The Stomatograph.

By W. Lawrence Balls, M.A., Fellow of St. John's College, Cambridge, Membre de l'Institut Egyptien, Botanist to the Egyptian Government Department of Agriculture.

(Communicated by Francis Darwin, F.R.S. Received December 22, 1911,—Read February 22, 1912.)

Introduction.

The appliance here described was the result of an attempt to devise a self-recording modification of Mr. Francis Darwin's "Porometer."*

This modification was designated the "Stomatograph," since it automatically records the changes which take place in the mean stomatal aperture of a given leaf, by measuring the velocity at which air under constant pressure can be passed through the tissues. Certain special mechanical difficulties had to be overcome in order to employ it for researches on stomatal movement in the open field, with particular regard to the cotton plant in Egypt.

The following article gives a brief account of the evolution of the appliance; a description of a "stomatograph" as made by the writer, with specimens of records actually obtained from it; a short discussion of the sources of error, indicating the modifications which I have suggested with the aim of making it into a precise and sensitive air-pump.

§ 1. *Development of the Appliance.*

The alarming deterioration of the Egyptian cotton crop, produced mainly† by unsuitable soil-water conditions, made it imperative to obtain some precise knowledge of the relationship of the plant to water. The stomata being of pre-eminent importance‡ in this respect, an investigation of their behaviour in the field crop appeared to be the first line of attack. Such an investigation was beyond the powers of our laboratory§ unless self-recording apparatus could be charged with the task. There was no existing appliance for the purpose, except the indirect method by means of leaf temperature,|| and this was quite unsuitable for field work, owing to the much greater

* Darwin, F., and Pertz, D. F. M., 'Roy. Soc. Proc.,' 1911.

† 'Report of Egyptian Government Cotton Commission,' 1910.

‡ See, however, Lloyd, F. E., 'Carnegie Inst.,' 1908.

§ Department of Agriculture, P.W.M., Cairo.

|| Darwin, F., 'Bot. Gaz.,' 1904.

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character.* www.jstor.org

magnitude of the temperature-changes produced by other causes,* such even as clouds by night.

The horn hygroscope and the cobalt-paper method were incapable of adaptation to automatic performance, but just as it seemed that there could be no alternative to personal observation, Mr. Francis Darwin showed the writer his Porometer. This simple appliance solved the difficulty.

The writer's first recording porometer consisted of a constant pressure aspirator attached to the porometer chamber ; the out-flowing water from this was collected in a syphon-bucket, supported on a spring and carrying a stylus. The slow descent of the bucket, followed by its sudden rise when emptied by the syphon, marked on a clock drum the time elapsing during the passage of a certain volume of air. Apart from some irregularity in the discharge of the syphon, this appliance was useless for field-work, since the aspirator acted as an air-thermometer, and the discharge of water was consequently irregular, even though the porosity of the inlet were constant. Certain data were obtained with it in the laboratory, and during limited periods of time in the field, which indicated the details to be considered.

A suggestion made by Mr. F. Hughes† led at this stage to the trial of a method in which a gas-holder was continually being charged with air-bubbles from a small water pump. The altitude of the gas-holder varied with the aperture of the exit, viz., the stomata, and an integrated pressure-graph was thus obtained. This method was not developed further, owing to the satisfactory performances of the appliance next described, which was more compact and portable.

§ 2. *The Stomatograph.*

Retaining the principle of the porometer, with modifications in the method of attaching the chamber to the leaf, an electrically-driven air-pump was constructed, which forced a fixed volume of air at constant pressure through the leaf at each stroke, and recorded the time taken in so doing. It was then easy to record the changes which were taking place in the porosity of the leaf which sealed the exit from the pump.

By arranging the pressure and pump capacity suitably, the stroke of the pump was made of sufficient frequency to prevent any notable error being caused by variations in temperature or barometric pressure during the occupation of the pump by any one charge of air.

The cotton plant possesses from 200 to 300 stomata on its lower leaf-surface, and about 100 on the upper, to each square millimetre. The aperture of these stomata, which are of moderate dimensions, varies from complete closure to widest distension during the twenty-four hours. Consequently, the flow of air from one side of the leaf to the other is extremely facile at certain times, and only a very low pressure is required. It was found that a pump discharging 5 c.c. of air at each stroke, under a pressure of 0·5 mm.

