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# Effects of Ensiling on the HCN Potential of Sorghum Plants

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# EFFECTS OF ENSILING ON THE HCN POTENTIAL OF SORGHUM PLANTS

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## ABSTRACT

The effects of ensiling on HCN potential (HCN-p) of sorghum plants were studied at Bowling Green, Kentucky in 1969-70. Four cultivars were sampled at various growth stages and ensiled in 1.8 liter glass containers fitted with gas release valves. The plant material was analyzed for HCN-p by the sodium picrate procedure prior to ensiling, immediately after being removed from the silo, and after 24 and 48 hours of air-drying. Gases released during ensiling, and gases flushed from the silos were analyzed for HCN. The level of HCN-p decreased during the ensiling period and during the first 24-hour drying period. Some HCN was found in the gases which were released during the aerobic respiration portion of the ensiling process. Low levels of HCN were detected when the silos were flushed with $CO_2$ at intervals during the ensiling period. Although the levels of HCN-p varied with cultivars and growth stages, the ensiling effects were relatively consistent for cultivars and growth stages.

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# CHAPTER I

## INTRODUCTION

Annual sorghums are an important source of forage, especially from mid-summer until frost. Through the use of sorghums, it is possible to fill the summer gap in cool season forage production with yields of nutritious forage. The development of higher yielding and disease resistant varieties, along with improved management practices, has brought increased interest and production of forage sorghums. They are valuable for use as pasture, hay, or silage.

The Sorghum genus contains several species of economic importance. All are believed to contain dhurrin, a glucoside, which occurs in root, stem, and leaf tissues (4). Under certain conditions it decomposes and hydrogen cyanide (HCN), a poisonous gas, evolves from the tissue (12).

When ruminant animals consume feed containing dhurrin, HCN is liberated in the rumen and absorbed by the blood where it interfers with oxygen utilization. If enough is absorbed, the poison acts with extreme rapidity and results in respiratory paralysis and death.

The fact that sorghum plants are sometimes toxic to animals has been known since the 1800's. Subsequent work has shown that the amount of dhurrin, the precursor of HCN, in sorghum plants is affected by variety, stage of maturity, and environment. The effects of ensiling on the HCN potential (HCN-p) of sorghum plants has been studied to a limited extent, but is not well understood (7).

The objectives of this study were to further elucidate, 1) the effects of ensiling processes on the HCN-p of sorghum plants, and 2) the effects of sorghum cultivars and stages of plant maturity on the fate of HCN-p during ensiling.

# CHAPTER II

# REVIEW OF LITERATURE

## Toxic Levels of HCN-p

With present information, it is not possible to accurately predict what effect different HCN-p levels in sorghum will have on the animal. Whether sorghum is safe for consumption by livestock depends on several factors, such as, HCN-p of the plant material, amount of material consumed, rate of HCN release, type of ration, and the animal's ability to detoxify HCN (7).

Dhurrin has the emperical formula $C_{14}H_{17}O_{17}N$. Mao (12) at Wisconsin proposed the enzymatic decomposition as given in Figure 1.

It has been shown that consumption of about 1 gram of HCN will kill a 1,000 pound cow (2). Couch (5) stated that sorghums containing 200 ppm of HCN could be fatal if grazed rapidly. Harrington (8) separated HCN-p levels of sorghums into three toxicity groups as follows: 1) safe, 0-500 ppm, 2) doubtful, 500-750 ppm, and 3) dangerous, greater than 750 ppm.

