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Investigation of Electric Potentials in Plants *

Badania nad potencjałami elektrycznymi u roślin

Исследования биоэлектрических потенциалов у растений

The electric potential differences in plants were measured by means of a Penick-bridge (PB) and Kaiser's electroencephalograph (EEG).

PB (Fig. 1) was constructed, using a 6Zh 1Zh penthode as the electrometer valve (2). A plate current $I_a = 10$ mikroA was given by a plate voltage $U_a = 6V$, heating current $I_ż = 100$ mikroA and grid voltage $U_s = -3V$. The plate current $I_a = 34$ mikroA was measured for $U_s = O$. The first grid of the valve 6Zh 1Zh was connected with the cathode, the second grid had the same potential as the plate, the third grid was grounded through a resistor $1.2 \cdot 10^8 \Omega$. The potential drop across this resistor was measured. The heating current was supplied by a d-c battery of large capacity. The zero position of the bridge was regulated by means of the 5kΩ and 38kΩ potentiometers, so that the galvanometer (type GES2-A2, sensitivity $5.4 \cdot 10^{-9}$A/div) showed no current without input voltage and was stable (zero drift 2—3 div/hour). The potentiometers of 1kΩ were used to adjust the zero position under working conditions. The relation between input voltage and current flowing through the galvanometer was plotted for different sensitivities. The voltages were measured as follows:

up to 17 mV with a precision 0.15 mV/div in the range (1)
„ „ 25 „ „ „ 0.20 „ „ „ „ (2)
„ „ 45 „ „ „ 0.30 „ „ „ „ (3)
„ „ 90 „ „ „ 0.75 „ „ „ „ (4)

Platinum wires were used as electrodes. On the plant under investigation the grounded electrode was below the measuring electrode,

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which was connected to the grid of the electrometer valve. The plant with the electrodes attached was placed in a Faraday cage. The EEG used in these investigations could detect a voltage in the range 0—200 mikroV, alternating in the range 0.3—70/sec.

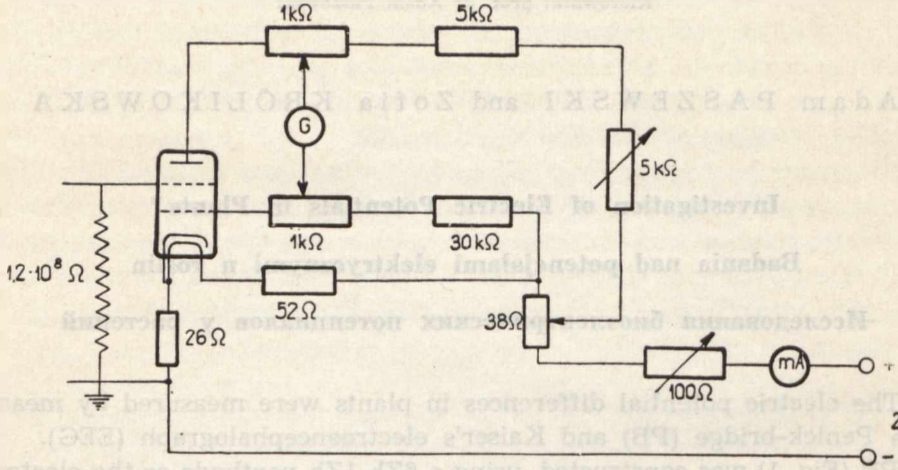


Fig. 1

THE EXPERIMENTAL METHODS

In measurements with the help of the Penick-bridge the plant-electrode contact was made as follows:

a. The electrodes were inserted into the plant, 2—3 mm deep

b. The electrodes were fixed to the plant, using leukoplast with cotton-wool soaked in water, or agar-agar between the plant and the electrode.

c. The electrodes were fixed on the supports and put in contact with the plants by the use of special manipulators.

a. Measurements with inserted electrodes

Twenty-nine measurements were made on *Pelargonium zonale* from 18th to 29th May 1960. The average room temperature was 18°C. After the insertion of the electrode into the plant, the monotonic changing potential (increasing or decreasing) was registered. This reached an end-value in 5 to 50 min. There were no potential jumps. The results are presented in Table 1.

The measurements from 1—12 were made on a plant of *Pelargonium zonale* with four lateral shoots, and the next on a *Pelargonium zonale* with one stem.

