To link to this article: **DOI:10.1023/A:1016131102232**

http://dx.doi.org/10.1023/A:1016131102232

Monitoring of water from the underground to the tree: first results with a new sap extractor on a riparian woodland

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Key words: conductivity, oxygen-18, riparian, sap extraction, stable isotopes, water consumption

Abstract

Riparian woodlands are characterized by variable hydrological conditions. Following the mapping of the complex underground water circulation of the wetlands, we studied the water uptake by trees. Although there are numerous analytical techniques available to monitor the water origin and water fluxes, no rapid technique for the extraction of xylem sap exists on the market. For this reason we designed and built a unique machine able to extract sap directly in the field from wood cores in a few minutes. A short description of the machine and its performance is given, prior to reporting the first experimental results obtained in a young riparian woodland along the Garonne River. The results compare the vertical water profile of the soil and the corresponding xylem sap at different roots horizons and in the trunk.

Introduction

Vegetation plays a key role in the vertical soil profile of water. Storage and plant use of water in riparian zones affects the hydrological regime of rivers and, therefore, affects downstream riparian areas (Hugues, 1990; Malanson, 1993). However, scientific disciplines typically separate the study of groundwater, surface water and vegetation. Also, it is only quite recently that people have started to review the knowledge available on the interactions between vegetation and groundwater (Le Maitre et al., 1999), or on the impact of riparian vegetation on hydrological processes (Tabacchi et al., 2000). The focus of our research was to investigate the link between water regimes and plant growth in riparian woodlands.

Numerous analytical techniques are available for studying the origin of water, but natural stable isotopes such as ^{18}O or deuterium seem to be the more precise approaches (Brunel et al., 1997; Hardegree et al., 1995), because they use natural variations of the stable isotopes of a given element. For instance, water (H_2O) movement can be followed by either the ratio $^{1}H/^{2}H$ (Hydrogen/Deuterium), or $^{16}O/^{18}O$. These ratios remain stable until the water evaporates.

Because the xylem sap in the tree is under lower than atmospheric pressure, the only way to remove the sap from the sapwood is by extraction. Our first idea was to use a pressure chamber to 'blow out' the sap from small branches. However, evaporation from leaves changes the isotopic signature, so leaf water is not representative of the water source (Dawson and Ehleringen, 1991). When reviewing the literature, three groups of sap extraction techniques were found. (i) In azeotropic distillation (Busch et al., 1992; Revesz and Woods, 1990), the sap is extracted from wood cores by an organic solvent, and then separated as an aqueous phase (the distillation takes about 15 min). (ii) In the quantitative freeze-drying technique (Cooper et al., 1989; Smith et al., 1991), the wood cores are distilled under pressure and the aqueous sap is trapped by ice formation. Here, also, the distillation must be undertaken and takes between 30 min to 2 h to be quantitative. (iii) In the last, less known technique, a direct extraction with 'a small brass piston squeezer'

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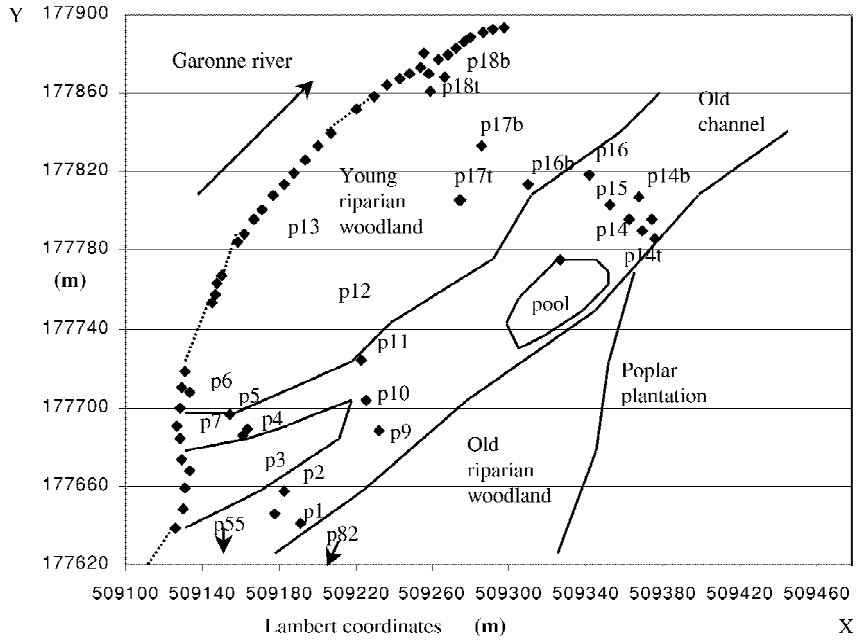

