XCI. THE ANTISCORBUTIC POTENCY OF APPLES. VI.

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PRELIMINARY observations made in connection with this inquiry demonstrated that, whilst Bramley's Seedling apples were antiscorbutically more active than King Edward apples, both varieties being typical late culinary sorts, their nitrogen content was definitely lower [Bracewell, Hoyle and Zilva, 1930, 1, 2]. In consequence of this observation we attempted [Bracewell, Wallace and Zilva, 1931] to ascertain whether by changing the nitrogen contents of fruits of these varieties their antiscorbutic activities could at the same time be influenced. It was found that in the case of the King Edward variety the vitamin C content was indeed higher in the apples in which the nitrogen content was lowered by cultural treatment, *i.e.* by substituting "grass" for arable culture. The difference in the antiscorbutic potency between the high and low nitrogen apples of the Bramley's Seedling variety was, however, very much less marked and its magnitude fell within the limits of the experimental error.

These results although not sufficiently clear-cut to be conclusive were suggestive enough to make it advisable to pursue the subject further. The experiments have therefore been repeated and extended during the last 3 years. In these further tests the nitrogen contents of the two varieties previously used have been varied by two methods; by substituting "grass" for arable culture and by the operation of bark-ringing vigorous trees. In these two cases the "grass" and bark-ringing practices lower the nitrogen contents of the fruits and at the same time usually increase the percentages of dry matter and total sugars [Wallace, 1930; Wallace, Charley and Jones, 1930], bark-ringing being generally more effective in increasing the latter two constituents. The results we have obtained are in our opinion definite enough to support the view that in the case of apple varieties an inverse relationship exists between the nitrogen content and the vitamin C activity.

EXPERIMENTAL.

A detailed description of the procedure followed in the vitamin C tests and their assessment has been given already in a previous communication of this series [Bracewell, Hoyle and Zilva, 1930, 1]. In all tests the fruits immediately after picking were placed in a low temperature store and kept at 1°. The humidity of the chamber was maintained at about 95 %. Samples for testing purposes were drawn from the store at weekly intervals. The chemical determinations in all cases were made at the time of placing the samples in store. The material for analyses, in every case, was obtained from 25 fruits per sample by removing a

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thin layer of peel and cutting thin slices across the fruits at right angles to the core, slices for nitrogen determinations always being taken at the equatorial regions and those for sugars, acid and ash from either side of the nitrogen regions in this order. The following are the cultural details relating to the trees from which the samples were obtained.

1930. Bramley's Seedling.

Arable, high nitrogen sample. The fruits were grown at Ledbury, Herefordshire. The soil is a heavy loam containing large proportions of the fine sand and silt fractions and is derived from marl deposits of the Old Red Sandstone formation. It contains a small supply of calcium carbonate. The trees are well grown half standards, from 25 to 30 years old, and since planting they have always been under high cultural conditions. Manuring until a year or two previous to 1930 had been very heavy, especially large dressings of nitrogen and phosphorus being applied, and the trees reflected the treatment. During the season of taking the samples, growth was vigorous and all trees carried a heavy crop of medium sized, green fruits. The fruits selected for the experiment were typical of the crop, being only of medium size, green with an occasional dull flush, juicy and of sharp flavour. Picking was carried out on October 15th. During the period of the test the fruits remained in a fresh condition and no storage breakdown occurred.

Grass, low nitrogen sample. This sample was obtained from an orchard at Marston Magna, Somerset. The soil is a heavy loam with a clay subsoil and is mainly derived from Lower Lias clay. There is a surface wash present consisting of mixed material of Middle Lias and Lower Lias origin, the sand of the former serving to lighten the surface layer. The soil is slightly calcareous. The trees are of the bush form and had been planted in 1912. Until 1917, the plantation was maintained under arable conditions but from then until the year of sampling the trees had been under grass culture with occasional stocking and had degenerated into typical low nitrogen specimens. No manures had been applied to the area while the trees were under grass.

In 1930, the trees were of poor vigour, there being practically no shoot growth, and the foliage was of yellowish-green colour. The crop was heavy and the fruits medium in size with pale ground colour and generally a brilliant red flush, typical of low nitrogen conditions. The flesh was white and firm and the flavour sweet. Picking was carried out on October 14th. The fruits remained in good condition during the test, and no breakdown was recorded.

Not ringed, high nitrogen sample. These fruits were grown near Wisbech under conditions typical of the district. The soil is a typical heavy silt-clay with a characteristic high water table. The trees are of the bush type and were approximately 20 years old at sampling. They had been grown under high cultural conditions until a season or two before, when cultivation was lowered to reduce the vegetative growth. Manuring had not been heavy in view of the natural vigour of the trees on this type of soil. In 1930 the trees were growing strongly and the foliage was dark green in colour indicating high nitrogen conditions. The trees selected for the test carried a medium crop, the size of the fruits being mostly medium, the colour dark green and the flesh juicy and acid to taste. Picking was done on October 17th. During the test all fruits remained fresh and sound in store.