Table 1

No	1	2	3 (cm)	4 (cm)	5 (mV)	6 (min)	7 (mV)
1	18.VI	at the base of 2 successive leaves (Fig. 2)	10	4	33	30	26
2	18.VI	„ „ „ „ „ „ „	14	3	76	5	97
3	18.VI	„ „ „ „ „ „ „	15	3.5	63	8	97
4	20.VI	„ „ „ „ „ „ „	13	2	6	6	11
5	20.VI	between two branchings (Fig. 3)	5	6	21	30	— 11
6	20.VI	perpendicular in the stem (Fig. 4)	13	2	— 6	26	— 10
7	20.VI	„ „ „ „ „	0	10	— 49	17	— 90
8	21.VI	„ „ „ „ „	6	11	— 88	5	— 96
9	21.VI	„ „ „ „ „	7	10.5	—121	8	—135
10	21.VI	„ „ „ „ „	6	5.5	— 90	30	— 41
11	21.VI	at the base of the leaf and at the base of the lamina (Fig. 5)	14	9	66	28	95
12	22.VI	„ „ „ „ „ „ „	10	7	46	18	50

No. — the test number, 1 — the date, 2 — the position of the electrodes, 3 — the distance of the electrode from the ground, 4 — distance between electrodes, 5 — the potential value 10 sec after switching on the bridge, 6/7 — the value of the potential in mV after minutes.

In the 9th measurement, when the potential had the value — 135 m V, the surrounding air was heated 8°C above average. The potential then achieved the new value —108 mV, which returned to the previous value — 135 m V 10 minutes after the removal of the heater. The following measurements (13—21 and 22—26), referred to in Table 2, show the dependence of the potential on the distance between the electrodes. The symbols 1—7 have the same meaning as in Table 1.

Table 2

No	1	2	3 (cm)	4 (cm)	5 (mV)	6 (min)	7 (mV)
13	24.VI	perpendicular in the stem (Fig. 4)	3	14	31	9	48
14	24.VI	„ „ „ „ „	3	12	11	10	61
15	26.VI	„ „ „ „ „	3	10	12	14	63
16	24.VI	„ „ „ „ „	3	6	20	15	79
17	24.VI	„ „ „ „ „	3	4	11	13	91
18	25.VI	„ „ „ „ „	3	2	16	18	95
19	25.VI	„ „ „ „ „	3	1.5	3	19	91
20	25.VI	„ „ „ „ „	3	1	18	7	91
21	25.VI	„ „ „ „ „	3	0.5	48	7	81
22	26.VI	„ „ „ „ „	3	8	18	19	48
23	26.VI	„ „ „ „ „	3	5	30	8	67
24	26.VI	„ „ „ „ „	3	3	9	18	71
25	26.VI	„ „ „ „ „	3	2.5	15	25	86
26	26.VI	„ „ „ „ „	3	1.5	31	35	60

The influence of chloroform ($CHCl_3$) on the potential value. Test 27: electrodes between the base of the leaf and the base of the lamina, distance above the ground 14 cm, distance between electrodes 8 cm. The potential 80 mV was reached in 4 min. after switching on the PB. Then a phial of chloroform was placed below the leaf, causing a decrease of the potential to the value 75 mV. After the removal of the phial of chloroform the potential reached the previous value 80 mV in 30 min.

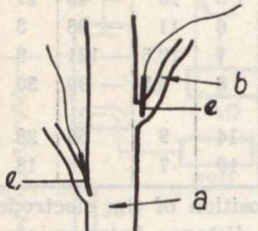


Fig. 2 a — stem, b — petiole, e — electrodes

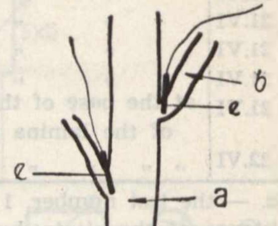


Fig. 3 a — stem, b — branch, e — electrodes

Test 28: method as in the previous experiments. The distance between the electrodes 6.5 cm, the height above the ground 20 cm. After 3 drops of $CHCl_3$ were pipetted on to the surface of the leaf, the potential decreased to the value 18 mV in 9 min.

b. Measurements using electrodes fixed with leukoplast

These measurements, summarised in Table 3, were carried out from 7th to 9th July 1960. *Phaseolus vulgaris* 6 weeks old, with 4 leaves, was used. The average temperature was 20°C. The electrodes were fixed on to the stalk (Fig. 3).

c. Measurements with electrodes fixed on supports, attached to the plants by means of manipulators. (10 July — 22 July 1960)

The following plants were used: *Phaseolus vulgaris* and *Drosera rotundifolia*.

Measurements using *Phaseolus wulgaris:* in the tests 1—5 the electrodes were fixed on the stalk. The potential difference was measured

a) between the base of the plant and the first node, and

b) between the base of the plant and the second node.

