STUDIES OF ONION ROOT RESPIRATION

V. EFFECT OF CULTURING TEMPERATURE AND SEED SAMPLE ON ROOT RESPIRATION AND DIAMETER*

by

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INTRODUCTION

Onion roots have been used rather widely for experimental material in studies of respiration, diffusion, bioelectric currents, water absorption and transport, etc. (For bibliographies see BERRY AND NORRIS[1] and ROSENE[2, 3]). In essentially all of these investigations roots have been cultured from bulbs partially immersed in tap water or in various nutrient solutions (HOAGLAND'S, TRELEASE'S, etc.) in darkness to inhibit shoot formation and root abnormalities. The conditions of culture have become somewhat uniform; however, there is still reason to believe that the tissue used from day to day has not been as comparable as it might be. There are some suggestions in previously conducted experiments that culturing temperatures may play a role in the subsequent behavior of the tissue[1], and with this in mind the present study was undertaken to provide quantitative data on this point, in the hope that such information might ultimately be useful in obtaining more standardized experimental material.

Investigations were also conducted to determine whether or not the temperature of root culture had an effect upon the diameter of the roots produced. Data are presented regarding the degree of variation shown in subsequent measurements by roots produced from onion sets of different "lots".

METHODS

Roots were grown from white onion sets (obtained from the Barteldes Seed Co., Lawrence, Kansas) in the manner described by BERRY[4]. There was included in each aquarium a glass covered heater, bimetallic thermoregulator, and circulating tap water cooling coil. Without a stirring device, but depending entirely upon conduction and the mixing provided by two aerators, it was found that the temperature of the nutrient solution remained constant to $\pm$ 1°C. In this manner roots were cultured at 20, 25, and 30°C for 42 $\pm$ 4 hours. The roots were grown, tissue prepared, and measurements conducted in a dark room in which the only source of illumination was from neon gas in ruby red tubing.

Roots were prepared for respiratory measurements by the method previously described[4], with the exception that the collecting funnels were supported in an aquarium containing growth solution maintained at the same temperature at which the roots were cultured. When the required number

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of root segments had been cut, the funnel was drained, the roots collected with a small spatula, and blotted on dry filter paper to remove excess liquid. The root segments were rapidly weighed to the nearest milligram on a Precision Torsion Balance, and transfered to the reaction vessels containing 3 ml of $M/15$ KH_2PO_4 ($p_H = 4.5$). Alternate vessels contained 0.2 ml 15% KOH and 0.2 ml buffer in the center well, which permitted determinations of Respiratory Quotients by the "direct" method. About one and one half hours were required to prepare six reaction vessels.

Roots were cut 5, 10, and 15 mm above the tip. The gas exchange of the 5–10 and 10–15 mm segments could thus be obtained by difference*. (This procedure was employed so that there would only be one cut per segment, the reasons for which are discussed in some detail elsewhere[4]). It may be noted that in general the apical 5 mm of the root includes the zone of mitotic activity, while the 5–10 mm segment represents the region of differentiation and elongation, and the 10–15 mm segment is made up predominantly of mature cells.

The Warburg constant volume respirometer was used to measure gas exchange. Reaction vessels were of approximately 15 ml capacity. In all cases the measurements were made at 30°C ± 0.05°C, with a shaking speed of 120 oscillations per minute (4 cm excursion) in an atmosphere of either air or pure oxygen. In using oxygen, the air in the reaction vessels was replaced by the evacuation technique described by UMBREIT, BURRIS, AND STAUFFER[5]. Control experiments showed that the actual process of evacuation did not bring about observable differences in gas exchange. As a further control, in many cases, acid was tilted in flasks used for measuring CO_2. Bound CO_2 was not present in significant quantities in any of these control experiments.

Results are reported as cubic millimeters of oxygen consumed and carbon dioxide produced per root segment per hour, and also per 150 mg**, of tissue (wet weight) per hour. In the case of the 0–10 and 0–15 mm segments, 150 mg of tissue was (to the nearest root segment) actually placed in each vessel, however, for the segments 5 mm in length, 100 mg of tissue was placed in the reaction vessel. This procedure was followed in order to shorten the time required to set up the experiment, and also in the case of the 0–5 mm segment to make the magnitude of gas exchange about the same as that of the longer segments. The values given in the Tables for the segments 5 mm in lengths (on the basis of weight) thus represent one and one half times the actual gas exchange measured.

Two series (A and B) of experiments were performed differing only in that the onion sets employed in the latter series were obtained (from the same source) about six months later than the sets used in Series A.