* Balls, W. Lawrence, 'Cairo Sci. Journ.,' 1911.

† Chemist to the Department of Agriculture, Cairo.

of mercury, through a circular area of leaf tissue of 5 mm. radius, was most convenient to employ; with these arbitrary constants, the time occupied in one stroke of the pump varied between extremes of 5 seconds and 50 minutes, according to the condition of the leaf. The pump had to show a maximum variation of not more than 5 per cent. in its capacity per stroke, and in the pressure exerted during the stroke. It had further to be constructed in such a manner as to be thoroughly protected, together with its recording drum, from wind, dust, and insects, when left in the midst of a field of cotton plants. Lastly, its dimensions had to be such that it should be easily portable, and should not damage nor interfere with the surrounding plants. All these requirements have been met by the appliance here figured and described, which is, moreover, very easy to use.

The Stomatograph, as set to fulfil the special purpose for which it was designed, consists of two parts; firstly, a small portable case containing the pump, its operating battery, and a relay; secondly, an electro-magnetic marker, writing on a chronograph, operated over a telegraph-wire by the relay, from a separate battery.

(a) *The Pump* (fig. 1A).—Since the low pressures required made the use of metal faces impracticable, the air was compressed by means of a gas-holder (*G*) floating in a liquid (*L*). This gasholder was suspended by a flexible cord, or metal strip, from the channelled quadrant arm of a short-beam balance (*B*), and counterpoised on the opposite side from a similar quadrant by a soft iron rod (*C*). The pressure was then regulated by adding weights to the gas-holder.

The exit-tube (*Ex*) from the top of the gas-holder was made of fine rubber of 1 mm. bore, fixed at a distance of a few centimetres to the wall of the enclosing box. Thus arranged, it interfered but slightly with the regularity of the gas-holder's movements, though a central vertical tube with three-point guides would be preferable.

The inlet valve which admitted air on the up-stroke of the gas-holder and at the same time defined its maximum content of air, consisted simply of two or three holes (*V*, *V*) in its wall, near the foot. When these holes were brought above the surface of the liquid in the beaker, the air rushed in suddenly, and the consequent sudden diminution in weight was utilised to knock off the electric contact mentioned below.*

To prevent the gas-holder from being jerked upwards too suddenly by the motive power, when taking in a fresh charge of air, a baffle-plate (*Bp*) was

* The insertion of a reversible electromagnetic valve on the exit would allow either positive or negative pressures to be employed, would define the pump-capacity with precision, and would maintain a constant pressure.

fitted to its lower end, which also served to carry the weights which determined the pressure.

Thus arranged, the gas-holder, having been freshly charged with air, and having settled down to temporary equilibrium, sank into the beaker more or

