Trees (1992) 6: 13–18

Structure and Function
Trees


# In vivo pH measurement in the xylem of broad-leaved trees using ion-sensitive field-effect transistors*

**Volker Herrmann and Manfred Tesche**

Institute of Forest Botany and Forest Zoology, Faculty of Civil Engineering, Water Management and Forestry, Dresden University of Technology, Pienner Strasse 7, O-8223 Tharandt, Federal Republic of Germany

Received December 18, 1990/Accepted September 10, 1991

**Summary.** A new method of in vivo pH determination in the xylem of broad-leaved trees using ion-sensitive field-effect transistors is developed and its suitability for use is studied. In the first few hours after the sensor had been implanted in the xylem signals could be detected which were generated in response to mechanical damage; particularly strong signal changes are detectable in *Populus balsamifera* L., *Tilia cordata* Mill. and *Aesculus hippocastanum* L. The pH values of the xylem sap extracted from branches corresponded to the values measured by the in vivo method only at certain times. Due to sensor drift the measuring accuracy of long-term experiments lasting up to 3 weeks is restricted. The in vivo measurement of pH in the xylem of poplar branches revealed the ability of the living xylem to buffer the pH of the sap to its own characteristic value.

**Key words:** pH value – Broad-leaved trees – Xylem – ISFET – Sensor – pH measurement – Long-term measurement – In vivo measurement

## Introduction

In order to get further insights into tree metabolism, to learn more about tree stress and to stop forest decline new experimental methods are required by tree physiologists. There is a lack of methods suited for long-term experiments and investigations into the whole tree (Tesche 1989). The pH value is one of the most fundamental values of biochemistry and its knowledge could be the key to the solution of many problems. The details of the reaction of tree metabolism to soil acidification are still unknown. The amount of soil and soil moisture acidification the tree is able to compensate by buffering mechanisms has still not been settled. The xylem water reaches all parts of the tree very quickly. If there were an actual acidification of the latter over long transport distances dramatic consequences for the metabolism of cells in all organs would result. Thus the availability of continuously recorded xylem pH values is of utmost importance for our understanding of the whole tree. Until now methods used for pH determinations in the xylem have been based upon the extraction of xylem sap and subsequent in vitro analysis. There are no in vivo methods available. We want to emphasize the need to develop new methods and equally to point out the difficulties implied by the introduction of new methods.

It is only during the spring, in which the sap flow occurs undisturbed, that the extraction of xylem sap from trees may be readily accomplished, but even in spring the sap can only be extracted without any difficulty from some trees (Essiamah 1982). The only exceptions are lianas having very large vessels with weak capillary adhesion forces, so that xylem may leak out under gravitational force during other seasons (Huber 1956). In the past various methods were developed for collecting the xylem sap independent of the season. Dixon and Atkins (1916) obtained low amounts of sap by centrifuging tiny branch pieces. Bennett et al. (1927) developed methods of sap collection using liquids or gases under pressure. Bollard (1953) reported about a method of sap extraction under vacuum. However, the branch to be extracted for sap should be successively cut, as only a small portion of xylem capillaries becomes empty due to infiltration of air. Zaerr (1982) used a pressure chamber to collect sap from larger stem pieces. Other methods, e. g. cooking or chemical disintegration of wood (Hart 1965) or binding of xylem sap to dextran gels (Glavec 1987) seem to be difficult, as xylem sap gets contaminated by the constituents of living or dead cells. Ferguson (1980) suggested that this problem could be avoided when using the vacuum extraction method of Bollard (1953).

Our present studies serve to investigate the possibilities of in vivo pH measurement using semiconductor sensors, in particular ion-sensitive field-effect transistors (ISFETs).

