

STUDIES ON PHLOEM EXUDATION FROM *YUCCA FLACCIDA* HAW. VIII. FLUID MECHANICS AND EXUDATION

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SUMMARY

Calculations on the linear velocity of the flow of exudate in sieve tubes were carried out, using measured average values for the length and cross-section of the sieve tubes, the surface area and the thickness of the sieve plate, and the number and radius of the sieve pores. Assuming sieve pores free of any obstruction a velocity of flow of 128 cm.hr^{-1} , atm^{-1} through one metre of sieve tube was found to be theoretically possible. With a pressure difference between the intact sieve-tube members and the exuding ones of at least 16 atm – at an assumed distance of one metre, the fluid mechanical capacity of the sieve pores of the *Yucca* inflorescence would consequently allow an exudate velocity of $20 \text{ metres. hr}^{-1}$. From the rate of bleeding a velocity of the exudate in the sieve tubes of 0.44 m.hr^{-1} was calculated, thus only a small part of the pores need to be unobstructed during bleeding. In a previous study sieve pores appeared to be often traversed by 10–20 protoplasmic filaments of approximately 250 \AA diameter. Assuming all the sieve pores obstructed by such filaments, spaced at the observed distance of 1000 \AA , calculations indicated that a pressure difference of $5\frac{1}{2} \text{ atm.metre}^{-1}$ would be sufficient to explain the earlier observed velocity of exudation.

It is concluded that the hypothesis of a mass flow of assimilates from the leaves to the top of the inflorescence of a bleeding plant does not encounter serious objections from the side of fluid mechanics.

1. INTRODUCTION

MÜNCH (1930) observed that following an incision in the phloem of red oaks a drop of exudate was produced, but that new incisions made above or below the wound did not deliver any exudate. This influence extended over several metres below and one metre above the first incision. He concluded that the sieve tubes of the red oak are delicate structures, a reversible plugging of the sieve pores apparently being easily effected. It is this quick reaction on wounding of the phloem that makes it difficult to obtain sieve tube sap from most plant species. In some plant species such a long distance inhibition of phloem transport seems absent. CRAFTS (1939) observed that cutting off thin slices of *Cucurbita* stems at short time intervals, each resulted in a rapid extrusion of a small amount of exudate from the sieve tubes, that soon stopped. He described the accumulation of protoplasm on the sieve plates near the wound and the rapid closing of the pores by callose.

In some respects the sealing of the wound in *Yucca* inflorescences resembles

the cucurbitous type as described by Crafts, but in *Yucca* two phases can be observed:

1. An expulsion for a few seconds of sap every time a new slice is cut from the wound surface, which is also observed at 0°C.
2. A continuous flow of exudate at a lower rate which stops gradually over approximately 24 hrs at 0°C (TAMMES *et al.* 1969). The closing mechanism of the sieve tubes in *Yucca* is sited very near the wound, at a distance of 1mm or less (probably at a distance of one sieve element).

In *Yucca* inflorescence stalks several types of sieve pores were found: pores without callose but with a small number of protoplasmic filaments running through them, and pores closed by callose with a plasmodesm in the middle together with some filaments traversing the callose plug (IE *et al.* 1966).

A number of investigators consider the often demonstrated presence of callose and proteinaceous material in the pores as a normal situation in the intact sieve tube rather than the result of wounding (*e.g.* SCHUMACHER 1967). Some go so far as to believe that this assumed presence of plugged pores in the intact sieve tube will make mass flow in these conduits highly improbable. For example WEATHERLEY & JOHNSON (1968) and FENSOM & SPANNER (1969) have made calculations on the resistance offered by the plugged pores, showing it to be far too high for a pressure-flow mechanism. Other investigators (*e.g.* ESAU & CHEADLE 1961; ESCHRICH 1965) believe that *in vivo* pores are essentially unobstructed structures. Support for this view comes from the work of CRONSHAW & ANDERSON (1969) and ANDERSON & CRONSHAW (1969): They consider the callose and protein-plugged pores as artefacts, as careful fixation after previous wilting can prevent their appearance in electron micrographs. The results reported by MILBURN (1970), who could induce sieve tube bleeding from *Ricinus* stems by massage, also provide strong evidence in favour of the mass-flow concept.

Moreover, well known facts like the translocation of viruses through sieve tubes and especially the spread of mycoplasmas in sieve tubes (DOI *et al.* 1967) can only be satisfactorily explained if the pores are not plugged by large numbers of filaments or massive deposits of callose. As elementary mycoplasma particles have a diameter of at least 800 Å, filaments in the sieve pores should be lying at least this distance apart.

