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Hugo de Vries and the plasmolysis method

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INTRODUCTION

Several authors (e.g. Faasse 1994) have noted that during the first half of the 19th century, plant science in The Netherlands was limited to visual investigation and classification. In the course of the century this descriptive approach was enhanced by the use of the microscope, by which inner structures could be added as tools for this floristic approach. The chairs in botany at the universities reflected this atmosphere. Outside the academic establishment plant science was practiced only by pharmacists and physicians, and the general knowledge about processes which are vital to living organisms was practically absent. This is illustrated by the fact that the foundation of a Society of Dutch Botanists in 1845 (the present Royal Botanical Society of The Netherlands) was the work of a small number of physicians and had as its goal 'to stimulate and expand on scientific research of the native vegetation, in order to realize from its results the publication of a Flora of The Netherlands, which answers the contemporary demands of knowledge of the botanical sciences' (Faasse 1994). However, in those days the educated public was apparently not excited by this typical floristic approach, since the society led an obscure existence. This changed only in 1871 when it started to organize meetings in which topics of general interest, including physiological issues, were discussed. This indicates that in the course of the century the time had come for a more comprehensive approach of plants, even for the general public.

A strong stimulus to modernizing plant science in those days was the Higher Education Act of 1876, which resulted in a considerable expansion of the Dutch universities. Hugo de Vries, appointed in 1877 as reader and soon afterwards as professor in plant physiology at the University of Amsterdam, was the first Dutch professor in botany without a medical qualification. His appointment resulted in the separation of the systematic/morphological and physiological direction of plant science. From then onwards, plant physiology was treated as a separate discipline, founded on physics and chemistry and with a strong practical orientation, which was appreciated by the public and by the government.

The development of plant physiology in The Netherlands in the 19th century must of course be seen in an international perspective. At the beginning of the century there was a considerable expansion of activity in the science of biology, especially in France and Germany (Morton 1981). The promotion of natural science was seen as the key to progress in industrial and agricultural methodology. This led, for example, to the

Fig. 14. Julius Sachs in his study. (From Ernst G. Pringsheim (1932): *Julius Sachs, der Begründer der neueren Pflanzenphysiologie* 1832–1897 (Jena).)

foundation of the University of Berlin in 1807, with a strong natural science faculty, soon followed by others throughout Germany, which resulted in a large number of universities where plant physiology was a regular part of the curriculum. The German botanist Matthias Jacob Schleiden published a most influential textbook in 1842, *Grundzüge der wissenschaftlichen Botanik*, in which the structure and development of plants, with physiology as an essential complement was treated, with strong emphasis on the chemical processes within the plant. He and his colleague Theodor Schwann (1839) demonstrated that both in plants and animals the cell is the fundamental unit of life, that it is organized with the nucleus as the major organelle and that it arises from previously existing cells. Its functioning is based on chemical and physical principles, as was emphasized vigorously by Liebig (1840), among others. Within a few years there was an enormous accumulation of facts and new insights in plant physiology, which received an additional impulse from the foundation of several agricultural research stations. Outstanding scholars, such as Hofmeister (Heidelberg, Tübingen), Sachs (Würzburg), De Bary (Strassbourg), Pfeffer (Leipzig) and Strassburger (Bonn), had a very strong influence on scientific progress, which extends to the present day. The most influential of them was certainly Julius Sachs (Fig. 14), whose studies on plant nutrition, carbon assimilation, growth and the morphogenetic effects of light had an enormous impact. His work culminated in a *Lehrbuch der Botanik* (1865) which together with his *Vorlesungen über Pflanzenphysiologie* (1882) and the textbook *Pflanzenphysiologie* (1881), written by his pupil W. Pfeffer, was the basis for teaching and research in plant physiology in much of the following century. Many students from all over Europe worked in his laboratory and spread his inspiration, knowledge and

advanced methodology; as did Hugo de Vries with interruptions, in the period 1872–1877.