* Dedicated to Prof. Dr. O. L. Lange to his 65th birthday

*Offprint requests to:* M. Tesche

**Table 1.** Characterization of trees used (soil site: sandy loam, good nutrient supply, pH value of 6.3 in the upper 10 cm of soil layers)

| Species | Sample size (*n*) | Age (years) | Height (m) | Diameter |
|---|---|---|---|---|
| Balsam poplar (*Populus balsamifera* L.) | 8 | approx. 25 | 15 | 25–35 |
| Small-leaved lime (*Tilia cordata* Mill.) | 1* | approx. 55 | 22 | 60 |
| European horse-chestnut (*Aesculus hippocastanum* L.) | 1* | approx. 45 | 17 | 60 |

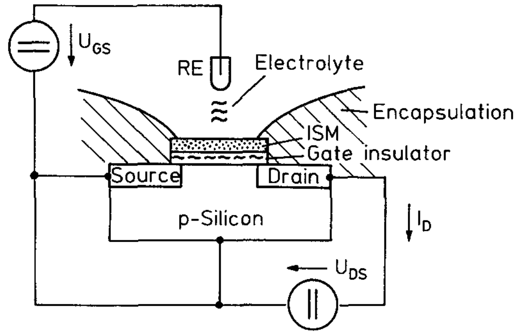


**Fig. 1.** Set-up of an ISFET
$U_{GS}$-Gate-source voltage; $U_{DS}$-Drain-source voltage; $I_D$-drain current; *RE*-reference electrode; *ISM* ion-sensitive membrane. The electrolyte is the liquid to be measured

Many of the problems encountered, e. g. the distribution of biopotentials or wound reaction following sensor implantation will arise similarily when ISFETs of various selectivity or biosensors based on ISFET are used. Therefore, these aspects are of fundamental importance. Publications dealing with the use of ISFET for research purposes into plant physiology do not exist as far as we know. Thus, this paper is intended to try add some facts to our former paper (Herrmann et al. 1989).

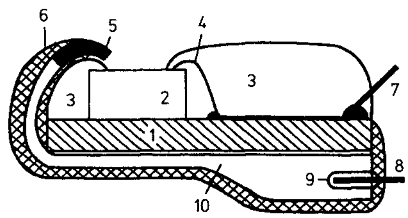


**Fig. 2.** Micro-RE installed on ISFET (sectional drawing) *1*-Cevausit-carrier material; *2*-Chips; *3*-ISFET-encapsulation; *4*-bondwire; *5*-diaphragm; *6*-epoxy resin; *7*-connections; *8*-silver wire; *9*-AgCl-coating; *10*-glass tube filled with molar KCl

## Materials and methods

*Trees.* Our investigations were performed exclusively on broad-leaved trees. Coniferous trees were not investigated, as the conifers readily exude resins at the measuring spot after the sensors have been implanted. Table 1 shows the characteristic data of the trees used in our studies.

*Measurement by ISFET.* The ion-sensitive field-effect transistor is a chemically sensitive semiconductor based on the metal-oxide field-effect transistor (MOSFET), which is modified by substituting the gate metal by an arrangement of reference electrode – electrolyte – ion-sensitive membrane.

Figure 1 shows the schematic set-up of the ISFET. When subjected to an electric field, a channel capable of conducting electric current builds up between the drain and source leading to the flow of a drain current ID. The driving electric field during measurement could be influenced by the surface potential of the ion-sensitive membrane (besides constants). The mechanism of formation of the surface potential is dependent upon the type of ion-sensitive membrane. The selected pH-ISFET consisting of $Si_3N_4$ membrane shows a satisfactory level of selectivity for hydrogen ions and a linearity between pH value and surface potential in the pH range of approximately 2–12.

An encapsulation protects the sensor from electrolyte contact, whereby the ion-sensitive membrane remains unprotected. The ISFETs when applied in practice show a number of disadvantages, e. g. temperature sensitivity and drift. The majority of errors originating from temperature and other factors could be corrected. However, it has not yet been possible to eliminate the errors attributed to drift.

Thus it follows that the accuracy of long-term in vivo pH measurements is limited (see Results and discussion). Detailed information on ISFET as a semiconductior sensor and on the formation of the surface potential is available from Bergveld (1981), Matsuo et al. (1981), Sibbald (1986), and Harame et al. (1987).

A stable measurement by ISFET requires a reference electrode to be employed. If not otherwise specified, silver/silver chloride RE (type SE20, Research Institute Meinsberg, FRG) was used. The connection of RE to the measuring electrolyte was made via a salt solution (0.1 mol KCL). Due to the presence of biopontentials and resulting disturbances micro-reference electrodes were made and glued to ISFET by applying epoxide resins (see Fig. 2).