Table 3

1	2	3	4	5	6	7
1	wet cotton — — wool	7	5	— 8 mV to — 18 mV in 10 min.	— 10 mV in foll. 20 min.	—
2	electrodes were put directly in contact with stalk	6.5	7	changes from 2 to 5 mV in 10 min.	8 mV in foll. 26 min.	—
3	agar in distilled water (2%)	8.5	5.5	6 — 1, 5 mV in 8 min; in-creased to 6 mV in foll. 5 min.	3 mV in foll. 3 min.	—
4	agar in tap water (2%)	7.5	7			the changes from — 5 mV to—9 mV with the amplitude of 1 mV

The symbols: 1 — test number, 2 — placed between electrode and plant, 3 — distance electrode-ground (cm), 4 — distance between electrodes (cm), 5 — time changes of voltage, 6 — voltage value, 7 — changing voltage.

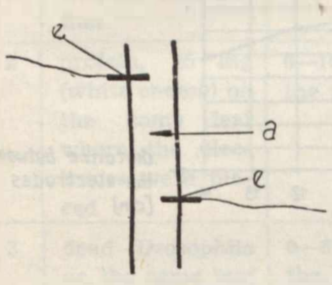


Fig. 4 a — stem, e — electrodes

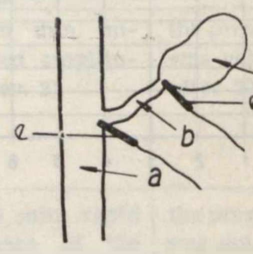


Fig. 5 a — stem, b — petiole, c — leaf, e — electrodes

The voltage changed with the amplitude up to 4 mV. The voltage values were higher in all cases a) in comparison with b) (Fig. 10).

In test 6 the electrodes were fixed on the stalk between the base of the plant and the first node. The distance between the electrodes was 6.5 cm. The measurements of the voltage changes lasted from 9 a.m. 19th July to 10 a.m. 20th July, 1960. The average values of the voltage over one hour are given in the diagram. The maximal frequency and amplitude of changes were observed from 4 p.m. to 9 p.m. (the amplitude

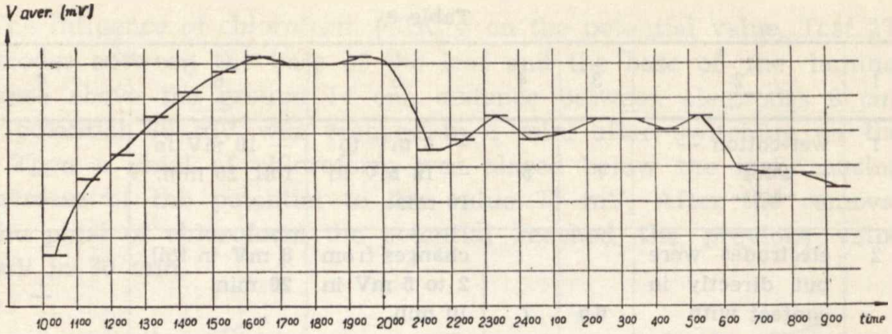


Fig. 6

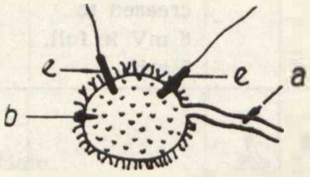


Fig. 8 a — petiole, b — leaf, e — electrodes

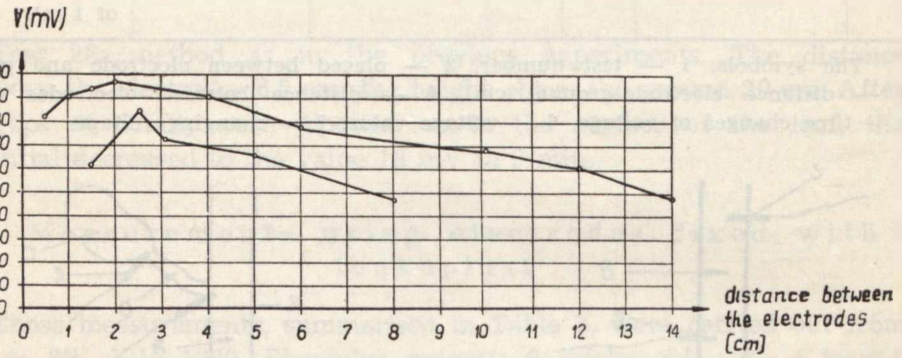


Fig. 9

Table 4

1	1	3	4	5
7	4	in 10 min. from 4 to 6 mV, amplitude 15 mV	45 mV after 10 minutes	—
8	5	from O to—4 mV, amplitude up to 2 mV		dead fruit-fly *Drosophila* has no effect on the potential changes

The symbols: 1 — test number, 2 — distance between electrodes (mm), 3 — changes of voltage with time, 4 — steady value of voltage, 5 — on the surface of the leaf.

