

**REVIEW ARTICLE: FIELD TECHNIQUES** 

# Instrumental methods for studies of structure and function of root systems of large trees

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## Abstract

New methods using different physical principles have been successfully applied in studies of root systems of large trees. The ground-penetrating radar technique provides 3D images of coarse roots (starting with a diameter of about 20 mm) from the soil surface down to a depth of several metres. This can even be done under layers of undisturbed materials such as concrete, asphalt and water. Fine roots cannot be visualized by this method, but the total rooted volume of soil can be determined. The differential electric conductance method has been used for fast measurement of conducting (absorbing) root surfaces. However, more testing is needed. Both these methods are noninvasive. The results can be verified by an almost harmless excavation of whole root systems, including fine roots, using the ultrasonic air-stream (air-spade) method. This method is suitable for all studies, as well as practical operations on roots or objects in their vicinity, where a gentle approach is required. Sap flow measurements on their own or in tandem with soil moisture monitoring play a leading role in studying root function and hydraulic redistribution of flow in the soil. The water absorption function of roots can be studied by measuring sap flow on individual root branches directly (as on crown branches) and also indirectly, by measuring the radial pattern of sap flow in different sapwood depths at the base of a stem. Root zone architecture can also be estimated indirectly by studying its functionality. The heat field deformation method with multi-point sensors has been found to be very convenient for this purpose. A combination of several such methods is recommended whenever possible, in order to obtain detailed information about the root systems of trees.

Key words: Air-spade excavation, electric conductance, ground-penetrating radar, radial flow pattern, sap flow.

## Introduction

Studies of large trees in the field used to be limited by the availability of instrumentation, with the exception of those rare research plots equipped with towers, scaffolding or cranes to permit access to crowns, and special chambers, with TV monitors and similar sophisticated technical means for root studies. However, the situation is now improving due to the application of several new principles and technologies, especially for studies of root systems. These consider root system architecture and the quantification of physical parameters, as well as absorbing functions and their ability to transport water long distances. For studies of root architecture there is good experience with the non-invasive techniques of groundpenetrating radar, differential electric conductivity, and the excavation technique using a supersonic air stream. The absorption functions of roots as well as root architecture can be studied through the sap flow technique.

## **Root architecture**

## Ground-penetrating radar

Ground-penetrating radar, GPR, (Hruška, 1997; Conyers and Goodman, 1997) has been utilized in co-operation with Geofyzika Inc. Brno for root system studies in several previous investigations (Hruška *et al.*, 1999; Čermák *et al.*, 2000). The measuring system consists of a portable signal transmitter and receiver, a type pulse EKKO 1000<sup>TM</sup> GPR system (Sensors & Software Inc., Mississauga, Ontario, Canada). It operates at a signal frequency of 450 MHz and the antenna is gradually moved over the soil surface along

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specified grid lines marked by tape. The grid is usually  $0.25 \times 0.25$  m, but can be denser for more detailed studies. The total length of lines depends on tree size and can reach 300-500 m, which corresponds to about  $30 \text{ m}^2$  of ground surface and 6000-10 000 radar images per tree. The effective velocity of the radar signal to the soil is typically 0.07–0.08 m n s<sup>-1</sup>, which can be checked by direct measurements of velocity in individual soil layers, providing that there are previously known values for soils of different texture (Fig. 1, upper panel). Recorded electronic data are analysed by the software packages Ekko Tools 4.22 (Sensors & Software Inc., Mississauga, Ontario, Canada) and Reflex 3 (KJ Sandmeier, Karlsruhe, Germany). Both packages are standard geophysical, georadar and seismic programs. This method of analysis allows the most interesting characteristics of a soil profile from a given viewpoint to be analysed, and irrelevant information to be suppressed, as in the studies cited above. For example, images of stones and roots of other trees are suppressed in order to minimize the interfering influence of these soil objects.

