CCXXII. STUDIES IN ZYMASIS.

VI. ZYMASIS BY APPLES IN RELATION TO OXYGEN CONCENTRATION.

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(Received July 26th, 1933.)

THE ensuing subject matter deals principally with the problem of determining the concentration of oxygen at which the anaerobic respiration of apples becomes extinguished. Pure nitrogen has been used in most of the recent work on the anaerobic respiration of plants, and we propose to follow Blackman [1928] and Parija [1928] in describing the CO_2 component of anaerobic respiration as nitrogen respiration (N.R.). We shall refer to the concentration of oxygen that extinguishes N.R. (*i.e.* anaerobic respiration) as the extinction point of N.R.

Bramley's Seedling apple was selected as a subject for experiment because much important work has been performed on this variety at the Botany School and at the Low Temperature Station at Cambridge; but experiments have also been performed with the Newton Wonder apple.

The scope of this research was widened to test Blackman's contention (see below) that, even for a single variety for a given season, the extinction point of N.R. is not a fixed magnitude throughout the storage period but shifts to higher values as the fruit ages. The extinction points of N.R. for Newton Wonder and Bramley's Seedling apples have therefore been determined late as well as early in the storage season. Further, as our experiments extended over 2 years, we can consider whether seasonal factors affect the time at which the shift of the extinction point begins in stored fruit.

The novelty of our work resides essentially in the method adopted for finding the extinction point for N.R. The method is based on the facts, established by earlier quantitative researches [Thomas, 1925], first that healthy apples do not normally undergo zymasis in air, and secondly that under anaerobic conditions ethyl alcohol accumulates in the flesh tissue until this is dead. It is argued that zymasis probably also occurs in low concentrations of oxygen, and that there is for any apple in a given physiological state a limiting concentration of oxygen that extinguishes this process.

By our methods of analysis, ethyl alcohol, acetaldehyde (which also accumulates in small amounts) and possibly traces of other compounds which are volatile in steam, are measured together to give what we term the "alcohol number" [see Fidler, 1933, 1]. This number may be taken as a measure of the zymasic products which have accumulated in the flesh tissue. Under anaerobic conditions CO_2 (Blackman's N.R.) is simultaneously produced, and there is evidence for Bramley's Seedling and Newton Wonder apples that the greater part of this N.R. is due to zymasis, and that the ratio alcohol number/N.R. for a given variety is constant during a single season [Fidler, 1933, 2]. From

this constancy we infer that the alcohol number may also be taken as a measure of N.R., and that the extinction point of N.R. will be that concentration of oxygen at which the alcohol number becomes zero.

Earlier workers by other methods have aimed at determining the extinction point of N.R. Thus Stich [1891], and several workers quoted by him, measured the effect of lowered oxygen concentration on the respiratory quotient of certain plant tissues, the general principle being that this quotient is increased when zymasis (*i.e.* N.R.) begins. None of these workers, however, measured alcohol.

Blackman [1928] inferred from CO₂ output curves that the extinction point of N.R. for Bramley's Seedling apples is below 5 % in early storage, and that it increases as the apples age. Again, no measurements of alcohol were made. The results we have obtained by using the alcohol number as an index are unambiguous, and they permit us to test whether the arguments based on data collected by other methods are valid. In order to compare the behaviour of the apples used by us with the behaviour of those used by Blackman and Parija [1928], we have also measured the rate of CO₂ production.

So far as we know, this is the first time that CO₂ and the alcohol number (or any other measure of accumulating zymasic products) have been simultaneously determined in gas mixtures of nitrogen and oxygen. Some of the advantages accruing from this procedure are developed later (see Section III). Thus, even when N.R. forms one component of the total CO₂ produced, what has been termed the total carbon loss [Blackman, 1928] has been calculated. Further, it is also possible by calculation to distribute total CO₂ between N.R. and normal respiration (Blackman's O.R.). The results of a few calculations are given in Section II in Table II, but we have not yet sufficient data for critical discussion. In fine, we consider that no analysis of glycolysis in which N.R. CO₂ is a product can approach completion unless cognisance is taken of the accumulating products of zymasis, should it be known that this process occurs under anaerobic conditions.

I. MATERIALS AND METHODS.

An account has already been given [Fidler, 1933, 1] of the times of gathering, and of the modes of storing and dispatching the fruit used in the experiments we are about to describe. The apparatus and methods of analysis employed have also been described by Fidler [1933, 1, 2].

Upon receiving the apples from storage the CO₂ output in air at 23° was determined. The air stream was then changed for one of pure nitrogen or of a nitrogen-oxygen mixture¹ at the rate of 3 litres per hour when the sample was a single apple, or 6 litres per hour when larger samples were used. Analyses of the issuing gas showed that the composition of the gas stream at this speed was not appreciably altered by the respiration of the fruit. Exposure to the artificial atmosphere lasted for about 4 days.

Sets of experiments were carried out at intervals during the storage season, the apples used in any one set being roughly of the same age. Each set consisted of two groups of four separate experiments, each of which was performed in a different concentration of oxygen. Six weeks to 2 months elapsed between each set of experiments. Thus, in addition to showing the effect of varying oxygen concentrations on apples of roughly the same age, it was also possible to deter-

¹ The nitrogen-oxygen mixtures were obtained in cylinders from the British Oxygen Company, Ltd., Walker Gate, Newcastle-on-Tyne, their composition being determined accurately by means of a Haldane gas analysis apparatus before use.

mine the effect of increasing age on zymasis by the fruit in any given concentration of oxygen. Work on a set of experiments lasted about 2 weeks, a new consignment of fruit for the second group of four experiments being timed to arrive on the day the apples used in the first four were distilled.