In this paper, I wish to draw attention to the role of osmosis in De Vries' thinking. Osmosis is the phenomenon of spontaneous flow of water from a dilute to a concentrated solution of some substance, which can be demonstrated by separating both solutions by a suitable membrane. It was discovered by Abbé Nollet in 1748 and studied extensively by Dutrochet (1837). This author showed that water flow depends on the difference in concentration of solute, and particularly on the nature of the membrane separating the two solutions. Using natural membranes, Dutrochet set up the first osmometer and observed that osmosis can result in a hydrostatic pressure. Although he could not explain the phenomenon, he was a pioneer in stating that osmosis might play a role in water movements in plants. His ideas had a strong impact on later developments. In particular, a few decennia later his ideas were the basis for theoretical considerations by Sachs, supported experimentally by De Vries, who demonstrated the selective permeability of the protoplast. Pfeffer (1877) was the first to make accurate measurements of the osmotic potential of solutions using semipermeable membranes made of fine-grained, unglazed porcelain, impregnated with copper ferrocyanide and of living cells. The pressures he measured were much larger than expected, which was possibly the reason why, according to Van 't Hoff (cited by De Veer, 1969), his work was somewhat ignored by his colleagues. It is to the credit of De Vries that he brought it to the attention of Van 't Hoff, which led to the classical publication in 1887 in which Van 't Hoff pointed out the analogy between gases and solutions, and also applied, for the first time, thermodynamic methods to the colligative properties of solutions vapour phase at the same activity and temperature. Pfeffer's results indicated that osmotic potential at constant temperature is directly proportional to the concentration of the solution; Van 't Hoff showed that this was the analogue of Boyle's law for gases.

Osmosis plays an essential role in water relations in plants. Present-day textbooks state that free energy of water in a given compartment is defined by water potential, which is essentially the sum of the osmotic potential, dependent on the concentration of solutes present, and the pressure in that compartment. Thus in a living cell the water potential is the sum of a positive turgor pressure and a negative osmotic potential; in a xylem vessel the pressure may take a negative value, dependent on the relative humidity of the environment. The water potential is rarely zero in any compartment of the cell.

HUGO DE VRIES AND THE MECHANICS OF PLANT GROWTH

De Vries' doctoral thesis work

In the middle of the 19th century interest in osmosis was in line with a general interest in the physical aspects of plant behaviour. This is reflected by the contents of De Vries' dissertation in 1870 on the influence of temperature on living plants. He studied the influence of temperature on general characteristics, such as vitality and germination rate, but he concentrated on physical aspects. He showed, for example, that a well-watered plant wilts when its roots are cooled by ice, showing that the roots control the uptake of water to some extent, and that ground temperature is of great importance for the wellbeing of the plant. He gave an explanation for this phenomenon much later, when describing the endodermis and in particular the route taken by water through this part of the root (De Vries 1886). In view of his later work, De Vries' experiments on what he called 'imbibition' are revealing. He used longitudinal segments of internodes

Table 1. The effect of different concentrations of NaCl on the number of windings of longitudinal sections of *Oenanthe fistulosa* at 20° (De Vries 1870; Opera I p. 45)

Salt concentration	0·25%	0·5%	0·75%	1%
Before incubation	1·7	2·1	2·2	1·9
After 1 h incubation	8·7	7	4·5	4·4
After 2 h incubation	9·2	8·2	4·7	4·6

of a variety of plants. When incubated in water the internal parenchyma swelled by the uptake of water, contrary to the epidermis, which resulted in a curvature of the segment with the parenchyma at the convex side. De Vries used the degree of curvature as a measure for the influx of water, and found, not unexpectedly, that in all cases the rate of water uptake was correlated with the temperature. However, De Vries also described the finding that the segments take up *less* water when incubated in a solution of salt (Table 1). Salt concentrations exceeding 5% resulted in a release of water from the segments, instead of an uptake. It is unclear why he included these results in his dissertation, since they were hardly related to the subject; it looks like an accidental finding which intrigued him. However, this is probably not true. Much later (1884), De Vries acknowledged that Pringsheim (1854) and Nägeli & Cramer (1855) had subjected cells to hypertonic solutions. Actually, they had already described the resulting contraction of the protoplast: De Vries' 'plasmolytic method' is based on their description. Also, Pfeffer (1881) mentions that Nägeli's work was the basis for subsequent work on osmotic properties of the cell[1]. So, during de Vries' thesis work, this topic was in the air.

