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IN
MIMOSA PUDICA.

Diss Utrecht 1935

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE WIS- EN NATUURKUNDE AAN DE RIJKSUNIVERSITEIT TE UTRECHT, OP GEZAG VAN DEN RECTOR-MAGNIFICUS Dr. H. BOLKESTEIN, HOOGLEERAAR IN DE FACULTEIT DER LETTEREN EN WIJSBEGEERTE, VOLGENS BESLUIT VAN DE SENAAT DER UNIVERSITEIT TE VERDEDIGEN TEGEN DE BEDENKINGEN VAN DE FACULTEIT DER WIS- EN NATUURKUNDE OP MAANDAG 4 FEBR. 1935 DES NAMIDDAGS TE 4 UUR

DOOR

ADRIËN LOO HOUWINK
GEBOREN TE UTRECHT

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AAN MIJN OUDERS
AAN MIJN AANSTAANDE VROUW

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THE CONDUCTION OF EXCITATION IN MIMOSA PUDICA

by

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Contents.

CHAPTER I.

Introduction.

Of all sensitive plants, *Mimosa pudica* has always been considered as the most interesting, owing not only to its complicated and rapid reaction but also to the fact that the excitation is not confined to the stimulated part itself but can be conducted through the whole plant.

The mechanism of movement and excitatory conduction was beyond the methods of the earlier investigators. What they did do was to study the means by which the plant could be stimulated, the contraction of the pulvini serving as an indicator only.

In the 19th century a number of theories were suggested to explain the spreading of the excitation. The further experimental research advanced, however, the less these theories fitted the facts. In 1900 not one was considered to be really plausible.

Until this time conduction of excitation was thought to be so rare in plants that no one supposed the sensitive plants to be equipped with more than one conducting system. In the last decade it has been shown that there are several modes of conduction, but the correlation between them remained completely unknown. The object therefore of the present investigation has been an inquiry into the relations between the several methods of conduction of stimulus.

> "Sir Paul Neile mentioned, that the King had, within four days past, desired to have a reason assigned, why the sensitive plants stir and contract themselves upon being touched; it was resolved that Dr. Wilkins, Dr. Clarke, Mr. Boyle, Mr. Coelyn and Dr. Goddard, be curators for examining the fact relating to those plants".
>
> (l. c. Royal Society, July 17, 1661).

One of these fellows of the Royal Society, Dr. CLARKE, made a visit to a garden in St. James Park, where such a plant *(Mimosa spec.)* was growing under glass. A full account of these investigations can be found in Micrografia by R. HOOKE (1667).

CLARKE (1661) stimulated the plant by various chemical agents and by cutting the leaf. In the latter case he saw that a green droplet was exuded, which offered, he thought, an explanation for the conduction of the stimulus. If a system of vessels throughout the plant contained the afore-mentioned green fluid, cutting a vessel should lessen the pressure in all parts, whereupon the pulvini, if sensitive to a change of pressure, would react. The transport of a stimulating substance by circulating sap was also suggested. Both assumptions involved the existence of vessels, which CLARKE could not see, but which, he hoped, would be observed afterwards. HOOKE discovered the vessels in other plants and he connected this observation with CLARKE's theory.

In 1736 DU FAY and DU HAMEL published some observations on *Mimosa.* They found that moderate heating, as well as cooling with ice, stimulated the pinnae. The excitation was conducted through the pinnae, but did not pass on to other parts of the leaf. A hundred years later, the stimulating effect of cooling was found a second time by FEE, and more recently BOSE was the third to discover it.

Sometime in the 18th century DESFONTAINES observed the behaviour of a plant which he had taken with him in a carriage. At first the plant reacted, but during the drive it recovered. He pulled up for a quarter of an hour and then drove on. The plant then reacted again; so he concluded that the excitability had become normal again during the rest.

PFEFFER (1873) made some exact investigations of the matter by stimulating the lower side of the pulvinus mechanically at regular intervals. If these were less than 2 min. the plant recovered, but the excitability did not return. According to LINSBAUER (1923) PFEFFER's statement holds only for certain frequencies of stimulation.

By leading off action currents, UMRATH (1927) found that if the lower side of the pulvinus is stimulated mechanically every 10 sec., some cells react to every other stimulus, but only by giving an electrical response. He supposed that the refractory period of the motor cells is lengthened by the frequent stimulation.

DUTROCHET (1824) tried to solve the problem of the movement by dissecting parts of the pulvinus. In this way he found that the movement is inhibited if the lower side of the pulvinus is lacking. This was confirmed in 1827 by BURNETT and MAYO, who published LINDSAY's experiments, at that time only available as a M.S. (1790) in the Library of the Royal Society.

By turning a plant upside down before and after reaction, and measuring each time the divergence of the petiole and the stem, BRÜCKE (1848) proved conclusively that the fall of the leaf is effected by the loss of rigidity of a group of cells at the underside of the pulvinus. The liquid which is thrust out by the cells into the intercellulair canals expels the air from them and thus brings about the change of colour which can be seen in the contracting pulvini, especially in those of the leaflets (1865). By exact measurements PFEFFER (1873) ascertained that the volume of the lower side of the pulvinus is much reduced after a reaction whereas that of the upper side remains nearly constant.

DUTROCHET also studied the conduction of the stimulus. When the stem was stimulated with a flame, the excitation passed regions where a ring of bark had been removed. When a wood cylinder was removed and the bark left intact as much as possible, the stimulus did not pass the zone. The conduction thus was confined to the wood. In 1916 LINSBAUER confirmed these results. DUTROCHET thought it improbable that a living tissue effected the propagation of the stimulus since the rapidity seemed independent of the temperature.

I have probably overlooked a number of researches, since in 1839 MEYEN mentioned the existence of an extensive literature, and remarked that a new experiment could hardly be devised. Yet he discovered an unknown and very fast method of conduction in the stem, which could be observed when the plant was stimulated by cutting into the stem just to the wood. The high speed of conduction reminded him of that of a nervous system, for example that of the lower animals. Some, who saw him perform his experiment, pitied the plants and asked if it did not hurt them.

The modern investigations on the conduction of the stimulus in *Mimosa* began with PFEFFER (1874). Three different ways of conduction could be suggested at that time.

First, a stimulating substance might be transported in some way or other through the plant, but so many adverse arguments could be raised, that no special experiments were needed to disprove it. For example by simply bending two leaflets on the basal or apical end of a pinna, all other leaflets of this pinna can be made to react in succession, the excitation moving basipetally as fast as acropetally, which, if no wound is made, no substance should do.

Secondly a nervelike system, though not observed anatomically, might exist. If a nerve is narcotised by ether or chloroform it does not conduct an excitation. PFEFFER, therefore, narcotised the middle part of a pinna and stimulated it by cutting a leaflet above the treated part. The leaflets in the ether did not react, but those below them did, showing that the conduction was not suppressed.

A third possibility remained, the one which CLARKE had already mentioned and which HOFMEISTER and SACHS had described very extensively in their manuals, namely a change of pressure which could spread along the petiole and stimulate the pulvini.

PFEFFER's theory is different from CLARKE's, but the guiding idea is still the explanation of the appearance of a droplet after cutting the plant, and much reasoning was required to account for conduction following stimulation without wounding, for example electrically (RITTER, 1809 (see STERN, 1924) and KABSCH, 1861).

By microscopic examination HABERLANDT (1890) found a network of wide tube-shaped cells in the bark, which he named "Schlauchzellen". They contained a substance which was stained red by ferric chloride. This was the same reaction which FEE (1850) had found for the much discussed droplet. Thus it was clear that at least part of the liquid must result from the bark, as already observed by MEYEN (1839). The latter had not attached any importance to the appearance of the droplet, as the plant could still be stimulated after the removal of the bark. HABERLANDT, however, supposed that MEYEN left some of the bark intact.

As the opposing leaflets, which react in pairs, are connected by the "Schlauchzellen", but not by the xylem, HABERLANDT concluded that the excitation was conducted by a change of pressure in the former turgescent cells and he endeavoured to explain all stimuli in terms of pressure changes. The stimulating effect of a flame should thus be due to steamproduction.

HABERLANDT's theory has much been critisized, first by CUNNINGHAM (1895): H. himself showed that the stimulus could pass a zone of the petiole which was killed by a jet of steam. CUNNINGHAM pointed out, however, that the "Schlauchzellen" could not have remained turgescent after such a treatment.

According to BORZI (1899) "Schlauchzellen" are lacking in *Neptunia*, though a stimulus can be conducted in it.

By mounting a cut plant on a pressure pump and diminishing or increasing the pressure very suddenly, MAC DOUGAL (1896) did not get any reaction. He therefore attacked HABERLANDT's as well as SACHS' theory.

This experiment was repeated by FITTING (1904), who in many ways attempted to check H.'s idea. A detached leaf recovers if placed in water. Cutting off a bit of its petiole should not change the pressure in the vessels nor in the "Schlauchzellen", if these are connected to one another as is supposed by H.'s theory. Nevertheless the leaflets reacted to it.

To ascertain whether the stimulus is conducted by the action of living cells, FITTING cooled a part of the petiole to 2° C. No delay in the conduction of the stimulus was found. Thus it was not effected by living cells. FITTING stimulated the leaf by cutting the petiole, below the cooled part, and the rapidity of the conduction was measured by noting the time needed for reaction of the leaflets.

This is in contradiction with a finding of BOSE (1914). He stimulated the petiole electrically, and found the reaction of the main pulvinus to be retarded when the excitation had to pass a cooled zone, through which it travelled downwards in this experiment. At 2° C. there was no conduction at all.

FITTING supported PFEFFER's view though he was all but satisfied by it, whereas BOSE convinced himself by many experiments that the stimulus is conducted in a plant in the same way as in a nerve. As in a nerve the excitation starts from the cathode and is stopped by an electrotonic block. Above all it is important that he found that an action current accompanies the excitation and travels at the same rate as the latter along the petiole as well as along the stem. Also in other plants, for example ferns, a thermal stimulus was observed to cause a propagated change of potential.

In 1916 it was observed by RICCA that if the lower part of the stem is stimulated by applying a flame, the stimulus can be conducted to the younger leaves not only through a killed zone, but also through a short water-filled tube, inserted between the upper and the lower part of the shoot. This could not be due to a change of pressure since a small manometer, attached to the tube, did not show any change. He concluded that a substance must have passed the tube to stimulate the pulvini above it. This substance must have been carried along with the transpiration stream. He made an extract out of slices of the stem, and found that when the basal end of the petiole of a detached leaf was dipped in this extract, the leaflets closed after some time. In this way the existence of such a stimulating substance was proved conclusively. RICCA used *Mimosa Spegazzinii* for his investigations.

FITTING (1930) tried to determine the nature of this stimulating substance by testing various chemicals as to the stimulating effect. It appears that the extract can be much diluted without losing its stimulating effect, but the more diluted it is, the more time it takes to make the leaflets react.

Since the excitation can also pass downwards, RICCA supposed that, by the stimulation, some of the substance is set free and is sucked in through the vessels by the negative pressure in the wood. He thought it very improbable that yet another method of conduction could exist, so he endeavoured to prove that HABERLANDT's theory was erroneous. He stimulated the leaflets by dipping them in water at 70° C., to disprove H.'s supposition that the stimulating effect of heating depends upon the formation of steam. The bark of the stem was found to be insensitive. He then divided the basal part of a cut shoot into separate strips of xylem and phloem. Only when the xylem was stimulated were the leaves affected. Yet these facts did not prove conclusively that there is but one way of conduction.