II. RESULTS.

A. *The alcohol number.*

Preliminary work [Fidler, 1933, 1] showed that the alcohol number of storage controls was not the best basis for evaluating change in the alcohol number owing to oxygen shortage; for it appears that zymasis may be induced by other factors connected with the experimental conditions. For gas mixtures containing oxygen, interest resides chiefly in the figures in column 11 of Table I. These have been obtained by deducting the corresponding conditions control values [Fidler, 1933, 1, Table II], from the alcohol numbers¹ found by analysis. There is no evidence that conditions as such increase zymasis in pure nitrogen, and for this gas the storage control has been used.

B. *The rate of CO₂ production, distributed between O.R. and N.R. Carbon loss.*

Only the results for the rate of production of CO₂ by Bramley's Seedling apples in 1931-2 will be considered here².

In Section III we compare our results for CO₂ output with those obtained by Parija [1928] who used Bramley's Seedling apples from a different locality. Our experiments differ from his in one important respect. To investigate the extinction point of N.R. he used the same apple in different concentrations of oxygen. Since we determined the alcohol number as well as the CO₂ output, we necessarily had to use different apples for every experiment.

NOTES TO TABLE I.

(a) *Weight of apple.* Up to experiment 62, the final weight was used in calculating the percentage concentration of products of zymasis. After this time the initial weight has been used. The loss in weight was never more than 6 % in a sound apple and was usually of the order of 1 to 3 %. As all the numbers in any series have been calculated to the same basis, the error is not serious.

(b) *Exps. 106, 107 and 108 on Bramley's Seedlings* were allowed to continue for about 8 days and then analysed. The alcohol numbers given in the table have been calculated by dividing the alcohol numbers obtained in the analysis by the quotient: CO₂ output per 8 days/CO₂ output during the first 100 hours.

(c) The results given for experiments in pure nitrogen are taken from Fidler [1933, 2], and are included for the sake of comparison with those obtained in 0.8 % oxygen.

(d) The storage control has been used in experiments where the alcohol number was greater than that in a conditions control.

¹ The ratio alcohol/acetaldehyde in these experiments was intermediate between 45/1 (the highest recorded in air) and 75/1 (the lowest recorded in nitrogen). It is not proposed to analyse these figures further since the research was not planned to determine the effect of oxygen tension and age of apples on this ratio. It is proposed, however, to carry out experiments to test these points in the near future.

² Results for Bramley's Seedlings in another season and for Newton Wonders in three seasons are given in a Ph.D. thesis [Fidler, 1932], where they have been treated in the manner adopted here for this selected set of results for Bramley's Seedlings.

Table I. *Changes in the percentage alcohol number for Newton Wonder and Bramley's Seedling apples in nitrogen-oxygen mixtures at 23°. (See notes to Table I.)*

