PHOTOSYNTHESIS IN RELATION TO STOMATAL FREQUENCY AND DISTRIBUTION

R. O. FREELAND

(WITH ONE FIGURE)

Received May 4, 1948

Introduction

The concept that stomates play an all-important role in controlling the diffusion of carbon dioxide into leaves during photosynthesis seems to be quite generally accepted by teachers and authors of botany texts. In a plant physiology text by MAXIMOV (**6**) may be found the following, "That the carbon dioxide enters the leaf mainly through the stomates may be shown by a simple experiment. If on a certain portion of the leaf the stomata are coated with vaseline, and the leaf is then exposed to light and afterward treated with iodine, the blue color reaction will be observed only in those portions where the stomata remained open." Also in the text by MEYER and ANDERSON (**7**) one finds "Critical experiments have shown, however, that the proportion of this gas (CO_2) entering the leaves by this route (directly through epidermis) is relatively small, and that practically all of the carbon dioxide entering leaves diffuses in through the stomates." Similar statements may be found in many other botanical books.

It seems that this concept stems mainly from the research of Blackman and his contemporaries. BLACKMAN (**1**) in a review of the research regarding this problem concludes that "Under normal conditions, practically the sole pathway for carbon dioxide into or out of the leaf is by the stomata." Further support and impetus were given to this idea by the classical experiments of BROWN and ESCOMBE (**2**) which demonstrated and partially explained the enormous diffusive capacity of small pores and stomates. More recently MASKELL (**5**) working with *Prunus lauro-cerasus* and NUTMAN (**9**) studying *Coffea arabica* have reported that photosynthesis in these plants is directly related to stomatal movement.

On the other hand considerable evidence has appeared in the literature which indicates that the rate of diffusion of carbon dioxide through epidermal cells of leaves may be quite appreciable and should not be minimized or ignored. Blackman mentions that Boussigault and Barthelmy reported data to the effect that the carbon dioxide exchange during "assimilation" was independent of stomatal distribution. MITCHELL (**8**) found that the leaves of tomato and Pelargonium absorbed carbon dioxide and accumulated carbohydrates in appreciable quantities although the stomates appeared to be closed. Furthermore, the amount of carbon dioxide absorbed by the leaves in which the stomates appeared to be closed was approximately equal to the amount absorbed by the same leaves when the stomates were open. HEINICKE and CHILDERS (4) and SCHNEIDER and CHILDERS (10) in their extensive research with apple trees, found many occasions when the rate of photosynthesis was quite high even though the stomates were closed. In this laboratory, many measurements of the apparent photosynthesis from the upper and lower surfaces of leaves of various plants have been made. As often as not the results have shown little or no direct correlation between photosynthesis and stomatal distribution. Some of these data will be presented in this paper.

Experimental procedure

The plants to be included in this report are yellow-green coleus, *Coleus blumei;* avocado, *Persea americana;* poinsettia, *Euphorbia pulcherrima;*

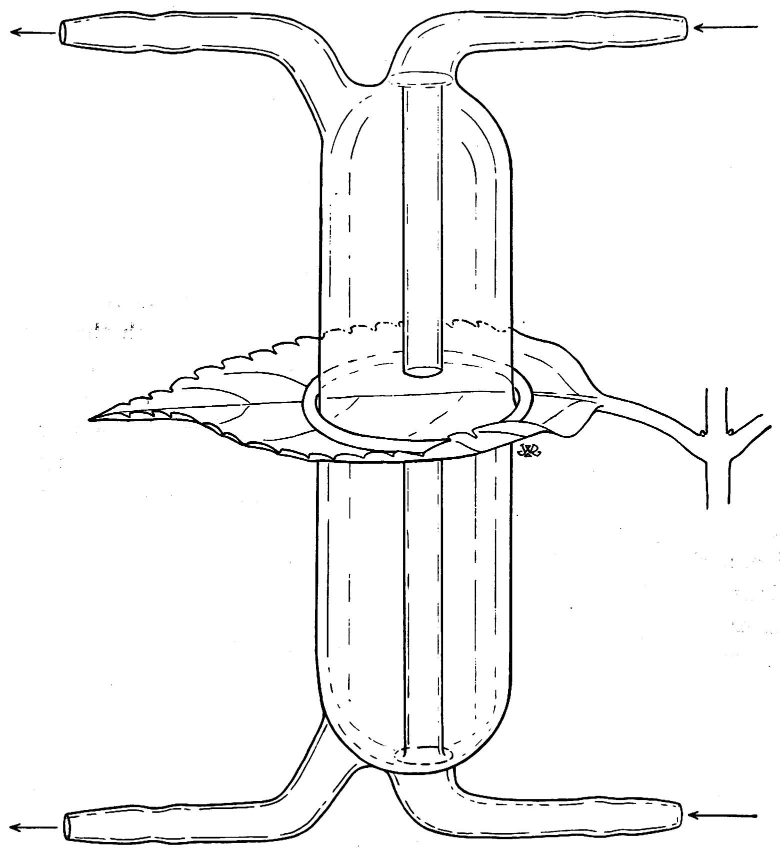

FIG. 1. Glass cups clamped on the top and bottom of a leaf. Description in text.