To test RICCA's theory SEIDEL (1923) made some observations on the rate of ascent of a Lithium salt as compared with the rate of conduction of the excitation in a cut shoot. In this way he found that the transpiration stream is too slow to account for the conduction. But SNOW, in the following year, made nearly the same experiment with methylene blue, which he proved to ascend as fast as the excitation. Thus it was indicated that it is possible that the conduction of the excitation in the stem is effected by a substance.

Beside the normal conduction Snow found a much faster form, the "high-speed conduction". When he incised a shoot as far as the cambium, the next leaf fell almost instantaneously, but its leaflets did not close. This was discussed by Dixon (1924), who considered it to be a special form of the normal conduction. Because of the smallness of the wound, too little of the substance should be sucked up to stimulate the leaflets, and the extraordinarily fast transport of the substance should be due to the high negative pressure in the vessels. Since Ball (1927) has demonstrated the "high-speed conduction" in submerged shoots, where a high negative pressure is not to be expected, Dixon's view is probably not correct.

A third form of conduction has been discovered by Ball in submerged shoots. He called it "rapid conduction". When such a shoot was stimulated electrically, a reaction of all main pulvini could be observed, the excitation travelling at a rate of 2—3 cm. per sec. in either direction. It could not pass a killed zone, but was not stopped when a ring of bark was removed. The pith and the wood, however, had to be intact. The longer the shoot had been submerged, the faster the rapid conduction travelled. It resembled the high-speed conduction in the fact that the leaflets never closed, but the latter affected only the next leaf or at best the next two leaves, and was never observed except after cutting the shoot. The rapid conduction, however, followed both upon an electric shock and burning.

One can easily observe a form of conduction in the pinnae, which resembles the rapid conduction in the stem. After applying a stimulus (electric shock or cutting a leaflet), the excitation travels along the pinna basipetally as fast as it does acropetally. This is the reason why Pfeffer rejected the idea of conduction by a stimulating substance, and more recently Snow (1924 and 1925) has attacked Ricca's theory on this ground.

Furthermore Snow compared the rate of this conduction with that of the transpiration stream. When a leaftip was submerged in a solution of methylene blue and then cut off, the excitation travelled many times faster than the stain. (This was denied later by Ricca, 1926). Snow also killed a zone of the petiole by steam and after that no conduction to the main pulvinus was found. Snow always used cut shoots, while Fitting and Haberlandt performed the same experiment but obtained the opposite results on intact plants. The difference between the plants of these investigators consists in the fact that the plants of SNOW had no negative pressure in the vessels. From these experiments one can conclude that under special conditions (a killed zone of the petiole) the particular form of conduction which is normal in the stem, can also be observed in the leaf.

In a few cases where the continuous xylem cylinder was broken but some part of the phloem left intact, rapid conduction of the excitation through the petiole to the main pulvinus took place. HERBERT (1922) had already observed this, and considered it to be in support of HABERLANDT's theory on the conduction by "Schlauchzellen". These experiments do not agree at all with RICCA's view that the excitation can be conducted in the xylem only.

All modern investigators have observed that the rapidity of the conduction of the excitation in the leaf depends upon the intensity of stimulation. When a terminal leaflet is cut through, conduction is much slower than when the end of a pinna is cut. LINSBAUER (1908) made a careful investigation of this matter. He measured the rapidity of the conduction in the petiole after its stimulation (1) by touching with a heated platinum wire (rate of conduction 5—9 mm. per sec.), (2) by incision (30 mm. per sec.), (3) by cutting it through (130 mm. per sec.). These experiments were continued bij UMRATH (1925a and b), who found several rates in each part of the plant and completed the work by measuring the electrical changes accompanying and following the excitation (1928 and 1929). When the petiole was stimulated by the discharge of a condensor, the slowest system of conduction reacted and only a simple action current was found. When it was stimulated in any other way, however, the result was generally much more complicated.

CHAPTER II.

Methods.

BOSE has shown that, when an electrode is stuck into the petiole or into the stem of *Mimosa*, its potential with respect to the earth is changed when the excitation, being conducted through the plant, affects the tissue into which the electrode has been stuck. In the recording of these potential changes, one has a method for tracing the excitation in the stem and in the petiole. The fact that the nature of these electric phenomena is unknown should be no reason not to use them. The cause of the contraction of the cells in the lower side of the pulvinus is not known either.

The electric response [1]) is much more variable than the visible reaction of the plant, as was shown by the work of UMRATH. Owing to this fact it is possible to disentangle eventual several ways of conduction, which all result in the only visible and always identical reaction namely the movement of the pulvinus. Most of the conclusions, which will be derived from the present investigation, might have been arrived at, even if no potential changes had been recorded. It would, however, have been a much more difficult task.

Of course the electric phenomena are also interesting on their own account. Yet it is questionable whether *Mimosa* is suited to the study of action currents in general. Some factors, by which the potential changes are complicated, will be discussed in chapter III.

The apparatus, which was used to amplify and to record the potential changes is shown schematically in Fig. A. The electrode in the earth is connected to the filament of valve I, that on the petiole, to the grid. Since this is insulated as much as possible, only a very weak current can flow through the plant. The valve functions best, when the grid is slightly negative to the filament. To effect this the potential of the filament can be varied by means of a potentiometer.

The electrode on the plant was of the silver-silverchloride type. It could have been stuck in the petiole or in the stem, but I preferred to make no wound and hung a hook-shaped electrode on the plant, connection being made by a drop of water. The silver wire was connected to the grid of valve I by a thin and flexible copper wire. Thus the petiole was free to move.

When the electrode on the plant becomes 0,1 V. negative, the plate current of valve I decreases, the potential difference in the resistance falls, the grid of valve II becomes more positive and its plate current increases by ± 3 mAmp. The deflection of the mirror of the recording galvanometer is registered in the usual way. For reproduction the photographic records have been copied bij means of transparent paper. In order to refrain from tedious descriptions a scheme of each experiment is given next to the record and the reactions are indicated by uniform symbols. *The curves have to be read from right to left.*

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[1]) The reaction of the cells under the electrode, which accompanies the conduction of excitation, can be observed by the electric response. It will be shown, however, that not all changes of potential are caused by a reaction of these cells.

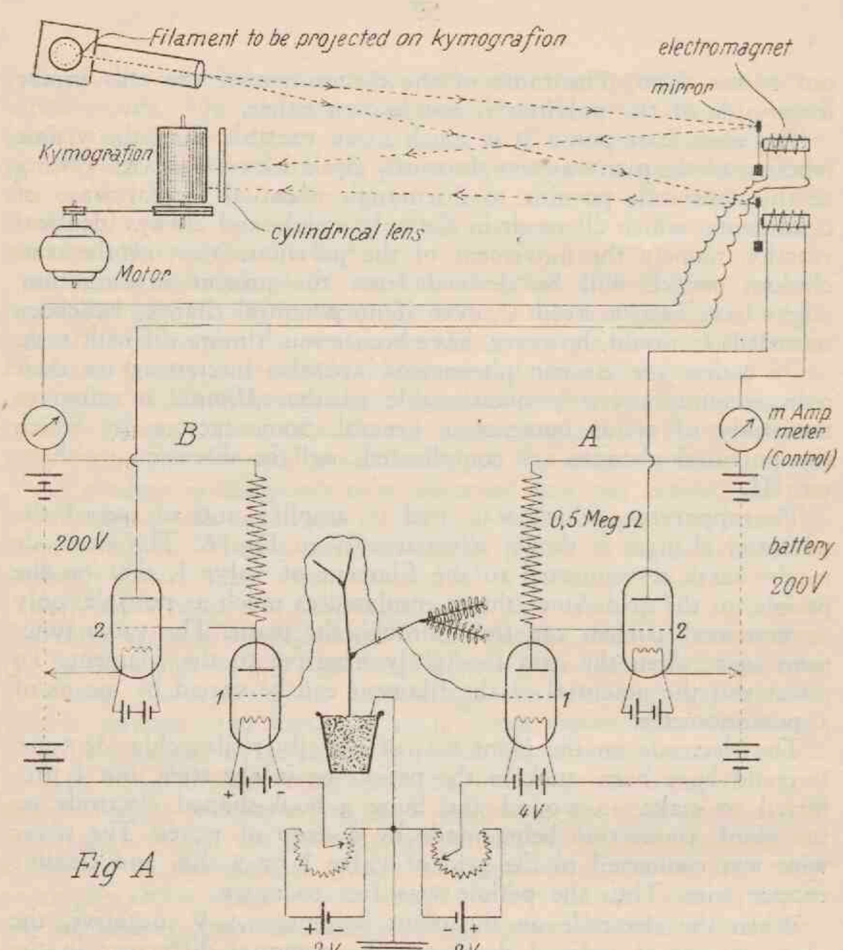
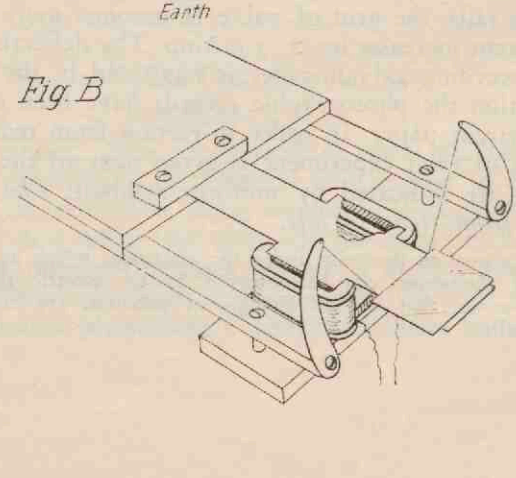

Filament to be projected on kymografion
electromagnet
mirror
Kymografion
cylindrical lens
Motor
B
A
m Amp meter (Control)
200 V
0,5 Meg Ω
battery 200V
2
1
1
2
+
-
4 V
+
-
2 V
-
+
2 V
Earth

Fig A

Fig. B



To record the potential changes of two points of the plant at the same time, two identical instruments were used. Part of the experiments have been made with an apparatus, slightly different from that which is represented by Fig. A. The valves I A and I B were connected to one accumulator, II A and II B likewise, and only one anode battery was used. The drawback was, that the potential of the battery lessened as soon as the plate current of one of the valves II increased, which caused the plate current of the other valve II to decrease a little. The result was that in the experiments which were made with this apparatus, the rise of one curve is accompanied by a slight fall of the other (indicated in the figures by an asterisk).

The recording galvanometers (Fig. B) were made for the purpose and have the adventage of being very simple and costing practically nothing. The zero-point is subject to shifting by magnetic hysteresis, but there is no objection against using them for qualitative work such as the present. Their period is only about 0.01 sec. This proved to be of great importance.

The action current in the petiole is about 0.1 Volt. No quite exact measurements can be made with the apparatus; moreover, they would be of no importance to the present investigation.

CHAPTER III.

Introduction to the Experiments.

By means of the experiments which will be discussed, it can be shown that there are at least two distinct ways of conduction, both in the stem and in the leaf. When the plant is stimulated without wounding, the excitation can only be propagated by one method, i.e. by the action of living cells. This action is accompanied by a change of the electric potential at the conducting place. There can be no objection against calling this change of potential an action potential. The name action current has also been applied. The former name is the more correct, but the latter one has always been used in the literature on this subject.

When a plant is stimulated by wounding, the excitation spreads in two ways. The first is the action of living cells mentioned above, the second is due to a stimulating substance, set free at the wound and transported in the vessels. In these vessels a negative pressure is commonly found, which accounts for the fact that the substance can be sucked in basipetally, whereas the transpiration stream transports it acropetally. It does not merely stimulate the pulvini, but also those cells which, by their action, can propagate the excitation. When the substance moves on faster than the excitation can be conducted by the action of the cells, these are stimulated by the substance, instead of by their neighbours' activity.

