CORRELATION BETWEEN THE ELECTRIC CURRENT GENERATED BY A BEAN ROOT GROWING IN WATER AND THE RATE OF ELONGATION OF THE ROOT

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Summary

Methods of measurement of the electric fields produced by plants have been developed which eliminate artefacts commonly present in such investigations.

The normal potential pattern in the water surrounding actively growing bean roots is described.

When rates of elongation of roots are controlled by two methods which produce very different types of overall metabolic change, the potential pattern is shown to be correlated with the rate of elongation.

Values are given of the electric power dissipated in water in which a bean root is growing, and of the current generated by the root in the water.

I. Introduction

Experiments made here and elsewhere show clearly that electric potentials measured on the surface of plant organs depend on a number of causes. In addition to those due to the plant itself when in its normal steady state of metabolism, there are a number of other sources of potential difference, some of them generated by the plant itself owing to treatment it receives during measurement, and others introduced from outside.

In this paper a new approach is made to the problem of the bioelectric phenomena associated with plant metabolism. The effect of the process of measurement on the observations made is entirely eliminated by measuring the potential fall down currents produced by the plant in an external medium.

When a bean root grows actively in aerated water it generates an electromotive force which passes current through the water. This paper describes measurements which have been made of the ohmic potential differences due to these currents in the water adjacent to the root. The pattern of these potentials is characteristic and reproducible when the root is growing strongly.

The paper further describes experiments in which the growth of the root has been controlled and in which a correlation has been found between the change in potential and the rate of elongation.

By addition of auxin of suitable concentration to the bath in which the root is grown, it is possible to arrest growth and observe the change in potential pattern. This process proves to be reversible, as removal of the auxin allows the plant to grow again and the potential pattern to recover.

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A similar correlation is observed when the temperature at which the roots are grown is raised, but the root cannot be restored to its original condition of normal growth by lowering the temperature.

II. Some Causes of the Conflicting Results Obtained in Previous Investigations of the Electric Fields of Plants

A number of investigations of the electric potential differences associated with plants have been made in the past. An examination of the literature shows very little agreement between the results obtained.
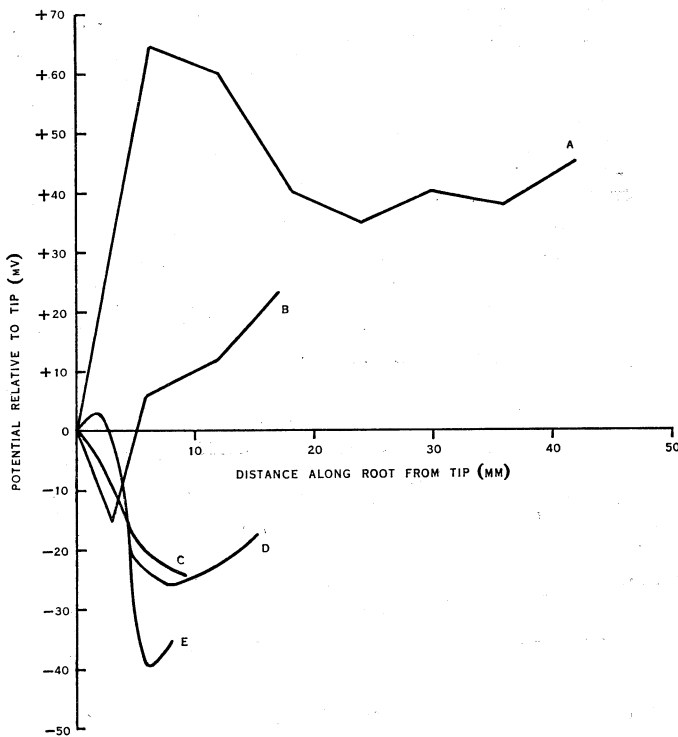

Fig. 1.—Comparison of the results obtained in several investigations of the relation between the external potential of the root relative to the tip and distance from tip along the root. Data obtained from A, Thomas (1939) (bean); B, Lundegårdh (1940) (wheat); C, McAulay, Ford, and Hope (1951) (maize); D, Lund and Kenyon (1927) (onion); and E, Ramshorn (1934) (bean).

