

## Comparative Study of the Content of Starch and Sugars of *Tribulus terrestris*, Lucerne, some Gramineae and *Pentzia incana* under different Meteorological, Edaphic and Physiological Conditions.

### Paper No. 2.—Carbohydrate Nutrition.

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In a paper dealing with the assimilates of lucerne (Henrici, 1949) it has been shown that the physiological condition of the plant, particularly its water content, has a large effect on the ratio of starch and sugars present in leaves and stems. These carbohydrates themselves, according to Quin (1943), have a far-reaching influence on such processes and diseases as bloating and dikkop in the animal. *Lucerne* is the plant which mostly causes bloating, *Tribulus terrestris* generally causing dikkop under specific conditions. Yet, particularly in drought, *Tribulus* may cause bloating if no other food is available in the veld, and vice versa, *lucerne* may lead to dikkop. Certain Gramineae like *Panicum laevifolium*, are the root of the less severe dikkop; according to the views of the veterinarians these diseases and occurrences are related, much more than is thought *a priori*, in such a way that all these plants contain one common chemical principle. Whilst there are quite a number of plants causing one of the phenomena mentioned above, it can be definitely stated that no Karoo bush ever caused bloating or dikkop. It was therefore thought advisable to make a comparative study of the assimilates of the different plants.

The principle was accepted that all the plants investigated contain starch, sucrose and reducing sugars (fructose and glucose), perhaps also some other sugars like fructosan, and that the main difference would be in the ratio of the various assimilates.

#### PLANTS INVESTIGATED.

The plants investigated were *Tribulus terrestris*, *Panicum laevifolium* var. *minus*, Algerian oats and *Pentzia incana* var. *Klein gansie*. The grasses and Karoo bush were grown on plots of the Veld Reserve, Fauresmith, and had rain as the only source of water. *Tribulus terrestris* was also collected from the Veld Reserve. Yet, as it became obvious in the course of investigation that in its case a soil factor played a large rôle, *Tribulus* was collected from very different soils

outside the Reserve, for instance at Oranjezicht, between Colesberg and Philippolis and at Havenga bridge, etc. These places were known to produce poisonous *Tribulus*; the sampling at Oranjezicht was actually done whilst sheep were dying in hundreds from dikkop.

As in the past, the poison of *Tribulus* had disappeared as soon as the plant was transported from an outbreak to the nearest laboratory and the author was convinced, as will be outlined later, that a soil factor played an important rôle. Soil from places which were known for bad dikkop outbreaks, was brought in tons to the Veld Reserve, Fauresmith, and large beds were filled with soils from Calvinia and from two wellknown farms in the Fauresmith district, Waagkop and Leeuwfontein. The beds will be described later. These beds were broadcast with seed of local *Tribulus terrestris*. It is quite likely, however, that some seeds from the original places were present in the soil and germinated as well. According to Schweickerdt (1937) however, all the small *Tribulus* of the different areas are of the same species viz. *terrestris*. The different growth habits of *Tribulus* have been described earlier (Henrici 1938).

Most work described in this paper was done with *Tribulus*, the other plants being chiefly for comparison. It was a very heterogeneous collection of species consisting of an annual like *Tribulus* growing very quickly, a perennial grass like *Panicum laevifolium* with no live aerial parts in winter, an annual *Gramineae* like *Algerian oats*, and a slow-growing perennial bush like *Pentzia*. Apart from the different types of slow and quick growers, plants were chosen which vary very widely as regards their resting periods.

In the Karoo bush a resting period is enforced in winter more by drought than by low temperature without the dying off of the aerial parts; another one often occurs in summer, caused by drought alone. *Tribulus* naturally dies off after the first cold days and has no resting period at all. In grass the aerial parts are lifeless in winter caused by drought as well as by cold.

#### WORKING HYPOTHESIS.

Botanically speaking, dikkop and bloating are only produced by plants growing quickly, which in one season may even have more than one generation, or by frequent cutting of irrigated plants (lucerne), but never by a slow-growing Karoo plant. It is therefore feasible that annuals and quick growers have a common physiological or bio-chemical condition which is absent in slow growers. For the single species special complications may occur, aggravating the primary cause of the disease, like the appearance of phylloërythrin in the bowels of animals after the intake of *Tribulus* (Rimington, 1933).

When the physiologist is asked what properties would be common to the annuals mentioned above and what properties would differ from the Karoo plant, a few things could be mentioned: (a) The type of growth, flush growth alternating with a complete standstill of growth, (b) a large chlorophyll content, and (c) the easy wilting and recovery of the aerial parts. There may be other points, but these will suffice for the present. The Karoo plant does not wilt easily, has a smaller chlorophyll content and has no flush periods of growth. One might ask whether these differences would cause changes in the fundamental metabolism of the plants. It is likely that they do, particularly the wilting. The plants were therefore tested either in the fresh or drooping state or when permanently wilted. *Pentzia incana* which does not show signs of wilting even with a heavy loss of water, was collected fresh or after a few weeks' drought, when the water content of the leaves had dropped considerably and the leaves were getting brittle.



From practical experience it was clear, however, that *wilting alone* was not causing the appearance of a poisonous substance in the annuals. It must be remembered that all the investigated plants are good fodder plants. In 1932 three sheep were kept on half a morgen plot of *Tribulus* on the Veld Reserve, Fauresmith, for 2 months. All sheep put on weight and were in excellent condition, although the *Tribulus* wilted and drooped more often than not, recovered again and faded away so that it could hardly be noticed. In the same way our trampled and wilted lucerne and grass did not cause a single sheep to get ill. On the other hand it could not be denied *a priori* that wilting might aggravate a disturbed metabolism (Henrici, 1946, II).

Although the author subscribes to the view of the veterinarians that the direct assimilates are in some way connected with the occurrence of the disturbance and diseases mentioned above, she is not of the opinion that they are the primary cause. From all her experience and from reports in the literature of the veterinarians she is convinced that a soil factor is the primary cause of the involved phenomena of dikkop and bloating. In the following paragraph a hypothesis will be expounded how, in the opinion of the author, the whole mechanism works. It must be understood that owing to war conditions the particular factor in mind, viz. zinc deficiency at the time of flush growth, could not be investigated spectrographically. This part of the work was supposed to be undertaken by a specialist at Stellenbosch, but no results are as yet available.

It may or may not be true that a soil factor is of paramount importance, as the results will show later. The theory of a temporary zinc deficiency is based on descriptions and observations recorded by Hoagland (1944). He mentions that generally speaking there is enough zinc in the soil, but different plants cannot all take it up in the same way, some are able to take more, others much less. A deficiency may occur in some plants especially in flush periods of growth. This deficiency may be remedied very quickly as soon as the flush period is over. Zinc deficiency only occurs under high illumination and with high temperatures, never in a foggy country. The rôle of zinc itself in the plant is not fully understood, only the results of the deficiency are known. They are: Stoppage of growth, resulting in dwarf plants; increase of peroxidase in the leaves; destruction of the auxin and chlorophyll, accelerated by the peroxidase activity; dissolution of starch, but no decrease of sugars; the appearance of phytosterine and polyphenolic substances (Reed and Dufrénoy, 1942).

Anybody who has worked for any length of time with *Tribulus* has seen many of these changes, but it must not be forgotten that similar changes may be caused by wilting alone. The appearance of the phytosterine, however, has never been recorded.

In spite of the fact that owing to war conditions the validity of the hypothesis of deficiency of zinc could not be proved, there was no reason to delay in continuing the research on plants from different soils. For this purpose beds 3 feet deep in cement frames, 8 × 8 feet were established at the Veld Reserve, Fauresmith, and soil from Calvinia and 2 other farms of the Fauresmith district, all renowned for their dikkop outbreaks, was carted into these beds. Drainage was facilitated by a layer of coke between Fauresmith soil and foreign soil. Only rain or distilled water was used for irrigation, water which was definitely free of zinc. Coke and cement proved to be free of zinc. As considerably more water was needed than was provided by rain in a drought, only *Tribulus* could be established on all 6 beds in the first year. (*Tribulus* seeds collected at Fauresmith were broadcast and germinated very well). A zinc-free water supply roof and

tank will be established later to enable lucerne and grass planting as well. Lucerne was also planted in Calvinia soil in pots and irrigated only with zinc-free water. The beds with *Tribulus* enabled us to investigate the contents of assimilates and the eventual glucoside of *Tribulus* on different soils under the same conditions, which arrangement was apt to show up the influence of a soil factor, especially the zinc deficiency.

#### METHODS OF SAMPLING AND ANALYSES.

The normal daily march of the direct assimilates has to be known before the variations due to wilting or drying or due to the soil factor can be studied. To ascertain the daily march the plants were collected four and later five times during 24 hours and thrown directly into 96 per cent. alcohol to prevent enzymatic reaction. After 24 hours they were taken out and separated in stems, leaves, fruits and roots in the case of *Tribulus*, and in leaves and stems in the case of grasses and Karoo bush. In cases of outbreak of dikkop single samples were taken on farms away from Fauresmith. Part of the samples was immediately put into alcohol, part put on ice as soon as possible. In these cases it was more important to get the sample on ice as quickly as possible than to get a daily curve. Dry matter was determined on special samples.

In the case of progressive wilting which lasted for a long period, samples were taken at a definite time once a day. A thermohydrograph was put into the beds with the plants to get the necessary meteorological data.

The chemical methods used for the determination of starch and sugars were the same as outlined in the previous paper (Henrici, 1949). As it became apparent later in the investigation that it was not necessary to distinguish between fructose and glucose in their effect on the animal, Van der Planck's (1936) method was abandoned. For the sugars Bertrand's (Klein, 1932, II, pp. 783-786) method was solely used, a value for reducing sugars being obtained after filtering with yeast, and a value for total sugars after inversion with invertase. The difference of the two values gave the sucrose.

When it became evident that the soil factor (zinc deficiency) made itself felt in the constituents of the plant, in other words, that a saponin-like substance was found in plants of specific soils, the following methods for characterising the substance were used: The colour reaction of saponins with sulphuric acid (Tunmann, 1913, pp. 388-390); the haemolysis of blood gelatine (Kofler in Klein III/2, p. 1105; the determination of haemolysis in defibrinated blood of sheep (Kofler, 1927, p. 151 ff.); the inhibition of the haemolysis by addition of cholesterolin (Kofler, 1932, in Klein III/2 p. 1104); methods of isolation were based on Kofler (1927 and 1932), Sieburg (1923) and Kobert (1912). Perhaps Rosenthaler's (1923) guide for plant physiologists helped the author most in getting an idea what kind of substance was actually present. For further chemical information Legal (Meyer, 1931) and Brady's tests (Brady, 1931) were used. To determine melting points in the latter reaction, a heating stage on the microscope was used.

#### RESULTS.

##### A. TRIBULUS TERRESTRIS. (TABLES 1, 2, 3.)

##### I. Daily march of assimilates under different physiological conditions on Fauresmith soil. (Table 1, Graph 1.)



TABLE 1.—Sugar and starch: *Tribulus terrestris* from Fauresmith soil.

No.	Date.	Time.	PER GRAM DRY MATTER.						Water Content per gram Fresh Matter.	Organ.	Remarks.		
			Starch.	Total Sugars.	Sucrose.	Reducing Sugars.	Glucose.	Fructose.				Fibre.	
2457	22. 3. 43	12 a.m.	3.63	2.45	0.97	1.48	1.04	0.40	—	90.5	Stems.....	Fresh.	
2458			2.54	2.33	1.32	1.01	0.62	0.39	—	—	—	Leaves.....	Fresh.
2459		7 p.m.	4.08	2.39	1.05	1.34	0.62	0.72	—	—	Thorns.....	Fresh.	
2460			2.28	3.25	1.86	1.39	0.97	0.43	35.18	74.08	Stems.....	Fresh.	
2461		12 p.m.	1.76	3.90	1.73	2.17	1.01	1.16	0.43	—	—	Leaves.....	Fresh.
2462			3.99	2.59	0.53	2.06	0.00	2.06	—	85.8	Thorns.....	Fresh.	
2463	2.51		1.28	0.23	1.05	0.73	0.32	—	—	Leaves.....	Fresh.		
2464	5.08		2.90	0.77	2.13	0.14	1.99	—	—	Thorns.....	Fresh.		
2465													
2466	23. 3. 43	7 a.m.	2.02	2.50	0.95	1.55	0.36	1.19	—	90.5	Stems.....	Fresh.	
2467			2.24	2.00	0.38	1.62	0.27	1.35	—	—	Leaves.....	Fresh.	
2468			2.58	2.73	0.53	2.20	0.23	1.97	—	—	Thorns.....	Fresh.	
2469	29. 3. 43	12 a.m.	4.24	3.25	2.24	1.01	0.10	0.91	32.17	67.78	Stems.....	Temporarily wilted	
2470			1.70	1.85	0.78	1.07	0.17	0.90	17.37	—	—	Leaves.....	Temporarily wilted
2471		7 p.m.	2.90	3.17	2.13	1.04	0.31	0.73	—	—	Thorns.....	Temporarily wilted	
2472			6.37	2.50	1.62	0.88	0.06	0.82	56.1	—	—	Stems.....	Temporarily wilted
2473		12 p.m.	3.21	1.72	0.74	0.98	0.24	0.74	—	—	—	Leaves.....	Temporarily wilted
2474			3.36	3.33	2.26	1.07	0.10	0.97	—	—	—	Thorns.....	Temporarily wilted
2475	4.23		3.05	1.94	1.11	0.16	0.95	—	80.9	—	Stems.....	Fresh.	
2476	3.87		1.78	0.75	1.03	0.19	0.84	—	—	—	Leaves.....	Fresh.	
2477	30. 3. 43	7 a.m.	2.56	6.43	5.30	1.13	0.41	0.72	—	—	Thorns.....	Fresh.	
2478			5.15	6.35	5.26	1.09	0.24	0.85	—	74.83	—	Stems.....	Fresh.
2479			2.16	1.79	0.85	0.94	0.27	0.67	—	—	—	Leaves.....	Fresh.
2480			1.53	4.98	3.75	1.23	0.14	1.09	—	—	—	Thorns.....	Fresh.
2489	8. 4. 43	12 a.m.	0.93	1.76	0.94	0.82	0.00	0.82	38.40	43.11	Stems.....	Wilted.	
2490			1.05	1.50	0.62	0.88	0.00	0.90	19.36	—	—	Leaves.....	Wilted.
2491		7 p.m.	0.50	1.37	0.72	0.65	0.00	0.65	—	—	Thorns.....	Wilted.	
2492			1.23	1.45	0.70	0.75	0.02	0.73	—	44.64	—	Stems.....	Wilted.
2493			0.62	1.50	0.64	0.86	0.17	0.69	—	—	—	Leaves.....	Wilted.
2494			0.59	1.64	0.94	0.70	0.05	0.65	—	—	—	Thorns.....	Wilted.
2495	12 p.m.	1.72	1.52	0.78	0.74	0.00	0.75	—	—	44.63	Stems.....	Wilted.	
2496		1.42	1.39	0.45	0.96	0.06	0.90	—	—	—	Leaves.....	Wilted.	
2497			0.42	1.36	0.52	0.84	—	—	—	—	Thorns.....	Wilted.	

CARBOHYDRATE NUTRITION.

TABLE 1 (CONTINUED).

No.	Date.	Time.	PER GRAM DRY MATTER.						Water Content per gram Fresh Matter.	Organ.	Remarks.	
			Starch.	Total Sugars.	Sucrose.	Re- ducing Sugars.	Glucose.	Fruc- tose.				Fibre.
2498	8. 4. 43	7 a.m.	0.32	0.84	0.09	0.75	—	—	—	55.65	Stems.....	Wilted.
2499			1.01	0.89	0.03	0.86	—	—	—	—	Leaves.....	Wilted.
2499a			0.72	1.03	0.36	0.67	—	—	—	—	Thorns.....	Wilted.
2371	30.12.42	Midday	2.19	1.47	0.50	0.97	—	—	—	—	Aerial parts	Fresh.
2412	10. 2. 43	Midday	1.37	0.55	0.19	0.36	—	—	—	—	Aerial parts	Wilted and recovered
2415	11. 2. 43	Midday	1.73	1.11	0.26	0.85	0.20	0.65	—	—	Aerial parts	Wilted.
2650	21.12.43	12 a.m.	6.66	2.34	0.78	1.56	—	—	—	68.42	Stems.....	Fresh.
2651			4.31	1.40	0.32	1.08	—	—	—	—	Leaves.....	Fresh.
2652			3.33	3.07	1.04	2.03	—	—	—	—	Thorns.....	Fresh.
2653			5.22	2.13	1.20	0.93	—	—	—	69.82	Roots.....	Fresh.
2654		7 p.m.	5.88	1.90	0.00	1.90	—	—	—	—	Stems.....	Fresh.
2655			4.84	1.77	0.39	1.38	—	—	—	—	Leaves.....	Fresh.
2656			3.15	3.13	0.97	2.16	—	—	—	—	Thorns.....	Fresh.
2657			5.49	2.75	1.47	1.28	—	—	—	64.48	Roots.....	Fresh.
2658		12 p.m.	8.02	2.39	1.87	0.52	—	—	28.90	—	Stems.....	Fresh.
2659			5.56	1.24	0.68	0.56	—	—	20.52	—	Leaves.....	Fresh.
2660			8.03	6.15	5.80	0.35	—	—	—	—	Thorns.....	Fresh.
2661			7.95	4.25	3.84	0.41	—	—	—	—	Roots.....	Fresh.
2662	22.12.43	7 a.m.	11.53	2.71	1.57	1.14	—	—	—	62.76	Stems.....	Fresh.
2663			3.40	1.65	1.02	0.63	—	—	—	—	Leaves.....	Fresh.
2664			4.79	5.25	4.76	0.49	—	—	—	—	Thorns.....	Fresh.
2665			8.70	3.61	3.18	0.43	—	—	—	68.40	Roots.....	Fresh.
2666		12 a.m.	6.90	2.12	0.63	1.49	—	—	—	—	Stems.....	Fresh.
2667			3.40	1.40	0.00	1.40	—	—	—	—	Leaves.....	Fresh.
2668			2.28	4.55	0.03	4.52	—	—	—	—	Thorns.....	Fresh.
2669			6.70	3.73	1.89	1.84	—	—	—	—	Roots.....	Fresh.
2670	19. 1. 44	12 a.m.	3.39	0.49	0.07	0.42	—	—	—	59.40	Stems.....	Wilted.
2671			2.34	0.34	0.23	0.11	—	—	—	—	Leaves.....	Wilted.
2672			3.16	1.27	0.80	0.47	—	—	—	—	Thorns.....	Wilted.
2673			5.76	0.69	0.47	0.22	—	—	—	61.96	Roots.....	Wilted.
2674		7 p.m.	5.59	1.03	0.59	0.44	—	—	—	—	Stems.....	Wilted.
2675			2.81	1.04	0.79	0.25	—	—	—	—	Leaves.....	Wilted.