It is clear that the rapidity of the propagation of the action by a living tissue depends on the condition of the cells. Since it was found that the rate of the propagation of this action does not depend on the intensity of the stimulus, one might call it an all-or-none reaction. On the other hand, when a wound is made, the stimulating substance will be sucked in the faster the larger the wound, subject to the negative pressure in the vessels.

Now the phenomenon of conduction in *Mimosa pudica* becomes still more complicated by the fact, that even in the most healthy plant the propagation by cell action generally is stopped at several places. When a wound is made in a leaflet and circumstances are such that the action is travelling faster than the substance, the main pulvinus of the stimulated leaf will be affected by the action, thus reacting before it has been reached by the substance. After that, however, the action is stopped. In fact I have never observed it to pass through the main pulvinus into the stem. Hence the other main pulvini will not be affected, unless the stimulating substance can get into the stem and stimulate it. The excitation, being once begun in the stem, may be propagated by the action of cells to the pulvini. Frequently, however, it is not propagated and they do not stir before they are stimulated by the substance itself.

CHAPTER IV.

The Conduction by Action of Cells.

It is convenient to study the conduction of the excitation by the action of cells, in a plant, which is stimulated by applying a drop of ice water, briefly called "ice" hereafter. It is not thus wounded and it recovers in a short time. Electric stimulation has a similar effect, but it causes much more difficulty, especially if action currents are to be led off.

Action in the Stem. The simplest action currents are found in the stem. One can stimulate it above or below the electrode. In both these cases the action currents are identical and due to the same cause, i.e. the reaction of the tissue on which the electrode is fastened (Fig. 1 and 2).

Fig. 1

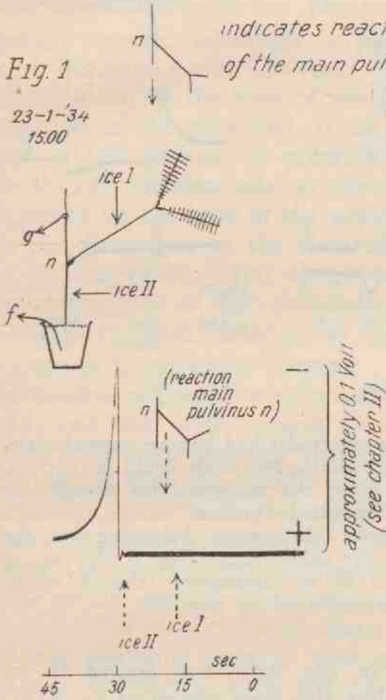
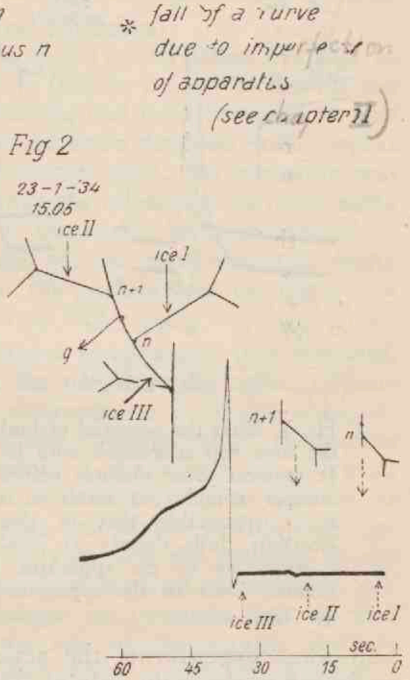


Fig 2



In these experiments I endeavoured to let the excitation pass from the petiole into the stem. The petiole was stimulated with ice, whereupon the main pulvinus reacted, but no change whatever was found at the electrode on the stem.

The exact moment of stimulation was not recorded, nor that of the reaction of the pulvinus. This is indicated in the figures by a *dotted* arrow.

Leading off action currents from two electrodes A and B at the same time is a convenient way of determining the rate of conduction (Fig. 3).

Fig 3

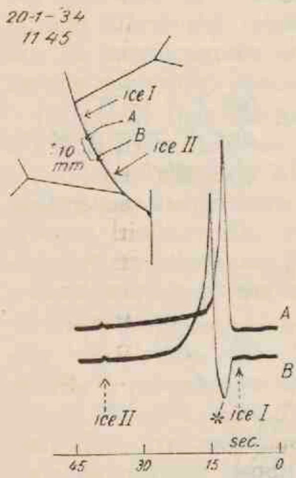


Fig. 4

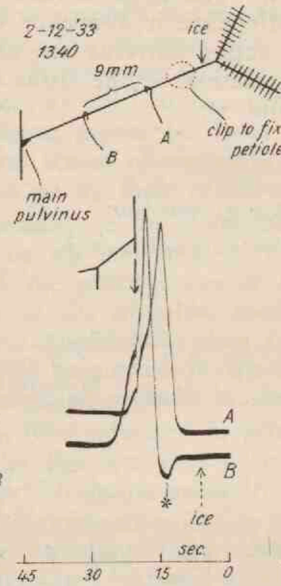


Fig 5

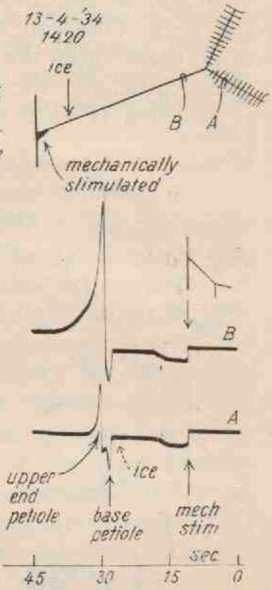


Fig. 3. After the potential of both the electrodes had become normal again, the stem was stimulated with ice once more. No result could be noticed. It requires some minutes before a plant has recovered far enough for another stimulus to result in a propagated reaction.
It is regrettable that in this figure the curve, belonging to one electrode, falls slightly as soon as the other rises. This is due to an imperfection of the apparatus. It is of no consequence though inconvenient. Later on the apparatus was improved (s. page 61).

In some experiments the action in the stem was found to be conducted to the main pulvini. This has also been observed by Ball, who called it "rapid conduction". I tried to let the action be conducted along a decorticated zone, in which I was not successful, unlike Ball, who found "rapid conduction" in the stem if only the pith and the xylem were left intact. Possibly his plants were in a better condition than mine.

Action in the Petiole. Not only the stem of *Mimosa* can respond to a stimulus in this way, but the hypocotyl and the petiole can do the same. In the latter the action current has a more complicated form. At the moment of the reaction of the main pulvinus, the curve suddenly falls. When there are two electrodes on the petiole, both curves naturally fall at the same time (Fig. 4). This is also found when the petiole is tied up so that it cannot move.

The main pulvinus can be stimulated by touching its lower side. Then the action of its reacting cells can be propagated to those in the petiole. In a young leaf this is commonly found. In some experiments, however, the action was confined to the pulvinus (Fig. 5). The curves show the effect of the reaction of the pulvinus on the potential of the petiole. After that, the lower part of the petiole was stimulated with ice. The next change of potential was a weak action current, seemingly a positive variation.

A Positive Action Current? This positive variation was due to the reaction of the base of the petiole (not to be confused with the reaction of the pulvinus). If an electrode had been fastened on this part of the petiole, its potential would have dropped with respect to that of an electrode on the undisturbed part. No electrode was fastened on the base of the petiole, but the adjoining pulvinus acted as an elongation of the electrode in the earth, being connected to it by the stem. Thus, the potential of the earth-electrode being constant, that of the electrodes on the petiole and on the pinna must have risen, causing the curves to fall.

Action Currents in General. When a tissue reacts to a stimulus, its electric potential with respect to the neighbouring tissue is changed, and that with respect to the electrode on it is also changed. The difference between these potential changes causes the action current. When the middle of a petiole is stimulated, its potential drops as much with respect to that of its basal, as to that of its apical part; an electrode on the upper end of the petiole does not show the intervening disturbance. The fact that the reaction of the base of the petiole causes the potential of such an electrode to rise proves that this reacting tissue changes its potential less to that of the pulvinus than to that of the rest of the petiole. Perhaps this is correlated with the fact, that when a cell of *Nitella* is stimulated and potential changes are led off, these changes depend on the concentration of KCl in the drop by which the electrodes are connected to the cell. (HARRIS and OSTERHOUT, 1929).

In the experiment of Fig. 5 one of the electrodes was fastened on a pinna and the other on the upper part of the petiole. The basal end of the petiole was stimulated with ice. The passing of the action at electrode B was accompanied by a normal action current (curve B). This case it is absolutely certain that the action did not pass into the pinna. The action was occasionally found to pass into the secondary pulvini. Passage of the action into the pinnae is only passable in a young leaf and is very rare. Curve A shows an action current even in this case, however. This must be due to the reaction of the upper end of the petiole. It seems that this changes its potential with respect to the rest of the petiole more than to the secondary pulvinus. In some other experiments this action current of curve A was much weaker.

Fig 6

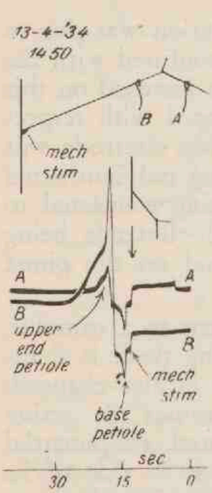


Fig 7

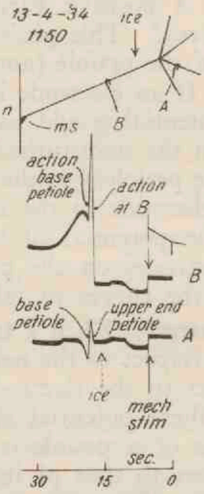
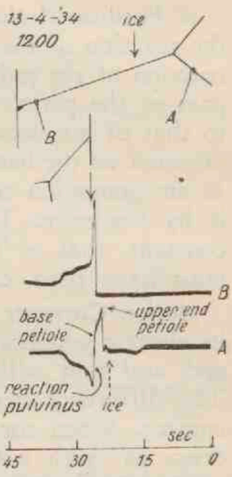


Fig 8



In the experiment of Fig. 6, in contrast to that of Fig. 5, the action of the pulvinus was propagated to the petiole. The action current of its base is seen to start about one second after the reaction of the pulvinus.

The experiment of Fig. 7 is much like that of Fig. 5. In the former the petiole was stimulated close to its upper end. The action travelled downwards to electrode B (normal action current in curve B), and upwards to the upper end of the petiole (small action current in curve A). Long before these action currents have finished, both curves show a rapid fall, due to the action of the base of the petiole.

In the experiment of Fig. 8. approximately the same may be seen. In this case the pulvinus was not stimulated beforehand, so its reaction, indicated by the very sudden fall of the curves, follows immediately upon the beginning of the action of the base of the petiole.

A Monophasic Action Current in the Petiole. Since apparently the action current in the petiole is more or less diphasic if led off in the way I have described, one may inquire whether it is not possible to get a really monophasic response. This was found indeed to be practicable by letting the action be stopped by a cooled zone. Fig. 9 represents such an experiment. First the basal part of the petiole was stimulated (action current in curve B) and the pulvinus reacted (which is shown by the curves though the petiole was prevented from falling). The action does not pass on to electrode A, as it cannot pass through a zone which is at a temperature of below ± 10° C. or above ± 50° C. Thereafter it was stimulated near the secondary pulvini, and the excitation travelled down to electrode A. It appears that the potential of the reacting cells between the unreactive cooled portion and the reactive non-treated portion, drops as much with respect to the former (cooled cells) as to the latter (untreated cells). Therefore a monophasic action current was found in this experiment.

