289.

6. Collison, S. E. 1919. Prussic acid in sorghum. Florida Agr. Exp. Sta. Bul. 155.

7. Conn, E. E., and B. Colette. 1959. Metabolism of a cyanogenic glycoside. Proc. IXth Inter. Bot. Cong.

8. Coop, I. E., and R. L. Blakley. 1948-49. N. Z. J. Sci. Technol., Sect. A, 30:277.

9. Couch, J. F. 1934. Poisoning of livestock by plants that produce hydrocyanic acid. U. S. D. A. Leaflet 88.

10. Dunstan, W. R., and T. A. Henry. 1902. Cyanogenesis in plants. Part II. The great millet, Sorghum vulgare. Phil. Trans. Roy. Soc. London V, 199 Sec. A., pp. 399-410.

11. Finnemore, H., and C. B. Cox. 1932. The amount of hydrocyanic acid in sorghum, Sudangrass and some hybrids. J. Proc. Roy. Soc. N. S. Wales 65:145-152.

12. Franzre, C. J., L. F. Phur, and A. N. Hume. 1932. A study of sorghum with reference to the content of HCN. South Dakota State College Tech. Bul. 1.

13. Fribourg, H. A. 1963. Summer annual forage grasses for Tennessee. Tennessee Agr. Exp. Sta. Bul. 373.

14. Garner, R. J. 1963. Veterinary toxicology. Williams and Wilkins Co., Baltimore, pp. 78-82.

15. Hadley, F. R., and F. L. Kozelka. 1935. Antidotes for hydrocyanic acid poisoning. Vet. Med. 30:79-81.

16. Hogg, P. G., and H. L. Ahlgren. 1942. A rapid method for determining hydrocyanic acid content of single plants of Sudangrass. J. Amer. Soc. Agron. 34:199-200.

17. Hughes, H. D., M. E. Heath, and D. S. Metcalf. 1953. Forages. Iowa State College Press, Ames, Iowa, pp. 400-411.

18. Manges, J. D. 1935. Cyanide poisoning. Vet. Med. 30:347-349.

19. Mao, C. H. 1964. Cyanogensis in Sorghum vulgare. Ph. D. thesis, Univ. of Wisconsin.

20. Martin, J. H., J. F. Couch, and R. R. Briese. 1938. Hydrocyanic acid content of different parts of the sorghum plant. J. Amer. Soc. Agron. 30:725-734.

21. Moodie, A. W. S., and A. A. Ramsey. 1929. Sorghum Sudangrass hybrids. Agr. Gaz. N. S. Wales 40:731-735.

22. Nowosad, F. S., and R. M. Mac Vicar. 1939-40. Adaptation of the "Picric-Acid Test" method for selecting HCN-free lines in Sudangrass. Sci. Agr. 20:566-569.

23. Peters, A. T., S. Avery, and H. B. Slade. 1903. Nebraska Agr. Exp. Sta. Bul. 77, pp. 1-16.

24. Pickett, R. C. 1954. Sudangrass in Kansas. Kansas Agr. Exp. Sta. Cir. 311.

25. Siegmund, O. H. 1961. Merck veterinary manual, 2nd ed. Merck and Co., Inc., N. J.

26. Slade, H. B. 1902. A study of the enzymes of green sorghum. Nebraska Agr. Exp. Sta. 15th Ann. Rpt., pp. 55-62.

27. Swanson, C. O. 1921. Hydrocyanic acid in Sudangrass and its effect on cattle. J. Amer. Soc. Agron. 13:33-36.

28. Vinall, H. N. 1921. A study of the literature concerning poisoning of cattle by the prussic acid in sorghum, Sudangrass and Johnsongrass. J. Amer. Soc. Agron. 13:267-280.

29. U. S. D. A. 1964. Agriculture statistics. U. S. Government Printing Office. Washington. 1964.

30. U. S. D. A, and Tennessee Department of Agriculture. 1963. Tennessee Agriculture Statistics Annual Summary. Bul. T-1.

31. van der Walt, S. J. 1944. Onderstepoort J. Vet. Sci. 19:79.

32. Willaman, J. J., and R. M. West. 1915. Notes on the hydrocyanic acid content of sorghum. J. Agr. Res. 4:179-185.

33. _____. 1916. Effect of climatic factors on the hydrocyanic content of sorghum. J. Agr. Res. 6:261-272.

34. _____. 1917. J. Biol. Chem,, 29:39-41.