De Vries' postdoctoral work in Würzburg

Why do salts inhibit the uptake of water by plant cells? Initially, De Vries did not grasp the role of osmosis. He followed the opinion of Hofmeister (1867), stating that it is the cell wall which absorbs water by 'imbibition'. As a result it expands, by which it endows the cell with a firmness, designated as 'turgor'. Sachs, however, in the first edition of his *Lehrbuch* (1865) had a completely different opinion: '... hervorgerufen wird der Turgor dadurch, dass die im Zellsaft gelösten Stoff endosmotisch einwirken auf das die Zelle umgebende oder ihr überhaupt von aussen zuführbare Wasser; indem sich das Wasser durch die endosmotische Anziehung im Zellraum anhäuft, drückt es von innen

[1] In historischer beziehung bemerke ich, dass Nägeli, welcher zuerst die diosmotischen Eigenschaften des lebendigen Protoplasmas in ihrer vollen Bedeutung würdigte, auch das Zustandekommen des Zellenturgors durch die osmotische Wirkung der im Zellsaft gelösten Stoffe in bekannter klarer Weise darlegte. Wie schon Nägeli, hat in noch bestimmterer Weise Sachs die Bezeichnung Turgescenz fur die Spannung zwischen Zellhaut und Zellinhalt reservirt, während Hofmeister, bei übrigens unklaren Vorstellungen über die Bedeutung osmotischer Drückkraft, die Steifheit (Elastizität), welche imbibirte Zellhäute an sich bieten, Turgor nannte. Durch meine eigenen Untersuchungen wurde dann, wie schon früher bemerkt, die Plasmamembran als diosmotisch massgebende Schicht erkannt, und damit das erweiterde Bild über das osmotische System in der Zelle gewonnen, wie es hier entwicklet wurde. Ebenso gelang es mir, die physikalischen Ursachen zu ermittlen, vermöge welcher schon verdünnte Lösungen der Krystalloide sehr bedeutende osmotische Drückkrafte erzeugen (Pfeffer 1881).

her auf die Zellhaut, diese wird gespannt und drückt vermöge ihrer Elasticität auf das in der Zell enthaltene Wasser'. In other words, the turgor arises by the osmotic action of solutes in the cells, which results in an internal hydrostatic pressure and expansion of the cell through the elasticity of the cell wall. Obstinately, De Vries wrote in the margin of his copy of the first edition of the Sachs' *Lehrbuch* (now in the library of the University of Amsterdam), that he did not agree, 'since *Nitella* internodes remain sturdy when cut. The imbibition of the cell wall determines, independently of the cellular contents, the turgor of the cell.'

Within a year De Vries rehabilitated himself with the work on the permeability of red beet cells (1871). With this tissue he demonstrated the phenomenon which he later called 'plasmolysis', using salt solutions of various strengths. From this work he concluded that (1) externally applied solutes (salts and sugars) withdraw water from the vacuole of the cell; (2) such solutes present in the vacuole attract water; and (3) the protoplasm is impermeable to solutes, in other words, is semipermeable. This experimental work lent solid support to Sachs' statements.

Meanwhile the third edition of the *Lehrbuch der Botanik* had been published, and here Sachs expanded his ideas about the role of the turgor in the mechanics of cell expansion. He stated that turgor is the driving force for growth, i.e. cell elongation: 'jede Ursache, welche die Turgescenz der Zelle ersteigert, wird ihr Wachstum fordern, jede, die sie hindert, wird ihr Wachstum verlangsamen'. By relaxation and incorporation of new cell-wall components the internal hydrostatic force results in the irreversible expansion of the volume of the cell. Again, De Vries (1874, 1877) tried to acquire experimental evidence for this—in retrospect—brilliant idea. In line with his past experience, he argued that a cell or tissue exhibits shrinkage when its turgor is reduced to zero by incubation in a salt solution. The degree of shrinkage should be a measure of the former turgor and thus be correlated with growth rate (Table 2). Implicit in this argument was the (incorrect) supposition that cell walls have a constant elasticity. Also unjustified was the idea that cell expansion is not only driven but also controlled by