Some of the results obtained using roots as material are compared in Figure 1, in which the potential of the root relative to the tip is plotted against the distance from the tip. It is evident that there is a wide diversity between the results of different observers. It is true that these results are not directly comparable since they have been obtained under a variety of conditions, and some

of the variation between them is certainly due to such factors as the type of plant used, its age, and the state of its environment. Nevertheless, a critical examination of the experimental techniques employed in the investigations shows that in most cases the measured potential differences were due not only to the plant e.m.f.'s but also to factors introduced by the measuring techniques. In particular, the following causes can be recognized as responsible for many of the variations in the results recorded.

The effect of variations in salt concentration at the point of contact has been almost invariably overlooked in previous investigations. Salt concentration changes are produced by drying and are due also to salt uptake by the plant. The resulting potential changes can be quite large, and are often greater than the plant potential it is desired to measure.

To illustrate the magnitude of the potentials set up in this way, it is found that if the concentration of KCl at one point of contact on a bean root is changed by a factor of 10 (the concentration at the other contact remaining unchanged) the potential difference between the contacts changes by more than 30 mV (Hope 1951).

Other factors which may obscure the true steady state electric pattern generated by the plant are variations in environment, either of the plant as a whole, or local variations at the point of contact. These may include temperature, humidity, salt concentration, the amount of aeration, and light and gravitational influences. In addition, stimulation or injury by the measuring probes can produce large potential differences.

III. Experimental Methods

Most of the sources of error listed above are liable to occur if the measuring probes are in mechanical contact with the plant. To avoid this, three methods have been developed and used to measure plant potentials without making mechanical contact with the surface of the plant.

Two of these methods are suitable for measuring electric potentials of plant surfaces in air, while the other is suitable for use with plants grown in water. In the first, which has been described elsewhere (Blüh and Scott 1950), a probe is set into vibration close to the plant, and its potential is adjusted until there is no field between the probe and plant. This condition is reached when no alternating current flowers in the probe circuit. In the second method, air in the neighbourhood of a probe and plant surface is made conducting by α-particle irradiation and a current flows between probe and plant to bring them to electrical equilibrium.

The third method, which forms the subject of the present paper, will now be described in more detail.

A fortunate property of the root is its ability to grow strongly and healthily in aerated water. If the conductivity of the water is sufficiently low, potential differences between points in the water adjacent to the root can be observed, owing to the currents generated by the root e.m.f.'s. For this reason, it was decided to grow bean roots vertically in a 10^{-4}N KCl solution under controlled

conditions and observe the potential pattern in the water adjacent to the root and its relation to the rate of elongation of the root.

Since the measuring tubes are not in contact with the plant, no effects due to injury by them can occur. As the salt concentration in the tubes and in the bath is the same, the point of measurement on the plant is not in a different condition from neighbouring points resulting from local diffusion or other local variations.

Errors introduced in the measuring circuit have been prevented by the use of a suitable valve electrometer and mercury-calomel electrodes. The whole input circuit is insulated with polystyrene and shielded to prevent A.C. pick-up. The plants are left in the measuring bath for at least an hour before measurements are begun to avoid stimulation effects which may occur in setting up the plant for measurement. The bath is aerated, stirred, and its temperature controlled to avoid changes in environment. No effect was observed on either rate of growth or the potential pattern of bean roots due to light.

As the potential measured is an ohmic drop along a current, its source is evidently an active e.m.f. involving energy change in the plant organ.

IV. MATERIAL

For experiments described in this paper, the broad bean, *Vicia faba* L., Johnson's Long Pod variety, was used. After soaking the seeds overnight in water, the seed coats were removed and a number of seeds impaled on stainless steel rods 1/16 in. dia. Mounted in this way, the seedlings could be handled easily for growing in water-baths and could be transferred to the measuring bath with a minimum of stimulation. Impaling the seed did not appear to affect the development of the plant in any way.

For most experiments the beans were prepared by growing in baths of aerated distilled water at 25°C. The water in the tank was slowly changed from a reservoir tank. The rods supported the plants so that the shoot was above the water-level and the roots were submerged and grew vertically. Plants 2-3 days old with roots 20-30 mm long were used in the experiments.

In one series of experiments discussed later, the roots were prepared by growing in air saturated with water vapour and minute water droplets. The fine spray was produced by a simple atomizer using compressed air. In this bath the roots grew quite strongly but the root surface appeared rather different from that of a root grown in water and more like what is observed for a root grown in dry soil or sphagnum moss.

V. APPARATUS

The "Perspex" measuring bath is shown in Plate 1. It was 7 in. long, 6 in. wide, and 3 in. deep and was insulated with polystyrene. The bath was aerated and the water in the tank circulated by passing compressed air through a sintered glass plug P mounted in the tank.