rubber plant, *Ficus elastica;* begonia, *Begonia sp.?; Rhoeo discolor;* bean, *Phaseolus vulgaris* (var. stringless green pod); tobacco, *Nicotiana tabacum,* and geranium, *Pelargonium zonale.* These plants were selected because of variation in stomatal distribution and thickness of cuticle. Six of the species have stomates in the lower epidermis while the other three have stomates in both the upper and lower surfaces of their leaves as shown in table I. The amount or thickness of cuticle progresses from very little in such plants as coleus and tobacco to much in poinsettia and rubber plant.

Apparent photosynthesis, in terms of milligrams of carbon dioxide used, was measured from the upper and lower surfaces of the leaves. Glass cups of the design shown in figure 1 were clamped opposite each other on several leaves of the experimental plant. These cups each had a diameter of 4 cm. and a depth of 8 cm. A little grafting wax applied around the rim of each cup and warmed slightly at the time the cup was applied to the leaf provided an air-tight seal. Natural air passed through these cups to absorption towers containing 0.1N KOH for carbon dioxide determination after the method of HEINICKE and CHILDERS (**4**). Using flowmeters and constant suction, FREELAND (**3**), the rates of air flow were so regulated as to prevent excessive depletion of the carbon dioxide of the air and extreme temperature rise in the cups. A rate of air flow of about 0.5 cu. ft. per hour was adequate. Experimental plants were placed near a laboratory window in direct sunlight where on hot days they were shaded with one or two layers of cheesecloth. No other attempt was made to control the temperature in the cups or around the plants. Temperatures were recorded several times during the course of each experiment using thermocouples and a Leeds and Northrup thermopotentiometer. Little or no difference in temperature was ever found in the cups attached to the opposite sides of a leaf. The duration of each experiment was four hours.

To test the accuracy of the apparatus and methods, a large number of determinations of the carbon dioxide content of the air in the laboratory have been made. From these measurements it has been found that the milligrams of carbon dioxide per cubic foot of air can be determined with a standard deviation of 0.2.

Results and discussion

The experimental results obtained for the various plants are summarized in table I. The data presented for each plant were obtained by averaging the results from three or more determinations made upon different days.

An examination of the data for those plants which have stomates only in the lower epidermis provides a basis for evaluating the importance of stomates in the absorption of carbon dioxide during photosynthesis. For example, in coleus, avocado, and begonia, carbon dioxide diffused through the upper epidermis in very significant amounts. In no case was the rate of diffusion of carbon dioxide through the upper epidermis alone as great as it was through the lower epidermis plus stomates. However, from the known structure of leaves, it would appear to be a reasonable assumption that carbon dioxide could diffuse through the lower epidermal cells, excluding stomates, at a rate approximately equal to that through the upper epidermis. On this basis the apparent photosynthesis calculated for the total leaf epidermis, excluding stomates, would really be twice the amounts presented for the upper epidermis in table I. Applying this principle to coleus, as an example, the apparent photosynthesis in mg. of CO_2 used per hour per square decimeter would be 6.2 through epidermal cells alone and 7.0 through stomates alone. The data for the other plants in this group, poinsettia, rubber plant, and *Rhoeo discolor*, indicate that they belong in a separate class. In these plants there is little or no diffusion of carbon dioxide through the epidermal cells and the stomates are the primary pathway through which carbon dioxide diffuses during photosynthesis. A partial explanation for the difference between these two groups of plants with respect to carbon dioxide exchange through the astomatous epidermis may be found in the thickness of the epidermis or cuticle. There probably are other factors as indicated by the data for begonia, which has a rather thick cuticle. At any rate the data for these plants, having stomates in the

TABLE I

SUMMARY OF THE DETERMINATIONS OF APPARENT PHOTOSYNTHESIS IN TERMS OF THE AMOUNT OF CARBON DIOXIDE WHICH DIFFUSED INTO THE LEAF THROUGH THE UPPER AND LOWER EPIDERMIS