Fig. 9

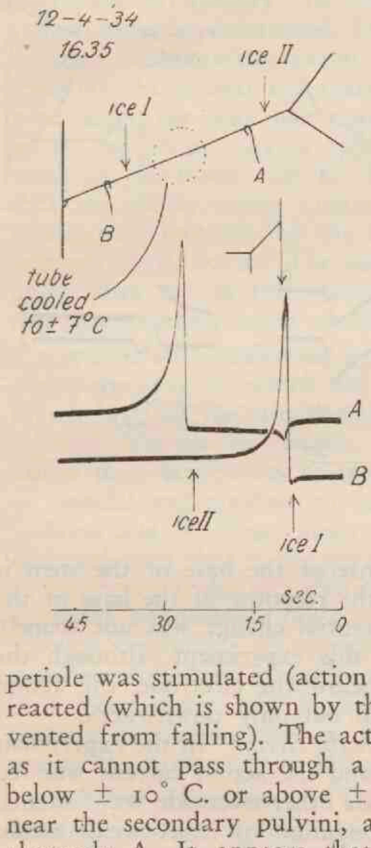
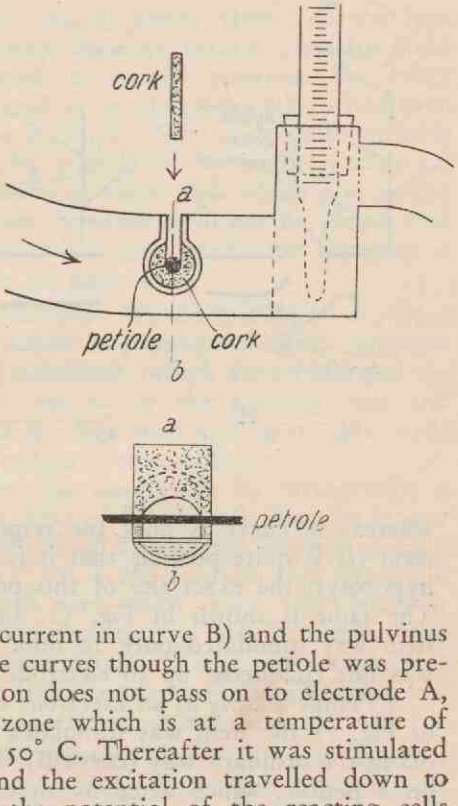


Fig. 9a



The Action of the Base of the Stem. Generally the action in the stem is stopped by some unknown cause before it reaches the earth, but sometimes a diphasic action current may be obtained from it. Fig. 10 shows such a diphasic action current in curve B, whereas in curve A only the response of the base of the stem is seen. (It is quite possible that it is the response of the base of the hypocotyl; the exact site of this potential change was not found). The same is shown in Fig. 11. In this experiment, although the stem was stimulated only 10 mm. below the electrode, the action was not conducted up to electrode B but only downwards.

Fig 10

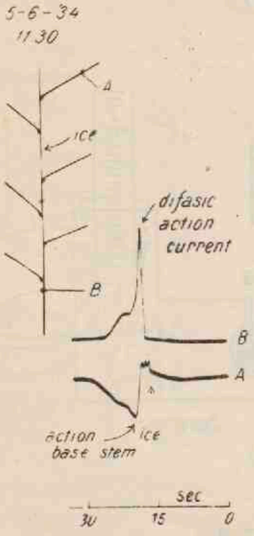


Fig 11

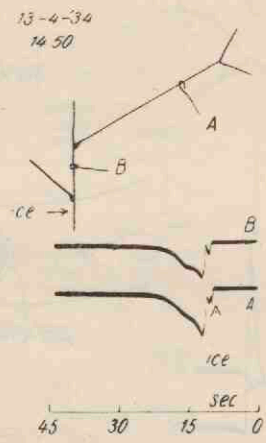
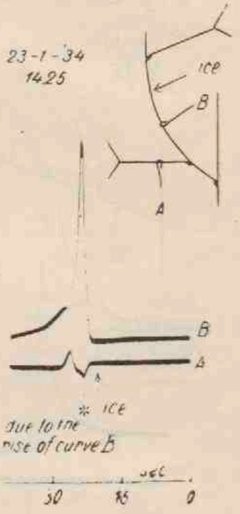


Fig 12



A Petiole Acting as an Electrode on the Stem. In the experiment of Fig. 12 the stem was stimulated and the action current was led off in the ordinary way (curve B). The other electrode was fastened on a petiole, which acted as an elongation of it. A weak action current appears in curve A at the moment that the action passes the pulvinus without stimulating it. At first one wonders why this action current is so weak, but it should be remembered that it only expresses the difference between two potential changes. On the one hand we have the change in potential of the reacting tissue to that of the still undisturbed part of the stem and on the other hand the change to that of the pulvinus.

Refractory Period. After a reaction, no conduction is possible for some minutes. To conduct the action from the stem to the pulvinus and from the pulvinus to the petiole a still longer rest is needed. This does not mean that the single cells cannot react during this period, but only that they cannot propagate the excitation to one another, either by the weakness of their reaction or by a decreased excitability. In this connection the work of UMRATH (1927) must be mentioned. He found that when a pulvinus is stimulated mechanically every 10 sec., it reacts visibly to the first stimulus. After that the reactivity does not return, even for some time after the leaf has resumed its normal position. Yet every 20 sec. an action current appeared at an electrode, which had been stuck in the lower side of the pulvinus. Now it is quite possible that the motor tissue cannot be stimulated by slight mechanical stimulation, but only by the action of those cells which give action currents every 20 sec. The action, however, will not be propagated unless they are in full "tonus", which can hardly be expected if they are reacting every 20 sec. (s. page 53).

Theories on Conduction by Action. We do not know anything about the way in which the action is propagated. BALL supposes that a little of the stimulating substance, which RICCA showed the cells of *Mimosa* to contain, is ejected by the reacting cells and causes their neighbours to react in their turn and so on. He made no special experiments to investigate this problem.

According to BOSE, however, the conduction by living cells is identical with that in the nerve. One of BOSE's arguments in favour of his theory is the fact that the rate of conduction depends greatly upon the temperature ($Q_{10} = \pm 2$). I can confirm this statement. Though conduction in a nerve is very much faster and though it is accomplished in a single cell, whereas it is almost certain that in a plant many cells have to cooperate, it is a remarkable fact that the conducting cells of a plant react by an action current and are at the same time sensitive to electric stimulation.

Electrical Stimulation. To stimulate a nerve one may apply a constant voltage during a comparatively long time, for example 1 sec. The weakest, still stimulating voltage is called the rheobase. Stimulating the nerve by twice the rheobase, one finds that to excite it, the potential (twice the rheobase) must be applied during a certain minimum time, the so-called chronaxy, (which is not 0,5 sec. but many times less). The excitability of a nerve is now usually expressed by its chronaxy, since it has been found that this is much more typical than its rheobase because of the dependence of the latter on such casual factors as the resistance of the circuit. Moreover the chronaxy was found to be related to the rate of conduction.

In view of these facts I made a few measurements of the chronaxy of the petiole of *Mimosa*. It was found to be approximately 0,05 sec., which is about 100 times as great as that of some nerves. As this investigation is barely started, one should not attach much value to it. Probably the chronaxy is not related in the same manner to the rate of conduction in a tissue, consisting of many cells, as it is related to that in a single axon of a nerve.

UMRATH (1925 c) made a great many experiments concerning the chronaxy of all parts of *Mimosa*. That of the petiole was found by him to be 0,2—0,4 sec. He compared these results and those of his investigations on nerves and muscles with a theory on conduction of excitation. Part of his work on this subject can be found in Planta *5* (1928).

Since HILL and OSTERHOUT (1930) have shown that one part of a cell of *Nitella* can be stimulated by the action current of the other part in case an intervening zone is killed, and even that the action can pass on in this manner from cell to cell, valuable supporting evidence has been advanced in favour of BOSE's theory.

CHAPTER V.

The Conduction by a Stimulating Substance.

When a wound is made, by burning or cutting a leaflet, not only an action current can be derived from an electrode on the petiole, but in addition a very irregular variation of potential, which may last several minutes, and which cannot be observed after mechanical stimulation. To distinguish this potential change from the action current it will be referred to as "the variation". It will be demonstrated in this chapter, that the variation is due to the effect of RICCA's stimulating substance.

"The Variation". Figs. 13 and 14 represent experiments in which a leaf was stimulated by cutting a leaflet. The action currents show that the action was conducted downwards to the pulvinus resulting in the fall of the leaf. Before the action itself was finished, the variation started. To disentangle the two kinds of

Fig 13

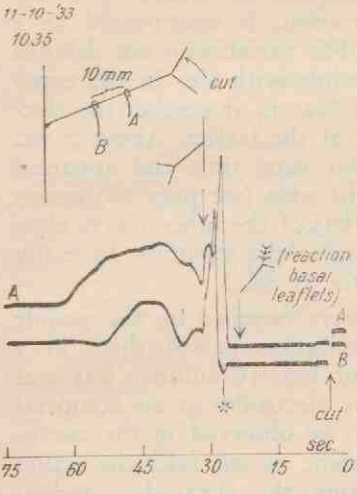
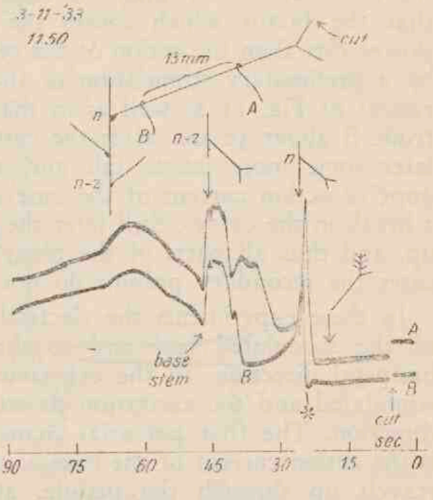

11-10-'33
1035

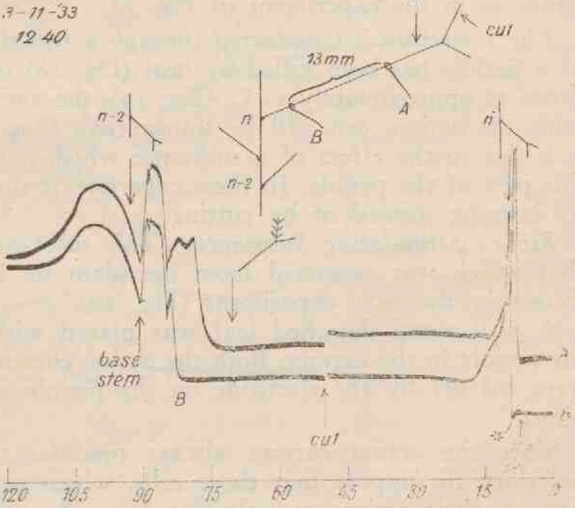
Fig 14


3-11-'33
11.50

indicates the closure of the basal leaflets of the stimulated pinna

indicates the closure of the basal leaflets of the pinnae when the excitation travelles acropetally

Fig. 15


3-11-'33
12 40

potential changes the petiole should be stimulated beforehand with ice. (Fig. 15). Then the two components of the curves are shown apart and one may notice that in this experiment the variation travels much slower than the action current, or strictly speaking, that the factor which causes the variation, is transported at a slower rate than the action of the cells. The variation is not delayed by a preliminary stimulation of the petiole with ice. In the experiment of Fig. 15 as well as in that of Fig. 14 it reaches the electrode B about 32 sec. after the cutting of the leaflet. About 7 sec. later some more leaves fall and at the same time the apparent positive action current of the base of the stem (see page 68) causes a break in the curves. Still later the leaflets of the other leaves close up, and thus all parts of the plant react to the stimulus. In many cases the secondary pulvini do not react at all.