TABLE 1 (CONTINUED).

No.	Date.	Time.	PER GRAM DRY MATTER.						Water Content per gram Fresh Matter.	Organ.	Remarks.
			Starch.	Total Sugars.	Sucrose.	Reducing Sugars.	Glucose.	Fructose.			
2676	19. 1. 44	7 p.m.	2.63	1.23	0.74	0.49	—	—	—	Thorns.....	Wilted.
2677			8.03	0.87	0.00	0.87	—	—	—	Roots.....	Wilted.
2678	12 p.m.		5.49	1.36	1.03	0.33	—	—	—	Stems.....	Wilted.
2679			2.70	1.32	0.08	0.24	—	—	61.16	Leaves.....	Wilted.
2680	7 a.m.		2.91	1.55	1.38	0.17	—	—	—	Thorns.....	Wilted.
2681			5.97	0.87	0.39	0.48	—	—	—	Roots.....	Wilted.
2682	12 a.m.		5.62	1.48	1.34	0.14	—	—	—	Stems.....	Wilted.
2683			2.32	1.09	0.92	0.17	—	—	65.51	Leaves.....	Wilted.
2684	12 a.m.		1.87	1.85	1.56	0.29	—	—	—	Thorns.....	Wilted.
2685			3.87	0.63	0.00	0.63	—	—	—	Roots.....	Wilted.
2686	12 a.m.		4.89	1.57	1.34	0.23	—	—	—	Stems.....	Wilted.
2687			2.38	1.07	0.87	0.20	—	—	53.05	Leaves.....	Wilted.
2688	3. 4. 44	12 a.m.	1.93	1.61	1.39	0.22	—	—	—	Thorns.....	Wilted.
2689			4.11	1.38	1.06	0.32	—	—	—	Roots.....	Wilted.
2822	3. 4. 44	12 a.m.	3.67	2.39	1.19	1.20	—	—	—	Stems.....	Temporarily wilted*
2823			2.56	1.09	0.08	1.01	—	—	56.06	Leaves.....	Temporarily wilted*
2824	7 p.m.		2.23	1.92	1.14	0.78	—	—	—	Thorns.....	Temporarily wilted*
2825			2.29	1.74	0.49	1.25	—	—	—	Roots.....	Temporarily wilted*
2826	12 p.m.		2.79	1.99	0.75	1.24	—	—	—	Stems.....	Temporarily wilted*
2827			2.59	1.22	0.12	1.10	—	—	62.34	Leaves.....	Temporarily wilted*
2828	12 p.m.		1.91	1.65	0.77	0.88	—	—	—	Thorns.....	Temporarily wilted*
2829			1.99	1.64	0.31	1.33	—	—	—	Roots.....	Temporarily wilted*
2830	12 p.m.		2.80	1.97	1.35	0.62	—	—	—	Stems.....	Temporarily wilted*
2831			2.39	1.27	0.57	0.70	—	—	60.60	Leaves.....	Temporarily wilted*
2832	4. 4. 44	7 a.m.	1.82	1.37	0.80	0.57	—	—	—	Thorns.....	Temporarily wilted*
2833			1.74	0.67	0.39	0.28	—	—	—	Roots.....	Temporarily wilted*
2834	12 a.m.		4.30	1.75	1.14	0.61	—	—	—	Stems.....	Temporarily wilted*
2835			2.29	1.20	0.45	0.75	—	—	67.23	Leaves.....	Temporarily wilted*
2836	12 a.m.		1.59	1.49	0.85	0.64	—	—	—	Thorns.....	Temporarily wilted*
2837			2.11	0.50	0.24	0.26	—	—	—	Roots.....	Temporarily wilted*
2838	12 a.m.		4.15	2.04	1.38	0.66	—	—	—	Stems.....	Temporarily wilted*
2839			2.96	1.24	0.51	0.73	—	—	62.89	Leaves.....	Temporarily wilted*
2840	4. 4. 44	7 a.m.	1.54	1.07	0.52	0.55	—	—	—	Thorns.....	Temporarily wilted*
2841			3.15	1.98	1.51	0.47	—	—	—	Roots.....	Temporarily wilted*

\* Temporarily wilted 4 times and allowed to recover.

CARBOHYDRATE NUTRITION.

TABLE 1 (CONTINUED).

No.	Date.	Time.	PER GRAM DRY MATTER.						Water Content per gram Fresh Matter.	Organ.	Remarks.
			Starch.	Total Sugars.	Sucrose.	Re- ducing Sugars.	Glucose.	Fruc- tose.			
3216	23. 2. 45	12.55 p.m.	5.59	3.47	2.57	0.90	—	—	36.00	Stems.....	Fresh.
3217			4.97	3.93	3.10	0.83	—	—	12.78	Leaves.....	Fresh.
3218			4.77	3.76	3.03	0.73	—	—	—	Thorns.....	Fresh.
3219			5.20	3.19	2.55	0.64	—	—	—	Roots.....	Fresh.
3301	4. 4. 45	12.20 p.m.	3.89	2.92	2.43	0.49	—	—	35.94	Stems.....	Badly wilted.
3302			3.63	2.43	2.05	0.38	—	—	—	Leaves.....	Badly wilted.
3303			3.89	2.74	2.23	0.51	—	—	—	Thorns.....	Badly wilted.
3304			4.04	2.91	2.49	0.42	—	—	—	Roots.....	Badly wilted.
3442	22. 2. 46	12 a.m.	2.23	4.52	2.91	1.61	—	—	51.76	Leaves.....	Wilted.
3443			0.00	2.99	2.47	0.52	—	—	—	Stems.....	Wilted.
3444			1.40	3.92	2.93	0.99	—	—	—	Roots.....	Wilted.
3454			1.56	1.32	0.97	0.35	—	—	60.10	Leaves.....	Wilted.
3455	23. 2. 46	0.40 a.m.	2.12	2.15	1.19	0.96	—	—	—	Stems.....	Wilted.
3456			2.29	2.32	1.28	1.04	—	—	—	Roots.....	Wilted.
3478			1.83	1.78	0.81	0.97	—	—	71.90	Leaves.....	Fresher.
3479			2.86	1.70	0.91	0.79	—	—	—	Stems.....	Fresher..
3480	23. 2. 46	6.15 a.m.	2.00	3.53	2.65	0.88	—	—	—	Roots.....	Fresher..
3469			0.00	2.22	1.22	1.00	—	—	71.70	Leaves.....	Fresher*.
3470			6.01	1.85	1.19	0.66	—	—	—	Stems.....	Fresher*.
3471			0.94	2.22	0.96	1.26	—	—	—	Roots.....	Fresher*.
3487	23. 2. 46	11.30 a.m.	1.29	2.37	1.37	1.00	—	—	38.30	Leaves.....	Wilted.
3488			2.10	2.70	0.49	2.21	—	—	—	Stems.....	Wilted.
3489			1.83	2.46	0.84	1.62	—	—	—	Roots.....	Wilted.

\* Fresher than day before.



TABLE 2.—*Tribulus terrestris* from Fauresmith soil. Progressive wilting.

No.	Date.	Time.	PER GRAM DRY MATTER.				Water Content per gram Fresh Matter.	Organ.	Remarks.	Ratio.	
			Starch.	Total Sugars.	Sucrose.	Reducing Sugars.					Fibre.
3152	14. 2.45	12 a.m.	8.06	4.32	2.91	1.41	32.74	71.50	Stems.....	Fresh.....	Leaves 1 Stems 0.75
3153			8.65	3.45	2.48	0.97	16.78	—	Leaves.....	Fresh.....	—
3154			6.92	3.95	2.56	1.39	—	—	Thorns.....	Fresh.....	—
3155			5.37	4.32	2.59	1.73	—	—	Roots.....	Fresh.....	—
3156	15. 2.45	11.50 a.m.	6.91	3.47	2.10	1.37	—	74.1	Stems.....	Fresh.....	—
3157			5.26	3.07	1.95	1.12	—	—	Leaves.....	Fresh.....	—
3158			5.81	4.65	3.01	1.64	—	—	Thorns.....	Fresh.....	—
3159			6.08	4.37	2.97	1.40	—	—	Roots.....	Fresh.....	—
3160	16. 2.45	11.50 a.m.	9.35	3.61	0.69	2.92	—	55.1	Stems.....	Fresh.....	—
3161			6.37	3.30	0.19	3.11	—	—	Leaves.....	Fresh.....	—
3162			4.75	3.86	0.67	3.19	—	—	Thorns.....	Fresh.....	—
3163			7.43	3.62	0.80	2.82	—	—	Roots.....	Fresh.....	—
3164	17. 2.45	11.45 a.m.	5.43	3.62	3.04	0.58	—	57.9	Stems.....	Beginning to wilt.....	—
3165			6.12	2.81	2.47	0.34	—	—	Leaves.....	Beginning to wilt.....	—
3166			5.66	3.76	3.09	0.67	—	—	Thorns.....	Beginning to wilt.....	—
3167			6.42	3.40	2.83	0.57	—	—	Roots.....	Beginning to wilt.....	—
3168	19. 2.45	12.20 p.m.	6.51	4.10	3.48	0.62	29.12	49.45	Stems.....	Wilted.....	0.75
3169			6.34	2.61	2.42	0.19	18.56	—	Leaves.....	Wilted.....	—
3170			7.73	4.90	3.63	1.27	—	—	Thorns.....	Wilted.....	—
3171			7.30	4.57	3.39	1.18	—	—	Roots.....	Wilted.....	—
3172	20. 2.45	12.15 p.m.	8.45	4.84	3.21	1.27	—	50.40	Stems.....	Wilted.....	—
3173			6.27	3.54	2.66	0.88	—	—	Leaves.....	Wilted.....	—
3174			5.16	4.15	2.90	1.25	—	—	Thorns.....	Wilted.....	—
3175			9.33	5.10	4.05	1.05	—	—	Roots.....	Wilted.....	—
3176	21. 2.45	12.15 p.m.	9.45	3.40	2.54	0.86	—	52.88	Stems.....	Wilted.....	—
3177			6.28	3.83	3.33	0.50	—	—	Leaves.....	Wilted.....	—
3178			6.89	3.52	2.85	0.67	—	—	Thorns.....	Wilted.....	—
3179			7.93	3.39	2.39	1.00	—	—	Roots.....	Wilted.....	—

CARBOHYDRATE NUTRITION.

TABLE 2 (CONTINUED).

No.	Date.	Time.	PER GRAM DRY MATTER.				Water Content per gram Fresh Matter.	Organ.	Remarks.
			Starch.	Total Su-gars.	Suc-rose.	Redu-cing Su-gars.			
3180	22. 2. 45	12. 15 p.m.	9.02	3.37	2.85	0.72	—	Stems.....	Improving after rain of previous night. Improving after rain of previous night. Improving after rain of previous night.
3181			8.16	2.95	2.15	0.80	—	Leaves.....	
3182			6.24	2.95	2.34	0.61	—	Thorns.....	
3183			5.88	3.52	2.95	0.57	—	Roots.....	
3184	23. 2. 45	12. 20 p.m.	13.43	3.25	2.62	0.63	28.10	Stems.....	Fresh sample of bigger plants. Small plants wilted, were not sampled.
3185			8.20	2.95	2.24	0.71	17.80	Leaves.....	
3186			7.21	3.40	2.68	0.72	—	Thorns.....	
3187			6.67	3.35	2.80	0.55	—	Roots.....	
3188	24. 2. 45	11. 45 a.m.	7.36	3.30	2.60	0.70	—	Stems.....	Fresh. Fresh. Fresh. Fresh.
3189			5.81	3.11	2.56	0.55	—	Leaves.....	
3190			9.29	1.76	0.83	0.93	68.79	Thorns.....	
3191			7.17	2.96	1.96	1.00	—	Roots.....	
3192	26. 2. 45	12. 30 p.m.	10.8	4.12	2.99	1.13	24.68	Stems.....	Fresh. Small plants, wilted, not collected.
3193			6.19	2.73	2.18	0.55	16.95	Leaves.....	
3194			4.68	3.30	2.80	0.50	—	Thorns.....	
3195			7.31	3.75	2.78	0.97	—	Roots.....	
3196	27. 2. 45	12. 40 p.m.	6.98	2.93	2.11	0.82	—	Stems.....	Wilted, more than ever before. Leaves drooping. Wilted. Wilted.
3197			4.54	3.43	2.94	0.49	—	Leaves.....	
3198			5.07	3.38	2.65	0.73	44.90	Thorns.....	
3199			5.77	3.75	2.90	0.85	—	Roots.....	
3200	8. 3. 45	12. 15 p.m.	7.08	3.79	3.20	0.59	—	Stems.....	Wilted. Leaves..... Wilted. Wilted.
3201			5.96	4.43	3.84	0.59	—	Leaves.....	
3202			4.66	4.22	3.58	0.64	51.81	Thorns.....	
3203			5.01	3.16	2.63	0.53	—	Roots.....	

TABLE 3.—Assimilates of *Tribulus terrestris* on different soils in different climates.

No.	Date.	Time.	Soil.	Climate.	PER GRAM DRY MATTER.				Water Content per gram Fresh Matter.	Organ.	Condition.	Remarks.
					Starch.	Total Sugars.	Sucrose.	Reducing Sugars.				
3024	15. 11. 44	2 p.m.	Ruigtevlei red sand	Ruigtevlei	—	2.96	1.82	1.14	—	Roots.....	—	—
3025					4.88	2.19	1.62	0.57	—	Stems.....	Fresh....	Poisonous.
3026					3.23	1.52	0.76	0.76	—	Leaves.....	Fresh....	Poisonous.
3027					3.75	3.42	2.25	1.17	—	Thorns....	Fresh....	Poisonous.
3028	15. 11. 44	2 p.m.	Ruigtevlei lands	Ruigtevlei	5.24	3.24	1.18	2.06	—	Roots.....	Fresh....	—
3029					4.01	2.72	1.15	1.57	—	Stems.....	Fresh....	—
3030					5.79	1.40	1.24	0.16	—	Leaves.....	Fresh....	—
3032	15. 11. 44	2 p.m.	Red sand with lime on Ruigtevlei	Ruigtevlei	—	2.67	0.35	2.32	—	Roots.....	Fresh....	—
3033					5.29	2.63	2.35	0.28	—	Stems.....	Fresh....	—
3034					4.99	2.24	1.97	0.27	—	Leaves.....	Fresh....	—
3035					4.67	2.26	2.15	0.11	—	Thorns....	Fresh....	—
3204	23. 2. 45	12. 45 p.m.	Leeuwfontein	Fauresmith Veld Reserve	5.39	2.48	2.00	0.48	29.82	Stems.....	Fresh....	—
3205					4.91	2.60	1.64	0.96	14.60	Leaves.....	Fresh....	—
3206					4.66	3.34	2.81	0.53	—	Thorns....	Fresh....	—
3207					5.46	2.97	2.40	0.57	—	Roots.....	Fresh....	—
3289	4. 4. 45	2. 20 p.m.	Leeuwfontein	Fauresmith Veld Reserve	3.61	2.92	2.08	0.84	30.96	Stems.....	Wilted...	—
3290					4.11	1.53	1.02	0.51	17.02	Leaves.....	Wilted...	—
3291					4.04	4.15	3.08	1.07	—	Thorns....	Wilted...	—
3292					3.69	3.07	2.18	0.89	—	Roots.....	Wilted...	—
3208	23. 2. 45	12. 50 p.m.	Waagkop....	Fauresmith Veld Reserve	5.62	3.87	2.97	0.90	36.51	Stems.....	Fresh....	—
3210					5.84	3.79	3.04	0.75	14.48	Leaves.....	Fresh....	—
3211					4.34	3.95	3.37	0.58	—	Thorns....	Fresh....	—
					5.26	4.74	3.97	0.77	—	Roots.....	Fresh....	—
3297	9. 4. 45	12. 20 p.m.	Waagkop....	Fauresmith Veld Reserve	3.86	2.63	2.22	0.41	36.36	Stems.....	Bad. wilt.	—
3298					3.55	2.76	2.27	0.49	15.84	Leaves.....	Bad. wilt.	—
3299					3.95	2.66	2.22	0.44	—	Thorns....	Bad. wilt.	—
3300					3.86	2.87	2.34	0.53	—	Roots.....	Bad. wilt.	—
3448	22. 2. 46	2 p.m.	Waagkop....	Fauresmith	1.40	2.58	2.01	0.57	69.50	Leaves.....	—	—
3449					9.75	3.56	2.71	0.85	—	Stems.....	—	—
3450					4.85	5.06	1.87	3.19	—	Roots.....	—	—



