Phyton (Horn, Austria)	**Vol. 42**	Fasc. 1	79–97	29. 7. 2002

Determination of the Osmotic Potential in Secondary Phloem of Norway spruce (*Picea abies* (L.) KARST.) Using Tissue Discs – A Technical Report

By

Sabine ROSNER*)**) and Silvia B. KIKUTA

With 4 figures

Received October 10, 2001

Accepted November 13, 2001

Key words: Disc method, relative water content, saturation kinetics, vapor pressure osmometry, water content.

Summary

ROSNER S. & KIKUTA S. B. 2002. Determination of the osmotic potential in secondary phloem of Norway spruce (*Picea abies* (L.) KARST.) using tissue discs – A technical report. – Phyton (Horn, Austria) 42 (1): 79–97, with 4 figures. – English with German summary.

Methods for determining relative water content, the osmotic potential at full saturation (Ψo (sat)) and the in situ osmotic potential (Ψo (in situ)) on secondary phloem discs of *Picea abies* (L.) Karst. are presented.

Osmotic potential at full saturation and relative water content (R), parameters enabling comparisons within the tree or between different trees, were measured on the same sample. Small water losses during manipulation steps between weighing after saturation and weighing after cryo-storage did not influence correct determination of Ψo (sat) values. Saturation periods of 5 to 10 min were sufficient to reach full turgor. With these short saturation times, oozing or respiratory loss of osmotically active solutes was negligible.

An improved disc method, termed SFD method, was developed to measure Ψo (in situ) on saturated, frozen discs that were dehydrated to the initial fresh weight on a micro-balance before measurement of the potential. Thus, the osmo-

*) S. ROSNER, S. B. KIKUTA, Institute of Botany, University of Agricultural Sciences, Vienna, Gregor Mendel Str. 33, A-1180 Vienna, Austria. Telephone: +43 1 47654 3198. Fax: +43 1 47654 3180. E-mail: rosner@edv1.boku.ac.at

**) To whom correspondence should be addressed.

tic potential was measured exactly at the water content operative at sample collection.

Ψo (in situ) determined with the conventional disc method (tissues frozen without saturation) were significantly lower (more negative) because of water loss during manipulation steps. The SFD method gives more reliable values for the in situ osmotic potential compared to the conventional disc method. Furthermore, it is possible to measure several different parameters of water status, such as water content, relative water content, and osmotic potential at different water contents, on the same sample. Further advantages of the SFD method over the press-sap and conventional disc methods are discussed.

Zusammenfassung

ROSNER S. & KIKUTA S. B. 2002. Bestimmung des osmotischen Potentials von Fichtenbast (*Picea abies* (L.) KARST.) mittels Gewebescheibchen – Ein technischer Report. – Phyton (Horn, Austria) 42 (1) : 79–97, mit 4 Abbildungen. – Englisch mit deutscher Zusammenfassung.

In der vorliegenden Arbeit werden Methoden präsentiert, den relativen Wassergehalt, das osmotische Potential bei voller Sättigung (Ψo (sat)) und das in situ osmotische Potential (Ψo (in situ)) an Bastscheibchen von Fichte zu bestimmen.

Mit der beschriebenen Methode ist es möglich, R und Ψo (sat) an derselben Probe zu messen. Geringe Wasserverluste während nötiger Manipulationsschritte hatten keinen Einfluss auf die korrekte Bestimmung von Ψo (sat). Sättigungszeiten zwischen 5 und 10 Minuten waren ausreichend, um volle Turgeszenz zu gewährleisten, und garantierten keine Verluste von osmotisch aktiven Substanzen oder respirative Verluste.

Die Bestimmung von Ψo (in situ) erfolgte an gesättigt eingefrorenen Gewebescheibchen, die zuvor auf einer Mikrowaage auf das ursprüngliche Frischgewicht dehydriert wurden, wodurch die Potentialmessung exakt beim ursprünglichen in situ Wassergehalt ermöglicht wurde (SFD Methode). Die Bestimmung von Ψo (in situ) mit der herkömmlichen Methode, bei der die Scheibchen ungesättigt eingefroren werden, führte zu signifikant negativeren Werten, welche durch unkontrollierbaren Wasserverlust bedingt waren.

Die Autoren erachten deshalb die SFD Methode als eine Methode, die repräsentative Werte für die Bestimmung von Ψo (in situ) liefert. Darüber hinaus ist es möglich, verschiedene Wasserhaushaltsparameter wie den (relativen) Wassergehehalt und das osmotische Potential an derselben Probe bei verschiedenen Sättigungsgraden zu messen.

Abbreviations

Ψo	osmotic potential
Ψo (in situ)	in situ osmotic potential of secondary phloem discs at sample collection
Ψo (sat)	osmotic potential of fully saturated secondary phloem discs
C	water content
C in situ	in situ water content at sample collection
C sat	water content at full saturation
CB	crown base

D	dry weight
F	fresh weight
R	relative water content
R in situ	in situ relative water content at sample collection
S	saturated weight
SFD method	method for measuring the osmotic potential at a given fresh weight (e.g. Ψo (in situ)); discs were Saturated, Frozen, and Dehydrated to pre-set fresh weights, for instance to the in situ values
3/10h	3/10 of tree height

Introduction

Münch's pressure-flow model (MÜNCH 1930) states that phloem osmotic relations play a predominant role in the translocation of photosynthates in the plant body. Therefore, an osmotically dynamic system has to be expected, with solutes constantly moving from sources (sites of photosynthate supply) to sinks (sites of metabolism or storage of photosynthates). Along this pathway, pronounced pressure gradients are generated, associated with differences in the solute concentration. Osmotic potential (Ψo) of symplastic solutions in plant organs is an important component of plant water relations describing the effects of dissolved solutes. Measurement of Ψo should thus provide information on the amount of assimilates supplied to, transported in and removed from the secondary phloem, as well as on storage, mobilisation, and distribution of reserve material.

Two standard methods for the direct measurement of osmotic potential in bark tissues, such as cortex (defined as the tissue between secondary phloem and periderm: ESAU 1969, TROCKENBRODT 1990), secondary phloem, and cambium are widely used today: Ψo is either measured on press saps or on killed plant tissue. Cryoscopy of press saps (DIXON & ATKINS 1910) has been the method most frequently used for these tissues. Thermocouple psychrometry allows a more rapid measurement of osmotic potential with fewer preparatory steps (SPANNER 1951), but only scarce data have been published on stem tissues. KAUFMANN & KRAMER 1967 measured osmotic potentials of secondary phloem discs from various deciduous tree species.