Heating the bath presented some problems. Electrical interference had to be avoided and an arrangement giving rapid response to the thermostat must

be used. It was decided to heat the bath by means of a coil of nichrome wire stretched across the top of the bath just above water-level. A 12-V A.C. supply was connected to the coil and heat radiated downwards warmed the water. Surprisingly little A.C. pick-up was experienced, and this arrangement, with some shielding of the heater from the measuring circuit, proved very satisfactory. The thermostat was a mercury-alcohol switch, C, which controlled the heater through a relay. Temperature stability was $\pm 0.5°C$.

The $10^{-4}N$ KCl solution used in the measuring bath was changed from a reservoir. The water was made to drip into the bath, and to drip out at the overflow to facilitate electrical insulation of the bath.

The beans were set up in the measuring tank on a stainless steel rod passing through the cotyledon with only the roots immersed and held vertically.

The electrometer used in these experiments employed a pair of matched M.E. 1400 Mullard electrometer valves in a balanced circuit. The instrument was designed to be insensitive to fluctuations in the high tension voltage and in the valve heater current. A Cambridge spot galvanometer (full scale deflection about 2 μA) was used and the maximum sensitivity of the electrometer was such that an input of 1 mV produced a deflection of 3 cm on the scale. Currents flowing in the input circuit of the electrometer under the conditions of operation were not greater than 10^{-12} A.

The connections to the measuring bath were made using mercury-calomel electrodes. The reference electrode was connected directly to the bath. The other electrode made contact with the bath through a measuring tube, T, containing $10^{-4}N$ KCl agar. This tube was mounted on a micromanipulator, M, allowing it to be moved easily in the vicinity of the plant root. Since $10^{-4}N$ KCl has a low conductivity (about 1.5×10^{-5} mho cm^{-1} at 25°C) the tip of the measuring tube had to be coarse or hand capacity effects became troublesome owing to the high resistance of the input circuit. In most of these experiments the diameter of the tip was about 0.5 mm, corresponding to a tip resistance of about 15 MΩ.

Elongation of the root was measured by projecting a greatly enlarged image on a wall. A 5-in. Waterworth projection lens was used, mounted in front of the box with its axis horizontal. For most experiments back lighting was employed, giving an enlarged shadow of the root, but in some experiments the root was marked, and strongly illuminated from the front so that the regions of elongation of the root could be found. In this way, changes in length of the order of 0.02 mm could be observed.

The whole box could be moved laterally on a metal slide, G, so that each bean in the box could be placed in turn in front of the lens for measurement.

VI. Results

The present paper consists of a study of the potential pattern close to a bean root actively growing in water contrasted with that which appears when growth is inhibited.

The aspect of growth that has been selected is rate of elongation and attempts have been made to control this by a variety of means, and study the resulting potential changes.

Several treatments have been applied to the growing root, such as subjecting it to mechanical vibration, controlling its oxygen supply, growing it at varying temperatures, and inhibiting growth by the application of a suitable concentration of auxin.
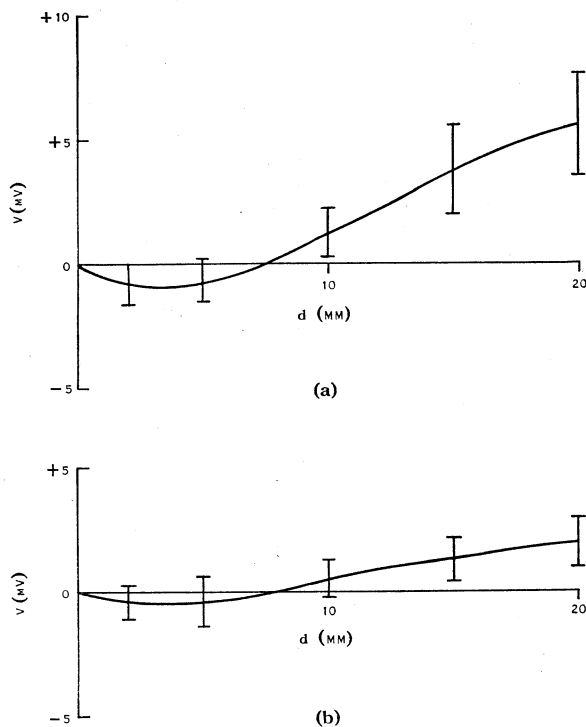
Fig. 2.—Control of growth and potential pattern of bean roots by the application of indoleacetic acid (IAA). (a) Roots at 25°C untreated with IAA. Mean growth rate 0.82 ± 0.060 mm/hr. (b) Same roots at 25°C 2 hr after treating with IAA (2 mg/l). Mean growth rate 0.027 ± 0.077 mm/hr. V is the potential relative to that of the root tip and d is the distance along the root from the tip.

