633.491:581.12

MEDEDELINGEN VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN/NEDERLAND
52 (3) 93–104 (1952)

APPLICATIONS OF THE DIAFEROMETER TECHNIQUE TO STUDIES ON THE GAS EXCHANGE AND THE CARBON DIOXIDE CONTENT OF POTATO TUBERS

by

F. H. SPIERINGS, G. P. HARRIS [1]) AND E. C. WASSINK

Laboratory of Plant physiological Research,
Agricultural University, Wageningen, The Netherlands; 101st communication

(*Received 8.7.'52*)

§ 1. INTRODUCTION

The techniques described in this paper have been developed in the course of studies on the respiration of potato tubers during storage. The investigation entails: 1) the study of rapid changes in rates of respiration, 2) the simultaneous measurement of the oxygen uptake and carbon dioxide evolution of one sample of plant material, 3) the control of the gaseous composition of the atmosphere surrounding the plant material during measurements, 4) the comparison of the carbon dioxide contents of potato tubers which have undergone different pretreatments. A method of measuring gas exchange which meets the first three of these requirements is described in the first part of the paper. The second part is concerned with a method of comparing the carbon dioxide status of tubers.

§ 2. THE MEASUREMENT OF RATES OF GAS EXCHANGE

The method adopted involves the determination of changes in the composition of gas mixtures by measuring the corresponding changes in their thermal conductivity.

A thin platinum wire is heated by a uniform electric current. The wire is surrounded by the gas mixture under investigation, to which it loses heat. A change in the thermal conductivity of the mixture causes a change in the rate of heat loss of the wire and consequently in its temperature. The resistance of the wire, which is measured by means of a galvanometer, is directly related to its temperature.

NOYONS (1922) was the first to use these methods in biological investigations and later adopted the name „diaferometer" for a modified form of his apparatus (1937). STILES and LEACH (1931) made use of the same principle in their katherometer and have described similar methods used in earlier biological and non-biological studies. More recently AUFDEMGARTEN (1939) and VAN DER VEEN (1949) have studied gas exchange in photosynthesis by the thermal conductivity method.

[1]) Present address: Department of Botany, University of Manchester.

The diaferometer of NOYONS, is well adapted to the work in progress here in that it measures both oxygen and carbon dioxide. The thermal conductivity of the latter gas differs markedly from that of air, so that small changes in concentration are readily detected. The difference between the thermal conductivity of oxygen and that of air, however, is relatively small. Therefore, it is preferable that the sensitivity of the galvanometer employed for oxygen determinations is higher than that for carbon dioxide, so that similar changes in the concentration of the two gases yield galvanometer deflections of the same order of magnitude. Before making the oxygen determinations, all carbon dioxide is removed, so that changes in the concentration of this gas cannot interfere with the measurements.

§ 3. THE DIAFEROMETER

An instrument supplied by Messrs. KIPP, Delft, Netherlands, has been used in this work. It is similar in construction to that used by NOYONS (1937), and a brief description is given for purposes of convenience.

The electrical circuit (see fig. 1) includes two WHEATSTONE bridges each with a galvanometer. One bridge is used for measuring oxygen, the other for carbon dioxide. The galvanometers are of the MOLL-D type, and the sensitivity is such that a tension of 10^{-5} V produces a deflection of 37.2 mm at 1 metre.

Platinum wires (P_{1-4}) the resistance of which is determined by the composition of the surrounding gas mixture form two sides of each bridge. These wires are extended through cylindrical holes in a copper block through which the gas mixtures pass. The other two sides (R_{1-4}) of each bridge are of fixed resistance during an experiment. Two „sensitivity knobs" control variable resistances (S_1, S_2) in parallel with the galvanometers. In addition there are two „control knobs" (C_1, C_2) by means of which a portion of one of the fixed resistances in each of the WHEATSTONE bridges may be bypassed. Thus it is possible to obtain a reproducible change in each bridge and to check the sensitivities of the galvanometers. The galvanometer spot is brought onto the scales by means of a potentiometer arrangement (P) controlled by an „adjustment knob". The electric current passing through the wires is produced by a 6 volt