RESULTS

Respiration measured in air

Table I presents the results of gas exchange measurements made in air at 30°C with different root segments grown at three different temperatures. All experiments are of one hour duration. The results are averages with standard deviations shown for Series A. The number of measurements averaged varies between 11 and 16 for this series. Standard deviations are not shown for Series B, due to the fact that fewer measurements were averaged (from 3 to 8).

An examination of Table I reveals the following points: a. At any given temperature at which roots are grown, the oxygen uptake and carbon dioxide output per unit weight decrease with increased length of root segment. This is also apparent in Columns II and III in that doubling or tripling the length of the root segment does not double or triple the rate of O_2 uptake or CO_2 output per segment. This is in accord with previously reported data showing that the meristem is the region of greatest respiratory activity[1, 4, 6, 7]. Columns II and III show results obtained in the A series only. Corresponding values for series B may be calculated from Table IA if desired. b. The roots which were cultured at the higher temperatures show decreased rates of gas exchange. Furthermore, this decrease in O_2 uptake and CO_2 output is not proportional as is shown

* In the second series (Series B) of experiments a few measurements were conducted directly on 5–10 and 10–15 mm segments.

** 150 mg was arbitrarily chosen as this weight contained about the same number of root segments per reaction vessel as had been used previously[1].

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by reference to the Respiratory Quotients (Columns IV and VII). (If they were proportional the R.Q. values would remain the same.) For those roots cultured at the higher temperatures the percentage decrease in the rate of carbon dioxide production is greater than the percentage decrease in rate of oxygen consumption. c. As a result of this disproportionate decrease in rate of oxygen consumption and carbon dioxide production an R.Q. nearer unity is shown by those roots grown at the higher temperatures. d. With regard to series B the magnitude of O_2 uptake is considerably greater, while the CO_2 output is slightly greater than in the first series, resulting in respiratory quotients somewhat nearer unity.

TABLE I

(Measured in Air at 30°C)

Root segment	O_2 consumed/ root segment/ hour	CO_2 evolved/ root segment/ hour	R. Q.		O_2 consumed/ hour/150 mg tissue		CO_2 evolved/ hour/150 mg tissue		R. Q.	
I	II	III	IV		V		VI		VII	
mm	mm^3	mm^3			mm^3		mm^3			
			A	B	A	B	A	B	A	B
Roots cultured at 20°C										
0–5	1.49 ± 0.14	1.88 ± 0.16	1.26	1.17	152 ± 14	172	193 ± 18	198	1.27	1.15
0–10	2.40 ± 0.13	2.94 ± 0.18	1.22	1.16	119 ± 6	126	144 ± 8	152	1.21	1.21
0–15	3.27 ± 0.22	3.95 ± 0.32	1.21	1.17	100 ± 5	116	125 ± 7	136	1.25	1.17
Roots cultured at 25°C										
0–5	1.46 ± 0.07	1.67 ± 0.12	1.14	1.05	148 ± 6	158	169 ± 12	171	1.14	1.08
0–10	2.28 ± 0.11	2.64 ± 0.20	1.16	1.11	109 ± 6	118	124 ± 7	130	1.14	1.10
0–15	3.05 ± 0.23	3.58 ± 0.28	1.17	1.15	96 ± 7	104	113 ± 9	118	1.18	1.13
Roots cultured at 30°C										
0–5	1.30 ± 0.14	1.39 ± 0.14	1.07	1.06	134 ± 11	146	145 ± 10	152	1.08	1.04
0–10	2.02 ± 0.15	2.18 ± 0.13	1.08	1.05	98 ± 6	109	107 ± 5	113	1.09	1.04
0–15	2.71 ± 0.18	3.01 ± 0.24	1.11	1.09	84 ± 5	97	96 ± 7	106	1.14	1.09

There existed the possibility that in some cases the high R.Q. might be due to inadequate liquid-gas exchange within the reaction vessels. However, experiments conducted at lower shaking speeds (96 oscillations/minute) did not show a reduced rate of gas exchange such as would be found if diffusion between gas and liquid were a limiting factor. This is in agreement with previously reported evidence[5] that the shaking speed employed is adequate for a gas exchange of the order of magnitude shown in these experiments.

Table IA presents values for the different zones of the root tip.

