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# Seasonal Variation in Leaf Hydrocyanic Acid Potential of Low- and High-Dhurrin Sorghums

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# Seasonal Variation in Leaf Hydrocyanic Acid Potential of Low- and High-Dhurrin Sorghums[1]

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## ABSTRACT

**The KS8 and N32 sorghum [*Sorghum bicolor* (L.) Moench] lines are low and high, respectively, in the hydrocyanic acid potential (HCN-p) of mature leaves. This difference is conditioned primarily by a single pair of alleles. The main objective of this study was to determine, at various stages of plant growth and various times during the growing season, the HCN-p of upper leaves and tillers of field-grown plants of these two parental lines and of two low-HCN-p $F_3$ lines derived from crosses between KS8 and N32. The four entries were grown in a randomized complete block design with three replications in 1985. Samples of leaf tissue were dried, ground, and extracted, and cyanide in the extracts was assayed colorimetrically. Using a mean HCN-p level of 500 mg kg$^{-1}$ dry wt to separate safe from unsafe sorghum forage, all samples of KS8 mature leaves and tillers would be considered safe, and all N32 samples would be considered potentially dangerous. Values for most of the samples of the $F_3$ lines fell within the safe range, but some samples of young regrowth exceeded the 500 mg kg$^{-1}$ limit. Regressions of HCN-p on height for upper leaves of main stems and of tillers indicated a significant negative relationship for all entries except for leaves from the main stems of KS8. However, the relationship was not close enough to support the use of plant or tiller height as a reliable indicator of HCN-p. Levels of HCN-p also were determined for mature leaves and young regrowth of hybrids involving KS8, N32, and 'Redlan' sorghums as seed parents and NP25, 'Piper,' and 'Greenleaf' sudangrasses [*S. sudanense* (Piper) Stapf] as pollinators. Results indicated that for minimizing the risk of cyanide poisoning, KS8 would be the seed parent of choice, and NP25 and Piper would be the preferred pollinators.**

***Additional index words:*** **Cyanogenesis, Prussic acid, *Sorghum bicolor* (L.) Moench, *Sorghum sudanense* (Piper) Stapf, Sorghum × sudangrass hybrids.**

A RECENT COMPARISON of the hydrocyanic acid potential (HCN-p) of the grain sorghum [*Sorghum bicolor* (L.) Moench] lines, KS8 and N32, indicated that seedling leaves of both lines were high in HCN-p, but mature leaves from field-grown plants differed greatly, flag leaves of N32 being at least 10 times as high in HCN-p as KS8 flag leaves (3). The large difference in HCN-p between KS8 and N32 was detected in field-grown plants within about 5 weeks after planting (5), and was found to be conditioned primarily by a single pair of alleles (3). The objective of the present study was to determine, at various times during the growing season and at various stages of plant development, the HCN-p of upper leaves and tillers of KS8, N32, and two low-HCN-p $F_3$ lines derived from crosses between KS8 and N32. Determinations of this type are needed for appropriate management decisions concerning the safety of sorghum forage for grazing livestock. It would be unusual for farmers to use grain sorghums such as KS8 and N32 as pasture except during the period after grain harvest. However, sudangrass [*S. sudanense* (Piper) Stapf] and sorghum × sudangrass hybrids are often used for pasture and greenchop, and the HCN-p of these forages at various growth stages is important to the livestock producer. Therefore, several other sorghum and sudangrass lines and their $F_1$ hybrids also were included in this study.

## MATERIALS AND METHODS

### Plant Material

Parental sorghum lines used in the main part of this study were the B-lines of KS8 (10) and N32 (11). Previously published HCN-p values for mature leaves of KS8 and N32 are approximately 24 and 850 mg kg$^{-1}$ dry wt, respectively (3). Reciprocal crosses between BKS8 and BN32 were made in 1982 by hand emasculation and hand pollination, and the resulting $F_1$ plants were self-pollinated in 1983 to produce $F_2$ seed. From numerous $F_2$ plants that were assayed and self-pollinated in 1984, two low-dhurrin plants were selected for carrying into the $F_3$ in 1985. One of these $F_2$ plants, from the BKS8 × BN32 cross, had a mature-leaf HCN-p of 18 mg kg$^{-1}$; the other, from BN32 × BKS8, had a value of 15 mg kg$^{-1}$.

