NOTES.

SPORANGIA OF LITOSIPHON, HARV.—A CORRECTION.

—I regret that I fell into error in stating (Annals of Botany, Vol. VIII, p. 459) that the plurilocular sporangia of *Litosiphon* are unknown. Dr. Bornet has called my attention to the following paragraph (which I ought to have remembered) in the *Etudes Phycologiques* of Thuret and Bornet, p. 15: 'A côté du *Punctaria* se place le *Litosiphon*, Harv., dont la fructification est tout à fait semblable. Dans les deux genres les sporanges uniloculaires sont globuleux, irrégulièrement épars sur la fronde, à la surface de laquelle ils ne font qu'une légère saillie. De part et d'autre les sporanges pluriloculaires sont disposés en groupes, et résultent de la division des cellules de la couche corticale. . . Les cellules corticales se divisent simplement, sans que leur forme soit modifiée, en quatre ou huit logettes dont chacune renferme un gros zoospore.' As the same error is made in Reinke's Atlas deutscher Meeresalgen (II. 3-5, S. 61) its correction is the more necessary.

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EXPERIMENTAL RESEARCHES ON VEGETABLE ASSIMILATION AND RESPIRATION. NO. 1. ON A NEW METHOD FOR INVESTIGATING THE CARBONIC ACID EXCHANGES OF PLANTS¹.

By F. F. BLACKMAN, B.Sc., Demonstrator of Botany in the University of Cambridge.—All the processes hitherto available for the estimation of carbon dioxide in its biological relations are open to serious objections, either on the score of the amount of time involved in their performance or of their inadaptability to the estimation of small quantities of carbon dioxide when slowly evolved.

The present communication describes an apparatus in which, as a result of two years' work, I have succeeded in combining a high degree of chemical accuracy with special adaptability to biological research.

¹ Abstract of a paper read before the Royal Society.

Thus by its aid the evolution of CO_2 , by a single germinating seed or by a small area of a foliage leaf, can be accurately estimated from hour to hour without a break for any desired time, while for the same area of leaf the more active absorption of CO_2 in assimilation can be easily determined for such short periods of time as fifteen minutes, and that at the same time separately for the two surfaces of one and the same leaf area. Further, for the purposes of this assimilation, a current of air containing any desired proportion of CO_2 , however small, can be supplied continuously to the tissue under investigation, while, if desired, estimations of the CO_2 evolved in respiration by some other part can be carried on simultaneously in a separate current of air freed from CO_2 . This is made possible by the apparatus being practically in duplicate; strictly comparative experiments can thus be carried out.

The actual estimation of the CO_2 is accomplished by the well-known method of absorption by baryta solution and titration with hydrochloric acid. The novelty consists in this—that only a very small quantity of baryta solution (under 15 c.c.) is employed in each experiment, and that after the absorption the whole of this is titrated with acid in the tube in which the absorption has taken place. Further, the burettes containing the standard solution are always in air-tight connexion with this absorption chamber, and no air beyond the current under investigation is ever admitted to the chamber, except such as has been carefully freed from CO_2 . The special arrangements for stirring and emptying, by means of this air freed from CO_2 , and stored under pressure, cannot be entered into here.

The two currents of air passing continuously through the apparatus are generated by two aspirators of a special type, which, worked on the principle of Mariotte's bottle, give a practically constant outflow in drops, whatever the level of the water within them, and are adapted to work steadily with small rates of flow (50 to 100 c.c. per hour). These currents enter the apparatus, either through an arrangement for removing the CO_2 when working on respiration or when working on assimilation through one for adding CO_2 . Both these are so constructed that the current never has to bubble through a layer of liquid, and so is supplied to the plant at strictly atmospheric pressure, thus avoiding any risk of drawing gases mechanically out of the part under investigation. The remover of CO_2 is a 'tower' full of beads, over which a stream of strong potash