In these experiments the electrodes were fastened on the petiole of the stimulated leaf and so the excitation passed them in a basipetal direction. In the experiment of Fig. 16 another leaf was stimulated and the excitation passed the electrodes in an acropetal direction. The first potential change to be observed in the curves is the action current of the base of the stem. 10 sec. later the action travels up through the petiole, affecting the electrodes, and is followed by the variation. Fig. 17 represents a similar experiment, the difference being that the petiole was stimulated with ice beforehand, as in the experiment of Fig. 15.

The Variation is Conducted through a Killed Zone. When part of a petiole has been killed by heat (Fig. 18) or when it is cooled down to approximately 5° C. (Fig. 19), the variation, as contrasted with the action, can still be transmitted. One may conclude that it is due to the effect of a substance which is transported through this part of the petiole. In these experiments the leaf was wounded by burning instead of by cutting.

Ricca's Stimulating Substance. This substance is identical with that which was extracted from the plant by RICCA (1916), as is shown in the next experiment (Fig. 20).

A well-rested detached leaf was placed with the basal end of the petiole in the extract. Both the action current and the variation were led off by the electrode on the petiole and by that on the pinna.

Since the action current always precedes the variation, it is necessary to suppose that those cells, which propagate the excitation by their action, are stimulated by the substance. It happens occasionally that they do not propagate it in the normal way. In

Fig. 16

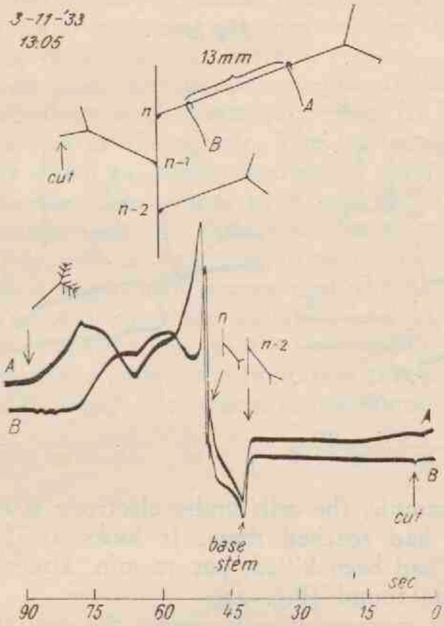

3-11-'33
13:05
13 mm
n
A
B
n-1
n-2
cut
n
n-2
A
B
A
B
cut
base stem
sec
90
75
60
45
30
15
0

Fig 17

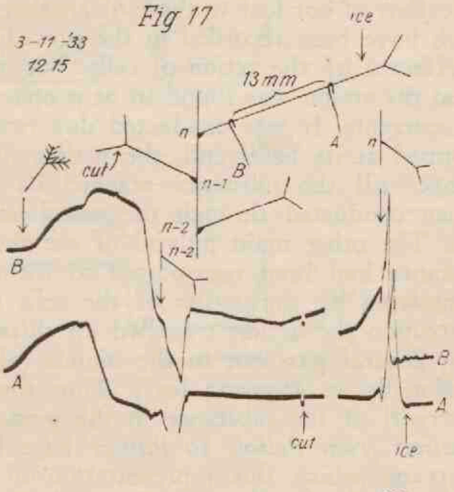

ice
3-11-'33
12.15
13 mm
n
A
B
cut
n-1
n-2
n
n-2
B
A
B
A
cut
ice
sec
105
90
75
60
45
15
0

Fig 18

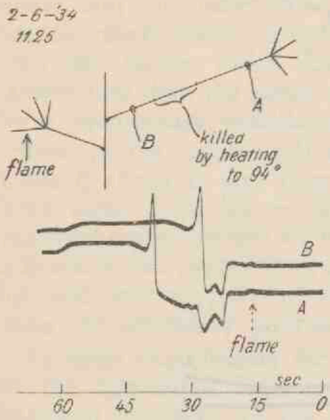


Fig. 19

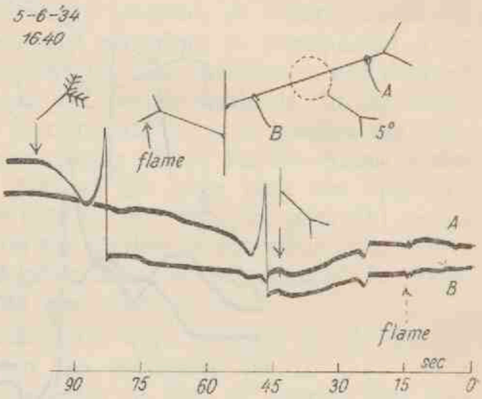


Fig. 21 for example, the cells under electrode A only reacted after the substance had reached them. It looks as if a zone between the electrodes had been killed, but 30 min. afterwards an excellent conduction was found (Fig. 22).

In the above mentioned experiments the excitation was conducted from the leaflets of one leaf to those of another. It is clear from the facts which have been recorded in the preceding chapter, that this was not effected by the action of cells. Yet in all the petioles and in the stem the action was found to be propagated as is shown by the action currents. It was conducted downwards through the pinna but stopped at its basal end, the petiole not reacting with potential changes till the substance reached its distal end. The action was then conducted through the petiole and affected the main pulvinus. The other main pulvini of the shoot only reacted after the substance had been transported to them (in some cases they were stimulated by the action of the cells in the stem; see page 64). Thereupon the action travelled up through the petioles, but did not in general pass out to the pinnae (although this was observed occasionally in a young leaf). Thus the closing leaflets indicate the arrival of the substance in the pinnae.

The Conduction from Petiole to Pinnae Vice Versa. Most of the experiments on which this representation of the conduction in *Mimosa* is based, have still to be discussed. Some of the other experiments have only partly been explained. In those, represented by Figs. 16, 17, 21 and 22 the leaflets only closed after the variation appeared at the distal electrode, the interval between the moment of stimulation and the reaction of the leaflets being wholly independent of the action current.

In Fig. 23 the transition from pinna to petiole is illustrated. One electrode was fastened on a pinna 2 mm. from its base and the other on the upper end of the petiole. The leaflets on the tip of the pinna were cut, which caused the leaflets to close in pairs. The excitation then travelled downwards to A and the action current was found to coincide with the closure of the adjoining leaflets. In this experiment it took a very long time before the variation appeared at A. This experiment shows that the action does not appear in the petiole until after the variation had passed A. If we assume that the variation is due to the stimulating substance, it follows that this substance carries the excitation through those parts of the plant, through which the action is not conducted.

Fig. 20

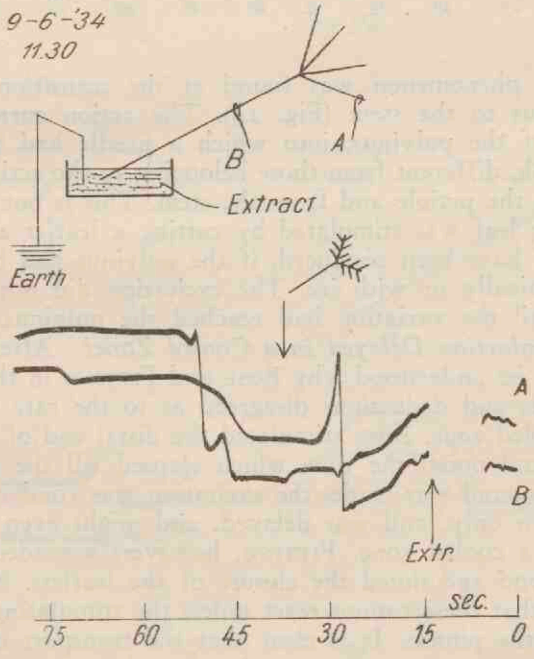

9-6-'34
11.30

Fig. 21

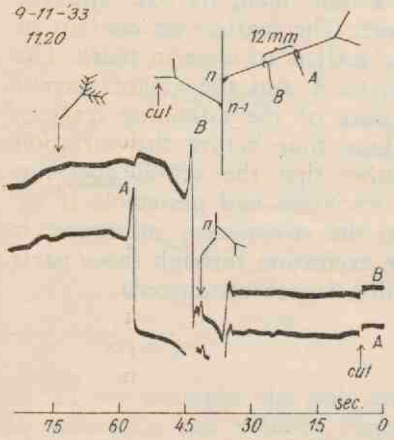
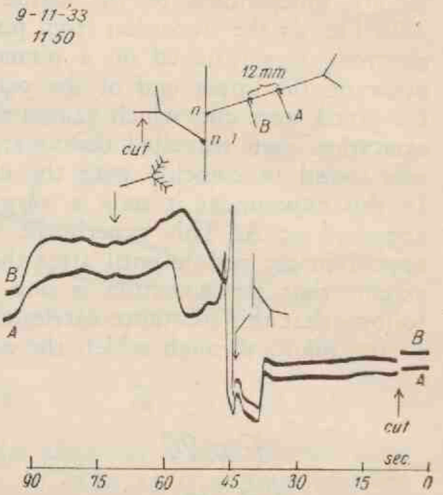


Fig. 22



The same phenomenon was found at the transition from the main pulvinus to the stem (Fig. 24). The action current of the lower side of the pulvinus, into which a needle had been stuck, shows a graph, different from those belonging to the action currents led off from the petiole and from the stem. This is not due to the fact that the leaf was stimulated by cutting a leaflet as the same graph would have been produced, if the pulvinus had been stimulated mechanically or with ice. The excitation did not appear in the stem until the variation had reached the pulvinus.

Is the Conduction Delayed in a Cooled Zone? After the foregoing it may be understood why Bose and Fitting in their experimental results and deductions disagreed as to the rate of conduction in a cooled zone. Bose stimulated the distal end of the petiole electrically and noted the time which elapsed till the fall of the leaf. As no wound was made, the excitation was conducted by the action of cells only, and was delayed, and might even have been stopped, by a cooled zone. Fitting, however, wounded a petiole at its basal end and noted the closure of the leaflets. Now it has been shown that these cannot react unless the stimulating substance has reached the pinnae. It is clear that the transport of the substance by the transpiration stream does not depend upon the temperature of the petiole. Hence Fitting did not find any correlation of the temperature and the rate of conduction. He concluded that the excitation is not conducted by the action of living cells.

Instead of cutting a petiole as did Fitting, I cut the leaflets of one leaf and noted the closure of those of another. In some experiments a zone of the petiole, either of the cut or of the uncut leaf, was cooled. In neither case was the reaction found to be delayed. The conduction of action was beyond doubt suppressed in the cooled zone, but in the experiments in which no zone was cooled, it stopped at the distal end of the petiole, so that the conduction of action was of no importance to the result of the experiment. The transport of the substance takes place independently of the cooling of any particular part of the petiole.

