

On a Method of Studying Transpiration.

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Transpiration is, perhaps, more directly under the rule of external physical conditions than any other physiological function. Yet proofs of this conclusion are wanting, at any rate in regard to the transpiration of leaves.

Thus, as far as I know, we have no complete experimental determination of the relation between the loss of water-vapour from leaves and the relative humidity of the air. Nor again have we any complete evidence as to the effect on transpiration of variation in the illumination to which the leaf is subjected.

These lacunæ in our knowledge depend on the fact that in leaves, transpiration is largely dependent on the behaviour of the stomata, being relatively large when they are wide open, and diminishing as they close. And since the aperture of the stomata depends on external condition, it is clear that no distinction can be made between the diminution in evaporation resulting from increased relative humidity of the air, and the diminution in the transpiration-rate due to stomatal closure. In fact it is impossible to learn anything accurately concerning transpiration until the varying aperture of the stoma is excluded from the problem. This might possibly be done by estimating the transpiration of leaves of aquatic plants in which the stomata vary but slightly in aperture; but the experiment would not be easily made in a trustworthy form.

The method I have actually employed is to block the stomata with a fatty substance,* and then to place the intercellular spaces of the leaves in communication with the external air by means of incisions.

Most of the experiments were made on laurel (*P. laurocerasus*). The lower surface of the leaf was smeared with melted cocoa-butter or with vaseline rubbed in with the finger, and four to six cuts were made with scissors or a razor, reaching from the periphery to the midrib between the large veins. Other plans were also tried, *e.g.* pricking the leaf with a needle or making numerous small incisions by stabbing with a scalpel.†

The method is similar to that of Stahl,‡ who showed that greased leaves pierced with holes assimilate and form starch in the tissues surrounding

* Cocoa butter in the earlier experiments, vaseline in all the later ones.

† The method was described in a paper read at Section K. of the Sheffield meeting of the British Association, 1910 (title alone published).

‡ 'Bot. Zeit.,' 1894.

the wounds, whereas greased leaves without such artificial stomata formed none or hardly any.

It may be objected that the stomata are not completely or uniformly closed by greasing, that some remain open, and that it is to the opening and closing of these in light and darkness that the rise and fall of the transpiration of the incised leaves is due. I find it difficult to believe that the general objection here discussed is sound, because experiments with the porometer* have convinced me that even a careless application of vaseline absolutely closes the stomata. It may be urged that in Experiment LO 2 (p. 271) the effect of grease is only to reduce transpiration from 379 to 10·9, *i.e.*, from 100 to 2·88. It must be remembered, however, that fatty substances are not impermeable to water, and that at any rate part of the 2·88 per cent. must be due to cuticular transpiration.

Another source of error should be guarded against. Mr. Blackman† has shown that a process of healing occurs in wounded laurel leaves. The beginning of the process is, however, marked by the edges of the wounds becoming translucent. As soon as this occurs the specimen should be discarded or fresh incisions made.

The following experiment, LO 2, October 2, 1912, gives an idea of the effect of greasing and slitting. It seems clear that the result is comparable (as far as magnitude is concerned) with normal stomatal transpiration :—

Experiment LO 2. October 2, 1912. *P. laurocerasus*.

A laurel branch cut under water with 10 leaves (one being small) having a stoma-bearing area of 600 cm.².

Fitted to a potometer (diameter of tube 0·95 mm.). At a north window, where the temperature during the observations varied between 13·6° and 15·2° C. and the relative humidity between 59 and 69 per cent. In the following abbreviated record of the experiment the potometer readings are corrected for differences in relative humidity.

A.M.	Transpiration.
11.10	439‡
27	427
53	379

P.M.

12.18 Finished vaselining leaves on both surfaces.

* See F. Darwin and D. F. M. Pertz, 'Roy. Soc. Proc.,' 1911, B, vol. 84, p. 137, for a description of the porometer.

† F. Blackman and G. Matthæi, 'Annals of Botany,' 1901, vol. 16.

