BRIEF PAPERS

A MODIFICATION OF THE WARBURG RESPIROMETER TO MEASURE THE RESPIRATION RATE OF TOMATO LEAF DISCS[1]

J. EDWARD KLINKER

Received January 19, 1950

In connection with a physiological study of six tomato varieties, the need for a method of measuring simultaneously the respiration rate of the leaves of several replicates of these varieties arose.

BONNER and WILDMAN (1) have reported on the use of the Warburg apparatus for measuring the respiration of spinach leaves. They obtained highly reproducible rates of gas exchange from 200 milligram samples of fresh spinach leaf fragments 5–10 mm^2 in area, when mixed with two milliliters of a phosphate buffer and measured in the Warburg respirometer.

Preliminary attempts were made to measure respiration of tomato-leaf discs by the oxygen absorption method discussed by UMBREIT, BURRIS and STAUFFER (3). Twenty discs were cut from a tomato leaf with a one-quarter inch cork borer. Oxygen uptake measurements of these discs were made in a darkened room in Warburg flasks. Two-tenths milliliter of 20% potassium hydroxide was used in the center well of each flask to absorb the carbon dioxide. All measurements were made at 25° C, using an M/15 potassium-dihydrogen-phosphate buffer with a shaker rate of 120 strokes per minute. Extremely variable rates of oxygen uptake were obtained between replicates of discs taken from the same leaf.

It seemed reasonable to suspect that the buffer might be the principal cause of these erratic rates of oxygen absorption. The buffer being in close contact with the leaf discs, would greatly impede the normal exchange of gases in respiration. This interference would be especially great in the absorption of oxygen since its solubility in water is quite low. From Lange's Handbook of Chemistry (2) the α value for oxygen at 25° C is 0.0283; whereas the α value for carbon dioxide at 25° C is 0.759, or carbon dioxide is approximately 27 times more soluble in water than is oxygen.

Subsequently a modification of this method was tried in which the buffer was eliminated and filter paper of uniform size was cut and fitted in the bottom of the Warburg flasks. One-tenth milliliter of water was pipetted into the bottom of each flask to moisten the filter paper. Twenty tomato leaf discs were then placed in regular order on the filter paper. The flask atmosphere was kept sufficiently moistened by the wet filter paper to maintain turgor of the leaf discs. Two-tenths milliliter of 20% potassium hydroxide was used in the center well to absorb the carbon dioxide. With the Warburg apparatus available for this work, simultaneous respiration determinations could be made of leaf discs from two complete replicates of six varieties each plus a thermobarometer. All measurements were made at 28° C in a darkened room.

[1] Paper No. 331, Department of Vegetable Crops, Cornell University, Ithaca, New York.

Two runs of two replicates each were made, each run being one hour in duration. After measurements of oxygen uptake were completed, the disc samples were dried to constant weight and their weights recorded. Calculations were made to convert the oxygen uptake values to microliters uptake of oxygen per milligram dry weight of the leaf discs. A summary of these data is given in table I.

TABLE I

OXYGEN CONSUMPTION OF LEAF DISCS OF SIX TOMATO VARIETIES. (MICROLITERS O_2/MG. DRY WT./HR)

VARIETY	REPLICATION				MEAN
	1	2	3	4	
46–31	2.26	1.62	2.31	2.21	2.10
Gem	3.02	2.25	2.22	2.92	2.60
John Baer	2.48	2.37	2.24	2.22	2.33
Longred	2.14	1.90	1.75	2.71	2.12
Rutgers	2.39	2.09	1.87	2.17	2.13
Improved Wasath Beauty	2.12	1.64	1.92	2.07	1.94

The data in table I show fairly uniform readings between replicates of the same variety. The statistical analysis revealed that the calculated F value for varieties exceeded the required F value at the .05 level of significance. Hence, the varieties, as measured by this method, had significantly different rates of leaf respiration. This would indicate that determinations made by this modified method were much more consistent than were those made when a buffer was used.

One obvious source of error in this method involves wounding. Discs cut from leaves probably do not carry on respiration at the same rate as intact leaves due to wounding. In the case of measuring the rate of respiration of one plant relative to another, as was required for this study, this error does not seem serious.

The author wishes to express his appreciation to Dr. G. Fred Somers and Dr. William C. Kelly of the U. S. Plant, Soil and Nutrition Laboratory, Ithaca, New York, for their many suggestions and aid in the development of this modified method.

DEPARTMENT OF VEGETABLE CROPS
CORNELL UNIVERSITY
ITHACA, NEW YORK

LITERATURE CITED

1. BONNER, JAMES and WILDMAN, S. G. Enzymatic mechanism in the respiration of spinach leaves. Arch. Biochem. **10**: 498. August, 1946.
2. LANGE, N. A. Handbook of Chemistry. Handbook Publishers, Inc. Sandusky, Ohio. 1944.
3. UMBRIET, W. W., BURRIS, R. H., and STAUFFER, J. F. Mannometric Techniques and Related Methods for the Study of Tissue Metabolism. Burgess Publishing Co., Minneapolis, Minnesota. 1945.