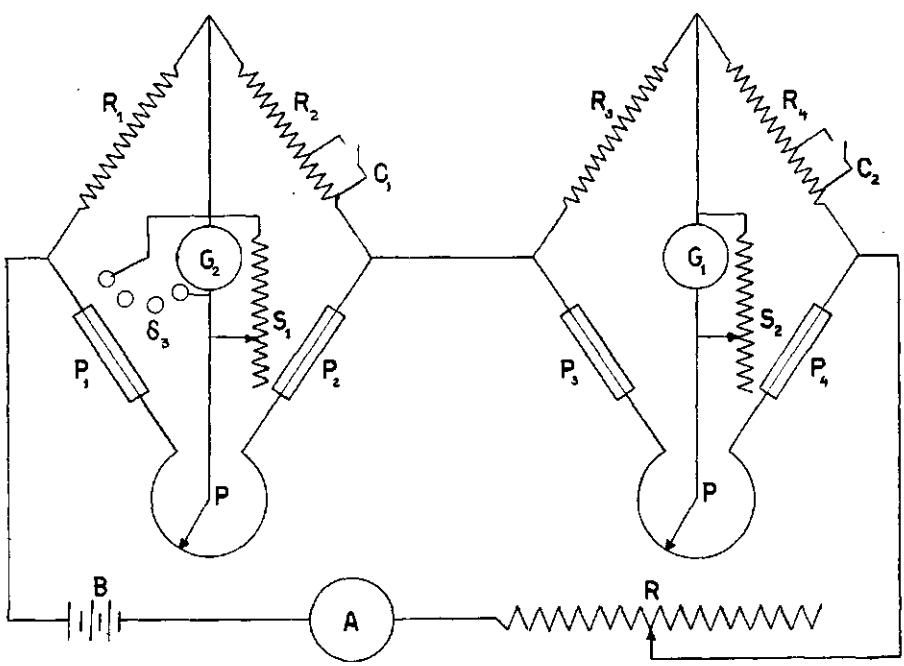


Fig. 1. The electrical circuit. Explanation of lettering in the text.

battery (B) and is regulated by means of a rheostat (R) used in conjunction with a sensitive milliammeter (A). The copper block, electrical circuit and galvanometers are built into a single compact casing.

The apparatus has two gas inlets and four gas outlets. Each inlet divides inside the apparatus casing and leads to one platinum wire in each of the two WHEATSTONE bridges. By means of a three-way tap it is possible to divert the flow of gas entering by one inlet to all four of the platinum wires, but this arrangement is not utilised in the experimental procedure described below. The branch conduits of the gas inlets are exposed at the surface of the instrument casing before entering the copper block. At these points are situated needle valves and flowmeters by means of which the rate of gas flow around each wire may be regulated.

§ 4. MODIFICATIONS OF THE DIAFEROMETER TECHNIQUE FOR USE WITH PLANT MATERIAL

a. The electrical circuit

The type of galvanometer supplied with the apparatus has been found sufficiently sensitive for the carbon dioxide measurements, provided a shunt, built in by the manufacterer is removed. A circular scale is fitted behind the sensitivity knob, by reference to which the sensitivity of the galvanometer (G_1) may be varied by fixed amounts. When a shunt is changed, the relative sensitivity of the galvanometer can be determined by use of the control knob (C_2). In order to make comparable measurements of oxygen a more sensitive galvanometer (G_2) has been installed, giving a deflection of 67.2 mm at 1 metre with a tension of 10^{-5} V. It is a double coil type (Nr A 54, type Kc, supplied by Messrs. KIPP, Delft). The sensitivity of this instrument can be varied by means of a universal shunt box (S_3). The wide range of sensitivities obtainable allow for measurements over a considerable range of changes in gas concentration.

b. The gas system

In the technique used by NOYONS (1937) and MAAS (1938), air from the outside of the laboratory was sucked through the apparatus by a pump. The sensitivity of the method was limited since only part of the air passing over the respiring tissue was drawn through the copper block. We have found that a cylinder of gas under pressure forms a satisfactory source for the gas stream, provided measures are taken to control rates of flow. The method has the advantage that different gases and gas mixtures may be used. Moreover, all the gas flowing over the plant material passes along the platinum wires during a measurement. This is important in cases where the amounts of carbon dioxide and oxygen exchanged are small.

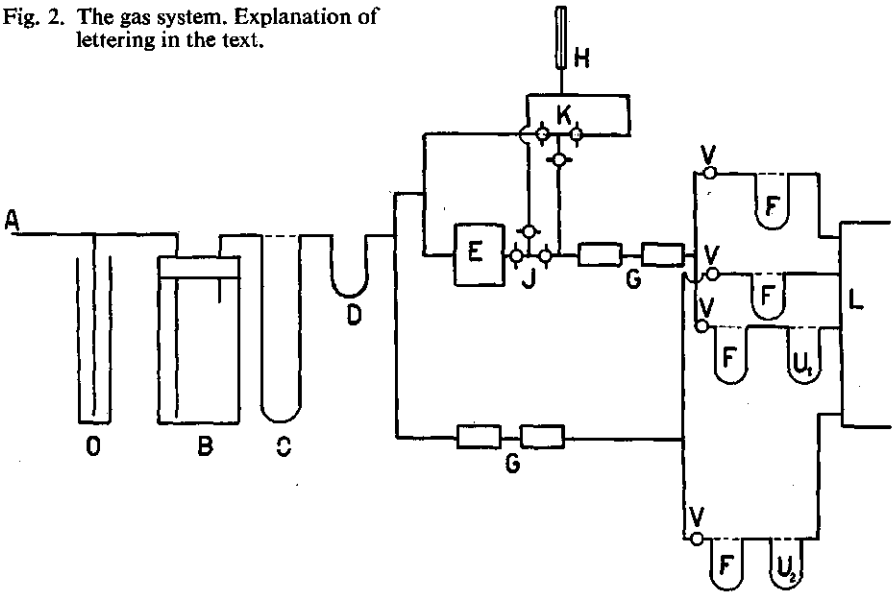
Fig. 2 shows the arrangement adopted. The gas stream is conducted through glass tubing of 0.38 cm inside diameter. The length of the tubing between the respiration chamber (E) and the copper block (L) is reduced to a minimum so that changes in gas concentration are transmitted very rapidly. Rubber connections are used only at points where screw clips are situated or where the operation of the apparatus requires a frequent renewal or rearrangement of sections of the gas circuit.