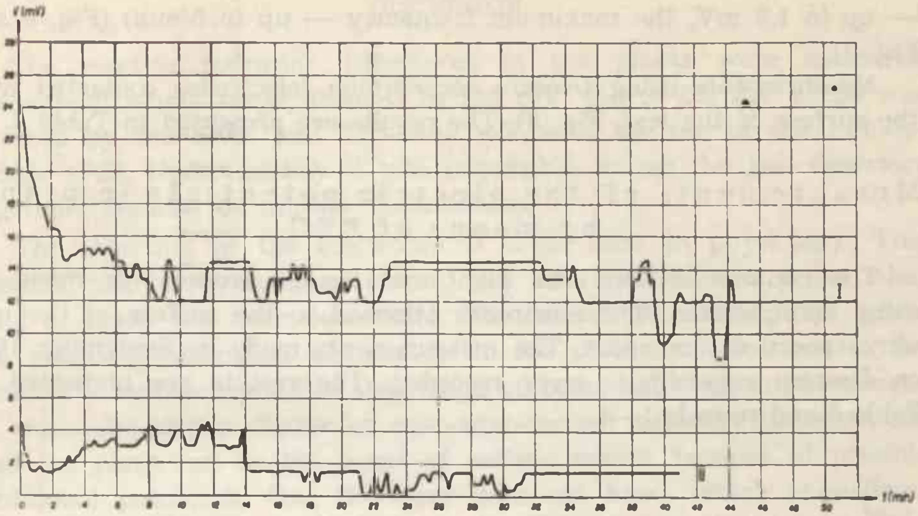


Fig. 10

Table 5

1	2	3		
1	without stimulation	all the time as on the record No. 1		
2	protein, 25 mg (white cheese) on the same leaf where the electrodes were placed	0—10 min. as on the record No. 1	10—20 min. increased amplitude (rec. 2)	the previous state was achieved after 20 min.
3	dead *Drosophila* on the same leaf where the electrodes were placed	0—6 min. as on the record No 1	6—36 min, rapid increase of the amplitude see record No. 1	the previous state was not achieved after 50 min.
4	dry sand, 25 mg, on the same leaf where the electrodes were placed	all the time as on the record No. 1		
4	dead fly *(Drosophila)* on another leaf	the distance between the leaf with the fly and the leaf with the electrodes — 60 mm. Reaction after 14 min, see record No. 4		

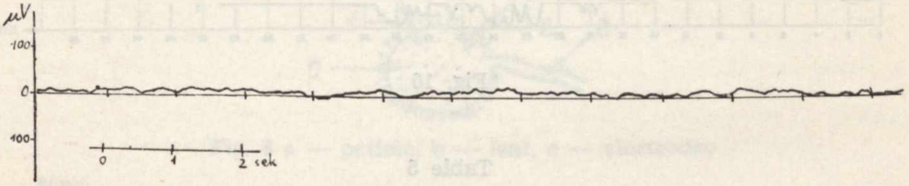
1 — test number, 2 — stimulation, 3 — potential changes in time

— up to 1.5 mV, the maximum frequency — up to 5/min) (Fig. 6 and Fig. 7).

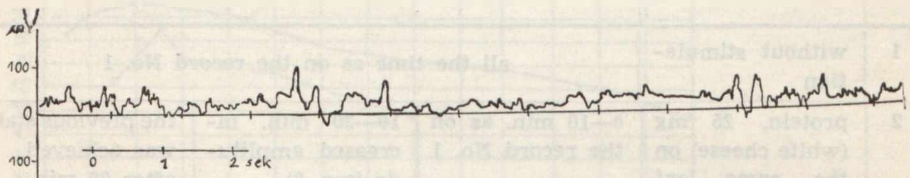
Measurements using *Drosera rotundifolia* (electrodes contacted with the surface of the leaf, Fig. 8). The results are presented in Table 4.

Measurements of the electric potentials in plants by means of EEG

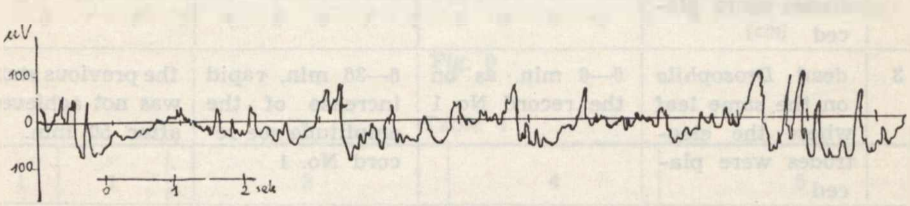
The contact between the plant and the electrodes was made by using manipulators. The electrodes attached to the surface of the leaf were spaced 5 mm apart. The measurements made in September 1960 on *Drosera rotundifolia* were recorded. The results are presented in Table 5 and records 1—4.