The last of these methods, auxin treatment, was considerably the most successful. By this means, elongation could be inhibited, and the inhibition subsequently removed and growth restored. The method of temperature control also provided results of interest which are described below, but preliminary results by the other methods were not so promising and study of these has not yet been followed up.

In both cases studied, the pattern of active e.m.f. was correlated with the rate of elongation. The fact that the correlation was the same in both cases suggests a direct link between elongation and potential pattern. Of course, it is possible that both elongation and potential pattern are more dependent upon some third change common to both methods of control.
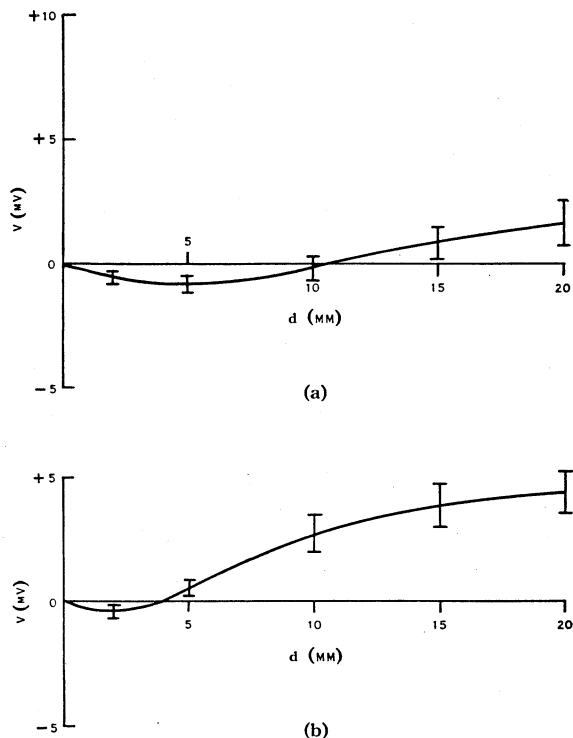

Fig. 3.—Recovery of growth and potential pattern on removal of IAA. (*a*) Roots at 25°C 2 hr after treating with IAA (2 mg/l). Mean growth rate $0 \cdot 037 \pm 0 \cdot 043$ mm/hr. (*b*) Same roots at 25°C 2 hr after removal of IAA from bath. Mean growth rate $0 \cdot 36 \pm 0 \cdot 072$ mm/hr.

(*a*) Control of Growth with Auxin

(i) Bean seedlings with roots approx. 25 mm long were transferred to the 10^{-4}N KCl measuring bath at 25°C and allowed to reach an equilibrium with their environment.

After about 2 hr, the potential pattern and rate of elongation of the roots were measured, and those whose growth rate was less than $0 \cdot 4$ mm/hr discarded. Indoleacetic acid (IAA) (2 mg/l of solution) was then added to the bath containing the rapidly-growing beans. After about an hour the potential pattern had reached a new steady state and the average potential and rate of elongation over the next 2 hr were recorded.

The results of this experiment are shown in Figure 2.   In this and sub-sequent diagrams the vertical lines through the points mark the 95 per cent. confidence limits; that is, the probability is 0·95 that the mean of the popula-tion represented by the sample lies within the limits given by the ends of the line.   The limits for the mean growth rate also are the 95 per cent. confidence limits.
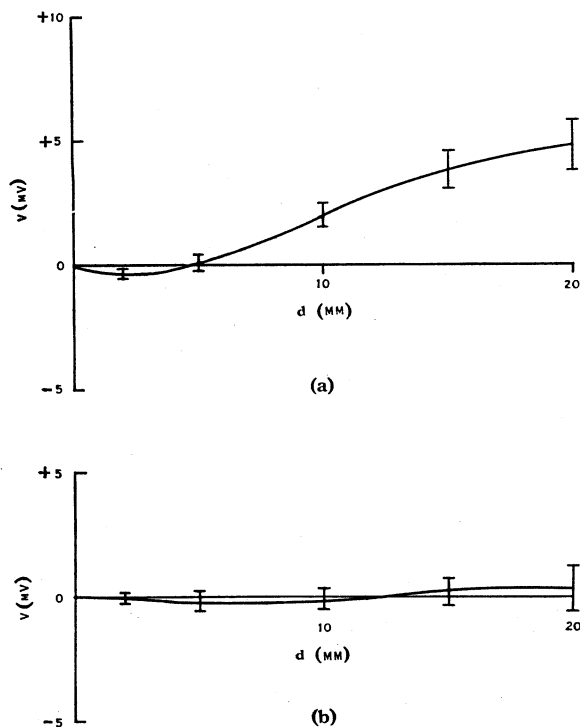

