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The Hydrocyanic Acid Content of *Cynodon plectostachyum* Pilger (Giant Star Grass) and its Suitability as a Pasture Grass.

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THE Director of Veterinary Services, Onderstepoort, Pretoria, was approached by Dr. I. B. Pole-Evans, Technical Adviser, Division of Botany and Plant Pathology, Pretoria, for information regarding the toxicity of *Cynodon plectostachyum* as this grass was considered to be an ideal one in combating soil erosion.

Since species of *Cynodon* are of major importance in the causation of hydrocyanic poisoning ("geilsiekte") in the Union of South Africa (Steyn, 1934), it was imperative to determine whether this grass could possibly be dangerous to stock.

TECHNIQUE.

Roots of the following four strains of *Cynodon plectostachyum* were obtained from the Officer in Charge, Rietondale Pasture Research Station, Pretoria:—

1. Star Mubende strain.
2. Star Fort Portal strain.
3. Star Kozinga Channel strain.
4. Star Mount Elgon strain.

These were planted in a reddish-brown sandy-loam in the Onderstepoort Poisonous Plant Garden in April, 1940. The grass remained dormant during autumn and winter. Growth was first observed in spring, i.e. September, 1940. The Mubende strain showed an excellent growth. The Fort Portal and Kozinga Channel strains grew less luxuriantly while the Mount Elgon strain showed a tardy growth. To assist the growth of the latter three strains they were irrigated until 24.10.40, by which date they also had attained good growth.

The specimens of *Sorghum verticiliflorum* Stapf [=*S. halepense* Nees (non Pers)] (Johnson grass) were collected from an already existent plot of this grass in the Onderstepoort Poisonous Plant Garden.

EXPERIMENTAL.

For analysis specimens of leaves were collected from the plots and immediately taken to the laboratory where they were minced as rapidly as possible and placed in weighed flasks containing the buffer and mercuric chloride solutions referred to below. The flasks were then again weighed, the difference in weights being the weight of the plant material added. On an average 20 gm. of leaves were taken for each analysis.

The enzymatic hydrolysis of cyanogenetic glucosides requires time and to prevent any destruction of hydrocyanic acid in this period mercuric chloride was added to the specimens as advocated by Briese and Couch (1938). Since cyanogenetic grasses may develop very large quantities of hydrocyanic acid when wilted it was considered advisable to add a larger quantity of mercuric chloride than used by Briese and Couch (1938), viz. 1 per cent. by weight of the plant matterial. Accordingly 1·0 gm. of mercuric chloride was added to each specimen. From the results of our analyses it is apparent that the above quantity of mercuric chloride did not greatly inhibit the enzyme action. In this respect it must be remembered that the mercuric chloride was present in a concentration of somewhat less than 0·5 per cent.

Couch and Briese (1939) and other workers quoted by them have demonstrated the influence of the hydrogen-ion concentration on the enzymatic hydrolysis of cyanogenetic glucosides and that for this process an optimum hydrogen-ion concentration exists which varies with different plants. These two authors also point out that the hydrogen-ion concentration may change during the process of hydrolysis and that the average hydrogen-ion concentration during the process, considered the optimum where the highest yield of hydrocyanic acid is obtained, may differ somewhat from the initial hydrogen-ion concentration. Therefore it was not exactly the optimum hydrogen-ion concentration for the liberation of hydrocyanic acid in *Cynodon plectostachyum* which was determined in the following tests, since specimens of the grass were placed in buffer solutions of varying hydrogen-ion concentrations and the buffer solution, in which the highest yield of hydrocyanic acid was obtained, was used for the subsequent analyses. For this purpose the Mubende strain was used and the results are given in Tables 1, 2 and 3. The material for the analyses was collected on three different occasions.

TABLE 1.

pH.	mgm. HCN per 100 gm.	pH.	mgm. HCN per 100 gm.
4·3	54·1	5·6	57·1
4·6	50·2	5·9	63
4·9	53·0	6·2	72·7
5·2	53·3		

TABLE 2.

pH.	mgm. HCN per 100 gm.	pH.	mgm. HCN per 100 gm.
6·1	44·4	6·8	59·9
6·3	44·4	7·0	65·3
6·5	53·2		

TABLE 3.

pH.	mgm. HCN per 100 gm.	pH.	mgm. HCN per 100 gm.
6·6	60·4	7·2	40·4
6·8	61·8	7·4	49·0
7·0	63·5		

It was assumed that the above results would also be applicable to the other three strains of *Cynodon plectostachyum* so that for all subsequent analyses a buffer solution with a hydrogen-ion concentration of 7·0 was used. The buffer solution for each specimen analysed consisted of 50 c.c. $\frac{M}{5}$ monobasic potassium phosphate and 29·63 c.c. $\frac{M}{5}$ sodium hydroxide diluted to 200 c.c. with water (Clark, 1922). The addition of mercuric chloride solution hardly affected the hydrogen-ion concentration.