Gas is led from a cylinder (A) into a large empty flask (B). There is a gas overflow (0) immediately after the valve of the cylinder in the form of an open side tube dipping into water in a glass cylinder. After the flask B is a flowmeter (C) consisting of a glass U-tube, the arms of which are connected at their upper ends by a glass capillary. The U-tube contains paraffin oil and is set against a scale. The capillary is connected into the gas circuit. The difference in the level of paraffin oil in the two arms of the tube is taken as a measure of the rate of flow. The gas then passes through a U-tube (D) containing soda-lime which frees it from carbon dioxide, after which it is divided into two streams. Each of these streams supplies one platinum wire in each of the two WHEATSTONE bridges. One of the streams, however, may be diverted through the respiration chamber, and consequently be modified in composition before entering the copper block.

When the stream is not diverted, gas of the same composition passes along each of the two platinum wires in either WHEATSTONE bridge and a certain galvanometer deflection (control reading) is produced. As a result of diverting the stream, the composition of the gas passing along one of the two wires is changed by passage through the respiration chamber, and a different galvanometer deflection (experimental reading) results. The difference between the two readings is a measure of the change in composition.

The diversion of the gas stream is carried out by adjustment of screw clips at J and at K. At all times gas is allowed to escape through the glass capillary H. During control readings

Fig. 2. The gas system. Explanation of lettering in the text.



however, the clips are adjusted so that this gas first passes through the respiration chamber in order to provide uninterrupted ventilation of the latter.

The actual arrangement of the system inside the instrument casing is simplified for the purposes of the diagram.

The needle valves (V) mentioned above have been retained but the four flowmeters have been replaced by some of our own construction (F). They are the same in principle as that used for the main gas flow. The four capillaries are made so as to be equal in resistance. The U-tubes are of thick walled capillary tubing of 1.6 mm inside diameter. The arms nearest the copper block have a small reservoir at the top in order to prevent fluid entering the block.

A U-tube containing soda-lime is introduced into each of the two branches used for the oxygen measurements. One of the tubes (U_1) serves to remove carbon dioxide produced by the plant material. The other (U_2) is not connected with the respiration chamber and serves merely to keep the two gas conduits comparable as regards resistance to gas flow.

The construction of the respiration chamber is adapted to the size and nature of the material under investigation. It is made largely of glass and is connected in the gas circuit by glass inlet and outlet tubes through a cork stopper.

§ 5. Problems associated with the use of the apparatus

a. Control of the rate of flow of gas

It was found that uncontrollable changes in the setting of the reduction valve of the gas cylinder affected the rate of flow through the apparatus. For this reason the overflow was introduced as an additional pressure regulator. This was essential because a change in the rate of gas flow along the platinum wires causes a change in their temperature and consequently influences the deflection of the galvanometers. The supply of gas is sufficiently constant provided there is no observable change in the reading of the flowmeter C (fig. 2) during an experiment.

The flask B was introduced in order that any small fluctuations in pressure resulting from the formation and release of gas bubbles at the end of the overflow tube might cancel each other out. Subsequently it has been found that the resistance of the capillary of the flowmeter C is itself sufficient to cancel out the pressure fluctuations.

In preliminary experiments some difficulty was experienced in avoiding a change in the rate of flow when the gas stream was diverted into, or away from, the respiration chamber. The arrangement shown in fig. 2 was devised to give a minimum difference in resistance between the two gas circuits involved. The system of screw clips replaces two three-way taps which were used originally. Different positions of the taps were found to give differences

in resistance sufficient to cause deflections of the galvanometers. This was due to slight differences in the diameters of the holes in each tap.