Fig. 4.—Control of growth and potential pattern by temperature. (a) Roots grown and measured in water at 25°C. Mean growth rate 0·532 ± 0·076 mm/hr. (b) Roots grown in saturated air and measured in water at 37°C. Mean growth rate 0·007 ± 0·041 mm/hr.

(ii) Bean seedlings were transferred to the measuring bath at 25°C con-taining 10^{-4}N KCl and 2 mg/l IAA.   After about 2 hr elongation had practic-ally ceased.   The potential pattern was then observed, and the water in the bath replaced with KCl solution without IAA.   At 1-3 hr after the removal of the IAA, when the root was again elongating, the mean potential pattern and growth rate were measured.

The results of this experiment are given in Figure 3.

An examination of Figures 2 and 3 shows that the inhibited roots have a markedly different potential pattern from those growing strongly.   The normal potential pattern of a growing root shows a region 2-5 mm from the tip most negative, with the base of the root approx. 6 mV more positive.   Suitable

treatment with IAA inhibits the elongation of the root and reduces the potential gradients along the root by a significant amount. Removal of the auxin allows the root to grow again (although the recovery is not complete in 2 hr, the growth rate being well below that of untreated roots) and the potential gradient along the root returns almost to its value prior to auxin treatment.

(b) Control of Growth by Temperature

Figure 4 (a) shows the normal potential pattern for roots grown in 10^{-4}N KCl at 25°C. In this case, and in the following one at 37°C, the growth and potentials were averaged for a 24-hr period following the initial settling down.

Figure 4 (b) shows the potential pattern for roots measured in 10^{-4}N KCl at 37°C. Inhibition of growth at this temperature was most marked in roots pre-treated by growth in a saturated atmosphere for 24 hr before exposure to 37°C. The graph refers to roots treated in this way. It is seen that the roots have almost entirely stopped elongating and the potential differences along the root are not significantly different from zero. . Roots measured at this temperature for 24 hr and then returned to a 25°C bath do not recover normal growth. The activity of the primary meristem is suppressed and the main root stops growing, but pronounced initiation of secondary roots quite close to the primary root tip is observed (see Plate 2). Electromotive forces are once more produced by the root when it is returned to the 25°C bath, but they are not the same as those for a root which has not been treated at 37°C.

(c) Power and Current

More detailed experiments have been made from which the current density and current direction in the neighbourhood of the root can be deduced. In addition, the total power dissipated in the solution due to current produced by the root can be calculated.

These data have been obtained by measuring the radial and longitudinal components of the potential gradient in different orientations around the root. With this information, it is possible to map the equipotential surfaces and hence the current paths throughout the external medium. A typical simplified pattern is shown in Figure 5, in which the full lines are current paths and the dotted lines are equipotentials.

For a root growing actively in 10^{-4}N KCl at 25°C, the total current leaving the root (which, of course, must be equal to the total current entering it, since leakage paths have been eliminated) is of the order of 5×10^{-8} A. If the root is taken to have a surface area of $2 \cdot 5$ cm^2 immersed, the mean current through unit area is 4×10^{-8} A/cm^2, although, of course, it varies considerably over the whole root surface. In certain regions of an active root it may be as high as 2×10^{-7} A/cm^2. The total power dissipated in the surrounding medium is of the order of 2×10^{-10} W.

It is found that small variations with time of the potential pattern occur even when all environmental factors known to affect the plant are controlled.

Under certain conditions, rapid rhythmic oscillations of potential take place. These oscillations can be induced experimentally.