No. of exp.	Variety of fruit	State of fruit	Date exp. begins	Hours' exposure to gas	Per-centage oxygen in gas	No. of apples in sample	Wt. of sample (g.)		The percentage alcohol number after 100 hours	
							Initial	Final	deduction of storage control value	After deduction of conditions control value
15	Newton Wonder	Small. Green. Very young	22. vii. 30	123½	5.7	5	—	152.0	0.002	—
From cool storage										
17	Newton Wonder	Green. Hard	24. ix. 30	97½	Nil	1	—	112.5	0.19	—
18	Newton Wonder	Green. Hard	24. ix. 30	97½	3.6	1	—	93.5	—	Nil
19			24. ix. 30	99½	5.7	1	—	113.5	—	Nil
20		" Hard. Young	1. x. 30	142	21.0	1	—	100.5	0.01	—
30		Green to green-yellow	27. xi. 30	97	Nil	1	—	193.0	0.25	—
31		Green-yellow	27. xi. 30	97	2.6	1	—	200.5	0.14	0.14
32			27. xi. 30	99½	3.6	1	—	200.0	0.08	0.08
37		Yellow. Slightly wilted	8. i. 31	99½	Nil	1	—	105.0	0.35	—
38		Green-yellow. Slightly wilted	8. i. 31	99½	2.6	1	—	142.5	0.28	0.27
39		"	8. i. 31	99½	3.6	1	—	139.5	0.25	0.23
Season 1931-2. From orchard										
73	Newton Wonder	Green. Hard. Very young	1. vii. 31	97½	Nil	12	103.0	96.5	0.70	—
74	Newton Wonder	Green. Hard. Very young	1. vii. 31	97½	0.8	12	102.0	98.5	0.67	—
C		Green. Hard. Very young	17. vii. 31	113	21.0	6	134.5	132.5	0.01	0.65
79		Green. Hard. Very young	17. vii. 31	95	Nil	9	174.5	167.0	0.73	—
80		"	17. vii. 31	72½	0.8	8	178.5	174.5	0.63	0.60
From cool storage										
81	Newton Wonder	Green. Hard. Very young	26. viii. 31	98½	Nil	3	208.0	201.0	0.38	—
82		"	26. viii. 31	98½	3.0	3	213.0	212.5	—	Nil
83		"	26. viii. 31	98½	5.0	3	203.0	198.0	—	Nil
84		Green. Hard. Very young	26. viii. 31	122	21.0	3	212.0	207.0	0.02	—
89		Green. Hard. Very young	10. ix. 31	99½	9.5	2	213.0	209.5	—	Nil
90		"	10. ix. 31	99½	40.0	3	207.5	203.5	—	Nil
91		"	10. ix. 31	99½	100	3	200.0	194.5	—	Nil
95		Green	28. x. 31	99	Nil	2	252.5	246.0	0.32	—
96		"	28. x. 31	99	3.0	2	288.0	284.0	—	Nil
97		"	28. x. 31	99	100	2	259.0	254.5	—	Nil
98		"	28. x. 31	140	21.0	2	321.0	312.5	0.01	—
99		Green to yellow-green	4. xi. 31	98½	8.0	2	329.0	324.0	—	Nil
100		"	4. xi. 31	98½	15.2	2	336.0	331.5	—	Nil
101		Yellow-green	4. xi. 31	98½	28.0	2	286.0	285.5	—	Nil
109		Green to yellow-green	10. xii. 31	98½	Nil	2	298.5	293.5	—	Nil
110		Yellow-green	10. xii. 31	98½	2.4	2	295.0	289.0	0.50	—
111		"	10. xii. 31	98½	2.9	2	293.0	290.0	—	Nil
112		Green to yellow-green	10. xii. 31	121½	21.0	2	279.0	270.0	—	Nil
113		Yellow-green to green-yellow	16. xii. 31	99	8.5	2	299.0	295.5	0.45	—
114		Yellow-green	16. xii. 31	99	15.2	2	280.5	279.0	—	Nil
115		Green-yellow	16. xii. 31	99	100	2	281.0	278.5	—	Nil
116		Yellow	16. xii. 31	99	60	2	264.5	256.5	—	Nil
125		Green-yellow	27. i. 32	99	Nil	1	84.0	81.5	0.50	—
126		Green-yellow.	27. i. 32	99	8.3	1	83.5	81.0	0.08	0.06
127		"	27. i. 32	99	2.4	1	74.5	72.0	0.07	0.05
128		"	27. i. 32	140½	21.0	1	97.5	97.0	0.02	—
129		"	10. ii. 32	98	2.9	1	106.5	105.0	0.17	0.11
130		"	10. ii. 32	98	5.3	1	92.5	91.5	0.19	0.13
131		"	24. ii. 32	96½	60.0	1	86.5	84.5	0.02	0.02
133		"	24. ii. 32	138	21.0	1	87.0	83.5	—	—

First sign of wrinkling

Season 1930-31. From cool storage

25	Green. Hard.	Young	19. xi. 30	97 $\frac{1}{2}$	Nil	1	—	148.5	0.34	—
26	Bramley's		19. xi. 30	97 $\frac{3}{4}$	2.6	1	—	147.5	0.05	0.05
27	Seedling		19. xi. 30	99 $\frac{1}{2}$	5.3	1	—	149.0	0.03	0.004
34	"	Firm	10. xii. 30	97	Nil	1	—	124.5	0.34	—
35	"		22. i. 31	99 $\frac{1}{2}$	2.6	1	—	153.5	0.17	0.16
43	Green to green-yellow		22. i. 31	98 $\frac{1}{2}$	Nil	1	—	134.5	0.37	0.17
44	Green-yellow		22. i. 31	98 $\frac{3}{4}$	2.6	1	—	150.0	0.25	0.10
45	Green-yellow. Wilted		22. i. 31	98 $\frac{3}{4}$	5.0	1	—	118.5	0.18	—
46	Green-yellow. Very slightly wilted		29. i. 31	116	21.0	1	—	170.0	0.06	—
50	Green-yellow		5. ii. 31	99 $\frac{1}{2}$	9.5	1	—	188.5	—	Nil
51	Yellow-green. Slightly wilted		5. ii. 31	99 $\frac{1}{2}$	11.0	1	—	187.5	—	Nil
52	"		5. ii. 31	99 $\frac{1}{2}$	15.3	1	—	193.0	—	Nil
53	"		11. ii. 31	98	28.0	1	—	142.0	—	Nil
54	Yellow-green. Badly wilted		11. ii. 31	98	100	1	—	90.0	—	Nil
55	Yellow. Badly scalded and wilted		5. iii. 31	116	21.0	1	—	147.0	0.15	—
57	"		5. iii. 31	98	69.0	1	—	121.5	—	Nil
58	"		5. iii. 31	98	40.0	1	—	139.5	—	Nil