In work of this nature it is necessary to have the results available as soon as possible and for this purpose the minimum period necessary for hydrolysis was determined.

TABLE 4.

Period of Storage.	mgm. HCN per 100 gm.	Period of Storage.	mgm. HCN per 100 gm.
1 day	53	2 weeks	68·1
3 days	68·2	3 weeks	65·2
1 week	68·8		

According to the results in Table 4 storing specimens for periods longer than 7 days does not greatly increase the yield of hydrocyanic acid obtained. This finding is in agreement with the work of Briese and Couch (1938), in which it is stated that the increase in the yield of hydrocyanic acid from specimens stored for periods longer than 7 days was gradual and small. The yield obtained by allowing hydrolysis to proceed for 7 days was therefore considered sufficiently near the maximum yield for our purposes.

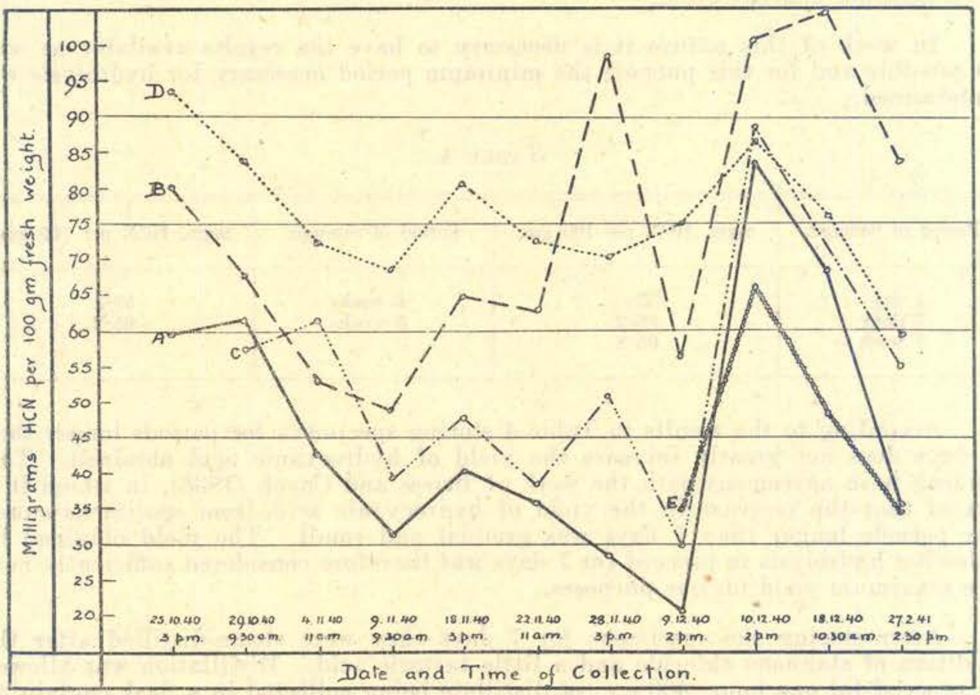
After storing the specimens for 7 days they were steam-distilled after the addition of stannous chloride and a little tartaric acid. Distillation was allowed to proceed for one hour, 400 c.c. of distillate being collected in a flask containing 10 c.c. of a 10 per cent. solution of sodium hydroxide. Further distillation of the specimens yielded very little, if any, hydrocyanic acid.

If distilling apparatus provided with rubber connections is used it is essential, as pointed out by Rimington (1932), to steam the apparatus for a few hours if the rubber connections are new, since, new rubber stoppers and tubing yield small quantities of hydrogen sulphide which interfere with the subsequent titration. Furthermore the ends of the glass tubing of such a connection should be in contact with each other so as to minimize the area of exposed rubber, since, as shown by Morris and Lilly (1933), rubber adsorbs hydrocyanic acid.

The hydrocyanic acid in the distillate was determined by the alkaline titration method as advocated by Green (1936).

