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Measurement of the Potential Across the Sieve Plates in *Vitis vinifera*

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Summary. The electrical potential difference across the sieve plates in the primary phloem of *Vitis vinifera* was measured by inserting micro-electrodes into the sieve-tubes. The values obtained ranged from 4—48 mV. The potential across the transverse walls of the phloem fibres was also determined and found to range from 1—11 mV. These results are discussed in relation to the theory of translocation based on electro-osmosis put forward independently by Fensom and Spanner.

Introduction

Electron microscope studies show that the pores in sieve plates are filled with parallel filaments linking adjoining sieve elements (Kollmann, 1960). The resistance of the pores to a flow of solution brought about by a hydrostatic pressure developed across them would therefore be expected to be high. Diffusion is inadequate to account for the velocities of translocation and the rates of mass transfer of sugar that have been observed in many species. This suggests that energy must be expended at each sieve plate to transport the sugar from cell to cell. Fensom (1957) and Spanner (1958) independently put forward theories to explain translocation based upon mass flow across the sieve plate by electro-osmosis. Electro-osmosis has the attraction of reconciling the presence of an active transport at the sieve plate with the evidence that sugar moves in the sieve tubes as a solution (Weatherley, Peel and Hill, 1959; Ziegler and Vieweg, 1961). However, for electro-osmosis to occur a potential must be generated across the sieve plate. This paper describes an attempt to detect such a potential in the primary phloem of the vine.

Materials and Methods

Hand cut longitudinal sections from the current season's stems of *Vitis vinifera* (var Black Hamburg) were taken during August and September. The freshly cut sections were immersed in 0.25 M sucrose following the procedure of Esau, Currier and Cheadle (1957). Sections about 1 cm long with a band of primary phloem along one edge were selected for investigation.

Two rubber strips from a rubber band were fixed with "Bostik" adhesive about 5 mm apart across a 2 cm sq. coverslip. About 1 mm of each strip was left without adhesive at the edge of the coverslip and the selected section was inserted between

the strips and the coverslip at this point. The section was thus held a little way in from the edge of the coverslip with the longitudinal band of phloem outermost. The coverslip was then placed on a slide and the open ended chamber thus formed between the rubber strips was flooded with 0.25 M sucrose solution. The slide was then placed under the microscope with the section facing the open side of the stage.

Micropipettes with a tip diameter of 1—2 μ were made using a Palmer electrode puller and were filled with 3 M KCl by heating them in the potassium chloride to boiling point and then allowing them to cool under reduced pressure in a vacuum dessicator. Suitable micropipettes were then connected to a pair of calomel electrodes by nylon tubing filled with 3 M KCl. The complete electrodes were connected to a "Vibron" electrometer. If the zero error of the electrodes was greater then 5 mV the micro-pipettes were discarded.

The micro-electrodes were inserted into the phloem using a pair of Singer mark III micro-manipulators at a microscope magnification of × 800. Satisfactory illumination of the specimen was achieved using a substage condenser with a focal length of 11 mm.

Results

A direct measurement of the potential across the sieve plate was achieved by inserting an electrode into the sieve element on each side of a sieve plate as shown in Fig. 1. The results from eight sieve plates are shown in Table 1.

Unfortunately it was not always possible to insert two electrodes into the phloem at once because the insertion of the second electrode

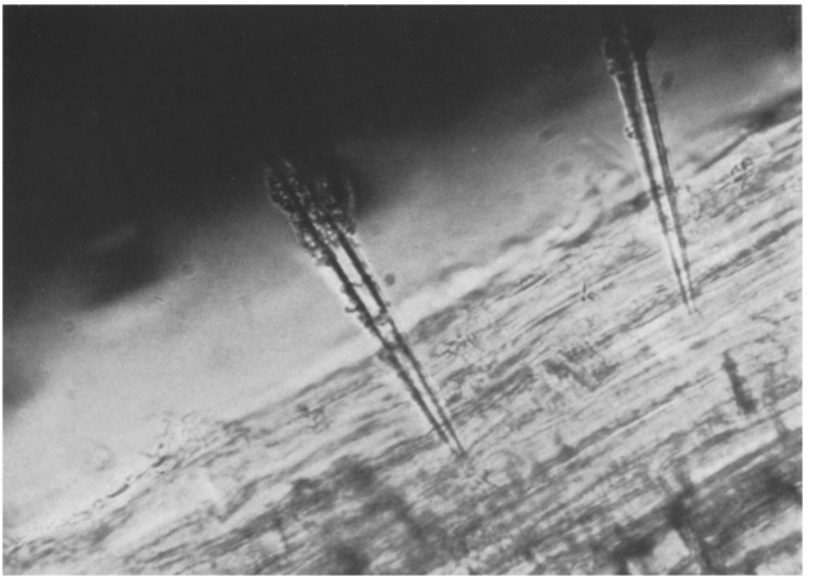

Fig. 1. Two micro-electrodes inserted into a sieve-tube on each side of a sieve plate in the primary phloem of *Vitis*. × 300

often dislodged the first. When this happened the potential between each sieve element and the bathing solution was measured separately and the potential across the sieve plate obtained indirectly from the difference between the two values. Calculated potentials for ten sieve plates and the sieve element potentials from which they were obtained are shown in Table 2.

Table 1. *Potentials (mV) across 8 sieve plates in the primary phloem of Vitis vinifera*

18	10
4	14
22	35
30	17

The direct measurements of the sieve plate potential range from 4—35 mV and the calculated values from 9—48 mV. It can be seen from Table 2 that whilst most of the potentials between the sieve element and the bathing solution were negative, some positive values were recorded. The latter were closely checked before they were accepted. They remained positive and steady even when the electrode was moved further into the cells.