*Measurement amplifier.* The operation of ISFET as a pH-sensor requires special amplifier circuits. For the purpose of long-term measurements a self-made device was used. The ISFET and the MOSFET on the sensor chip were connected as a differential amplifier, each with a drain current of about 100 μA. The signal was amplified and up to 4 channels multiplexed to an Analog-Digital-Converter. Special interface circuits effect the communication between the converter and the computer (KC 85/2, Mühlhausen Electronics Co., FRG). A p/n junction on the sensor chip is used as temperature sensor, and: its data were transferred to a computer. All corrections were made in software. For short-term measurements a hand-operated pH-meter (type 5155, made by Central Institute of Nuclear Research, Rossendorf) was used.

*Reference measurements.* For reference measurements, xylem sap was extracted from branches near the place of implantation by applying a method after Bollard (1953). The pH value was measured by a pH glass electrode (Research Institute Meinsberg). Because the knowledge on buffering the xylem sap is not sufficient in this respect, the measurement was carried out immediately after the extraction, in order to avoid a change of the pH due to the reaction with $CO_2$ in the air.

*Implantation of ISFET.* For the in vivo pH measurement of the xylem of trees, the ISFET has to be implanted. The wounding resulting from implantation induces abrupt changes of the processes of transportation in this region. In case of wounding, the trees protect their water transport systems against air access by sealing the bordered pits and by thyllosis. In order to avoid the cut-off of transpiration flow in the undamaged vessels in the vicinity of implantation site, the sensors were implanted under water to avoid air infiltration. The employed ISFET catheter was 3 mm in diameter with a measuring field of approximately 0.2 mm × 0.5 mm. Soaking the stem under deionized water, a hole was drilled into the xylem up to a depth of 2 cm, the sensor was inserted and

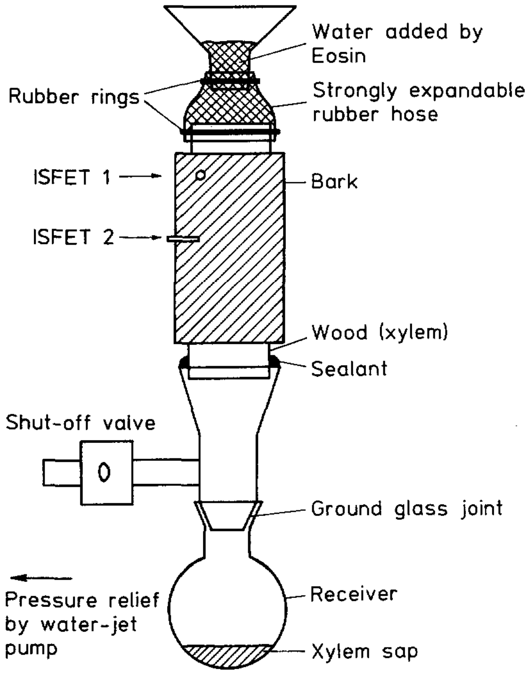


**Fig. 3.** Experimental set-up for the extraction of xylem sap

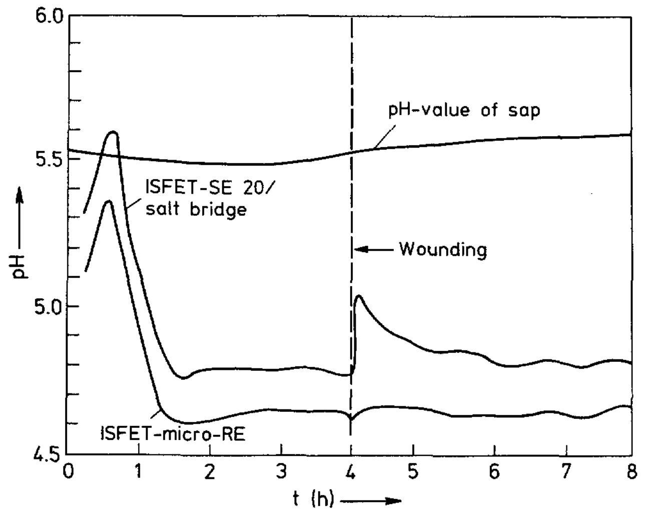


**Fig. 4.** pH measurement in *Populus* relative to one RE SE 20 and alternative to one Micro-RE in comparison with the pH value of the sap extracted from a neighbouring branch

subsequently the wound was sealed by tree wax. While using the SE20 type reference electrode, the salt bridge was implanted in the vicinity of the sensor at the same depth.