Seeds of BN32, BKS8, and the two $F_3$'s were planted in a soil mixture in growth chambers (27°C, continuous cool white fluorescent light at about 150 $\mu$mol m$^{-2}$ s$^{-1}$) in April 1985, and first leaves of week-old seedlings were sampled for HCN-p assay. The seedlings were then transplanted to the greenhouse where they were allowed to grow until 17 May 1985 when they were transplanted to the field [soil type: Kennebec silt loam (fine-silty, mixed, mesic Cumulic Hapludoll)] at the University of Nebraska Agronomy Farm, Lincoln, NE. In both the greenhouse and the field, the four entries were arranged in a randomized complete block design with three replications. Row spacing in the field was 0.76 m with a 0.61-m spacing between plants within rows. These spacings were designed to reduce interplant competition and encourage tillering. Each of the three blocks consisted of 40 plants (a four-row plot with 10 plants per row) of each of the four entries; thus, a total of 480 plants were available for sampling during the season. To provide plants at various stages of development throughout the season, the first row in each plot was clipped (stubble height 10 to 15 cm) on 3 July, the second row on 15 July, and the third row of each plot on 26 July. The fourth row was left unclipped.

Four samples were harvested from each of the 12 plots on each sampling date. Two of the samples usually consisted of blades of upper leaves from main stems; each sample included one leaf blade, with midrib removed, from each of three plants. The other two samples usually consisted of upper portions (distal to the collar of the youngest leaf with a collar) of young tillers, one tiller from each of three plants per sample. A conscious effort was made to select tillers for height uniformity across entries at each sampling. Exceptions to the foregoing sampling procedure occurred on 11 and 27 September when all four samples consisted of flag

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**Table 1. Hydrocyanic acid potential (HCN-p) of upper leaves from main stems of BN32, BKS8, and two $F_3$ lines resulting from crosses of BN32 and BKS8. Sampled in 1985 at indicated growth stages and plant heights.**

| Growth stage | Sampling date | Plant height (mean ± SE)† | HCN-p‡ | | | | |
|---|---|---|---|---|---|---|---|
| | | | BN32 | BKS8 | $F_3$, BKS8 × BN32 | $F_3$, BN32 × BKS8 | SE§ |
| | | cm | mg kg⁻¹ dry wt | | | | |
| Preboot | 14 June | 52 ± 0.5 | 861 | 43 | 71 | 48 | 33 |
| | 20 June | 61 ± 0.7 | 1033 | 27 | 68 | 37 | 19 |
| | 28 June | 86 ± 1.4 | 1031 | 32 | 46 | 29 | 13 |
| Boot | 11 Sept.¶ | 127 ± 2.3 | 1149 | 24 | 42 | 28 | 8 |
| Heads partially emerged | 3 July | 104 ± 1.8 | 748 | 37 | 48 | 35 | 5 |
| | 11 July | 127 ± 2.8 | 773 | 37 | 55 | 44 | 38 |
| | 1 Aug.# | 126 ± 2.8 | 921 | 19 | 39 | 23 | 75 |
| | 27 Sept.¶ | 123 ± 4.5 | 743 | 29 | 41 | 24 | 25 |
| Heads fully emerged | 26 July | 127 ± 4.7 | 781 | 32 | 45 | 42 | 23 |
| | 8 Aug. | 149 ± 6.5 | 565 | 37 | 46 | 35 | 13 |
| Flowering | 29 Aug.# | 154 ± 5.8 | 618 | 29 | 35 | 30 | 12 |
| | 11 Sept.†† | 145 ± 5.0 | 741 | 23 | 34 | 29 | 10 |
| Grain filling | 27 Sept.# | 149 ± 5.8 | 514 | 26 | 35 | 19 | 6 |
| | | SE‡‡ | 23 | 2 | 2 | 2 | |

† Measured from soil surface to tips of heads for samples taken following full head emergence, and to tips of leaves extended along ruler for samples taken at earlier stages. Each mean represents 24 plants.
‡ Each mean represents six plants.
§ SE values calculated using error term from sampling analysis of variance.
¶ Regrowth from plants that were clipped 26 July.
# Regrowth from plants that were clipped 3 July.
†† Regrowth from plants that were clipped 15 July.
‡‡ SE across blocks and samplings.