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TABLE IA

CALCULATED AND EXPERIMENTAL VALUES FOR EACH ROOT ZONE IN AIR

Root segment	O_2 consumed/root segment/hour		CO_2 evolved/root segment/hour		R. Q.	
I mm	II mm^3		III mm^3		IV	
	A	B	A	B	A	B
Roots cultured at 20°C						
0–5	1.49	2.05	1.88	2.40	1.26	1.17
5–10	0.91	1.11 (0.82)*	1.06	1.25 (1.28)	1.16	1.13 (1.56)
10–15	0.87	0.95 (0.83)	1.01	1.16 (1.06)	1.16	1.22 (1.28)
Roots cultured at 25°C						
0–5	1.46	1.84	1.67	1.93	1.14	1.05
5–10	0.82	0.99 (0.77)	0.97	1.21 (1.00)	1.18	1.22 (1.30)
10–15	0.77	0.81 (0.78)	0.94	1.05 (0.86)	1.22	1.30 (1.10)
Roots cultured at 30°C						
0–5	1.30	1.75	1.39	1.85	1.07	1.06
5–10	0.72	0.90 (0.76)	0.79	0.92 (0.96)	1.10	1.02 (1.26)
10–15	0.69	0.76 (0.75)	0.83	0.94 (0.78)	1.20	1.24 (1.04)

* In all cases the 0–5 mm values are the results of direct measurements. The values in parentheses are those obtained by direct measurement on the 5–10 and 10–15 mm segments, while the other values are calculated by differences.

The values in parentheses represent averages of about four measurements in each case.

These data make more obvious the fact that the meristem shows the greatest respiratory activity when calculated on the basis of root segment. They also show that the rate of oxygen uptake and carbon dioxide production of these segments is decreased when the roots are cultured at the higher temperatures. In general, it would appear that the most significant change in respiratory quotient shown by the roots cultured at different temperatures takes place in the 0–5 mm segment. In addition, there is a considerable increase in the rates of gas exchange shown by roots used in the B series of measurements as compared to series A. There are given in Table IA (series B) the results of some direct measurements made on the 5–10 and 10–15 mm segments. In general, these show lower values than those obtained by difference. Better agreement was obtained by BERRY[4] between calculated and direct measurements on the 5–10 and 10–15 mm segments.

It is of interest to consider these results with reference to those previously obtained by BERRY AND NORRIS[1], some of which are comparable. Values of O_2 uptake at 30°C are given, which differ from the present data in that the temperature of root culture was not as precisely controlled (however it was near 25°C). They found an oxygen uptake of 1.96 cu. mm/root segment/hour for the apical segment, 3.38 for the 0–10 mm segment, and 4.09 for the 0–15 mm segment. These values of oxygen uptake are much

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higher than those shown in Table I. Explanations of these differences are considered later.

Respiration measured in oxygen

Critical pressure of oxygen at 30°C for the three different regions of the root under consideration has been determined[1]. The critical pressure of oxygen was found to be 45% for the 0–5 mm zone, 21% (air) for the 5–10 mm zone, and 10% for the 10–15 mm zone*. To eliminate the possibility of oxygen as a limiting factor, measurements identical with those reported in Table I were made in an atmosphere of pure oxygen and are recorded in Table II. The values for series A represent averages of from 8 to 11 measurements, while those for series B are averages of from 3 to 7 experiments.

TABLE II

(Measured in 100% O_2 at 30°C)

Root segment	O_2 consumed/ root segment/ hour	CO_2 evolved/ root segment/ hour	R. Q.		O_2 consumed/ hour/150 mg tissue		CO_2 evolved/ hour/150 mg tissue		R. Q.	
I	II	III	IV		V		VI		VII	
mm	mm^3	mm^3			mm^3		mm^3			
			A	B	A	B	A	B	A	B
Roots cultured at 20°C										
0–5	1.77 ± 0.09	2.05 ± 0.15	1.16	1.02	176 ± 15	213	207 ± 17	219	1.18	1.03
0–10	2.72 ± 0.20	3.13 ± 0.26	1.15	1.03	132 ± 10	148	157 ± 9	162	1.19	1.09
0–15	3.62 ± 0.36	4.18 ± 0.24	1.15	1.04	112 ± 6	129	134 ± 8	137	1.20	1.06
Roots cultured at 25°C										
0–5	1.63 ± 0.06	1.79 ± 0.14	1.10	1.01	169 ± 8	177	187 ± 12	179	1.11	1.01
0–10	2.49 ± 0.10	2.73 ± 0.13	1.10	1.01	117 ± 8	127	128 ± 6	132	1.09	1.04
0–15	3.26 ± 0.18	3.60 ± 0.26	1.10	1.06	103 ± 6	106	118 ± 11	113	1.15	1.07
Roots cultured at 30°C										
0–5	1.47 ± 0.07	1.53 ± 0.09	1.04	1.00	147 ± 8	160	155 ± 4	160	1.05	1.00
0–10	2.22 ± 0.23	2.35 ± 0.25	1.06	1.01	105 ± 7	118	111 ± 6	123	1.06	1.04
0–15	2.93 ± 0.19	3.15 ± 0.27	1.07	1.03	90 ± 4	100	97 ± 6	106	1.08	1.06

The oxygen uptake and carbon dioxide output (with two exceptions in the CO_2 column) are greater in pure oxygen than in air, however, with regard to the latter the increase shown in pure oxygen is not large in some instances. Data presented in Table III make this more evident.