The Variation Marks the Presence of the Stimulating Substance. It has been mentioned repeatedly that the variation is an indication of the presence of the stimulating substance and in fact another explanation of the above mentioned results can scarcely be suggested. Nevertheless it is hard to visualize the substance being

Fig. 23

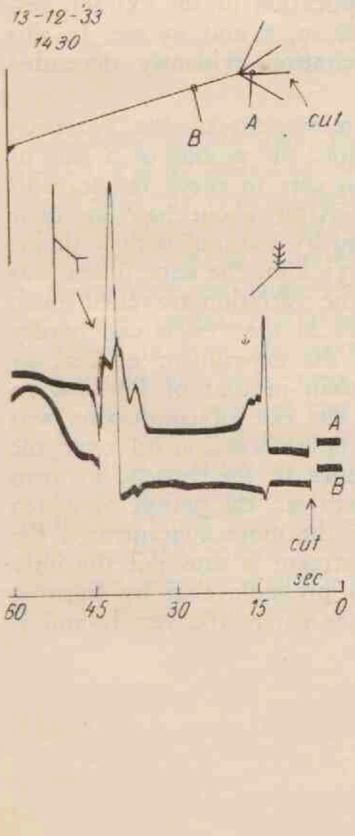
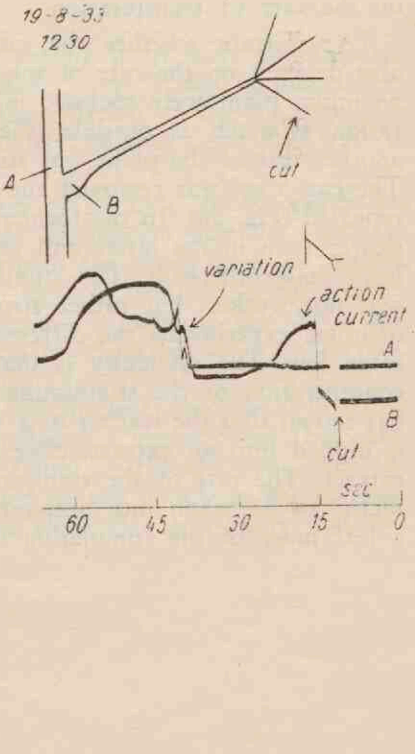


Fig 24



sucked in through the vessels as fast as the variation was found to travel downwards. Experiments were next made to ascertain whether the two are really correlated.

Acropetal Conduction. RICCA and SNOW have observed that in the stem the rate of excitatory conduction depends upon the rate of transpiration. They found that a stain, when sucked in by a cut shoot, is transported at the same rate as that with which the excitation is conducted when a stimulating extract is sucked in.

When the basal end of a detached leaf is dipped in the extract, an action current is aroused in the petiole, but it does not pass out to the pinnae and the leaflets. These react after the variation has reached the pinnae. According to our supposition the substance should also have reached them at that time. To determine if this were the case I added a concentrated solution of methylene blue to the extract. The moment the leaflets started closing, the petiole was cut through at its upper end and in 8 out of 16 cases the stain was found at the cut. As it was found in only a few vessels it may readily have been overlooked in the other 8 cases.

In another similar experiment 4 leaves were kept in damp air and another 4 in air of 50% humidity. The leaflets of the former closed 50, 35, 75 and 85 sec. after application of the extract, respectively. Those of the latter after 10, 20, 8 and 13 sec. In this experiment the conduction of the excitation evidently depended on the rate of transpiration.

To ascertain whether the rate of movement of the variation also depends on the rate of transpiration, the pinnae of a leaf of an intact plant were enclosed in a glass case to check the transpiration as much as possible (Fig. 25). After about half an hour another leaf of the plant was stimulated by burning with a flame. The case then was removed and after 35 min. the experiment was repeated (Fig. 26). In the former case the variation travelled much slower than in the latter and the water in the vessels can hardly have done otherwise. The stimulus in the experiment of Fig. 25 was not weaker, but rather stronger than in that of Fig. 26. In identical experiments the difference in the rate of conduction was often less. The difficulty is that one never has control over the concentration of the stimulating substance in the vessels. FITTING has shown that the leaflets of a detached leaf, the petiole of which is dipped into an extract, close sooner the more concentrated the extract. The rate of the transpiration stream is thus not the only factor to be taken into account. It is probable that by burning a leaf more of the stimulating substance enters the vessels and is

Fig. 25

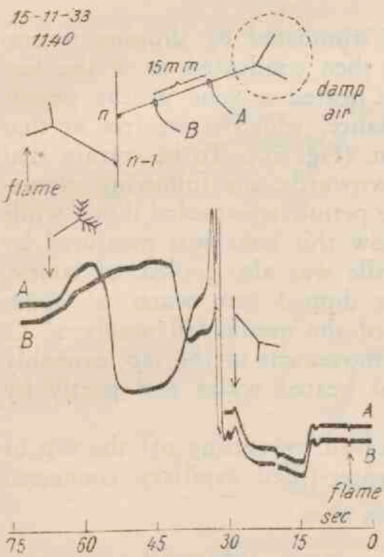
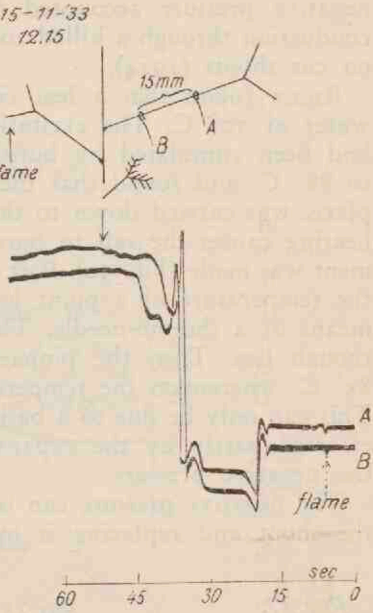


Fig. 26



transported to the stem, than by cutting it. A more concentrated extract will be sucked up by the other leaves. It can be thus understood that the interval between the reaction of the main pulvini and the closure of their leaflets may depend upon the way in which the plant is stimulated.

Basipetal Conduction. The part of RICCA's theory which has been most generally attacked is the assumption that the substance is sucked in from the wound through the pinna and the petiole. This could be caused only by a negative pressure in the vessels. This negative pressure can be lessened by cutting the shoot and placing it in a dish with water and by checking the transpiration.

A leaf was stimulated by burning (Fig. 27). After this the stem was cut below the main pulvinus and the leaf was placed in damp air. Some hours later it was stimulated a second time (Fig. 28). This time the variation was not found at the electrode. Yet the substance must have reached the top of the petiole as the conduction by action was observed at the electrode. It is likely that this is due to the expansion of the sap upon burning the leaflets. The result of this experiment confirms SNOW's supposition that lack of negative pressure accounted for his failure to get any basipetal conduction through a killed zone of the petiole. SNOW experimented on cut shoots (1924).

RICCA found that a leaf can be stimulated by dipping it into water at 70° C. The excitation is then conducted as if the leaf had been stimulated by burning. I heated a zone of the petiole to 88° C. and found that the substance, which is set free at that place, was carried down to the stem. (Fig. 29). To be certain that heating causes the sap to move downwards, the following experiment was made (Fig. 30). Part of the petiole was cooled down while the temperature of a point just below this zone was measured by means of a thermo-needle. The needle was also cooled, of course, though less. Then the pinnae were dipped into water of about 85° C. whereupon the temperature of the needle fell nearly 2° C. This can only be due to a basipetal movement of the sap, probably effected partly by the expansion of heated water and partly by the negative pressure.

The negative pressure can be observed by cutting off the top of the shoot and replacing it by a water-filled capillary connected

Fig 27

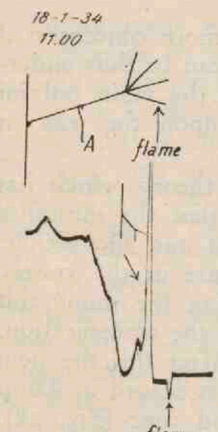


Fig 28

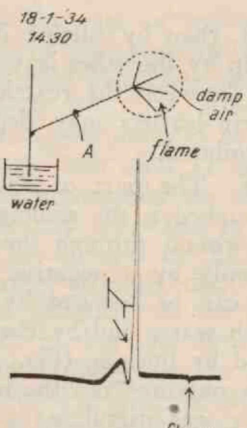


Fig 29

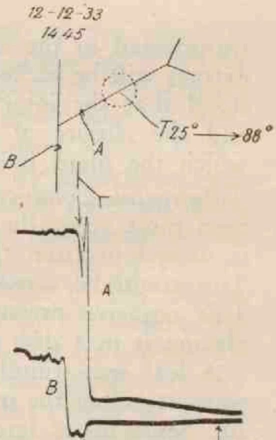
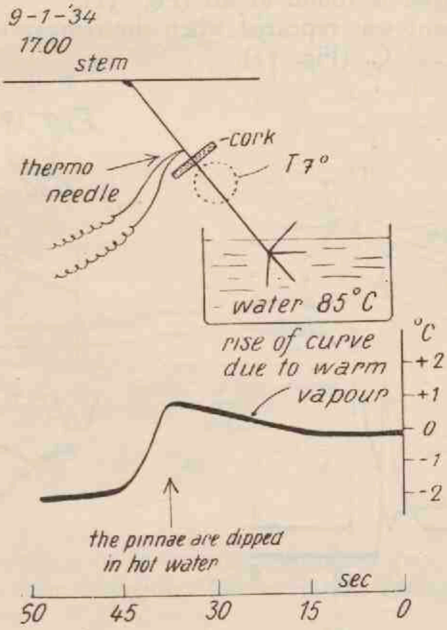


Fig. 30



with a piece of bicycle-valve tubing. In most cases the water was sucked in.

It is quite possible that the roots of potted plants are in a worse condition than those of plants, rooted in the ground. I do not know whether the pressure in the vessels of the latter is ordinarily positive or negative.

Positive pressure inside a potted plant can be obtained by heating its roots to about 40° C., while the air is damp. When a positive pressure has been established in this way, it often can be detected by the abnormal appearance of the younger leaves, the pinnae being curved and even twisted, the leaflets bending their tips downwards. This remarkable appearance of the plant may be due to the change of pressure from negative to positive as a result of which different torsioneffects develop.

When such a plant is stimulated by wounding, the excitation is hardly ever conducted to the other leaves, and at the electrodes on the petiole of the stimulated leaf the variation is found to be lacking or to be smaller and to travel at a lesser rate. In some cases no variation is found at all (Fig. 31).

The experiment was repeated when the temperature of the roots had fallen to 22° C. (Fig. 32).