Investigations of these phenomena are continuing and it is proposed to make them the subjects of later papers.
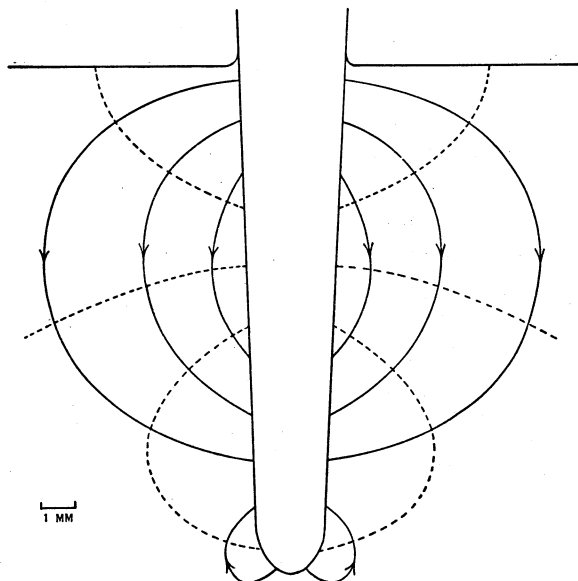

Fig. 5.—Typical simplified pattern of the current paths through the $10^{-4}N$ KCl solution in which bean root is growing. The full lines are typical current paths (lines of force) and the dotted lines are equipotentials.

VII. Discussion

The experiments described in this paper were made with the object of providing definite reproducible information about the electric currents produced by a plant organ acting as a unit. The long-range object is to obtain information about the morphology and behaviour of the electric patterns that characterize integrated plant structures and their relationship to the morphology and development of the material structure.

It is found that the most negative part of the potential pattern for a rapidly growing root corresponds with the region which is elongating most rapidly (2-7 mm from the tip). A possible mechanistic explanation is now given.

The greatest uptake of salt might be expected to take place in the region of greatest rate of elongation, where newly vacuolated cells must fill up to a salt concentration which is many times greater than that of the external solution.. It is known that the mobility of K^+ is greater than that of Cl^- in tissue (Hope 1951). The K^+ will penetrate the root more rapidly, forming an electric double layer, and a slight excess of Cl^- will build up in the region outside the elongating part of the plant. This will make the region electrically negative. With this

hypothesis it would also be expected that a root which had not 'elongated for some time would be absorbing salt at a slower rate and more uniformly over the whole root surface. Under these conditions the observed reduction in the external field is to be expected.

The mechanisms which produce electromotive forces in plant tissues may be such that some can supply more power than others. Whether the field produced by one of these will be observed outside the plant in a particular case depends on the magnitude of the current generated in the conditions of measurement. If this current is too large, the field may not be measured, either because the e.m.f. itself is polarized, or because the potential drop in the tissue is so great that the external field is insignficant.

The present method selects for measurement only those bioelectric processes which are capable of supplying a relatively large amount of power to the external medium. By employing other methods which were mentioned earlier in this paper (Section III) it is possible to investigate less powerful bioelectric processes taking place in the organ and so gain a more complete picture of the bioelectric behaviour of plants.
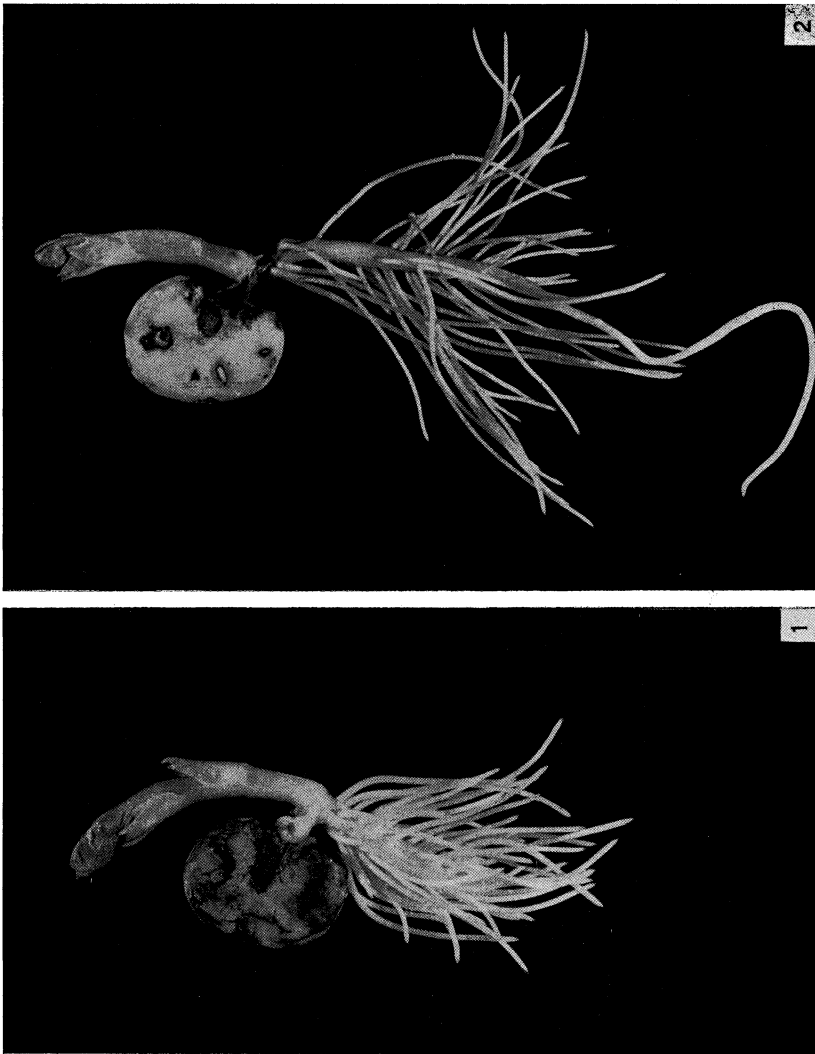
VIII. Acknowledgment