Only the in situ osmotic potential (Ψo (in situ)), defined as the Ψo of freshly harvested tissues, has been measured on cortex, secondary phloem and cambium of Norway spruce (*Picea abies* (L.) Karst.) trees. Water loss from these tissues during sampling, killing, and preparation of press saps for the determination of Ψo (in situ) was not taken into account (KRAEMER 1953, MERKER 1956, DÜNISCH & al. 1996). Considerable water loss from cut edges occurs even in leaf discs (WALKER & al. 1984), thus influencing the measured water potential significantly. Discs of secondary phloem will loose even more water during preparation, since the entire surface is ex-

posed to air. Therefore, it has to be tested if the conventional disc method is reliable for determining in situ osmotic potential.

Ψo (in situ) per se offers limited information on water relations in the plant, since it is influenced by active and passive processes. An active change of osmotic potential is caused by an increase or a decrease of osmotically active solutes at a given water content. A passive change originates solely from a difference in water content, thus from a change in volume of the symplast. To distinguish between active and passive changes, parameters such as the in situ relative water content (R in situ), that is the actual water content as a fraction of the water in the fully hydrated tissue, or the osmotic potential at full saturation (Ψo (sat)) have to be determined. These parameters permit comparisons of water relations in the secondary phloem within a tree and between trees, as well as the measurement of seasonal courses and reactions to drought stress.

Considering the importance of assimilate distribution and utilisation for plant growth and defence, data on the osmotic potential of the phloem can be helpful in understanding differences in the vitality of trees. Drought stressed trees may be easierly colonised by aggressive phloem feeding bark beetle species (Coleoptera: Scolytidae) due to their less effective terpene-based defence system (CHRISTIANSEN & al. 1987, LEWINSOHN & al. 1993, BRYANT & RAFFA 1995, PAINE & al. 1997). Also, they may offer high nutritional quality for the beetles, because of the increased levels of minerals, soluble N and sugars in the secondary phloem (CLANCY & al. 1995). Thus, for a better understanding of these plant-insect interactions, osmotic parameters should be measured in the secondary phloem and not in the xylem.

The purpose of this study was to develop reliable methods for determining the in situ relative water content and the osmotic potentials both in situ and at full saturation on secondary phloem tissue of *Picea abies*. The conventional disc method introduced by KAUFMANN & KRAMER 1967 was improved. Since no data on saturation kinetics for secondary phloem have been available, saturation experiments were done to find out how to reach full turgor with a minimum loss of osmotically active solutes.

Material and Methods

Plant material

Samples of secondary phloem were taken from Norway spruce trees (*Picea abies* L. KARST.) harvested at four low-elevation sites in Lower Austria: Manhartsberg, Prinzersdorf, Rosalia, and Amstetten. Samples were also taken from a high-elevation stand in Styria: Murau. For further details of study sites, trees, and time of sample collection see table 1.

Samples were either taken in the field immediately after harvesting or from spruce logs (60 cm length) transported to the laboratory and stored in a cold room (4 °C, 80% r.h.).

Table 1.
Characterisation of study sites and harvested trees

Study site	Height a.s.l. (m)	E	N	Tree age (y)	Tree height (m)	Time of harvesting
Murau	1200	14°10'	47°05'	90	25.0	August 4, 1995
Manhartsberg	500	15°46'	48°33'	95	24.7	August 17, 1995
Prinzersdorf	250	15°31'	48°12'	65	20.0	June 21, 1995
				80	23.5	August 26, 1996
				60	19.0	November 5, 1996
Rosalia	600	16°17'	47°42'	75	24.9	May 30, 1996
				80	23.6	
				75	23.5	
				75	23.7	July 22, 1996
				75	21.8	
				75	21.1	
Amstetten	300	14°50'	48°05'	20	12.0	September 28, 1999

Preparation of discs

A core of bark plus one or two annual increments of wood was punched out with a cork-borer of 6 mm diameter. To avoid water loss during transport to the laboratory the cores were tightly wrapped in Parafilm® (American National Can™, Greenwich) and stored in a styrofoam box kept cool with ice packs.

Starting from the vascular cambium, discs were cut tangentially (in direction to the periderm) from the core with a scalpel. On the disc cut next to the cambium the water status of the conducting secondary phloem, consisting of uncollapsed sieve cells and parenchyma cells, was determined. In *Picea abies*, the sieve cells of the last and next-to-the-last annual rings remain uncollapsed during summer and transport assimilates axially (HOLDHEIDE 1951). The thickness of the discs had to be chosen according to the specimen and varied from 0.2 to 0.5 mm, since the width of the conducting secondary phloem depends on genetics, growth conditions, and the physiological age of the bark (BÄUCKER & al. 1998, FRANCESCHI & al. 1998, ROSNER 1998). The width of secondary phloem (conducting and non-conducting) of the trees investigated was 4 to 6 mm.

About 0.5 mm thick discs were also cut from the central part of the non-conducting secondary phloem 1.5 to 2.5 mm distant from the vascular cambium. Both sampling positions proved suitable for delivering water status parameters comparable within or between trees. Radial gradients of osmotic potential and relative water content in secondary phloem were be discussed in another paper (ROSNER & al. 2001).