**Table 2. Hydrocyanic acid potential (HCN-p) of upper portions of tillers of BN32, BKS8, and two $F_3$ lines resulting from crosses of BN32 and BKS8. Sampled on indicated dates in 1985, at tiller heights as shown.**

| Height range† | Sampling date | Tiller height (mean ± SE)† | HCN-p‡ | | | | |
|---|---|---|---|---|---|---|---|
| | | | BN32 | BKS8 | $F_3$, BKS8 × BN32 | $F_3$, BN32 × BKS8 | SE§ |
| cm | | cm | mg kg⁻¹ dry wt | | | | |
| <25 | 14 June | 22 ± 0.2 | 1518 | 260 | 406 | 261 | 42 |
| | 20 June | 22 ± 0.3 | 1566 | 140 | 261 | 213 | 79 |
| | 11 July¶ | 24 ± 0.6 | 2271 | 256 | 612 | 600 | 63 |
| 25–34 | 3 July | 32 ± 1.3 | 1080 | 82 | 180 | 138 | 60 |
| | 18 July¶ | 29 ± 0.5 | 1343 | 259 | 318 | 371 | 47 |
| | 26 July# | 30 ± 0.5 | 1497 | 303 | 298 | 351 | 124 |
| | 8 Aug.†† | 26 ± 0.2 | 2183 | 411 | 630 | 599 | 57 |
| | 15 Aug.†† | 26 ± 0.5 | 1591 | 313 | 461 | 348 | 71 |
| 35–44 | 28 June | 41 ± 1.1 | 1067 | 46 | 62 | 48 | 46 |
| | 1 Aug.# | 43 ± 0.7 | 1145 | 94 | 125 | 64 | 22 |
| >44 | 18 July¶ | 83 ± 1.2 | 993 | 30 | 45 | 41 | 13 |
| | 15 Aug.†† | 71 ± 1.4 | 1166 | 45 | 138 | 53 | 55 |
| | 29 Aug.†† | 50 ± 1.1 | 1105 | 43 | 179 | 58 | 35 |
| | | SE‡‡ | 53 | 16 | 25 | 25 | |

† Measured from soil surface to tips of leaves extended along ruler. Each mean represents tillers from 24 plants.
‡ Each mean represents tillers from six plants.
§ SE values calculated using error term from sampling analysis of variance.
¶ From plants that were clipped 3 July.
# From plants that were clipped 15 July.
†† From plants that were clipped 26 July.
‡‡ SE across blocks and samplings.

leaves from stems at two stages of growth (Table 1), and on 18 July and 15 August when only tillers, representing two different heights, were sampled (Table 2). As previously described (3), an insulated box was used to transport samples to the laboratory where the leaf tissue was cut into pieces 1 to 2 cm² in area, each sample was thoroughly mixed, and a 2.50-g portion was removed for drying. These portions were dried at 75°C for at least 3 h, dry weights were determined, and the dry tissue was ground to pass a 1-mm screen. Ground samples were stored in small plastic vials in a laboratory freezer at about −18°C until they were extracted for assay.

Separate analyses of variance were calculated for each sampling. This allowed testing for significant differences among entry means and calculation of the standard errors of entry means within samplings. Also, HCN-p of upper leaves from main stems and of tillers was regressed on height. Two separate regression analyses were calculated. The first was a simple linear regression; the second included the second-order term for height, height², as well as the first-order term, height. The sequential analyses provided a means of determining whether or not a linear model was adequate in describing the relationship between HCN-p and height.