*Short-term experiment.* In the short-term experiment during June/July 1989 the measurement using the micro-reference electrode (see Results) was continued for 4 h only. The main aim of this experiment was to detect principal reaction types as well as to compare the pH values determined by this method with the values obtained from the extracted sap of the same branches. Fifteen measurements were carried out using eight poplars, and five measurements per tree using lime and horse-chestnut trees (see Table 1). The ISFET sensor was implanted in the stem as described below and the sap was collected from a lower twig of the tree, possibly in the close neighbourhood of the implantation site.

*Measurement of pH in branches.* The measurement of pH in the *Populus* branches was performed in order to compare the pH values measured by the ISFET with those of the extracted xylem sap from the same branch. To this end a poplar branch was cut at a height of 4 m before sunrise and re-cut under water in order to avoid formation of air bubbles. Two ISFET sensors were inserted radially into a branch of about 25 cm in length and about 3 cm in diameter at an angle of 90° to each other. Figure 3 shows a cut branch with ISFET implanted, and the experimental equipment. The first ISFET (ISFET 1 in Fig. 6) and the second ISFET (ISFET 2) were implanted into the branch at distances of 15 and 10 cm from the lower cut surface. The branch was positioned in a way that the lower cut-end was subjected to the vacuum. The upper-end was soaked fully under water to enable the displacement of xylem sap. Without this measure air embolism would prevent the sap from being sucked out of the vessels. For interpreting the results at least two periods are needed: one during which ISFET is measuring the xylem sap and another during which the sucked water is measured. This is possible by adding colour to the water. It could be shown that Eosin-colour solution is transported at a speed that is 20% below that of water.

## Results and discussion

### *Problems and errors due to biopotentials*

In preliminary experiments, the presence of biopotentials, or in other words electric potential differences, could be detected in the tree stem; this is also known from literature (e.g. Tattar and Blanchard 1976). In the *Populus* stems, radial potential gradients of up to 100 mV could be detected. In the stem's axial direction minor potential differences occur, however, a jump of potential of up to 100 mV could be detected over the root/soil interface. When located between ion-sensitive membrane and reference electrode, these potential differences affect the measurement. As a potential difference of 50 mV causes an error of approximately 1 pH unit, the biopotentials should be avoided by positioning the reference electrode properly (that means the tip being very close to the sensitive area of the ISFET).

Figure 4 shows the alternative measurement of ISFET using the SE20/salt bridge reference electrode and the micro-reference electrode (Fig. 2). A difference of 0.2 pH between the both curves could be detected, whereby a potential difference of about 10 mV between the both reference electrodes can be calculated. In the first 1–2 h following implantation of the sensor typical initial wounding reactions occurred. Similar changes could not be detected in the xylem sap extracted from the neighbouring branches.

A further mechanical wounding due to drilling a hole close to the implantation site causes an additional jump of potential, probably resulting from rupturing of a number of cell membranes possessing membrane potential. Figure 4 shows that this jump in potential could be readily detected while using the SE20 reference electrode, but this magnitude is negligibly small while using the micro-reference electrode. The remaining difference of potential between the micro-reference electrode and the ion-sensitive membrane is presumably so small, that the resulting error could be neglected. In the following experiment, the micro-reference electrode was used.