## CARBOHYDRATE NUTRITION.

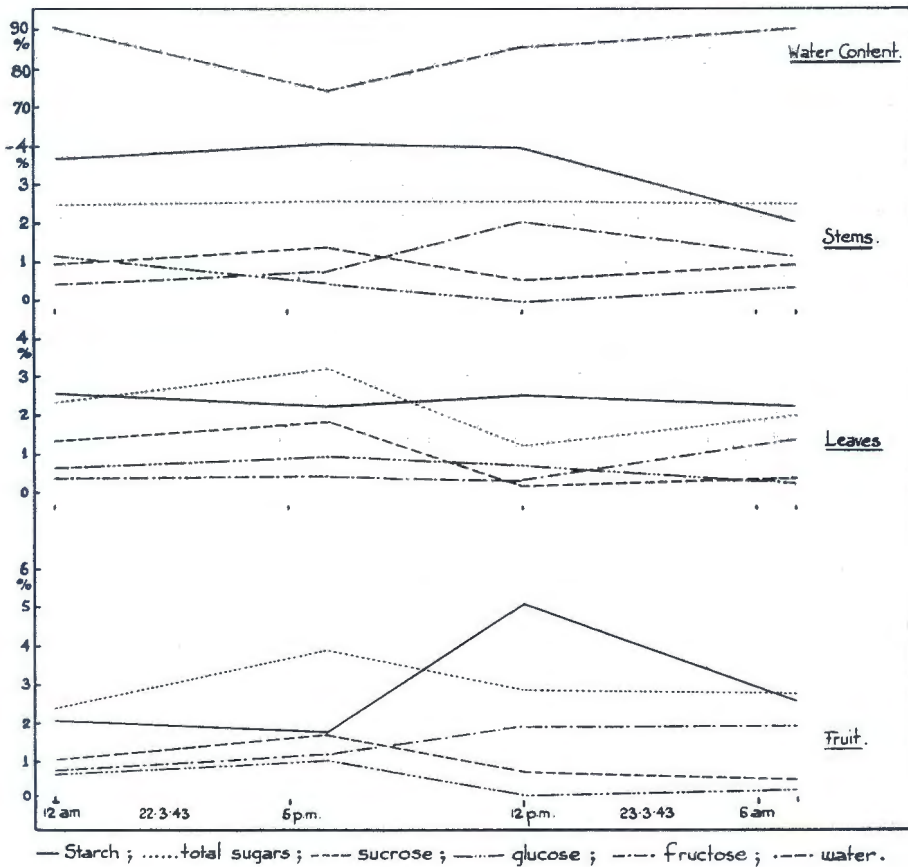
TABLE 3 (CONTINUED).

No.	Date.	Time.	Soil.	Climate.	PER GRAM DRY MATTER.				Water Content per gram Fresh Matter.	Organ.	Condition.	Remarks.
					Starch.	Total Su-gars.	Suc-rose.	Re-duc-ing Su-gars.				
3460	23. 2. 46	5. 55 a.m.	Waagkop....	Fauresmith..	1.95	2.64	1.38	1.26	—	Leaves.....	Fresh....	—
3461					4.60	2.04	1.24	0.82	—	Stems.....	Fresh....	—
3462					0.86	1.93	0.93	1.00	—	Roots.....	Fresh....	—
3429	10. 12. 45	2. 30 p.m.	Oranjezicht..	Oranjezicht..	4.59	1.55	0.03	1.58	—	Leaves.....	Slightly wilted	Poisonous
3430					9.14	2.86	1.39	1.47	—	Stems.....	On brak	—
3431					7.26	4.20	1.05	3.15	—	Roots.....	soil	—
3432	10. 12. 45	3. 10 p.m.	Oranjezicht..	Oranjezicht..	4.45	3.05	1.55	1.50	—	Leaves.....	Fairly spread on	Poisonous
3433					10.49	5.06	1.09	3.97	—	Stems.....	sandy	—
3434					7.31	5.29	0.42	4.87	—	Roots.....	limestone fresh.	—
3533	10. 12. 45	3 p.m.	Oranjezicht lands	Oranjezicht..	7.28	6.99	0.99	6.90	—	Total aerial	—	Poisonous
3530	8. 1. 46	11 a.m.	Oranjezicht..	Oranjezicht..	4.42	2.56	0.13	2.37	—	Roots.....	On sandy brak-	Poisonous
3531					5.85	2.45	0.49	1.96	—	Stems.....	brak-	—
3532					6.11	2.57	1.44	1.13	—	Leaves.....	veld	—
3534	29. 4. 46	11 a.m.	Calvinia.....	Fauresmith..	0.53	0.35	0.0	0.35	—	—	—	Not hae-molytic
3535	5. 5. 46	5 p.m.	Havenga bridge	Havenga bridge	5.18	1.62	1.13	0.49	—	Leaves.....	—	—
3536					4.34	1.38	0.02	1.36	—	Stems and roots	—	Not hae-molytic
3445	22. 2. 46	12 a.m.	Leeuwfontein	Fauresmith..	2.19	2.82	2.15	0.67	—	Leaves.....	Wilted....	—
3446					6.64	4.67	3.60	1.07	—	Stems.....	—	—
3447					4.59	4.82	4.15	0.67	—	Roots.....	—	—
3457	23. 2. 46	7. 15 p.m.	Leeuwfontein	Fauresmith..	1.15	4.18	2.73	1.45	—	Leaves.....	Wilted....	—
3458					8.67	4.00	1.34	2.66	—	Stems.....	—	—
3459					2.61	6.35	5.36	0.99	—	Roots.....	—	—
3472	23. 2. 46	0. 45 a.m.	Leeuwfontein	Fauresmith..	0.87	3.64	1.42	2.22	—	Leaves.....	Wilted....	—
3473					5.94	3.85	2.74	1.11	—	Stems.....	—	—
3474					2.66	5.51	3.66	1.85	—	Roots.....	—	—

TABLE 3 (CONTINUED).

No.	Date.	Time.	Soil.	Climate.	PER GRAM DRY MATTER.				Water Content per gram Fresh Matter.	Organ.	Condition.	Remarks.
					Starch.	Total Su-gars.	Sucrose.	Reducing Su-gars.				
3463	23. 2.46	5.50 a.m.	Leeuwfontein	Fauresmith..	1.12	2.51	0.54	1.97	—	Leaves....	—	Wilted.
3464					0.72	3.48	2.74	0.74	—	Stems....	—	—
3465					0.53	3.44	2.10	1.34	—	Roots....	—	—
3481	23. 2.46	11.30 a.m.	Leeuwfontein	Fauresmith..	1.21	2.67	1.97	0.70	—	Leaves....	—	Wilted.
3482					3.97	5.30	3.68	1.62	—	Stems....	—	—
3483					3.30	4.51	3.46	1.05	—	Roots....	—	—
3212	23. 2.45	12.40 p.m.	Calvinia....	Fauresmith..	5.76	2.61	2.19	0.42	34.64	Stems....	Fresh....	—
3213					5.72	3.08	2.56	0.52	14.58	Leaves....	—	—
3214					4.97	3.30	2.52	0.78	—	Thorns....	—	—
3215					4.34	3.26	2.53	0.73	—	Roots....	—	—
3293	4. 4.45	12.20 p.m.	Calvinia....	Fauresmith..	5.22	3.49	2.89	0.60	33.92	Stems....	Wilted....	—
3294					3.24	2.26	1.60	0.66	17.16	Leaves....	—	—
3295					3.89	3.28	2.81	0.47	—	Thorns....	—	—
3296					3.74	2.68	2.31	0.37	—	Roots....	—	—
3439	22. 2.46	12 a.m.	Calvinia....	Fauresmith..	1.91	2.76	2.07	0.69	—	Leaves....	Wilted....	—
3440					3.96	8.84	8.17	0.67	—	Stems....	—	—
3441					1.41	4.45	0.63	3.82	—	Roots....	—	—
3451	22. 2.46	7.30 p.m.	Calvinia....	Fauresmith..	1.80	4.88	3.48	1.40	—	Leaves....	Wilted....	—
3452					7.62	3.80	3.16	0.64	—	Stems....	—	—
3453					2.61	4.04	1.58	2.46	—	Roots....	Wilted....	—
3475	23. 2.46	0.50 a.m.	Calvinia....	Fauresmith..	0.62	1.58	0.58	1.00	—	Leaves....	Wilted..	—
3476					1.68	2.31	1.52	0.79	—	Stems....	—	—
3477					1.13	3.94	2.82	1.12	—	Roots....	—	—
3466	23. 2.46	5.50 a.m.	Calvinia....	Fauresmith..	2.27	3.91	0.36	3.55	—	Leaves....	Fresh....	—
3467					2.27	2.73	1.30	1.43	—	Stems....	Fresh....	—
3468					4.73	5.21	4.39	0.82	—	Roots....	Fresh....	—
3484	23. 2.46	11.30 a.m.	Calvinia ...	Fauresmith..	1.13	1.95	0.48	1.47	—	Leaves....	Wilted....	—
3485					2.27	3.03	1.91	1.12	—	Stems....	Wilted....	—
3486					1.29	4.10	2.25	1.85	—	Roots....	—	—
3534	29. 2.46	11.30 a.m.	Calvinia ...	Fauresmith..	0.53	0.35	0.0	0.35	—	—	—	—

GRAPH 1.—*Tribulus terrestris*, fresh. Fauresmith soil.



1. *Fresh plants.*

The graphs of the assimilates of fresh *Tribulus* of which No. 1 is an example, distinctly show that the stems contain more starch than the leaves, and that the starch content of the leaves does not follow the pattern of assimilation with a starch maximum either in the afternoon or early evening. These graphs rather resemble those of wilted lucerne (Henrici, 1949) with a maximum of starch at midnight. Whilst sugars are more or less present in the same quantity as in fresh summer lucerne, starch occurs in much larger amounts, particularly in the stems. In fresh *Tribulus* there is a tendency of monosaccharides prevailing; yet there are a few instances where there is more sucrose than reducing sugars. The sugar content of roots and fruits is very high. The roots give the most regular graphs of the assimilates in *Tribulus*.

2. *Wilted plants.*

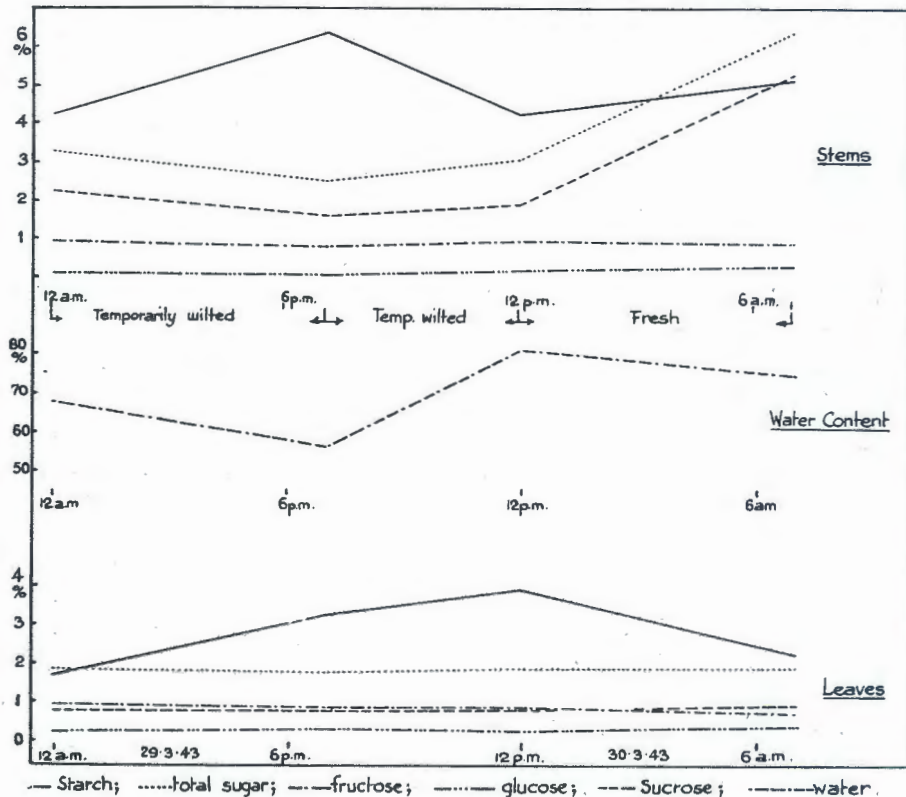
As the graphs of the temporarily wilted *Tribulus* are very similar to those of the fresh plants, the question arises whether even the so-called fresh *Tribulus* has the leaves in the state of incipient drying, with the stems taking over the

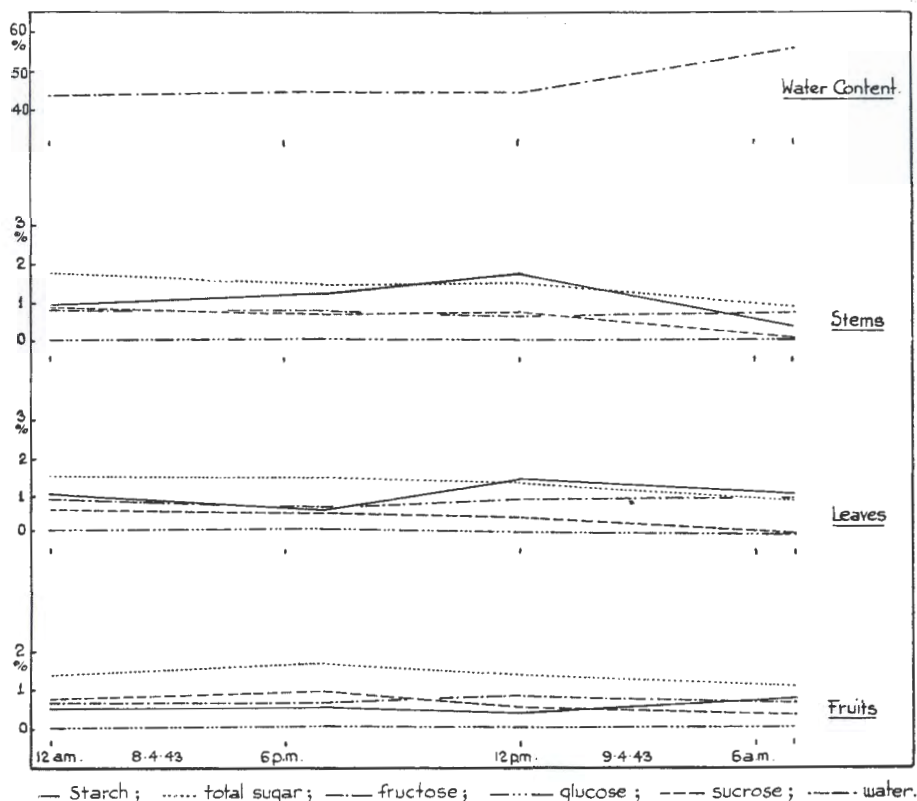


process of photosynthesis as in the case of lucerne. It must not be overlooked that fresh *Tribulus* can have 90 per cent. of water, and that on a windy, sunny day as much as 15 per cent. may be lost, quite enough to warrant incipient drying.

No definite rule can be given at what time *Tribulus* may have a high starch content. In fresh and temporarily wilted plants it varies considerably in different years. Yet it is really the exception even in so-called fresh *Tribulus* to be high in the leaves; but in the stems it is always from medium to very high, except when the plant is completely wilted. In completely wilted leaves and stems the content of starch is very low. It is likely that the maximum for starch formation is at much lower temperature than the optimum for photosynthesis (Czapek, 1921); actually at high temperature less starch may be found than at 25° C. There is no doubt that the permanent wilting decreases the starch content of the leaves, but not in stems or roots, as in these organs, except in extreme cases, plenty of starch is found. Temporary wilt scarcely affects stems and roots. From the point of view of carbohydrate food stems and roots must be excellent; the large content of starch kept animals going in the 1933 drought as stated previously.

GRAPH 2.—*Tribulus terrestris*, temporarily wilted. Fauresmith soil.



GRAPH 3.—*Tribulus terrestris*, wilted. Fauresmith soil.

Wilting also decreases the content of di- and monosaccharides in the leaves, but not invariably in stems and roots. On Fauresmith soil the sucrose and reducing sugars are very seldom over 2 per cent. in roots and stems, except in experiments of progressive wilting. Contrary to the lucerne experiments the stage of predominating sucrose lasts a long time.

In fresh *Tribulus* there is plenty of fructose and a good amount of glucose, and rather less sucrose. In temporarily wilted plants there is little reducing sugar, but, particularly towards the end of the experiment, plenty of sucrose. In the progressive wilting experiment (Table 2) the small amount of monosaccharides towards the end of the experiment combined with the large amount of sucrose is absolutely striking and completely different from lucerne. Even in permanent wilt, although not present to the extent of over 1·1 per cent., sucrose preponderates. In roots, stems and at times in the fruits there is more sucrose than in fresh or temporarily wilted leaves. Also in this respect *Tribulus* differs from lucerne.

The trend of the sugars in a progressive wilting experiment (Table 2) is quite unexpected. The experiment begins with a very high starch content in all organs, and a high sugar content, the sucrose being about double that of the reducing sugars. On the 3rd day the water content drops to about 56 per cent. without any proper wilting to be seen. Starch content of leaves and fruits decreases appreciably,

but not the total sugars; yet the ratio of sucrose to reducing sugar is totally changed, as at times sucrose in leaves is more than 10 times the amount of reducing sugars, and in other organs 4-5 times. An increased water content, by rain or milder meteorological conditions, again immediately increases the starch content of all organs, but the ratio of the sugars does not return to that of the starting point.