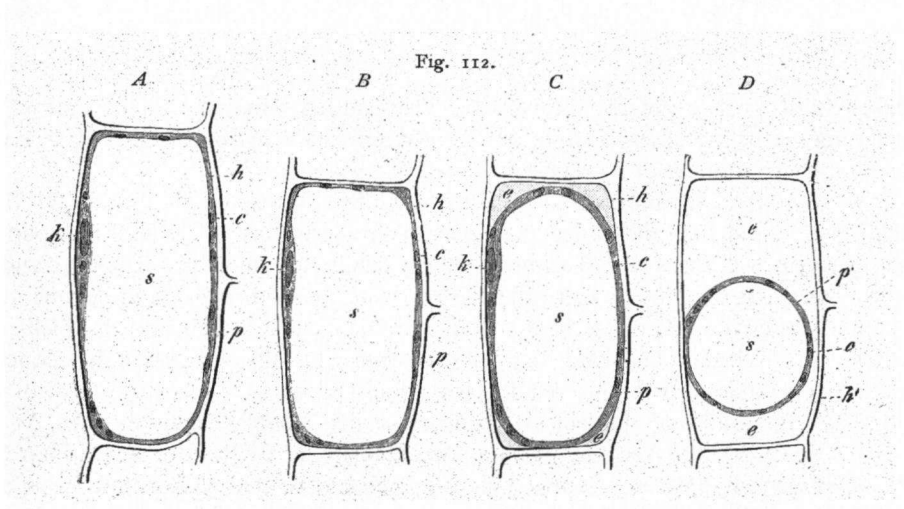

Fig. 15. A picture of plasmolyses in a young parenchyma cell of a flower stem of *Cephalaria leucantha*. From left to right: normal situation; immersed in a solution of 4% salpeter; in 6% salpeter; in 10% salpeter. (From Hugo de Vries (1880): *Leerboek der Plantenphysiologie* (Amsterdam) (149).)

Table 2. Young flower stems of *Cephalaria leucantha* were cut in two halves longitudinally, which were subsequently incubated in sugar solutions of different concentrations. After 2 h the contraction and after the following 46 h the elongation of the top 80 mm were measured (De Vries 1877; Opera I p. 417)

Sugar concentration (%)	Length after 2 h	Length after 48 h	Contraction after 2 h	Elongation in the next 46 h
5	81·5	83·5	—	2
10	81	82·5	—	1·5
15	79·5	81	0·5	1·5
20	75·5	74·5	4·5	—

Table 3. Young flower stems of *Butomus umbellatus* were divided with Indian ink into zones of 20 mm. After measuring the elongation per zone after 12 h, the stems were cut longitudinally into two halves which were incubated in 10% KNO_3 for 2·5 and 4·5 h. (De Vries 1877; Opera I, p. 462)

Segment	Growth (mm) per zone in 12 h before incubation	Contraction (mm) after 2·5 h	Contraction (mm) after 4·5 h
1 (top)	3·1	1·8	1·8
2	4	1·8	1·9
3	4·9	1·8	1·8
4	**5·6**	1·8	**2·1**
5	5·3	1·8	1·8
6	4·2	1·6	1·7
7	3	1·7	1·8
8	1·7	1·5	0

the hydrostatic pressure inside the cell. De Vries underestimated wall plasticity as a determining factor in cell expansion, and considered cell function more from a mechanical than from a metabolic viewpoint.

With Indian ink, he marked segments on young flower stems, measured their elongation rate and then incubated the stems in salt solutions. After a certain period, the contraction was measured. Examples of his results are shown in Tables 3 and 4. However, such experiments showed a strong variability, and although De Vries concluded that 'Im Grossen und Ganzen beobachten wir also einen deutlichen Parallelismus zwischen Turgorausdehnung und Langenwachstum', he also expressed his concern about the reproducibility of his results, for which he blamed the variability of his experimental material and the lack of accuracy of his (visual!) measurements. At the end of his study he also recognized that the extensibility of the cell wall is hard to grasp experimentally. We now know that turgor has a relatively constant value and that elasticity of the cell wall is strongly dependent on the age of the cell and is hardly connected with cell expansion (Steudle *et al.* 1977). By applying his plasmolysis method on press sap of various tissues and plant species, De Vries claimed later (1884) that