Selected images of root systems derived by computer are shown on the screen and can be printed (Fig. 1, lower panel). Quantification of information from the images is then usually performed by methods of image-analysis. Radar can only distinguish roots with a diameter greater than 20 mm. Thus, thin conductive roots or fine absorption roots (with a diameter of 0.1–1 mm) and other tiny structures are not visible. The frequency of signal used can



Fig. 1. Upper panel: an example of the primary radar image (radar reflections in the soil along a defined path across analysed roots), and lower panel: the resulting picture of the root system of large winter oak—*Quercus petraea* (Mattusch) Liebl. (modified after Hruška *et al.*, 1999).

distinguish large vertical and horizontal structures (e.g. big stones or walls) down to a depth of 30 m or even more (Hruška and Klablena, Geofyzika Inc. Brno, 1997: personal communication). This method can substantially contribute to studies of root systems, which are otherwise difficult to access. Measurement can even be performed through impermeable materials such as concrete or asphalt layers or beneath water or a river-bed. Distances are detected with about 50 mm error and roots can be detected down to a depth of about 2.5 m. In general, the radar has an accuracy of about 80%. Errors originate due to the presence of a great number of reflections from other linear objects in soils. For example, it is difficult to distinguish whether roots are crossing or just touching each other (Sustek et al., 1998; Stokes et al., 2002). A great advantage of this method is the possibility of characterizing the root dynamics by repeating measurements over time.

#### Differential electric conductance

Several systems for the measurement of electric conductivity have been routinely used for geophysical studies in soils (al Hagrey and Michaelson, 2002). A similar approach, but combining conductivity measurements of tree stems or coarse roots, and soils, has been used to estimate the area of conducting root surface (in m<sup>2</sup> per tree), irrespective, of their morphological parameters (Staněk, 1998).

This method is based on differences in the conductivity of the materials and the fact that the zones in which roots absorb soil water are practically identical to the zones through which the electric current passes when the tree becomes part of an electric circuit, supplied from an external voltage source. A discrete conductor of a definite size (represented by a tree) is introduced into the soil, which has practically an infinitesimal cross-section (this is the principle of an earthing technique). The contact of such a conductor with the soil is achieved through earth electric resistance, which is the total electric resistance of the soil half-space, where the current passes from a discrete conductor to earth. The shape and size of this half-space is presumably dependent on dimensions of the grounding electrode. The magnitude of the soil resistance in plants is a function of the conducting area of their roots. The halfspace resistance of the ground electrode is typified by a pronounced non-linearity in the potential characteristics. The ground resistance of the tree,  $R_z$ , is found by dividing the voltage U, measured between the tree base and the location in the soil, where the potential characteristics become effectively constant, by current I, which flows through the measured circuit from the external source. The 'earth resistance' is measured using the additional current electrodes in the soil. The area of root conducting (=absorbing) zones,  $S [m^2]$  is then calculated from the equation:

$$S = \frac{\rho L}{R_z} = \frac{\rho L I}{U} \xi \eta v \tag{1}$$

where  $\rho$  is the resistivity of the woody tissues, which must be estimated separately using a series of thin metallic electrodes and *L* is the distance between the tree and the feeding electrode. This equation can be applied when the earth resistance of the measured tree is the largest series resistance in the circuit. Under natural conditions empirical corrections need to be applied by introducing three dimensionless coefficients: coefficient  $\xi$  takes account of the mutual electric shading of root tips of absorption zones occurring in close proximity ( $\xi > 1$ ). Coefficient  $\eta$ characterizes the impact of root mechanical damage (1< $\eta$ <1) and v takes account of the existence of outer current pathways (v <1). The measurement error is typically about ±15%.

The first results obtained with this method on seedlings and large trees of a range of species are promising. Root surfaces taken by parts (entire root systems, half of that and the part remaining after most of the roots were cut by a bulldozer) and measured on large Norway spruce trees were linearly related (Fig. 2). They represent about 20– 40% of the tree 'social area' (calculated from mean distances to neighbouring trees, weighted by the ratio of their basal areas. Measured root surfaces were not expected to correspond exactly to the tree 'social area' shown in the figure (more important are the relations for individual trees), but no alternative method providing similar data was available against which to compare the results. Therefore, more experience is needed before recommending this method for more general use.

#### Root excavation by the supersonic air stream

In some cases it is not sufficient to visualize root systems, and it is necessary to excavate them, preferably without



**Fig. 2.** Relationships between electronically measured conducting (i.e. absorbing) root surface area in large Norway spruce trees (*Picea abies* (L.) Karst.) and their arbitrary estimated 'social area'. The remaining part of the root system was smaller in a tree with DBH=27.1 cm when compared to others.

causing any significant damage. The classical approach is the archeological method (carefully using simple hand tools), but this is extremely time-consuming and expensive. Another approach is represented by the laser-like thin supersonic air stream. The corresponding tool, the so called 'air-spade', is produced by, for example, Air-Spade Technology, Verona, PA, USA (ceg@air-spade.com) or by Dave Leonard Consulting Arborist, Lexington, KY, USA (dave7oaks@aol.com). An air-spade model 150/90 connected to an Ingersoll-Rand compressor supplying air at 0.8 m<sup>3</sup> s<sup>-1</sup> and pressure of 0.6 MPa gives a stream with a speed of Mach 2.