The method used for the determination of hydrocyanic acid in *Cynodon plectostachyum* can therefore be briefly summarised as follows:—The plant material is rapidly minced and placed in a weighed flask containing 20 c.c. of a 5 per cent. mercuric chloride solution and 200 c.c. of a buffer solution having a hydrogen-ion concentration of 7·0. The flask is weighed again in order to calculate the weight of the plant material. The flask is set aside for 7 days after which 2·0 gm. of stannous chloride and a little tartaric acid are added to the contents of the flask which are then steam-distilled. Four hundred c.c. of distillate are collected in a flask containing 10 c.c. of 10 per cent. sodium hydroxide solution. The hydrocyanic acid is determined in the distillate, using a 100 c.c. aliquot, by the alkaline titration method (Green, 1936).

GRAPH I.



A=Star Mubende Strain; B=Star Fort Portal Strain; C=Star Kozinga Channel Strain; D=Star Mount Elgon Strain; E=Johnson Grass.

Graph I shows the hydrocyanic acid content of the four strains of *Cynodon plectostachyum* and of Johnson grass (*Sorghum verticilliflorum* Stapf), on which a few determinations were made for purposes of comparison.

Table 5 briefly gives data concerning the weather conditions on the days when the specimens were collected and on the preceding days.

TABLE 5.

Date.	Weather Conditions.	Date.	Weather Conditions.
21–24/11/40	Very warm.	21/11/40...	Cloudy, warm, a little rain.
25/10/40...	Very warm.	22/11/40...	Cloudy, warm, a little rain.
28/10/40...	Very warm, cloudy in the afternoon and rain from 7–8 p.m.	23–24/11/40	Cloudy and cooler.
		25–27/11/40	Warmer.
29/10/40...	Cool, cloudy.	28/11/40...	Fairly warm.
30/10/40–1/11/40	Very warm.	6–8/12/40..	Cloudy, warm.
		9/12/40....	Warmer.
2–3/11/40..	Cloudy, cool, large amount of rain.	10/12/40...	Extremely warm, grass wilted.
4/11/40....	Cloudy, cool.	11/12/40...	Cloudy, rain towards evening.
5–7/11/40..	Very warm.	12–13/12/40	Slightly cloudy, fairly warm.
8/11/40....	Cloudy, cool, large amount of rain.	14/12/40...	Rain, fairly cool.
9/11/40....	Cool.	15–16/12/40	Fairly warm.
10–13/11/40	Warm.	17/12/40...	Cool, raining all day.
14–15/11/40	Fairly warm.	18/12/40...	Warmer, sun shining.
16–17/11/40	Very warm.	22–23/2/41.	Raining, cool.
18/11/40...	Very warm, thunder clouds.	24–26/2/41.	Warm.
19/11/40...	Warm.	27/2/41....	Raining, cool.
20/11/40...	Warm, thunder clouds during night.		

Graph I clearly demonstrates the following:—

(1) As far as the hydrocyanic acid content is concerned the four strains of *Cynodon plectostachyum* can be placed in the following order, beginning with the strain having the highest hydrocyanic acid content: Mount Elgon strain, Fort Portal strain, Kozinga Channel strain, Mubende strain.

(2) Although on the whole the hydrocyanic acid content of the Mount Elgon strain was the highest, that of the Fort Portal strain was the highest under conditions of wilting.

(3) On the whole the hydrocyanic acid content of Johnson grass was lower than that of any strain of *Cynodon plectostachyum*. Johnson grass * has been the cause of poisoning in stock (Mathews, 1932), and contains dangerous quantities of hydrocyanic acid (Winks, 1940).

(4) Under different climatic conditions the hydrocyanic aicd content of the four strains of *Cynodon plectostachyum* and Johnson grass show a similar variation.

(5) In warm, dry weather the hydrocyanic acid content increases whereas in cool, humid weather it decreases.

(6) The effect of wilting is clearly shown by the tremendous increase in the hydrocyanic acid content of Johnson grass and all four strains of Star grass on 10.12.40 as compared with that on 9.12.40. This phenomenon is in close agreement with that found in our indigenous species of *Cynodon*. It should be mentioned here that in all our tests with fresh (unwilted) specimens of our indigenous species of *Cynodon* and the four strains of Star grass, the former species of grass have in all cases been found to contain much less hydrocyanic acid than the Star grass.

* Botanists in the different countries are not agreed that *Sorghum verticilliflorum* and *Sorghum halepense* are identical species.

(7) The difference in the hydrocyanic acid content of the four strains of Star grass is less marked when the grass is wilted than at other times.

Is *Cynodon plectostachyum* a Dangerous Pasture Grass?