‡ The figure 439 is obtained from the number of seconds (*viz.* 22·8) in which the column of water in the potometer tube travels 1 cm., which means the absorption of

P.M.	Transpiration.
12.20	285
34	64.7
43	Surface of branch vaselined.
59	47.3
4.36	18.1
Oct. 3—	
A.M.	
10.23	Fresh surface cut to branch.
11.21	10.9
32	Four incisions made per leaf, <i>i.e.</i> two on each side of midrib.
36	94.3
P.M.	
12.5	One more slit per side.
7	196
12	One more slit, making four per side
44	255
Oct. 4—	
A.M.	
10.11	Fresh surface cut to branch.
11.5	234

It will be seen that the coating of vaseline on the leaves and surface of the branch does not completely check transpiration. Thus, as above mentioned, on the second day (October 3), when the original negative pressure must have disappeared, transpiration had only been reduced from 379 to 10.9 or from 100 to 2.88. This fact is in the present instance of little importance, as my object is to illustrate the effect of incisions on the transpiration rate.

It is obvious (i) that when the lamina is cut into strips the transpiration rises with great rapidity; (ii) that although in this instance it does not obtain the rate of transpiration observed when the stomata were open, the two are comparable for practical purposes.

In the case of these slit leaves it is of some interest to know the amount of connection between the external air and the intercellular spaces. This was estimated from the observations on the laurel twig (Experiment LO 2) just described. Each leaf had eight incisions (four per side), varying in

0.00708 c.c. The figure 439 is the reciprocal of 22.8 multiplied by 10,000. To convert the number 439 into cubic centimetre it is only necessary to multiply it by 2.55 mm.³ which gives the rate, in this case 1.12 c.c. per hour per 600 cm.² or 18.7 c.c. per square metre of stoma-bearing area.

length from about 25 to 40 mm. The sum of the lengths of the incisions = 2437 mm. The thickness of the leaves was taken as 0.38 mm., and since each incision exposes two leaf-sections to the air, the total area of section exposed by the experiment is

$$2 \times 2437 \text{ mm.} \times 0.38 \text{ mm.} = 1852 \text{ mm.}^2 = 18.52 \text{ cm.}^2.$$

The stoma-bearing area of the 10 leaves, omitting the mid-ribs, was 600 cm.², so that the amount of surface exposed by incision is 18.52 per 600 or 3.09 per cent. Unger* gives for *P. laurocerasus* the intercellular spaces as 21.9, say, 22 per cent.† of the volume of the leaf. Therefore of the transverse section exposed by incision only 22 per cent. is intercellular space. We may therefore say that in a laurel leaf having four incisions on each half of the lamina the transpiratory apertures connecting the intercellular spaces with outer air are $22 \times 3.09/100$ or 0.68 per cent. of the area of the leaf. Since these correspond in function to stomata it is worth while comparing them with actual stomata.

A rough calculation gave the area of the laurel stomata as 0.88 per cent. of that of the leaf. The transpiring area of the slit leaves is, therefore, much the same as that of the stomatal apertures under ordinary conditions.

The Effect of Changes in the Humidity of the Air.

The method of incision has been used in studying the effects, on transpiration, of variations in the relative humidity of the air; and this has led to a rough plan for reducing transpirations at varying humidities to a common standard. The method of producing a damp atmosphere was a simple one. At first the plant was covered with a large bell-jar resting on a ground-glass plate, and so arranged that a current of air, dry or moist, could be drawn through it. But finally I came to the conclusion that a simpler method was preferable, namely, to change the relative humidity by raising or lowering the bell-jar; in this way—assuming that the laboratory air is fairly dry—it is easy to change the relative humidity from 50 per cent. to 95 per cent., which is sufficient for my purpose.‡ The wet and dry

* 'Sitzb. K. Akad. Wien,' 1854, vol. 12, p. 367.

† Microscopic examination of a transverse section led me to estimate the air spaces as roughly 25 per cent.

‡ It is unfortunate that these observations, with the exception of Experiment 8, were not made in darkness or in constant light. The experiments which are most likely to be vitiated by this fault are Nos. 3, 4, and 7. Experiment 4 might be expected to give an especially bad result from the effect of dull light at the end of the experiment. But the diagram, fig. 4, shows rather striking uniformity in the relation between transpiration and humidity of air. In Experiment 3 the diagram is not very satisfactory in any case, but omission of the last two readings (the ones under suspicion) would not alter the

bulb thermometers were in the upper part of the jar, while the branch had leaves in both lower and upper regions. I did not find this to be a serious source of error, and it is one which might be avoided by fitting an apparatus by which the air in the bell jar could be stirred and thoroughly mixed, as indeed was done in some of the later experiments.