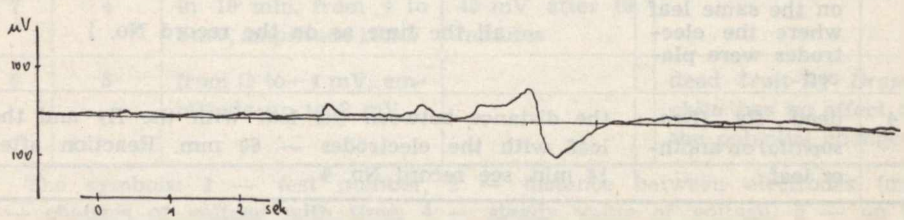
Record 1



Record 2



Record 3



Record 4

DISCUSSION

The electric potential differences in the plants were measured throughout whole range, from O to 150 mV; the 0—0.2 mV range was covered by the EEG, and 0.15—150 mV with the use of the Penick-bridge (PB). Unfortunately it was impossible to use the two detectors together, because of mutual disturbances.

The insertion of the electrodes is often used in physiology. This method can, however, cause errors. One does not know *a priori* what kind of tissue has been penetrated. This makes it difficult to relate the electro-physiological phenomena to the particular processes in the plant. The results are rather dependent on some pathological phenomena of traumatic origin. Water or agar-agar-contact between the electrode and the plant can be the cause of serious errors, because of possible additional potentials. One therefore does not know which phenomena are bioelectrical, and which from a physiological viewpoint are artefacts. The possibility still exists of damaging the surface tissue layer of the plant by the platinum contact electrode.

Drosera rotundifolia is an interesting object for electrophysiological investigations, because it does not require special treatment in laboratory conditions (high temperature and humidity) and it behaves normally, in contrast to *Mimosa pudica*, which loses its sensibility rather quickly. The measurements carried on with precision up to 1 mV had shown that the old leaf of *Drosera rotundifolia* had a positive potential while the young one had a negative potential, independent of the exciting of the plant by the dead fly. The course of the EEG records shows that a measuring apparatus with a sensitivity greater than 0.15 mV/div cannot record the potentials arising after stimulus by the dead fly. Only after the use of EEG could these potentials be measured. After the dead fly or the protein had been put on the leaf, potentials were registered by EEG. The record did not show any changes after dry sand had been put on the leaf. The sand had been heated and washed with HCl. It is not known if the potential changes are connected with the bioseismic motions caused by the bending of the *Drosera*-hairs, or with chemical processes. In both cases the currents in the *Drosera*-leaf are present in the living plant. In the case of mechanical shock the changes of the electric potentials in the plants are registered in some tenths of a second; in our experiments, however, the changes are recorded after some minutes (5).

Great caution is needed in interpreting the EEG record. The EEG is not adapted to the examination of plants. In particular the input resistance of EEG is low in comparison with the changeable resistance of plant tissues and this might cause artefacts.

We were not able to determine if changes of the potentials are caused by movements of the papillae, or by biochemical processes, which would be very interesting, if true. The movements of the papillae may cause changes in the contact of the electrodes and the changes may be recorded, because the EEG is a very sensitive apparatus.

We got records even when the fly and the electrodes were on different leaves. A similar stimulation of *Drosera* leaves with IAA was transmitted to another leaf at a distance of 60 mm in 2 min.

What is the relation of the potentials measured in milliV to potentials measured in microV, in so far as we were in fact able to observe microV potentials in plants?

It is probable that each stimulus at first causes microV potentials which may then become milliV or fade away still as microV. We think that the latent period could be studied by using microvolts records. In addition the microV curve might be regarded as a changing component of milliV oscillations.

On the basis of theoretical considerations we think that the existence of microV potentials in plants is very likely; also our measurements were not completely convincing.

As can be seen from the literature, electric potentials can be regarded as a summary indication of the physiological state of the plant. This is why examination of these phenomena is so important.

CONCLUSIONS

Measurements by means of a Penick-bridge.

The potentials measured by means of inserted electrodes were in the range of some tens of mV, while those measured by means of contact electrodes were in the range of 10—20 mV.

The potential of the stalk between the bases of two leaves (Fig. 2) was positive, as was the potential between the bases of the leaves and the base of the lamina (Fig. 5). The electrodes inserted at any point in the stalk of a one-stalked plant (Fig. 4) showed a negative potential, while the potential of the stalk in a plant with four shoots above the earth was positive. The potential of the stalk between two lateral shoots (Fig. 3) was negative. In the two series of measurements of potentials carried out on the stalk of *Pelargonium zonale*, regularity was observed. When the distance between the electrodes was increased from 1 cm to 2—2.5 cm the potential rose, and with a further increase of distance it diminished (Fig. 9).