Sieve tube exudation from inflorescence stalks makes calculations possible on the velocity of flow in the sieve tubes during bleeding. For *Yucca* a value of 44 cm.hr⁻¹ has been found (VAN DIE & TAMMES 1966) and for the *Arenga* palm even 7 metres.hr⁻¹ has been reported (TAMMES 1952). In the present paper calculations have been made on the pressure difference needed in the sieve tube system to explain the observed velocity of flow. They are based on the assumed presence of open pores as well as on that of pores filled with filaments. The distance between the filaments in the pores is approximated as 1000 Å, their thickness as 250 Å, values derived from a previous paper (IE *et al.* 1966). Additional parameters needed for the calculations are presented in this paper.

2. PARAMETERS USED

The calculations were made for flow through tubes of 100 cm length at a pressure difference of 1 atmosphere = 10^6 dynes.cm⁻².

The viscosity of a 16% sucrose solution at 20°C is 0.017 poise or $\eta = 59$ poise⁻¹.

The diameter of the sieve tubes measured in cross sections is 10–30 μ with an average of 21 μ ; measured in longitudinal sections values were found between 8 and 40 μ with an average of 22 μ (30 measurements). For the radius a typical value of 10^{-3} cm was taken. For convenience of calculation the tubes were assumed to be cylindrical, although they actually are polyangular in cross section.

The average distance between the sieve plates in sieve tubes was found in the following way:

Inflorescence stalk sections of 3 mm length were placed into a droplet of a carbon black suspension in water at 2°C. Because of the evaporation of the upper surface of the sections, suspension was sucked into intercellular cavities, xylem vessels and sieve elements. In the latter the carbon particles were sieved out by the first sieve plate encountered. The statistical half length of the sieve elements could thus easily be assessed by measuring the distance between the lower surface of the section and the agglomerates of carbon particles against the plates. 30 measurements gave 464 μ as the average length of a sieve element or 2155 plates per metre sieve tube.

The minimum thickness of the sieve plates found in the electron micrographs was 0.2 μ . As sieve tubes are usually not cut at right angles and the walls thus appear to be thicker than they really are, the minimum value must be taken for the calculations. But as this is the thickness of the dehydrated wall, a thickness of twice this value has been used in the calculations as an approximation of the thickness of the plate in the living sieve tube. The total thickness of the plates in 1 metre sieve tube is $2155 \times 0.4 \mu = 0.086$ cm.

The sieve plates are at an oblique angle to the walls of the tubes. The surface area of a sieve plate (S_p) is therefore much larger than the cross section of the tube, approximately at least three times this value, thus $S_p = 3\pi 10^2 \mu^2 = 942 \mu^2$.

The radius of the pores was found to be 0.2–0.3 μ , with an average of 0.26 μ . Or $r = 26 \times 10^{-6}$ cm. The surface area of the average pore is $0.21 \mu^2$.

The number of pores per sieve plate was calculated from the percentage of the surface area occupied by the pores on the plates in the electron micrographs. It amounts to 1760 pores per plate. Thus the total surface area of the pores in one plate can be estimated as $370 \mu^2$, compared with a cross section of the average sieve tube of $314 \mu^2$.

3. CALCULATIONS

The rate of laminar flow¹ through a tube (without plates) can be expressed by the Poiseuille equation (CASEY 1962):

$$Q = \varnothing \frac{\pi r^4}{8L} \Delta P \text{ (cm}^3 \cdot \text{sec}^{-1}\text{)} \quad (1)$$

where L is the length of the tube = 100 cm, r is the radius = 10^{-3} cm, ΔP is the difference in pressure between the ends of the tube = 10^{-6} dynes. cm^{-2} , \varnothing is the fluidity factor, $\frac{\text{cm} \cdot \text{sec}^{-1}}{\text{dynes} \cdot \text{cm}^{-1}}$, or $\text{poise}^{-1} = 59 \text{ poise}^{-1}$ ($\varnothing = \eta^{-1}$ by definition, where η is the viscosity in poise). In order to obtain the total resistance of the tube parts and the plates of the sieve tube the Poiseuille equation can be written as:

$$Q = \frac{\Delta P}{8L/\varnothing \pi r^4} \quad (2)$$

Here the factor $8L/\varnothing \pi r^4$ can be regarded as the flow resistance of either the tube parts or the plates. According to this, the resistance in the tube parts in one metre of sieve tube will be:

$$4.3 \times 10^{12} \frac{\text{dynes} \cdot \text{cm}^{-2}}{\text{cm}^3 \cdot \text{sec}^{-1}}$$

The resistance of one pore channel in one metre of sieve tube ($L = 2155 \times 0.4 \mu = 86 \times 10^{-3}$ cm and $r = 26 \times 10^{-6}$ cm) is

$$0.8 \times 10^{16} \frac{\text{dynes} \cdot \text{cm}^{-2}}{\text{cm}^3 \cdot \text{sec}^{-1}}$$