The supersonic air stream operates by its velocity. When the stream touches a smooth object (such as a stone or root) it will slip over and nothing will happen. However, when the stream hits any tiny pore, air is compressed in it (it cannot blow out under such a high speed) and the pore will explode. Soil is thus blown away and the roots and other smooth objects remain untouched (Fig. 3). Fine roots, as well as coarse roots, can be opened almost harmlessly (however, not such tiny cellular structures as mycorrhiza). The technique works extremely quickly on light soils (hundreds of times faster than hand-excavation), but more slowly on heavy soils, which are less porous. In heavy soils, the soil first cracks and then is dispersed in small particles. The occurrence of some lesions was observed on roots in places where they were damaged earlier, or where they were slightly damaged by fast-moving soil particles. When the roots create a dense network on stony soils it is better gradually to remove loose stones in order to prevent any damage, which may be caused by their movement. This method is suitable for scientific purposes such as studies of root infection by fungi (Rizzo and Gross, 2000) and verification of root description by other methods (Šustek et al., 1998; Stokes et al., 2002). It also serves well for biotechnical purposes, for example, when it is necessary to prune roots in urban greenery (Smiley, 2001; Nathenson and Jarabak, 2001) or when laying cables or pipes in places where there are roots of valuable trees, which must not be seriously damaged. The method could also be used for temporary opening of large areas of the root zone in order to install sensors (sap flow, water potential, etc.) on separate roots at defined positions.

#### **Root function**

All the methods described above characterize root structure in varying parameters and details. By contrast, sap flow, as the physiological process reflecting the activity of root systems, has been found to be a good indicator of the functional state of roots in different environmental conditions or subjected to different treatments.



**Fig. 3.** Upper panel: excavating root systems in a group of trees using the supersonic air stream method. Middle panel: surface roots of Norway spruce (*Picea abies* (L.) Karst.) on a 60 m<sup>2</sup> section of heavy soil of excavated to 200–400 mm (the procedure took 6 h). Lower panel: a view from below of the excavated central part of the root system of a single black pine tree (*Pinus nigra* Ait.)

In routine sap flow studies at the tree and stand levels, sap flow sensors have usually been installed at breast height (1.3 m above ground), where the behaviour of a large number of roots and leaves is integrated. Practically, any commercially available method can be used for this purpose. Moreover, if more detailed information about the spatial distribution of flows within the crown or root zone is required, sap flow sensors can be installed on the crown and root branches of any size.

Simultaneous measurements of soil water content and the amount of water absorbed by trees from soils (through sap flow) have been used for the estimation of rooting depth and the horizontal extent of root systems since the 1980s (Čermák et al., 1980, 1993a, b; Čermák and Kučera, 1990a, b; Cabibel and Do, 1991; Cabibel, 1994; Clothier and Green, 1997). Calculations can be made by analogy with the effective crown projected area (Čermák et al., 1982). Soil water content is measured and calculated as the water storage below 1.0 m<sup>2</sup> of soil surface ( $M_w$ ) down to the maximum effective rooting depth. Under optimum soil water content and very dry soils, this can be estimated from, for example, leaf and soil water potential, and sap flow measurements (Čermák et al., 1980). Watering of the soil surface together with sap flow measurements can be used for similar purposes (Čermák et al., 1993a, b). Differences in water storage  $(dM_w = M_{w1} - M_{w2})$  occurring during a short period of time (one or several days) can be described as

$$dM_{\rm w} = \frac{Q_{\rm wt}}{A_{\rm ref}} \tag{2}$$

where  $A_{\rm ref}$  is effective root area and  $Q_{\rm wt}$  is sap flow per tree.

If the sample tree only absorbs water from the top 100 mm of the soil, it will require a rather large ground area in order to supply enough water for the measured daily transpiration. If the sample tree absorbs water from the whole rooting depth, a much smaller ground area will be sufficient for the same amount of transpiration. The approximate effective root distribution can be calculated similarly, when soil water is measured in the different soil horizons (Fig. 4).