IX. References

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ELECTRIC CURRENT GENERATED BY ROOT GROWTH
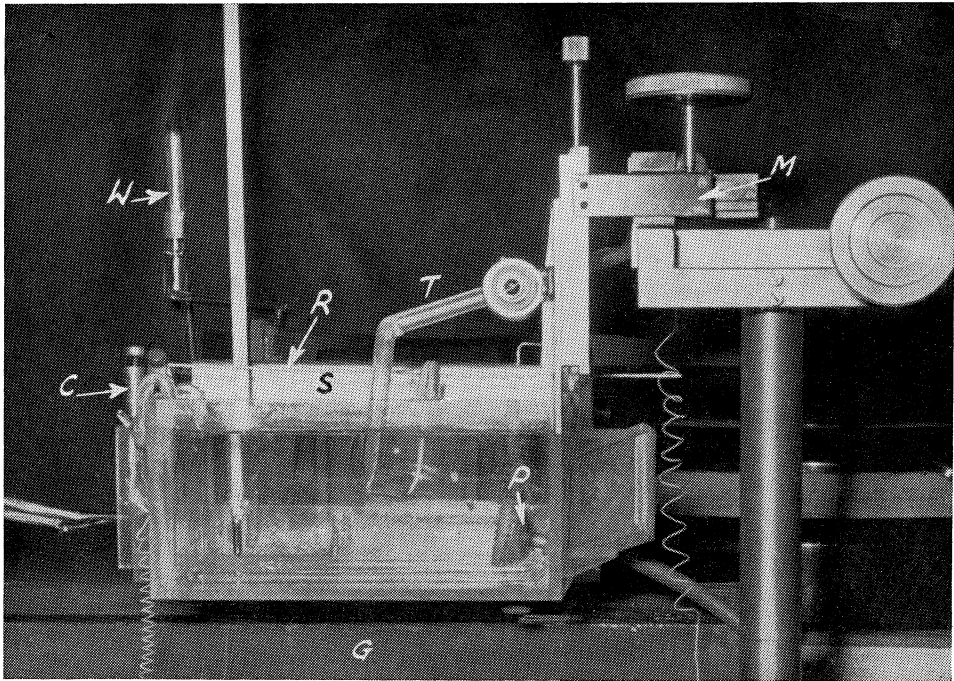
The measuring bath used in the experiments desribed in the paper. The follow-
ing parts are referred to in the text: P, sintered glass plug; S, shield for A.C. heater; C,
mercury-alcohol switch control; R, stainless steel rod supporting plant; M, micromanipulator;
T, measuring tube; W, water inlet; G, metal slide for moving box.

ELECTRIC CURRENT GENERATED BY ROOT GROWTH

Fig. 1.—Root one week after treatment at 37°C. Notice lack of development of primary root with secondary roots appearing quite close to primary tip.

Fig. 2.—Untreated root of same age.

Abnormal subsequent growth of roots treated for 24 hr at 37°C.