Season 1931-2. From orchard

71	Green. Hard.	Very young	1. vii. 31	97 $\frac{1}{2}$	0.8	11	249.0	239.5	0.45	0.44
72	Bramley's		1. vii. 31	97 $\frac{1}{2}$	Nil	11	236.5	233.0	0.43	—
75	Seedling		8. vii. 31	116 $\frac{1}{2}$	21.0	8	172.0	161.5	0.01	—
77	Green. Hard.	"	17. vii. 31	72 $\frac{1}{2}$	0.8	5	183.5	179.0	0.58	0.57
78	Green. Hard.	Very young	17. vii. 31	95	Nil	5	200.0	192.0	0.60	—
85	Green. Hard.	Very young	2. ix. 31	99	Nil	2	273.5	271.5	0.40	—
86	Green. Hard.	Very young	10. ix. 31	99	3.0	2	215.5	214.0	—	Nil
87	Green. Hard.	Very young	2. ix. 31	99	5.0	2	236.0	235.5	—	Nil
88	Green. Hard.	Very young	2. ix. 31	140 $\frac{1}{2}$	21.0	2	218.5	218.0	0.01	—
92	Green. Hard.	Very young	18. ix. 31	98	8.0	2	183.5	181.0	—	Nil
93	Green. Hard.	Very young	18. ix. 31	98	40.0	2	186.0	182.0	—	Nil
94	"	"	18. ix. 31	98	100	2	208.5	205.5	—	Nil

Season 1931-2. From cool storage

102	Green		11. xi. 31	98 $\frac{1}{2}$	Nil	1	223.0	218.0	0.50	—
103	Bramley's		11. xi. 31	97	3.0	1	211.0	206.0	—	Nil
104	Seedling		11. xi. 31	98 $\frac{1}{2}$	100	2	376.5	372.0	—	Nil
105	"		11. xi. 31	115 $\frac{1}{2}$	21.0	1	207.5	202.0	0.05	—
106	"		18. xi. 31	—	8.5	1	234.5	—	—	Nil
107	"		18. xi. 31	—	28.0	1	206.5	—	0.06	0.005
108	"		18. xi. 31	—	69.0	1	164.5	—	0.06	0.003
117	"		12. i. 32	99	Nil	1	187.5	185.5	—	Nil
118	"		6. i. 32	99	8.3	1	180.5	180.5	—	Nil
119	"		6. i. 32	99	2.4	1	112.0	110.0	—	Nil
120	"		6. i. 32	138 $\frac{1}{2}$	21.0	1	158.0	157.5	0.06	—
121	"		13. i. 32	99	0.8	1	181.0	177.0	0.34	0.28
122	"		13. i. 32	99	2.9	1	191.0	191.0	—	Nil
123	"		13. i. 32	99	5.2	1	148.0	146.0	—	Nil
124	"		13. i. 32	99	8.5	1	173.5	173.0	—	Nil
134	Green-yellow		6. iii. 32	97 $\frac{1}{2}$	Nil	1	138.0	134.5	0.50	—
135	"		16. iii. 32	97 $\frac{1}{2}$	8.3	1	247.5	244.0	—	Nil
136	"		16. iii. 32	97 $\frac{1}{2}$	2.4	1	160.5	157.5	0.10	0.02
137	"		16. iii. 32	140	21.0	1	167.5	164.0	0.09	—
138	Green-yellow. First sign of wrinkling		31. iii. 32	97 $\frac{1}{2}$	2.9	1	99.5	97.5	0.11	0.07
139	"		31. iii. 32	97 $\frac{3}{4}$	5.3	1	160.5	158.0	0.05	0.004
140	"		31. iii. 32	97 $\frac{3}{4}$	60.0	1	152.0	150.5	—	Nil
141	"		31. iii. 32	116 $\frac{1}{2}$	21.0	1	127.5	126.0	0.05	—

Table II. *Rate of CO₂-production, N.R., O.R., carbon loss, and CO₂/alcohol number of Bramley's Seedling apples during 1931-2. (For details of state of fruit, date of experiment and the alcohol number, see Table I.)*

g./100 g. apple/100 hours								
Corrected for conditions control values†								
Exp. No.	Oxygen in gas %	Actual carbon loss*	Actual CO ₂ output	CO ₂	N.R.	O.R.	CO ₂ /alcohol number	Total carbon loss
								Carbon lost as CO ₂
1	2	3	4	5	6	7	8	9
From orchard:								
71	0.8	0.42	0.680	0.67	0.50	0.17	100: 63	2.3
72	Nil	0.42	0.714	—	0.71	Nil	100: 58	2.2
75	21.0	0.15	0.542	0.54	—	0.54	—	1.0
77	0.8	0.50	0.727	0.72	0.64	0.07	100: 76	2.6
78	Nil	0.50	0.667	—	0.67	Nil	100: 93	2.7
85	Nil	0.33	0.455	—	0.46	Nil	100: 83	2.6
86	3.0	0.05	0.176	0.16	Nil	0.16	—	1.0
87	5.0	0.07	0.239	0.22	Nil	0.22	—	1.0
88	21.0	0.13	0.446	0.43	—	0.43	—	1.0
92	8.0	0.06	0.200	0.19	Nil	0.19	—	1.0
93	40.0	0.06	0.203	0.19	Nil	0.19	—	1.0
94	100	0.10	0.337	0.32	Nil	0.32	—	1.0
From cool storage:								
102	Nil	0.46	0.592	—	0.59	Nil	100: 80	2.6
103	3.0	0.14	0.390	0.33	Nil	0.33	—	1.0
104	100	0.18	0.563	0.50	Nil	0.50	—	1.0
105	21.0	0.18	0.545	0.49	—	0.49	—	1.0
106	8.5	0.16	0.477	0.42	Nil	0.42	—	1.0
107	28.0	0.17	0.469	0.41	0.005	0.41	100: 3	1.0
108	69.0	0.20	0.605	0.55	0.004	0.54	100: 1	1.0
117	Nil	0.30	0.456	—	0.46	Nil	100: 67	2.4
118	8.3	0.15	0.419	0.35	Nil	0.35	—	1.0
119	2.4	0.15	0.414	0.35	Nil	0.35	—	1.0
120	21.0	0.20	0.594	0.54	—	0.54	—	1.0
121	0.8	0.33	0.520	0.46	0.46	Nil	100: 58	2.2
122	2.9	0.14	0.384	0.32	Nil	0.32	—	1.0
123	5.2	0.17	0.465	0.40	Nil	0.40	—	1.0
124	8.5	0.19	0.539	0.47	Nil	0.47	—	1.0
134	Nil	0.45	0.631	—	0.63	Nil	100: 76	2.6
135	8.3	0.18	0.496	0.44	Nil	0.44	—	1.0
136	2.4	0.20	0.490	0.42	0.02	0.40	100: 4	1.0
137	21.0	0.21	0.567	0.57	—	0.52	—	1.0
138	2.9	0.22	0.499	0.44	0.09	0.36	100: 14	1.6
139	5.3	0.18	0.493	0.44	0.006	0.43	100: 1	1.0
140	60.0	0.22	0.615	0.57	Nil	0.57	—	1.0
141	21.0	0.22	0.638	0.59	—	0.59	—	1.0