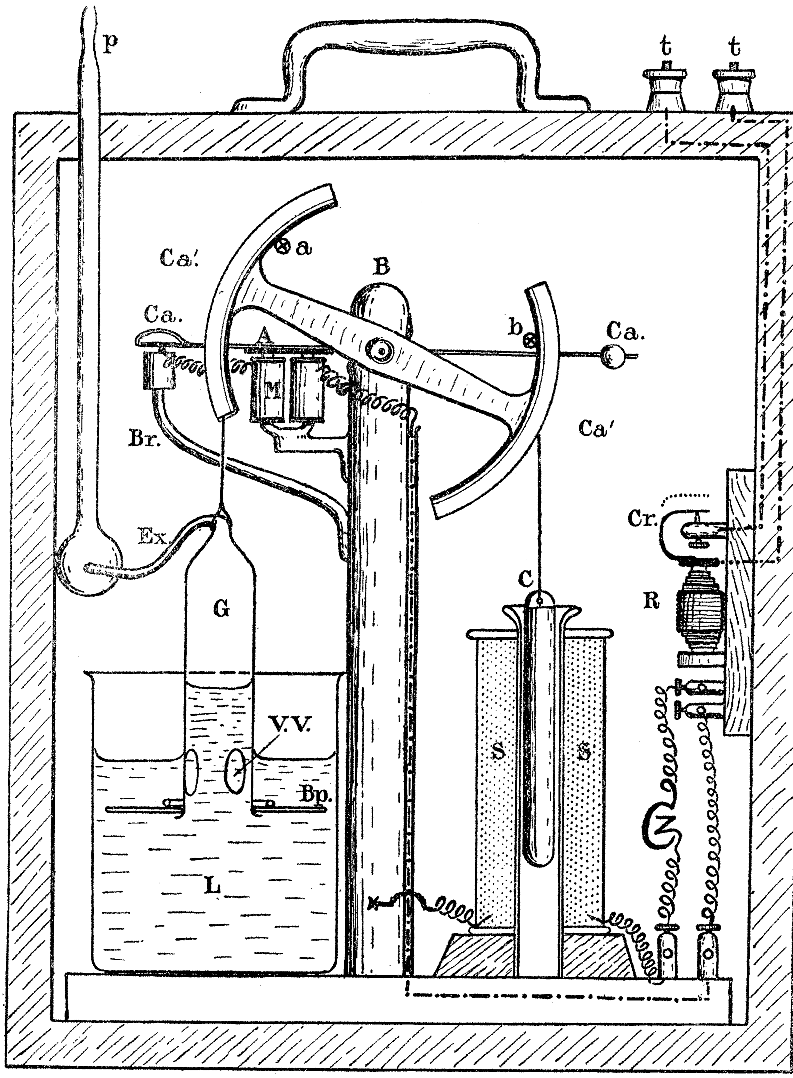


Fig. 1a.

less rapidly according to the aperture of its exit. Having descended to a certain point, with no friction to overcome excepting that of the pin-bearing of the balance, it made an electric contact, which sent a current through a solenoid (S, S) surrounding the iron rod previously mentioned.

This rod was then drawn down into the solenoid, raising the gas-holder rapidly until a fresh charge of air had been taken in, when the contact was broken. The figure shows the position of the pump at a fraction of a second before the admission of a fresh charge, while the solenoid circuit is still intact.

The use of a solenoid and core in this way has several advantages, the chief of which is that its lifting power is greatest when most required, namely, at the stage shown in the figure. The only other difficulty then left was to provide a prolonged contact, which should be broken when the fresh charge had entered the gas-holder, and not until then. This was effected by a separate light swinging arm (*Ca*) behind the main balance, but on the same pivot, shown also in plan in fig. 1B.

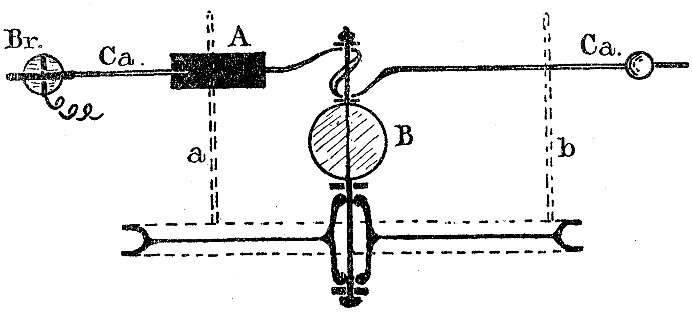


FIG. 1B.

This arm was counterpoised by a sliding weight so as to rest normally at an inclination of 30° to the horizontal (*Ca′*, *Ca′*), the elevated end being that which bore a platinum wire contact. As the gas-holder side of the balance descended, a projecting rod (*a*) which it bore, pressed upon this elevated end, and carried it down also, until the platinum wire came into contact with the platinum-tipped surface of a fixed bracket (*Br*) projecting from the side of the balance-pillar but insulated from the latter. This contact completed the circuit through the solenoid and caused the gas-holder to begin to rise; in order to prevent the contact beam from swinging up immediately from the bracket, and so breaking the contact, a small electro-magnet (*M*) was placed under it, near its pivot, and included in the same circuit; this magnet was wound so as to be only sufficiently powerful to keep the contact arm in contact, by means of a ferro-type armature (*A*).

Contact was thus held until the new charge of air bubbled into the gas-holder, when the sudden diminution in weight of the latter allowed the core to jump further into the solenoid. At this moment a similar projecting rod (*b*) on the core side of the balance-beam engaged with the other end of the contact arm and knocked it off from the holding-down magnet.

The contact being thus broken, the arm swung up into equilibrium, the gas-holder dropped back to temporary equilibrium in the beaker, and a fresh stroke of the pump commenced. A slight alteration in pressure is involved by the necessity for depressing the contact arm, but this is reduced to a negligible amount by making the contact arm as light as possible.