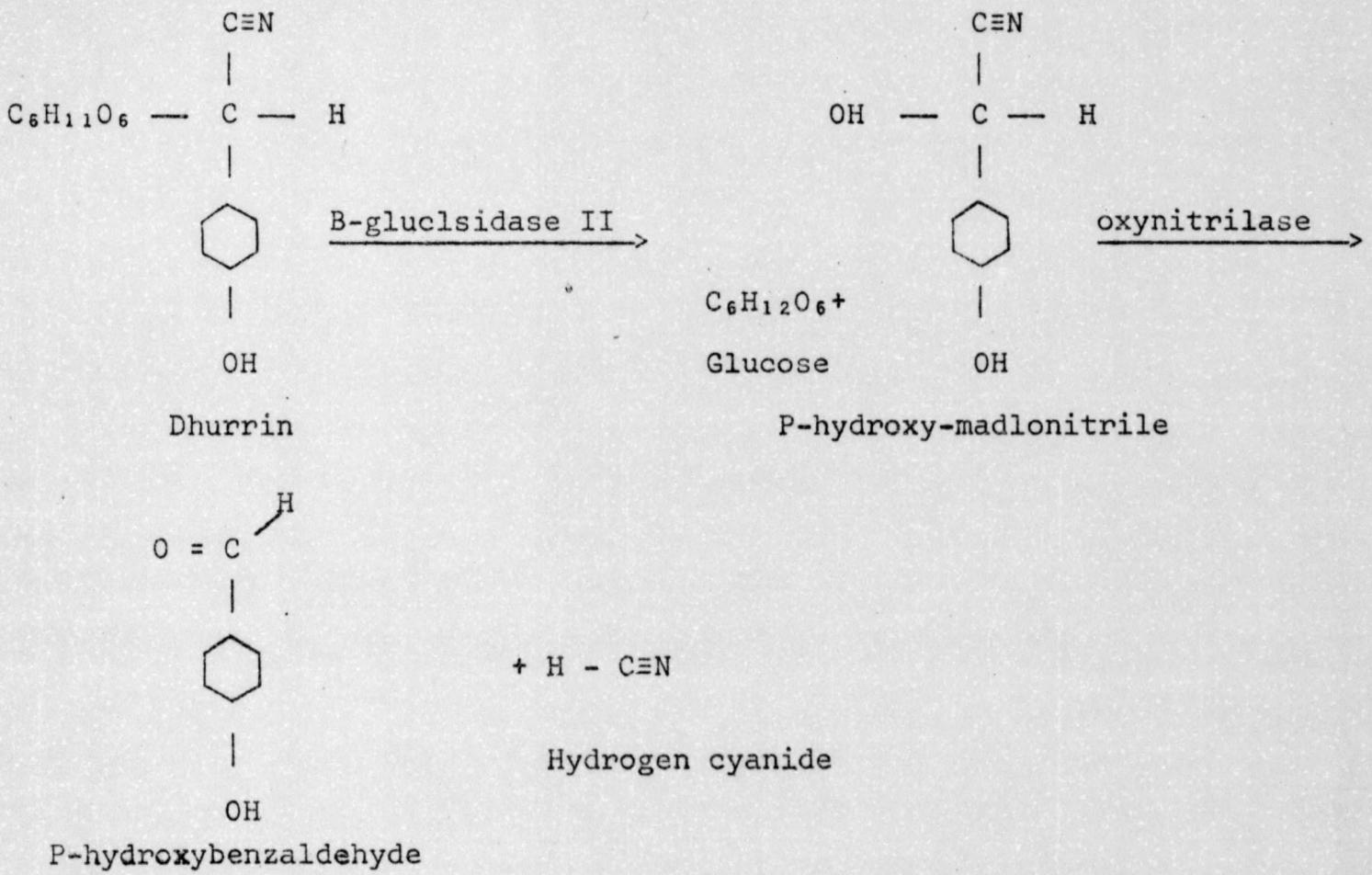


Figure 1. Proposed enzymatic decomposition of dhurrin (Mao 1967).

## HCN-p of Sorghum Varieties

Willaman and West (19) in 1915 reported differences in HCN-p among varieties. Benson (1) reported that sudangrass, sorghum x sudangrass hybrids, and grain sorghums were low, intermediate, and high in HCN-p, respectively. Sudangrass varieties such as 'Cumberland' (17) and 'Piper' (9) have been developed for low HCN-p.

## Effect of Stage of Growth on HCN-p

Maxwell (13) in 1903 reported HCN-p was greatest in sorghums in the early stages of growth. Several studies have revealed that HCN-p decreases as plant height and maturity increase. Harrington (8) stated that plant height affected HCN-p of sorghum x sudangrass hybrids more than that of sudangrasses. Hybrid plants which were two feet or taller had less than one-half as much HCN-p as plants which were approximately one foot high. The HCN-p of sudangrasses decreased slightly, or remained at about the same level, as plant height increased.

Hein (9) stated that prussic acid is present in appreciable quantities only in the rapidly growing part of the plant which is a very small proportion of a plant 18 inches or more in height. Wolf and Washko (20) attributed the decrease in HCN-p of larger plants to the increased percentage of plant parts which were lower in HCN.