The rate of transpiration was estimated by a potometer, not one of the type formerly used by me,* in which an air bubble is timed as it passes rapidly along a narrow capillary tube, but one in which the free end of the water-column is timed with a stop-watch as it passes along a horizontal tube of about a millimetre internal diameter.† It is, in fact, like Kohl's potometer, or that figured in Pfeffer's 'Physiology,' though the method of bringing the column back to zero is not identical with either. I have not thought it necessary to give the actual quantities of water absorbed by the plant per hour, but merely a series of numbers proportional to the rate of absorption.

In all experiments (except No. 8) the plants were placed close to the north windows of the laboratory; the action of the stomata was in all cases excluded by a coating of grease, transpiration taking place only by incisions, as above described.

In the following tables T means temperature, ψ stands for relative humidity:—

Experiment 1.—November 6, 1909. *P. laurocerasus*. Fig. 1. Cut branch in potometer.

Time.	Period.	Rate.	T.	ψ .
			° C.	per cent.
10.13 A.M.	i	36	13·8	74
10.39 "	ii	38	13·6	74
10.49 "	iii	36	13·6	74
	Bell-jar over plant.			
11.16 "	iv	16	14·2	91
11.28 "	v	20	14·7	92
11.37 "	vi	14	15·0	93
11.51 "	vii	17	15·2	94

transpiration curve. Experiment 7, in which the last reading was taken at sunset, gives, nevertheless, a good straight diagonal, as seen in fig. 7. A number of experiments were made (like Experiment 8) in the dark room. I cannot see that they differ as a whole from those illustrated in the present paper.

* F. Darwin and R. Phillips, 'Camb. Phil. Soc.,' 1886, vol. 5; see also F. Darwin and Acton, 'Physiology of Plants,' 1901, 3rd Edit., p. 79.

† In all the later experiments the diameter was either 0·95 mm. or 1·1 mm.

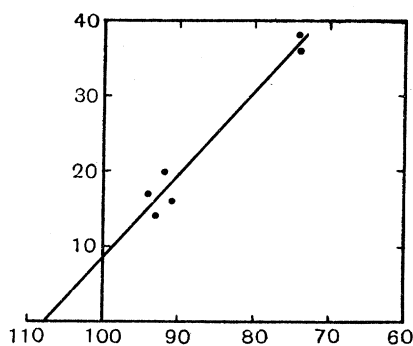


FIG. 1 (Experiment 1).

In the figures the ordinates represent transpiration rates, while the relative humidity (ψ) is given on the horizontal axis. Thus if transpiration varies directly as the relative humidity, the diagram should give a straight diagonal line. The fact that the diagonal does not pass through the intersection of the axes will be discussed later.

Experiment 2.—November 8, 1909. *P. laurocerasus*. Fig. 2.

Time.	Period.	Rate.	T.	ψ .
11.48 A.M.	i	56	° C.	per cent.
12.3 P.M.	ii	56	15.8	53
	Bell-jar over plant.		15.4	57
12.24 "	iii	35	15.1	89
12.35 "	iv	32	15.2	92
12.44 "	v	27	15.4	93
2.7 "	vi	17	15.6	94

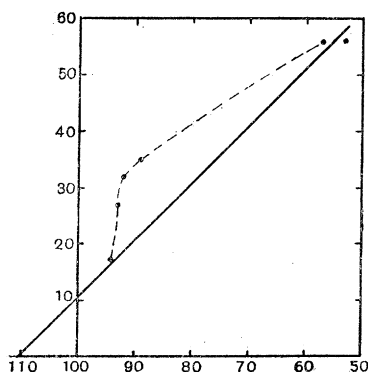


FIG. 2 (Experiment 2).