Determination of saturation time for discs (disc saturation method)

Osmotic potential at full saturation (Ψo (sat)) and relative water content (R) were measured on discs prepared as follows: After determining the fresh weight (F) on a micro-balance (ME/BE22, resolution: 0.001 mg, Mettler, Zürich, CH), the discs were saturated with distilled water according to the method described by SLAVIK 1974 for leaves. A water-saturated polyurethane foam sheet with holes of 6 mm in

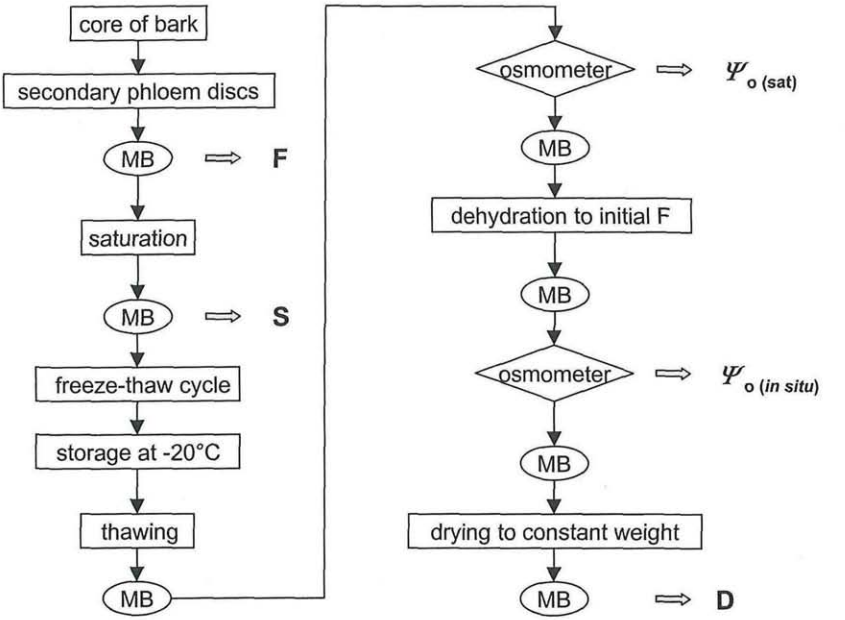

Fig. 1. Protocol of sample preparation and measurement procedure for secondary phloem discs. Osmotic potential at full saturation (Ψo (sat)) and in situ osmotic potential (Ψo (in situ)) were measured on the same sample. Ψo (in situ) was measured with the SFD method. MB = micro balance. For details see materials and methods ("Correlation between osmotic potential and water content").

diameter was put into a small plastic box (7 × 3 cm) with a tightly fitting lid. The discs were placed with forceps into the holes (one disc per hole), thus providing direct contact between the foam sheet and the edges of the discs. After 5 to 120 min of saturation the discs were removed from the box, quickly blotted with filter paper and weighed again to obtain the saturated weight (S). Water loss from the discs was kept at a minimum by performing all steps of manipulation very rapidly. Saturation times of more than 2 hours lead to no further increase in S (unpublished data).

Saturated discs were put in 0.7 ml micro-tubes (Roth, Karlsruhe, D), killed in a repeated freeze-thaw cycle (KIKUTA & RICHTER 1992) to eliminate turgor, and stored at –20 °C (Figure 1).

Before measurement of the osmotic potential, the closed vials with fully saturated and killed discs were warmed up to 37 °C, the operating temperature of the Wescor 5500 Vapor Pressure Osmometer (Logan, Utah, USA). After weighing, discs were transferred immediately into the sample holder of the osmometer, and the osmotic potential was measured. The osmolality values (mosmol kg-1) obtained were converted into osmotic potentials at 25 °C using conversion tables (Lang 1967). As it was not advisable, according to the manufacturers' recommendations, to run the micro-balance and the osmometer under high air humidity in a closed chamber, sample manipulation was performed under laboratory conditions. Time elapsed during the manipulation steps did not exceed 5 seconds. For a high degree of accu-

racy, discs were also weighed after measurement of the potential. The mean value of the two weighings was used to calculate R (equation [3]) for the potential measured.

On average, the osmometer measurement of one sample required 45 min, but with some specimens took up to 90 min till vapor pressure equilibrium was reached (for equilibration times of leaves see KIKUTA & RICHTER 1992). Therefore, to process a large number of samples it was absolutely necessary to store the discs frozen until the measurement of potentials.

Discs were dried to constant weight for 12 h at 85 °C (LITVAY & MCKIMMY 1975). The values of fresh weight (F), saturated weight (S), and dry weight (D) were used to calculate the following parameters:

$$C = (F - D) / D \quad [1]$$

$$C\ sat = (S - D) / D \quad [2]$$

$$R = (F - D) / (S - D) \quad [3]$$

Percentage values were obtained by multiplying C and R by 100.

Determination of osmotic potential at full saturation (Ψo (sat)) on bark pieces (bark piece saturation method)

To detect a possible loss of osmotically active substances during saturation, the disc saturation method was compared to an alternative saturation approach: Bark pieces (5 × 5 cm) were saturated by placing them between two wet sheets of polyurethane. Cores of bark were punched out after saturation for 25, 40, 60, and 130 min. Discs were then cut from the cores and killed in a repeated freeze-thaw cycle before measurement of the osmotic potential. Parallel measurements of Ψo (sat) were done with the disc saturation method, using 10, 20, 30, and 40 min of saturation.

Determination of in situ osmotic potential (Ψo (in situ)) with the SFD method

The SFD method allows measurement of the osmotic potential at different water content levels above, at or below the in situ values.

For measurement of Ψo (in situ), discs prepared as described in "Preparation of discs" were weighed to determine their initial fresh weight, then saturated (method see "Determination of saturation time for discs") for 5 min (conducting secondary phloem) or 10 min (non-conducting secondary phloem), and frozen. Non-conducting secondary phloem required longer saturation because of changes occurring in the tissue at the end of its functional period, such as collapse of sieve cells, dilatation growth, sclerification of parenchyma cells, and accumulation of calcium oxalate crystals (ESAU 1969).

The stored discs were thawed in closed vials at room temperature, transferred to the micro-balance and allowed to dehydrate to the initial fresh weight plus a few tenth of a milligram, exactly 2.5% of the initial fresh weight. The initial fresh weight of each disc was about 10 mg. The potential was measured, and the sample was weighed again. The mean between the weighings prior to and immediately following the Ψo measurement should correspond to the initial fresh weight. Weighing before and after Ψo measurement on a number of samples provided sufficient information on the weight losses during the period between weighing and potential measurement. Therefore, it was possible to estimate the weight to which the samples had to be dehydrated, so that:

(Weight before Ψo measurement + Weight after Ψo measurement) / 2 = Initial weight

Consequently, we assumed that the potential measured was a reasonable approximation to Ψo (in situ).

Comparison between the conventional disc method and the SFD method

To validate the SFD method, in situ osmotic potential was also measured on the same samples with the conventional method: After determining their fresh weight, discs (method see "Preparation of discs") were killed in a freeze-thaw cycle and stored at –20 °C for a few days. After thawing, the discs were weighed again and the osmotic potential was measured. Then the overall decline of water content was calculated (conventional disc method).