Loyd and Gray (10) reported that the HCN-p of stems and leaves of 'Piper', 'Greenleaf', and 'Suhi-1' plants was highest one week after emergence and then decreased as plants matured. The HCN-p of roots generally decreased for three weeks after plant emergence and then fluctuated around this level for the remainder of their study. The HCN-p of plant heads was low for all varieties and did not change markedly during their period of observation.

## Effect of Drying on HCN-p

There are conflicting reports on the effects of drying on HCN-p of sorghum plants. Franzke et al. (6) found that drying in the sun caused a larger decrease in HCN-p than drying in the shade or in an oven. Boyd et al. (2) found that neither sun curing nor air-drying appreciably lowered the HCN-p of sorghum plants when steam distillation analysis was used. Swanson (16) and Manges (11) reported that air-drying did not decrease the cyanide content. Plants that were high in HCN-p at the time of cutting did not lose appreciable amounts of cyanide due to air-drying or sun curing. However, when plants were oven-dried at 115°C the content of cyanide decreased. Gray et al. (7) reported that air-drying reduced the HCN-p to about one-third to one-half that of fresh material.

## Effect of Frost and Freezing on HCN-p

The effects of frost and freezing on HCN-p of sorghum plants have been difficult to explain. Boyd et al. (2) reported no increase in HCN-p of frosted material. Swanson (16) reported that freezing of sudangrass did not cause a decrease in the cyanide content if the plants were assayed before thawing and wilting. When the plants were analyzed after thawing and wilting, the HCN-p dropped rapidly. The Merck Veterinary Manual (15) states that freezing does not ordinarily increase the glucoside concentration of HCN producing plants, but it may tend to increase the quantity of free HCN in the plants. Boyd et al. (2) suggested that the increase in cyanide after frost results from the production of new leaves and shoots which are higher in HCN-p than the older plant material. Other recent studies suggest that the magnitude of increase in HCN-p after frost depends on the stage of growth, the variety, and the intensity of the freeze (8).

Wattenbarger et al. (18) reported an increase in HCN-p after most frosts when the frost did not kill the plant. In their findings, increases were most evident from one to six days after frost. They reported that freezes (-5°C or lower) resulted in death of the plants and caused a decrease in HCN-p which continued until little or none was detected.

## Effect of Ensiling on HCN-p

Following an extensive study, Boyd et al. (2) concluded that plants which were high in HCN-p at the time of ensiling would remain high for several weeks after ensiling. Franzke et al. (6) found that the amount of HCN lost during ensiling varied with year and stage of maturity. Harrington (8) found that HCN-p of sudangrass which had been frosted remained about the same level for eight weeks after preservation as simulated silage; whereas, the HCN-p of similarly treated sorghum-sudangrass hybrids increased to more than twice that before ensiling. Rohweder et al. (14) suggested that sorghum silage could be fed safely, because HCN escapes during silage making. Harrington (8) stated that air-drying of silage reduced HCN-p, and that silage made from young sorghum-sudangrass hybrids should be air-dried for at least three days before feeding. Brise and Couch (3) suggested that HCN was in free form and escaped during feeding.

# CHAPTER III

## MATERIALS AND METHODS

This study was conducted at Western Kentucky University, Bowling Green, Kentucky in 1969 and 1970. The forage and grain sorghum cultivars were seeded at the rate of 28 and 10 kg/ha, respectively, in rows spaced 92 cm apart on May 19, 1969. The soil was Huntington silt loam. The area was fertilized with 134.6, 29.6, and 55.8 kg/ha of N, P, and K, respectively. Weed control was accomplished with a pre-emergence treatment of 2.2 kg/ha of propazine.

### Cultivars and Stages

The forage sorghum cultivars included: 'Piper' sudangrass (Sorghum bicolor var. sudanense (L.) Moench.), and 'Sudax' and 'NB 280S' sorghum (Sorghum bicolor (L.) Moench.) X sudangrass hybrids. First growth of piper and NB 280S was ensiled at the early head stage. First aftermath of these cultivars was ensiled at 50 and 100-cm stages. First aftermath of Sudax was ensiled at the 50-cm stage. The grain sorghum, 'Funk's Br 814', was taken from an experiment adjacent to the forage sorghum experiment. The ensiled material consisted of tillers approximately 50-cm in length

These tillers were removed when the main culm had reached the early seed stage.