Fig 31

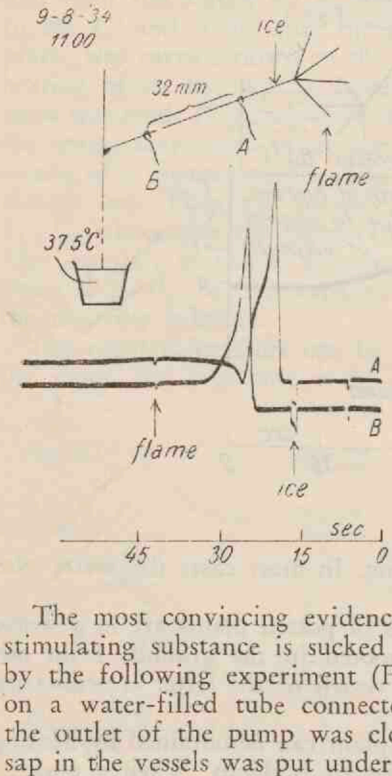
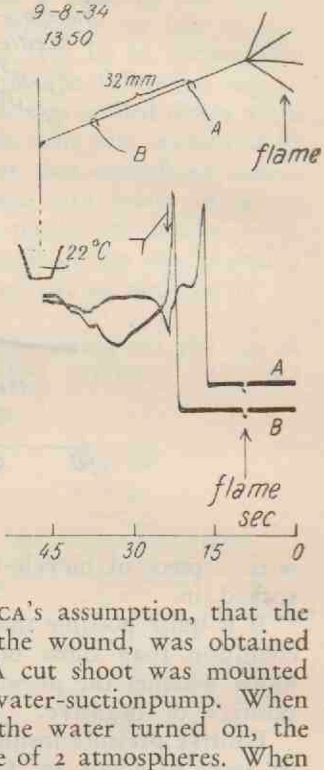


Fig 32



The most convincing evidence for RICCA's assumption, that the stimulating substance is sucked in from the wound, was obtained by the following experiment (Fig. 33). A cut shoot was mounted on a water-filled tube connected to a water-suctionpump. When the outlet of the pump was closed and the water turned on, the sap in the vessels was put under a pressure of 2 atmospheres. When one of the leaves was now stimulated with a flame, the burned leaflets only reacted. In this experiment the action did not travel through the entire pinna [1]). When afterwards the outlet of the pump was opened, the pressure became negative, and shortly thereafter the remaining open leaflets of the stimulated pinna closed in pairs. Thereupon the petiole was reached by the substance and

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[1]) From other observations it seems possible to ascribe this to the age of the leaf.

Fig 33

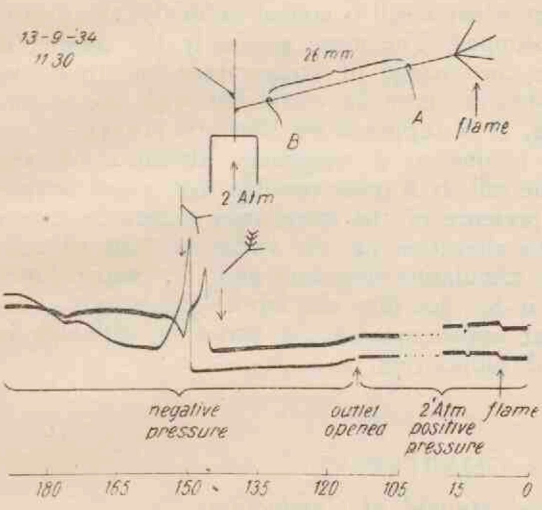
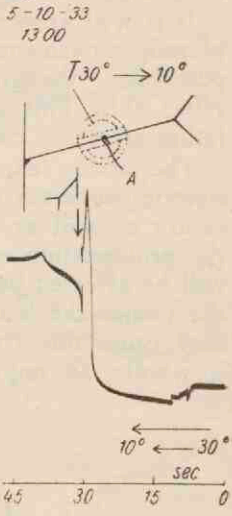


Fig 34



the action was aroused in it. It was conducted past the electrodes and affected the main pulvinus. The variation followed the action current.

As has been shown in all experiments the appearance of the variation depends upon the transpiration stream. It is certainly not due to a slow form of conduction by action of living cells as has been suggested by Umrath (1928).

The electrical variation must, however, be due to some change in the cells under the electrode. These cells may be identical to those which conduct the action. It is remarkable that, though the electric phenomena enable us to study the conduction by action of living cells, the anatomical path of this conduction is still unknown. Bose supposes the phloem to be the path of conduction (1925). I have made no investigations into this matter.

The way in which the potential is changed in a part of the petiole, while it is being cooled, is shown in Fig. 34. The first top of the curve is probably identical with the action current. The second part is reminiscent of the variation.

In a few similar experiments a part of the petiole was cooled gradually in the course of some minutes. The potential of the electrode in the cooled part remained constant during the cooling until the treatment itself caused stimulation. It then changed as in the experiment of Fig. 34.

It is well known that when a cell is cooled suddenly, the protoplasmic movement is stopped. The more gradually the cooling is performed, the greater the change of temperature has to be to effect this. This phenomenon may be correlated with the stimulating effect of cooling, as is supposed by UMRATH (1934).

The action current is due to a temporary alteration of the electric properties of the cell. It is quite possible that a low temperature as well as the presence of the stimulating substance causes the prolongation of this alteration i.e. the variation. Not all cells will be affected by the stimulating substance and they will recover the sooner the less of it has got into the sap of the vessels. This may cause the irregular appearance of the variation, although it is wholly an unproved supposition.

CHAPTER VI.

A Third Method of Conduction.

In some experiments a leaf was stimulated by cutting a pinna. Occasionally the main pulvinus was observed to react almost at once, i.e. after 1—3 sec. The fall of the leaf preceded the closure of the basal leaflets of the cut pinna. Neither an action current nor a variation was found to accompany the conduction (Fig. 35). They follow it and travel through the petiole in their usual way.

It has been mentioned above that the action of a pulvinus, which has been stimulated mechanically, can pass out to the petiole. Sometimes the same can be observed in these experiments. The pulvinus is then stimulated by some unknown change brought about by cutting a pinna, and the action, being thus aroused in it, is conducted acropetally. The basal electrode therefore is the first to react, whereas the variation moves basipetally (Fig. 36). UMRATH (1928) was deceived by this phenomenon. Since he led off from only one electrode on the petiole, he did not observe that the action is conducted up instead of downwards. He concluded from the appearance of the action current, that this rather fast method of conduction is effected by the action of some special cells, which can be stimulated in no other way than by cutting a pinna or a petiole.

As this method of conduction was most frequently observed in plants with little or no negative pressure in the vessels, it seems

.g 35

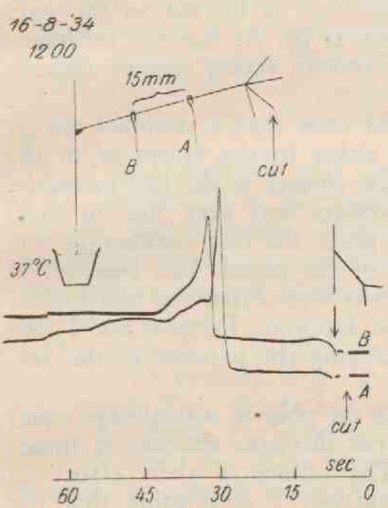


Fig 36

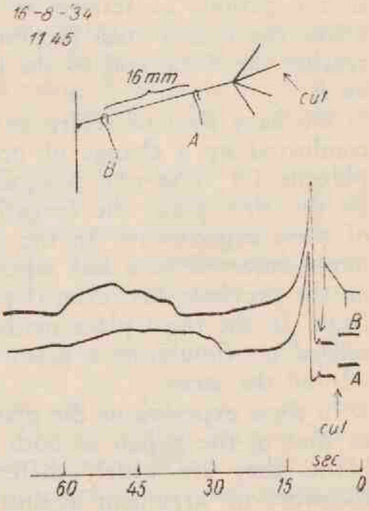
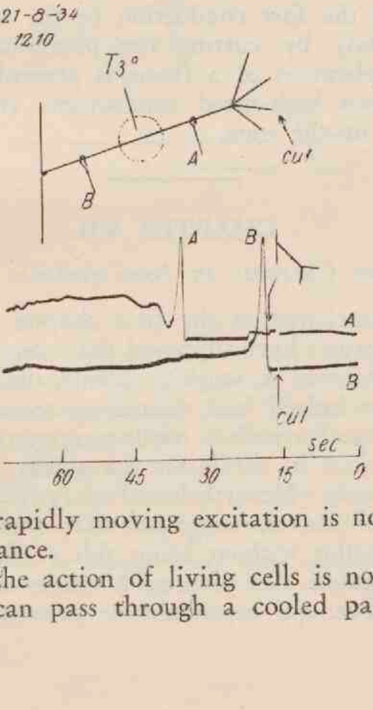


Fig. 37



probable that the rapidly moving excitation is not due to the movement of a substance.

Conduction by the action of living cells is not probable either, as the excitation can pass through a cooled part of the petiole, through which the action is not conducted (Fig. 37). The action in the petiole, as started at the pulvinus, is limited to the part below the cooled zone (action current at B). At last the substance reaches the distal end of the petiole and an action current appears at A.

We have then to return to the old view that a stimulus can be conducted by a change of pressure either in the xylem or in the phloem. Of these two possibilities the former is the less probable. In the first place the (negative) pressure was very low in some of these experiments. In the second place the fast conduction was never observed in a leaf when part of the petiole had been killed on the previous day, even if the leaflets were expanded and looked fresh. In the third place neither MAC DOUGAL, FITTING nor I succeeded in stimulating a plant by changing the pressure in the vessels of the stem.

In these experiments the pressure in the phloem was always equal to that in the xylem as both were cut through, whereas in intact plants they are widely different. The failure of these efforts is therefore no argument against HABERLANDT's assumption that the stimulus can be conducted through the "Schlauchzellen".

The "high-speed conduction" in the stem (SNOW, 1924) has much in common with the fast conduction in the leaf which was discussed above. Only by cutting the plant can both be found. Although the application of a flame is generally a more efficient stimulus, these two high-speed conductions are not obtained by burning the leaf or the stem.

CHAPTER VII.

Action Currents in Non-sensitive Plants.

It has been mentioned in the first chapter that until recently nearly all investigators have supposed that conduction of excitation such as can be observed in sensitive plants, does not exist in other plants. The latter indeed had, according to scientific opinion of earlier times, no use for such a conducting system. In itself, this is not remarkable, since in the latter the action can be detected by electric changes only. Nevertheless FEE (1858), in a treatise on the movements of leaves, suggested that some plants might be sensitive to a stimulus without being able to show it: "Aussi pensions nous qu'il existe des plantes, à tissues aussi excitables que ceux de la Sensitive, qui cependant ne peuvent se mouvoir faute d'organes appropriés aux mouvement. Ce n'est pas assez que d'avoir la faculté, il faut encore avoir l'instrument" [1]).

Bose has led off action currents from plants, which do not react in any visible way to the stimuli to which he subjected them.

Umrath did the same (1929), but some of these potential changes differ greatly from the action currents which I have found in *Mimosa*.

As I had observed that wounding *Mimosa* causes a substance to be transported in the vessels, which by its stimulating effect, conducts the excitation without the assistance of living cells, I thought it desirable to ascertain whether the potential changes in other plants are real action currents, due to conduction by action of cells.

The stem of *Vitis discolor* can be stimulated with ice. The action is propagated at a rate of 9 mm. per sec., up as well as downwards (Fig. 38). It was never observed to pass out to another internode. When the same internode was stimulated another time, after 1,5 min., the action travelled more slowly. When the interval between two stimuli was only 20 sec., the action was not conducted at all.

Fig. 38

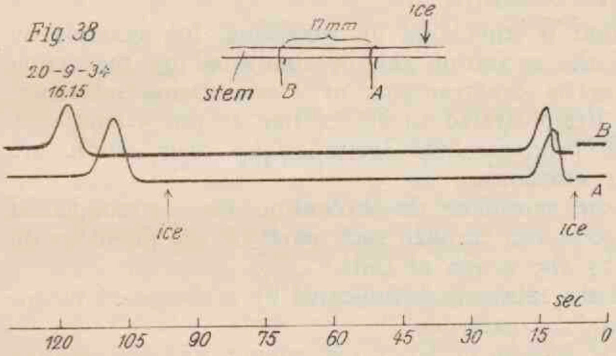


Fig 39

Also in the tendril of *Vitis gongylodes* an action current was found when it was stimulated with ice. When the tendril was cut, the potential change was much like the variation, which in *Mimosa* is due to the stimulating substance (Fig. 39).