Such calculations will be erroneous or impossible when the effective rooting depth cannot be estimated or when trees absorb significant amounts of water from below the rooting depth. Application of this calculus is also limited by the sensitivity of the soil water measuring device, especially in wet soils. A period of preceding drought is most suitable for such studies. Similarly, the functional root distribution can be estimated if the soil hydraulic conductivity is known and the response of sap flow measured in the stem during watering. However, this is not possible when the conductivity is high or if water penetrates irregularly along root surfaces.

#### Measurements of flow redistribution in soil

Further investigations, together with the development of improved instrumentation for both sap flow and soil moisture measurements, have shown that water can move passively from wet to dry zones via roots following gradients of water potential (Dawson, 1993; Richards and Caldwell, 1987; Caldwell and Richards, 1989; Burgess et al., 1998, 2001a; Smith et al., 1999; Scholz et al., 2002). As this phenomenon operates bi-directionally, methods for such studies must allow sap flow measurements to be made in both directions. Such methods include the constant power heat method, CPHM (Lightbody et al., 1994; Lott et al., 1996; Sakuratani et al., 1999), the heat ratio method, HRM (Burgess et al., 1998, 2000, 2001b; Scholz et al., 2002) and the heat field deformation method, HFD, (Nadezhdina et al., 1998, 2002; Nadezhdina and Čermák, 1998). The possibility of using the modified thermal dissipation technique for bi-directional measurements in roots has also been reported recently (Brooks et al., 2002).

Both vertical upward transfer of water (hydraulic lift) from deep to more dry shallow soil layers (Caldwell *et al.*, 1998; Horton and Hart, 1998) and downward transport (inverse hydraulic lift) have been reported (Schulze *et al.*, 1998; Smith *et al.*, 1999). Evidence of a possible substantial horizontal redistribution of water via relatively



Fig. 4. Schematic diagram of a tree root system, the functional distribution of which was estimated through measurement of total sap flow in stem and soil moisture at different depths and distances around the tree before and after watering.

superficial lateral roots was presented most recently by Brooks et al. (2002). Jackson et al. (2000), in their review of root water uptake and transport, highlighted the potential importance of hydraulic lift for ecosystem water balance. Brooks et al. (2002) suggested that the redistribution of water by roots could increase the survival of understorey seedlings during severe drought. This issue is regarded as a high priority for further investigation. The long-term (5 months) sap flow measurements on roots of oak and lime trees, show high competition for water between neighbouring trees (N Nadezhdina, unpublished data). Application of multi-point sensors allows the functionality of different sapwood layers of big roots to be studied and their role in the redistribution of soil water to be assessed. Measurements of sap flow patterns in small roots and at different depths below bark in large lateral maple roots under natural and experimental conditions (cutting branches, local irrigation), demonstrated a rapid hydraulic redistribution of flow in accordance with gradients of water potential and pathway resistances (N Nadezhdina, unpublished data).

## Estimation of spatial root distribution from measurements of the radial pattern of sap flow in stem bases under natural conditions—studying root architecture through stem xylem function

Trees of a range of species can be supplied by water equally from surface or deep roots (or both), or alternatively by different parts of their root systems depending on soil water and root distribution and overall site characteristics (Čermák et al., 1980; Lott et al., 1996; Howard et al., 1996; Čermák and Prax, 2001). More recent sap flow measurements confirm earlier findings (Boucherie, 1840; Arcikhovskiy, 1931), that sapwood is not functionally homogenous over its entire cross-sectional area (Swanson, 1971; Čermák et al., 1984, 1992; Čermák and Nadezhdina, 1998). This is associated with the long-term development of xylem conduits (Mark and Crews, 1973), but is also attributed to non-homogeneous root and leaf distributions (Čermák and Kučera, 1990a). Some methods (e.g. HPV, CPHM or HFD) allow more detailed determinations of flow rates at different sapwood depths, that improves the ability to observe the link between whole tree architecture and function as well as between root zone form and root functioning. There is considerable experience in this laboratory with the application of the HFD method, based on the measurement of temperature gradients around a linear heater in axial and tangential directions for this purpose (Nadezhdina et al., 1998, 2002).

