Apparent Fallacies of Electrical Response in Cotton Plants.

BY

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With a Diagram in the Text.

THE present note describes briefly some results, negative in themselves, but none the less interesting, obtained in an abortive attempt to use the electric reactions of plant tissue, described by Waller,¹ as a general test for healthiness in Egyptian cotton plants.

The significance of these electrical responses or 'blaze currents' is obscure, but they at least differentiate between dead and living tissue, and their intensity might be expected to decline along a gradient as the tissue became less healthy. It seemed, therefore, that they should provide a simple and rapid means of obtaining a rough expression of the progress of the injury done to cotton plants by the root-asphyxiation which takes place when the Nile rises, through the direct and indirect rise of the water-table. Study of the injury to the root itself at least should be facilitated, as samples of roots could be taken up with a soil-borer and tested, whereas it is practically impossible to duplicate the field conditions of a root-system and at the same time to conduct observations upon it.

These expectations have been quite falsified, and though the method holds good as a 'death-test', it does not seem to be a 'vitality-test' in a quantitative sense. Moreover, it failed of its object with regard to the testing of root-samples, because the small roots give most insignificant responses.

¹ A. D. Waller: Signs of Life, and references. London, 1903.

J. Chunder Bose: Electrical Response in Plants. London, 1910.

The writer only made acquaintance with this later work of Mr. J. C. Bose after these notes had been put together in their present form, when he found that most of the matter had been anticipated by Bose on a much wider scale; notably, the reversal of polarity in fatigue, and the dependence of response upon anisotropic structure. These two phenomena seem to the writer to be of supreme importance in the elucidation of electrical responses, and as Mr. Bose's book contains much other matter, the present writer may perhaps be excused for maintaining the restricted scope of this article, without the amplification which should have been made in the light of Bose's researches.

Switchboard with appendages in diagrammatic plan. $\circ$ = Mercury cups $\diamond$ = Bridge switch

Resistance RR Post Office box and cell
 GR Unipivot galvanometer
 r Leads to switch
 r' r² Bridges (r' r' to E¹ E¹, or r² r² to E² E²)

After testing resistance, the bridges are swung round to positions b' b' or b² b²

Inductorium PI Primary coil and cell
 SI Secondary coil
 CC Commutator in primary circuit
 PKI Key breaking primary circuit (and in its fall closing blaze circuit at KB)
 ii Secondary leads to switch, through raised wire arches

Electrodes E Paired U-tubes
 A Tested object
 C Control
 b' b² Leads from electrodes to switch. Also acting as leads for secondary from switch to electrodes

Blaze GB Ayrton-Mather galvanometer
 bb Leads from switch to galvanometer
 KB Key closing blaze circuit at a regular interval after break at PKI

Note. After testing E¹ E¹, lift bridges from b' b' and close PKI before inserting bridges in b² b² for the testing of E² E². The make-shock is thus evaded

Bearing in mind that the experiments were undertaken simply in order to develop a Testing Method rather than as a real investigation of these phenomena in themselves, the following data may possibly be of use for the reference of other plant physiologists who also have been sceptical as to these apparently lawless outbreaks of energy.

Apparatus.—The home-made apparatus employed was based on that described by Waller, with modifications. Its only notable feature was a mercury switchboard, arranged in such a way that two pairs of electrodes—the tested object and a control—were *always handled alternately* (Fig.). By bridging over from cup to cup, it was possible to obtain in a very few minutes the resistance of the tissues and a series of antidrome and homodrome responses from both alternately, with occasional observations of the circuit zeros, of the galvanometer zero, and of the deflexion of either circuit by a standard voltage.

Another set of bridges on the same board enabled a Method of Balance to be employed whereby the control and the test-object were connected in series and then in parallel, and stimulated with a single shock from the inductorium; if the two were identical, no response resulted from stimulation in parallel, and any fatigue effects were equally shared by both, while an increased fatigue on the part of one over the other led to an increase in the compound response with each successive stimulation. Since there are numerous risks of manipulation in this method—which I believe is original—it was used as a supplement to the simple alternate testing.