The four flowmeters in front of the copper block provide a means whereby the rate of flow along each wire may be checked. This rate, which also determines the rate of flow through the respiration chamber, must be reproducible on different occasions. A given rate of gas exchange will then produce the same percentage change in the composition of the gas stream passing through the chamber. The resistances of the four flowmeters are made equal so that the same reading on any one of the four corresponds to the same rate of flow.

b. Control of the moisture content of the gas stream

Changes in the moisture content of the gas streams produces deflections of the galvanometers which interfere with the measurements. Consequently it is necessary to maintain constant the moisture content of the gas passing through the blocks. Two methods have been used to attain this. In the first the gas is dried as completely as possible; in the second it is saturated with vapour directly before entering the measuring blocks. In the experiments described in this paper the former method was employed. Calcium chloride, silica gel and phosphorus pentoxide were tried as drying agents, but only the latter proved to be effective.

Before entering the copper block each gas stream passes through two glass tubes (G) containing phosphorus pentoxide. The tubes are 12 cm in length and 1,2 cm in diameter. They are in a horizontal position and filled for half their height with phosphorus pentoxide. The inlet and outlet of each tube is so situated that the gas stream flows over the surface of the powder.

The phosphorus pentoxide is renewed when half the powder in the first of a pair of tubes appears to be saturated with moisture. The efficacy of the method had been demonstrated by substituting a flask containing water for the respiration chamber and showing that the galvanometer deflection remains constant irrespective of whether air passes through the flask or bypasses it.

Paraffin oil is used in preference to water in the flowmeters next to the copper block because water was found to vapourise during a measurement and interfere with the determinations.

c. Control of other factors interfering with measurements

The apparatus is installed in a cellar room where no rapid fluctuations in temperature occur. This ensures that the gas surrounding and entering the copper block remains at a constant temperature during an experiment.

The instrument and battery are placed on a concrete bench and covered with a glass casing in order to avoid mechanical disturbance and air draughts.

It is necessary to establish the nature of the gas exchange to be studied before using the diaferometer, as changes in the concentration of gases other than carbon dioxide and oxygen will also influence the galvanometer deflections.

§ 6. Experimental procedure

A respiration chamber is chosen into which the plant material will conveniently fit, leaving as little surplus space as possible. This reduces any time lag due to the equilibration of gas concentrations throughout the chamber. The current passing through the circuit is adjusted to 100 milliamps several hours before the commencement of an experiment. A further fine adjustment follows when the wires have reached temperature equilibrium with the gas streams passing along them. The valve on the gas cylinder is opened so that a steady flow of bubbles escaped from the overflow tube. When normal respiration is being studied a cylinder containing air is used. The needle valves in front of the copper block are adjusted to give equal readings on the four flowmeters. The slower the rate of flow the smaller the rates of gas exchange which may be detected, always providing the rate of flow is maintained constant and the flowmeters can be read accurately. In experiments with single potato tubers a rate of approximately 70 ml per min. through the respiration chamber has been found satisfactory.

The gas is allowed to flow for a period of thirty minutes before any readings are taken in order to ensure a condition of equilibrium in the system. During this period the screw clips are adjusted so that the respiration chamber is ventilated through the capillary H. (fig. 2).

Five minutes before readings are to be taken the galvanometer spots are centred on the scales by turning the adjustment knobs. If large changes in composition are to be determined the sensitivities of the instruments are reduced by means of the shunts.

The method of making the measurement is similar to that described by Noyons (1937).

Reading of both galvanometers are taken at one minute intervals and plotted as shown in fig. 3. When about six readings lie on a horizontal or slightly inclined line, the balance of the bridge is sufficiently constant. The screw clips are then adjusted for the experimental readings, so that gas is diverted through the respiration chamber before reaching the platinum wires.

An equilibrium between the gas composition in the plant chamber and that along the wires is established in about two minutes after adjusting the clips. After this time, if respiration is proceeding at a steady rate, the experimental readings show the same degree of constancy as the control readings. After making about six readings the clips are again adjusted for a second set of control readings. In this way it is possible to detect any change in the drift of the galvanometers which is due to factors other than the gas exchange of the plant material.

The vertical distance between experimental and control lines (see fig. 3) is measured and may be converted into absolute units by reference to the calibration graphs.

When rapid changes are occurring in the rates of gas exchange the experimental line will be inclined to the control line. Under these conditions experimental readings may be taken over longer periods, in order that the respiration drift may be followed in detail. Control readings are taken at suitable time intervals so that the position of the control line may be accurately determined. The vertical distance between the control line and any of the experimental readings is a measure of the gas exchange rate at that time.