* *I.e.* total zymasis in carbon units (carbon equivalent of column 4, *plus* the carbon equivalent of the conditions control alcohol number).

† Except in Exps. 72, 78, 85, 102, 117, 134, where O₂ % = Nil.

NOTES TO TABLE II.

(a) *Distribution of CO₂ between O.R. and N.R.* Before distributing the CO₂ given off in gas mixtures of nitrogen and oxygen between O.R. and N.R., allowance must be made for the CO₂ component of zymasis which is induced by conditions as such (see p. 1631). This part of the total CO₂ produced may be calculated from the alcohol number of the conditions control.

For this calculation, instead of using the conventional equation for alcoholic fermentation, we prefer to use the formula experimentally determined for the variety of apple under observation. Strictly, this formula should be determined each season, but there are insufficient data for this refinement. Instead we take the mean value for the given variety for all the seasons during which

this apple has been used. Thus the preliminary work over two seasons [Fidler, 1933, 2] permits us to write for Bramley's Seedlings: respirable substrate $\rightarrow 100 \text{ CO}_2 + 85 \text{ C}_2\text{H}_5\text{O}$. By subtracting this CO_2 component of zymasis occurring in air at 23° under experimental conditions from the total CO_2 absorbed by the baryta, we arrive at a number representing the CO_2 which must be distributed between O.R. and N.R.

If the alcohol number obtained in any experiment is of the same order as that of a conditions control, we conclude that there is no N.R. due to oxygen shortage. All the CO_2 produced is then attributed to O.R.

When, however, zymasis due to oxygen shortage is detected, the CO_2 component is calculated as we have described above. Thus, for Bramley's Seedlings, in calculating N.R. due to oxygen shortage, 100 g.-mol. equivalents of CO_2 are taken as equivalent to 85 g.-mol. equivalents of zymasic products accumulating in the tissues. The O.R. occurring simultaneously may be found by subtracting the calculated value for N.R. from the total CO_2 output corrected for experimental conditions.

(b) *Calculation of the percentage carbon loss.* The percentage carbon loss, given in Table II, is the sum of the weights in g. of carbon given off as CO_2 and excreted in the tissues as ethyl alcohol and acetaldehyde.

(c) *The quotient Total carbon loss/Carbon lost as CO_2 .*

In the paper dealing with zymasis in nitrogen [Fidler, 1933, 2], a column was included giving Glycolysis in C units/N.R. in C units. Such a column cannot be included in the present paper, since total carbon loss in nitrogen-oxygen mixtures is not equal to glycolysis in carbon units. The difference is due to O.A. (oxidative anabolism [Blackman, 1928]). This, however, cannot be evaluated from our data. A column has been added here giving the quotient Total carbon loss/Carbon lost as CO_2 . When there is no N.R. the value of this quotient is unity. As N.R. forms an increasingly important component of respiration, the quotient rises to a maximum of 2.7 (for an anaerobic CO_2 /alcohol ratio of 100/85).

III. DISCUSSION AND GENERAL CONCLUSIONS.

(A) *The effect of varying oxygen concentrations on the alcohol number in Newton Wonder and Bramley's Seedling apples of different ages and in different seasons.*

(i) *The alcohol number as a measure of N.R., and the possible relations between N.R. and oxidation processes.* The graphs in Figs. 1a and 1b, and 2a and 2b¹, in which are represented the results given in Table I for Newton Wonder and Bramley's Seedling apples, show the concentrations of oxygen that extinguish N.R. at different times in the storage season, and what is the relative extent of N.R. when it occurs. When the alcohol number points lie above the x -axis, zymasis takes place to a greater extent than in a conditions control [see Fidler, 1933, 1]. It follows first that N.R. then forms one component of the respiratory process, and, secondly, that this N.R. occurs owing to a deficiency of oxygen in the gas mixture passed over the fruit. Further, the relative magnitudes of N.R. in the total respiration in different gas mixtures² may, of course, be

¹ To avoid crowding of graphs we have assembled in Figs. 1a and 1b the results for apples before they became pronouncedly yellow, and in Figs. 2a and 2b the results for the same populations when they were rapidly becoming or had become yellow. This colour change criterion of the ageing of the flesh tissue of apples has been used before [see e.g. Blackman and Parija, 1928]. Evidence given later in this section suggests, however, that other phenomena of ageing do not necessarily march in line with this colour change, i.e. apples of the same variety, from the same population and of the same colour are not necessarily in the same physiological state in all respects.