Lastly, the connections were arranged as follows: from the battery to the solenoid, then to the metal balance-post, and through its pivot down the contact-arm to its platinum tip. When the circuit had been completed as described above, the current passed into the corresponding platinum wire, which it met at right angles on the insulated end of the bracket, and from there passed by insulated wires into the holding-down magnet, down the side of the balance pillar to the relay (*R*) and back to the battery.

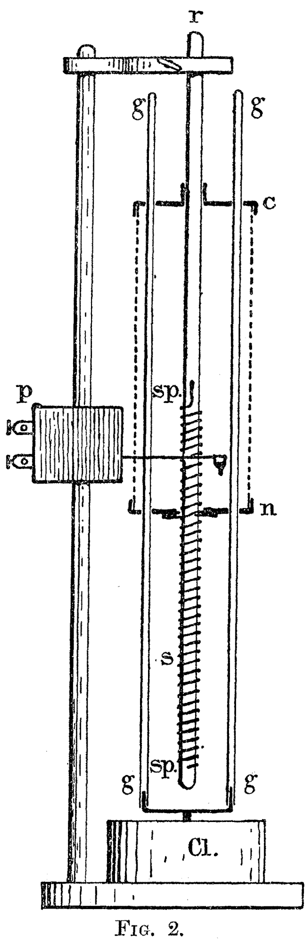


Fig. 2.

(b) *The Relay.*—This was simply an electro-magnet, wound with thick wire, and attracting an armature against a spring. When the circuit was closed, and the armature drawn down to the magnet, a contact (*Cr.*) was made by the armature, which closed the separate circuit of the telegraph recorder. When short lengths of telegraph wire are employed, as when working indoors, the relay can be omitted.

(c) *The Battery.*—This consisted of three "W.-O." medium cells, which worked the appliance almost continuously for over two months.

(d) *The Case.*—The box containing pump, relay, and battery, was 30 cm. high, and 20 cm. in width and depth. It was provided with a dust-proof door in front, a handle for transport, and bore on its top two binding-screws (*t, t*) from the relay, for the telegraph wire, together with a glass nozzle (*p*) leading from the exit tube of the pump. When placed on the ground beneath the plant to be examined, care was taken to level it approximately, so as to avoid friction of the core on the glass solenoid-lining.

(e) *The Recording Appliances* (*Fig.* 2).—These were placed in the laboratory

50 metres away, and connected with the binding-screws from the relay by double flexible wiring.

The Marker.—This (*p*) was simply a single-beat electric bell, bearing a pen in place of the hammer, and so marking a vertical dash across its trail on the drum whenever the circuit was closed and broken.

The Battery.—A single " W.-O." cell was amply powerful for this circuit.

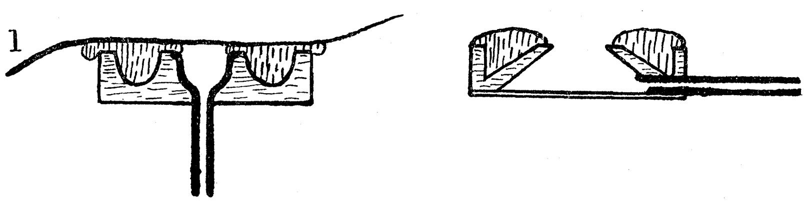
The Drum.—The drum was required to differentiate between marks made at intervals of only 5 seconds, and to record for 30 hours continuously. A vertical chronograph was therefore adopted, descending on a screw, with one rotation of 15 cm. circumference in every hour.

A brief note on its construction, which was remarkably cheap, may perhaps be included. The proper screw was replaced by a spiral (*sp*) of stout brass wire, extended to the requisite pitch, and fitted closely over a glass rod (*r*). The nut (*n*) consisted of three needle-points, chocked to their correct relative heights round a hole in the centre of a piece of tin, which clipped the lower end of the drum ; their points bore on the central glass rod, and their lower sides on the brass wire. With the screw-pitch and weight of drum employed, the clock (*Cl*) was more nearly a brake than a motor.

The hour spindle of the clock was fitted with a double "L" piece, over the upturned ends of which were dropped the lower ends of two vertical glass tubes (*g*, *g*). These passed upwards, parallel with the screw-axis and inside the drum, through two guide holes in the tin nut, and then through two more in the top cover (*c*) of the drum, which was thus free to move vertically down these two driving rods, though constrained to a spiral motion by the screw.