## Ensiling

At the specified stage of growth, plants of each cultivar were cut and brought to the laboratory where they were immediately chopped into 0.5 to 1.0 cm lengths and packed in 1.8 liter experimental silos (Figure 2). The silos were glass jars with air tight covers. Each silo was fitted with two copper tubes, one extending to near the bottom (bottom tube); the other tube (top tube) extended through the silo cover but not into the silage. Plastic tubes, with release valves, were attached to the copper tubes outside the silo covers. The plant material was ensiled for a minimum of 28 days.

## HCN Determinations

The level of HCN-p in plant material was measured prior to ensiling, immediately after being removed from the silo, and after being air-dried for 24 and 48 hours. Ten samples weighing approximately 1-g were analyzed separately for each cultivar at each sampling. Samples were dried at 70°C for converting HCN-p from a fresh- to a dry-weight basis. Measurements of HCN-p were made using the sodium picrate method as developed by Anderson (Anderson, Laurens. 1960. Precise estimation of hydrocyanic acid in sudangrass and sorghum.

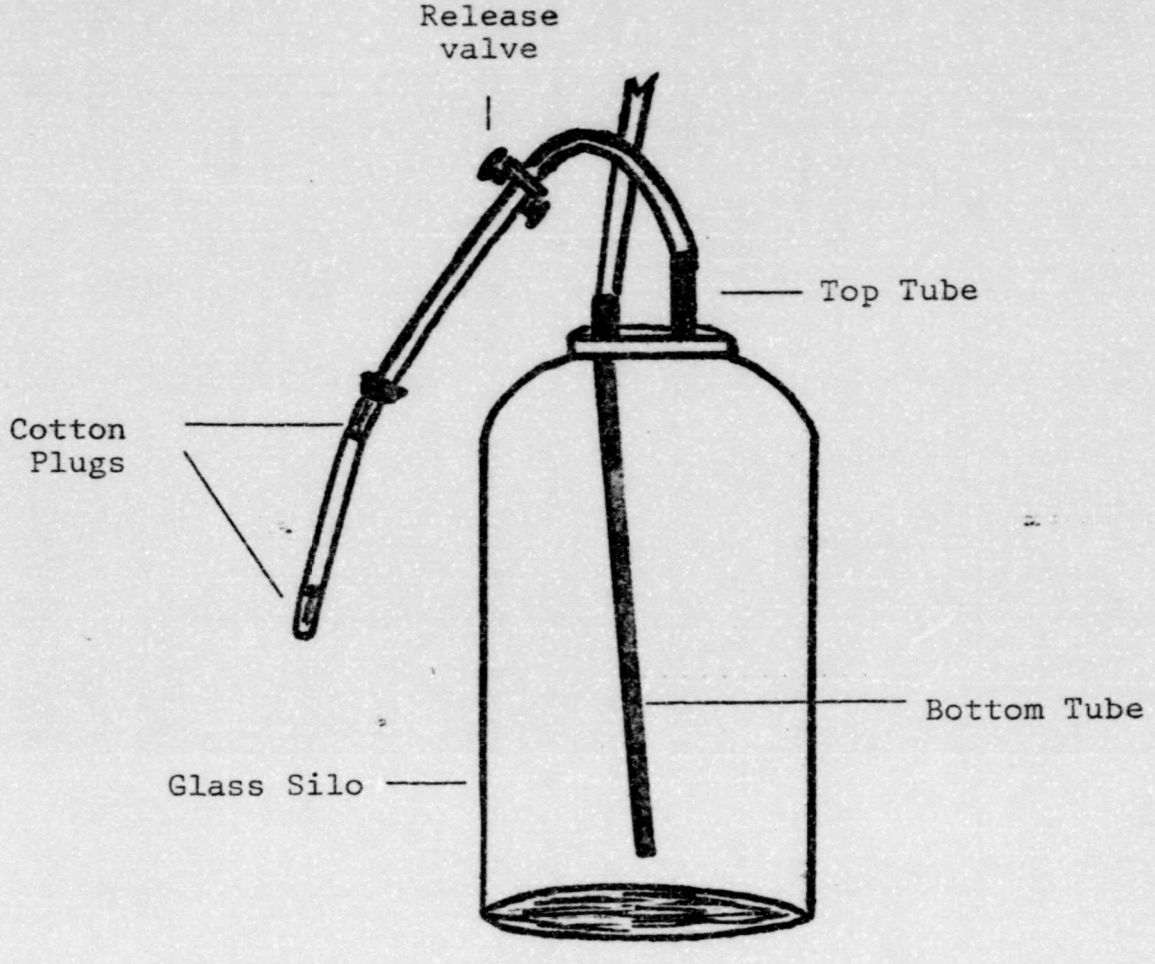


Figure 2. Experimental silo used in study.