Ringed, low nitrogen samples. This sample was gathered from trees in the same orchard as the previous "not ringed" sample and all conditions for growth *etc.* were comparable in the two cases with the exception of the "ringing"

treatment which had been performed during May 1930. The effect of the ringing was typical and very marked; shoot growth was greatly restricted and the leaves were pale green, and during the latter part of the season showed high yellow, red and purplish tints. The fruits had the appearance of low nitrogen specimens, possessing a very pale ground colour with bright red flush, while the flesh was white, hard and notably sweet for the variety. The crop was again medium although slightly less than the "not ringed." Picking was carried out on October 17th. Towards the end of the test, many fruits showed symptoms of "Flesh Breakdown" and at the conclusion of the experiment an examination was made with the following results; 30 fruits cut showed 19 with "Flesh Breakdown" to a medium extent and 1 with "Bitter Pit."

1931. *Bramley's Seedling.*

Not ringed, high nitrogen sample. This sample was secured from the same plantation at Faversham, Kent, as the 1929 Bramley, high nitrogen fruits [see Bracewell, Wallace and Zilva, 1931].

In 1931, the cultivation differed from that of 1929, the cultural system having been changed in 1930 from "arable" to "grass" with a view to lowering the highly nitrogenous growth of the trees. Nevertheless, in 1931, the trees were still growing vigorously and bearing excellent green foliage. The crop was heavy, the fruits being of medium size, green in colour with an occasional dull brownish-red flush. The flesh was juicy and fairly acid. Picking was carried out on September 19th. The fruits appeared fresh throughout the test and at the conclusion a storage examination showed the following results; 50 cut fruits gave 4 very slight "Core Flush" and 1 very slight "Flesh Breakdown."

Ringed, low nitrogen sample. These fruits were from trees comparable in every way with those of the previous sample with the exception that the trees in this case had been "ringed" in May, 1931. The effect of the ringing treatment was again very pronounced, both on the growth characters of the trees and the quality of the crop. The effects of the ringing were as described in the 1930 experiment. The crop was heavy, fruit size was slightly larger than in the "not ringed" sample and the fruits were highly coloured, white fleshed and very sweet to taste. Some storage breakdown and a certain amount of slight "Superficial Scald" developed during the later stages of the experiment, an examination at the conclusion yielding the following results; 50 fruits cut showed 32 slight, 1 medium and 2 severe cases of "Flesh Breakdown."

It should be noted that the flavour of "breakdown" specimens was fairly fresh and suggested plentiful supplies of acid and sugar.

1932. *King Edward VII.*

Not ringed, high nitrogen sample. The fruits of this sample were taken from the same group of trees at Long Ashton Research Station as those used for the arable, high nitrogen series in the 1929 experiments. The cultural and other treatments practised on the plot previous to 1929 had been maintained until 1931 without any change, and the trees had continued to grow vigorously.

The 1932 crop was medium and the fruits were of medium size and bright green in colour with an occasional dull brownish-red flush. The flesh was fairly juicy and the flavour medium acid and typical of the variety. Picking was done on October 14th. No storage breakdown occurred during the period of the test.

Ringed, low nitrogen sample. These fruits were from the same group of trees as those of the previous sample, the only difference being the ringing treatment. In order to obtain a large effect, the trees were "ringed" in two consecutive

Table I.