² In column 9, Table II in Section II, it will be seen that the ratio Total carbon loss/Carbon lost as CO_2 decreases from 2.7 to unity when the oxygen concentration is increased. The extinction point is the minimum oxygen concentration at which this ratio becomes unity.

judged from the differences in the heights of the points above the x -axis. When, however, the alcohol number points lie on the x -axis, there is no zymasis beyond that occurring in a conditions control. In these cases there is sufficient oxygen in the gas mixtures to extinguish N.R. completely.

Fig. 1a.

Fig. 1a. Percentage alcohol numbers of green to yellow-green Newton Wonder apples.

Season 1930-1. From store: I, 24. ix. 30. ●
 Season 1931-2. From orchard: II, 17. vii. 31-26. viii. 31. ×
 From store: III, 28. x. 31. ○
 From store: IV, 10. xii. 31. △

Fig. 1b.

Fig. 1b. Percentage alcohol numbers of stored green to yellow-green Bramley's Seedling apples.

Season 1930-1. I, 19. xi. 30-10. xii. 30. ●
 Season 1931-2. II, 2-10. ix. 31. ×
 III, 11. xi. 31. ○
 IV, 6-13. i. 32. △

The graphs afford unquestionable evidence that at all times in the storage season, as the oxygen concentration outside the apples is increased, N.R. gradually falls from the maximum value attained in pure nitrogen. In most of our experiments this fall in N.R. continued until zymasis completely ceased: then, of course, the extinction point of N.R. is the minimum oxygen percentage at which the alcohol number reaches zero. This percentage is not the same for all apples (see later sub-sections).

We have insufficient data for low concentrations of oxygen to calculate the changing quantitative relations between N.R. and oxidation processes as the oxygen concentration rises (see also footnote on p. 1640). The most obvious hypothesis is that N.R. is substituted step by step by oxidation processes in rising oxygen concentrations. There are two conflicting contemporary views that would then have to be borne in mind in explaining the inhibitory action of oxygen on zymasis. According to one view, N.R. and oxidative processes are cognate. Accepting this view, we might infer from the experiments and arguments of Meyerhof [1925] and Blackman [1928] that, as nitrogen is progressively replaced by oxygen, the products of glycolysis are diverted from ethyl alcohol and CO_2 (N.R.) to water and CO_2 (O.R.) and O.A. (oxidative anabolism,

or the Meyerhof-Pasteur reaction). According, however, to the alternative view (see for example Lundsgaard [1930] and Boysen-Jensen [1922; 1931], the enzyme systems governing N.R. and oxidation processes are quite distinct. Should this view become established it would mean that the contiguous processes of N.R. and oxidation occurring in low concentrations of oxygen are unconnected although they affect the same substrate. It would then follow

Fig. 2a. Percentage alcohol numbers of green-yellow to yellow Newton Wonder apples.

Season 1930-1. I, 27. xi. 30. ●
 II, 8. i. 31. ×
 Season 1931-2. III, 27. i. 32-24. ii. 32. ○

Fig. 2b. Percentage alcohol numbers of green-yellow to yellow Bramley's Seedling apples.

Season 1930-1. I, 22. i. 31-5. ii. 31. ●
 Season 1931-2. II, 16-31. iii. 32. ×

that in rising oxygen concentrations zymasis is increasingly inhibited and that simultaneously oxidation processes, which are independent of anaerobic glycolysis, are activated to an equivalent extent.

(ii) *The extinction point of N.R. early in the storage season.* In Fig. 1a are recorded the alcohol numbers for Newton Wonder apples during two seasons at the beginning of the cool storage periods. When these experiments were started we thought that zymasis would occur in all concentrations of oxygen

less than 5 %. But our results for this variety indicate that early in storage zymasis, owing to oxygen shortage, does not occur even in 2.5 % oxygen¹. For Bramley's Seedlings, in 1931-2, it will be seen from graphs II, III and IV in Fig. 1*b*, that this same concentration of oxygen completely inhibited zymasis in September and November 1931, and even as late as January 1932. At this last date the apples which we used were yellow-green². Our results for this same variety at the corresponding time in the previous season cannot be used in the present analysis; for before the middle of December 1930 (see graph I, Fig. 1*b*) the apples, whilst still green, underwent zymasis in all gas mixtures containing less than 5 % of oxygen. It is probable, however, that had we experimented on Bramley's Seedlings at an earlier date in the autumn of 1930, zymasis would have been stopped by a lower concentration of oxygen (see next subsection).

We conclude, therefore, that the extinction point of N.R. for apples of these two varieties whilst they are still on the tree or early in storage is not greater than 2.5 % oxygen. This is a surprisingly low figure. And it must be remembered that in a bulky organ, such as an apple, the internal concentration of oxygen in the intercellular spaces around the respiring cells is always less than the concentration of oxygen outside. Questions concerning the oxygen relations of possible respiratory systems in cells suggest themselves, but must for the present be left unanswered.