flows continuously, and through which the air is drawn. The CO_2 generator is constructed on a new principle, and consists of a tall tube containing fragments of marble, through which the air current passes at a constant rate, while very dilute HCl trickles down it at an extremely slow rate, which is made constant and independent of external variations of temperature by special arrangements. Thus a constant amount of CO_2 is being continually generated, and is carried off by the air current. The amount of CO_2 formed can be controlled by the strength of the acid employed. When generating amounts below 2 per cent. of the air current this arrangement works very constantly. From the CO_2 generator or remover, as the case may be, the current of air passes to the receivers, in which the parts of the plant under investigation are situated. These receivers are of various forms, according to the material experimented on, but are all constructed on the cardinal principle of making them as small as possible consistent with the well-being of the part, in order that changes in the composition of the gas shall, as soon as possible, be felt by the current which passes thence through narrow tubes to the absorption chambers. When titrations are being made, and the air current can no longer be allowed to pass through the absorption chambers, it passes through a column of water equal in its resistance to that of the baryta solution in the absorption chamber. This enables the rate of flow to be kept constant between, as well as during, the actual experiments. Numerous other details, such as the special method of refilling the burettes, &c., and above all those small points by which constancy is, as far as possible, attained, many of which have involved weeks of special experiment, cannot be described here.

Simplification of technique by complication of apparatus has been the guiding principle, and the result is that, although the whole consists of at least eight separate pieces of apparatus, many being further in duplicate, and all connected together by a plexus of tubes, yet the working is so automatically arranged that the operator, beyond reading the burettes and occasionally working a finger bellows, has nothing to do but turn stopcocks.

If only one series of estimations is being made, these can be kept absolutely consecutive, the current being led through one of the absorption chambers, while the solution in the other one is being titrated and renewed, and so on alternately. When two series of comparative estimations are being made at once, a small interval

must be allowed after each double estimation, during which the titrations are performed; the currents of air in connexion with the plants then pass through bye-paths, still at their previous rate. This interval, in which a double titration, emptying and refilling of the absorption chambers, is accomplished can be reduced to ten minutes.

Delicacy of estimation sufficient for present work is obtained by the use of half-decinormal, $N/20$, standard solutions. Phenolphthalein is used as indicator, and specially delicate end-reactions can be obtained, since atmospheric CO_2 is excluded, and moreover the burettes containing *both* the solutions can be drawn upon.

The burettes, narrow and graduated in $1/10$ c.c., are read to $1/100$ c.c., with a simple arrangement for avoiding parallax. All other usual precautions are taken, and series of control titrations, with an error of observation of not more than 0.1 per cent., are often obtained. This corresponds to $1/200$ c.c. CO_2 .

In experiments of short duration, $1/50$ c.c. CO_2 is found to be sufficient for a trustworthy estimation from which definite conclusions may be drawn.

The communication immediately following the present one, illustrates the applicability of this apparatus to the investigation of minute quantities of carbon dioxide.

EXPERIMENTAL RESEARCHES ON VEGETABLE ASSIMILATION AND RESPIRATION. NO. 2. ON THE PATHS OF GASEOUS EXCHANGE BETWEEN AËRIAL LEAVES AND THE ATMOSPHERE¹. By F. F. BLACKMAN, B.Sc., Demonstrator of Botany in the University of Cambridge.—On the question of the path by which carbonic acid passes out of the leaf in respiration and into it in assimilation, whether this takes place by the stomatal openings or through the continuous surface of the cuticle, all possible extreme and intermediate views have been expressed in recent textbooks of botany. On account of the smallness of the quantities of gas involved practically no attempt has hitherto been made to determine this question by direct estimation. The existing experimental evidence is all of an indirect nature, and tends rather to support the view that the exchange is a cuticular phenomenon.

An ingenious synthesis of Graham's observations on the com-

¹ Abstract of a paper read before the Royal Society.

Blackman, Frederick Frost. 1895. "Experimental researches on vegetable assimilation and respiration. No. 1. On a new method for investigating the carbonic acid exchanges of plants." *Annals of botany* 9, 161–164.

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