Table II discloses the same facts as Table I, *i.e.*: a. at any given temperature the

* It should be emphasized that these critical pressures of oxygen were determined on root segments showing a significantly higher rate of gas exchange than that shown by the roots presently employed.

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oxygen uptake and CO_2 output per unit weight decrease with the longer root segments, b. those roots cultured at the higher temperatures show decreased rates of gas exchange, c. the decrease in gas exchange is disproportional as indicated by the decreasing R.Q.'s, and d. series B shows a considerably higher rate of oxygen uptake than series A, while with two exceptions the latter series shows only a slightly higher rate of CO_2 production, which results in respiratory quotients nearer unity. It seems somewhat odd that the R.Q.'s yielded by series B are nearer unity than those shown by series A, in that the data presented later show that the diameter of the roots used in series B were greater than those used in series A.

Table IIA shows essentially the same points as Table IA, *i.e.*, the meristem proves to be the region of greatest respiratory activity on a per root basis; a decreased respiratory rate is shown for roots cultured at the higher temperatures; and, for series A the most marked changes in R.Q. seem to be exhibited by the apical segment.

TABLE IIA

CALCULATED AND EXPERIMENTAL VALUES FOR EACH ROOT ZONE IN O_2

Root segment	O_2 consumed/root segment/hour		CO_2 evolved/root segment/hour		R. Q.	
I	II		III		IV	
mm	mm^3		mm^3			
	A	B	A	B	A	B
Roots cultured at 20°C						
0–5	1.77	2.55	2.05	2.60	1.16	1.02
5–10	0.95	1.12 (0.87)*	1.08	1.18 (1.18)	1.14	1.05 (1.36)
10–15	0.90	0.98 (0.87)	1.05	1.07 (1.01)	1.17	1.09 (1.16)
Roots cultured at 25°C						
0–5	1.63	2.14	1.79	2.16	1.10	1.01
5–10	0.86	1.04 (0.83)	0.94	1.06 (0.97)	1.09	1.02 (1.17)
10–15	0.77	0.86 (0.83)	0.87	1.07 (0.86)	1.13	1.24 (1.04)
Roots cultured at 30°C						
0–5	1.47	1.91	1.53	1.91	1.04	1.00
5–10	0.75	0.96 (0.83)	0.82	1.00 (0.91)	1.09	1.04 (1.10)
10–15	0.71	0.83 (0.79)	0.80	0.89 (0.86)	1.13	1.07 (1.09)

* See footnote on Table IA. The values in parentheses represent averages of about four measuerments in each case.

In general, for the first series, respiratory quotients nearer unity seem to be shown by the roots cultured at 30°C. The results of some direct measurements conducted on the 5–10 and 10–15 mm segments are recorded in Table IIA. As was the case in Table IA (series B) these show lower values than those values obtained by difference.

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Comparing these results to those previously obtained by measurements made at 30°C in pure oxygen[1], it is evident that the rates of gas exchange are considerably lower in the series of experiments reported here. This is especially true for the 0–5 mm segment. The previous results show an oxygen uptake of 2.75 cu. mm/root segment/hour for the apical segment, 4.20 and 4.95 for the 0–10 and 0–15 mm segments.

The data in Table I (both series) and Table II (series A) show a lower R.Q. for roots cultured at the higher temperatures. Upon examination of the corresponding Table IA and IIA it becomes apparent that the greater portion of this change in R.Q. is attributable to the behavior of the apical segment. For the most part, the decrease in R.Q. values shown upon comparing Table I and IA to Table II and IIA are readily explained by recalling that the measurements made on the meristem in air at 30°C represent respiration measured below the critical oxygen pressure. This will not account for changes shown in R.Q.'s by the more basal segments; however, for the most part these changes are of less magnitude than those shown by the apex and may be of little significance.