The question whether the electric phenomena in *Vitis* may be identified with those in *Mimosa* remains to be investigated.

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1) And he added: „Ainsi le phoque (seal), aussi intelligent peut-être que le chien, ne peut, faute de pieds, s'éloigner du bord de la mer, n'accomplissant que des actes peu nombreux, purement instinctifs".

SUMMARY.

A. When *Mimosa pudica* is stimulated without being wounded, for example by applying a drop of water at less than 10° C., the excitation is conducted by the action of living cells and accompanied by potential changes, referred to as action currents.

The action of the cells can be propagated through the stem, the petioles, the pinnae and the pulvini. In most plants it is stopped at several places, for example at the transition from pinna to secondary pulvinus.

The rate at which it is conducted depends upon the temperature. It does not pass through a killed part of the petiole nor through a zone which is cooled to approximately 5° C.

The conduction by action of cells naturally depends upon the condition of these cells. The action is best conducted in damp air in a young shoot.

The conduction by living cells may be identified with BALL's "rapid conduction" in the stem and with UMRATH's "slow conduction" in the leaf. Most of BOSE's work is related to it. He observed the action current and found most of the above mentioned properties of this kind of conduction.

B. When a plant is stimulated by wounding, for example by burning a leaflet, the excitation can be conducted by the action of cells as well as by the transport of a stimulating substance, which RICCA has demonstrated to be set free at the wound.

The cells which propagate the excitation by their action, are stimulated by this substance.

By means of the substance the excitation can be conducted through a killed zone and through such parts of the plant, as do not propagate it by the action of cells.

The presence of the substance is indicated by a change of potential, referred to as "the variation".

The substance is sucked in from the wound by the negative pressure in the vessels, and is transported by the transpiration stream. By changing the pressure from negative to positive it can be prevented to be sucked in.

C. When a plant is stimulated by cutting a pinna, the excitation can be conducted by the action of cells, by the transport of the stimulating substance, and also by a third, very fast mechanism of conduction by which only the main pulvinus is affected. It is not accompanied by potential changes. It was observed in young leaves, especially in damp air.

It passes a cooled zone of the petiole but was never found to be conducted through a killed part.

It may be comparable to Snow's "high-speed conduction" in the stem, and to Umrath's "fast conduction" in the leaf.

The probability of a relation between Haberlandt's "Schlauchzellen" and this fast conduction has been discussed.

No mechanism of conduction should be referred to as "normal conduction".

The author wishes to take this opportunity to thank Prof. F. A. F. C. Went, at whose suggestion this work was commenced, and Prof. V. J. Koningsberger, the present Director of the Institute, for their valuable aid and criticism.

BIBLIOGRAPHY.

1. Ball, N. G., Rapid Conduction of Stimuli in Mimosa pudica. The New Phytologist, 26, p. 148. (1927).
2. Borzi, A., L'Apparato di moto delle Sensitive. Rivista di Scienze Biol. 4. (1899).
3. Bose, J. C., An Automatic Method for the Investigation of Velocity of Transmission of Excitation in Mimosa. Phil. Trans. B, 204, p. 63. (1914).
4. ——— and G. P. Das, Physiological and Anatomical Investigations on Mimosa pudica. Proc. Roy. Soc. B, 98, p. 290. (1925).
5. ——— The Nervous Mechanism in Plants. London. (1926).
6. Brücke, E., Ueber die Bewegungen der Mimosa pudica. Archiv f. Anatomie und Physiologie, 1848, p. 434.
7. ——— Die Interzellularräume des Gelenkwulstes der Mimosa pudica. Sitzber. Wien. Akad. Math. Nat. Kl. II, 50, p. 203. (1865).
8. Burnett, G. and H. Mayo, Observations upon the Motion of the Leaves of Mimosa pudica. Quart. J. Sc. Lit. Art. New Series, III, p. 76. (1827).
9. Candolle, A. P. de, Physiologie végétale. p. 866. (1832).
10. Clarke, see Hooke.
11. Cunningham, D. D., The Causes of Fluctuations in Turgescence in the Motor Organs of Leaves. Ann. Roy. Bot. Garden, Calcutta. 6, part I. (1895).
12. Desfontaines, R. L., see de Candolle.
13. Dixon, H. H., Transmission of Stimuli in Plants. Nature, 114. (1924) and Nature, 115. (1925).
14. Dutrochet, M. H., Mémoires pour servir à l'histoire anatomique et physiologique des végétaux et des animaux. Cap. 9. Paris. (1837).
15. Fay, C. F. du et H. L. du Hamel, Observations sur la Sensitive. Mém. de l'Acad. d. Sc. Paris. (1736).
16. Fée, A. L. A., Mimosa pudica. Mémoire physiologique et organographique sur la Sensitive et les plantes dites sommeillantes. Mém. Soc. Mus. hist. nat. Strasbourg. 4, p. 69. (1850).

17. ——— Notices sur les plantes dites sommeillantes et en particulier sur Porliera hygrometrica. Bull. Soc. Bot. France. 5, p. 451. (1858).
18. Fitting, H., Weitere Untersuchungen zur Physiologie der Ranken. Cap. 5. Jahrb. wiss. Bot. 39, p. 424. (1904).
19. ——— Untersuchungen über endogene Chemonastie bei Mimosa pudica. Jahrb. wiss. Bot. 72, p. 700. (1930).
20. Haberlandt, G., Das Reizleitende Gewebesystem der Sinnpflanze. Leipzig. (1890).
21. Harris, E. S. and W. J. V. Osterhout, The Death Wave in Nitella. II. Applications of Unlike Solutions. J. Gen. Physiol. 12, p. 355. (1929).
22. Herbert, D. A., Anaesthesia in Plants. Philippine Agricult. 11, No. 5. (1922).
23. Hill, S. E. and W. J. V. Osterhout, Salt Bridges and Negative Variations. J. Gen. Physiol. 13, p. 547. (1930).
24. Hofmeister, W., Die Lehre von der Pflanzenzelle. p. 315, Leipzig (1867).
25. Hooke, R., Micrografia. London. (1667).
26. Kabsch, W., Anatomische und physiologische Untersuchungen über einige Bewegungserscheinungen im Pflanzenreiche. Bot. Zt. 19, p. 362. (1861).
27. Linsbauer, K., Ueber Reizleitungsgeschwindigkeit und Latenzzeit bei Mimosa pudica. Wiesner Festschrift. p. 396. (1908).
28. ——— Zur Kenntnis der Reizleitungsbahnen bei Mimosa pudica. Ber. D. Bot. Ges. 32, p. 609. (1914).
29. ——— Ueber die Interferenz der Stossreizen und über Ermüdungserscheinungen an Blattgelenken von Mimosa pudica. Jahrb. wiss. Bot. 62, p. 283. (1923).
30. Mac Dougal, D. T., The Mechanism of Movement and Transmission of Impulses in Mimosa and other „Sensitive" Plants: A Review with some Additional Experiments. Bot. Gaz. 22; p. 293, (1896).
31. Meyen, F. J. F., Pflanzenphysologie. p. 516 seq. (1839).
32. Pfeffer, W., Physiologische Untersuchungen: Mimosa pudica. Leipzig (1873).
33. ——— Ueber Fortpflanzung des Reizes bei Mimosa pudica. Jahrb. wiss. Bot. 9, p. 308. (1874).
34. Ricca, U., Solution d'un problème de physiologie: la propagation de stimulus dans la Sensitive. Arch. Ital. d. Biol. 65. (1916).
35. ——— Transmission of Stimuli in Plants. Nature, 117, p. 654. (1926).
36. Sachs, J., Handbuch der Experimental Physiologie. p. 479. Leipzig. (1865).
37. Seidel, K., Versuche über die Reizleitung bei Mimosa pudica. Beitr. z. allg. Bot. 2, p. 557. (1923).
38. Snow, R., Conduction of Excitation in Stem and Leaf of Mimosa pudica. Proc. Roy. Soc. B, 96, p. 349. (1924).
39. ——— Conduction of Excitation in the Leaf of Mimosa Spegazzinii. Proc. Roy. Soc. B, 98, p. 188. (1925a).
40. ——— Transmission of Stimuli in Plants. Nature, 115, p. 82. (1925b).
41. Stern, K., Elektrophysiologie der Pflanzen. Berlin. (1924).
42. Umrath, K., Ueber die Erregungsleitung im Blatte von Mimosa pudica. Sitzber. Wien. Akad. Math. Nat. Kl. I, 134, p. 29. (1925a).
43. ——— Ueber die Erregungsleitung bei Mimosen Sitzber. Wien. Akad. Math. Nat. Kl. I, 134, p. 189. (1925b).

44. ——— Zur Theorie der elektrischen Erregung. Biol. Gen. 1, p. 396. (1925c).

45. ——— Ueber Refraktärstadien. Zs. f. Biol. 87, p. 85. (1927).

46. ——— Ueber die Erregungsleitung bei sensitiven Pflanzen, mit Bemerkungen zur Theorie der Erregungsleitung und der elektrischen Erregbarkeit im. Allgemeinen. Planta, 5, p. 274. (1928).

47. ——— Ueber die Erregungsleitung bei höheren Pflanzen. Planta, 7, p. 174. (1929).

48. ——— Der Einflusz der Temperatur auf das Elektrische Potential, den Aktionsstrom und die Protoplamaströmung bei Nitella mucronata. Protoplasma, 21, p. 329. (1934).

A general survey on the subject can be found in:

Dassen, M., Verhandeling ter beantwoording van de vrage: Wat weet men met zekerheid van de bewegingen die men aan de bladen van vele planten waarneemt? Nat. Verhand. d. Holl. Maatschappij d. Wetenschappen Haarlem. Oct. 22. (1835).

Goebel, K., Die Entfaltungsbewegungen der Pflanzen und deren teleologischen Deutung. Cap. 9. Die Sensitiven. Jena. (1920).

STELLINGEN

I.

De reactie is bij de seismonastie geen maat voor de prikkelbaarheid.

II.

De theoretische voorstelling van de plagiotropie, zoals die door Metzner werd gegeven, houdt geen rekening met het feit dat de geonastie (Rawitscher) onder invloed van de lengtekracht langzaam toe- of afneemt, zodat ook de stand, waarin geotropie en geonastie in evenwicht zijn, met de tijd verandert.

III.

Uit een verandering van de water-permeabiliteit alléén kan geen volumenverandering van de cel resulteren.

IV.

De theorie van Selig Hecht over de correlatie van de lichtsterkte en de gezichtsscherpte bij insecten wordt door het experiment niet afdoende bewezen.

V.

Ten onrechte trekt Winterstein uit zijn proeven de conclusie dat de cH van het bloed en niet de CO_2 spanning de ademhaling reguleert.

Pflüger's Archiv, 234, 1934.

VI.

Het voorkomen van Nothomonokotylen onder de Polycarpicae wijst niet op een verwantschap van de Monokotylen met deze groep.

VII.

De geographische verspreiding van Mercurialis perennis wordt niet in de eerste plaats door het gehalte van de grond aan vrije kalk bepaald.

De Silva, J. of Ecology, 22, 1934.

VIII.

Er is geen grond om met Hasselbaum aan te nemen dat de Mycorrhiza van Empetrum voor de stikstofvoeding van deze plant van betekenis is.

Hasselbaum, Bot. Archiv 31, 1931.

IX.

Het is door de proeven van Menon niet bewezen dat de op appel en de op aardappel parasiterende schimmels éénzelfde protopectinase afscheiden; zijn proeven bewijzen echter wel dat de specialisatie op andere gronden moet berusten.

Menon, Ann. of Bot. 48, 1934.