The influence of chloroform on the size of the electric potential of the plant was discovered. The placing of a phial of chloroform under the leaf (electrodes in position as in Fig. 5) caused a drop in the potential. When chloroform was removed, the potential reached its original value after 30 min. After the direct application of chloroform to the leaf, the potential changed considerably (about 60%) and did not return to its original value.

If cotton-wool soaked in tap-water, or agar in tap-water was placed between the electrode and the plant, the potential was negative. If distilled water, or agar in distilled water, was applied as contacts, the potential was positive.

In the measurements of the potential of *Phaseolus vulgaris*, regularity was observed. The potential between the base of the stalk and the first node was greater than that between the base of the stalk and the second node.

The twenty four hour measurements of the potential carried out on *Phaseolus vulgaris* showed the maximum value of the potential and the greatest frequency and amplitude changes between 4 p.m. and 8 p.m. then a marked fall in the value of the potential, and a small increase about 3 a.m.

Measurements by means of EEG.

It is likely that the EEG records action potentials arising in *Drosera rotundifolia* in the digestive process if the records are not artefacts. We got also records, when the plant was stimulated by a fly on another leaf. 6—10 min after nutriment had been placed on the leaf, a marked increase in frequency and amplitude changes occurred. Putting sand on the leaf produced no change in potentials.

We feel that we are under a friendly obligation towards Professor W. Stein for having so kindly allowed us the use of the electroencephalograph of the Neurological Clinic of the Academy of Medicine, we would also like to thank mgr B. Darwaj for helping us to carry out the electroencephalographic recordings. Our thanks also go to the Botanical Committee of the Polish Academy of Sciences for their financial contribution.

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STRESZCZENIE

Pomiary potencjałów elektrycznych u roślin dokonywane były dwiema metodami: przy pomocy mostka Penicka (układ na lampie 6Ż, 1Ż pracującej w układzie elektrometrycznym) i przy pomocy elektroencefalografu Kaisera. Metoda mostkowa pozwalała na pomiary z dokładnością do mV, metoda elektroencefalograficzna do mikroV. Jako elektrody stosowane były druciki platynowe. W pierwszej fazie badań elektrody były wbijane w roślinę, następnie umocowywane leukoplastem, a między rośliną i elektrodą znajdowała się watka nasycona wodą lub agar, wreszcie elektrody były przytykane bezpośrednio do rośliny przy pomocy manipulatorów. Obiektami badań były: *Pelargonium zonale*, *Phaseolus vulgaris* i *Drosera rotundifolia*.

Przy pomiarach elektrodami wbijanymi po dołączeniu elektrod do mostka potencjał monotonicznie spadał lub wzrastał i ustalał się na pewnej wartości po czasie od 5 do 35 min.

Przy pomiarach elektrodami wbijanymi znaleziono zależność wielkości potencjału od odległości między elektrodami. Przy zwiększaniu odległości między elektrodami od 1 cm do 2—2,5 cm potencjał wzrastał, a przy dalszym oddalaniu elektrod malał. Działanie na liść par chloroformu lub bezpośrednie podanie chloroformu na liść powodowało spadek potencjału.

Przy stosowaniu między elektrodą a rośliną kontaktu w postaci agaru na wodzie destylowanej albo watki nasyconej wodą destylowaną potencjał był dodatni, natomiast w przypadku agaru na wodzie wodociągowej albo watki z wodą wodociągową — ujemny.

Potencjały mierzone przy pomocy elektrod stykowych były rzędu kilkunastu mV i zmieniały się z amplitudą do 4 mV, maksymalna częstość wahań wynosiła 5 na 1 min. Przy pomiarach elektrodami stykowymi zauważono, że potencjał między nasadą łodygi a pierwszym węzłem okazał się większy niż między nasadą łodygi a drugim węzłem.

Przeprowadzony dobowy pomiar potencjału wykazał maksimum wartości potencjału i największą częstość oraz amplitudę wahań w godz. od 16.00 do 20.00, potem spadek wartości potencjału i mały wzrost około godz. 3.00.

Pomiary przy pomocy elektroencefalografu były przeprowadzane na *Drosera rotundifolia.* Zmiany potencjału rzędu mikroV notowane były przez pisak elektroencefalografu. Przy położeniu na liść pokarmu elektroencefalograf rejestrował zmienną różnicę potencjałów (amplituda do 150 mikro V), po podaniu na liść piasku kwarcowego zapis pozostawał bez zmiany w stosunku do zapisu zmian różnicy potencjałów liścia nie podrażnionego.

РЕЗЮМЕ

Изучение биоэлектрических потенциалов у растений производилось с помощью мостика Пеника (Penick bridge). Измерительная установка выполнена на лампе 6Ж1Ж, работающей в электрометрическом режиме и при помощи электроэнцефаллографа Кайзера. Первый метод позволил регистрировать потенциалы в милливольтах, второй — в микровольтах. Биотоки отводились с помощью платиновых электродов. В первой части исследований электроды вкалывались в растение, во второй — прикреплялись с помощью лейкопласта, а для улучшения контакта между электродом и растением иногда помещался кусочек ваты, смоченной дестиллированной водой, или агар. В других случаях электроды приводились в в соприкосновение с растением при использовании микроманипуляторов.