In all the experiments stems and roots often behave very similar with regard to their contents of assimilates and have a similar percentage of the single constituents. If changes occur they are in both organs in the same direction and often contrary to those in the leaves. That is a notable difference from lucerne (Henrici, 1949).

It has already been stated that the aerial parts do not show a seasonal trend in their assimilates. Yet for the roots it can be said, what is natural for an annual plant, that the highest starch values are found in the height of summer and are decidedly much lower in April. The first frost generally kills the plants.

## II. Daily march of assimilates in *Tribulus terrestris* under the same climatic conditions on different soils. (Table 3.)

On the 22/23.2.46 *Tribulus* from 3 different soils was collected under the same climatic conditions. The plants in day-time were all wilted but they were fresh or at least fresher between 5 and 6 in the early morning on the 23rd. To look at, no exterior difference could be seen between plants of Calvinia and Leeuwfontein soil, the plants of Fauresmith soil were slightly larger, but as Fauresmith had a bad drought, wilting and zinc deficiency accumulated their effect. The question was whether any physiological or chemical changes could be detected in these plants.

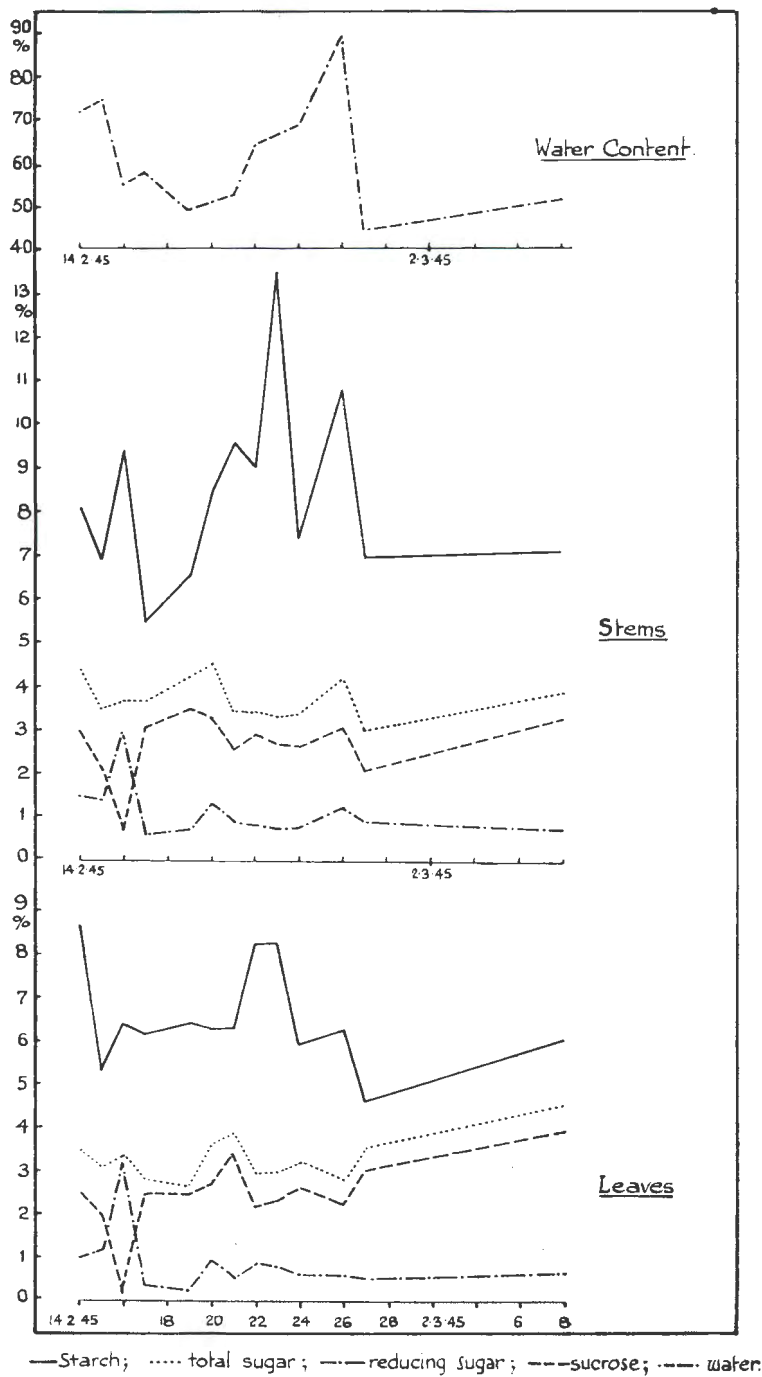
First of all the water content of the Fauresmith plants during the night was considerably higher than that of plants of the other two soils, Leeuwfontein having the lowest water content. All the leaves had a low starch content, Leeuwfontein the lowest in day time. For the sugars differences could scarcely be seen; the sucrose in the leaves was everywhere higher on the first day than on the second. The reducing sugars on Calvinia soil had a maximum early in the morning on the second day. The analyses of the stems offered more diversities, though Leeuwfontein and Calvinia were rather similar. They had a high starch content on the first day with a maximum late in the evening, the starch maximum for Fauresmith being early in the morning. Compared with the Fauresmith plants, the abundance of sugars, particularly sucrose, was noticeable. In the Calvinia plants a maximum was reached some hours before the starch maximum on the first day, although the roots were rich in sugar. The preponderance of sugars in the roots in place of starch, may be due to the season which was extraordinarily dry, and practically only allowed wilted *Tribulus* under the Fauresmith climate. The enormous quantity of starch particularly in the roots of *Tribulus* was a characteristic feature in less severe seasons.

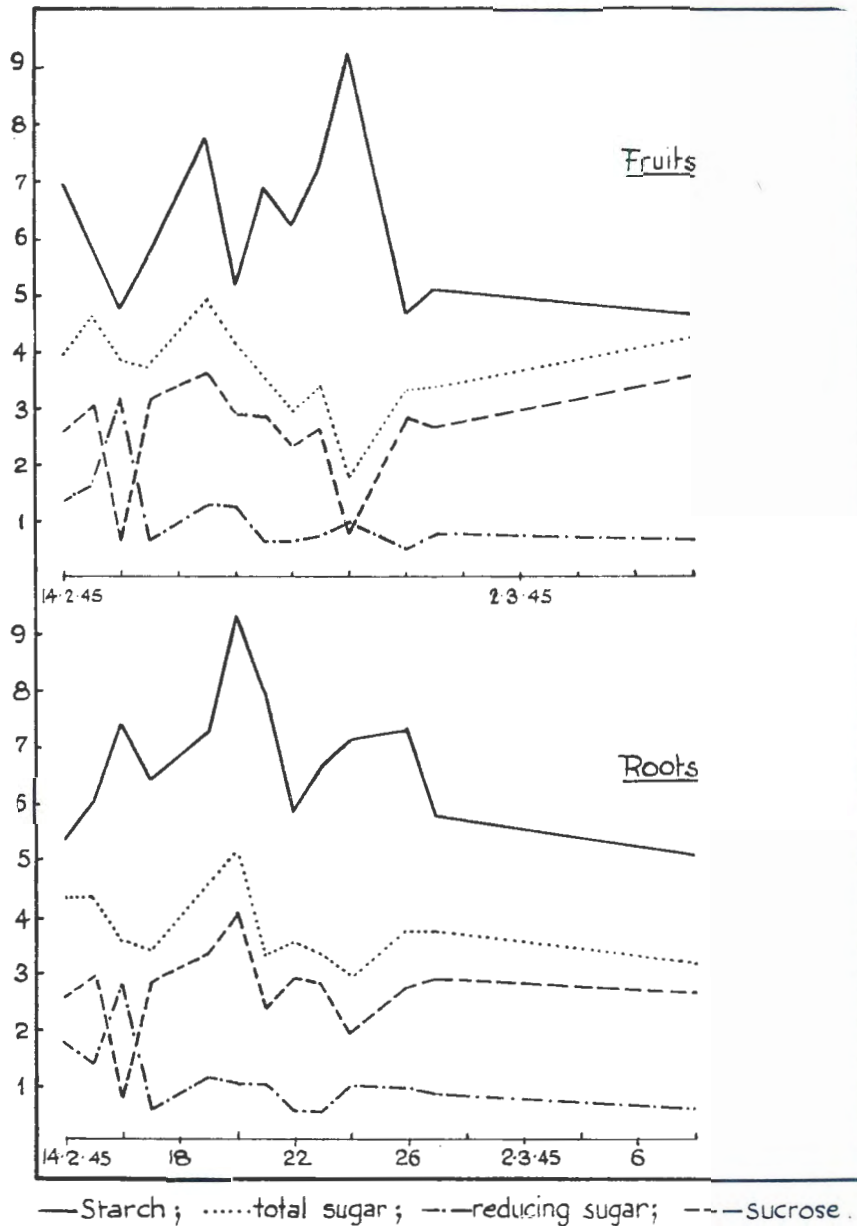
Table 3 gives some data on the assimilates of *Tribulus* on various soils under different physiological conditions. As was pointed out before, Oranjezicht had rains much earlier than Fauresmith, but on the whole a similar drought climate. For the aerial parts with the exception of Oranjezicht, the sucrose stage predominated. Oranjezicht under the worst dikkop outbreak, however, had lots of reducing sugars in fairly fresh plants.



CARBOHYDRATE NUTRITION.

GRAPH 4A.—Progressive wilting of *Tribulus terrestris* on Fauresmith soil.

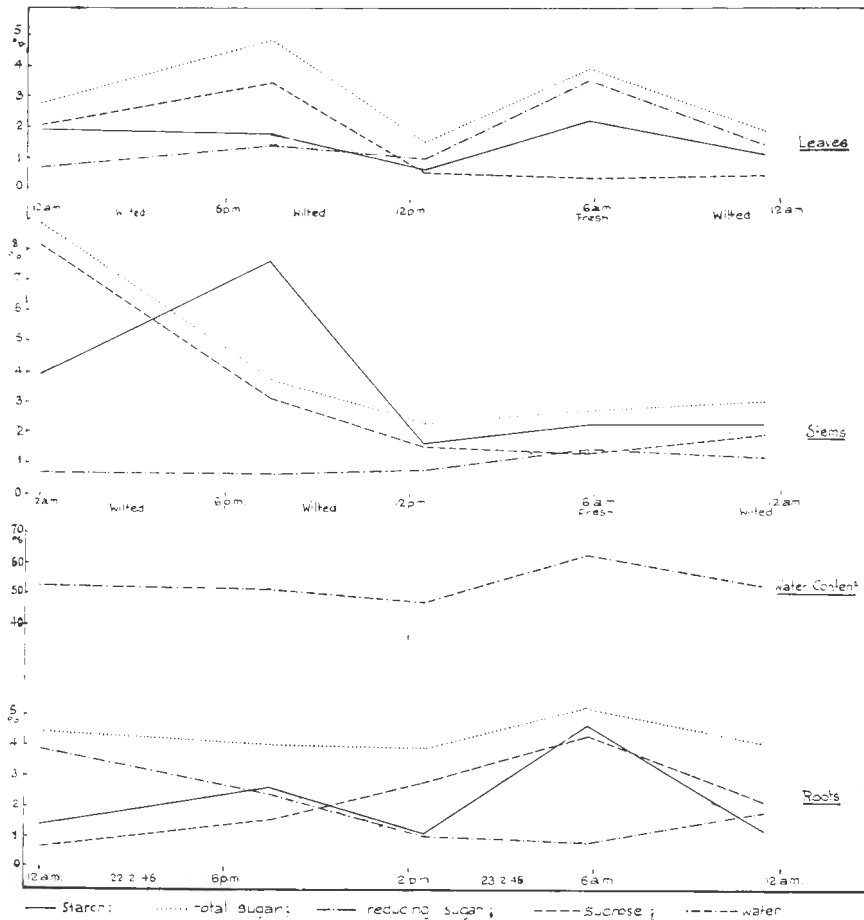


GRAPH 4B.—Progressive wilting of *Tribulus terrestris* on Fauresmith soil.

Conclusively it may be said that the analyses of the sugar and starch in *Tribulus* from different soils and climate do not offer anything unexpected apart from occasional very high sugar values. It is therefore obvious that the sugars as such are not the primary cause of the disease, but may very likely come into the picture secondarily.

CARBOHYDRATE NUTRITION.

GRAPH 5.—*Tribulus terrestris*. Calvina soil, Fauresmith climate.



*The presence of a glucoside in Tribulus from certain soils.*

In extracting different samples of *Tribulus* giving dikkop, a persistent foam was observed on some samples. The samples from Oranjezicht for example, foamed like beer. The matter seemed worth while investigating further.

It appeared that extractions of all poisonous *Tribulus* (Ruigtevlei, Oranjezicht) and of most of the suspected soils (Calvinia, Leeuwfontein, Waagkop) produced this foam. Beating the extraction of Oranjezicht *Tribulus*, more foam was obtained. Dissolved in 0.9 per cent. NaCl, the foam proved haemolytic within 1 second, and within a couple of hours if diluted to 1:40,000. But on standing more than 40 minutes, the solution became suddenly inactive.

As, by the time the material of Oranjezicht was collected, it was expected to find an unstable poisonous factor, some material was not as usual killed in alcohol, but rushed in a fresh state to a refrigerator and hardened by freezing. The



hardened material kept constantly in a refrigerator was pounded in mortars and extracted for all investigations with cold reagents. Material from the other soils were treated in a similar way. Even keeping in a refrigerator reduced the haemolytic qualities and foaming by the day. Yet foaming persisted for some months.

Later it proved advisable to extract the material alternately with 96 per cent. alcohol and ether. This removed all chlorophyll, subsequently reducing enzymatic reaction without much affecting the haemolytic factor.

The following reactions were done: Test with  $H_2SO_4$ ; haemolytic test; precipitation with normal and basic lead acetate; precipitation of the surplus lead with  $H_2S$ , and testing the filtrate dissolved in 50 per cent. alcohol again for saponin; binding of the haemolytic factor with cholesterin; breaking up of the compound with xylol, and removing the cholesterin in ether; testing for the haemolytic factor; filtering of the pre-extracted water extract with  $Mg(OH)_2$  and elution of the column with 50 per cent. alcohol; treating a similar water extract with an  $Al(OH)_3$  and talc, or sugar column. Salting out was tried. The different extractions and elutions were tested with Legal solution and Brady's reagent. Some water extractions, after preliminary treatment with alcohol and ether, were kept on the waterbath, others were evaporated in vacuum. The results will be discussed separately.

#### *Haemolysis.*

All roots, leaves and stems of *Tribulus* from Oranjezicht and Ruigtevlei, and from soils of Calvinia and Leeuwfontein and some of Waagkop gave haemolysis in dilution of 1:20,000, if collected before March, 1946. No haemolysis was obtained with material from Havenga bridge, collected in May, 1946, with some samples from Waagkop soil, and with all Fauresmith soil plants whether wilted or fresh collected between January and April, 1946.

The haemolysis could be stopped by addition of cholesterin and renewed again by removing the latter by boiling in xylol.

The haemolytic factor of the leaves and stems could be precipitated by basic lead acetate. In further treatment with  $H_2S$  an active factor remained, possibly slightly yellow.

The factor of the roots could be precipitated with neutral lead acetate. In later extractions it was partly precipitated by neutral, partly by basic lead acetate.

#### *Absorption analysis.*

In the absorption analysis  $Mg(OH)_2$  worked exceedingly well, absorbing the haemolytic factor which could be eluted with 50 per cent. alcohol leaving behind a lot of impurities. Of course for any further treatment the alcohol was evaporated and the substance taken up with water. Evaporation was best done *in vacuo*, as even heating on the water bath greatly diminished the activity of the factor.  $Al(OH)_3$  and sugar column did not give any satisfactory results.

#### *Solubility.*

The factor was soluble in water, and from 56 per cent. alcohol downward soluble in alcohol, but not soluble in 96 per cent. methyl- or ethylalcohol, nor in ether or xylol.

*Colour with sulphuric acid.*

The extract plus 1 drop of concentrated  $H_2SO_4$ , produced a yellow, later red, then violet, colour.

*Brady's reagent.*

Brady's reagent (2-4-Dinitrophenylhydrazine in sulphuric acid, see Brady, 1931) as reagent for a keto-group reacts not only with a keto-group of the aglucone of the saponin, but also with the reducing sugars of the saponin. For this reason a number of sugars which might occur in saponins according to Klein (1932, II/2, II p. 1103) (maltose, glucose, fructose, galactose, arabinose and lactose) were tested and crystals were obtained with 2-4-Dinitrophenylhydrazine, except for lactose. The same or very similar crystals as with maltose, glucose, fructose and galactose were found in the saponin solutions when treated with Brady's reagent.

The Brady's solution, being acid and applied warm, seemed to break up the saponin molecule and to give crystals in the first line with the sugar components. Literature on this subject seems to be non-existing, thus the melting points discussed later could not be compared.