Unpublished mimeograph, University of Wisconsin Department of Biochemistry, College of Agriculture and Agricultural Experiment Station) and modified by Wattenbarger et al. (18).

During the aerobic respiration portion of the ensiling period, the bottom tube was closed and the top tube was opened to permit gases to escape. The top tube was fitted with a 15 cm length of plastic tube containing 2 cotton plugs saturated with picrate solution to absorb any escaping HCN. The plugs were removed after 7 days and eluted in 10 ml of distilled water.

Following the aerobic respiration period, both tubes of most silos were closed until the end of the ensiling period. In some silos $CO_2$ gas was flushed through the bottom and up through the silage. Gases escaping through the top tube were bubbled into a beaker containing 4 ml of picrate solution to absorb any escaping HCN. The silos were flushed for 2 minutes each day beginning the day after the cotton plugs were removed and continuing for 21 consecutive days.

The picrate solutions in which the gases had been bubbled and those which were eluted from the cotton plugs were heated in a water bath until no further color changes were observed. The solutions were cooled and read in a colorimeter as prescribed in the sodium picrate method.

As suggested by Wattenbarger et al. (18) and Benson, Gray, and Fribourg (1), confidence intervals ($t_{.05}S_{\bar{x}}$) were used to compare treatment means.

# CHAPTER IV

## RESULTS AND DISCUSSION

Sudangrass, sorghum-sudangrass hybrids, and grain sorghum were low, intermediate, and high in HCN-p, respectively, (Tables 1, 2, 3). This is in agreement with reported results by Benson et al.(1). The younger and shorter plant material was consistently higher in HCN-p than the older, taller material. All material studied was within the recommended range for safe consumption. These results support the findings of Loyd and Gray (10). The HCN-p of plants in the early head stage was low for all cultivars. This is the stage of maturity most often used for silage.

The level of HCN-p decreased for all cultivars and stages of maturity during the ensiling period (Tables 1, 2, 3). The decreases were greatest in the cultivars ensiled at the 50-cm stage (Table 1). The decrease was not significant for piper and sudax in the early head stage (Table 3). The amounts of measured HCN-p in the silage were near zero for all cultivars and stages of maturity, except for Funk's Br. 814 at the 50-cm stage (Table 1). It contained about 62 ppm of HCN-p which is well below the toxic level. This decrease in HCN-p during ensiling in in agreement with some

Table 1. HCN-p (ppm dry weight) of plants of four sorghum cultivars ensiled at 50 cm.

| Cultivar | Fresh plants | Released during aerobic respiration | When removed from silo | After being air-dried | |
|---|---|---|---|---|---|
| | | | | 24 hours | 48 hours |
| Funk's Br. 814 | 304.17±17.57* | 0.10±0.00 | 62.50±8.95 | 41.10±4.07 | 51.04±4.51 |
| Piper | 6.79± 3.39 | 0.03±0.01 | 0.31±0.01 | 0.00 | 1.10±0.82 |
| NB 280S | 125.46±13.45 | 0.03±0.01 | 0.31±0.67 | 0.01±0.01 | 0.30±0.86 |
| Sudax | 67.29±13.02 | 0.06±0.01 | 0.01±0.12 | 0.05±0.05 | 0.24±0.29 |

*Means and confidence intervals ($t_{.05}S_{\bar{x}}$). The means are based on different numbers of determinations as follows: fresh plants, 10 determinations; aerobic respiration, 3 determinations (1 per silo); and all other treatments, 30 determinations (10 per silo).