A few trials were made on plants *in situ* in the field along a telegraph wire, but the difficulty of avoiding electrode fallacies makes this method impracticable, and it was only tried in order to make sure that the response of an organ was much the same before and after its removal from the plant.

Resistances were measured by a Post Office box with a Paul Unipivot galvanometer. Induction shocks—always breaks—were given by a small Ruhmkorff coil, purchased locally, whose description I am unable to give; with one Leclanche cell it gave a spark about one-fiftieth of a millimetre long, which was the usual stimulus, as being strong enough to induce marked fatigue after four or five repetitions; tetanization with this stimulus was bearable by wetted fingers on the electrode zincs.

The galvanometer employed was the Ayrton-Mather, made by the Cambridge Scientific Instrument Co., with a resistance of 22 ohms at 15° C., giving a deflexion of 155 mm. for one microvolt at a distance of one metre. This instrument is by no means an ideal one for the purpose, being somewhat insensitive, but this fact does not vitiate the particular results obtained, since the method employed was a comparative one throughout, as described above.

The maximum 'response' deflexion obtained in some 2,000 stimulations

was slightly over 200 mm. The inertia of the moving coil in this instrument is fairly high, so that it is not easy to decide what the real momentary values of the response might be. Since the experiments were all strictly comparative this is unimportant. An ordinary 'good response' gave about 40–50 mm. deflexion.

Three methods of stimulation were used in the preliminary work, namely, mechanical injury, induction shocks through independent electrodes applied over one or the other non-polarizing electrodes, and induction shocks through the latter themselves with the galvanometer out of circuit. In the latter method, which was the only one employed regularly, a falling key broke the primary and then closed the galvanometer circuit in rapid succession.

The non-polarizable electrodes were of the U-tube pattern, mounted in pairs with an interval of 15 mm. between the centres of their contacts with the tissues. It was found that all trouble from their polarization was best avoided by dismounting them after an hour's work and cleaning them; the zincs were dipped in weak sulphuric acid and washed in running water, the glass plugs removed, cleaned and washed, and stuffed afresh with filter paper and a cap of clay, all wetted with 0.6 per cent. saline. After the electrodes had been used for four or five hours, fresh saturated zinc sulphate was placed in the U-tubes.

EXPERIMENTAL RESULTS.

Feebleness of response from the root.—The first disappointment of these trials was the weak response obtained from young roots of about a millimetre in diameter, however the electrodes were placed. Old woody roots responded freely.

Since it seemed likely that the effect of a break shock through the electrodes might be less effective than a local stimulation over one electrode, such stimulation was tried, but with no better results. A notable blow from a falling lever was required to produce a slight response, and in no case—either with roots or other tissues—was it found possible to obtain a larger response by any other method than the simple one of a break shock through the electrodes.

Response dependent on structure.—It seemed then that the magnitude of the response was in part due to the *non-homogeneity or anisotropy* of the length of tissue tested. Thus, with one electrode on the hypocotyl and the other on the root, large responses were usually obtained, and next in magnitude to these were the responses from the last two internodes of the main axis, where one electrode was on semi-meristematic tissue and the other on partly differentiated tissue (Table I). The direction of the initial response with moderate shocks was a function of the structure; thus, with

one electrode on the stem near the seed-leaf stalks and the other nearer the ground, the former point was always 'zincative' in the beginning.

Currents of injury.—With ordinary care in handling the material the current of injury was insignificant, and all the experiments were, in fact, conducted without the use of a compensating current. The exception to this was provided by the tender tissues of the apical bud and youngest internodes of the stem. In these the injury current was frequently of fair magnitude and of some hours' duration.

Fatigue.—Using the moderate induction shock already mentioned, it was found that a very definite fatigue, or progressive numbing of the response, was exhibited, ending in a *reversal of polarity* in the tissue (Table IV). Some attempts were made to ascertain whether the form of this fatigue-curve could be in any way utilized, but without result. Attention was also directed to a possibility that the weakness of root-responses might be due to the employment of too powerful a stimulus, but no indication of such an effect was obtained; the absence of a sledge-coil hampered this inquiry.