As there are 1760 pore channels in a sieve tube the total resistance offered by them will be:

$$4.6 \times 10^{12} \frac{\text{dynes} \cdot \text{cm}^{-2}}{\text{cm}^3 \cdot \text{sec}^{-1}}$$

¹ As already shown by HORWITZ (1958) the possibility of a turbulent flow in sieve tubes may be excluded. The critical Reynolds number corresponding to the transition from a laminar to a turbulent flow is $N_R = 2000-3000$ according to the equation given by PAO (1961).

$$N_R = \frac{V D \rho}{\eta}$$

where V is the linear velocity of flow in $\text{cm} \cdot \text{sec}^{-1}$, D the diameter of the tube in cm, η the viscosity in poise and ρ the mass density. For *Yucca* $N_R = 7 \times 10^{-3}$.

The added resistance of tube parts and plates will then be:

$$8.9 \times 10^{12} \frac{\text{dynes} \cdot \text{cm}^{-2}}{\text{cm}^3 \cdot \text{sec}^{-1}}$$

In Poiseuilles equation (2) $Q = \frac{10^6}{8.9 \times 10^{12}} \text{ cm}^3 \cdot \text{sec}^{-1}$ for $\Delta P = 1 \text{ atm}$.

This is the value for a single tube. To obtain the velocity of flow (exudation) it has to be divided by its cross section $\pi r^2 = 3.14 \times 10^{-6} \text{ cm}^2$. A linear velocity of $0.036 \text{ cm} \cdot \text{sec}^{-1}$ or $128 \text{ cm} \cdot \text{hr}^{-1}$ is thus found for a sieve tube of 100 cm length and a pressure difference between both ends of 1 atm . As the pressure difference between the intact sieve tube members in the plant and the site of exudation is approximately 16 atm ,² fully unobstructed pores in the sieve plates would allow a linear velocity of exudate flow through the sieve tubes of $16 \times 128 \text{ cm} \cdot \text{hr}^{-1} = 2048 \text{ cm} \cdot \text{hr}^{-1}$. The actually measured velocity of exudate flow through the sieve tubes was $0.44 \text{ m} \cdot \text{hr}^{-1}$ (VAN DIE & TAMMES 1966). This means that only a small part of the pores need to be unobstructed by callose or protoplasmic material in order to explain the observed rate of bleeding. Unobstructed pores are rare in electron micrograph of *Yucca* sieve plates. Actually observed are pores traversed by 10–20 filaments of approximately 250 \AA thick. If regularly distributed over the pore cross-section they will be lying approximately 1000 \AA apart.

If one considers a sieve pore with strands spaced at 1000 \AA apart being built up of a number of separate channels with a diameter of 1000 \AA a modified form of the Poiseuille equation can be applied to calculate its resistance to flow.

Formula (2) can be written as:

$$\Delta P = \frac{Q \cdot 8L}{\pi r^4 v} = \frac{v \pi r^2 \cdot 8L}{\pi r^4} = \frac{8vL}{r^2}$$

as the rate of flow $Q (\text{cm}^3 \cdot \text{sec}^{-1})$ is $v \cdot \pi r^2$, in which v is the linear velocity of flow ($\text{cm} \cdot \text{sec}^{-1}$). For $L = 1 \text{ cm}$ the equation becomes:

$$\Delta P = \frac{8v}{r^2} = \frac{8v\eta}{r^2} \quad (3)$$

ΔP is the pressure drop in $\text{dynes} \cdot \text{cm}^{-2} \cdot \text{cm}^{-1}$, and η the viscosity in poises. For a velocity of $44 \text{ cm} \cdot \text{hr}^{-1}$ or $v = 0.012 \text{ cm} \cdot \text{sec}^{-1}$ through the channels application of formula (3) gives $\Delta P = 6.5 \times 10^7 \text{ dynes} \cdot \text{cm}^{-2} \cdot \text{cm}^{-1}$. For a total *in vivo* length of the pore channels of 0.086 cm in one metre of sieve tube the necessary pres-

² The bleeding sap contained per ml 0.47 – 0.53 millimoles of sucrose, approximately 0.1 millimoles of amino acids and approximately 0.1 milliequivalent of inorganic ions. In fully turgescer tissue this together amounts to approximately 16 atm (TAMMES & VAN DIE, 1964).

sure drop becomes 5.6×10^6 dynes.cm⁻² or 5.6 atm. This value is well within the theoretically available pressure of approximately 16 atmospheres.