It will be seen that the dots representing transpiration for various values of ψ are by no means in a straight line. This I take to be "lag," that

is to say, a relatively slow response to the change in the humidity of the air (ψ). When the air is drying instead of becoming damper the "lag" is of the opposite character, as seen in fig. 6.

Experiment 3.—November 15, 1909. *P. laurocerasus*. Fig. 3.

Time.	Period.	Rate.	T.	ψ .
11.30 A.M. }	i	57.0	° C.	per cent.
12.6 P.M. }			15.4	54
12.12 " }			Bell over plant.	
12.19 " }	ii		50.0	15.4
12.44 " }				
2.54 " }	iii	23.5	15.4	83
3.19 " }				
3.28 " }	Water poured on floor of bell.			
3.44 " }	v	13.0	15.5	94
3.56 " }				
3.56 " }	vi	11.0	15.4	94
4.10 " }				

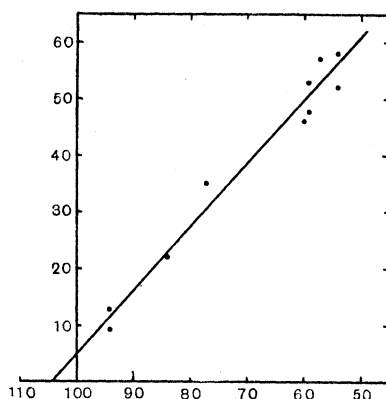


FIG. 3 (Experiment 3).

Experiment 4.—January 1, 1910. *P. laurocerasus*. Fig. 4.

Time.	Period.	Rate.	T.	ψ .
12.35 P.M.	i	42.7	° C.	per cent.
12.51 "	ii	37.9	14.4	53
12.59 "				
2.30 "	iii	33.3	14.2	61
3.12 "	iv	30.7	14.7	68
3.22 "	v	25.8	14.8	72
3.35 "	vi	22.6	14.7	77
3.47 "	vii	19.2	14.7	81
4.4 "	viii	16.1	14.9	89

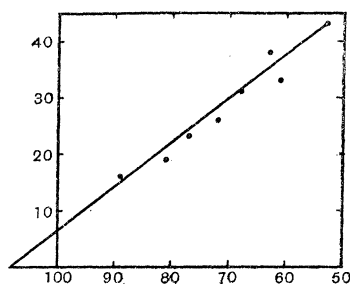


FIG. 4 (Experiment 4).

Experiment 5.—January 3, 1910. *P. laurocerasus*. Fig. 5.

Time.	Period.	Rate.	T.	ψ .
			° C.	per cent.
10.33 A.M.	i	39.4	16.3	61
10.57 „	ii	29.6	15.8	72
11.52 „	iii	28.1	16.0	74
12.19 P.M.	iv	22.3	16.4	78
12.37 „	v	21.3	16.4	82
12.46 „	vi	17.2	16.4	87
1.33 „	vii	16.5	16.0	85
1.40 „	viii	14.3	16.1	89
1.54 „	ix	10.6	16.1	94

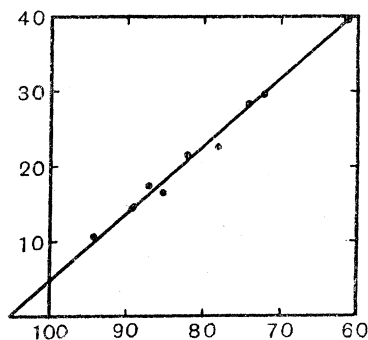


FIG. 5 (Experiment 5).

During the above observations the bell-jar had been gradually lowered, *i.e.* the supports replaced by smaller ones until only a crack, 1 or 2 mm. in height, remained. The bell was now (1.57) raised to 7 mm. and a current of air drawn through. The supports were gradually increased in height and finally (3.20) the bell was removed altogether.

Experiment 6 (= 5 continued).—Fig. 6.