Similarity of plants at various times of the day.—The physiological condition of cotton plants in Egypt at dawn, at noon, and at midnight are so extraordinarily different, especially in May and June,¹ that they might be expected to show some difference in electrical response. Comparable plants removed from the field at these times, and immediately tested in various parts, showed no clear differentiation (Table I), excepting that the terminal bud (which at noon is not growing) gave feebler responses in the middle of the day. Even this result was not constant.

Immersion of the root-system in water.—Comparable pots of seedlings of the same age were tested, whereof one pot had been immersed in water over the soil surface for varying periods, while the other was watered as usual. The first pair of pots tested over a period of four days gave definite results (Table III), showing deterioration of the blaze-current from the water-logged pot, beginning at the root-hypocotyl junction and moving upward to the first and second internodes. The result was, however, fallacious; for instance, the second pair tested reversed this behaviour by giving markedly stronger blazes at all times from the water-logged pot. These water-logged plants showed that they were not in normal health, in spite of their free responses, by subsequently shedding their lower leaves.

Desiccation.—Pots of seedlings were allowed to dry up and tested against controls. Two days before their death the blaze from them was scarcely inferior to that from the controls (Table IV), when they were unable to stand erect and when some were only just able to recover on watering.

¹ The author: *The Cotton Plant in Egypt*. London, 1912.

CONCLUSIONS.

This last failure made it abundantly clear that if the electrical test could not conveniently differentiate between such very sickly plants and their normal brethren, the primary object of the experiments—namely, a test for *partial* damage by root-asphyxiation—was doomed to failure, and the work has been for the time abandoned.

The subject is evidently more important than it is commonly believed to be, and the writer hopes to re-examine it in the future with the desire of finding some clue to the apparent contradictions of the present data. The appliances will remain in use as a routine test for life and death.

A possible hypothetical explanation of these negative results seems to lie in the form of the curve which may be plotted to represent the diminution of the response with loss of vitality. Thus the writer at first imagined that this curve would be fairly uniform, or even straight. If, however, it were logarithmic in form, the loss of vitality might progress steadily for some time without becoming noticeable by these comparatively rough experimental methods, while at a stage somewhat beyond that to which these tests have usually been carried, the slope would become rapid and noticeable, finally diving down to the death-point.

On the other hand, the curve of fatigue (Table II) does not seem to admit of such an explanation, and fatigue from repeated shocks ends ultimately in death. Here the diminution in response is very marked between the first and second stimuli, then tolerably uniform until well beyond the reversing point—which might well be due to differential fatigue—and finally dying out until the response is merged in polarization errors.

Lastly, the critical point at issue is the nature of 'vitality' itself, and it would seem to the writer that the present apparent contradictions offer a fair ground for physico-chemical inquiry.

SUMMARY.

1. This note describes an unsuccessful attempt to utilize electrical response as a test for health in Egyptian cotton plants.

2. During the tests it was discovered (*a*) that the initial magnitude and sign of the response obtained was dependent on the degree and direction of the asymmetry, anisotropy, or non-homogeneity of the two points of electrode contact; (*b*) that fatigue by repetition of stimulation usually led to a gradual reversal of the direction of response before complete insensitivity was reached.

3. The currents recorded as responses were the result of genuine differences provoked between the two portions of a tissue on which the electrodes rested. They were not pre-existent in the electrodes or tissues, and were not due to polarization.

That they were, moreover, contingent on the 'livingness' of the tissue is shown by the fact that boiling, fatigue, or tetanization abolished the response entirely.

4. Two improvements in method were devised and used:

(a) Method of Control, consisting in duplication of electrodes and leads, with examination of test-subject and a control alternately throughout.

(b) Method of Balance, being an alternating connexion of text-subject and control in series or in parallel, with consequent augmentation or neutralization of the response.

My thanks are due to Dr. Wilson, of the Cairo School of Medicine, for advice and interest, to my colleague, Mr. F. Hughes, for the loan of the Post Office box, and to Professor A. D. Waller for kindly and stringent criticisms on the manuscript of this note.