Three kinds of crystals were obtained which were most likely due to the sugars. Two were beautifully red in the dark field under the microscope, brownish red in transparent light. The one kind (picture 1. B) is a coral-like crystal, mostly in chains, presumably due to a maltose component (or glucose?). The crystal of pure maltose or pure glucose had a m.p. of  $135^\circ C.$ , the m.p. of the crystal in the saponin was in a few instances between  $99$  and  $102^\circ C.$ , but generally much lower,  $48^\circ C.$

The second crystal obtained was a red (dark field) aggregate of plates with dents, probably originating from fructose. Its melting point was much lower,  $48-51^\circ C.$  on cold days,  $87-91^\circ C.$  on warmer days. Pure fructose gave with 2-4-dinitrophenylhydrazine crystals melting at  $\pm 100^\circ C.$  The third crystal, very similar to the 2-4-dinitrophenylhydrazine of galactose, was a hooked irregular plate, its melting point was also lower than that of the pure sugar. The melting point of the pure sugar with Brady's reagent proved to be  $130^\circ C.$

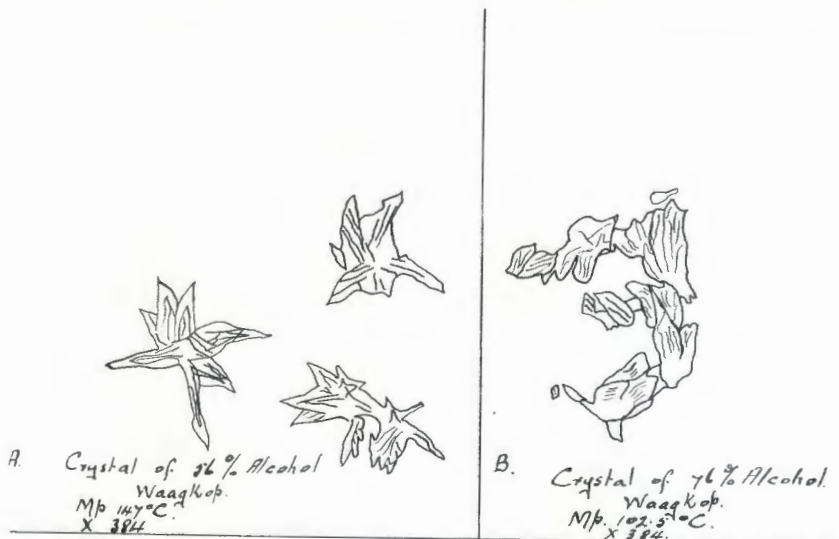
All hydrazones known in literature of these sugars have a much higher melting point. But they were obtained mostly by p-nitrophenylhydrazine. It may be that the presence of a second nitro-group lowers the melting point. On the other hand it is possible that part of the saponin-aglucon is still attached to the sugar component and influences the melting point. Further investigations must show which hypothesis is correct. Moreover, solutions were not pure.

Two more crystals found in the preparation were definitely not due to any sugar tested. They were: (a) Long yellow hexagonal prismatic needles with a sharp point at one end. They were generally lying together in stars. On the whole they were much scarcer than the crystals due to sugars; it is noteworthy, however, that they generally had the same melting point as the sugar component.

(b) A spider-like red crystal was found as well, more common than the needle, but much less frequent than the crystals due to sugar. Its colour was red brown and generally it melted together with the crystals of the sugar component.

The five crystals described above appeared mixed in the same preparation, the hydrazones always predominating; in determining the melting point on a heating stage of a microscope it was noteworthy that although the melting point

in preparations made on different dates changed as will be described later, all the crystals obtained in one preparation melted at the same temperature, the sugar crystals explosively, the needles showing first some corrosion. The melting occurred within a degree at the utmost, generally within  $\frac{1}{2}^{\circ}$  C. In many instances when the sugar crystals melted, some smaller, nearly colourless needles were observed to form within the former crystal masses, but these disappeared again in the explosion. The melting points cannot be considered as absolutely accurate,



Crystal of 26% Alcohol.  
From Lecuwfontein  
Mixed with A Like spiders Crystals longer and thinner than A  
Mp.  $53^{\circ}$  C. X 384.



as the temperature on the glass over the condenser was definitely lower than on the metal object-table where the thermometer was lying. At temperatures of 150° C. the difference may be as much as 3° C., at the lower temperature at which work was performed, it was less, but still it existed.

In colder weather none of these well-defined crystals were obtained, only crystal sand could be seen. The crystals themselves were not stable and seldom lasted longer than an hour on a microscopical slide. In solution of a reagent glass good crystals broke up to crystal sand within a week. I am indebted to Mr. W. A. Lombard for doing a number of tests on the Brady's crystals and for some suggestions on extraction of the factor. The melting point of the crystals was determined under the microscope on a heating stage. It appeared that all the above crystals had more than one melting point when prepared on different days, to such an extent that in cold weather at the end of the season a lower melting point, 48-51° C., was obtained whilst in warmer weather the m.p. was  $\pm$  87° C. In a few instances right at the start with crystal B and spider and needles a m.p. of 151° C. was realised.

Preparations made on the same day from different material generally had the same melting point; this means that other conditions like room temperature had a lot to do with the particular melting point.

Tests were done with acetone and formaldehyde to ascertain their form of crystals with Brady's reaction. Their needles were distinctly different in form and colour from the one obtained with the saponin extraction and it is therefore unlikely that any known aldehydes or ketones caused these crystals.

Considering that different sugars react with Brady's solution, it was of importance to know that the solution tested, did not contain any free sugars before Brady's solution was added. It is true that the melting points differ in the free sugars and in the components from the saponin. But before this fact was realised, solutions were only tested with Brady's solution, and the presence or absence of crystals and their forms noted, but their melting points were not always determined. Water extracts of *Tribulus*, pretreated with alcohol and ether, then run through the magnesium hydroxide column and eluted with alcohol, the latter evaporated and replaced with water, no longer contained free sugars, only the saponin in a toffee-like substance. The toffee-like substance, dissolved in water, did not show reducing properties, but on addition of acetic acid and warming, the solution showed reducing properties within a quarter of an hour, the saponin being broken up. Fresh water extracts of *Tribulus* without any further treatment cannot be tested with Brady's solution for saponins, as any such solution will contain fructose and glucose reacting with it. This fact eventually explained a few contradictory results obtained at the start of the work with local wilted *Tribulus*. Thus in the discussion below only solutions which were previously freed of sugar are included.

The Brady's test was positive in all *Tribulus* material of leaves and stems of Oranjezicht, Ruigtevlei, Calvinia and Leeuwfontein soil, as well as in some of the *Tribulus* from Waagkop soil. It was negative in all fresh material from Fauresmith soil. A few crystals were found under the microscope in wilted Fauresmith *Tribulus*. The reaction of the roots was parallel with that of the corresponding leaves.

Whether the crystals obtained with the different melting points were isomers, had to be left for future investigation. Considering the crystals were not due to sugars, one might conclude that there was a keto-group present.

The samples which gave positive Brady's tests were haemolytic and vice versa. There were very few instances where there was a very poor Brady's reaction and haemolysis could not be observed within 24 hours.

#### *Legal test.*

Contrary to the Brady's test, the Legal test was only positive in very few instances and only in material which had not been heated at all. In roots and aerial parts of *Tribulus* from Oranjezicht, a positive Legal test was obtained, but not the expected red colour in alkali; after about a minute a red brown precipitate was obtained which dissolved in mineral acid with a yellow colour. In acetic acid it gave a deep amethyst blue colour. Only one other sample of roots and leaves from Calvinia soil gave the same positive reaction in high summer.

In other samples from Calvinia the colour in alkali was only deep yellow, no precipitate was formed or only after long standing and gentle heating. All colour vanished when mineral acid was added; in  $\text{CH}_3\text{COOH}$  the colour was either blue or blue-green. The  $\text{Mg}(\text{OH})_2$  column after a month still gave positive tests with Legal solution when extracted with water; this may be due to an aldehyde.

The  $\text{C}=\text{O}$  group which gives the Legal reaction in the saponin is definitely not the same as the group which gives the Brady's reaction in the saponin, as many more positive reactions with Brady's solution were recorded. The  $\text{C}=\text{O}$  group of the Legal reaction seems to be the group which is destroyed the easiest, simply by raising the temperature.

No positive Legal tests were obtained with any fresh or wilted material of *Tribulus* from Fauresmith soil. Of samples from Waagkop soil only ice-stored material was tested, all proving negative. A few samples from Leeuwfontein soil in high summer gave the reaction described for Calvinia plants. Neither plants from Calvinia soil nor from Havenga bridge gave positive results in April or May.

The fact that the Legal test, except in two or three instances, took some time to accomplish, does not speak in favour of the keto group belonging to a  $\beta$ - $\gamma$  unsaturated lactone ring, as was first thought. The actual presence of a lactone ring had to be proved by titration with alcoholic KOH. No undamaged material was available any longer when this was perceived.

It must be emphasised that the saponin described here is obtained in the foam of a cold, even iced, water extraction and can completely be extracted by cold water. For all the first determinations of haemolysis, this foam was mixed with 5 c.c. 0.9 per cent. NaCl. For later work the NaCl extractions in the cold were used. There is another saponin present in the *Tribulus*. According to Mr. Lombard, however, this second saponin was present in all plants collected between October and December, 1946, and had very different qualities. It was very stable in water solution; it was extractable in large quantities by boiling water under the reflux cooler, but only sparingly by cold water; it scarcely foamed in cold water; it could be split in aglucon and sugar by boiling for hours under the reflux cooler with sulphuric acid. From the description of the saponin obtained from Oranjezicht material it was obvious that there were two different substances. Whether they were similar in structure, the future must show.



*Resumé of the chemical qualities of the saponin-like glucoside in Tribulus.*

During the height of the summer 1945/46 a saponin-like glucoside was shown to be present in the aerial parts and roots of *Tribulus terrestris* collected whilst poisonous or from suspected soils. The glucoside produced persistent foam, but was quickly destroyed in a pure water or NaCl extraction of the plant. The NaCl extraction was strongly haemolytic. Even in the plant kept on ice the glucoside diminished by the hour. The haemolytic quality was suspended by the addition of cholesterol. The glucoside of the aerial parts of the fresh plant was precipitated by basic lead acetate, that of the roots by neutral lead acetate. Later, part of the root glucoside was also precipitated by basic lead acetate. The glucoside contained several C=O groups, giving a positive reaction with Legal and Brady's reagent, the group giving the Brady's test generally running parallel with positive tests for haemolysis. The glucoside was soluble in water, and sparingly so in 50 per cent. alcohol, insoluble in ether, methyl-, ethylalcohol and xylol.

The glucoside was most unstable as simple heating on a waterbath or even storing on ice caused it to break up. The first group to deteriorate was presumably the C=O group positive in the Legal test.

The glucoside was definitely not present in the fresh plants from ordinary Fauresmith soil, between January and March, 1946. Minute amounts of Brady's crystals (the sugar component) were, however, seen under the microscope in wilted *Tribulus* from Fauresmith soil, yet the extraction was not haemolytic. The glucoside was also not present in plants from Calvinia soil and Havenga bridge, collected late in the season.

*The dependence of the saponin-like substance on a temporary zinc deficiency.*

In the older literature (Kofler, Sieburg and Kobert) this substance in the leaves would be called a neutral saponin, the primary substance in the root an acid saponin. Up to the present it is impossible to say whether the saponin belongs to the terpene or phenanthrene saponins of the new literature (Fieser, 1936). Seeing that different sugars are present in the molecule or at least in the solution of the saponin, the possibility exists that there is more than one saponin present. Therefore the terms "the factor" or "the saponin" of *Tribulus* will be used in the discussion.

In the first instance the saponin is only formed on special soils, presumably those which are deficient in zinc or which are unable to supply zinc in sufficient quantity to *Tribulus* in flush periods. At the end of the season no zinc deficiency occurs, as growth is slow, and no saponin is formed (tests on material from Havenga bridge and Calvinia soil). Slow-growing Karoo bushes do not produce such a substance. Already in older literature (Sieburg) the possibility of the saponin being a phytosterol was expressed, and Dufrenoy and Reed (1942) definitely made mention of a phytosterin being formed as effect of zinc deficiency under strong illumination.

When the newer literature on the distribution of saponins is studied (Roberg and Marchal, 1937), stress is laid on the point that the same plant species known to contain saponin may contain such different amounts of saponin that some do not give any haemolytic effect whilst others haemolyse within minutes. Roberg stated that the causes of this discrepancy are unknown but recorded by several authors, and that an edaphic factor was suspected, not inheritance, as plants and seeds from one locality generally behaved the same.



That is very little, yet it suggests much. Plants which contain saponin occasionally, can be found without any at all on certain soils. This would correspond to *Tribulus* on Fauresmith soil. Plants with little saponin would correspond to *Tribulus* on Waagkop soil and plants with lots of saponin as coming from Oranjezicht and Calvinia soils.

Another important observation is mentioned in older literature (Kofler, 1927, Sieburg, 1923). Plants containing ethereal oils do not contain saponin, and the idea is expressed that the plants either develop the nucleus of terpene to the volatile oils or, according to the later conception, the phenanthrene nucleus to the phytosterins. Most of our Karoo plants, except the *Atriplex*, contain volatile oils, the terpene nucleus. Most grasses do not contain volatile oils, neither does *Tribulus* nor lucerne.

#### *Probable physiological place of the glucoside.*

Kofler (1927) was the only author who foresaw that a saponin could under certain unfavourable conditions be formed in the place of starch. The possibility of it being a reserve substance which has again been drawn into the metabolism is more generally accepted by the older authors (Kobert, Kofler, Sieberg).

As has been pointed out, zinc deficiency and wilting are factors dissolving starch and leading to the accumulation of sugars (Hoagland, 1944, Henrici, 1949). Without these the amount of appearing sugars corresponds quantitatively to the disappearing starch, even if an increased respiration at wilting is accepted (Iljin, 1923). The possibility that another sugar-containing product may be formed, is given. Both zinc deficiency and incipient drying are "unfavourable conditions". Now it has to be remembered that dikkop also occurs on fresh *Tribulus*, which is simply stunted in growth, but not wilted. At both times when *Tribulus* was collected during dikkop outbreak, at Ruigtevlei and Oranjezicht, most plants were perfectly turgid with a high water content. On the other hand dikkop may well occur on *Tribulus* in the state of incipient drying, temporarily wilted.

It may be assumed that the glucoside appears primarily in zinc deficient fresh plants, but the amount may be increased during incipient drying, the latter being the second unfavourable factor. It is not thought that permanently wilted *Tribulus* wherein all metabolism has stopped will produce the glucoside. Actually between October and December, 1946, it was found that saponin decreased and even disappeared as wilting progressed.

The quick disappearance or destruction of the glucoside in water solution is presumably due to the peroxidase which is very much more active under the influence of zinc deficiency (Hoagland, 1944, Reed and Dufrenoy, 1942). Investigations into this effect are undertaken at the Veld Reserve, Fauresmith, where the isolation of the glucoside is contemplated.

That the glucoside is again drawn into the metabolism, is certain, as in older fresh *Tribulus* on Calvinia soil and at Havenga bridge, no trace of it can be seen. When and how it happens is of course not yet clear, but happen it does.

### B. LUCERNE.

#### *I. Additional data on the assimilates during progressive wilting, after frost and under the influence of grazing. (Tables 4, 5, 6, 7, 8.)*

As in the case of *Tribulus* it has never been possible to produce lucerne which causes bloat on the soil of the Veld Reserve, Fauresmith. The various conditions which lead to an increase of the contents of sugars, and a decrease in

starch have been discussed in an earlier paper (Henrici, 1949). Here some additional data will be given on the effect of progressive wilting, frost and grazing (Tables 4, 5, 6, 7 and 8) as well as on lucerne cultivated in pots. The latter experiments are of importance in connection with the migration of the carbohydrates. It was suggested in the earlier paper that under the influence of frost soluble carbohydrates might migrate from the roots to the aerial parts as the high content of sugars could not be explained through possible photosynthesis. As roots were necessary for these analyses, it was easier to handle plants in pots. They reveal that there is a large amount of starch in the thick roots, as well as a large amount of total sugars, but little reducing sugar; the small thin roots contain little starch and a medium amount of sucrose and little monosaccharides. From these additional data it appears that in a drought year as well as in October and towards the end of the growing season there is more starch and sucrose in the leaves than during the hot summer months. (Table 7, Pot lucerne). The two experiments on plot lucerne (Table 8) confirm earlier findings that during wilting there is more reducing sugars than sucrose, but if the plant recovers sucrose prevails.

In the progressive wilting experiment (Table 4) the starch is decreased as well as the total sugars. The maximum of the reducing sugars is found on the 4th day, afterwards the monosaccharides decrease. From the 11th day onward the sucrose decreases rapidly.

In the grazing experiments (Table 6) the small amount of sugar is striking, particularly the small amount of sucrose. The same applies to frosted lucerne, at the end of the winter, which is grazed immediately. (Table 5.)

#### *Presence of saponin in Lucerne.*

Some veterinarians explain bloating as the disastrous effect of the gas originating from rapidly fermenting sugars of the lucerne in the stomach of the animal; the frothy mass in the rumen is unable to escape and finally suffocates the animal. The foamy consistence of the ruminal mass is attributed to the presence of saponin in the lucerne (Quin, 1943). It must be understood that saponin used in this sense has not the chemical meaning of to-day (Fieser, 1936) and may be Jacobson's glucoside (1919) which is mentioned elsewhere in this article.

During all the grazing experiments on the Veld Reserve, Fauresmith, not a single positive result of bloat occurred, in spite of large amounts of sugars being present by frosting or wilting, and the author of this paper therefore is inclined to suggest that here also the soil factor plays a decisive rôle apart from the condition of the animal. The soil factor may in this case either be simply another (presumably zinc deficient) soil or a badly cultivated soil where the zinc is out of reach of the plant. The suggestion seems the more timely, as sugar and starch analyses from lucerne from different soils under similar physiological conditions (fresh or drooping) do not differ in such a way as to warrant an explanation (see Table 9).