Water loss during the conventional procedure could be simulated by dehydrating saturated, frozen, and stored discs on the micro-balance: After thawing, Ψo (in situ) was measured with the SFD method (in situ, SFD). Then the discs were dehydrated to a pre-set weight less than the initial fresh weight, and Ψo was measured ($<$ in situ, SFD). The calculation of this weight was based upon the mean percentage loss of initial fresh weight when discs were prepared for Ψo measurements with the conventional disc method. If the osmotic potentials show no significant differences between the two methods compared, it is evident that the SFD method gives correct values for the in situ osmotic potential and that no physiological changes influencing Ψo occurred during saturation or dehydration at room temperature.

Correlation between osmotic potential and water content

Regressions between Ψo and R or C can be used for calculating the osmotic potential at a given water content. In leaf samples, calculation of Ψo for a pre-set R is generally not necessary, if Ψo (sat) is determined exactly at 100% R. In secondary phloem discs, determination for a given R might be relevant because small water losses occur after thawing during transfer and weighing. For a high degree of accuracy it is possible to calculate R at the measurement of Ψo, and, on the other hand, Ψo for a uniform R, for instance at 90 or 95%, provided that S is correctly determined before freezing. In our experiments, a correct determination of S was insured by placing the saturation boxes at a distance of only 10 cm from the micro-balance and working very rapidly.

For calculating the regression, two or three measurements of potential at different water contents are required on the same sample. Figure 1 shows the protocol for determining the osmotic potential in situ and at full saturation. The mean of the weights before and after the measurement of Ψo (sat) was used to determine the corresponding water content exactly. With the SFD method, measurements of the osmotic potential at several C-levels above, at or below the in situ values are possible. Data of osmotic potential and reciprocals of C or R were used for calculating linear regressions, according to the theory of pressure-volume (pV) curves (type I transformation, Tyree & Richter 1981, 1982). The linear portion of a pV curve, reached after the turgor loss point, represents the osmotic behavior of the vacuolar sap. By extrapolating this straight line to R values above the turgor loss point, the osmotic potential for any given water content can be estimated, for instance Ψo at an R of 100%, which is defined as osmotic potential at full saturation (Ψo (sat)). Since in our experiments the osmotic potential of killed tissue was measured (and not the water potential of living tissue), a linear regression line can be calculated directly from the equations: $\Psi o = a + b * 1/C$ or $\Psi o = a + b * 1/R$.

Short term storage of spruce logs

To detect any possible influence of log storage on osmotic potential and water content of secondary phloem, samples were taken immediately after tree harvest, and periodically thereafter, from the stored log.

After harvesting, a log of 60 cm length was cut at breast height from the tree and cores of bark were punched out with a cork borer. Discs of conducting secondary phloem were cut from the core and saturated for 5 min. Afterwards, the discs were quickly blotted with filter paper, put in vials and frozen in dry ice. Then, the log was transported to the laboratory, and samples were processed as described in Figure 1. The log was stored for four days in a cold room (4 °C, 80% r.h.). Every day, samples were taken for measurement of C in situ, C sat, R in situ, Ψo (in situ) and Ψo (sat). For samples collected immediately after tree harvest, only data of Ψo (sat) and C sat are available, since it was not possible to use the micro-balance under field conditions.

Statistical analyses

Statistical data analyses were carried out with SPSS 7.5.1 for Windows. Mean values of normally distributed data were tested for significance of differences either with the t-test for independent samples or with one-way ANOVA and subsequent Student-Newman-Keuls multiple range test. Associations between two variables were examined using linear regression analyses. Differences or correlations were accepted as significant if P was $\leqslant 0.05$. *: $P \leqslant 0.05$, **: $P \leqslant 0.01$, ***: $P \leqslant 0.001$.

Box plots show the distribution of data. The lower boundary of the box indicates the 25^{th} percentile, the upper boundary the 75^{th} percentile. A line within the box marks the median. The "whiskers" lines extend from the box to the highest and lowest values.

Results

Saturation time for calculating relative water content (R) using tissue discs

For samples of conducting secondary phloem (n = 5) taken from the central part of the crown at 19.4 m height of a tree harvested on August 4, 1995 at Murau 5 min were sufficient to reach full saturation, leading to a calculated R in situ of 45.7 $\pm$ 2.7%. Saturating the samples for 10 min led to values of 44.7 $\pm$ 4.5%, and for 20 min to 48.5 $\pm$ 5.7%.

Discs of non-conducting secondary phloem of samples taken in 3/10 of tree height (7.4 m) from a tree harvested on August 17, 1995 at Manhartsberg, were fully saturated after 10 min. R in situ was 48.6 $\pm$ 2.2% and did not differ significantly from values of discs saturated for 120 min (50.8 $\pm$ 4.5%).

Osmotic potential of secondary phloem at full saturation (Ψo (sat)) obtained with the disc saturation method compared to the bark piece saturation method

Bark pieces of 5×5 cm took six times longer for saturation than discs (Figure 2). Using the disc saturation method, 10 min of saturation were sufficient to determine Ψo (sat) for conducting secondary phloem. With the

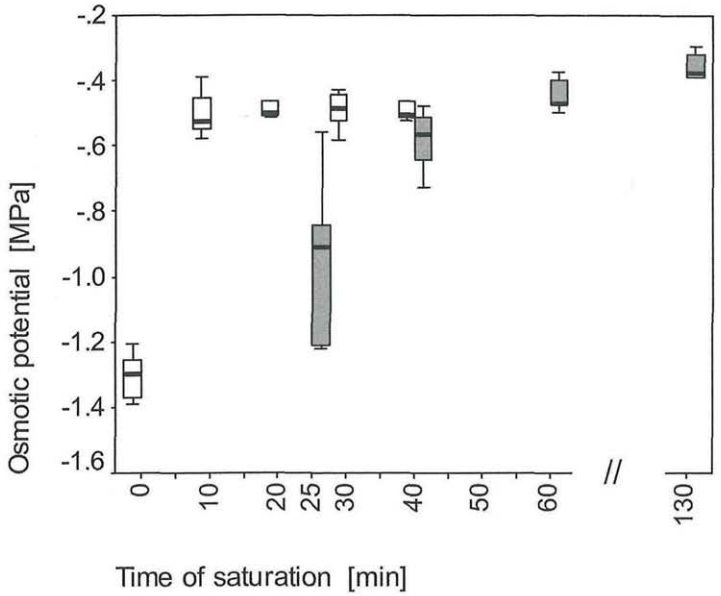

Fig. 2. Osmotic potentials of conducting secondary phloem, duration and mode of saturation varying. In situ osmotic potential (time of saturation = 0 min) was obtained with the SFD method. Dark filling: bark pieces (5 × 5 cm); white filling: secondary phloem discs (∅ 6 mm). Time of harvesting: June 21, 1995 in Prinzersdorf; sample collection on June 26; n = 5.