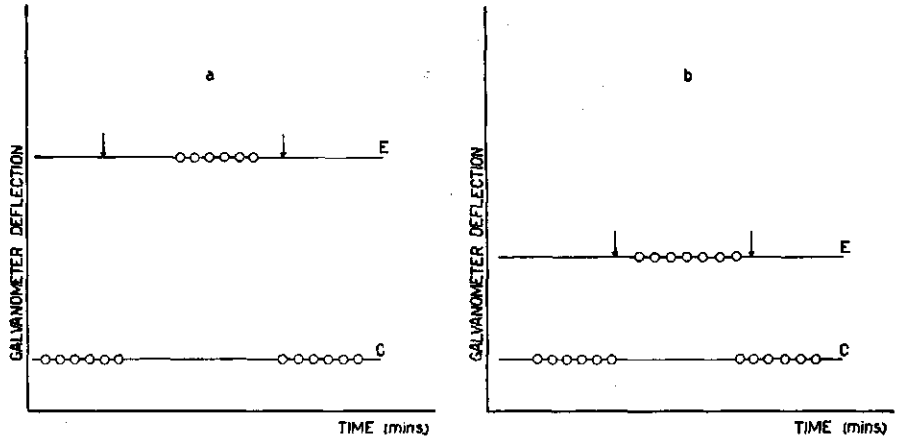


Fig. 3. Method of recording galvanometer readings. Oxygen (a) and carbon dioxide (b) exchange of a single potato tuber in air. Arrows indicate points at which gas stream is diverted; E, experimental line; C, control line. For further details see text.

§ 7. Calibration of the diaferometer

The apparatus is calibrated separately for oxygen and for carbon dioxide by injecting measured quantities of these gases into a container which takes the place of the respiration chamber in the gas circuit.

The gas to be injected is derived from a cylinder and the rate of flow is maintained constant by an overflow and a large capacity flask, as employed in connection with the main gas stream. Different rates of injection are obtained by raising or lowering the level of liquid in the overflow.

The rate of flow is measured by a flowmeter with a capillary of high resistance and a U-tube with long arms, so that low and widely differing rates of flow can be employed.

The flowmeter is calibrated over the range of rates of flow used in the calibrations of the diaferometer by collecting the gas in an inverted burette over paraffin oil. The oil is contained in a trough of large surface area in order to minimise changes in pressure resulting from a rise in the level of the liquid during the collection of gas. The volume of gas collected in a given time is plotted against the reading of the flowmeter.

The galvanometer deflections corresponding to given rates of flow are recorded following the normal procedure for measuring rates of gas exchange. In this way the instrument is calibrated for different quantities of both oxygen and carbon dioxide.

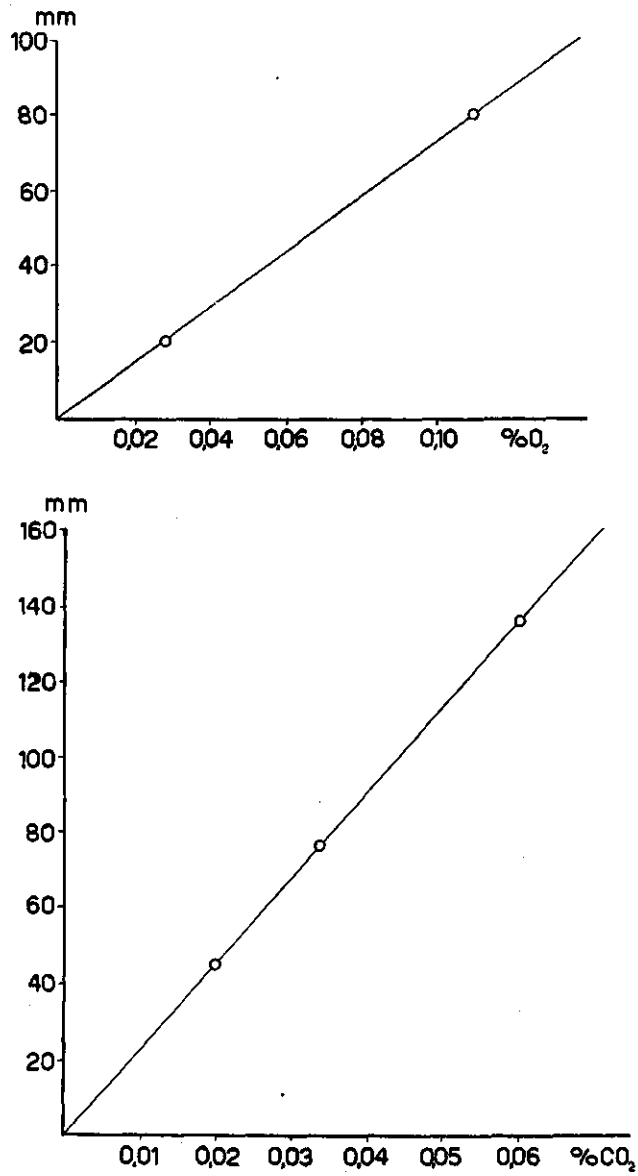


Fig. 4.

Oxygen and carbon dioxide calibration of the diaferometer.
Ordinate: galvanometer deflection.