A retort-stand served to hold the axial screw, and a cheap American clock, placed centrally beneath it, drove the drum. The timing was corrected by marking the time at the beginning and end of a record, and cutting the paper off the drum along the hour-line.

(f) *The Chamber.*—Some difficulty has been experienced by workers with the porometer in the cementing of the leaf to the chamber. A method devised by the writer appears to be an improvement, since a tight joint can be made or broken in a few seconds, and may be maintained for several days without direct injury to the leaf. The flange of the chamber (fig. 3A) contains a deep groove, concentric with the chamber itself, this



A Fig. 3. B

groove being filled to overflowing with paraffin of some melting-point suitable to the particular circumstances, from 50° C. to 30° C. The wax

is slightly softened by the heat of the hand, and the leaf (l) merely pressed upon it. When the leaf is to be exposed to strong sunlight, a white ring of cardboard is centred over that portion in contact with the wax, at a distance of a millimetre or two, by a three-point support. The chamber employed to obtain the records here reproduced is shown in fig. 3A. On the suggestion of Mr. Francis Darwin this has been modified to fig. 3B, in which the back is made of glass, and so provides a nearly normal illumination to the lower side of the leaf.

When a leaf is to be left for many hours in the open air on such a chamber, it is well to bind it lightly with wool in order to prevent the wind from stripping it off, and so wasting battery power by leaving a free exit from the gas-holder, besides interrupting the record.

§ 3. *Records of Stomatal Movement.*

The appliance here described nominally records the velocity with which air escapes through the stomata. The square root of this velocity (when below a certain value) appears to represent more closely the actual stomatal aperture.* Discussion of the difficult physical aspects of the matter is beyond the purpose or the power of the present writer, but attention may be drawn to one systematic error in the appliance.

For low velocities, and until some such upper limit as a "fifteen-second stroke" with the constants of pressure, capacity, and area given above, an increase in the frequency of the stroke is probably directly proportional to increases in mean stomatal aperture; beyond some such upper limit, however, the friction of the out-flowing air along the exit-tube and skin-friction of the liquid on the gas-holder become noticeable, so that when there is no leaf on the chamber the strokes still take about five seconds each, which is the minimum time for leaves with fully-opened stomata. The correction for this would have to be worked out empirically for each appliance.

Neglecting this for the present, the stomatograph can be used for almost any kind of stomatal investigation, either in the laboratory or in the field. Comparison of various leaves on the same plant can be effected rapidly, by substituting one leaf after another on the chamber, as soon as some five strokes of the pump have been recorded. One particular advantage of the original porometer is that its results show the mean condition of many hundreds of stomata in the experimental area; with the stomatograph this advantage can be extended to obtaining the mean of many leaves on the same plant, by employing a number of chambers, all of which

* Darwin and Pertz, *loc. cit.*

are connected to the same pump. Such records might be of interest to students of ecology.

The chief interest attaches, however, to continuous field records, since these are unique, excepting for microscopic examinations made by Lloyd.* Fig. 4 shows the general nature of stomatal behaviour on a single adult leaf of