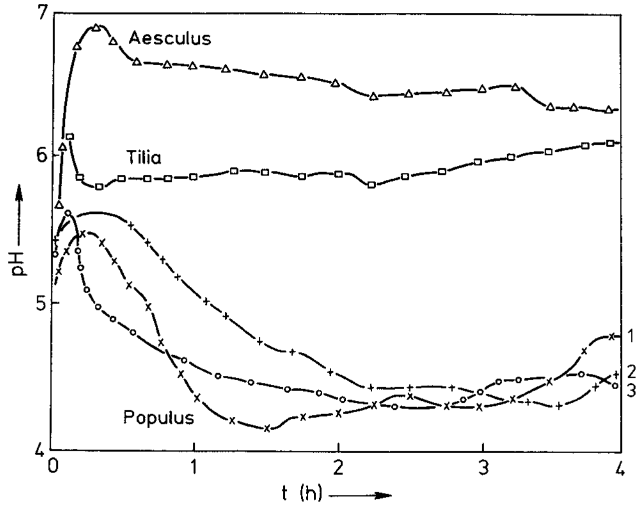


**Fig. 5.** pH values of different trees immediately after implantation. The pH values of the sap extracted from a branch adjacent to the place of implantation was with:
*Aesculus*: 6.3; *Populus* 1: 5.5
*Tilia*: 6.1; *Populus* 2: 5.6
*Populus* 3: 5.6

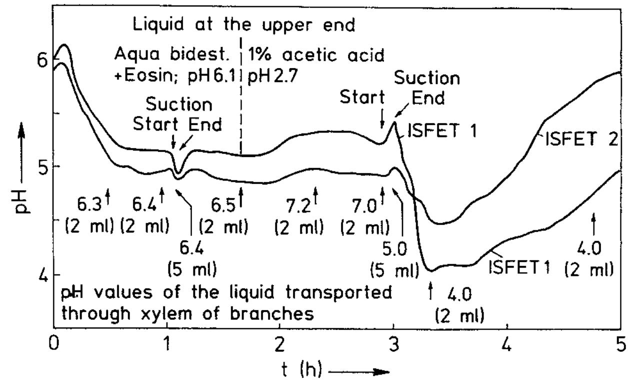


**Fig. 6.** pH values measured in a branch of Populus by means of two ISFETs

*Short-term experiment*

Figure 5 shows typical measurement curves of different tree species. It could be shown that a strong variation of the measured values occurred during the first few hours in all the trees investigated. The peak seen in the first few minutes may be ascribed to the stabilisation of pH regulated by the tree in the remaining water of the wound after the sensor had been implanted. The subsequent decrease may be assumed to be due to the wound reaction of the tree. The pattern according to which the values changed remained equal in the same species; however, strong differences occurred among different species. However, the time scale of the wound reaction is not exactly the same for all experiments; even with regard to only one species. See for instance the three curves of poplar trees. The pattern according to which the value changes remains the same, but the respective time periods required for reaching top and bottom of the curves varies from about 12 min (top) to 2 h (bottom). The top values have small variances (of approximately 0.2 pH), and so do the bottom values (approximately 0.4 pH). In lime and horse-chestnut during each experimental period 5 measurements were carried out successively in the same tree but at different places around the stem. The reactions are similar to those shown with poplar. There is a typical curve for every tree species, the time scale of reaction, however, is not exactly the same. To simplify matters we showed only one curve for both of these trees. The top values of the horse-chestnut trees were found to be about 0.7 pH above, and those of the poplar 0.1 pH below the extracted sap of a neighbouring branch.

The differences occurring between the measured top values which are assumed to correspond to the sap values, and the sap values may be triggered by the wounding reaction. This might in part be due to the natural pH gradient between the site of implantation and the site of sap extraction within the branch. Essiahmah (1982) reported an axial maximum pH gradient of 0.3 pH over a distance of 2.5 m. The course of the measured curve was governed mainly by the wound reaction of the tree and possessed a specific character. This reaction may be assumed to have originated in the immediate vicinity of the wounded zone, whereby a micromilieu builds up at the site of implantation. This assumption may be supported by the fact that the brown coloration of the wood in the vicinity only 1 to 2 mm from the wounded zone was observed after the sensor had been removed, although air was excluded from the process. An increase in the number of experiments including comparative measurements by other methods near the spot of implantation should improve our understanding of this problem.