Table 2. HCN-p (ppm dry weight) of plants of two sorghum cultivars ensiled at 100 cm.

| Cultivar | Fresh plants | Released | | When removed from silo | After being air-dried | |
|---|---|---|---|---|---|---|
| | | During aerobic respiration | Following aerobic respiration | | 24 hours | 48 hours |
| Piper† | 15.90± 6.92* | 0.04±0.03 | | 0.06±0.01 | 0.21±0.01 | 0.18±0.01 |
| Piper§ | 15.90± 6.92 | 0.02±0.01 | 0.17±0.12 | 0.00 | 0.01±0.01 | 0.00 |
| NB 280S† | 19.48±16.46 | 0.01±0.01 | | 0.00 | 0.03±0.01 | 0.00 |
| NB 280S§ | 19.48±16.46 | 0.02±0.01 | 0.21±0.02 | 0.00 | 0.00 | 0.00 |

*Means and confidence intervals ($t_{.05}S_{\bar{x}}$).

†Means are based on different numbers of determination as follows: fresh plants, 10 determinations; aerobic respiration, 4 determinations (1 per silo); and all other treatments, 40 determinations (10 per silo).

§Means are based on different numbers of determinations as follows: fresh plants, 10 determinations; aerobic respiration, 2 determinations (1 per silo); following aerobic respiration, 42 determinations (21 per silo); and all other treatments, 20 determinations (10 per silo).

Table 3. HCN-p (ppm dry weight) of plants of three sorghum cultivars ensiled at the early head stage.

| Cultivars | Fresh plants | Released | | | | |
|---|---|---|---|---|---|---|
| | | During aerobic respiration | Following aerobic respiration† | When removed from silo | After being air-dried | |
| | | | | | 24 hours | 48 hours |
| Piper | 0.30±0.34* | 0.00 | 0.04±0.02 | 0.00 | 0.00 | 0.00 |
| NB 280S | 8.30±2.12 | 0.00 | 0.06±0.05 | 0.47±0.35 | 0.00 | 0.01±0.05 |
| Sudax | 9.40±5.83 | 0.00 | 0.20±0.11 | 4.07±0.59 | 0.05± .03 | 0.70±0.30 |

*Means and confident intervals ($t_{.05}S_{\bar{x}}$). The means are based on different numbers of determinations as follows: fresh plants, 10 determinations; aerobic respiration, 3 determinations (1 per silo); and all other treatments, 30 determinations (10 per silo).

†Mean accumulative amounts measured when flushed with $CO_2$ daily for 21 days.

reports (Franzke et al. (6); Rohweder et al. (14), but is not in agreement with other reports (Boyd et al. (2); Harrington (8).

Rohweder et al.(14) suggested that HCN escaped as a gas during ensiling, and Briese and Couch (3) suggested that the HCN gas escaped during feeding of the silage. In this study, small amounts of HCN gas were released during the ensiling period. Funk's Br. 814 (50-cm tillers) released 0.10 ppm of HCN during aerobic respiration. Smaller amounts were measured from the other cultivars and stages (Tables 1, 2, 3). Tests for HCN in the gases released during and following aerobic respiration were strongly positive, but when the amounts released were expressed as ppm on a silo weight basis, the values were less than 1 ppm.

The levels of HCN gas released during aerobic respiration (7 days) was not generally related to the level of HCN-p in the ensiled material (Tables 1, 2, 3). However, the level of HCN-p in plants of Piper and NB 280S at the early head stage was low (0.30 ppm and 8.30 ppm, respectively) and no HCN gas was detected during aerobic respiration (Table 3).

Several silos of 100-cm stage and early head stage material were flushed with $CO_2$ following aerobic respiration. Some HCN was detected in the gases flushed from the silos during this period (21 days). The release of HCN continued over the 21-day period. For Piper and NB 280S the amounts

of HCN measured in the period following aerobic respiration were greater for plants at the 100-cm stage (0.17 and 0.21 ppm, respectively) than for plants at the early head stage (Tables 2, 3). Within the early head stage, more HCN was detected during the period following aerobic respiration in the Sudax plants than in Piper or NB 280S plants. Levels of HCN-p in the silages made from Piper and NB 280S plants at the 100-cm stage were about the same whether or not the silos had been flushed. Only 0.06 ppm of HCN was detected in the Piper material that was not flushed; none was measured from the other samples (Table 2). The amounts of HCN detected during the ensiling period were small in comparison to the levels in plants prior to ensiling.

Release of HCN gas during the ensiling period supports the suggestion by Rohweder et al. (14) and Briese and Couch (3) that HCN is in a free form and escapes; thereby, resulting in a decrease in HCN-p of the silage. However, in the present study, the escape of HCN gases during the ensiling period was prevented. Since HCN is highly soluble in water and the silages contained between 70 and 85 per cent water, the HCN was most likely absorbed in the silage moisture. The HCN flushed from the silage would constitute the free HCN, i. e., the HCN which had been released by the plant cells, but which had not been reabsorbed by the silage moisture. Apparently the absorbed form was not detected by the sodium picrate assay for HCN.