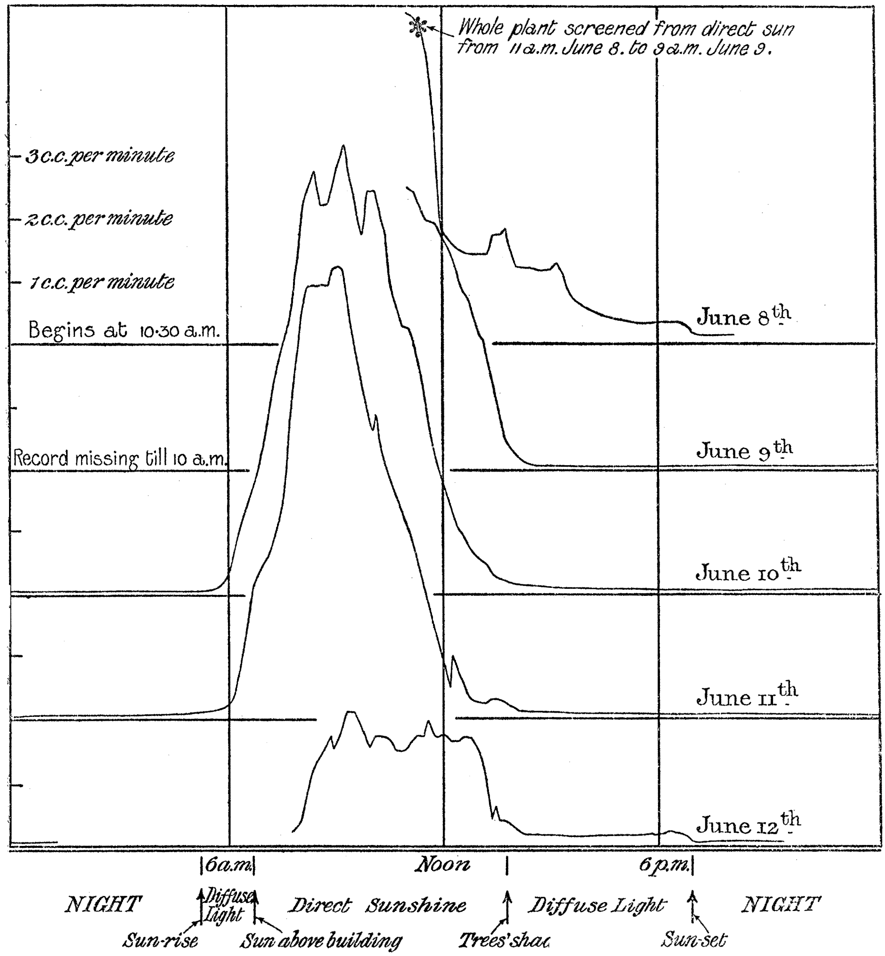


Fig. 4.

Egyptian cotton, growing under field conditions excepting for the presence of the chamber. The behaviour of *Helianthus* in Egypt appears to be closely similar. The curve gives the number of cubic centimetres of air passing through the leaf per minute in each ten-minute period.

The circumstances under which this record was taken were roughly as follows: Sun temperature with black bulb *in vacuo*, 65° C. Maximum shade temperature

* *Loc. cit.*

at 2 P.M., 35° C. Minimum night temperature at 5 A.M., 22° C. Humidity, varying, from 20 per cent. of saturation by day to 100 per cent. by night. The soil had been irrigated seven days before the record begins. Sunshine was direct and continuous, between the limits indicated on the graph, during all five days.

The leaf showed slight signs of injury on the last day, in the portion which touched the wax, but this slight browning had disappeared within a week after its removal from the chamber. The record for the fifth day is plainly abnormal.

The general nature of these and of other similar records is as follows :—At sunrise the stomata open slightly and continue to do so until the direct sun strikes them. They then increase their aperture very rapidly to a maximum at about 9 A.M. After remaining wide open for a longer or shorter time, which appears to depend on the development of the root-system and on the humidity of the air and soil—provided, of course, that sunshine is continuous—they begin to close more and more quickly till their aperture is less than it was before the direct sun reached them. The explanation of this closure seems to be provided by an hypothesis of "water-starvation"; the root-absorption being insufficient to cope with the heavy loss by transpiration, the latter is reduced in consequence.

The closure continues until, on some days, the stomata are practically shut by noon. Preliminary investigations indicate that, in consequence of this, photo-synthesis only takes place during the early part of the day, and that the plant is in a quiescent condition during the afternoon—neither growing* nor feeding, but merely waiting for release from the tyranny of the sun.

In the particular site where these records were taken, the sun passes behind trees at 1.30 P.M., when the stomata close completely. A portion of this effect might be due to the construction of the chamber (3A), and will have to be re-examined next year with the new chamber (3B).

The effect of shading the whole plant from direct sun, though not from bright diffuse light, and so reducing the water loss, is shown in fig. 4 (June 8). The shade was applied before rapid closure had begun, and provoked immediate partial closure, till the aperture was about the same as during pre-sunshine period of the early morning. This aperture was maintained well into the afternoon, and was followed by slow closure until sunset, when the final closure took place almost suddenly.

The stomatograph has thus begun immediately to justify the purpose of its invention by throwing a flood of light on the important problems of water-relationships of the cotton-plant under the very severe conditions of the Egyptian summer. The investigations suggested by considerations of such records as those reproduced here are almost endless, and they have in

* Balls, W. Lawrence, 'Cairo Sci. Journ.,' September, 1911.

addition the most direct relation to irrigation matters, which are of great economic importance for Egypt.