bark piece method, conducting secondary phloem reached full saturation after 60 min only: Ψo was significantly different from potentials measured after 25 ($P \leqslant 0.01$) and 40 min ($P \leqslant 0.05$) saturation, respectively, but not significantly different from results obtained on discs saturated for 10 to 40 min. In bark pieces saturated for 130 min osmotic potentials became less negative, indicating dilution of osmotically active substances: Mean values of Ψo (sat) after 130 min saturation differed significantly from results of discs saturated for 10 to 40 min ($P \leqslant 0.05$, Figure 2).

In conclusion, we think the saturation of discs preferable to the saturation of bark pieces, because the disc method offers, in addition to shorter saturation periods, the advantage of determining osmotic potential, water content and relative water content on the same sample.

Comparison between the conventional disc method and the SFD method for determination of in situ osmotic potential (Ψo (in situ))

With discs treated by the conventional disc method (frozen without saturation), water loss till potential measurement was 26.2% (fresh weight loss of 15.7%) for the experiment done in August, and 11.1% (fresh weight loss of 7.2%) in November.

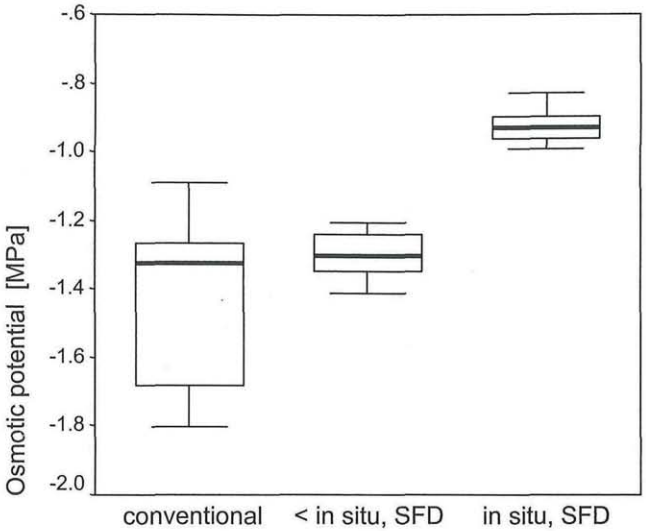

Fig. 3. Osmotic potentials of secondary phloem discs measured with different methods: Discs were (a) non-saturated, frozen, and stored ([conventional] n = 9); (b) saturated, frozen, stored, and dehydrated below initial fresh weight (F) ([< in situ, SFD] n = 8); (c) saturated, frozen, stored, and dehydrated to initial F ([in situ, SFD] n = 8). Samples from the central part of secondary phloem were taken on August 28, 1996 at 3/10 tree height (7.1 m) from a tree harvested on August 26 in Prinzersdorf.

Water loss caused by the conventional disc method could be well simulated with the SFD method by dehydrating saturated discs to a pre-set weight below the initial fresh weight. Water loss till measurement of the potential was not significantly different between the two methods, with values for the SFD method of 25.1% (14.8% fresh weight loss) in August, and 11.7% (7.7% fresh weight loss) in November [< in situ, SFD].

In August, the osmotic potential was –1.43 $\pm$ 0.25 MPa for the conventional disc method [conventional] and –1.30 $\pm$ 0.07 MPa for the SFD method [< in situ, SFD], with the values not differing significantly (Figure 3). Again, in the November experiment, no significant differences were found between the osmotic potentials measured with the conventional disc method (–1.27 $\pm$ 0.09 MPa [conventional]) and the SFD method (–1.23 $\pm$ 0.08 MPa [< in situ, SFD]) (Figure 4).

Since it is possible to simulate the water loss of discs treated in the conventional way by dehydrating saturated discs on the micro-balance below the initial fresh weight, it is assumed that the in situ osmotic potential could be restored by the same method.

The osmotic potentials of discs measured with the conventional method were in both experiments significantly more negative than the in situ osmotic potentials determined with the SFD method ($P \leqslant 0.001$,

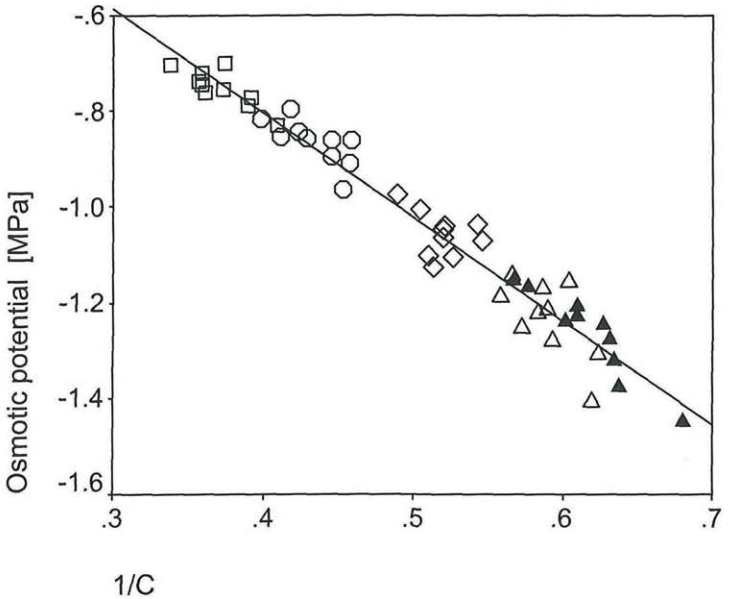

Fig. 4. Correlation between osmotic potential and water content of the central part of secondary phloem. Samples were taken on November 7, 1996 at 3/10 tree height (5.7 m) of a tree harvested in Prinzersdorf on November 5. Osmotic potential was determined at different water contents. Discs were (a) fully saturated (❑, Ψo (sat), disc saturation method), (b) exposed to short dehydration on the micro-balance (○, SFD method), (c) dehydrated approximately to initial F (◇, Ψo (sat), SFD method), (d) dehydrated to a pre-set value below initial F (Δ, SFD method). In situ osmotic potential was also measured with the conventional disc method on not-saturated, frozen discs (▲). Linear regression is given by Ψo (MPa) = 0,0642 – 2,1718 * 1/C, r = 0,98***, n = 50.

