Preliminary Report upon the Occurrence of Hydrocyanic Acid in the Grasses of Bechuanaland.

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It is generally known that compounds of hydrocyanic acid are present in many edible plant leaves (see for instance Rosenthaler, 1921), but continental European observers have, in general, not regarded these hydrocyanic acid containing plants as poisonous when fed to cattle. In all probability the amount present in Europe is insufficient to produce any visible effects.

In America, however, Swanson, 1921, published a paper in which he showed that Sorghum and Andropogon produced hydrocyanic acid upon wilting, whereas the fresh material contained no free acid, although it may contain glucosides. Cattle fed with some of this wilted grass became ill; horses proved much less susceptible.

In a series of experiments dealing with carbohydrate metabolism, it was incidentally observed that hydrocyanic acid was frequently present in wilted grasses; in some cases in such quantities as to be easily detected by its characteristic smell in the aequeous solutions used in the studies. Before carrying out further investigations into the toxicity of the wilted plants it was thought advisable to ascertain the frequency of the occurrence of the acid by some simple method.

METHOD.

The method used in this investigation was the "vest-pocket test" for cyanogenesis, adopted by Green for field work of this Division. It is based upon the Guiguard reaction as used by H. E. Armstrong (1911, pp. 471–483), and is described in a footnote of a paper by Green in the ninth and tenth reports of this Division, in words which may be repeated here for the benefit of any botanists unfamiliar with this exceedingly simple test—

> "Picrate solution and papers, 5 gm. sodium carbonate and 0·5 gm. picric acid in 100 c.c. water. Wet ordinary filter-paper with this, hang up to dry until only just 'perceptibly moist,' and cut into convenient strips, about 1 cm. by 4 cm. Papers should be made up fresh every week as sensitiveness decreases with time. The solution keeps well for months in a stoppered bottle.
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> "*Test.*—Into a stout glass tube, about $1\frac{1}{2}$ cm. by 7 cm. or other convenient vest-pocket size, push a few grammes of the moist shredded plant (or moist pulverized seed). Add two or three drops of chloroform to hasten autolysis, insert a slip of 'perceptibly moist' picrate paper at the top, and cork tightly. Incubate in a vest-pocket, examining at intervals. Liberation of HCN is indicated by reddening of the yellow picrate paper—within a few minutes if the amount is large, after twenty-four hours if only traces are present. If the paper remains lemon-yellow

it either means that a cyanogenetic glucoside is absent or that a hydrolytic ensyme is not intimately associated with it. In the latter case chemical analysis may still show hydrocyanic acid, but with the majority of plants analysis will not show much if the simple test fails. It may be added that the test is so delicate that cyanogenesis is revealed in a large number of common non-toxic edible plants, an easily comprehensible fact in view of the significance of cyanogen in normal plant anabolism."

As it was inconvenient to adopt the vest-pocket procedure for attaining warmth, and as the daily temperature when the determinations were made was over 30° C., the reactions were allowed to take place at room temperature.

Results.

A previous analysis of one of the Armoedsvlakte grasses, *Chloris petraea*, had shown the presence of a perceptible amount of hydrocyanic acid. This grass was therefore tested at various stages of its development, and at each test a considerable amount of liberated hydrocyanic acid was found, i.e. in less than one minute the picrate-paper turned red.

This perennial is not abundant at Armoedsvlakte, and in the last few years it has gradually become scarcer, even although it regularly produces seeds. On account of its present scarcity there is little chance of stock poisoning taking place.

It is interesting to note that the annual *Chloris virgata*, which only appears with the rains and dies down soon afterwards, does not contain a trace of hydrocyanic acid, so that the presence of hydrocyanic acid cannot be taken as a generic characteristic.

Further investigations were carried out at intervals from September, 1923, to January, 1925, when the following grasses were tested :—

Digitaria eriantha,
Anthephora pubescens,
Eragrostis superba and *lehmanniana*,
Chrysopogon serrulatus,
Fingerhuthia africana,
Sporobolus fimbriatus,
Themeda triandra,
Cymbopogon plurinodis,
Aristida uniplumis,
Aristida congesta, and
Pogonarthria falcata.

As a general rule, it was found that none of these grasses while fresh yielded any hydrocyanic acid, but that practically all did so under certain conditions. One of the conditions is wilting, or at least incipient drying, in spring. It is peculiar that hydrocyanic acid (i.e. its compounds) occurs more frequently and more abundantly before the rains in spring, or after the spring rains if conditions are such as to cause wilting of the plants, than it does during the summer drought periods.

The various grasses were found to contain different amounts of cyanogenetic substances. *Anthephora pubescens*, *Digitaria eriantha*, and *Chrysopogon serrulatus* when in a wilted or withered state, contained large quantities in the sense that an intense reaction was given by picrate papers. Chrysopogon, when in its typical drought condition, contains as much as, or even more than, Chloris. Anthephora and Digitaria generally contain less, but they show their hydrocyanic acid at earlier stages of wilting. Even if they were only slightly withered, the paper turned red within two hours, whereas in Chrysopogon the reaction did not occur at all unless the plant was strongly withered, but the paper then reddened immediately.

Aristida congesta always contained some cyanogenetic substances when the leaves were "rolled," and occasionally the amount present was so large as not only to cause the paper to turn red immediately, but also to redden any drops formed on the sides of the tube in contact with the paper.

Aristida uniplumis, *Pogonarthria falcata*, and, on very dry days, *Themeda triandra* and *Sporobolus fimbriatus*, yielded a little hydrocyanic acid. In September, 1923, *Themeda triandra* showed more, whereas in January, 1924, during a drought, it showed none at all. As the season 1924–25 was not dry, all further tests on Themeda and Sporobolus gave negative results.