RUHLAND AND RAMSHORN[8] working with *Vicia faba* report that the R.Q. of young, growing (mitotically active) tissues may be in excess of unity and record values well above 1. They state that the oxygen consumption of dividing tissues is less than that of elongating or mature tissues, which they attribute to an "active aerobic fermentation" occuring in dividing tissues, and mention that meristem not severed from the plant may show fermentation without respiration.

A metabolic gradient has been demonstrated in various kinds of roots by numerous investigators (onion[1, 4, 6, 9]; wheat[10, 11]; barley[9, 12]; corn[9]; rape[9]; *Vicia faba*[9]). In fact, GREGORY AND WOODFORD[13], using an apparatus for the simultaneous study of oxygen, salt, and water uptake in the various zones of the intact root, found a steep gradient of metabolic activity and nitrogen uptake along the root of *Vicia faba*, with the apical segment being the most active in all respects. These results appear to be contrary to those obtained by RUHLAND AND RAMSHORN, but conform with most of the experimental evidence.

Comparison of Respiratory Rate in Air and Oxygen

The increase in rate of gas exchange in pure oxygen over the rate in air shown by the different segments cultured at different temperatures is tabulated in Table III.

Note that for the apical segment there is a considerable increase in rate of oxygen uptake in pure oxygen. This is in accordance with the previously determined fact that the oxygen content of air is well below the critical pressure for the meristem when measured at 30°C[1]. There is also an increase in the rate of CO_2 output; however, the increase is not as large as that for oxygen uptake, thus resulting in the lower R.Q. values obtained when respiration was measured in 100% oxygen. In series B the increase in oxygen uptake shown by the apical segments in pure oxygen over the rate shown in air is greater than in series A (roots cultured at 30°C prove to be an exception). This is no doubt related to the thicker roots used in this case (see following section on root diameter). There is also a proportionately smaller increase in CO_2 production in pure oxygen in series B (on the basis of increase in oxygen uptake), resulting in lower respiratory quotients. This too may be related to the greater root diameter.

With regard to the 5–10 and 10–15 mm segments, there are minor increases in the rate of oxygen uptake, while in about one half the cases there are decreases shown in

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CO_2 production. These relatively minor changes are of doubtful significance, except that they verify the fact that the partial pressure of oxygen in air is not limiting to these segments at the temperature of measurement.

TABLE III

INCREASE IN RATE OF O_2 UPTAKE AND CO_2 PRODUCTION IN 100% O_2 AS COMPARED TO RATE IN AIR BY ROOT SEGMENTS CULTURED AT DIFFERENT TEMPERATURES*

Root segment	Temperature of root culture	Increase in O_2 uptake		Increase in CO_2 output	
I	II	III		IV	
mm	°C	mm^3/root segment/hour		mm^3/root segment/hour	
		A	B	A	B
0–5	20	+ 0.28	+ 0.50	+ 0.17	+ 0.20
0–5	25	+ 0.17	+ 0.30	+ 0.12	+ 0.23
0–5	30	+ 0.17	+ 0.16	+ 0.14	+ 0.06
5–10	20	+ 0.04	+ 0.01 (+ 0.05)**	+ 0.02	— 0.07 (— 0.10)
5–10	25	+ 0.04	+ 0.05 (+ 0.06)	— 0.03	— 0.15 (— 0.17)
5–10	30	+ 0.03	+ 0.06 (+ 0.07)	+ 0.03	+ 0.08 (— 0.05)
10–15	20	+ 0.03	+ 0.03 (+ 0.04)	+ 0.04	— 0.09 (— 0.05)
10–15	25	0.00	+ 0.05 (+ 0.05)	— 0.07	+ 0.02 (0.00)
10–15	30	+ 0.02	+ 0.07 (+ 0.04)	— 0.03	— 0.05 (+ 0.08)

* Obtained by taking the difference in Columns II and Columns III in Tables IA and IIA.
** See footnote on Table IA.

Root diameter

For several reasons it was thought to be of interest to measure diameter of the roots cultured at different temperatures. In the first place BERRY AND NORRIS[1] have pointed out as a qualitative observation that when the culture temperature of onion roots falls below 25°C, thicker roots are obtained. They used a value of 0.73 mm as an average diameter of an onion root in certain of their calculations[14]. This value was taken from data previously obtained by BERRY[4]. Secondly, the importance of measuring root diameter was emphasized after several preliminary experiments in this series, which indicated that the respiratory exchange obtained in these measurements was much less than previously obtained values[1].