Figures 3 and 4, [conventional], [in situ, SFD]). The mean value of in situ osmotic potentials was –0.92 ± 0.05 MPa in the August experiment, and –1.06 ± 0.05 MPa in November, which yielded differences of 0.2 to 0.5 MPa between the two methods compared.

Correlation between osmotic potential and water content

The correlation between osmotic potential and water content or relative water content was very close. Equations of linear regression analysis and correlation coefficients are given for samples taken at the Rosalia (Table 2) and Prinzersdorf (Legend of Figure 4) sites. In each case we used three values of Ψo (sat) and three of Ψo (in situ), and the corresponding C or R. Six data pairs proved sufficient to achieve high correlation coefficients. Linear equations can be used for calculating osmotic potential at any C or R selected. With the regression equation Ψo versus R it was pos-

sible to calculate the influence of the water loss between weighing after saturation and weighing after storage (at potential measurement) on Ψo (sat) values. Water loss, which varied between 2 and 8%, had no significant influence on Ψo (sat) (Table 2).

Water status of stored logs

In the conducting secondary phloem of logs stored for four days in a cold room, no significant changes in water status parameters, such as C in situ, C sat, R in situ, Ψo (in situ) and Ψo (sat), were observed (Table 3). For Ψo (sat), almost identical values were measured throughout the experiment.

Discussion

In our opinion, the SFD method presented has various advantages compared to the conventional disc and the classical press sap methods.

To obtain correct values for Ψo (in situ) it is essential that water and solute loss of the samples is prevented. BENNETT 1990 reviews various problems occurring when the water status of plant tissues is measured with thermocouple psychrometers. An important source of error is the water loss between sample collection and transfer into the chamber of the osmometer, which leads to a passive concentration of solutes. WALKER & al. 1984 found a negative correlation between the ratio leaf area/leaf volume and the measured value of total water potential. Water potential became more negative with decreasing radius of the leaf discs or with increasing ratio leaf area/leaf volume. In leaf discs, severe water losses may occur at cut edges without cuticle. In secondary phloem discs the entire surface is exposed to air without any barrier in between, thus water losses have to be expected. The question arose, if these losses have an impact on the correct determination of the in situ osmotic potential. Water loss during manipulation steps (weighing after saturation – killing – weighing at potential measurement) from rehydrated discs had no impact on the determination of Ψo (sat), because the loss of water in relation to the total water content was small (2 to 8%). Thus, it is not necessary to correct measured Ψo (sat) values for water loss.

Our experiments showed that it is not possible to obtain reliable values for in situ osmotic potential with the conventional disc method where measurements are done on discs frozen without prior saturation (Figures 3 and 4). Water loss during manipulation steps, such as weighing and killing or transfer to vials and osmometer, lowers osmotic potentials considerably. From disc preparation until determination of the initial fresh weight, small water losses can occur to the same extent with the conventional disc method and the SFD method. With the SFD method, further water loss during the next steps of manipulation was prevented (Figure 1). With the

Table 2.

Correlation between osmotic potential (Ψ_o) and water content (C), and relative water content (R) of the central part of secondary phloem calculated for trees from the Rosalia site (n =6). The linear regression is described by the equations: a + b*1/C. $\Psi_{o\ (sat)}$ is given as measured value and as calculated value for R = 100% using the equation a + b*1/R. Trees were harvested on May 30, 1996 (number R1–R3), and on July 22, 1996 (number R7–R9). Immediately after harvesting, samples were taken at 3/10 h) and at brown case (CB).

Tree	Relative height	Absolute height (m)	Linear regression Ψ_o and 1/C			Linear regression Ψ_o and 1/R			$\Psi_{o\ (sat)}$ (MPa)	$\Psi_{o\ (sat)}$ (MPa)
			a	b	r	a	b	r	measured	calculated
R1	3/10 h	7.5	0.0493	-1.5286	**0.96****	0.0019	-0.5225	**0.92*****	-.51	-.48
	CB	16.1	-0.0467	-1.4544	**0.98*****	-0.0182	-0.6277	**0.98*****	-.64	-.60
R2	3/10 h	7.1	-0.0704	-1.1576	**0.92****	-0.0266	-0.4339	**0.98*****	-.46	-.43
	CB	14.2	-0.0375	-1.3717	**0.99*****	-0.0563	-0.5008	**0.97*****	-.55	-.52
R3	3/10 h	7.1	-0.0398	-1.2230	**1*****	-0.0593	-0.4499	**0.99*****	-.50	-.48
	CB	14.2	-0.0106	-1.2735	**0.98*****	-0.0559	-0.4625	**0.99*****	-.52	-.48
R7	3/10 h	7.1	-0.0314	-1.1968	**0.96****	-0.0247	-0.4636	**0.98*****	-.49	-.45
	CB	15.7	0.0226	-1.3664	**0.99*****	0.0313	-0.5023	**0.99*****	-.47	-.43
R8	3/10 h	6.5	0.0991	-1.4664	**0.98*****	0.0539	-0.5796	**0.93****	-.52	-.48
	CB	12.3	0.0187	-1.3867	**0.99*****	0.0090	-0.5606	**0.94****	-.54	-.51
R9	3/10 h	6.3	-0.0809	-1.1457	**0.97*****	-0.0818	-0.4601	**0.96****	-.54	-.51
	CB	13.3	0.0111	-1.2678	**0.97*****	0.0249	-0.5136	**0.99*****	-.49	-.45

Table 3.

Water status parameters of discs from conducting secondary phloem of a spruce tree harvested on September 28, 1999 in Amstetten. Samples were collected immediately after tree harvest and from a log (taken at breast height) stored up to 4 days in a cold room (4 °C, 80% r.h.). Same letter indicates no significant differences; n = 5.