Therefore an attempt was made to obtain lucerne from a farm whilst bloating actually occurred. A first sample was taken in September, 1945, on the farm Grapfontein, Fauresmith district, where early in the morning the preceding day sheep were suffering from bloat. The lucerne had been frosted when the sheep were put in, but no accident occurred. However, the lucerne collected before 8 a.m. proved to be haemolytic and the watery extract was frothing. A positive test with Brady's reagent was obtained later. In November a really bad case of bloating occurred on the farm Merwede in the district and the owner had the



TABLE 4.—Assimilates in progressive wilting of lucerne. Provence lucerne.

No.	Date.	Time.	PER GRAM DRY MATTER.				Water Content per gram Fresh Matter.	Ratio.	Organ.	Conditions.
			Starch.	Total Sugars.	Sucrose.	Reducing Sugars.				
3111	13. 2. 45	2.17 p.m.	2.00	2.52	0.82	1.70	60.2	Stems...	Fresh.	
3112			2.22	2.02	0.41	1.61	—	Leaves...	Fresh.	
3113	14. 2. 45	2.15 p.m.	1.49	2.99	1.41	1.58	55.8	Stems...	Drooping.	
3114			1.60	2.81	1.31	1.50	—	Leaves...	Drooping.	
3115	15. 2. 45	2.15 p.m.	2.03	3.09	1.06	2.03	55.1	Stems...	Wilted.	
3116			1.46	2.16	0.69	1.47	—	Leaves...	Wilted.	
3117	16. 2. 45	2.25 p.m.	1.08	3.25	0.99	2.26	51.8	Stems...	Drooping badly.	
3118			0.86	2.24	0.21	2.03	—	Leaves...	Drooping badly.	
3119	17. 2. 45	11.55 a.m.	1.21	2.95	0.68	2.27	51.1	Stems...	Getting dry, badly wilted.	
3120			0.49	1.78	0.18	1.60	—	Leaves...	Getting dry, badly wilted.	
3121	19. 2. 45	2.25 p.m.	1.44	2.44	0.68	1.76	49.45	Stems...	Badly wilted; leaves folding.	
3122			0.30	1.62	0.41	1.21	—	Leaves...	Badly wilted; leaves folding.	
3123	20. 2. 45	2.30 p.m.	1.02	2.02	0.13	1.89	48.64	Stems...	Leaves dry, lower leaves falling off.	
3124			0.58	1.84	0.91	0.93	—	Leaves...	Leaves dry, lower leaves falling off.	
3125	21. 2. 45	2.30 p.m.	0.33	2.62	1.20	1.42	50.00	Stems...	Cut in rain; leaves dry; falling off.	
3126			0.96	2.01	0.52	1.49	—	Leaves...	Cut in rain; leaves dry; falling off.	
3127	22. 2. 45	2.15 p.m.	0.58	2.18	0.86	1.32	50.74	Stems...	Badly wilted, most leaves brown.	
3128			0.00	1.50	0.49	1.01	—	Leaves...	Badly wilted, most leaves brown.	
3129	23. 2. 45	2.45 p.m.	0.53	1.70	0.53	1.17	52.89	Stems...	Badly wilted, only top of stems still green.	
3130			0.00	1.41	0.46	0.95	—	Leaves...	Badly wilted, only top of stems still green.	
3131	24. 2. 45	11.45 a.m.	0.49	2.11	0.58	1.53	46.52	Stems...	Very bad.	
3132			0.00	1.65	0.35	1.30	—	Leaves...	Very bad.	
3133	26. 2. 45	2.30 p.m.	0.00	1.76	0.45	1.31	46.98	Stems...	Brown leaves predominant.	
3134			0.00	1.67	0.31	1.36	—	Leaves...	Brown leaves predominant.	
3135			0.00	1.61	0.11	1.50	—	Leaves...	Brown only.	



TABLE 5.—Assimilates of lucerne after frost. Provence lucerne.

No.	Date.	Time.	PER GRAM DRY MATTER.				Water Content per gram Fresh Matter.	Ratio.	Organ.	Conditions.
			Starch.	Total Sugars.	Sucrose.	Reducing Sugars.				
3311	3. 9. 45	6. 40 a.m.	2.04	2.16	0.77	1.39	80.7	Leaves 1.0.... Stems 1.4.... Roots 5.9....	Leaves..... Stems..... Roots.....	Fresh. Fresh. Fresh.
3312			2.72	1.50	0.32	1.18				
3313			5.30	3.66	1.26	2.40				
3314	3. 9. 45	12 a.m.	2.78	1.62	0.11	1.51	78.9	— — —	Leaves..... Stems..... Roots.....	Fresh. Fresh. Fresh.
3315			1.89	1.70	0.13	1.57				
3316			4.48	3.51	0.91	2.60				
3317	3. 9. 45	6. 45 p.m.	0.89	1.73	0.45	1.28	75.6	— — —	Leaves..... Stems..... Roots.....	Fresh. Fresh. Fresh.
3318			1.37	1.75	1.02	0.73				
3319			6.21	4.09	1.99	2.10				
3320	3. 9. 45	12 p.m.	1.98	2.20	0.68	1.52	78.3	— — —	Leaves..... Stems..... Roots.....	Fresh. Fresh. Fresh.
3321			1.39	1.13	0.22	0.91				
3322			11.84	3.72	1.22	2.50				
3323	4. 9. 45	6 a.m.	1.43	2.05	0.50	1.55	77.5	— — —	Leaves..... Stems..... Roots.....	Frosted. Frosted. Frosted.
3324			3.55	1.62	1.60	0.02				
3325			5.43	4.37	3.00	1.37				

TABLE 6.—Assimilates in grazing experiments of Provence and Hunter's River lucerne.

No.	Date.	Time.	PER GRAM DRY MATTER.				Water Content per gram Fresh Matter.	Ratio.	Organ.	Conditions.
			Starch.	Total Sugars.	Sucrose.	Reducing Sugars.				
3326	10. 9. 45	8. 25 a.m.	1.19	1.33	0.22	1.01	Leaves 1.0....	Leaves.....	Cut before grazing. 3 Sheep put in. In good condition.	
3327			1.48	2.25	0.72	1.53	Stems 0.9....	Stems.....		
3328	10. 9. 45	11. 55 a.m.	1.39	1.60	0.18	1.42	—	Leaves.....	Still fresh, but a little trampled.	
3329			2.16	2.04	0.55	1.49	—	Stems.....		
3330		4. 10 p.m.	3.64	1.31	0.00	1.31	—	Leaves.....	Tops eaten off, otherwise still fresh.	
3331			1.60	2.37	1.63	0.74	—	Stems.....		
3332	11. 9. 45	12. 10 p.m.	2.72	2.07	1.23	0.84	—	Leaves.....	Fresh, but grazed down.	
3333			2.64	2.24	1.44	0.80	—	Stems.....		
3334	12. 9. 45	12. 05 p.m.	1.82	2.29	1.36	0.93	—	Leaves.....	Eaten down.	
3335			3.21	2.52	1.66	0.86	—	Stems.....		
3136	23. 2. 45	2. 45 p.m.	2.71	2.53	0.86	1.67	1:3.1.....	Stems.....	Cut before and let grow plus minus 4 ins. high, growing well, sheep put into it just after sample was taken.	
3137			7.41	1.94	0.53	1.41	—	Leaves.....		
3138	26. 2. 45	2. 30 p.m.	2.17	2.15	0.31	1.84	—	Stems.....	Fresh, but trampled by sheep.	
3139			5.06	2.13	0.00	2.13	—	Leaves.....		
3140	27. 2. 45	2. 20 p.m.	2.09	2.06	0.94	1.12	—	Leaves.....	Trampled by sheep for 2 days.	
3141			4.05	1.43	0.09	1.34	—	Stems.....		
3142	28. 2. 45	2. 45 p.m.	2.67	1.38	0.17	1.21	—	Stems.....	Much eaten, only bottom part of plant left. Tops eaten.	
3143			5.20	0.81	0.11	0.70	—	Leaves.....		

TABLE 6 (CONTINUED).

No.	Date.	Time.	PER GRAM DRY MATTER.				Water Content per gram Fresh Matter.	Ratio.	Organ.	Conditions.
			Starch.	Total Sugars.	Sucrose.	Reducing Sugars.				
3144	7. 3. 45	2. 20 p.m.	1.44	—	—	—	80.53	Stems.....	Trampled and eaten down by sheep, allowed to grow again 2-3 inches high.	
3145			3.28	1.60	0.18	1.42	—	Leaves.....		
3146	13. 3. 45	9. 20 a.m.	1.79	1.52	0.52	1.00	80.25	Stems.....	Fresh and growing well, plus minus 4 inches high. Cut before sheep were put into it.	
3147			2.73	1.51	0.19	1.32	—	Leaves.....		
3148	15. 3. 45	9. 15 a.m.	0.97	1.72	0.64	1.08	73.57	Stems.....	Trampled and tops eaten off by sheep.	
3149			4.07	1.67	0.86	0.81	—	Leaves.....		
3150	16. 3. 45	9. 25 a.m.	3.14	1.56	0.45	1.11	72.83	Stems.....	Grazed for 2 days; very short.	
3151			3.78	3.20	1.08	2.12	—	Leaves.....		
3337	20. 9. 45	8. 20 a.m.	4.53	1.47	1.06	0.41	79.5	Leaves.....	Grazed down and allowed to grow fresh.	
3338			4.06	1.58	0.89	0.69	—	Stems.....		
3339	20. 9. 45	12. 05 p.m.	2.05	1.04	0.28	0.76	74.0	Leaves.....	Very short.	
3340			4.71	1.46	0.88	0.58	—	Stems.....		
3341	20. 9. 45	4. 00 p.m.	7.55	1.01	0.19	0.82	74.4	Leaves.....	Very short, but fresh.	
3342			3.32	1.33	0.75	0.58	—	Stems.....		
3343	20. 9. 45	7. 49 p.m.	5.61	1.03	0.47	0.56	76.2	Leaves.....	Eaten down very short.	
3344			4.54	1.37	0.72	0.65	—	Stems.....		

TABLE 7.—Series II. Assimilates of lucerne in pots.

No.	Date.	Time.	Variety.	PER GRAM DRY MATTER.				Water Content per gram Fresh Matter.	Ratio.	Organ.	Conditions.
				Starch.	Total Sugars.	Sucrose	Reducing Sugars.				
3362	11.10.45	5 a.m.	P	2.52	2.15	1.92	0.23	71.7	Leaves 1.0	Fresh.	
3363				2.39	4.15	4.04	0.11		Stems 0.5	Fresh.	
3364				1.93	2.31	1.95	0.36		Small roots 15.2	Fresh.	
3365				7.10	6.36	5.95	0.41		Large roots.....	Fresh.	
3366	11.10.45	12 a.m.	P	5.10	2.14	2.01	0.13	68.2	Leaves.....	Fresh.	
3367				4.20	4.00	3.80	0.20		Stems.....	Fresh.	
3368				1.98	3.16	2.63	0.53		Small roots	Fresh.	
3369				6.33	7.04	6.60	0.44		Large roots.....	Fresh.	
3370	11.10.45	7.30 p.m.	P	4.42	2.23	2.12	0.11	68.8	Leaves.....	Fresh.	
3371				2.34	4.14	3.66	0.48		Stems.....	Fresh.	
3372				2.52	2.37	1.79	0.58		Small roots	Fresh.	
3373				13.61	5.23	4.71	0.52		Large roots.....	Fresh.	
3374	11.10.45	12 p.m.	P	5.17	3.37	2.46	0.91	69.4	Leaves.....	Slightly wilted.	
3375				3.67	3.66	3.16	0.50		Stems.....	Slightly wilted.	
3376				0.95	2.92	2.24	0.68		Small roots.....	—	
3377				9.43	6.73	6.23	0.50		Large roots.....	—	
3378	12.10.45	5.40 a.m.	P	4.48	2.64	2.31	0.33	66.6	Leaves.....	Slightly wilted.	
3379				3.45	3.34	2.62	0.72		Stems.....	Slightly wilted.	
3380				0.39	3.45	2.85	0.60		Small roots.....	—	
3381				9.00	7.18	5.09	2.09		Large roots.....	—	
3405	1.11.45	8.05 p.m.	P	1.61	1.92	0.42	0.50	74.0	Leaves.....	Fresh.	
3406				2.20	1.22	0.76	0.46		Stems.....	Fresh.	
3407				1.16	2.31	2.13	0.18		Small roots.....	—	
3408				5.78	1.83	1.35	0.48		Large roots.....	—	

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CARBOHYDRATE NUTRITION.

TABLE 7 (CONTINUED).

No.	Date.	Time.	Variety.	PER GRAM DRY MATTER.				Water. Content per gram Fresh Matter.	Ratio.	Organ.	Conditions.
				Starch.	Total Sugars.	Sucrose	Re- ducing Sugars.				
3409	2.11.45	12.30 a.m.	P.	3.33	1.34	0.27	1.07	69.2	—	Leaves..... Stems..... Small roots..... Large roots.....	Slightly withered..... Slightly withered..... — —
3410				1.91	1.47	1.17	0.30				
3411				1.35	1.14	1.04	0.10				
3412				1.73	5.30	5.02	0.28				
3413	2.11.45	5.15 a.m.	P.	2.31	1.07	0.75	0.32	76.0	—	Leaves..... Stems..... Small roots..... Large roots.....	Fresher than in the night.
3414				0.23	0.70	0.01	0.69				
3415				1.49	1.09	0.75	0.34				
3416				3.49	2.72	2.49	0.23				
3417	2.11.45	12.30 p.m.	P.	1.29	0.84	0.15	0.69	69.4	—	Leaves..... Stems..... Small roots..... Large roots.....	Slightly wilted. Slightly wilted. — —
3418				2.18	1.10	0.63	0.47				
3419				1.35	1.45	1.04	0.41				
3420				6.31	2.27	1.96	0.31				
3421	2.11.45	7.30 p.m.	P.	1.73	1.79	0.98	0.81	73.2	Leaves 1... Stems 1... Roots 4-6.	Slightly wilted. Slightly wilted. — —	
3422				1.61	2.08	1.61	0.47				
3423				1.67	2.11	1.73	0.38				
3424				2.54	2.22	1.87	0.35				
3490	19.12.45	12 a.m.	P.	1.35	2.85	2.14	0.71	75.4	Leaves 1... Stems 1.16 Roots 2.08	Fresh. Fresh. — —	
3491				1.18	2.54	2.04	0.50				
3492				1.66	5.98	4.13	1.85				
3493				2.31	4.10	3.35	0.75				
3494	19.12.45	8 p.m.	P.	1.74	2.75	1.99	0.76	74.6	Leaves..... Stems..... Small roots..... Large roots.....	— — — —	
3495				1.52	3.25	2.54	0.71				
3496				0.81	2.73	1.02	1.71				
3497				1.90	3.52	2.81	0.71				
3498	19.12.45	12 p.m.	P.	1.98	2.00	1.65	0.35	76.6	Leaves..... Stems..... Small roots..... Large roots.....	— — — —	
3499				1.99	2.40	1.92	0.48				
3500				3.32	1.08	0.82	0.26				
3501				3.27	4.47	4.30	0.17				

TABLE 7 (CONTINUED).

No.	Date.	Time.	Variety.	PER GRAM DRY MATTER.				Water Content per gram Fresh Matter.	Ratio.	Organ.	Conditions.
				Starch.	Total Sugars.	Sucrose	Re- ducing Sugars.				
3502	20.12.45	5.15 a.m.		1.81	1.21	0.74	0.47	72.8	Leaves 1...	—	
3503				2.31	0.87	0.55	0.42	—	Stems 1.16.	—	
3504				2.06	3.84	3.64	0.20	—	Small roots....	—	
3505				5.32	1.90	1.62	0.28	—	Large roots....	—	
3506	20.12.45	12 a.m.		2.36	1.99	1.33	0.66	74.0	Leaves.....	Fresh.	
3507				0.22	1.62	1.29	0.33	—	Stems.....	—	
3508				0.00	1.92	1.64	0.28	—	Small roots....	—	
3509				4.39	4.83	4.51	0.32	—	Large roots....	—	
3510	29. 4.46	11.45 a.m.	P.	1.48	1.43	1.09	0.34	78.3	Leaves.....	Fresh.	
3511				1.52	2.22	1.85	0.37	—	Stems.....	—	
3512				2.52	3.66	3.27	0.39	—	Small roots....	—	
3513				7.24	3.70	3.25	0.45	—	Large roots....	—	
3518	29. 4.46	7 p.m.		3.05	1.99	1.50	0.49	73.9	Leaves.....	—	
3519				7.77	4.09	3.15	0.94	—	Stems.....	—	
3520				5.71	2.31	1.56	0.75	—	Small roots....	—	
3521				10.56	3.60	3.25	0.35	—	Large roots....	—	
3514	30. 4.46	12.20 a.m.	P.	1.52	1.46	0.89	0.57	73.5	Leaves.....	—	
3515				4.68	2.66	2.16	0.50	—	Stems.....	—	
3516				1.47	3.25	2.63	0.62	—	Small roots....	—	
3517				3.24	1.82	0.88	0.94	—	Large roots....	—	
3522	30. 4.46	6.20 a.m.	P.	2.68	2.04	1.45	0.59	72.2	Leaves.....	—	
3523				2.44	3.08	2.16	0.92	—	Stems.....	—	
3524				0.81	3.21	2.63	0.58	—	Small roots....	—	
3525				10.7	7.42	7.20	0.22	—	Large roots....	—	
3626	30. 4.46	12.20 p.m.	P.	3.46	2.44	1.49	0.95	74.1	Leaves.....	—	
3527				5.74	3.47	3.03	0.44	—	Stems.....	—	
3528				6.55	3.23	2.61	0.62	—	Small roots....	—	
3529				12.54	7.32	6.24	1.08	—	Large roots....	—	

TABLE 8.—Series II. Assimilates of lucerne in plot.