Season	Plantation	Variety	Special treatment	As % fresh weight						Ash in dry matter	Ash constituents as % ash				Ratio total sugars/nitrogen
				Dry matter	N	Acidity as malic acid	Reducing sugars	Sucrose	Total sugars R+S		CaO	MgO	K ₂ O	P ₂ O ₅	
1929	Faversham, Kent	Bramley's Seedling	Arable (high N)	11.9	0.0388	1.135	5.84	2.27	8.11	1.8	2.4	2.6	53.7	9.6	209
	Faversham, Kent	Bramley's Seedling	Grass (low N)	11.5	0.0169	0.838	6.69	1.97	8.66	1.6	3.8	2.5	50.9	12.0	512
1929	Long Ashton, Somerset	King Edward VII	Arable (high N)	13.5	0.0389	0.968	6.80	2.37	9.17	1.6	2.4	2.1	53.0	9.0	236
	Bratton, Wiltshire	King Edward VII	Grass (low N)	16.8	0.0307	0.750	10.28	1.97	12.25	1.1	3.1	3.1	50.4	10.7	399
1930	Ledbury, Herefordshire	Bramley's Seedling	Arable (high N)	11.4	0.058	0.79	7.26	1.28	8.54	1.29	3.02	3.38	53.5	12.8	147
	Marston Magna, Somerset	Bramley's Seedling	Grass (low N)	12.5	0.036	0.73	7.57	1.86	9.43	1.29	—	—	47.1	16.2	262
1930	Wisbech, Cambs.	Bramley's Seedling	Not ringed (high N)	10.9	0.059 (Dupl. sample)	0.99	5.92	1.79	7.71	1.98	—	—	55.3	9.62	131
	Wisbech, Cambs.	Bramley's Seedling	Ringed (low N)	13.8	0.044	1.00	6.75	3.38	10.13	1.47	—	—	56.5	7.45	230
1931	Faversham, Kent	Bramley's Seedling	Not ringed (high N)	12.0	0.039	1.18	6.59	2.28	8.87	1.45	2.50	—	50.2	14.1	227
	Faversham, Kent	Bramley's Seedling	Ringed (low N)	14.7	0.031	1.14	7.00	3.43	10.43	1.06	2.44	—	54.6	10.0	336
1932	Long Ashton, Somerset	King Edward VII	Not ringed (high N)	12.0	0.031	0.93	6.29	2.26	8.55	1.52	2.48	2.39	53.9	10.15	276
	Long Ashton, Somerset	King Edward VII	Ringed (low N)	13.1	0.025	0.77	7.83	2.76	10.59	1.09	2.77	2.72	52.0	11.23	424

years, in May 1931 and 1932. The treatment produced very marked effects both on growth characters and fruit quality during 1932. Shoot growth was entirely checked, the foliage was pale and became highly tinted with yellow, red and purplish shades. The crop was slightly heavier than on the "not ringed" trees, and the fruits were smaller and with pale ground colour and good pinkish-red flush. The flesh was white and hard, and the flavour sweet and much less acid than that of the comparable fruits. Picking also took place on October 14th. No storage breakdown was found during the test.

DISCUSSION OF RESULTS.

The results of the biological tests show consistently a higher antiscorbutic activity in the case of the apples in which the nitrogen is lowered whether it be by cultural means or by bark-ringing (Tables I and II). This increased activity

Table II.

Dose g. daily	Bramley's Seedling 1929. High nitrogen	Bramley's Seedling 1929. Low nitrogen	King Edward VII 1929. High nitrogen	King Edward VII 1929. Low nitrogen	Bramley's Seedling 1930 not ringed. High nitrogen	Bramley's Seedling 1930 ringed. Low nitrogen
3	+++↓	++++	No protection	↓	+++	++++
5	++++	++++	↓	+↓	++++	++++
10	++++	++++	++↓	++++↓	++++	++++
20	*	*	++++	++++	++++	++++
	Bramley's Seedling 1930. High nitrogen	Bramley's Seedling 1930. Low nitrogen	Bramley's Seedling 1931 not ringed. High nitrogen	Bramley's Seedling 1931 ringed. Low nitrogen	King Edward VII 1932 not ringed. High nitrogen	King Edward VII 1932 ringed. Low nitrogen
3	+++	++++	+++↓	++++	↓	+↓
5	++++	++++	++++	++++	+↓	+++↓
10	++++	++++	++++	++++	+++	++++
20	++++	++++	++++	++++	Not tested	Not tested

* Test not finished but fully protected for 55-60 days.

although not so very marked in some Bramley's Seedling samples, namely those of 1929 and 1931, falls definitely outside the limit of the experimental error of the prophylactic method in the other samples of this variety. Quite possibly lower test doses might have revealed a more marked difference. Considering that the results obtained during several years have pointed always in the same direction little doubt can be entertained concerning their significance. Moreover, the lowering of the nitrogen content in the King Edward apples by cultural treatment or by ringing raised the vitamin C content of the fruit as much as 1.5 to 2 times. It is quite likely that the Bramley's Seedling variety being antiscorbutically one of the most active, possesses almost the maximum potency possible in the apple. The chemical data and the general biological characteristics of the apples tested leave no doubt that the differences observed in the nitrogen content are also significant.

Although the nitrogen content and the activity of these apples were always inversely related, it is evident that there was no strict proportionality between them. When it is borne in mind that the nitrogen fraction of the apple consists in all probability of various nitrogenous compounds this would not be expected.

A survey of other analytical data shows that acidity, sucrose and ash constituents show no correlation with the vitamin activity. On the other hand the

decreases in nitrogen content in all cases are also associated with increases in the ratio total sugars/nitrogen, the increases being due in all instances both to lower values for nitrogen and higher values for total sugars. It is difficult at this stage of the inquiry to interpret the significance of these observations, but it is obvious that a connection cannot be excluded between some nitrogenous constituent or constituents and vitamin C in the metabolism of the apple.

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