Sample collection	$\Psi_{o\ (sat)}$ (MPa)	C_{sat} (%)	$\Psi_{o\ (in\ situ)}$ (MPa)	$C_{in\ situ}$ (%)	$R_{in\ situ}$ (%)
At the moment of harvesting	-0.746 ± 0.070 a	360.29 ± 24.36 a			
After 3 h transport to the laboratory	-0.748 ± 0.067 a	359.60 ± 18.68 a	-0.926 ± 0.059 a	287.11 ± 26.18 a	79.79 ± 5.11 a
After storage for					
24 h	-0.739 ± 0.071 a	352.57 ± 35.26 a	-0.936 ± 0.039 a	287.08 ± 24.37 a	79.24 ± 5.58 a
48 h	-0.737 ± 0.027 a	353.78 ± 17.50 a	-0.952 ± ß.064 a	286.24 ± 14.60 a	80.92 ± 1.29 a
72 h	-0.743 ± 0.065 a	351.69 ± 35.44 a	-0.965 ± 0.079 a	280.38 ± 25.42 a	78.98 ± 2.27 a
96 h	-0.743 ± 0.064 a	356.33 ± 24.52 a	-0.964 ± 0.047 a	284.36 ± 33.20 a	79.75 ± 7.00 a

conventional disc method, water loss during manipulation steps after determination of the initial fresh weight (such as sample killing and transfer to the vials, transfer of thawed samples to the osmometer), lowered the osmotic potential significantly. Therefore, we suppose that the conventional disc method does not measure in situ osmotic potentials correctly. This is especially true for samples frozen and stored for subsequent measurements of potential. Storing specimens at low temperature is unavoidable if one has to manage a large number of samples. In this case, the SFD method gives obviously more reliable values for in situ osmotic potentials than the conventional disc method.

Compared to the press-sap method, the SFD method is superior, since several water relations parameters can be measured on the same sample. These parameters are water content (C), relative water content (R), in situ osmotic potential (Ψo (in situ)) and osmotic potential at full saturation (Ψo (sat)).

A prerequisite for correct Ψo (in situ) – measurements with the SFD method is the exact determination of the initial fresh weight, which should be done either directly in the field or immediately after sample transport to the laboratory. It is also possible to store spruce logs under low temperature and high humidity, and take the disc samples within the following days. For plant material harvested in late summer, our results showed that up to the fourth day of storage no changes in water status occurred (Table 3). Thus, we consider short term storage of logs acceptable, especially when a large number of samples has to be handled.

Osmotic potential at full saturation permits comparisons of the content of osmotically active solutes within the tree or between different trees at the same relative water content. Saturation times suggested by SLAVIK 1974 for leaf discs (more than 8 h) turned out to be far too long for secondary phloem discs. Oversaturation of samples may result in excess water uptake into the apoplast, including the interfibrillar spaces of the cell wall, conduits and intercellular spaces, and in a loss of symplastic solutes. Therefore, the shortest resaturation period sufficient to bring tissues to a Ψo level corresponding to full hydration in the field should be used (PARKER & PALLARDY 1987, ABRAMS 1988, KUBISKE & ABRAMS 1991). For secondary phloem discs, 5 to 10 min of saturation were sufficient to reach full turgor. As our experiments show, oversaturation resulting in excess apoplastic water or leakage of solutes from the sieve cells did not occur during this short rehydration period: Non-saturated, frozen discs had, at equal water contents, similar osmotic potentials as SFD discs (Figures 3 and 4). If any leakage from the sieve cells happened during saturation, SFD discs would have less negative Ψo values compared to non-saturated frozen discs. In addition, data obtained with the conventional disc method also fit perfectly in the regression between osmotic potential and 1/C of

SFD method data (Figure 4). This indicates that 'plateau effects', such as observed in pV-curve studies (LADIGES 1975, PARKER & PALLARDY 1987, KUBISKE & ABRAMS 1990, ABRAMS & KUBISKE 1994, PARKER & COLOMBO 1995, 1996,), did not occur with the SFD method. Compared to the pV-procedure, the osmotic potential at full saturation can be determined directly and the values need not be corrected (e.g. with the correlation between fresh weight and total water potential).

Another advantage of the short saturation time is that errors in the measurement of Ψo (sat) or R in situ caused by respirative losses of carbohydrates can be avoided.

Due to the very close correlation between osmotic potential and water content or relative water content, when using the SFD method, it is possible to calculate values of osmotic potential at any pre-set C or R.

In conclusion, with the SFD method for the measurement of osmotic potential on secondary phloem discs, several different water status parameters can be measured on the same sample and probably more reliable values are given compared to the conventional disc method.

Acknowledgments

This work was supported by Project P 10485-Bio and the Special Research Program Forest Ecosystem Restoration (SF008), funded by the Austrian Science Foundation and the Austrian Ministry of Agriculture and Forestry.

Experiments were done at the Institute of Forest Entomology, Forest Pathology, and Forest Protection, University of Agricultural Sciences, 1190 Vienna. The authors thank Hanno RICHTER for critical comments on the manuscript and Birgit KARTUSCH for expert advice concerning plant anatomy. Thanks are due to Erwin FÜHRER and Peter BAIER (Institute of Forest Entomology, Forest Pathology, and Forest Protection) for project coordination and helpful discussions.

References

ABRAMS M. D. 1988. Sources of variations on osmotic potentials with special reference to North American tree species. – For. Sci. 34: 1030–1046.

— & KUBISKE M. E. 1994. Synchronous changes in tissue water parameters of mature foliage from well-watered and periodically droughted tree seedlings. – J. Exp. Bot. 45: 171–177.

BÄUCKER E. BUES C.-T. & VOGEL M. 1998. Radial growth dynamics of spruce (*Picea abies*) measured by micro-cores. – IAWA Journal 19: 301–309.

BENNETT J. M. 1990. Problems associated with measuring plant water status. – HortScience 25: 1551–1554.

BRYANT J. P. & RAFFA K. F. 1995. Chemical antiherbivore defense. – In: GARTNER B. L. (Eds.), Plant stems: Physiology and functional morphology, pp. 365–381. – Academic Press. San Diego, New York, Boston, London, Sydney, Tokyo, Toronto.