The diameter of approximately one thousand roots (series A) cultured at each of the three temperatures was measured with an ocular micrometer in a low power microscope. No more than 100 roots selected at random were measured in any one day. Those roots cultured at 20°C showed an average diameter of 0.56 ± 0.09 mm; those cultured at 25°C, 0.59 ± 0.06 mm; and those cultured at 30°C, 0.55 ± 0.09 mm. The diameter of approximately 300 roots cultured at each of the three temperatures was measured in series B. In each of the three cases the average diameter was 0.68 ± 0.08 mm. Thus the roots studied in the second series of measurements were approximately 0.1 mm (19%) thicker than those formely employed, but still fall short of the diameter of the roots used by BERRY AND NORRIS[1].

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Apparently then, the culture temperature does not affect the diameter of the roots within the observed range. The differences in diameter of the roots employed in series A are probably of no significance as such, however, as will be shown later the discrepancies existing in different series of respiratory measurements (reported on the "per root" basis) can be accounted for largely on the basis of different diameter.

Table IV further emphasizes the fact that there is no significant difference in weight or diameter of roots produced at different controlled temperatures from a single "lot" of onion sets.

TABLE IV

NUMBER OF ROOT SEGMENTS REQUIRED TO WEIGH 150 mg

Root segment	Number of samples		Number of root segments weighing 150 milligrams	
I	II		III	
mm	A	B	A	B
Roots cultured at 20°C				
0–5	52	28	102±6	84±5
0–10	44	14	50±4	42±3
0–15	44	24	31±2	28±2
5–10	—	18	—	78±5
10–15	—	16	—	77±5
Roots cultured at 25°C				
0–5	50	20	103±7	85±6
0–10	46	16	48±4	41±4
0–15	44	12	31±2	27±2
5–10	—	18	—	79±5
10–15	—	16	—	75±3
Roots cultured at 30°C				
0–5	40	26	103±7	84±5
0–10	42	20	48±4	42±3
0–15	48	18	31±2	28±1
5–10	—	16	—	78±3
10–15	—	18	—	74±5

These data furnish additional evidence that the difference in rates of gas exchange shown by roots cultured at different temperatures are not a result of increased weight or diameter of the roots.

DISCUSSION

The foregoing data show that onion roots grown at different temperatures exhibit different respiratory behavior, which indicates the desirability of controlling the temperature of root growth in future investigations in order that more comparable data might

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result. This fact has implications not only for onion roots, but very likely for all metabolic studies involving plant material.

The fact that for growth, the temperature of the root's environment is more important than that of the air seems to have been recognized about thirty years ago by CANNON[15] and by BRENCHLEY[16]. This has a direct bearing on these experiments since room temperature was not accurately controlled. Also, this is of practical importance with respect to future experimentation in that as a rule aquaria temperature may be controlled much more accurately and easily than room temperature.

The values for oxygen uptake shown by the 0–5 and 5–10 mm root segments in both series of experiments reported here are notably lower than those previously obtained by BERRY AND NORRIS[1]. It is also apparent that the diameter of the roots used previously was greater. The reasons for this difference in diameter are obscure, but it would seem to be largely responsible for the different results. The only change in the method of growing the roots was that constant temperature was maintained in the aquaria in these experiments, while in the earlier measurements they were cultured at a somewhat less constant temperature (however this temperature varied only a few degrees from 25°C).

In Table V comparable data from three investigations showing O_2 uptake by the various root segments in air and O_2 are recorded. Results are shown both on the basis of O_2 consumption per root segment per hour and per gram wet weight per hour.

These data provide an excellent opportunity for observing the merits of recording measurements of gas exchange on the per root per hour basis as compared to gas exchange on the per unit weight per hour basis. Column V shows the maximum difference in comparable values based on the percentage of the largest figure. Note that in every case agreement in gas exchange is much better on the basis of unit weight*. The large discrepancy existing in the values of O_2 uptake for the 5–10 mm segment between the earlier measurements and the current values is unaccounted for. For this reason the figures in parenthesis in Column V represent the differences in the values obtained in the two latest series of measurements.

It is of interest with regard to the 0–5 mm segment that the partial pressure of O_2 in air is limiting (below the critical pressure) when measurements are made at 30°C. This is related to the diameter of the roots, as it has been suggested that diffusion may be limiting in respiratory rate when the partial pressure of oxygen is below the critical value[14]. For example, the critical pressure of oxygen has been found to be 45% at 30°C for roots 0.73 mm in diameter[1]. Obviously the critical pressure would be somewhat less for a root having a diameter of 0.59 mm. This is evident in Table III where in making the transition from air to oxygen the increase in oxygen uptake is less for the roots of smaller diameter than for the larger ones. Of course this factor does not influence the measurements made in 100% O_2.