In order that the percentage of carbon dioxide or oxygen corresponding to a given galvanometer deflection may be calculated, the total rate of gas flow through the container has to be measured. This gas escapes from two of the four outlets of the instrument, which are connected to a single tube and the amount of gas passing in a given time is measured in a manner similar to that described above. We have found it convenient to use the same rate of flow as that normally used in experiments (70 ml per min.).

Fig. 4 shows the calibration graphs for oxygen and carbon dioxide. The higher gas concentrations employed were measured at reduced sensitivities of the galvanometers and multiplied by the appropriate factors.

In measurements of the gas exchange in respiration the oxygen galvanometer responds to a decrease in oxygen concentration and a corresponding increase in nitrogen concentration. In the calibration the same conditions obtain, except that the oxygen concentration is increased and the nitrogen decreased. Thus, deflections of the oxygen galvanometer produced in experiments, may be referred directly to the calibration graph. In the case of carbon dioxide measurements, however, the position is complicated by simultaneous changes in oxygen concentration resulting from the absorption of this gas by the plant material. In order to overcome this difficulty the carbon dioxide galvanometer is calibrated for oxygen also. Thus a correction can be applied for the effect of the observed oxygen absorption on the carbon dioxide reading before reference is made to the carbon dioxide calibration graph. This assumes that in a calibration carbon dioxide is injected into pure nitrogen. The fact that it is actually injected into a mixture containing approximately one part of oxygen to four parts of nitrogen can only introduce errors of a very low order, as the values for the thermal conductivities of oxygen and nitrogen lie relatively close together and differ widely from the value for carbon dioxide.

§ 8. A COMPARATIVE METHOD FOR THE STUDY OF THE CARBON DIOXIDE STATUS OF POTATO TUBERS

a. Statement of the problem

One of us (F.S.) has found that when a potato tuber is cut in air there is a sudden release of carbon dioxide. No corresponding uptake of oxygen is observed and the release of carbon dioxide takes place in pure nitrogen as well as in air. This is taken as evidence that the carbon dioxide which is liberated is part of the gas which has accumulated in the intact tuber before cutting. RICHARDS (1896) studied the carbon dioxide production of tubers after they were cut, and noted a marked rise in production over the first two hours followed by a fall and then a second rise reaching a maximum after about twenty five hours. He concluded that the initial rise was „due to the escape of this gas (carbon dioxide), already absorbed or held in the tissue, in a physical rather than a chemical manner". In the experiments conducted here a second increase in the rate of carbon dioxide production is also observed, but this takes place some thirty minutes after cutting in air. At this time a corresponding increase in the rate of oxygen absorption occurs. This is interpreted as the result of an increase in respiration following injury, a reaction frequently reported in both fleshy and non-fleshy organs.

Thus in determining the carbon dioxide status of potato tubers, it is the period immediately after cutting that is of significance. If, during this period, the carbon dioxide content of the atmosphere surrounding a tuber is increased, the amount of carbon dioxide liberated is reduced. Still higher concentrations of carbon dioxide reverse the direction of the diffusion gradient and the gas is absorbed into the tuber. At some intermediate concentrations an equilibrium must be produced between the carbon dioxide in the atmosphere and that in the tuber. If a tuber were cut at this concentration no carbon dioxide exchange would be observed.

Using the diaferometer technique it is possible to determine this equilibrium value, which provides a measure of the carbon dioxide status of the tissues.

b. Technique and preliminary results

The method employed gives an average value for the internal carbon dioxide status of similarly treated potato tubers. Tubers used in the experiments are selected for similarity of size in order to reduce the number of replicate determinations required.

A tuber taken from the sample under investigation is placed in a respiration chamber. The chamber is closed by a rubber stopper and connected to the gas circuit of the diaferometer by an inlet and outlet tube. A metal rod, which carries a knife blade at its lower end, passes through a hole in the stopper. It is possible to manipulate the blade, without allowing the escape of gas, by moving the rod up and down in the stopper. The flow of gas through the chamber is only disturbed for such time as the manipulations are in progress. Gas of known carbon dioxide content is passed through the apparatus. This gas is produced by injecting carbon dioxide from a cylinder into a carbon dioxide-free air stream in a manner similar to that described for the calibration. The concentration of carbon dioxide in the gas leaving the mixing chamber is determined by reference to the flowmeter calibrations. This concentration may be varied by raising or lowering the overflows.

After about six experimental readings (see under Experimental Procedure), the tuber is cut in half by the knife blade at right angles to the axis and the two halves are allowed to fall away from each other. The reaction of the tuber in terms of gas exchange is then followed for about fifteen minutes.