Table 4. The epicotyl of etiolated *Phaseolus multiflorus* plants was divided with Indian ink into zones of 3 mm. After measuring the elongation per zone after 8 h, the epicotyl was cut off and incubated in 10% KNO_3. The length of the zones was measured after 2 h (De Vries 1877; Opera I, p. 469)

Zone	Elongation per zone before incubation	Contraction (mm) after incubation for 4 h
1 (top)	0·5	0·4
2	1·1	0·5
3	2·2	0·4
4	1·2	0·4
5	1·1	0·4
6	1·1	0·1

Table 5. Salpeter value of press sap of leaf stems of *Rheum officinale* and *Heracleum sphondylium*. The 'salpeter value' is the osmotic potential of the sap, measured with the plasmolysis method, and referred to the value of 0·1 M KNO_3 (De Vries 1884; Opera II, p. 258)

Part	Salpeter value of the press sap
Rheum officinale	
Stems of very young leaves	0·21
Stems of older leaves	0·21
Stems of still older leaves	0·215
Stems of older, non-growing leaves	0·215
Heracleum sphondylium	
Stems of young leaves	0·17
Stems of older leaves	0·165
Stems of older, non-growing leaves	0·165

turgor is indeed remarkably constant, as illustrated in Table 5, but this is not correct; his method would only yield osmotic potential values. Again, De Vries is hardly to blame for that. Only since Slatyer & Taylor's definition of water potential and its components in 1960 can such mistakes no longer be made.

De Vries as physical chemist

Although experimentally not very successful, his continuing interest in the effects of solutes after being appointed professor brought him fame. He compared the power of various solutes to induce plasmolysis in selected 'indicator plants' (particularly *Curcuma rubricaulis* and *Tradescantia discolor*), and used them as osmometers. He expressed this property as an 'isotonic coefficient' by comparison with a 1% KNO_3 (=0·1 M) solution. The concentration of a KNO_3 solution that had the same plasmolytic power as a particular solution was designated as the 'salpeter value'. Based mainly on data from Pfeffer (1877), the salpeter value of 0·1 M KNO_3 was estimated by De Vries to be approximately 3 atm (using the Van 't Hoff law, formulated a few years later, this value is 4·86 atm). The results of a large collection of data may be summarized as follows: (1) plasmolytic power is dependent on concentration but is independent of the nature of the dissolved substance; the isotonic coefficients of equimolar solutions of, for example, different

Table 6. Isotonic coefficients (=the power to induce plasmolysis, in comparison with that of 0·1 M KNO_3, which was given the arbitrary value of 3) of various compounds (De Vries 1884; Opera II, pp. 214, 215)

Compound	Isotonic coefficient
Sucrose	1·88
$NaNO_3$	3
KCl	3
NaCl	3·05
NH_4Cl	3
K_2SO_4	3·9
K_2HPO_4	3·96
K_3 citraat	5·01
$MgSO_4$	1·78
$MgCl_2$	4·33
$CaCl_2$	4·33

Table 7. Proportionality of isotonic coefficient (see legend to Table 6) and molecular lowering of the freezing point of various compounds (De Vries 1884; Opera II, p. 226)

Compound	Mol. lowering of freezing point	Isotonic coefficient	Ratio
KNO_3	27	3	9
$NaNO_3$	26·4	3	8·8
K_2SO_4	35	4	8·75
$MgSO_4$	18	2	9
KCl	33·6	3	11·2
NaCl	31·4	3	10·5
NH_4Cl	43·2	3	11·6
$CaCl_2$	43·2	4·33	10

monosaccharides has the same value; (2) compared with KNO_3, the isotonic coefficients of salts differ in the proportion of 2:3:4 ... (Table 6). The first conclusion led De Vries to search for other properties, in which the affinity of a solute to the solvent is expressed. The publications of Güldberg (1870), De Coppet (1871) and Raoult (1880) directed him to freeze-point and vapour-pressure decrease, and he demonstrated their similarity as shown in Table 7. The second property supported the theory of salt dissociation formulated by Svante Arrhenius. With these conclusions and with the wealth of data he produced, De Vries strongly supported the early developments of physical chemistry, as acknowledged extensively by Van 't Hoff in his Nobel lecture (1901).