Time.	Period.	Rate.	T.	ψ .
			° C.	per cent.
1.54 P.M.	i	10.6	16.1	94
2.24 "	ii	20.8	16.3	78
2.39 "	iii	24.7	16.3	72
3.6 "	iv	30.3	16.5	69
3.17 "	v	34.6	16.2	67
3.29 "	vi	41.7	17.0	59
3.33 "	vii	43.5	17.0	58

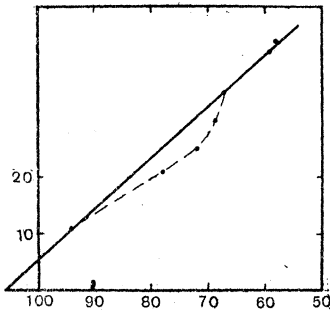


Fig. 6 (Experiment 6).

In Experiment 6 the air is drying instead of becoming damper, and the "lag" is of an opposite character to that in fig. 2.

Experiment 7.—November 15, 1909. *P. laurocerasus*. Fig. 7.

Time.	Period.	Rate.	T.	ψ .
			° C.	per cent.
11.30 A.M.	i	57.0	15.4	54
12.6 P.M.				
12.12 P.M.	Bell on.			
12.19 "	ii	50.0	15.4	60
12.44 "				
2.54 "	iii	23.5	15.4	83
3.19 "				
3.28 "	Water on floor of bell.			
1.44 "	iv	13.0	15.5	94
1.56 "				
3.56 "	v	11.0	15.4	94
4.10 "				
	Bell removed.			
5.14 "	vi	54.0*	15.9	50
5.36 "				

* The value of this observation is doubtful; it is marked with a x in fig. 7 and omitted drawing the diagonal.

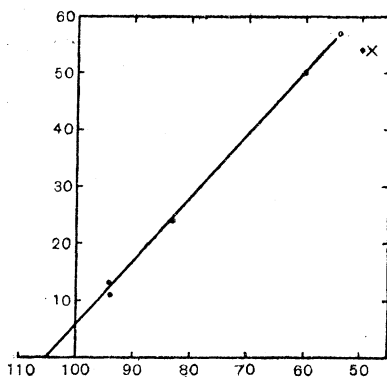


FIG. 7 (Experiment 7).

Experiment 8.—April 22, 1912. *P. laurocerasus*. Fig. 8. Apparatus fitted up in the Dark Room.

Time.	Period.	Rate.	T.	ψ .
10.45 A.M.	i	22.4	° C.	per cent.
to 11.30 "		(average)	17.3	60
11.32 "	Bell jar over plant/supported on blocks 25 mm. high.			
11.56 "	ii	15.1	17.8	74
12.8 "	Blocks reduced to 2 mm.			
12.14 p.m.	iii	12.7	18.0	77
12.24 "	iv	10.7	18.0	84
12.32 "	v	8.0	18.1	88
12.37 "	vi	7.8	18.1	88
12.44 "	vii	6.5	18.1	91
12.54 "	viii	5.3	18.2	93
12.59 "	ix	5.0	18.2	94
2.15 "	x	3.5	18.2	97
2.29 "	xi	3.3	18.3	98

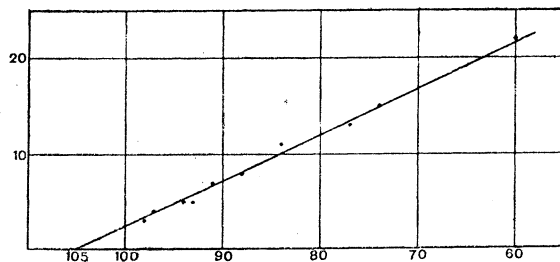


FIG. 8 (Experiment 8).

The general characteristics of the illustrations above given are

(1) The points which represent the transpiration for different degrees of relative humidity are roughly in a straight line—from which it follows that a definite relation of some sort exists between transpiration and relative

humidity. This conclusion, which is a physical necessity, does not seem to have been definitely proved or represented diagrammatically.

In some cases (*e.g.* figs. 2 and 6) the line of dots (*i.e.* the transpiration curve) is not straight—the change in rate of transpiration lags behind the change in ψ —for reasons not yet clear.