CHRISTIANSEN E. WARING R. H. & BERRYMAN A. A. 1987. Resistance of conifers to bark beetle attack: searching for general relationships. – For. Ecol. and Manage. 22: 89–106.

CLANCY K. M. WAGNER M. R. & REICH P. B. 1995. Ecophysiology and insect herbivory. – In: SMITH W. K., HINCKLEY T. M. (Eds.), Resource physiology of conifers, acquisition, allocation, and utilisation, pp. 125–180. – Academic Press, San Diego, NY, Boston, London, Sydney, Tokyo, Toronto.

DIXON H. H. & ATKINS W. R. G. 1910. On osmotic pressure in plants and on a thermo-electric method for determining freezing points. – Scientific Proceedings of the Royal Dublin Society (New Series) 12: 275–311.

DÜNISCH O. BAUCH J. PULS J. & MÜLLER M. 1996. Biological and chemical wood properties of long-term polluted spruce (*Picea abies* (L.) Karst.) at high-altitude stands of the Erzgebirge. – Holzforschung 50: 497–506.

ESAU K. 1969. The Phloem. – In: ZIMMERMANN W. OZENDA P. WULFF H. D. (Eds), Handbuch der Pflanzenanatomie. – Gebrüder Borntraeger, Berlin, Stuttgart.

FRANCESCHI V. R. KREKLING T. BERRYMAN A. A. & CHRISTIANSEN E. 1998. Specialized phloem parenchyma cells in Norway spruce (*Pinaceae*) bark are an important site of defense reactions. – Am. J. Bot. 85: 601–615.

HOLDHEIDE W. 1951. Anatomie mitteleuropäischer Gehölzrinden (mit mikrophotographischem Atlas). – In: Handbuch der Mikroskopie in der Technik, Bd. 5 Teil 1, pp. 193–314. – Umschau Verlag, Frankfurt.

KAUFMANN M. R. & KRAMER P. J. 1967. Phloem water relations and translocation. – Plant Physiol. 42: 191–194.

KIKUTA S. B. & RICHTER H. 1992. Leaf discs or press saps? A comparison of techniques for the determination of osmotic potentials in freeze-thawed leaf material. – J. Exp. Bot. 43: 1039–1044.

KRAEMER G. D. 1953. Die kritischen Grenzen der Brutbaumdisposition für Borkenkäfer an Fichte (*Picea excelsa* L.). – Z. Angew. Entomol. 34: 463–512.

KUBISKE M. E. & ABRAMS M. D. 1990. Pressure-volume relationships in non-rehydrated tissue at various water deficits. – Plant, Cell Env. 13: 995–1000.

— & 1991. Seasonal, diurnal and rehydration-induced variation of pressure-volume relationships in *Pseudotsuga menziesii*. – Physiol. Plant. 83: 107–116.

LADIGES P. Y. 1975. Some aspects of tissue water relations in three populations of *Eucalyptus viminalis* Labill. – New Phytol. 75: 53–62.

LANG A. R. G. 1967. Osmotic coefficients and water potentials of sodium chloride solutions from 0 to 40 °C. – Aust. J. Chem. 20: 2017–2023.

LEWINSOHN E. GIJZEN M. MUZIKA R. M. BARTON K. & CROTEAU R. 1993. Oleoresinosis in grand fir (*Abies grandis*) saplings and mature trees. Modulation of this wound response by light and water stress. – Plant Physiol. 101: 1021–1028.

LITVAY J. D. & MCKIMMY M. D. 1975. Determining moisture content and moisture sorption in douglas-fir bark. – Wood Science 7: 284–291.

MERKER E. 1956. Der Widerstand von Fichten gegen Borkenkäferfraß. – Allg. Forst- u. Jagdztg. 127: 129–45; 165–87.

MÜNCH E. 1930. Die Stoffbewegung in der Pflanze. – Jena, Gustav Fischer.

PAINE T. D. RAFFA K. F. & HARRINGTON T. C. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. – Ann. Rev. Ent. 42: 179–206.

PARKER W. C. & PALLARDY S. G. 1987. The influence of resaturation method and tissue type on pressure-volume analyses of *Quercus alba* L. seedlings. – J. Exp. Bot. 38: 535–549.

— & COLOMBO S. J. 1995. A critical re-examination of pressure-volume analysis of conifer shoots: Comparison of three procedures for generating PV curves on shoots of *Pinus resinosa* Ait. seedlings. – J. Exp. Bot. 46: 1701–1709.

— & 1996. Cut-shoot vs root rehydration: Effects on pressure-volume analysis of shoots and fascicles of *Pinus resinosa* seedlings. – Physiol. Plant. 97: 651–658.

ROSNER S. 1998. Vergleichende Untersuchungen der Anatomie und des Wasserhaushaltes der Rinde von Fichte (*Picea abies* (L.) Karst.). Doctoral Thesis, University of Agricultural Sciences, Vienna.

— , BAIER P. & KIKUTA S. B. 2001 Osmotic potential in secondary phloem of Norway spruce (*Picea abies* (L.) Karst.) in relation to anatomy. – Trees 15: 472–482.

SLAVIK B. 1974. Water content, water saturation deficit (relative water content). – In: JACOBS J., LANGE O., OLSON J. S., WIESER W. (Eds.), Methods of studying plant water relations, pp. 136–156. – Academia, Prague, Springer, Berlin, Heidelberg, New York.

SPANNER D. C. 1951. The Peltier effect and its use in the measurement of suction pressure. – J. Exp. Bot. 11: 145–168.

TROCKENBRODT M. 1990. Survey and discussion of the terminology used in bark anatomy. – IAWA Bulletin n.s. 11: 141–166.

TYREE M. T. & RICHTER H. 1981. Alternative methods of analysing water potential isotherms: Some cautions and clarifications. I. The impact of non-ideality and of some experimental errors. – J. Exp. Bot. 32: 643–653.

— & 1982. Alternate methods of analysing water potential isotherms: Some cautions and clarifications. II. Curvilinearity in water potential isotherms. – Can. J. Bot. 60: 911–916.

WALKER S., OOSTERHUIS D. M. & WIEBE H. H. 1984. Ratio of cut surface area to leaf sample volume for water potential measurements by thermocouple psychrometers. – Plant Physiol. 75: 228–230.