TABLES SHOWING TYPICAL RESULTS.

Two components are given for each tissue tested, namely, the resistance in ohms, and the value of the response as millimetres deflexion of the galvanometer.

The testing was in all cases done in duplicate, with a control on the same switchboard. The value of the response is given in pairs; thus in Table II the readings run :

A. 134, 62 : 41, 34 : 34, 24 : 13, 6 : -16, -23.
C. 88, 54 : 39, 42 : 35, 28 : 30, 15 : -9, -21.

These imply that an antidrome shock on A gave 134; during the recovery of A an antidrome shock on C gave 88; the primary circuit was then reversed and A was tested again with a homodrome shock, giving 62 mm. deflexion, and then B gave 54 with the same treatment. This succession continues to the end of the row of figures.

In all cases the interval between stimuli was only so long as was required for the previous response to die down. Correction is made for slight displacements of the circuit zero through injury currents.

Four response readings or more were usually taken when the initial one amounted to more than 10 mm. deflexion.

TABLE I.

Response from various parts of similar field plants at different hours of the day :

<i>Portion tested.</i>	Upper line = response.		Lower line = resistance.	
	<i>Time.</i>		<i>Time.</i>	
	<i>Noon.</i>		<i>10 p.m.</i>	
Root 10 cm. below soil-surface	8, 10 : 190,000		<i>7 a.m.</i> 67, 50 : 35, 48 : 115,000	
Hypocotyl-root junction	45, 40 : 55, 65 : 300,000		50, 53 : 45, 50 : 120,000	
First leaf midrib	1, 4 : 370,000		2, 5 : 300,000	
Fifth leaf midrib	9, 10 : 200,000		3, 6 : — 300,000	
Fifth leaf petiole	9, 8 : 250,000		12, 18 : 300,000	
Youngest leaf, i. e. 10th leaf	8, 9 : 170,000		9, 23 : 25, 11 : 180,000	
Youngest internodes of stem.	20, 15 : 12, 18 : 120,000		85, 110 : 99, 98 : 75,000	

TABLE II.

Fatigue.

Hypocotyls of two similar seedlings :

Resistance : A. 130,000 ; C. 160,000.*Response* : A. 134, 62 : 41, 34 : 34, 24 : 13, 6 : -16, -23 : &c.

C. 88, 54 : 39, 42 : 35, 28 : 30, 15 : -9, -21 : &c.

(Mean response : A. 37.7 ; C. 36.1.)

Direction of deflexion reversed as fatigue increased, up to about -40, and then died down to zero after about fifty more shocks.

TABLE III.

Damage by root-asphyxiation :

A. Seedlings from pot immersed in water.

C. Control.

Values of resistance and mean response (*b*) from A expressed as percentage of values from C.

<i>Root.</i>		<i>After 24 hours.</i>	<i>After 54 hours.</i>	<i>After 100 hours.</i>
<i>Hypocotyl junction</i>	<i>b</i>	20	38	50
	R	100	76	90
<i>Hypocotyl</i>	<i>b</i>	100	37	43
	R	90	22	180
<i>First internode</i>	<i>b</i>	—	70	50
	R	—	100	210
<i>Cotyledons</i> ¹	<i>b</i>	—	5	?
	R	—	150	100

The first repetition of this experiment gave, e.g. for first internode after 100 hours, the following result :

R = 350,000 in both A and C.

Response : A. 74, 58 : 45, 32.

C. 79, 54 : 54, 38.

TABLE IV.

Desiccation.

A. Plants had wilted, and died two days later.

C. Control.

Resistance : 400,000 in both.*Response* : First internode. A. 47, 36 : 35, 31.

C. 69, 62 : 51, 45.

Hypocotyl. A. 21, 30.

C. 47, 75.

¹ Cotyledons shed in following week.

Balls, W. Laurence. 1913. "Apparent fallacies of electrical response in cotten plants." *Annals of botany* 27, 103–110.

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