(2) The second characteristic of the diagrams is that the diagonal does not pass through the point of intersection of the axes—or, in other words, transpiration is not zero in saturated air. I have not hitherto seen this graphically represented as the result of experiment, although it might have been foretold. The fact that transpiration occurs in saturated air, and that it is due to the production of heat in plant-respiration was first made clear by Sachs*, who proposed that the fact should be utilised as a means of measuring the “Eigenwärme” of plants.† We shall see later that the diagram (fig. 9) may perhaps be applied to the same end. The position of

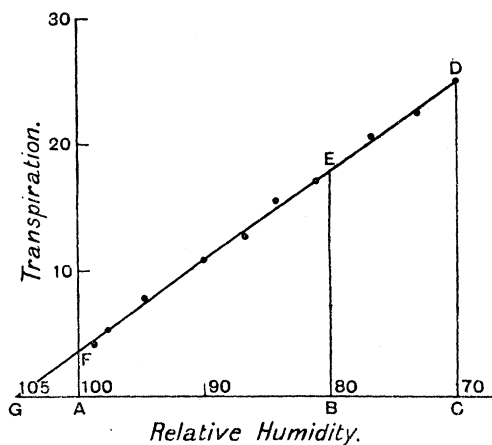


FIG. 9.

the point G varies in different cases. In the earlier experiments, I estimated $AG = 7$, but I now consider 5 a more reasonable average. The construction here given has been used throughout my work for the rough reduction of transpiration-rates to a common degree of relative humidity. Thus, supposing that in fig. 9 the transpiration-rates DC and EB have been obtained under different conditions of illumination, it is clear that we cannot estimate the effect of such conditions until the amounts have been corrected for the differences in relative humidity.

* ‘Sitzb. K. Akad. Wien,’ 1857, vol. 26, p. 326.

† See Sachs, ‘Physiologie Experimentale,’ 1868, p. 249 (the French translation of his book on plant physiology).

Now

$$\frac{DC}{EB} = \frac{CG}{BG} \quad \text{or} \quad DC = \frac{CG}{BG} \times EB. \quad \therefore \quad DC = \frac{105-70}{105-80} \times EB = \frac{35}{25} EB.$$

We will suppose that in an experiment on the effect of illumination we find the transpiration-rate in the light (relative humidity 70 per cent.) to be 120; while the rate in the dark (humidity 80 per cent.) is 75. We must multiply 75 by 35/25. The product 105 is the transpiration in the dark room (humidity 80 per cent.) reduced to humidity 70 per cent., and therefore now comparable with transpiration in the light, *i.e.* 120. Thus

$$\frac{\text{Transpiration in light}}{\text{Transpiration in dark}} = \frac{120}{105} = \frac{114}{100}.$$

Sir Joseph Larmor has been good enough to point out to me that it is possible to get a rough idea of the temperature of the leaf at full saturation, *i.e.* of the leaf temperature which in fig. 9 produces the amount of transpiration (or what may be perhaps called distillation) equal to AF. The oblique line, or curve of transpiration, cuts the horizontal at 105, *i.e.* at 5 per cent. above saturation. The figure shows that, in supersaturated air, *i.e.* 5 per cent. above saturation, transpiration is nil. The hypothetical degree of supersaturation should be a measure of the transpiration AF at the saturation point, and therefore of the internal temperature which can distil off water in saturated air. Assuming* the temperature of the air to be 16° C., the vapour pressure would be 13·51. If we add 5 per cent. to this we get 14·2, which is the vapour pressure corresponding to 16·8°, or 0·8° C. above the temperature of the air. There seems no improbability in leaf-respiration producing, under the conditions of the experiment, a temperature of roughly 1° C. above that of the atmosphere. In my earlier experiments I concluded that the transpiration curve DEFG cut the horizontal at 107. This would have given a temperature 1·1° C. above that of the air, instead of 0·8° C.

It should be noted that the distance AF, *i.e.* the amount of transpiration in saturated air, will depend on the general temperature, since respiration is greatly influenced by temperature. We have some evidence on this point, but the experiment needs careful repetition.

It is remarkable that, as far as I know, the method here used for plotting the relation between transpiration and relative humidity has not been employed. If Le Clerc† had treated his results in this way, he might, perhaps, have obtained a result like mine.

I cannot conclude without expressing my indebtedness to Miss D. F. Pertz for much kind help in the laboratory.

* The figure is a diagram not taken from any one experiment.

† Le Clerc, 'Ann. Sci. Nat.,' 1883, vol. 16.