(iii) *The shift of the extinction point in ageing apples.* The graphs in Figs. 2*a* and 2*b* show that zymasis is not completely inhibited by 2.5 % oxygen when senescent characteristics, such as the change to green-yellow and yellow, wilting and superficial scald, appear. Thus we conclude that although the extinction point of N.R. appears to be constant early in the storage season, it shifts to higher values when signs of ageing become more marked.

The charts for the preliminary experiments on green-yellow Newton Wonder apples in 1930-1 (Fig. 2*a*, graphs I and II) show that, notwithstanding the steep decline in the alcohol numbers with increasing oxygen concentrations, zymasic products accumulated in apples in 3 % oxygen in greater amounts than in the conditions controls. Early in 1932 experiments were carried out with a wider range of gas mixtures and we interpret graph III as showing that after January, in green-yellow air-stored Newton Wonder apples, (a) N.R. is not completely extinguished in any concentration of oxygen (*cf.* the complete extinction of N.R. in 3 % oxygen in December 1931—graph IV, Fig. 1*a*) and (b) for each increase in oxygen concentration up to a value that is less than 5 % oxygen, the corresponding decrease in N.R. is more rapid than when the oxygen concentration is increased beyond 5 %. We are not prepared, from this experiment alone, to state what is the precise form of the curve representing this fall in N.R. in senescent apples.

¹ Graph II in Fig. 1*a* shows that very young green apples picked from orchard trees behave similarly to fully grown green apples taken from store.

² Although colour is a useful guide for grouping apples, it is clearly not a rigid criterion of physiological state. Here we see that zymasis occurred in green apples in December 1930 in 2.5 % oxygen but was completely inhibited by this concentration in yellow-green apples in the next season. On a colour standard these latter apples would be considered to have been the more senescent of these two samples, but in terms of the respiratory events in the cells of the flesh tissue we consider that they were the less senescent. There is an element of hazard at present in arranging comparable experiments for stored apples in two different seasons. This might conceivably be lessened by using in successive seasons only those apples that have approximately the same extinction point of N.R.

In the comparable experiments with Bramley's Seedling apples (Fig. 2*b*), we note from graph I that in late January to early February 1931 as much as 9 % oxygen was needed to extinguish N.R. But when we examine graph I in Fig. 1*b*, we see that in December 1930, *i.e.* about 6 weeks earlier in the storage season, zymasis was nearly completely checked by only 5 % oxygen. For Bramley's Seedling apples ageing in air-stores during 1930-1, as well as for Newton Wonder apples in this same season, there is clear evidence, therefore, that in late storage greater concentrations of oxygen are needed to inhibit zymasis, *i.e.* to extinguish N.R., than in early storage.

In 1931-2, the results for Bramley's Seedlings, although not so striking, point to the same conclusion. Thus graphs II, III and IV in Fig. 1*b* show quite definitely that there was no zymasis in concentrations greater than 3 % oxygen before January 1932; and from graph II in Fig. 2*b* we see that by March 1932 the alcohol number had risen distinctly above the conditions control value in 3 % oxygen. At this latter date the slight rise shown in 5.3 % oxygen is by itself hardly significant, but as this rise is quite in accord with the behaviour of Bramley's Seedlings in the previous season, and with Newton Wonders in both seasons, it strengthens the evidence for a shift in the extinction point of N.R. to higher levels in the later phases of storage.

Blackman analysed a series of curves of CO₂ output by Bramley's Seedlings in gas mixtures containing different percentages of oxygen [Blackman, 1928] and inferred that such a shift occurs. Using the alcohol number as an index of N.R. we have verified Blackman's inference by our work on two varieties of apple.

(iv) *Varietal and seasonal differences as to the date at which the shifts of the extinction point begins.* We may first note that different varieties from the same orchard and picked at about the same time, and with a similar storage history, may behave differently in a given storage season. For example, graph II in Fig. 2*b* records but little zymasis in March 1932 in Bramley's Seedlings at concentrations of oxygen above 3 %. We conclude that the extinction point had just started to shift at this date. In striking contrast, the shift for Newton Wonders was well under way a month earlier. Indeed, for this variety, at this time, N.R. was not completely extinguished in quite high concentrations of oxygen. This may be because the senescent breakdown of the respiratory centres in the protoplasm of the cells had begun [see Fidler, 1933, 1]. This is possibly the inevitable fate of all apples kept in store, and it is conceivable that the first shift of the extinction point may be a sign of incipient disorganisation. Should this turn out to be the case, varietal differences in regard to this shift may prove to be related to the different keeping qualities of different varieties in a given season.

Secondly, variation has been observed in the behaviour towards oxygen of a given variety of apple picked from the same orchard in successive years at approximately the same time and stored and dealt with each year as nearly as was practicable in the same fashion. Fig. 2*b* clearly illustrates this seasonal difference for Bramley's Seedlings. A comparison of graphs I and II shows that the extinction point had shifted far more by February 1931 than by March in the following storage season. In fact this shift by March in the second season was no greater than that which had occurred by December in the previous season (see graph I, Fig. 1*b*). We infer that the date on which the seasonal shift in the extinction point begins is, for any given variety, determined by the impress made by the complex of significant environmental factors incident on the fruit whilst it is still on the tree. Kidd and West [1919-31] in their storage

trials have frequently reported that the impress of these factors persists in storage apples and governs their keeping qualities. There may be a relation between these qualities and the time at which the shift of the extinction point begins; and we found that Bramley's Seedlings kept better in 1931-2 than in 1930-1. The more rapid deterioration of this variety in the first season was possibly owing to the earlier shifting of the extinction point.