The HCN-p of the silage was also determined using steam distillation (Boyd, F. T. 1938. The determination of and factors influencing the amount of cyanide in sudangrass. Ph. D. Thesis. University of Wisconsin). Based upon limited results, the HCN-p estimates obtained from steam distillation were consistently higher than those obtained from the sodium picrate method. However, the relative changes in HCN-p were similar for the two methods.

In this study the effects of air-drying were inconsistent. With the exception of NB 280S ensiled at the 100-cm stage (Table 2), all silages decreased in measured HCN-p during the first 24 hours of air-drying. However, during the second 24 hours of air-drying, HCN-p increased in some of the silages. Funk's Br. 814 and Piper had a significant increase in HCN-p during the second 24 hours of drying (Table 1). There was also an increase in Sudax ensiled at the early head stage (Table 3). The HCN-p of other silages remained at the same levels or decreased. The decrease in HCN-p after air-drying supports the suggestions of Gray et al. (7) and Couch (5), but is in opposition to the results reported by Boyd et al. (2) and Manges (11).

Under the conditions of this study, none of the plant materials approached the danger level as defined by Harrington (8). The highest level of HCN-p in the material studied was about 304 ppm in the grain sorghum. According to Harrington (8) a level of 750 ppm is considered dangerous for consumption.

With the exception of the grain sorghum silage, the measured HCN-p of all silages was essentially zero ppm. This suggests that silage would be safer than fresh material for animal feeding. However, these results are based upon laboratory tests and not upon animal feeding trials. It is generally accepted that there is a valid relationship between results of the sodium picrate method and animal response.

In view of Harrington's report (8) that HCN-p of sorghum sudangrass hybrids which had been frosted increased during ensiling, perhaps animal feeding trials are needed to provide valid establishment of safety of ensiled sorghum.

# CHAPTER V

## SUMMARY

The effects of ensiling processes on the HCN-p of sorghum plants, plus the effects of different sorghum cultivars and stages of plant maturity on the fate of HCN during ensiling, were studied at Bowling Green, Kentucky in 1969 and 1970.

Funk's Br. 814 grain sorghum, Piper sudangrass, and Sudax and NB 280S sorghum-sudangrass hybrids were studied. Cultivars were sampled at three stages of growth ( 50-cm, 100-cm, and early head). They were ensiled in 1.8 liter, air-tight glass jars, fitted with gas release valves. HCN-p analyses were made prior to ensiling, during aerobic respiration (seven days), following aerobic respiration (21-days), upon removal from the silos, and after 24 and 48 hours of air drying. A modification of the sodium picrate assay procedure was used for assaying HCN-p.

HCN-p was found in the cultivars before ensiling, but all samples were below the reported toxic level. The concentrations of HCN-p were highest in 50-cm, intermediate in the 100-cm, and lowest in the early head plant material. The grain sorghum was highest in HCN-p, followed by sorghum-sudangrass hybrids and sudangrass, respectively.

Small amounts of HCN were found in the gases which were released during the aerobic respiration portion of ensiling. Low levels of HCN were detected when the silos were flushed with $CO_2$ at intervals during the ensiling period. In most cases, the HCN-p decreased after the material was removed from the silo and air dried. Although the levels of HCN-p varied with cultivars and growth stages, the decrease in HCN-p during ensiling was relatively consistent.

In view of the fact that detectable HCN decreased during ensiling, and HCN was present in the gases flushed from the silage, it appears that HCN is released during ensiling and is reabsorbed in a non-detectable form.

Further work is needed to determine what changes the HCN undergoes during ensiling and if the reabsorbed form is toxic to animals. Under the conditions of this study, silage would be safer than fresh material for animal consumption.

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# VITA

Glenn McCarty was born on September 7, 1942, in Henry County, Kentucky. He was reared on a farm in a rural community called Campbellsburg. Glenn graduated from Campbellsburg High School in 1960. He received the Bachelor of Science Degree in Agriculture from Western Kentucky University in June of 1969, and became a candidate for the Master of Science Degree in Agriculture at Western Kentucky University.