

## An attempt to interpret some electrophysiological phenomena in plants in terms of Pflüger's laws.

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### Abstract

In *Lupinus* shoots an electrical stimulus (DC) produces a voltage wave identical in character to the action potential wave (AP) in stimulated simple plant cells or in nerves and muscles. This fact served as basis for investigations undertaken to establish whether the dependences, known in neurophysiology as Pflüger's laws of contraction, are also true in the case of plant stimulation. Experiments were performed both in a system analogous to that of Pflüger (Variant I), and in more varied system (Variant II). The validity of these laws in plants has been fully confirmed. Other investigations (Paszewski and Zawadzki 1973a, 1973b, 1974) showed that such laws as the strength-duration relation and the all-or-nothing law prove correct in plants.

### INTRODUCTION

Burdon-Sanderson (1882) noted the presence of biopotentials in the insectivorous plant *Dionaea muscipula*. The investigations carried out on plants responding to stimulation by rapid movements e. g. *Mimosa*, proved the existence of typical phenomena of excitability in plants (Sibaoka 1969). Sibaoka was one of the first scientists to connect the problems of plant excitability with the accompanying AP. A review of the research on excitability in plants is given by Pickard (1973).

The methods of investigation of AP — applied in animal physiology for a long time — have not been accepted in plant physiology. Thus, under an identical term, plant physiology differs widely as regards methodology from animal physiology.

This gap is filled by investigations on biopotentials carried out on separate gigantic cells of *Characeae* (Dainty 1962). They deal first of all with the physical and chemical properties of plant cell membranes and with the mechanism and regulation of transport in those cells. The results are highly convergent which those obtained for animal cell membranes. The investigations of the present authors (Pa-

szewski and Zawadzki 1973a, 1973b, 1974) are the first to our knowledge in which square DC pulses were used as a stimuli for verification of basic neurophysiological laws in higher plants. The characteristics of an AP wave, the strength duration relation and the all-or-nothing law were studied. The occurrence of relationships known in neurophysiology as "Pflüger's laws of contraction" was also investigated. The aim of the present paper is to present results of these investigations.

### MATERIALS AND METHODS

Measurements were made on 40-70-day-old plants of *Lupinus angustifolius* L. cv. Wielkopolski. The plants were planted in pots in a greenhouse and exposed to artificial light for 16 hours a day. The temperature did not exceed 25°C and did not fall below 18°C. The pots were watered with tap water. Some hours before starting the experiments the plants were placed with the pots in a measuring chamber which allowed the attachment of electrodes, the stimulating electrodes being driven right through the shoot. The average diameter of a shoot was 2 mm. The height of the plants, depending on their age, varied between 35 and 45 cm.