B. The rates of CO₂ output and of carbon loss in relation to oxygen concentration.

(i) *CO₂ output.* Blackman [1928] stated that the extinction point of N.R. is reached in that concentration of oxygen where CO₂ production is at a minimum, and he placed this minimum at 5 % oxygen. Our results accord well with his. Thus the graphs in Fig. 3 show that at all dates in 1931-2, as the oxygen concentration in the external atmosphere was increased, the rate of CO₂ output by Bramley's Seedlings first fell from the high value attained in nitrogen to a minimum value and then rose to the normal rate for air¹. The extinction point again (see sub-section A) appears to be less than 5 % oxygen.

A few points are given for higher concentrations of oxygen. These suggest the continued upward drift reported by Blackman [1930].

Fig. 3. CO₂ output by Bramley's Seedling apples during 1931-2. Corrected for conditions control values.

I, 2-18. ix. 31. ●
II, 11-18. xi. 31. ×

III, 6-13. i. 32. ○
IV, 16-31. iii. 32. △

Blackman concluded from the seasonal variation in the oxygen concentration at which the minimum CO₂ output was observed, that the extinction point for N.R. would move to higher oxygen concentrations as the fruit aged. Our conclusions in sub-section A support Blackman's views. Further, we note here that we have found for Newton Wonders in 1930-1 and 1931-2, and for Bramley's Seedlings in 1930-1, that the oxygen concentration in which the minimum value for CO₂ output was observed rose from 3-5 % oxygen to 8-10 % oxygen as the apples aged². This rise affords further evidence that the behaviour of the apples we have used was substantially the same as that of the Bramley's Seedlings used by Blackman and Parija.

¹ The distribution of the CO₂ produced between N.R. and O.R. has been calculated, and the resulting values are given in Table II; but we have not collected enough data yet for gas mixtures containing less than 2.5 % oxygen to permit of critical analysis. We propose to perform further experiments in these low percentages of oxygen.

² The data on which this statement is based are included in Fidler's Ph.D. thesis [1932].

(ii) *Carbon loss.* In view of Blackman's calculations [1928] further data on the rate of carbon loss in relation to oxygen concentration may prove of interest.

We have obtained values for the rate of carbon loss under different conditions by simultaneously measuring CO_2 output and the alcohol number. We note that each carbon loss graph in Fig. 4 has the same form as the corresponding CO_2 output graph and has the same position for the minimum, *viz.* at about 3–5 % oxygen, the extinction point of N.R.¹ Carbon loss is greatest under anaerobic conditions. As the oxygen concentration passes from zero upwards, we observe that the first effect of oxygen is increasingly to restrict carbon loss. This restriction can be correlated with the gradual inhibition of zymasis as the oxygen concentration rises. Carbon loss appears to be at a minimum when zymasis has been completely inhibited. The second effect of oxygen, *viz.* to increase carbon loss by stimulating respiration, shows clearly after this minimum (see Blackman [1928]).

Fig. 4. Actual carbon loss by Bramley's Seedling apples during 1931-2.

I, 2-18. ix. 31. ●
II, 11-18. xi. 31. ×

III, 6-13. i. 32. ○
IV, 6-13. iii. 32. △

It will be noticed that the graphs I, II, III and IV lie at higher levels as the season advances. In all concentrations of oxygen a general seasonal stimulation of respiration probably contributes to this observed rise in carbon loss. In the lower concentrations of oxygen, moreover, the restricting effect of oxygen on zymasis may diminish.

SUMMARY.

Apples have been exposed to mixtures of nitrogen and oxygen at 23° at different times during the developmental and storage life of the fruit. Using conditions controls rather than storage controls, and, allowing for experimental error and for sampling variation, we have found that:

(i) Rising oxygen concentrations progressively retard zymasis. The high rate attained in pure nitrogen falls until at a certain concentration of oxygen no alcohol is produced. This concentration is termed the extinction point of N.R. (nitrogen respiration).

(ii) Early in the storage season in Newton Wonder and Bramley's Seedling apples the extinction point lies between 1 and 3 % oxygen. There is some evidence that it may be near to 3 % oxygen.

¹ It was shown by Fidler [1932] that the same agreement holds between the extinction point of N.R. and the minimum carbon loss, early and late in the season, for (a) Bramley's Seedlings 1930-1, (b) Newton Wonders during three seasons.

(iii) The extinction point shifts to higher concentrations of oxygen later in the storage season.

(iv) In old apples alcohol may accumulate even in 100 % oxygen, *i.e.* there is no extinction point for N.R.

(v) The time of the beginning of the shift of the extinction point may be different for different varieties in the same season and for a single variety in different seasons.

(vi) The rates of CO₂ production and of carbon loss fall from maximum values in nitrogen to minimum values in 3–5 % oxygen early in the storage season, but in 8–10 % oxygen for older apples. The rates increase gradually when the oxygen concentration is progressively raised above these critical concentrations.

This research was performed under the auspices of the Department of Scientific and Industrial Research [see Fidler, 1933, 1]. Grants by the Royal Society and by the Research Committee, Armstrong College to one of us (M. T.) have helped on the research.

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