This procedure is repeated at the one carbon dioxide concentration with other tubers from the same sample. Further groups of tubers are cut at different concentrations, so that at the lower values the carbon dioxide content of the gas stream is increased by cutting, while at the higher concentrations a reduction in carbon dioxide content results. Fig. 5 shows specimen determinations made at different carbon dioxide concentrations. At the lower concentration carbon dioxide is evolved from the cut surface of the tuber while at the higher concentration it is absorbed. At the intermediate concentration a condition of approximate equilibrium obtains.

Table 1. Relative rates of carbon dioxide exchange of potato tubers cut at different carbon dioxide concentrations

Tuber no.	Concentration of carbon dioxide			
	0.05 %	0.25 %	1.0 %	1.75 %
1	+22 [1])	+11	+1	−14
2	+20	+10	+2	−16
3	+20	+ 9	+1	−16
4	+18	+ 9	−2	−20

[1]) The values given represent the reading of the carbon dioxide galvanometer in mm, 10 minutes after cutting a tuber.

The results reported in Table 1 were obtained in experiments with potatoes of the variety Bevelander, harvested in the summer of 1950. Tubers with a diameter of 35–45 mm were selected for the determinations. They had been stored at a temperature of 2 °C for several months previous to their transfer to a temperature of 20 °C on 26th January 1951. Sprouts began to develop at the higher temperature but were rubbed off two days before the commencement of the determinations on 10th February.

Preliminary experiments had shown that a condition near to equilibrium as regards carbon dioxide exchange obtained when tubers were cut in a gas mixture containing 1% carbon dioxide. The four concentrations used in the experiments were selected on this basis. Four tubers were cut at each concentration.

The fact that the galvanometer deflections at 0.25% were opposite in direction

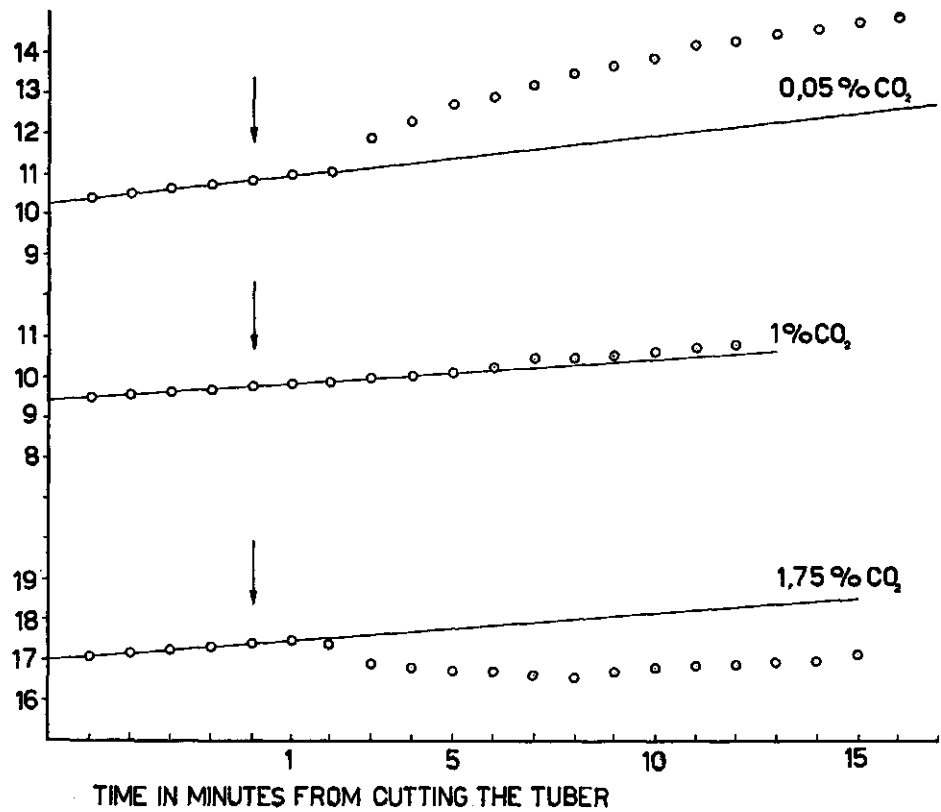


Fig. 5. The effect of three different concentrations of carbon dioxide on the carbon dioxide exchange of cut potato tubers. Arrows indicate the time at which tubers are cut. Ordinate: Galvanometer readings in cm.

to those at 1.75% (see Table 1) shows that the equilibrium value lay between these two concentrations. One percent was the only concentration at which both positive and negative deflections were recorded indicating that of the concentrations employed, this was nearest to the equilibrium value required.