In a related study, three sorghum A-lines (KS8, N32, and

**Table 3. Height and hydrocyanic acid potential (HCN-p, dry weight basis) of three sudangrasses (NP25, Piper, and Greenleaf), three grain sorghums (AKS8, AN32, and ARedlan), and their nine $F_1$ hybrids.**

| Entry | Samples from plants not previously clipped | | | | | |
|---|---|---|---|---|---|---|
| | Sampled 18 June, youngest leaf with collar | | Sampled 23 July, upper leaves of tillers | | Sampled 23 July, flag leaves | |
| | Height | HCN-p | Height | HCN-p | Height† | HCN-p |
| | cm | mg kg⁻¹ | cm | mg kg⁻¹ | cm | mg kg |
| NP25 | 44 | 48 | 22 | 18 | 155 | 24 |
| Piper | 58 | 126 | 35 | 48 | 192 | 61 |
| Greenleaf | 54 | 303 | 37 | 206 | 170 | 149 |
| AKS8 | 39 | 90 | 37 | 12 | 115 | 28 |
| AN32 | 40 | 647 | 36 | 697 | 100 | 697 |
| ARedlan | 43 | 714 | 47 | 231 | 108 | 95 |
| AKS8 × NP25 | 57 | 56 | 35 | 28 | 193 | 27 |
| AKS8 × Piper | 60 | 85 | 41 | 25 | 203 | 23 |
| AKS8 × Glf. | 62 | 148 | 39 | 52 | 180 | 56 |
| AN32 × NP25 | 57 | 206 | 38 | 120 | 213 | 102 |
| AN32 × Piper | 59 | 291 | 47 | 190 | 213 | 191 |
| AN32 × Glf. | 56 | 461 | 40 | 115 | 207 | 216 |
| ARedlan × NP25 | 56 | 178 | 36 | 192 | 210 | 82 |
| ARedlan × Piper | 65 | 190 | 37 | 69 | 217 | 45 |
| ARedlan × Glf. | 56 | 413 | 37 | 317 | 210 | 192 |
| SE‡ | 3 | 39 | 4 | 105 | 4 | 35 |

| Entry | Regrowth from plants clipped 26 July | | | | Regrowth from plants clipped 26 July and 13 Aug. | |
|---|---|---|---|---|---|---|
| | Sampled 6 Aug. | | Sampled 23 Aug. | | Sampled 21 Aug. | |
| | Height | HCN-p | Height† | HCN-p | Height | HCN-p |
| | cm | mg kg⁻¹ | cm | mg kg⁻¹ | cm | mg kg |
| NP25 | 29 | 59 | 87 | 25 | 34 | 39 |
| Piper | 28 | 344 | 95 | 82 | 30 | 81 |
| Greenleaf | 29 | 937 | 97 | 217 | 32 | 536 |
| AKS8 | 24 | 655 | 73 | 38 | 28 | 67 |
| AN32 | 25 | 2513 | 68 | 1160 | 27 | 1533 |
| ARedlan | 23 | 3204 | 71 | 908 | 28 | 1306 |
| AKS8 × NP25 | 26 | 364 | 104 | 98 | 29 | 87 |
| AKS8 × Piper | 28 | 638 | 112 | 60 | 32 | 40 |
| AKS8 × Glf. | 28 | 1044 | 107 | 107 | 29 | 189 |
| AN32 × NP25 | 28 | 1012 | 104 | 213 | 31 | 223 |
| AN32 × Piper | 28 | 1580 | 98 | 271 | 33 | 396 |
| AN32 × Glf. | 27 | 1852 | 105 | 633 | 30 | 840 |
| ARedlan × NP25 | 28 | 1089 | 109 | 116 | 31 | 101 |
| ARedlan × Piper | 28 | 1311 | 103 | 465 | 31 | 285 |
| ARedlan × Glf. | 28 | 2030 | 106 | 568 | 30 | 682 |
| SE‡ | 1 | 167 | 4 | 96 | 1 | 85 |

† Canopy heights. Other heights were measured to tips of leaves extended along ruler.
‡ SE values calculated using error term from sampling analysis of variance.