A question of considerable importance lies in an adequate accounting for the difference in diameter of the onion roots. Two factors, both of which are extremely difficult to evaluate, may partially account for this: a. the onion bulbs used were of different "lots", although most of them were obtained from the same source**, and

* Possibly still better agreement would result if respiratory rates were reported on the basis of milligrams nitrogen or milligrams phosphorous contained in the tissue.

** A few sets used by BERRY AND NORRIS[1] were obtained from a source other than the Barteldes Seed Company.

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TABLE V

(Measured at 30°C on two day old roots)

I Root segment	Berry and Norris[1] Cultured at 20–25°C Assumed diameter 0.73 mm	Series A (this report) Cultured at 25°C Measured diameter 0.59 mm	Series B (this report) Cultured at 25°C Measured diameter 0.68 mm	Maximum difference
	II mm^3 O_2 uptake/root/hour	III mm^3 O_2 uptake/root/hour	IV mm^3 O_2 uptake/root/hour	V % of highest figure
		Measured in Air		
0–5	1.96	1.46	1.84	25%
5–10	1.42	0.82	0.99	42% (17%)*
10–15	0.71	0.77	0.81	12%
		Measured in O_2		
0–5	2.75	1.63	2.14	41%
5–10	1.45	0.86	1.04	41% (17%)
10–15	0.75	0.77	0.86	13%
	mm^3 O_2 uptake/gram wet weight/hour	mm^3 O_2 uptake/gram wet weight/hout	mm^3 O_2 uptake/gram wet weight/hour	
		Measured in Air		
0–5	985	1000**	1045	6%
5–10	780	495	521	37% (5%)
10–15	420	445	428	6%
		Measured in O_2		
0–5	1380	1118	1215	19%
5–10	795	518	548	35% (6%)
10–15	443	445	455	3%

* The figure in parentheses represents the difference between the values in Columns III and IV. It is shown due to the large discrepancy between these figures and those shown in Column II.

** These values are determined by calculating the average weight of the respective segments from Table IV and converting the mm^3 O_2 uptake/root/hour values to mm^3 O_2 uptake/g wet weight/h.

b. these experiments were conducted during the fall, winter, and spring, whereas the earlier experiments were conducted in the spring and early summer. In barley roots, Hoagland and Broyer[17] noted seasonal effects on development when the plants were grown in a green house, but attributed these effects to illumination. Experimentally it would be virtually impossible to use the same "lots" of onion sets, and impractical to conduct all experimentation during the same season.

Several points of interest may be considered with regard to the respiratory quotients. In the first place, all of the R.Q.'s are above unity, which upon comparison with values for other tissues may seem unusual. Secondly, the respiratory quotients are nearer unity

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for those roots cultured at the higher temperatures. No adequate explanation for this observation is offered now; however, it may be that lower culture temperatures favor the development of anaerobic mechanisms, while the higher culture temperatures permit a greater development of oxidative mechanisms, or inhibit anaerobic processes. On the other hand, the type and quantity of substrate present within the roots at the time of excision might vary for the roots cultured at different temperatures, which could possibly account for differences in respiratory activity.

In conclusion, it may be said that the data presented indicate that roots grown at different temperatures show differences in subsequent respiratory behavior as measured under identical conditions. An elevation of the culture temperature causes a decrease both in oxygen uptake and carbon dioxide output, but the decrease in carbon dioxide output is greater than that of oxygen uptake, so that respiratory quotients become smaller (approach unity) at the higher culture temperatures.

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SUMMARY

Onion roots from two differents "lots" of sets were grown in nutrient solution at three different temperatures; 20, 25, and 30°C. Gas exchange measurements (in air and O_2) were made at 30°C on 0–5, 0–10, and 0–15 mm segments of the root using the WARBURG manometric technique; values for the 5–10 and 10–15 mm segments were for the most part obtained by difference, however, some few measurements were made directly.

Roots cultured at the higher temperatures show a decrease in rate of oxygen consumption and carbon dioxide production as compared to those cultured at 20°C. This decrease is not proportional in the case of the two gases exchanged as exemplified by the fact that the respiratory quotient exhibits a decreased value for the roots cultured at the higher temperatures. This indicates that carbon dioxide production is depressed to a greater degree than is oxygen consumption. The most marked changes of his nature are shown by meristematic tissue.

The diameter of the roots produced at the three temperatures is not significantly different for any one "lot" of onion sets. However, the diameter of roots produced from different "lots" of sets is markedly different. Because of this, the desirability of reporting measurements of gas exchange per unit weight of tissue rather than per root is shown; a practice which should result in more comparable data. Also, in any one series of investigations, it appears to be desirable to use onion sets from the same "lot".