Объектом исследований были *Pelargonium zonale* и *Drosera rotundifolia.* При измерениях с помощью вкалываемых электродов после присоединения электродов к электрометру потенциал постепенно уменьшался или возрастал и после 5 до 35 минут устанавливалась его какая-то средняя величина.

При измерениях с помощью вкалываемых электродов установлена зависимость между расстоянием между электродами и величиной разности потенциалов. При увеличении расстояния между электродами от 1 до 2,5 см. потенциал возрастал, а при дальнейшем увеличении расстояния — уменьшался.

Действие паров хлороформа на лист, или же его непосредственная подача на лист вызывали падение потенциала.

В случае наличия между электродом и растением агара на дестиллированной воде знак потенциала был положительный, а в случале агара на водопроводной воде — отрицательный.

Величина потенциалов, измеряемых с помощью стыковых электродов составляла от нескольких до нескольких десятков милливольт, они колебались с амплитудой 2 мв и максимальной частотой 5 периодов на минуту.

При измерениях с помощью стыковых электродов замечено, что разность потенциалов между основанием стебля и первым узлом ветвления оказалась выше чем между основанием и вторым узлом.

Проведенные измерения суточного хода потенциала показали, что его максимальная величина и частота а также амплитуда приходятся на время от 16,00 до 20,00 часов, затем наступает падение потенциала и небольшое увеличение около 3-х часов.

Измерения с помощью электроэнцефаллографа проведены на *Drosera rotundifolia*. Колебания биопотенциалов в микровольтах регистрировались автоматически. При нанесении пищи на лист были обнаружены колебания с амплитудой до 15 микровольт, а при подаче кварцевого песка таких колебаний не было.

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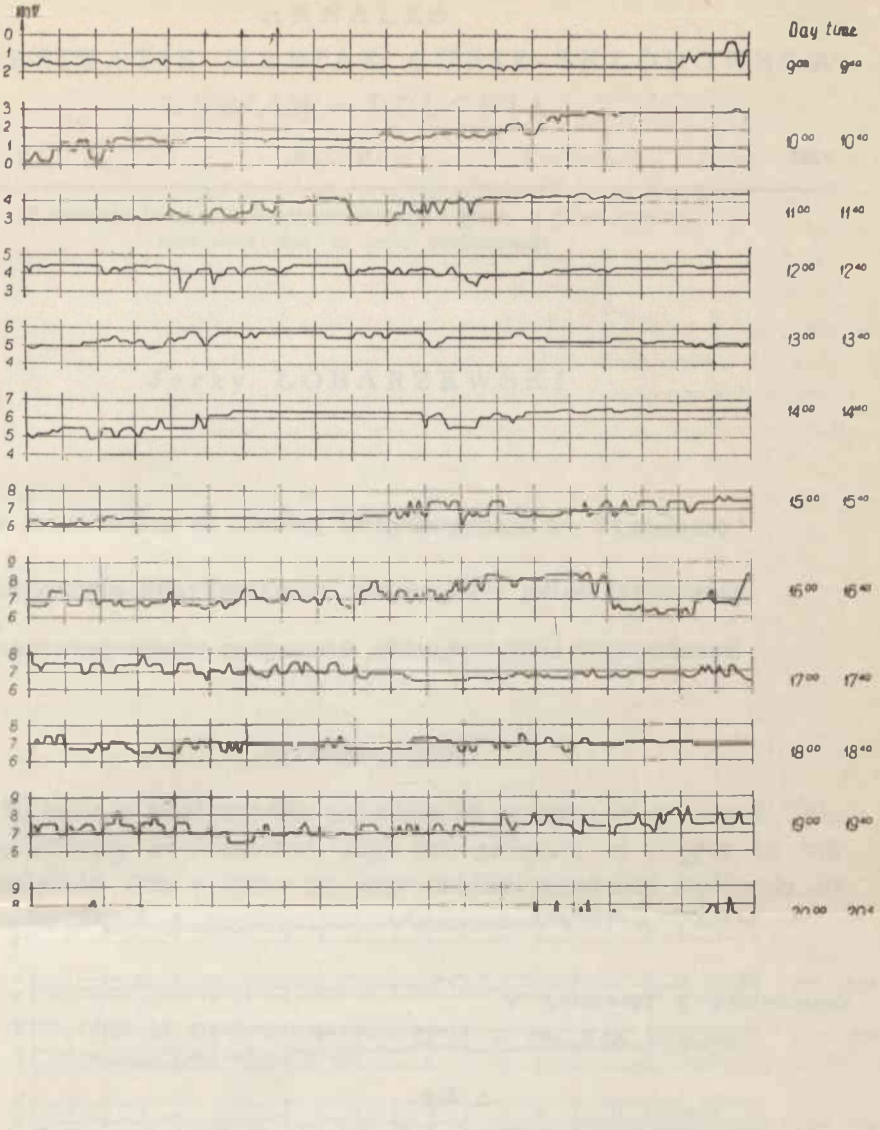
1100 + 120 odbitek. W-4 | Data otrzymania manuskryptu 11.9.61. | Data ukończenia druku 30.1.62.