SPANNER has developed a formula for calculating the resistance to flow of a filament-filled pore channel (WEATHERLEY & JOHNSON 1968). Application of this formula³ shows that a pressure drop of 6.2×10^7 dynes.cm⁻² per cm of sieve plate will be required to get a flow of 44 cm.hr⁻¹ through the filament-filled pores of *Yucca*. This corresponds with a pressure drop of 5.3 atm per metre of sieve tube, a value close to that found with the more simple Poiseuille equation⁴.

4. DISCUSSION

The translocation of viruses through the sieve tubes is often considered as strong evidence in favour of the mass-flow concept. But as viruses may be translocated as sub-units or in the form of infectious ribonucleic acid one cannot infer from their movement anything about the diameter of the sieve-plate pores. The recent discovery, however, of mycoplasma diseases in many plant species (CASPER 1969) and the spread of these organisms through the sieve tubes (DOI *et al.* 1967; CASPER 1969) allows conclusions about the effective pore diameter as the smallest infectious mycoplasma particles have a diameter (in electron micrographs) of approximately 800 Å (CASPER 1969). Filament-filled sieve pores, therefore, should possess interstices between the strands with dimensions of at least that order to allow a spread of the disease through the phloem. Filament-filled pores with much smaller interstices, e.g. 200 Å as assumed by WEATHERLEY & JOHNSON (1968) seem, therefore, improbable, except after injury. Apart from the question whether in the intact sieve-tube system translocation takes place by a pressure-driven mass-flow, such a mechanism seems the most plausible explanation in the case of bleeding from the cut distal end of the *Yucca* inflorescence: Assimilates formed in the leaves are translocated to the site of bleeding (VAN DIE & TAMMES 1965) while the translocation and bleeding processes proceed unhampered during local cooling at a temperature of 0°C (TAMMES *et al.* 1969). The present calculations support this view and show that fluid-mechanically such a pressure-driven mass flow is certainly possible. The calculated resistance of the sieve plates with open or obstructed pores is very low. This is caused partly by the

³ If the filaments are d cm in diameter and spaced at D cm apart, the velocity of flow being v cm.sec⁻¹, the viscosity of the liquid η poise, ΔP the pressure gradient along the filament-filled tube in dynes.cm⁻².cm.⁻¹, $A = 0.525 D$ and $\alpha = d/A$, then according to SPANNER (WEATHERLEY & JOHNSON, 1968):

$$\Delta P = \frac{8 \times \eta (1 - \alpha^2)}{A^2 (4 \ln \alpha + (1 - \alpha^2) (3 - \alpha^2))}$$

⁴ As the total surface area of the pores in one sieve plate ($370 \mu^2$) is approximately the same as the cross section of the sieve tube ($314 \mu^2$) the velocity of flow in tube parts and in the pores will also be approximately the same. During the passage of the pores the streaming fluid is forced to deviate slightly from its longitudinal direction of flow. But the energy losses caused by these deviations will be negligible compared with the total energy losses caused by the friction of solvent and solute molecules during their passage through the pores.

extreme thinness of the plates as found in some electron micrographs. The measured value of $0.2\ \mu$ is of course that of the dehydrated wall, but is nevertheless remarkable. It is different from measurements with the light microscope, where the plates are never quite parallel to the optical axis. But also in other studies thin sieve plates have been found: From the electron micrographs of ANDERSON & CRONSHAW (1969) a minimum value of $0.4\ \mu$ seems probable for tobacco; in tomato a thickness of $0.35\ \mu$ has been found (DAVID-FERREIRA, in CASPER 1969). Beside the thinness of the plates, their oblique position with regard to the axis of the sieve tube and their large number of pores, the form of the pores in *Yucca* is streamlined, having a wider mouth at both sides than in the middle of the plate. This means that the resistance offered to mass flow will be less than calculated.

If mass flow is possible in sieve tubes during exudation one wonders why it should not be the way in which assimilates are translocated in the intact plant too. Of course pressure differences between source and sink will be much less than 16 atm, but the assumed distance of one metre between source and sink is certainly not correct in *Yucca*. Probably each cell around the sieve tubes is able to secrete sucrose into the sieve tube lumen, even if it is localized in a sink region (VAN DIE & TAMMES 1966). Whether such a cell absorbs sucrose from its neighbouring sieve-tube member or secretes it into it might depend on the sucrose content in the lumen of the sieve tube member (TAMMES *et al.* 1967). A mass flow in the sieve tubes, driven by the summation of these absorption and secretion processes of individual phloem cells around the sieve tubes, might be the mechanism of assimilate translocation in the intact plant.

ACKNOWLEDGEMENT

The authors are indebted to Dr. W. J. Vredenberg and Ir. U. H. Banga for advice on calculations regarding fluid mechanics.

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