*Long-term experiment*

The possibility of conducting a long-term experiment (done in the same way as the short-term ones) in poplar was checked over a period of up to 3 weeks. Within this period stable values were obtained. Following an initial decrease (see Figs. 4, 5) there was an increase in pH values after 10 to 48 h up to the initial value of approximately pH 5.2–5.8. It has not been possible to detect any diurnal rhythm of pH change. In the period investigated there was a maximal fluctuation of 0.7 in the pH values obtained from healthy trees.

One of the major problems of long-term experiments is the correction of the measured values based on the assumption of a linear drift (approx. 0.1 pH per day). As our knowledge about the drift behaviour (linear or non-linear) of the sensor over the period of investigation is not adequate, the accuracy of our results from the long-term experiment is restricted.

*Measurement of pH in the branches*

Figure 6 shows a representative measurement of pH in the branches. The behavior of the ISFET 1 and ISFET 2 sensors in the starting phase was similar to that mentioned above (short-term experiments). However, the measured values were by pH 0.3–0.5 greater than the former ones. The pH value of the xylem sap dropping under gravity from the cut-surface was at 6.3 greater than the values measured by applying any other method. The reason for this discrepancy may lie in the wounding caused by two successive cuttings of the two ends and shortening of the branch under water.

The increase of transport velocity of the xylem liquid under vacuum led to a decrease of pH up to about 0.4 pH as indicated by the sensor display. After the normal pressure had been restored, the pH normalized to the initial value. A similar, but oppositely directed effect, could be shown when 1% acetic acid (pH 2.7) was transported through the xylem. A possible explanation of this effect may be the responses of accessory tissues to the pressure applied. Another reason for pH change may be that a sudden change is likely to lead to a potential difference between the micro-reference electrode and the ion-sensitive membrane, which subsequently could be detected as a pH change. However, this explanation contradicts the assumption that there is no potential difference between the micro-reference electrode and the ion-sensitive membrane. The initial value was restored within approximately 5 min. The stream potential should not be taken into account, as the effect of liquid stream with a comparable velocity (maximum 1 cm/min) and similar composition could not be reproduced in vitro.

The substitution of water at the upper branch-end by 1% acetic acid led to a weak increase in pH as indicated by the reaction of the sensor as well as to an pH increase of the transported liquid. It demonstrated the ability of the living xylem to actively buffer the pH of the capillary liquid to a definite magnitude. The weak increment of pH might be due to the excessive fluctuation of the regulatory systems in the plants. Clarkson et al. (1984) detected the pH buffering effect in the roots of onions. The rise in transport rate of the acetic acid through the xylem after a period of about 5 min of vacuum application at first led to an increase in pH as described above, and later on to an enhanced response of the two ISFETs showing increased acidification. The measured values continued to decrease even after the sucking process had been shut down. It seemed that the reaction did not take place when acetic acid reached the site, but only when the buffering capacity was fully utilized. However, at this stage the pH of the liquid sucked through the xylem changed from 2.7 to 4.0. The subsequent decline of the curve might result from the irreversible tissue damages leading to the death of the corresponding complex. Following the steep decline of pH values detected by the ISFETs no further reactions on the changes in transport velocity or pH values at the upper end of the branch could be verified.

If the acetic acid is used at a higher concentration (10%) there occurs simultaneously with a quick response of the ISFET a strong decrease of the transport rate to about 1/8th of the previous value. Although both the ISFETs responded qualitatively in the same way, quantitative differences could be identified. The reasons for this may lie in the absorption of $H^+$ ions through the vessel walls or through the accessory tissue, which may trigger a pH change over the distance from ISFET 1 to ISFET 2. These differences may also be attributed to the specific nature of the site in which the sensors had been implanted.

The results of these experiments show under the given conditions that the pH values measured by ISFET followed after the starting phase qualitatively those of the transported liquid, however with differences in the absolute values, sometimes exceeding 1 pH.

*Summary*

The method described opens up new possibilities of in vivo determination of pH in the trees and could be extended to cover measurement of other ion concentrations. The ISFET has got some advantages over other potentiometric sensors, e.g. it is light-weight, mechanically stable, and has a low-ohm output signal. As the ISFETs are produced in the microelectronics compatible standard, it is possible to manufacture compact sensors with multiple ISFET-arrays as well as integrated reference electrode (in exact terms reference field-effect transistor) and signal-processing electronics (e.g. see Wong and White 1989).