mV
Day time
9⁰⁰ 9⁴⁰
10⁰⁰ 10⁴⁰
11⁰⁰ 11⁴⁰
12⁰⁰ 12⁴⁰
13⁰⁰ 13⁴⁰
14⁰⁰ 14⁴⁰
15⁰⁰ 15⁴⁰
16⁰⁰ 16⁴⁰
17⁰⁰ 17⁴⁰
18⁰⁰ 18⁴⁰
19⁰⁰ 19⁴⁰

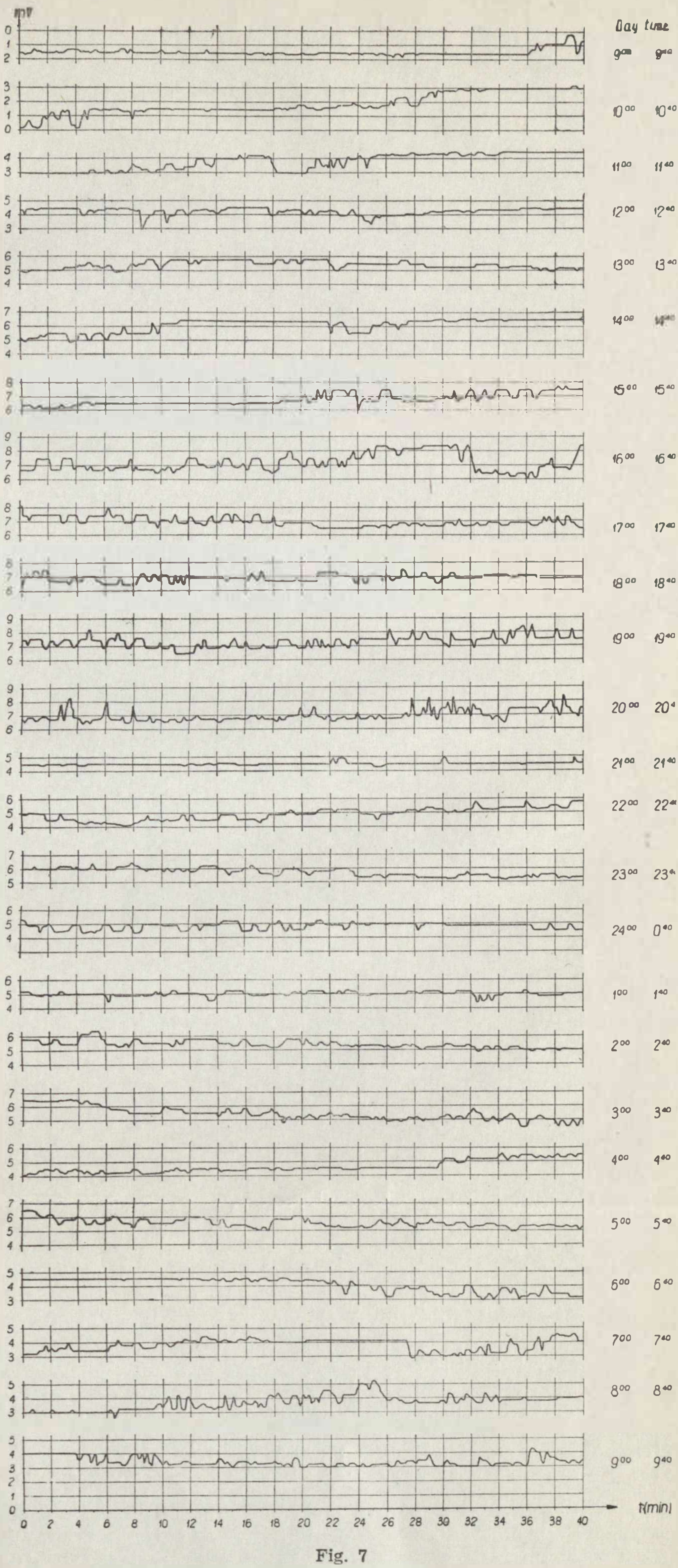


Fig. 7