The results are discussed in relation to earlier investigations. It is suggested that onion roots, as well as other plant tissue, be cultured at constant temperature, which should yield more reproducible results in future experimental work.

RÉSUMÉ

On a cultivé des racines d'oignon de deux groupes de plantes dans une solution nutritive à trois températures différentes, à savoir: à 20°C, à 25°C, et à 30°C. On a mesuré l'échange de gaz (dans l'air et dans O_2) à 30°C à l'égard des segments de racines de 0–5 mm, de 0–10 mm, et de 0–15 mm, employant la technique manométrique de WARBURG; on a déduit les valeurs des segments 5–10 et 10–15 par la différence: cependant, on a fait quelques mesures directement.

Les racines cultivées aux températures plus élevées montrent une diminution de la vitesse de consommation de l'oxygène et de la production du dioxyde de carbone, quand on les compare avec celles qui étaient exposées à une température de 20°C. Il est évident que cette diminution n'est pas proportionelle pour les deux gaz échangés, parce que le quotient respiratoire montre une valeur diminuée dans les cas des racines cultivées aux températures plus hautes. Cela indique que la production du dioxyde de carbone est diminuée plus que ne l'est la consommation de l'oxygène. Les changements les plus remarquables de ce type se rencontrent dans le méristème.

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Dans un même groupe de plantes d'oignon, le diamètre des racines produites aux trois températures ne varie pas essentiellement. Cependant, les diamètres des racines produites par des groupes différents sont remarquablement différents. Par conséquent, on voit que l'on doit donner les mesures de l'échange de gas par unité de poids de tissu plus tôt que par racine. De cette méthode doivent résulter des données comparables. De plus, dans la même série d'investigations il parait plus raisonable d'employer des plantes d'oignon du même groupe.

On examine les résultats par rapport à des investigations antérieures. L'on suggère que les racines d'oignon, aussi bien que des tissus d'autres plantes, soient cultivées à une température constante, procédé qui doit donner des résultats plus reproductibles dans les recherches futures.

ZUSAMMENFASSUNG

Zwiebelwurzeln wurden aus zwei verschiedenen "Gruppen" von Stecklingen in Nährlösung bei drei verschiedenen Temperaturen, 20, 25 und 30°C, gezogen. Der Gasaustausch wurde (in Luft und Sauerstoff) bei 30°C an Wurzelsegmenten von 0–5 mm, 0–1 omm und 0–15 mm mit Hilfe der WARBURG'schen Manometertechnik gemessen; die Werte für Segmente von 5–10 mm und 10–15 mm wurden grösstenteils durch Subtraktion errechnet, doch wurden einige wenige Messungen direkt durchgeführt.

Wurzeln, die bei höheren Temperaturen gezogen worden waren, zeigten, im Vergleich zu den bei 20°C gezogenen, eine Abnahme der Geschwindigkeit des Sauerstoffverbrauches und der Kohlendioxydbildung. Diese Abnahme ist für die beiden ausgetauschten Gase nicht proportional, wie aus der Tatsache hervorgeht, dass der Atmungskoeffizient für bei höheren Temperaturen gezogene Wurzeln einen erniedrigten Wert zeigt. Dies weist darauf hin, dass die Kohlendioxydbildung stärker als der Sauerstoffverbrauch herabgesetzt wird. Die auffallendsten Veränderungen dieser Art zeigen sich im Meristem.

Der Durchmesser der bei den drei Temperaturen gebildeten Wurzeln, variiert innerhalb einer "Gruppe" von Zwiebelstecklingen nicht bedeutend. Hingegen sind die Durchmesser von Wurzeln aus verschiedenen "Gruppen" von Stecklingen deutlich verschieden. Es ist daher wünschenswert, die Ergebnisse der Messungen des Gasaustausches lieber pro Gewichtseinheit des Gewebes als pro Wurzel anzugeben; auf diese Weise sollte man besser vergleichbare Resultate erhalten. Ausserdem ist es wünschenswert innerhalb einer Reihe von Untersuchungen Zwiebelstecklinge aus derselben Gruppe zu "benutzen".

Die Ergebnisse werden im Verhältnis zu früheren Untersuchungen erörtert. Es wird vorgeschlagen, dass Zwiebelwurzeln, sowohl wie andere Pflanzengewebe, bei konstanter Temperatur gezogen werden; auf diese Weise sollte man in späteren Versuchen besser reproduzierbare Werte erhalten.

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