In official correspondence with Dr. Pole-Evans, R. S. Ball, Plant Breeding Station, P.O. Njoro, Kenya, and D. C. Edwards, Department of Agriculture, Nairobi, Kenya, supplied information concerning *Cynodon plectostachyum* in Kenya. Ball states that except for scouring at the onset of the rains no trouble has been experienced with this grass and furthermore that no trouble has been caused by other species of *Cynodon*. Edwards also states that they had no trouble with Star grass and adds that the position in regard to hydrocyanic acid poisoning in Kenya is such that no authentic records of such poisoning on pasture exist. It would appear therefore that climatic conditions in Kenya do not favour at all the occurrence of hydrocyanic acid poisoning (" geilsiekte ") and it is therefore not surprising that under such conditions pastures of *Cynodon plectostachyum* can be grazed with impunity.

The Chief Research Officer, Department of Agriculture, Kuala Lumpur, Federated Malay States, in correspondence with the Chief, Division of Soil and Veld Conservation, Pretoria, and the Director of Veterinary Services, Onderstepoort, Pretoria, supplied the following analyses of *Cynodon plectostachyum*:—

Table 6

Strain.	Date of Planting Seedlings.	Moisture Content.	Hydrocyanic Acid.
		%	%
Fort Portal and Ripon Falls	5/3/40	72·8	0·004
Lake Shirva	5/4/40	71·6	0·0024

The average rainfall per annum is given as almost 100 inches. Under such conditions it is not surprising to find a low hydrocyanic acid content. The above figures are, however, surprisingly low, seeing that Fort Portal was one of the strains analysed. Unfortunately no details are given of the method used for determination of hydrocyanic acid.

In Australia *Cynodon plectostachyum* was recommended for possible use in pasture improvement and erosion control. Plots grown from seed obtained from Kenya were found to contain 0·14 per cent. of hydrocyanic acid and therefore seeds and cuttings of the plants were not being released (Editorial, 1940) as the plant was considered dangerous for stock.

Seddon and King (1930), on the results of their work on plant material containing sambunigrin or prunasin, regard a hydrocyanic acid content of 0·02 per cent. in the fresh plant as dangerous. Judged on this basis all four strains of *Cynodon plectostachyum* should definitely be regarded as dangerous.

Furthermore the hydrocyanic acid content of all four strains of *Cynodon plectostachyum* exceed, according to our analyses, that of Johnson grass which is a well-known cause of hydrocyanic acid poisoning.

In an endeavour to obtain definite information as regards the degree of toxicity of *Cynodon plectostachyum* sheep were, on several occasions, run on the plots of this grass in the Onderstepoort Poisonous Plant Garden. The sheep simply refused to eat the grass, even animals which had been starved for periods up to two days.

Dr. Merensky, Westfalia, Duiwelskloof, Pietersburg district, informed one of us (D.G.S.) that his animals would at the most eat the grass in the early morning but refuse to eat it from about 10 a.m. onwards. On the farm Westfalia we had the opportunity to compare the hydrocyanic acid content of *Cynodon transvaalensis* Burtt-Davy (" Transvaal kweek "), two strains of *Cynodon plectostachyum* (strains 469 and the " blue type "), and *Cynodon Bradleyi* Stent (Bradley lawn grass). The Guignard picrate paper test was employed for this purpose and all specimens were collected at 11 a.m. on 13.12.40. " Transvaal kweek " yielded negative results with the test, whilst Bradley lawn grass, and strain 469 and the " blue type " of the Star grass showed approximately 0·001, 0·01 and 0·03 per cent. of hydrocyanic acid respectively. Bradley lawn grass showed slight signs of wilting as it had encroached on to a cement furrow and was scorched by the sun, whilst the remaining three grasses appeared perfectly fresh. The annual rainfall on the above farm varies from 35 to 40 inches. It is of interest to note that, in the unwilted state, the hydrocyanic acid content of the two strains of Star grass growing at Westfalia is much lower than that of the strains growing at Onderstepoort, where the annual rainfall is much lower than in the Westfalia area.

Discussion and Conclusions.

From the results of our analyses it would definitely appear that, especially under certain climatic conditions, all four strains of *Cynodon plectostachyum* are capable of causing hydrocyanic acid poisoning. According to the effect of the weather conditions the danger would be least in parts with a high rainfall and which are cool, and greatest in parts with a low rainfall, which are at the same time very warm. This is especially so since in the latter parts wilting may easily occur. It is a well-known fact that " Geilsiekte " (hydrocyanic acid poisoning) occurs most frequently in the warmer and drier parts of the Union of South Africa (Steyn, 1934).