'Redlan'), three sudangrass pollinators [the low-dhurrin population, NP25 (6), and 'Piper' and 'Greenleaf'], and their nine $F_1$ hybrids were seeded at the University of Nebraska Agronomy Farm on 9 May 1985, in 6.4-m rows spaced 0.76 m apart. The 15 entries were arranged in single-row plots in a randomized complete block design with three replications. The west half of each row was clipped (stubble height 10 to 15 cm) on 26 July, and the west one-fourth of each row was clipped a second time on 13 August. Six samplings of upper leaves from main stems or of upper portions of tillers were made between 18 June and 23 August (Table 3). Samples were harvested, dried, ground, and stored as described above.

### HCN-p Assay

Assays of HCN-p in seedling leaves were done spectrophotometrically following autoclaving of the leaves to extract and hydrolyze dhurrin (2). Portions of the dried and ground, field-grown leaf samples were extracted 2 to 3 h with water at room temperature, and dhurrin in the extracts was hydrolyzed with NaOH as previously described (3). The released cyanide was determined colorimetrically by a modification of the procedure of Lambert et al. (7).

## RESULTS AND DISCUSSION

### N32, KS8, and $F_3$ Lines

First leaves of the plants used in the main part of this study, sampled 1 week after seeds were planted, had the following HCN-p values (mg kg$^{-1}$ fresh wt, mean ± SE, $n$ = 120): BN32—649 ± 13; BKS8—922 ± 19; $F_3$, BKS8 × BN32—826 ± 15; and $F_3$, BN32 × BKS8—1070 ± 17. As previously observed (3), the seedling HCN-p level of KS8 was considerably higher than that of N32 despite the fact that N32 had a much higher mature-leaf HCN-p than did KS8. Also, both $F_3$ lines were high in seedling HCN-p although both came from $F_2$ plants that were very low in mature-leaf HCN-p.

Heights of plants prior to full head emergence were quite uniform across entries, as shown by the relatively small standard errors for height means (Table 1). The increase in standard errors accompanying heading was largely the result of the characteristically short peduncles of N32. For fully headed plants, heights of N32, measured from the soil surface to the tip of the panicle, were only about two-thirds as great as those for the other three entries.

All HCN-p means for upper leaves of N32 exceeded the 500 mg kg$^{-1}$ dry wt value that has been used as the limit between safe and doubtful or dangerous sorghum forage (4) (Table 1). Values for upper leaf samples of the other three entries were well below the 500 mg kg$^{-1}$ limit, with individual sampling date/entry means ranging from 19 to 71 mg kg$^{-1}$.

Past research, some of which dates back to the early 1900s, has shown that young plants and new regrowth are higher in HCN-p than mature leaves (1, 8, 9). It is not surprising, therefore, that in the present study, HCN-p values obtained for leaves from tillers and young regrowth (Table 2) were much higher than those for older leaves (Table 1). The only entry for which all means were < 500 mg kg$^{-1}$ was KS8. All N32 tiller means were high; in two instances (11 July and 8 August) the 500 mg kg$^{-1}$ level was exceeded by a factor of at least four. Samples of both $F_3$ lines that were harvested on 11 July and 8 August exceeded the 500 mg kg$^{-1}$ limit; otherwise, tiller samples of these two lines appeared to be reasonably safe based on this limit. Any risk of using these $F_3$ lines for livestock feed could be greatly decreased, of course, if the forage were harvested and fed in such a way that the animals could not sort out the young tillers.