The measurement of the radial pattern of sap flow at the stem base is important for the detection of layers that are anatomically connected to roots of different ages and depths. Thus, the amount of water absorbed by different root sectors can be estimated by only measuring the sap flow at the base of the main stems (Nadezhdina and Čermák, 2000a). This method does not give a visual 3D image of root systems (as the radar does), but the approximate direction of the water source can be estimated according to the sensor orientation on the stems. These results (Nadezhdina and Čermák, 2000a; Nadezhdina et al., 2002) showed that the outer sapwood seems most important for water transport especially under conditions favourable for tree growth, when the majority of water comes from the surface soil layers and where there are also more fine roots assuring nutrient supply (Clemensson-Lindell and Persson, 1995; Persson and Ahlstrom, 2002). The dependence of radial patterns of sap flow on root distribution around the stem was evaluated on Black pine (Pinus nigra Arn.) and two mountain ash trees (Sorbus intermedia (Ehrh.) Pers.) in an urban location where root distribution was verified first by the ground-penetrating radar technique and then by root excavation using the airspade technique (N Nadezhdina et al., unpublished results). The authors have also performed experiments that involved severing different lateral and sinker roots. The results indicate that the magnitudes of sap flow rate in the corresponding stem sectors were clearly dependent on surface root distribution and density around the stems. Moreover, it was found that the radial pattern of sap flow in the stem reflected the relative vertical pattern of efficient root density/activity in the corresponding soil sector (Fig. 5).

Very narrow peaks of sap flow were recorded in the outer xylem layers around the stem, with the exception of the south-east side  $(140^\circ)$  where the roots were damaged



**Fig. 5.** Radial patterns of sap flow in the stem of a mountain ash tree plotted around the stem circumference (as if the stem were unrolled). The *x*-axis represents the circumference and *y*-axis the xylem radius. The additional (short) *x*-axis in the centre of the figure corresponds to the sap flow density. The thick arrow on the upper scheme indicates the northern side of the stem and thick lines correspond to the placement of sensors in the stem.

by fungi. Further excavation by the air-spade showed that the tree had a very dense network of fine roots in the surface soil layers, probably as a response to partial disfunctioning of coarse roots, which were infected by fungi in places. The authors therefore concluded that surface absorbing roots were closely connected with the shallow layers of sapwood. In that particular mountain ash tree, the more active layers of xylem axial transport reflected the large functional root zone close to the surface. Senock and Leuschner (1999) reported that daily flows of water through the surface roots were consistently 30% higher than through the deep roots of a Eucalyptus saligna Sm. tree. Green and Clothier (1998) found that when the surface soil layers were uniformly wet, 70% of tree water uptake occurred in the top 0.40 m of the root zone, in which approximately 70% of an apple tree's fine roots were located.

Maximum sap flow density in areas damaged by fungi was significantly lower and moved to deeper layers, in a similar way to an early investigation in laurel forest where flow was found to be very low in the outer xylem layers in trees damaged by fungi (Jimenez *et al.*, 2000). Fungal infection of roots, leading to root destruction, always restricts their water absorption (Čermák and Kučera, 1990*b*).

## Estimation of spatial root distribution through measurements of radial pattern of sap flow under experimental manipulations

Measurements of the radial pattern of sap flow in stem bases as well as in large roots, together with experimental manipulations, including locally watering the soil and severing roots below sensors, provide means by which both the changing spatial distribution of roots and their temporal functional pattern can be better observed.

(1) *Radial sap flow pattern and local irrigation*: Experiments with local irrigation have shown that it is a very simple, non-destructive and informative method for studying the functionality and segmentation of uptake around trunks, as well as the redistribution of the 'wet markers' in the soil or in the stem and canopy for losses according to gradients of water potential.

Many studies, based on direct measurements of soil water content and sap flow within individual roots under local irrigation, have shown that the spatial pattern of water uptake can rapidly change in response to the application of irrigation water (Green and Clothier, 1995, 1998; Clothier and Green, 1994, 1997; Green *et al.*, 1997; Fernandez *et al.*, 2001). Root activity quickly shifted to the regions where the soil had been watered. The role of near-surface roots as primary paths of uptake was often emphasized. Utilization of multi-point sensors along the stem or root xylem radius allows the tracks between sources and sinks within integrating stem pathways to be

recognized. During long-term measurements of the radial pattern of sap flow in stems of fig (Nadezhdina and Čermák, 2000*a*), pine (Nadezhdina *et al.*, 2002) and lime trees (N Nadezhdina, unpublished data), the sap flow in the outer xylem layers, which often connected the foliage exposed to the sun with the surface roots, was shown to be more sensitive to drought than flow in the deeper xylem layers which were anatomically connected with the deeper roots. Flow usually recovered to the level recorded before drought after artificial irrigation or natural soil watering by rain (Fig. 6, right).