Consequently, whereas the grass may not be very dangerous in those parts of the Union of South Africa with a high rainfall, the authors consider that *Cynodon plectostachyum* would constitute a very grave danger to stock in most parts of the Union of South Africa, especially in the more arid areas. It should be added that a number of factors play a rôle in the production of toxic quantities of hydrocyanic acid in plants, especially in grasses. The most important of these factors are: (1) the stage of development of the plant, e.g. the younger the grass the more likely it is, when wilted, to develop toxic quantities of hydrocyanic acid; (2) climatic conditions, e.g. spasmodic rains followed by hot and dry weather, and hot and dry winds are conditions which are very favourable to the production of fatal amounts of hydrocyanic acid; (3) soil types and fertilizers—dry sandy soil, poor soil and *nitrogenous fertilizers* (Burtt-Davy, 1912, and Couch, 1932) facilitate the production of hydrocyanic acid in plants; and (4) plants which are inclined to develop hydrocyanic acid during the process of wilting, are extremely dangerous when damaged by frost. Another important factor in the determination of the toxicity of cyanogenetic feeds is the rate at which they are ingested.

From the above information it is obvious that any grass, which in an unwilted state contains hydrocyanic acid (as in the case with the above four strains of " Star " grass) is extremely likely to develop highly toxic and even fatal quantities of this poison under certain soil and climatic conditions. Such grasses may constitute very valuable pasture in areas with a high rainfall and(or), under normal climatic conditions, but are extremely dangerous in areas with a low rainfall and(or) under abnormal climatic conditions as described above. The whole of the Union of South Africa is notorious for the irregularities of its climatic conditions. It should be noted that the " Star grass " will grow satisfactorily only in areas with a high and regular rainfall.

The following is a quotation from a publication by Ball and Robbins (1941): " Johnson grass. European species, introduced about 1884 as forage crop and widely planted until 1903, when planting was prohibited by State Law. Rapid spread in the State attributable to consignments of Sudan grass seed, containing as an impurity seeds of Johnson grass. Johnson grass now infests several thousand acres of agricultural lands in some 40 counties of the State." Comparative tests conducted at Onderstepoort by one of us (S. J. v. d. W.) have shown that under the same soil and climatic conditions, and when the different grasses tested were wilted, Johnson grass contained less hydrocyanic acid, and is therefore less dangerous, than the four strains of star grass with which it was compared. Twenty years after the introduction of Johnson grass as a forage crop into the State of California, the authorities realised its dangerous nature and declared it a noxious weed. According to the authors' tests Johnson grass is less dangerous than the four strains of Star grass and the experience with Johnson grass in California should be a well-founded warning to us as far as the introduction of Star grass on an extensive scale into all parts of the Union of South Africa is concerned. According to Ball and Robbins (1932) Johnson grass is one of the major noxious weeds of California.

It is for the above reasons that we cannot but feel that the introduction of " Giant Star grass " as a pasture grass into the Union of South Africa would constitute a grave danger to stock. Under normal climatic conditions it may prove a good pasture grass in areas with a high rainfall, but even in such areas there is grave danger of heavy losses among stock when abnormal climatic conditions prevail.

This article is a report on investigations which have been made at Onderstepoort into the toxicity of Star grass. Further investigations are being made at various agricultural colleges and pasture research stations and the results will be published in due course.

If the " Giant Star grass " is considered to be of outstanding importance in combating soil erosion it should be possible to breed, for this purpose and incidentally also for pastures, hydrocyanic acid-low or hydrocyanic acid-free strains on the same lines as is being done with Sudan grass in the United states of America (Nowosad and MacVicar, 1940) and in South America (Bonjour, 1937). There are many strains of Giant Star grass and they will definitely vary in their hydrocyanic acid content. It is possible that some of them may contain such small amounts of this poison that they will be much less dangerous than the four strains examined by us; and there is also the possibility that some of the strains may be so low in their hydrocyanic acid content that they may be harmless. Well-cured hay, prepared from cyanogenetic plants can as a rule be safely fed to stock. There is no reason why this should not be the case with Giant Star grass.

The efficacy of feeding sulphur in licks, or dosing it (Steyn, 1940), in the prevention of " geilsiekte " in stock, depends on the hydrocyanic acid content of the plants concerned. Under field conditions the feeding of sulphur yields excellent results in the prevention of " geilsiekte " caused by our indigenous species of *Cynodon* (and other grasses) and there is good reason to believe that similar results will be achieved in the case of Giant Star grass. On the other hand, it would appear very unwise to propagate plants which are so dangerous that we continually have to administer antidotes.

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