§ 4. *Summary.*

The stomatograph is an air-pump measuring and recording the quantity of air which it forces through a leaf on the chamber of Mr. Francis Darwin's porometer, and so recording any changes in stomatal aperture.

It is extremely easy to use, and having been so constructed as to be independent of weather changes, is specially adapted to obtaining records from plants under normal out-door conditions of environment, such as are required in agricultural and ecological studies.

Records obtained in this way with the Egyptian cotton crop show that the instrument may be of great utility in the study of purely economic matters connected with irrigation.

Apart from its use for the special purpose, the form of pump adopted seems likely to be of use in replacing aspirators for many kinds of scientific research.

My thanks are due to Mr. Francis Darwin for the interest which he has taken in this modification of his porometer, and to Mr. Horace Darwin for suggestions as to its improvement. My colleague, Mr. Frank Hughes, is responsible for many ideas, and for assistance in making the various working models.

DESCRIPTION OF THE FIGURES.

General: The drawings of apparatus are all semi-diagrammatic, sectional and superficial views being combined in the same figure. Figure 4 is plotted from the chronograph records.

FIG. 1A.—Interior of case containing pump, relay, and battery. Gas-holder and solenoid in section, remainder in diagrammatic view. Insulated wire represented by dotted lines, or by spirals. Battery (*CZ*) is actually stored at the back of the case, behind the pump.

- *p.* Pump nozzle for connection to chamber.
- *t, t.* Telegraph connections to recording apparatus.
- *a.* End of rod which depresses the contact arm, *Ca.*
- *b.* End of similar rod which knocks off the contact arm.
- *B.* Pillar of balance, bearing quadrant beam in front, and contact arm behind on same pivot.
- *Ca.* Contact arm, during contact.
- *Ca′, Ca′.* Normal position of contact arm.
- *A.* Light armature on contact arm.
- *M.* Holding down magnet, maintaining contact of *Ca* by means of *A.*
- *Br.* Bracket supporting insulated fixed contact at tip.

Cr. Contact closing relay circuit. (Dotted line represents position of same when relay circuit is open.)
R. Relay magnet.
Ex. Rubber exit tube from gas-holder to nozzle *p*, passing through a condensed-water trap-bulb.
G. Gasholder, with—
V, *V.* Inlet valve holes.
L. Liquid (paraffin, water, or mercury) in which gas-holder floats.
Bp. Baffle plate at foot of gas-holder, carrying ring-weights.
C. Soft iron core.
S. Solenoid.

FIG. 1B.—Plan of upper portion of pump to show arrangement of contact arm. Lettering as in Fig. 1A.

FIG. 2.—Semi-sectional view of chronograph. Drum represented by dotted lines.
P. Electro-magnetic marker, with pen.
r. Glass rod, bearing brass spiral.
sp. Brass wire spiral.
g, *g.* Driving rods, rotated by "L" pieces on hour spindle of clock.
Cl. Clock.
c. Upper detachable end of drum cylinder.
n. Lower ditto, with three-point nut.

FIG. 3.—New pattern of porometer chamber, connected in use to nozzle *p* of pump.

3A. Chamber made from cork and glass, with grooved flange containing paraffin wax, as used in obtaining fig. 4.

3B. Improved chamber, also with the waxed flange, but transparent at back.

FIG. 4.—Record for five consecutive days, converted to a curve, showing changes in stomatal aperture as indicated by the variation in volume of air under constant pressure which can be forced through the leaf-tissue. Plotted in terms of c.c. per minute, through an area of leaf of 80 sq. mm., under a pressure of 0·5 mm. of mercury. Classified in 10-minute intervals.

Excepting on the first and fifth days the results represent the normal behaviour of the Egyptian cotton plant (*Gossypium peruvianum?*) under conditions which are nearly identical with those of the field crop, excepting for some shading from direct sun in the early morning and after 2.30 P.M., on account of adjacent trees and buildings.

The leaf employed had just reached its full dimensions, on a plant with 10 leaves, growing in the garden attached to the laboratory of the Khedivial Agricultural Society, temporarily occupied by the Egyptian Government Department of Agriculture.