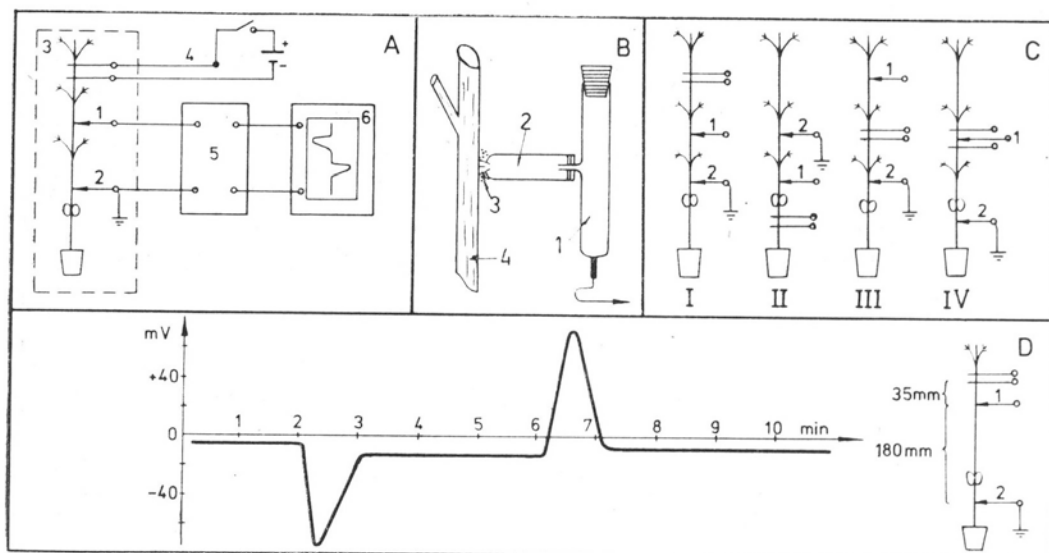


Fig. 1. Diagrams of the experimental arrangement

A. Diagram of the measuring apparatus. 1 and 2 — recording calomel contact electrodes, 3 — measuring chamber, 4 — pair of needle-shaped silver stimulating electrodes with the stimulating apparatus, 5 — electrometer with  $R_{ia} = 10^{14} \Omega$ , 6 — recorder (Kipp BD3)

B. Diagram of a connection of calomel contact electrode. 1 — calomel electrode, 2 — glass pipette filled with 0.1 per cent KCl, 3 — camel-hair brush which makes possible a liquid contact with the plant the brush is wetted by a solution from the pipette, 4 — plant shoot.

C. Four examples of the connections of the stimulating and recording electrodes (see Fig. 1A).

D. Typical AP curve obtained at electrical stimulation of a *Lupinus* shoot.

Fig. 1 shows diagrams of the experimental arrangement. Experiments were repeated with electrodes combined in various ways — see Fig. 1C. The recording and stimulating electrodes were placed at various distances and this made possible deter-

mination of speed of the AP and the point at which AP was started. The distance between the stimulating electrodes was 10 or 30 mm. Stimulating voltage ranging from 1 to 10 V were applied and it was possible to reverse the electrode polarity cathode — anode. The stimulation lasted for various periods of time from 0.1 to 360 sec. Fig. 2 shows a schematic comparison of the method used in Pflüger's experiments and that applied in the present investigations. Analysis of Fig. 1 and Fig. 2

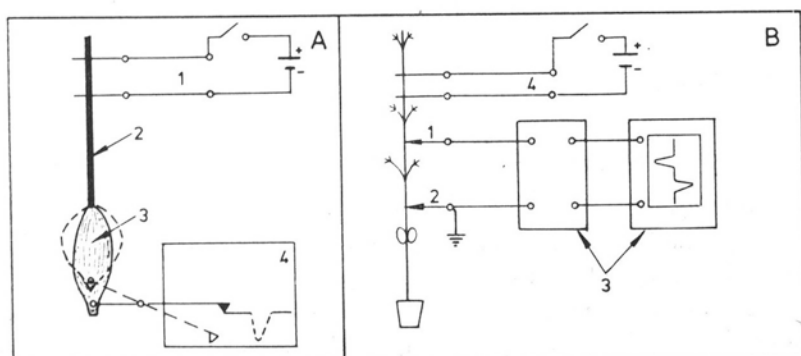


Fig. 2. Schematic comparison of the methods applied in Pflüger's experiments (A) and in the present investigations (B).

A. 1 — stimulating apparatus, 2 — sciatic nerve, 3 — muscle, 4 — mechanical registration of contraction.

B. 1 and 2 — measuring electrodes, 3 — electronic apparatus measuring AP, 4 — stimulating apparatus.

shows a complete analogy between the methods applied. The only difference consists in the fact that in Pflüger's classical experiments the AP was "registered" by muscle contraction while in the present investigation — by means of an electronic measuring system in view of the lack of evident contraction elements in *Lupinus*. From the point of view of the method, this difference is not essential. Fig. 1D presents a typical AP curve obtained in electrical stimulation of a *Lupinus* shoot in the experimental system presented in Fig. 1A.

## RESULTS AND DISCUSSION

The results of Pflüger's experiments and their interpretation are generally known. The results are usually presented in the form of a table 1 (Waławski 1971).