It is widely recognized that a generally negative relationship exists between HCN-p and plant height in sudangrass and sorghum. This relationship is the basis for the recommendation that sudangrass and sorghum × sudangrass hybrids not be grazed until the plants have reached a height of at least 38 cm (12). In the present study, HCN-p was regressed on height for upper leaves of main stems as well as for tillers. For upper leaves, the simple regression coefficients were significant ($P < 0.01$) for all entries except KS8. However, the $r^2$ values were low, ranging from 0.03 to 0.23. When height$^2$ was added to the model, the $r^2$ values were affected only slightly with the range from the second-order model being 0.03 to 0.24. All of the regression coefficients for the second-order term were nonsignificant. Thus, there was a weak linear relationship between HCN-p and height, and there was no evidence of a nonlinear relationship between these variables.

For tillers, simple linear regression of HCN-p on height produced coefficients that were significant for each entry, but, as was the case with upper leaves, the relationship was weak. The $r^2$ values obtained from linear regression ranged from 0.32 to 0.36. With addition of the second-order term, height$^2$, to the model, $r^2$ values increased slightly resulting in values ranging from 0.39 to 0.45. The regression coefficients for height$^2$ were significant for all four entries. For each entry, the coefficient for height was negative, whereas the coefficient for height$^2$ was positive. Thus, the HCN-p of tillers tended to decrease with increases in height, but the rate of decrease lessened as height increased. Height was not a reliable indicator of HCN-p for either tillers or upper leaves from main stems.

### Sorghum × Sudangrass Hybrids

Comparison of the HCN-p of upper leaves from unclipped plants of three sorghums, three sudangrasses, and their nine $F_1$ hybrids revealed that only N32 exceeded the 500 mg kg$^{-1}$ level at all three samplings (Table 3). The 18 June samples of Redlan exceeded this level, and mean values for AN32 × Greenleaf and ARedlan × Greenleaf were not far below the 500 mg kg$^{-1}$ level on this date. Otherwise, HCN-p means of the previously unclipped plants of all entries in this part of the study were within the 500 mg kg$^{-1}$ limit.

High HCN-p levels were observed in young regrowth harvested 6 August from plants of most entries that were clipped on 26 July (Table 3). Only NP25, Piper, and the KS8 × NP25 hybrid were below the 500 mg kg$^{-1}$ level. Based on the 500 mg kg$^{-1}$ limit, regrowth of N32, Redlan, N32 × Greenleaf, Redlan × Piper, and Redlan × Greenleaf was still potentially dangerous at heights of 70 to 105 cm (sampled on 23 August). Regrowth from plants clipped on 26 July and again on 13 August and harvested 21 August was lower in HCN-p, for unknown reasons, than regrowth from plants of generally comparable height that were clipped on 26 July and sampled on 6 August. However, HCN-p levels of Greenleaf, N32, Redlan, N32 × Greenleaf, and Redlan × Greenleaf still exceeded 500 mg $kg^{-1}$ in the 21 August sampling.

Large variation in HCN-p occurred among the six samplings within each entry (Table 3), but rankings of the 15 entries were similar across samplings. Correlation coefficients between pairs of samplings($n$ = 15 for each of the 15 pairs of means) ranged from 0.58 to 0.98. All $r$ values were significant at $P$ = 0.05; all except one were significant at $P$ = 0.01, and all except three exceeded an $r$ value of 0.75.

Overall mean HCN-p levels, including all six samplings of the hybrids with KS8, N32, and Redlan as female parents were 174, 495, and 462 mg $kg^{-1}$, respectively. Comparable means for the hybrids involving the three pollinators were: NP25—239; Piper—342; and Greenleaf—551. These results indicate that from the standpoint of minimizing the risk of cyanide poisoning by sorghum × sudangrass hybrids, KS8 would be the seed parent of choice, and NP25 and Piper would be the preferred pollinators.

The nine hybrids and six samplings included in this study provided a total of 54 HCN-p means. Examination of the data in Table 3 indicates that 43 of these hybrid means fell between their respective parental means. Six of the remaining 11 means involved the low-HCN-p A-line, KS8, as the female parent and either NP25 or Piper as the pollinator, and HCN-p values for these six hybrids, although not always intermediate between parental values, were low and similar to the parental values. In general, the sorghum × sudangrass hybrids were like the KS8 × N32 hybrid (3) in having HCN-p levels that were intermediate between parental values.

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