In this connexion, the investigations of Armstrong may be recalled (1911). He found for *Lotus corniculatus* that samples taken from the same habitat showed in one year a considerable quantity of cyanogenetic substance, in another year none at all. In the same year the cyanogenetic bodies were more abundant in stunted drought forms of the species than in luxuriantly growing samples. In the northern parts of Europe cyanogenesis could scarcely be detected in Lotus. Armstrong attributes the occurrence of cyanogenetic glucosides to strong and continuous insolation, although individual differences may play a certain rôle. For the data recorded here the same factors may apply. In the extreme spring of 1923, with frequent dwarf forms of grasses, the cyanogenetic substances were more abundant than in the more temperate spring of 1924.

Under no circumstances could the presence of compounds of hydrocyanic acid be demonstrated in *Eragrostis superba*, *Eragrostis lehmanniana*, *Cymbopogon plurinodis*, and *Fingerhuthia africana*. It is noteworthy that grasses which contain very little or no cyanogenetic compounds have a purplish-red colour during drought periods, whereas grasses with cyanogenetic compounds never show anthocyanin. The formation of the anthocyanin is due to the same factors as is the reddening of the leaves of tropical trees during droughts (Miyoshi, 1909). The grasses in Armoedsvlakte seem to show a correlation between the appearance of Anthocyanin and cyanogenetic substances. As stated above, hydrocyanic acid was not found in fresh grass, except in the case of *Chloris petraea*. Wilted grasses, tested again as soon as they were again turgescent after the rains, still showed cyanogenetic substances in varying measure, but some *hours later* the test was negative.

Nature and Rôle of the Cyanogenetic Substances in the Grasses.

Neither the nature nor the rôle of the cyanogenetic substances present in the grasses examined is definitely known. It is possible that the cyanogenetic substance present in the fresh grass is a different compound from that present in the wilted or withered grass. Swanson, 1921, definitely speaks of free HCN in wilted grasses.

It is probable that the cyanogenetic substance of Chloris is a cyanogenetic glucoside of the Dhurrin type, which includes the glucosides of p-oxy-amygdalenic acid (Czapek, III, 1921, p. 213). These glucosides have been found in various North African and South American grasses.

The part played by cyanogenetic substances in plant metabolism has been widely discussed of recent years, but there seems to be no unanimity in authoritative opinion. The view is generally accepted that they arise in the chloroplastids, and are most abundant when protein metabolism is most active (Czapek, III, 1921, pp. 217–218). This may be the case in *Chloris petraea*. Their appearance in a large variety of grasses during the process of wilting may, however, be due either to katabolic processes or to an accumulation of certain products of anabolism. It is not impossible that during wilting hydrocyanic acid may arise katabolically by oxidation of amino-acids (Czapek, 1920, II, pp. 57 and 288), although the process has never actually been studied in plants. Since, however, hydrocyanic acid may be regarded as an intermediary anabolic product, produced from absorbed nitrates and on its way up to plant proteins, an accumulation under conditions of wilting, in which the later anabolic phases of nitrogen metabolism are interfered with, seems quite feasible. In another paper, now in preparation for publication, it is shown that during wilting carbohydrates disappear, or are formed in less degree than in fresh plants. Ullrich (1924, p. 523) regards carbohydrates as essential for the purpose of protein formation. Without excluding the possibility of production in katabolism, it is therefore not unreasonable to suppose that the sudden appearance of cyanogenetic substances during wilting is due to accumulation of intermediary anabolic products not further built up by the plant as fast as usual. The fact that they disappear again a few hours after the wilted tissues have been rendered turgescent by rain might be regarded as lending colour to this view.

Summary.

The occurrence of cyanogenetic compounds in grasses which do not show such in the normal fresh state is common during wilting or withering of natural veld in Bechuanaland. The phenomenon is particularly noticeable at the time of the spring rains, and different grasses vary considerably in regard to stage of wilting at which it occurs. These compounds appear suddenly in considerable amounts and disappear again a few hours after the wilted tissues recover normal turgescense.

Their formation may be explained as due either to katabolic processes or to an accumulation of products of anabolism, the further disposal of which is interfered with by disturbance of the later phases of nitrogenous and carbohydrate metabolism.

LITERATURE.

1911.—Armstrong, Henry E.; Armstrong, Frankland E.; and Horton, Edward. Herbage Studies: *Lotus corniculatus*, a Cyanophoric Plant. *Proceedings of the Royal Society of London.* B. Vol. LXXXIV, pp. 471–484.

1913–1921.—Czapek, Friedrich. Biochemie der Pflanzen, Jena, G. Fischer.

1909.—Miyoshi. *Journal Coll. Sci.*, Tokyo. Art. 2; cited after Czapek, I, p. 593.

1919–1921.—Rosenthaler, L. Beiträge zur Blausäurefrage, Versuche in Tabellenform, Schweiz. *Apotheker-Zeitung*, 57, 1919 (pp. 267–270, 279–283, 295–297, 307–313, 324–329, 341–346); 58, 1920 (pp. 17–20, 137–142); 59, 1921 (pp. 10–13, 22–26, 465–469, 641–647).

1921.—Swanson, C. O. Hydrocyanic Acid in Sudan Grass. *Journal of Agricultural Research*, XXII, pp. 125–138.

1924.—Ullrich, Hermann. Die Rolle der Chloroplasten bei der Eiweissbildung in den grünen Pflanzen. *Zeitschrift für Botanik.* Vol. XVI, pp. 513–562.