Potentials in the phloem fibres immediately adjacent to the sieve tubes were also measured. These fibres have thin cross walls and the potential across some of these was measured by inserting micro-electrodes on each side of the cross wall as shown in Fig. 2. The potential between the fibre cells and the bathing solution was also determined. Table 3 shows the results for six fibre cells.

Table 2. *Calculated sieve plate potentials (mV) in the primary phloem of Vitis vinifera*

Sieve element		Calculated sieve plate potential
a	b	
−13	−44	31
−29	−11	18
−19	−10	9
−52	−14	38
− 8	−23	15
−19	+18	37
−25	−20	5
−12	+36	48
− 9	+22	31
−15	+ 5	20

Table 3. *Potentials (mV) from 6 phloem fibres of Vitis*

Potential across transverse walls	Potential between fibre and outside solution
1	+ 1
2	+ 2
11	− 5
4	−13
6	− 4
5	− 3

The potentials of the fibres were generally much lower than those for the sieve tubes and in most cells appeared to be zero after taking into account experimental error.

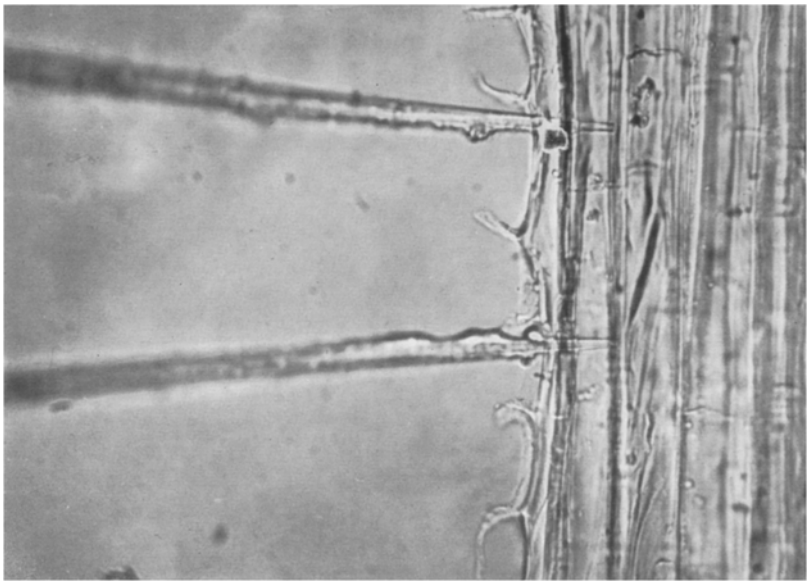

Fig. 2. Micro-electrodes on each side of a cross wall in a phloem fibre of *Vitis*. × 300

Discussion

Although the potentials across the sieve plates differed widely, the size of the potential seems to be too great to be accounted for by experimental error. This is clear from the results obtained from the phloem fibres which served as controls. The fibres eventually become lignified and die but the potentials were measured when they were apparently alive as they still contained cytoplasm. The presence of a potential across the sieve plate indicates a barrier to diffusion and so supports the evidence of Kollmann (1960), Hepton and Preston (1960) and later workers (see review by Weatherley and Johnson) that the sieve pores are filled with cytoplasm. The potential between the sieve element and the bathing solution is in accord with the results of Esau, Currier and Cheadle (1957). They were able to plasmolyse the sieve tubes of vine and other species and this is good evidence that at least one barrier to diffusion lines the longitudinal walls.

Spanner (1958) suggested that the potential required across the sieve plate for electro-osmosis to occur could be brought about by a local circulation of potassium ions. Subsequently, Peel and Weatherley (1959) found that the phloem sap obtained from willow using aphid mouth parts contained up to 0.5 M potassium. If a potassium concentration of 0.25 M in the phloem sap and a potential of 50 mV across the sieve plate are assumed it can be calculated that electroosmosis will

bring about a pressure difference of 0.1 atmospheres. This figure is similar to the values calculated by SPANNER (1962) and WEATHERLEY (1963) from the amount of respiratory energy which appears to be available. WEATHERLEY and JOHNSON (1968) have calculated, using a formula derived by SPANNER, that the pressure drop across each sieve plate would be 0.14 atmospheres if a translocation rate of 100 cm/h is assumed and also that the sieve pore is assumed to be traversed by parallel filaments 100 Å thick and 200 Å apart. The potentials in the phloem of vine appear therefore, to be of the right order to sustain an electro-osmotic flow at the rates required.

For electro-osmotic flow to occur in one direction over long distances however, Spanner's theory requires that all the sieve plates in a file of sieve elements be polarised in the same direction. I have so far not been able to ascertain if this is so in the phloem of vine but observed that where the potentials of a number of sieve plates were measured in the same section they were all polarised in the same direction. There does not appear to be an accumulation of potentials along the phloem as the potentials between the sieve elements and the surrounding medium were of the same order as those across the sieve plates. It seems that the sieve plate potentials must be cancelled out by a gradual decline in potential down each sieve element. Spanner suggested that the turgor pressure follows a similar sawtooth pattern and this is borne out by experiment as there does not appear to be a detectable gradient of turgor pressure down the phloem (KAUFMANN and KRAMER, 1967).

Although the results presented here, taken together with the evidence from electron microscopy, appear to lend support to the electro-osmosis theory, there are some disadvantages to the technique which must be borne in mind. The phloem is known to be a sensitive tissue and besides actually stopping translocation the cutting of the sections may have caused important changes in the tissue. Furthermore, due to the difficulty of inserting the micro-electrodes, a considerable time sometimes elapsed (up to 3 h.) from when the sections were taken to when the measurements were made. The positive potentials in some of the sieve elements must also be looked upon with some suspicion and they need to be repeated. If they are found to be natural then they may turn out to be important in our eventual understanding of the translocation mechanism.

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