In experiments on plants it was possible to obtain excitation by means of a switch with stimulus duration shorter than the time of passing of the impulse between the cathode and the anode. At anode — cathode distance of 30 mm the time of impulse propagation is about 20 — 40 sec. (Paszewski and Zawadzki 1973b). It is easy, therefore, to open the circuit before the impulse reaches the anode, what was not possible in Pflüger's experiments.

The results of investigations of plants can be presented in two variants. Variant I — Experimental arrangement as in Fig. 2B. Stimulation period longer than 60 sec. (time exceeding AP propagation time between stimulating electrodes). Experiment

Table 1

A nerve's reaction on direct current activity — Pflüger laws of contraction (after Walewski 1971)

Current intensity	Electrode arrangement			
	nearer the muscle			
	cathode		anode	
	Cl.	O.	Cl.	O.
Weak	+	—	+	—
Medium	+	+	+	+
Strong	+	—	—	+

Cl. — closing of the circuit, „O”. — opening of the circuit. + muscle contraction, — lack of contraction.

analogous to Pflüger's experiments. Variant II — Experimental arrangement applied in various combinations of mutual positions of electrodes (examples in Fig. 1C). Stimulation time ranging from 0.1 to 360 sec.

The results of experiments according to Variant I are presented in Tab. 2. An almost complete agreement of the results in both tables is striking. In two cases only for medium currents agreement was not reached. This can be explained by the fact that in the case of a muscle there was a possibility of two successive contractions because the refractory period is much shorter than the time of stimulus application. In a plant the refractory period is about 1 hour and a repeated passage of the AP could not be observed after the period of e. g. 2 min (Paszewski and Zawadzki 1973b). It may, therefore, be affirmed that Pflüger's laws have been proved valid in plants.

Table 2

A plant's reaction on direct current activity — examination of the AP in a *Lupinus* shoot

Current intensity	Arrangement of stimulating electrodes in relation to measuring ones:			
	anode-cathode-measuring electr.		cathode-anode-measuring electr.	
	Cl.	O.	Cl.	O.
Weak $10^{-5}$ A/cm <sup>2</sup>	+	—	+	—
Medium $10^{-4}$ A/cm <sup>2</sup>	+	—?	—?	+
Strong $10^{-3}$ A/cm <sup>2</sup>	+	—	—	+

Cl. — closing of the circuit, „O”. — opening of the circuit. + impulse propagation, — lack of impulse propagation.

The results will be discussed in detail in reference to the value of the stimulating current intensity and various combinations of electrode arrangement and time of stimulation (Variant II). As seen, Variant I, important from the methodological point of view, is only a special case of Variant II.

I. Weak currents of about  $5 \cdot 10^{-5}$  A/cm<sup>2</sup>, i.e. rheobase currents or with values slightly above the rheobase (Paszewski and Zawadzki 1974). Regardless of the

electrode arrangement and the time of stimulus application (periods longer than the useful time of pulse) the AP always originates from the cathode at the moment of onset of the stimulating current and propagates in two directions i. e. in the direction of the anode and in the opposite one. The useful time of pulse is about 0.2 to 0.5 sec. This is the time of "onset" necessary for the occurrence of excitation. Longer flow of the current does not influence the course of the phenomenon. The offset of current after 0.5 sec or after 6 min. does not change the fact of arising and propagation of AP in both directions. The anode, does not take part in the phenomenon, either in active state or after the offset of current. It can be interpreted, after Pflüger, that in the case of weak currents both hyperpolarization and depolarization of the anode are too weak either to inhibit or to initiate excitation.