No.	Date.	Time.	Variety.	PER GRAM DRY MATTER.				Water Content per gram. Fresh Matter.	Ratio.	Organ.	Conditions.
				Starch.	Total Sugars.	Sucrose.	Reducing Sugars.				
3347	11.10.45	5.55 a.m.	P.	1.91	1.11	0.00	1.11	66.0	Leaves 1.1	Leaves.....	Beginning to wilt.
3348				4.66	2.62	0.85	1.77	—	Stems 1.1	Stems.....	Beginning to wilt.
3349				7.31	5.74	0.57	5.17	—	Roots 9.5	Roots.....	Beginning to wilt.
3350	11.10.45	12.05 p.m.		4.57	1.22	0.00	1.22	61.6	—	Leaves.....	Slightly wilted.
3351				6.22	4.73	2.66	2.07	—	—	Stems.....	Slightly wilted.
3352				8.36	6.33	1.32	5.01	—	—	Roots.....	—
3353	11.10.45	7.35 p.m.		3.81	3.39	2.25	1.14	63.9	—	Leaves.....	Fresh.
3354				5.26	3.89	2.06	1.83	—	—	Stems.....	Fresh.
3355				10.83	7.85	7.52	0.33	—	—	Roots.....	—
3356	11.10.45	11.55 p.m.		1.15	2.60	2.11	0.49	63.8	—	Leaves.....	Fresh.
3357				3.41	5.13	4.92	0.21	—	—	Stems.....	Fresh.
3358				4.83	8.71	8.42	0.29	—	—	Roots.....	—
3359	12.10.45	5.50 a.m.		1.48	2.29	1.84	0.45	66.3	—	Leaves.....	Fresh.
3360				5.41	4.07	3.71	0.36	—	—	Stems.....	Fresh.
3361				8.73	7.92	2.56	5.36	—	—	Roots.....	—
3390	1.11.45	8.05 p.m.	P.	9.98	2.21	0.21	2.00	73.7	Leaves 1.2	Leaves.....	Slightly wilted.
3391				6.25	2.23	0.17	2.06	—	Stems 1.2	Stems.....	—
3392				9.75	4.72	0.71	4.01	—	Roots 6.0	Roots.....	—
3393	2.11.45	12.25 a.m.		4.59	1.62	0.01	1.61	71.8	—	Leaves.....	Slightly wilted.
3394				4.85	2.31	0.05	2.26	—	—	Stems.....	—
3395				12.42	4.46	0.88	3.58	—	—	Roots.....	—
3396	2.11.45	5.45 a.m.		3.86	2.06	0.29	1.77	73.2	—	Leaves.....	Fresh.
3397				5.03	2.44	0.08	2.36	—	—	Stems.....	—
3398				15.09	1.30	0.11	1.19	—	—	Roots.....	—
3399	2.11.45	12.10 p.m.		6.98	2.11	0.61	1.50	80.5	—	Leaves.....	Fresh.
3400				5.03	1.85	0.98	0.87	—	—	Stems.....	—
3401				13.47	4.82	3.94	0.88	—	—	Roots.....	—
3402	2.11.45	7.45 p.m.		3.49	1.67	0.49	1.18	57.9	—	Leaves.....	Fresh.
3403				2.18	1.26	1.26	0.92	—	—	Stems.....	—
3404				5.07	3.65	2.41	1.24	—	—	Roots.....	—



TABLE 9.—Series II. Sugars and starch of lucerne which caused bloating.

No.	Date.	Time.	PER GRAM DRY MATTER.				Water Content per gram Fresh Matter.	Organ	Origin and Conditions.
			Starch.	Total Sugars.	Sucrose.	Reducing Sugars.			
3382	25.10.45	—	1.92	2.82	1.68	1.14	Leaves.....	Received from Onderstepoort as baled lucerne. Originally from Upington. Fermented.	
3383			2.98	3.65	0.03	3.62	Stems.....		
3384	2.10.45	—	1.55	1.89	0.48	1.41	Leaves.....	Bale received from Onderstepoort; originally from Upington; mouldy and badly fermented.	
3385			1.13	5.82	1.71	4.11	Stems.....		
3386			0.63	3.37	0.72	2.65	Leaves.....	Bale received from Onderstepoort; partly mouldy and slightly fermented.	
3387			1.23	6.11	1.66	4.45	Stems.....		
3388			1.01	2.76	0.38	2.38	Leaves.....	Bale still good from Onderstepoort. Not fermented. All this lucerne caused bloating.	
3389			1.47	6.99	1.97	5.02	Stems.....		
3345	26. 9. 45	8 a.m.	4.26	1.37	0.75	0.62	Leaves.....	Grapfontein, 18 inches high. Gave bloating day before. No bloating when sampled.	
3346			3.14	1.72	0.50	1.22	Stems.....		
3425	13.11.45	4.25 p.m.	5.01	1.77	0.60	1.17	Leaves.....	Caused bloating on Mervede. Sampled whilst animals were dying. Put into Alcohol 2 hours after sampling.	
3426			1.43	2.18	1.45	0.73	Stems.....		
3427		5.30 p.m.	3.31	2.04	0.86	1.18	Leaves.....	Drooping slightly; causing bloating on Mervede. Put immediately in alcohol.	
3428			1.61	2.31	0.91	1.40	Stems.....		
3539	8. 9. 46	4 p.m.	0.20	4.15	3.84	0.31	Aerial parts	From same place as previous year.	

kindness to ring me and sample the lucerne whilst the sheep were dying. The material extracted with water foamed as much as the Oranjezicht *Tribulus*, was very strongly haemolytic and produced beautiful Brady's crystals, exactly the same forms (spider) as the *Tribulus*. The foam and extract were no longer positively haemolytic after 30 minutes standing. All in all the same picture was obtained as in the case of poisonous *Tribulus*. A saponin-like glucoside was present, and it was not in any case the same as Jacobson's saponin of Alfalfa (1919) which is not haemolytic and contains N.

A year later Merwede was visited on the 8th September when there was a heavy frost early in the morning and a cold wind blowing the whole day, the very day for bloating, as the farmer said. He had lost, during the preceding week, a sheep on lucerne and was afraid to put animals into the lucerne lands. Two samples were taken, one put into alcohol and the other fresh into a refrigerator. The one sample was from the same land as the year before, the second from an adjoining camp. Both, particularly the first, proved to be haemolytic although not as strongly so as in 1945.

In December, 1945, Onderstepoort sent Upington lucerne which had caused bloating in ten oxen at the Pretoria laboratory. All this lucerne proved to be haemolytic and gave Brady's reaction in later tests. A couple of months later, however, neither haemolysis nor crystals could be obtained. The Upington lucerne contained a large amount of  $\text{NO}_2$  as well, which tinted the blood brown by the formation of methaemoglobin (Quin and Rimington, 1933). A sample of baled Upington lucerne was given to me by the owner of Merwede, 3 months before it had caused bloating. When tested later it was neither haemolytic nor did it contain a substance giving crystals with Brady's solution. Other baled, rotten, lucerne supplied during the drought in 1945 from Upington to Fauresmith did not contain any saponin, but large amounts of  $\text{NO}_2$  and  $\text{NO}_3$ , tinting the blood brown within seconds and causing dozens of animals to die in agony.

The Legal test was always negative with lucerne.

None of our own lucerne grown on the Reserve and tested at the same time under similar meteorological conditions was ever haemolytic.

The saponin of the lucerne could be precipitated with normal lead acetate; when the material was stored for some time in the frigidaire the saponin could be precipitated by basic lead acetate. Only aerial parts were tested. The glucoside behaved really more like that of the roots of *Tribulus*. In spite of this difference, the Brady's crystals obtained from the lucerne leaves were the same in form as those obtained from *Tribulus* and had the same melting point. Yet there was one difference: Haemolysis and foaming and the quality to form Brady's crystals disappeared very quickly from lucerne even if stored on ice. In 1946 haemolysis and foaming disappeared within a week on iced lucerne, and no decent Brady's crystals could be obtained in the elution of  $\text{Mg}(\text{OH})_2$  after 10 days' storing.

Sugar and starch content of bloating lucerne is quite normal (Table 9). The figures for baled lucerne which contains a large amount of stem, reveal a large amount of reducing sugar. It is possible that the saponin just increases the permeability of the intestines to allow sugars and gases to pass with great velocity. On the other hand the possibility here also exists that the saponin is split up in the stomach and a large amount of sugars is freed, increasing the amount of sugars which ferment rapidly.

The presence of large amounts of saponin explains also why, if instead of invertase, weak hydrochloric acid is used to break up disaccharides, tremendous amounts of reducing sugars are obtained, of which there is no sign when an enzyme is used.

### C. GRAMINEAE. (TABLE 10.)

The Table (10) on the assimilates of the *Gramineae* shows the small content of starch in leaves and stems; the starch, however, does not decrease when the plants wilt. It may be the starch of the guard cells of the stomata (as under the microscope no other starch could be detected), and therefore it is not so easily dissolved as ordinary starch by wilting.

Frosting doubtlessly increases the sugar content of the grasses, particularly the hexoses, but this is not due to a breakdown of starch in leaves, but rather to a translocation of sugars from the roots, as the starch content is about the same as in ordinary times.

In fresh *Algerian oats* there is more fructose than glucose, and more sucrose than fructose. But in grazed *Panicum* the latter position is reversed.

With regard to daily graphs of the assimilates, the fresh *Algerian oats* do not seem to have any definite maximum of starch in the day time, but to have a definite maximum of total sugars in the evening; *Panicum*, however, shows a very small pronounced maximum of starch in the evening. Leaves and haulms are never quite empty of starch during the summer nights.

In the Fauresmith area "dikoor" caused by the eating of young, slightly wilted, grass is very rare, but it has occurred on the same soil when there were at times heavy dikkop outbreaks; no outbreak occurred, however, and no samples could be taken in recent years. Artificial conditions of cutting the grass and letting it grow two inches before being grazed and trampled did not produce any ill effects on sheep at the Veld Reserve, Fauresmith, though the experiment was repeated numerous times. One analysis is given as example in Table 10.

On the whole it can only be stated that in grazing experiments of this nature the photosynthetic activities seem to be restricted. The same is the case with lucerne.

It is of interest to compare the obtained data with those of Weinmann and Reinhold (1946). *Algerian oats* and *Panicum minus* apparently belong to the grasses whose chief carbohydrate is non-reducing sugar with starch present. Weinmann's criticism of the authors who uphold the negligible quantity of starch present in grasses can only be agreed to, as the amounts of starch found in the present investigation are even higher than his. Another important point is the unpublished results of Bunting, cited in the paper, of the variability and occasionally very high values of total sugars of the stem bases of indigenous grasses in the Transvaal winter, and his own data (Figs. 2 and 3) of increasing starch and reducing sugar from April to winter. They find their parallel in experiment 2517/24 of 18/19.6.43, where enormous quantities of sugar appear after frosting and in the lucerne experiments under the same conditions (Henrici, 1949), though the explanation given in the present instance differs, and is certainly not thought of in terms of material not used for growth.



TABLE 10.—Assimilates of Gramineae of Fauresmith.

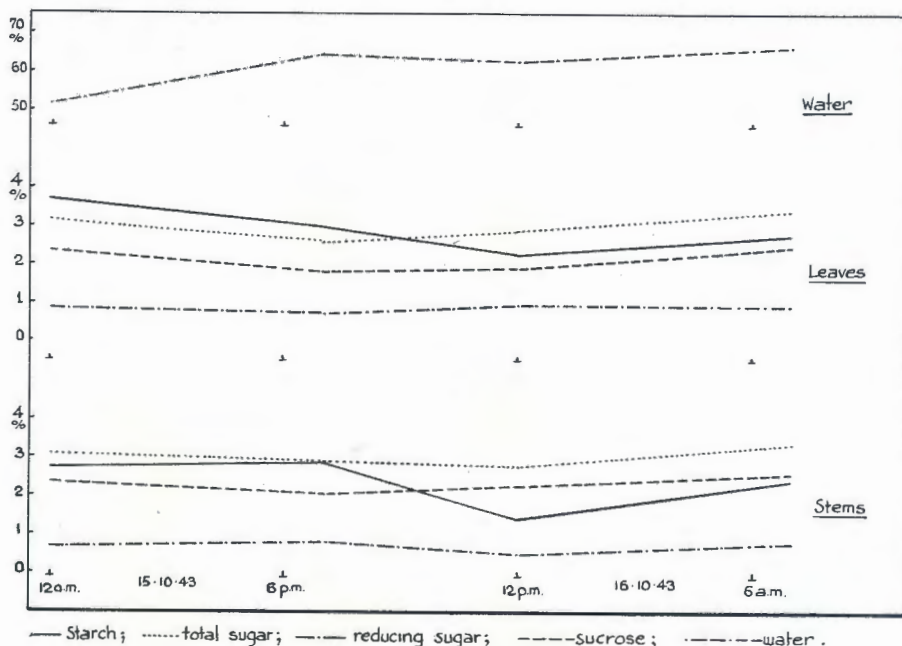
No.	Date.	Time.	Plant.	PER GRAM DRY MATTER.					Water Content per gram Fresh Matter.	Organ.	Conditions.	
				Starch.	Total Su-crose. gars.	Su-crose. gars.	Redu-cing Su-gars.	Glucose.				Fruc-tose.
2425	19. 3. 43	12 a.m.	Algerian oats.	1.02	2.12	1.33	0.79	0.14	0.69	70.9	Stems.....	Fresh.
2426			Algerian oats.	0.51	1.61	0.61	1.00	0.28	0.72	—	Leaves.....	Fresh.
2427	19. 3. 43	7 p.m.	Algerian oats.	0.75	1.76	1.00	0.76	0.20	0.56	77.7	Stems.....	Fresh.
2428			Algerian oats.	0.51	2.00	1.06	0.94	0.12	0.82	—	Leaves.....	Fresh.
2429	19. 3. 43	12 p.m.	Algerian oats.	0.63	1.50	0.73	0.77	0.10	0.67	77.8	Stems.....	Fresh.
2430			Algerian oats.	0.73	1.71	0.76	0.95	0.11	0.84	—	Leaves.....	Fresh.
2431	20. 3. 43	7 a.m.	Algerian oats.	0.90	1.12	0.11	1.01	0.12	0.89	56.4	Stems.....	Fresh.
2432			Algerian oats.	0.70	1.59	0.55	1.04	0.18	0.86	—	Leaves.....	Fresh.
2481	5. 4. 43	12 a.m.	Algerian oats.	1.09	3.15	2.01	1.14	0.23	0.91	64.36	Stems.....	Permanently wilted.
2482			Algerian oats.	1.71	3.24	1.90	1.34	0.37	0.97	—	Leaves.....	Permanently wilted.
2483	5. 4. 43	7 p.m.	Algerian oats.	1.24	3.41	2.02	1.39	0.40	0.99	63.95	Stems.....	Permanently wilted.
2484			Algerian oats.	1.62	3.59	2.36	1.23	0.30	0.93	—	Leaves.....	Permanently wilted.
2485	5. 4. 43	12 p.m.	Algerian oats.	1.24	2.51	1.07	1.44	0.57	0.87	67.95	Stems.....	Permanently wilted.
2486			Algerian oats.	1.19	3.29	1.81	1.48	0.01	0.87	—	Leaves.....	Permanently wilted.
2487	6. 4. 43	7 a.m.	Algerian oats.	0.84	2.72	1.20	1.52	0.46	1.06	61.42	Stems.....	Permanently wilted.
2488			Algerian oats.	1.27	3.42	1.86	1.56	0.48	1.08	—	Leaves.....	Permanently wilted.
2517	18. 6. 43	12 a.m.	Algerian oats.	1.70	6.68	0.59	6.09	—	—	72.9	Stems.....	Fresh frosted.
2518			Algerian oats.	2.73	7.27	2.29	4.98	—	—	—	Leaves.....	Fresh frosted.
2519	18. 6. 43	7 p.m.	Algerian oats.	2.33	7.38	1.58	5.80	—	—	69.66	Stems.....	Fresh frosted.
2520			Algerian oats.	1.30	5.73	1.37	4.36	—	—	—	Leaves.....	Fresh frosted.
2521	12 p.m.		Algerian oats.	2.02	7.18	1.82	5.36	—	—	68.69	Stems.....	Fresh frosted.
2522			Algerian oats.	0.89	10.08	5.81	4.27	—	—	—	Leaves.....	Fresh frosted.
2523	19. 6. 43	7 a.m.	Algerian oats.	0.78	7.79	3.10	4.69	—	—	72.67	Stems.....	Fresh frosted.
2524			Algerian oats.	1.09	7.12	3.44	3.68	—	—	—	Leaves.....	Fresh frosted.
2372	2. 1. 43	Noon	Algerian oats.	1.36	1.92	1.72	0.2	0.00	0.21	78.7	Leaves and stems.	Fresh.
2391	25. 1. 43	Noon	Algerian oats.	2.43	1.71	1.50	0.2	0.00	0.20	33.6	Leaves and stems	Wilted.
2416	13. 2. 43	Noon	Algerian oats.	2.73	1.70	1.32	0.38	0.00	0.38	41.0	Leaves and stems	Wilted and recovered.

TABLE 10 (CONTINUED).