Our present results should be regarded as preliminary. Detailed studies should be conducted that concentrate on the reaction of trees on wounding during implantation. The question that remains to be investigated is to what extent the signals detected represent the undisturbed region of the tree at times following the first dramatic wound reaction of the xylem. As regards the sensor technology, the drift behaviour of the ISFET as for long-term in vivo experiments should be studied in order to correct the pH values for the drift.

*Acknowledgements.* We should like to express ours thanks to Dr. M. T. Pham and his colleagues (ZfK Rossendorf) for providing us with the ISFET sensors, Prof. S. Kaiser for his technical advice and Dr. E. Hoque, GSF München, for his helpful discussion.

## References

Bennett JP, Andersen FG, Milad Y (1927) Methods of obtaining tracheal sap from woody plants. New Phytol 26: 316–323

Bergveld P (1981) The operation of an ISFET as an electronic device. Sens Act 1: 17–29

Bollard EG (1953) The use of tracheal sap in the study of apple tree nutrition. J Exp Bot 4: 363–368

Clarkson DT, Williams L, Hanson JB (1984) Perfusion of onion xylem vessels: a method and evidence of control of the pH of the xylem sap. Planta 162: 361–369

Dixon HH, Atkins WRG (1916) Osmotic pressure in plants. VI. The composition of the sap in the conducting tracts of trees at different levels and at different seasons of the year. Sci Proc R Dublin Soc 5: 51–62

Essiamah SK (1982) Frühjahrsaktivitäten bei einheimischen Laubbäumen. Dissertation University Göttingen, FRG

Ferguson AR (1980) Xylem sap from *Actinida chinensis*: apparent differences in sap composition arising from the method of collection. Ann Bot 46: 791–801

Glavac V (1987) Die Gewinnung des Xylemwassers von lebenden Bäumen und Analyse seiner Mineralstoffgehalte. Angew Bot 61: 453–466

Harame DL, Bousse L, Shott JD, Meindl J (1987) Ion-sensing devices with silicon nitride and borosilicate glass isolators. IEEE Trans on electr Dev ED-34: 1700–1709

Hart H (1965) Morphological and chemical differences between sapwood, discolored sapwood and heartwood in Black Locust and Osage Orange. For Sci 14: 335–338

Herrmann V, Tesche M, Kaiser S (1989) Versuche zur Applikation von Ionensensitiven Feldeffekttransistoren (ISFET) zur in vivo Messung von pH-Werten im Xylem von Bäumen. Wiss Z Tech Univ Dresden 38: 319–323

Huber B (1956) Die Gefäßleitung. In: Ruhland JW (ed) Handbuch der Pflanzenphysiologie vol 3. Springer, Berlin Heidelberg New York, pp 541–582

Matsuo T, Esashi M (1981) Methods of ISFET fabrication. Sens Act 1: 77–96

Rademacher P (1986) Morphologische und physiologische Eigenschaften von Fichten (*Picea abies* L.), Tannen (*Abies alba* Mill.), Kiefern (*Pinus sylvestris* L.) und Buchen (*Fagus sylvatica* L.) gesunder und erkrankter Waldstandorte. Dissertation University Hamburg, FRG

Sibbald A (1986) Recent advances in field effect chemical microsensors. J Mol Electr 2: 51–83

Stanislawek S (1987) Sugar content of xylem sap and susceptibility of willow to *Chondrostersumpurpureum*. New Zeal J Bot 25: 263–269

Tattar TA, Blanchard RO (1976) Electrophysiological research in plant pathology. Annu Rev Phytophathol 14: 309–325

Tesche M (1989) Distreß und Eustreß bei Gehölzen. 2. Symposium, Ausgewählte Probleme der Gehölzphysiologie, Techn. Univ. Dresden, Sekt. Forstwirtsch. Tharandt, Tagungsbericht 5–12

Wong HS, White MH (1989) A CMOS-Integrated ISFET-Operational amplifier chemical sensor employing differential sensing. IEEE Trans Electr Dev ED-36: 479–487

Zaerr JB (1982) Pressure chamber device to collect xylem sap from forest trees. For Sci 28: 219–222