II. Medium currents of about  $10^{-4}$  A/cm<sup>2</sup>. The value of these currents is a few times (3 to 10) higher than of rheobase current. The phenomenon of excitation has in this case a different course, to some extent, than in the case of weak currents. The anode begins to play an important part here. The AP always originates from the cathode at the moment of closure of the circuit. The useful time of pulse is shorter than in weak currents and is about 0.1 sec. AP propagates in both directions of the shoot. If we break the circuit before the anode is reached by the AP the latter may pass without being disturbed through the area of the anode which will be inactive by that time. In some cases, at the moment of opening the circuit, a new AP starts from the anode. The frequency of these cases increases together with the rise of the stimulating current intensity, e.g. from  $10^{-4}$  to  $3 \cdot 10^{-4}$  A/cm<sup>2</sup>. This anodic impulse begins to propagate also in two directions. The two impulses meet in the segment between the two stimulating electrodes and they disappear. In the parts of the stem beyond the stimulating electrodes the cathodic and anodic impulses propagate, respectively. If the stimulating circuit remains closed the cathodic impulse reaches the active anode and is arrested there. The phenomenon of "arrest" lasts 60 — 90 sec. After this time the impulse may spontaneously pass further. When the circuit is opened in the period of "arrest" of the impulse at the anode, its further progress is observed at the same moment. It is hard to tell whether it is the same impulse that was arrested or whether it is a new one of anodic origin. The characteristic of both these impulses (AP) is identical. They can only be distinguished by the time after which they reach the measuring electrode. The phenomena here described may be interpreted as a situation corresponding to the contractions occurring when the circuit is closed and reopened in the experiment of Pflüger with the use of medium current.

III. Strong currents of about  $10^{-3}$  A/cm<sup>2</sup>. The values of these currents are 20 — 50 times higher than a rheobase current. The phenomena discussed here are almost identical to those in the case of medium currents. The only difference is that every opening of the circuit before the cathode impulse reaches the anode evokes an anodic AP. When the circuit is kept closed, an identical arrest of the cathode impulse is observed on the anode; the impulse starts at the moment of opening of the circuit or spontaneously if the time of "arrest" on the active anode is sufficiently long. A tendency was noted towards a longer arrest at the anode about 100 — 150 sec.

It is often observed that if the impulse does not "break through" spontaneously after 3 — 4 minutes of closed circuit, the opening of the circuit no more produces a new impulse.

The foregoing results can be summed up as follows:

1. There is a possibility of a simultaneous flow of both the stimulating current and the AP through the same segment of the shoot. No mutual influence of these processes was observed.

2. Two or more impulses can arise and propagate in the same plant shoot on the condition that this takes place in different areas which are not in the refractory period. A meeting of two impulses causes their decay.

3. The regularities occurring in plant stimulation are identical (with the exception of the time factor) to those found in nerves which are called Pflüger's laws of contraction.

4. The applicability of the basic neurophysiological laws (Pflüger's laws, as well as strength-duration relation and all-or-nothing law — Paszewski and Zawadzki 1974) in plants can be the basis for the acceptance of the concept that excitability phenomena are essential for the mechanisms of regulation of vital processes both in animal and plants. They are qualitatively the same processes, while they differ only quantitatively i. e. in the time factor.

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*Próba zastosowania praw Pflügera do interpretacji pewnych zjawisk elektrofizjologicznych u roślin*

### Streszczenie

Bodziec elektryczny (prąd stały) wywołuje w łodygach *Lupinus* przemieszczającą się falę potencjału elektrycznego identyczną w kształcie do fali potencjału czynnościowego stymulowanych pojedynczych komórek roślinnych lub nerwów i mięśni. Fakt ten był podstawą podjęcia badań mających na celu określenie czy zależności znane w neurofizjologii jako "prawa skurczów Pflügera" występują analogicznie u stymulowanych roślin. Eksperymenty przeprowadzono w dwóch wariantach — analogicznym do doświadczeń Pflügera (Wariant I) oraz w układach rozszerzonych (Wariant II). Badania potwierdziły stosowanie się tych praw u roślin. Zjawiska zachodzące w obszarze elektrod stymulujących wykazują pełną analogię do zjawisk obserwowanych w nerwie. Inne badania (Paszewski i Zawadzki 1973a, 1973b, 1974) wykazały, że pobudliwość roślin można charakteryzować prawami stosowanymi dotychczas wyłącznie w fizjologii zwierząt, takimi jak "zależność: siła — czas trwania" oraz "wszystko albo nic".

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