No.	Date.	Time.	Plant.	PER GRAM DRY MATTER.					Water Content per gram Fresh Matter.	Organ.	Conditions.		
				Starch.	Total Su-gars.	Su-crose.	Redu-cing Su-gars.	Glu-cose.				Fruc-tose.	
2690	23. 12. 43	12 a.m.	Panicum minus varietas planifolium	1.79	1.90	0.75	1.15	—	—	64.68	Stems.....	Fresh.	
2691				1.49	1.64	0.32	1.32	—	—	—	—	Leaves.....	Fresh.
2692	23. 12. 43	7 p.m.		1.20	2.34	0.73	1.61	—	—	63.54	Stems.....	Fresh.	
2693			1.59	2.49	0.74	1.75	—	—	—	—	Leaves.....	Fresh.	
2694			1.13	2.85	1.46	1.39	—	—	—	60.31	Stems.....	Fresh.	
2695		12 p.m.	1.25	2.73	0.94	1.79	—	—	—	—	Leaves.....	Fresh.	
2696	24. 12. 43	7 a.m.	1.10	2.10	1.32	0.78	—	—	—	64.66	Stems.....	Fresh.	
2697			1.46	2.48	0.89	1.59	—	—	—	—	Leaves.....	Fresh.	
2698	24. 12. 43	12 a.m.	0.88	2.79	1.38	1.41	—	—	—	65.00	Stems.....	Fresh.	
2699			2.00	2.71	1.03	1.68	—	—	—	—	Leaves.....	Fresh.	
2700	12. 1. 44	12 a.m.	Panicum minus varietas planifolium	0.71	1.71	0.04	1.67	—	—	60.47	Stems.....	Cut, let grow about 2 inches, sheep put into it and then eaten and trampled.	
2701				0.81	1.85	0.72	1.13	—	—	—	Leaves.....		
2702	12. 1. 44	7 p.m.		1.21	1.26	0.10	1.16	—	—	57.14	Stems.....		
2703			1.58	1.38	0.26	1.12	—	—	—	—	Leaves.....		
2704	12. 1. 44	12 p.m.	1.15	1.28	0.25	1.03	—	—	—	59.66	Stems.....	Stems.....	
2705			1.25	1.17	0.08	1.09	—	—	—	—	Leaves.....		
2706	13. 1. 44	7 a.m.	0.86	1.45	0.14	1.31	—	—	—	55.85	Stems.....	Stems.....	
2707			1.21	1.16	0.05	1.11	—	—	—	—	Leaves.....		
2708	13. 1. 44	12 a.m.	1.01	1.75	0.59	1.16	—	—	—	56.55	Stems.....	Stems.....	
2709			1.16	1.36	0.13	1.23	—	—	—	—	Leaves.....		

## PENTZIA INCANA. (TABLES 11 AND 12.)

Even under the best meteorological conditions, *Pentzia incana* does not contain very much starch, 3.7 per cent. being the highest value observed. (Exp. 2640/47.) When drought sets in the starch values decrease considerably, particularly in the twigs. In a prolonged drought and after the rainless winter, the percentage of starch in leaves and twigs is indeed very small (see particularly Exp. 2311-2320 of 1941). In fairly normal leaves of *Pentzia* there is at midday a starch maximum and a minimum early in the morning; during drought the time of the maximum is at night, and that of the minimum during day time. It is noteworthy that of all the plants so far investigated this Karoo bush has at times 0 values of starch during day time, a fact never observed even at night time in the other plants. *Pentzia* has the reputation of being an excellent fodder plant in the fresh state, but deteriorates very quickly in drought. The observation about the starch gives an answer to the cause of this deterioration.

GRAPH 6.—*Pentzia incana*, fresh.

In fresh *Pentzia* total sugars are quite abundant, mostly consisting of sucrose. In dry *Pentzia* the total sugars have decreased at the expense of cane sugar, and reducing sugars prevail. The total sugars have a maximum during day time, generally in the late afternoon during drought, and early in the morning during rainy periods. Young twigs and leaves contain nearly the same amount of sugars when fresh, yet starch is more decidedly present in the leaves. Contrary to the soft-leaved lucerne or *Tribulus*, it does not seem as if the twigs in time of drought take over the assimilation; they contain less assimilates than the leaves!

Another important point of difference is the fact that absolutely young fresh leaves and twigs during times of drought contain very small amounts of assimilates,



TABLE 11.—Sugar and starch content of *Pentzia incana*.

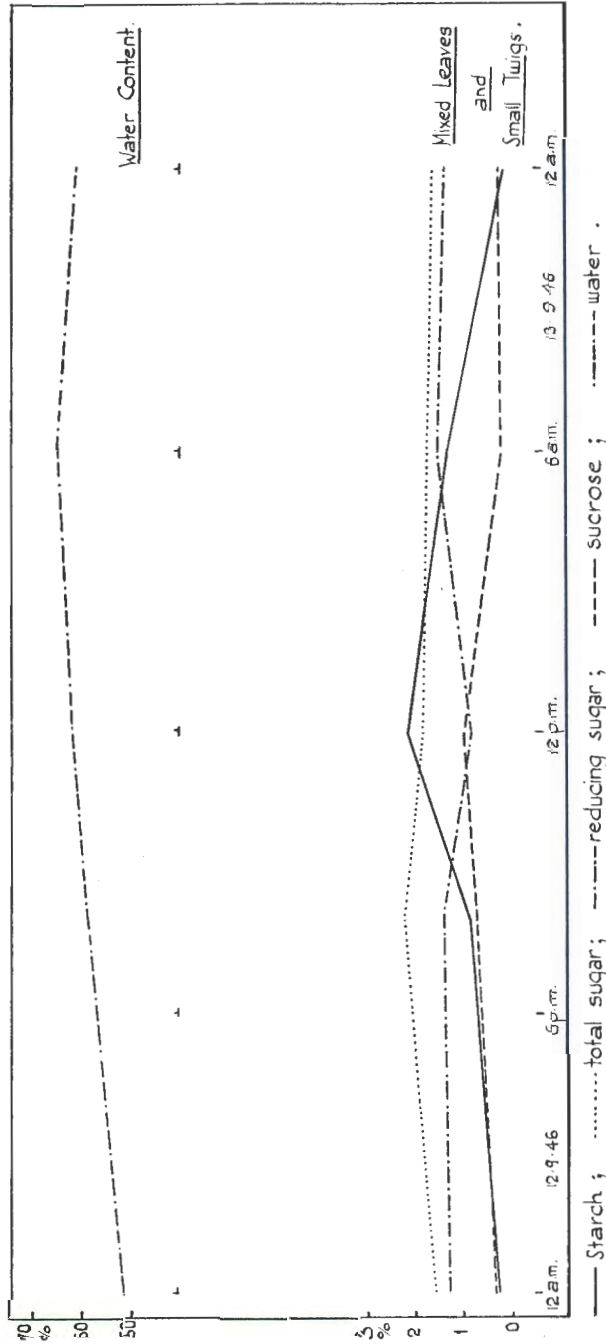
No.	Date.	Time.	PER GRAM DRY MATTER.				Water Content per gram Fresh Matter.	Organ.	Conditions.
			Starch.	Total Sugars.	Sucrose.	Reducing Sugars.			
2640	15.10.43	12 a.m.	3.65	3.17	2.34	0.83	51.74	Leaves.....	Fresh.
2641			2.71	3.03	2.34	0.69	—	Stem.....	
2642	15.10.43	7 p.m.	2.96	2.52	1.80	0.72	64.23	Leaves.....	
2643			2.81	2.81	2.03	0.78	—	Stem.....	
2644	15.10.43	12 p.m.	2.24	2.89	1.91	0.98	62.79	Leaves.....	
2645			1.40	2.72	2.23	0.49	—	Stem.....	
2646	16.10.43	7 a.m.	2.76	3.40	2.48	0.92	66.50	Leaves.....	
2647			2.39	3.30	2.54	0.76	—	Stem.....	
2842	18. 4.44	12 a.m.	1.52	1.81	0.77	1.04	39.69	Stem.....	After long drought, permanently wilted.
2843			3.29	2.29	1.07	1.22	—	Leaves.....	very dry
2844	18. 4.44	7 p.m.	1.88	1.34	0.31	1.03	34.77	Stem.....	
2845			1.45	1.74	0.69	1.05	—	Leaves.....	
2846	18. 4.44	12 p.m.	1.24	1.63	0.50	1.13	30.42	Stem.....	
2847			1.94	1.61	0.45	1.16	—	Leaves.....	
2848	19. 4.44	7 a.m.	1.13	1.71	0.62	1.09	46.63	Stem.....	
2849			2.12	1.98	0.90	1.08	—	Leaves.....	
2850	19. 4.44	12 a.m.	1.47	1.63	0.20	0.43	42.47	Stem.....	
2851			0.19	1.36	0.67	0.69	—	Leaves.....	
3036	4.12.44	12 a.m.	0.0?	—	—	—	41.17	Leaves and	Cut after long drought.
3037	4.12.44	7.30 p.m.	0.0?	2.31	0.81	1.50	35.45	small twigs.	Cut after long drought.
3038	5.12.44	5.45 a.m.	2.33	1.27	0.45	0.82	45.23	—	Cut after long drought.
3039	5.12.44	11.45 a.m.	0.92	1.74	0.27	1.47	57.94	—	Cut after long drought.
3546	12. 9.46	12 a.m.	0.26	1.55	0.29	1.26	51.20	Mixed leaves	After heavy drought.
3547	12. 9.46	8 p.m.	0.84	2.21	0.76	1.45	59.70	and small	After heavy drought.
3548	12. 9.46	12 p.m.	2.14	1.89	1.00	0.89	62.50	twigs.	After heavy drought.
3549	13. 9.46	6 a.m.	1.31	1.76	0.21	1.55	65.80	—	After heavy drought.
3550	13. 9.46	12 a.m.	0.19	1.67	0.27	1.40	61.10	—	After heavy drought.
3571	1.11.46	12 a.m.	1.07	1.60	1.41	0.19	77.00	Mixed leaves	Cut after heavy drought, new growth.
3572	1.11.46	7 p.m.	1.38	1.19	0.65	0.54	77.3	and small	Cut after heavy drought, new growth.
3573	1.11.46	12 p.m.	1.68	1.23	1.08	0.15	79.1	twigs.	Cut after heavy drought, new growth.
3574	2.11.46	6 a.m.	0.39	1.91	1.67	0.24	79.4	—	Cut after heavy drought, new growth.
3575	2.11.46	12 a.m.	1.22	1.44	1.13	0.31	75.7	—	Cut after heavy drought, new growth.

TABLE 12.—Sugar and starch content of *Pentzia incana*.

No.	Date.	Plot No.	PER GRAM DRY MATTER.				Water Content per gram Fresh Matter.	Organ.	Conditions.
			Starch.	Total Sugars.	Sucrose.	Reducing Sugars.			
2311	7. 10. 41	10/1	0.10	1.22	0.82	0.40	71.49	Leaves and young twigs..	—
2312		10/2	0.12	1.15	0.84	0.31	67.37	Leaves and young twigs..	Fair, no grass.
2313		10/3	0.19	1.13	0.48	0.65	70.12	Leaves and young twigs..	Some plants dry, plenty of grass.
2314		10/4	0.12	1.10	0.79	0.31	69.48	Leaves and young twigs..	Poor.
2315		10/5	0.32	1.70	0.99	0.71	71.31	Leaves and young twigs..	Good.
2316		10/6	0.26	1.13	0.37	0.76	69.62	Leaves and young twigs..	Fair.
2317		10/7	0.96	0.82	0.43	0.39	69.22	Leaves and young twigs..	Plants poor, but some much better.
2318		10/8	0.48	1.42	0.56	0.86	67.35	Leaves and young twigs..	Good, no grass.
2319		10/9	0.69	1.36	0.91	0.45	70.98	Leaves and young twigs..	Good.
2320		10/10	1.66	1.56	0.24	1.32	73.09	Leaves and young twigs..	Fair, green leaves and twigs.

particularly of starch, in spite of a water content of over 70 per cent. The ratio of sucrose to reducing sugars is in accordance with previous experience on fresh *Pentzia*, but the absolute amount of the sugar is much smaller. The plants in question (exp. 3571-2575 of 1/2.11.46) were defoliated and cut down a month previously to get completely new growth.

GRAPH 7.—*Pentzia incana*, after bad drought.



## DISCUSSION AND SUMMARY.

The daily march of assimilates in different fodder plants, *lucerne*, the Karoo bush *Pentzia incana*, the Gramineae *Algerian oats* and *Panicum minus* and *Tribulus terrestris*, is discussed. The assimilates were determined under different meteorological and physiological conditions. All plants were grown on ordinary Karoo soil on the Veld Reserve, Fauresmith, and had, except for lucerne, rain as the only source of water. The lucerne plots were regularly irrigated and the plot had been fertilized 3 years previously with a heavy dressing of superphosphate. Lucerne was also used in pots. *Lucerne*, *Tribulus* and the Gramineae were investigated in fresh, temporarily wilted and permanently wilted conditions or in condition of progressive wilting. *Pentzia* which does not show signs of wilting was collected fresh or dry after long droughts. Under favourable meteorological conditions and working with a fresh plant the curves of the assimilates of no plant show anything unusual, the maximum of starch being either in the afternoon or early evening. It is under unfavourable meteorological conditions, resulting in drooping or wilted plants, that disturbances in the carbohydrate metabolism occur such as dissolution of starch, increase of sugar and unusual times of the maximum of starch, particularly in leaves.

All the plants mentioned above contain starch, sucrose, fructose and glucose. But the ratio of the different carbohydrates varies with the genus. Gramineae at the best of times contain little starch, but rather more total sugars than lucerne. In the Gramineae therefore the daily variations of the sugar are more pronounced than those of the starch. The latter is most abundant in the guard cells of the stomata and may not be quite the same as ordinary starch produced daily during photosynthesis. *Pentzia*, too, never reaches amounts of starch as those present in fresh lucerne. Whilst it is generally accepted that the leaves are the organs for the formation of starch, it has been observed in lucerne and *Tribulus*, particularly in the latter, that the stems take over the photosynthesis or at least the starch formation in times of drooping and incipient drying; more starch is found in stems than in leaves at that time. *Pentzia* does not show such a phenomenon.

*Pentzia*, particularly in drought loses all its starch, whilst lucerne, except at the beginning of winter, always contains an appreciable amount.

In all the feeding experiments on the Veld Reserve, Fauresmith, no animal showed any disturbance; on the contrary, lambs thrived on all the plants under all meteorological conditions. When under similar meteorological conditions in the surroundings of Fauresmith disturbances such as bloating, dikoor and dikkop were observed, it seemed logical to think that another factor besides the animal played a rôle and that this factor was a soil factor. Actually it was suggested that this factor was a zinc deficiency in flush periods of growth in the sense of Hoagland (1944). Although this hypothesis could not be confirmed owing to war conditions, it seemed reasonable to accept that under the influence of different soils the plants might differ in some of their chemical constituents. Actually it was found that *Tribulus* and *lucerne* from farms where there was a dikkop outbreak or where bloating occurred contained a saponin during the height of summer. This saponin was absent in *Tribulus* and lucerne from the Fauresmith Veld Reserve during the summer of 1946. The glucoside was, however, absent in all plants either very early or very late in the season.

Another unfavourable condition for lucerne besides drought, which decreases the amount of starch and increases the sugar content in leaves and stems, is frost, but this occurrence is presumably due to migration from the root and not only to assimilation.



To make sure that this factor is conditioned by the soil and not by meteorological influence, soil of places renowned for dikkop outbreaks was brought to the Veld Reserve, Fauresmith, and beds were established with *Tribulus* (seeds from Fauresmith), getting only rain or distilled water. These plants on soil from Calvinia, Waagkop or Leeuwfontein contained the same saponin during the height of the season 1946, whilst in the surrounding *Tribulus* on Fauresmith soil it was absent. Having *Tribulus* from different soils near the laboratory it was possible to study the daily march of the assimilates as influenced by the soil. In principle there was no difference in the daily curve, although on some soils the plants wilted quicker. The usual changes in starch and sugars occurred in this case as described above.

Some chemical qualities of this saponin in question are described. It is possible that this glucoside is not the only constituent which varies in the plants from different soils. American authors have described a polyphenolic substance, a phytosterin, which is formed amongst other disturbances in the plants on zinc deficient soil. It is thought that the present saponin might be a related compound. In how far it is connected with dikkop, future animal experiments will have to show.

If the saponin conditioned by the soil factor proves to be a link in the chain of Tribulosis, it must be understood that it is only *one* of the conditions necessary to produce dikkop, but a primary one. There are quite a few others. First there is the question whether in high summer the glucoside is present throughout the plants on the particular soils, whether its effect is only obvious when the veld is bare, or whether saponin is only present at certain times and whether it is always absent on Fauresmith soil. If sheep are grazing on veld with few other plants but *Tribulus*, on which they are forced to live, the occurrence of dikkop is much more likely. If plenty of other plants are available an occasional mouthful of *Tribulus* with the saponin may prove absolutely harmless. Hamels may graze without any ill-effect for a long time whilst hungry ewes may immediately succumb.

Climatic conditions play as big a rôle, as they are the first to influence the veld, abundance of fodder being available with regular good rains, whilst plenty of "dubbeltjies" (*Tribulus*) is available with occasional small showers. Moreover, they also cause flush periods and incipient drying and wilting of the *Tribulus* itself. So, as mentioned before, it seems that the glucoside has a seasonal trend, being present in very small quantities at the start of the season, and absent at the end of the summer. Up to now other fluctuations within the summer are still unknown, but these will be investigated in the near future at the Veld Reserve, Fauresmith.

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