In plotting the data of Table 1 and drawing a smooth line through the points, we can establish rather accurately the value of the concentration at which no carbon dioxide exchange with the surroundings takes place which, in our experiments was found to be in the neighbourhood of 1%. We have preliminary evidence that this value is liable to variation with respect to season and pretreatment, which of course, appears quite logical.

c. Discussion

A number of methods have been devised for the determination of the carbon dioxide content of the intercellular spaces of fleshy plant organs, and in particular of the potato. These have been reviewed recently by GORTER and NADORT (1941) and SMITH (1947). A technique for studying the gaseous conditions inside an apple fruit was devised by SMITH (1947) and used in a modified form by

HULME (1951). Their method involves the withdrawal of a small sample of gas from the fruit through a hypodermic needle inserted into the tissues. The injury to the tissues is slight and the duration of the period of extraction has no effect on the composition of the gas withdrawn. The potato, however has a smaller intercellular space than the apple and the withdrawal of sufficient gas for analysis would involve a considerable disturbance of the equilibrium between gaseous and dissolved carbon dioxide in the tissues. Consequently, in the comparison of the carbon dioxide status of tubers, it would be difficult to ensure that the carbon dioxide measured had come from the same source in each determination. GORTER and NADORT discuss a method devised by MAGNESS (1920) for measuring the concentration of carbon dioxide in the intercellular space of potato tubers by subjecting the tissues to a negative pressure and analysing the gases which are extracted. They found that the values obtained in this way are dependent on the extent to which the pressure is reduced, and that when the pressure is relatively low a considerable volume of carbon dioxide is withdrawn from solution in the tissues.

The equilibrium values determined by the diaferometer technique are not subject to this source of error and are likely to be much closer to the actual concentration of the carbon dioxide in the intercellular space of the tubers. The fact that the carbon dioxide concentrations determined by MAGNESS are so much higher than those reported here might be explained by this.

The older method of DEVAUX (1890) does not employ negative pressures and for this reason has been judged preferable to that of MAGNESS (GORTER and NADORT, 1941). It involves the analysis of gas which diffuses from the tissues of the tuber at atmospheric pressure. In order that gaseous equilibrium might be established, however, periods of one or two days elapse between cutting the tissues and making the carbon dioxide determinations. Increases in respiratory rates especially during the latter part of this period, might contribute considerably to the concentration of carbon dioxide measured. In the method described in this paper determinations are made shortly after cutting the tuber before there has been any appreciable increase in the respiratory rate as a result of injury. This provides a possible reason why concentrations determined by DEVAUX for potatoes stored at room temperature are three to five times as great as the equilibrium values determined here.

With the diaferometer technique tubers remain in the external test tension of carbon dioxide during the introductory phase of the determinations. This must introduce a tendency towards equilibration of the internal and external carbon dioxide concentrations.

The error introduced in this way could only be considerable, however, when the difference in carbon dioxide tensions is well marked, a situation which could not arise at the equilibrium concentrations which are determined. Furthermore it has been shown that a reduction in the duration of this introductory phase from thirty to ten minutes does not produce any appreciable difference in the results obtained, even at carbon dioxide concentrations higher or lower than the equilibrium value.

§ 9. Summary

A method of measuring rates of oxygen and carbon dioxide exchange of potato tubers by a modification of the diaferometer technique is described.

The diaferometer may also be used for comparing the carbon dioxide status of tubers which have undergone different pretreatments. The method involves cutting the tubers in an atmosphere containing different concentrations of carbon dioxide. When the carbon dioxide concentration is relatively low carbon dioxide in the intercellular space diffuses out into the surrounding gas. When the carbon dioxide concentration is relatively high carbon dioxide diffuses into the tuber. It is possible to choose a concentration of carbon dioxide in the external gas phase such that no diffusion into or out of the tuber occurs. When this equilibrium is established the external concentration of carbon dioxide must correspond closely to the concentration of carbon dioxide in the intercellular space of the potato tuber. In the experiments reported we found that the carbon dioxide concentration in the internal gas spaces was near to 1.0%. We have preliminary evidence that this value is liable to variation with respect to season and pretreatment.

References

Aufdemgarten, H., Planta **29**, 643 (1939).
Devaux, H., Bull. Soc. Bot. France, **37**, 272 (1890).
Gorter, A. and Nadort, W., Proc. Nederland. Akad. Wetenschapp. **44**, 1112 (1941).
Hulme, A. C., Jour. Exp. Bot. **2**, 65 (1951).
Maas, J. A., Die visco-elastischen Eigenschaften von Kautschuk und glatten Muskeln, kontrolliert am Stoffwechsel des Helixfusses. Thesis, Utrecht, 1938.
Magness, J. R., Bot. Gaz. **70**, 308 (1920).
Noyons, A. K. M., Arch. néerlandaises physiol. **7**, 488 (1922).
Noyons, A. K. M., Ann. Physiol. **13**, 909 (1937).
Richards, H. A., Ann. Bot. **10**, 531 (1896).
Smith, W. H., Ann. Bot. N.S. **11**, 363 (1947).
Stiles, W. and Leach, W., Ann. Bot. **45**, 461 (1931).
Veen, R. van der, Physiologia Plantarum **2**, 217 (1949)