When water was applied widely round the stem (Fig. 6, right), sap flow increased in the shallow and medium sapwood layers and the total increase nearly reached the level recorded before drought. On the other hand, after local irrigation in a small zone around the stem, the total increase was less and occurred in the medium and deep layers suggesting that the roots near the stem were connected with the inner sapwood layers (Fig. 6, left). Experiments with local irrigation around stems could also be useful for determining the location or orientation of roots near the corresponding stem sector.

(2) Radial sap flow pattern and severing experiments: Source- and sink-severing experiments enable the association of anatomical architecture with function of the root system to be assessed (Nadezhdina and Čermák, 2000a). Sap flow in roots and the stem base declines immediately after damage to the conducting system, to an extent corresponding with the importance of the particular part of the root system that is injured. Some specific features found when applying sap flow measurements by multi-point sensors during severing experiments are as follows. (a) surface roots are presumably connected with the outer layers of sapwood, while intermediate and deep roots supply water uniformly over most of the sapwood (Fig. 7) (Nadezhdina and Čermák, 2000a). This agrees with Jackson et al. (2000): 'Localized failure in small, shallow roots would direct water uptake to deeper layers during drought without disrupting major arteries of axial transport.' (b) Conducting xylem in the stem from its base up to about breast height is strongly sectored and connected with the roots only from the corresponding side of the stem, showing strong segmentation of flow, especially in the outer xylem (N Nadezhdina, unpublished data). (c) A compensation mechanism may operate in roots, allowing an increase in the absorption of water by preferentially using from one part of the root system in cases where another part of the root system is damaged or loses its water source (Nadezhdina and Čermák, 2000b). This mechanism evidently represents an important safety feature for tree survival. However, in another cutting experiment, where sap flow was measured at breast height and big lateral roots were cut below, pathways that became free after their source was severed were not available for transporting



Fig. 6. Scheme of changes in the radial pattern of sap flow during differential irrigation. Left: water was applied only close to the stem. Right: water was applied over a wide range around the stem.



# Radial pattern of sap flow in roots after their severing

**Fig. 7.** Schematic diagram of a tree root system during sap flow measurement and severing of roots at different depths. Cut 1 (left) characterizes the situation where the intermediate root is severed. This is followed by rather uniform sap flow decrease over the whole sapwood depth. Cut 2 (right) characterizes the situation where the surface roots are severed. Sap flow decreases in the outer and intermediate sapwood layers.

water between other sources and sinks even after many days (N Nadezhdina, unpublished data). At the same time, the abrupt loss of a significant part of the water source under a constant high sink demand could be temporally compensated for by an increase of flow through other water pathways in all undamaged sapwood layers of the stem (N Nadezhdina, unpublished data). The amplitude and duration of such compensation could be limited by water availability in the soil. (d) Immediate responses of sap flow to source-severing can be used to characterize root properties such as the magnitude of root/soil resistance to water flow, their water capacity, etc. (Nadezhdina and Čermák, 2000*a*).

#### Combination approach—issues for the future

New physical principles and corresponding instruments allow relatively fast and detailed studies of architecture and absorption functions of roots in small as well as large trees to be easily be carried out in most field conditions. Each of the methods described has specific objectives and drawbacks. It is therefore recommended that root studies combine several methods in order to get the most complete picture of any particular situation. An example of one such combined experiment is presented in Fig. 8.

Applying sap flow measurements by radial profile HFDsensors in combination with air-spade techniques is very useful for studying root structure and function. The most recent investigations have shown the value of utilizing combined approaches. Applications have included the estimation of vulnerability of a spruce stand to heavy machinery load, when root systems were excavated after sap flow and other measurements had been made (see Fig. 3, upper and middle panels) and complex studies of urban trees (Čermák *et al.*, 2000; Stokes *et al.*, 2002; N Nadezhdina *et al.*, unpublished results).



Fig. 8. Scheme for the recommended combined approach to root studies showing opened main roots and the possible position of applied sap flow sensors.

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