Historical evaluation

We must conclude that De Vries has contributed substantially to the 19th century ideas about growth in plants, not so much by supporting Sachs' ideas, as by directing the attention to the predominant role of water in plant life. The physiological work of De Vries leaves the impression of being the result of a most energetic and dedicated experimentalist. This characteristic must have been most stimulating for colleagues and students. None the less, the time had not yet come for the elucidation of the mechanism

of plant growth. The ideas of water relations in a plant cell were to be preliminary, and too little was yet known about the structural and metabolic properties of the cell wall. It may be noted that not until 1992 were the expansins, proteins in the cell wall that induce wall expansion, discovered (McQueen-Mason *et al.* 1992). Perhaps the best illustration of De Vries' energy and perseverance is his contribution to the theory of colligative properties in which he used his plasmolysis method far beyond the realm of plant science. This forceful striving for the application of the scientific method and his ability to describe his results in both the scientific literature and in journals available to the general public gives him a remarkable place in the history of natural science. His interest in growth phenomena in plants was inherited by his student F.A.F.C. Went, whose son Frits Went eventually discovered the growth hormone auxin, which is the first real hallmark in plant growth physiology.

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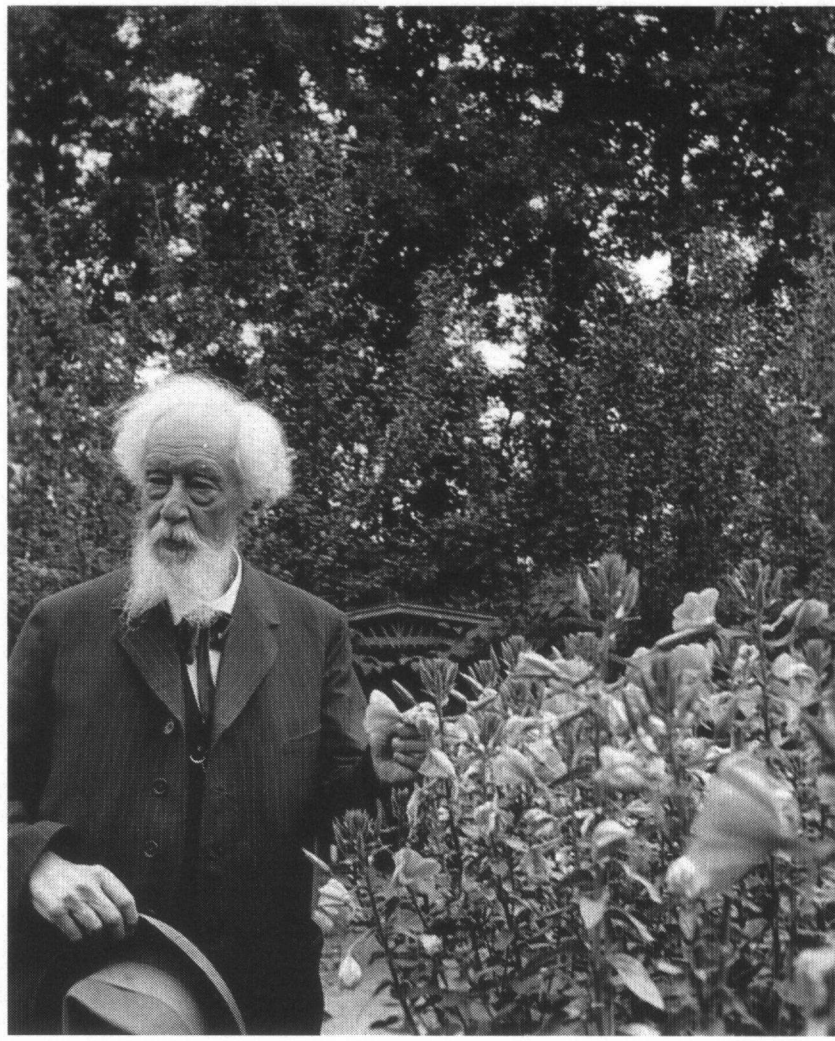

Fig. 16. Hugo de Vries with flowering *Oenotheras* in the experimental garden of his house in Lunteren, July 1932 (Library of the Biological Centre, University of Amsterdam: Archive Hugo de Vries).