PLANT	MG. OF CO_2/DM.^{2}/HR.		STOMATES/CM.^{2}	
	UPPER	LOWER	UPPER	LOWER
COLEUS	3.1	10.1	0	11,000
AVOCADO	4.1	5.9	0	15,200
POINSETTIA	0.6	4.1	0	13,000
RUBBER PLANT	*	2.3	0	17,000
BEGONIA	2.2	3.3	0	3,400
RHOEO DISCOLOR	*	3.5	0	1,700
BEAN	*	3.8	1,000	7,000
TOBACCO	3.1	3.6	3,000	3,000
GERANIUM	1.3	3.7	2,300	15,000

* Not significant.

lower surface of the leaves, indicate that the statement of Blackman and others that the stomates are practically the sole pathway for carbon dioxide into and out of leaves under normal conditions is not true in many cases. The data confirm the conclusions of Mitchell, Heinicke, and others to the effect that some plants may carry on a rather high rate of photosynthesis with the stomates closed.

The results for bean, tobacco, and geranium which have stomates in both the upper and lower epidermis of the leaves must be examined from a different point of view. For these plants the amounts of apparent photosynthesis related to stomatal diffusion and epidermal diffusion respectively cannot be separated. In tobacco there is a direct correlation between stomatal frequency and apparent photosynthesis for the upper and lower side of the leaves. As indicated above there is no way of determining how much, if any, of the carbon dioxide exchange was due to epidermal diffusion exclusive of stomates. For geranium the degree of correlation is much less and for bean it is probably nonexistent. Therefore, one must conclude from these data that for those plants having stomates in both the upper and lower epidermis of their leaves the diffusion of carbon dioxide into the leaves during photosynthesis may or may not show a direct correlation with stomatal frequency and distribution. It seems probable that in some plants, at least, some other factor (or factors) other than stomatal frequency and distribution plays a considerable role in determining the pathway of carbon dioxide exchange during photosynthesis.

Summary

1. This is a report of measurements made to determine the relative significance of stomates versus the epidermis, exclusive of stomates, as routes followed by carbon dioxide which diffuses into leaves during photosynthesis.

2. Plants were selected for the experiments to obtain wide variations in stomatal frequency and distribution, and thickness of cuticle.

3. For those plants having stomates only on one side of their leaves, considerable variation occurred with respect to the absorption of carbon dioxide through the epidermal cells during photosynthesis. In all cases the rate of apparent photosynthesis through the stomatal bearing epidermis was greater than through the astomatous side. In some plants the amount of carbon dioxide exchange through epidermal cells alone was very significant sometimes being approximately equal to the amount which diffused through the stomates. In other plants of this group, particularly those with a thick cuticle, little or no apparent photosynthesis could be detected through the epidermis without stomates.

4. The data for those plants having stomates in both the upper and lower epidermis indicate that apparent photosynthesis, in terms of CO_2 absorption, may or may not show a direct correlation with stomatal frequency and distribution.

Department of Botany
Northwestern University
Evanston, Illinois

LITERATURE CITED

1. Blackman, F. F. Experimental researches on vegetable assimilation and respiration. II. On the paths of gaseous exchange between aerial leaves and the atmosphere. Phil. Trans. Roy. Soc. B. **186**: 503–562. 1895.
2. Brown, Horace T., and Escombe, F. Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants. Phil. Trans. Roy. Soc. B. **193**: 223–294. 1900.
3. Freeland, R. O. Automatic electric switch for constant air pressure. Sci. **102**: 231–232. 1945.
4. Heinicke, A. J., and Childers, N. F. The daily rate of photosynthesis, during the growing season of 1935, of a young apple tree of bearing age. Cornell Univ. Agr. Exp. Sta. Bull. **201**. 1937.

5. Maskell, E. J. Experimental researches on vegetable assimilation and respiration. XVIII. The relation between stomatal opening and assimilation—a critical study of assimilation rates and porometer rates in cherry laurel. Proc. Roy. Soc. B. **102**: 488–533. 1928.
6. Maximov, N. A. Plant Physiology. McGraw-Hill Book Co. 1938.
7. Meyer, B. S., and Anderson, D. B. Plant Physiology. D. Van Nostrand Co. 1939.
8. Mitchell, J. W. Effect of atmospheric humidity on rate of carbon fixation by plants. Bot. Gaz. **98**: 87–104. 1936.
9. Nutman, F. G. Studies of the physiology of Coffea arabica. II. Stomatal movements in relation to photosynthesis under natural conditions. Ann. Bot. N. S. **1**: 681–694. 1937.
10. Schneider, G. W., and Childers, N. F. The influence of soil moisture on photosynthesis, respiration, and transpiration of apple leaves. Plant Physiol. **16**: 565–583. 1941.