Figure 1. Experimental field sites obtained from topographic data (black diamond). The X and Y scale are expressed in metres relative to Lambert coordinates (North: positive X). Three transects were chosen: *upstream* from piezometer Nos. 1 to 7 (p1 to p7), *middle* from p9 to p12 and *downstream* from p14 to p18. Some of them have duplicate or triplicate measurements to better assess local ground water variations. Also some piezometers have been added upstream on the gravel bar like p55 and p82.

(White et al., 1984) is undertaken and takes only a few minutes. This home-made piston type squeezer is powered by battery clamp pullers, and is light enough to be carried on the field side. We decided to try to reproduce this kind of extraction, and also wanted to test if such drastic compression of the wood cores would not release some intracellular liquid.

Materials and methods

Site description

The field site was a 2-km long gravel bar, 250 m wide along the Garonne River. The site was about 110 m above sea level and 50 km downstream of Toulouse, France (Figure 1). The site was located along an older riparian forest and had been progressively colonized over a period of 10 years by vegetation, mainly black poplar and white willow, with a density ranging from 20 to 0.5 trees per square meter. Three parallel transects were marked from the Garonne river up to the higher poplar plantation. More than 25 polypropylene piezometers (p), 1.5 to 3 m long, were installed, designated p1 to p18. Most of the piezometers were placed by following a microtopographic survey (plotted with a black diamond in Figure 1), as well as using the waterline of the Garonne River and some other geographical features. Some extra piezometers were added upstream on the gravel bar (e.g., p55 and p82) near older poplar trees. Initially, the experimental plot was screened for the possibility of underground water mixing into the gravel bar and surroundings (Lambs, 2000). It was found that some old river arms, even though filled with gravel and fine sediments, continued to link the gravel bar to the river. The aim of this study was to measure the origin of the water utilised by the riparian woodland trees on the gravel bar and also to assess the possible link between water availability and the corresponding ecological preferences of plant species. The equipment at the last transect (the furthest downstream) was completed with two sets of three tensionic ceramic tensiometers (tensionics, SDEC, France) able to sample water from the unsaturated areas in a porous ceramic by depression (Vigouroux, 1997). As the water table was about 1 m deep, three depths were sampled: 30, 60 and 90 cm. The trapped water was taken up with a 50-mL syringe. The underground water in the piezometers was cleaned out and sampled every week with a peristaltic pump (Geotech, CO, USA). A parallel study measured tree sap flow and tree growth on the same transect (Lambs and Muller, 2002).

Methods of sap extraction

In the first method we used a Scholander pressure chamber (Soil Moisture Equipment Corp., USA). This equipment is normally used to measure the water potential of leaves. For this extraction purpose, and to avoid any loss of sap, the chamber was placed on a special support so that the sap coming out of the small branch directly could drop into a 2-mL glass vial. Branches with a few leaves were harvested early in the morning near each selected piezometer. By sampling three twigs with a few leaves we could obtain about 0.5 mL of sap. The water table during the study (11/09/97) was between 0.5 and 1.3 m deep. A second experiment was done later (26/03/98) to see the influence of the pressure on the obtained sap.

In order to design the new machine to extract xylem sap from wood cores, we first trialed a French press cell, ref. no. FA-073 (SLM-AMINCO, Urbana IL, USA). This tool is traditionally used in biochemistry to extract liquid from soft tissues, and uses a heavy hydraulic press. Although this press gave us the pressure needed for a good extraction, it was not suitable for sampling fibrous tissues such as wood cores.

The design of the machine was undertaken in several stages (Figure 2). Firstly, there was the drawing and machining of an original pressure chamber in stainless steel adapted for woody samples and the extraction of small quantities of sap, and thick enough to resist up to 50 kN of pressure. The design of this part allowed the chamber to be easily opened for cleaning between samples. The second stage was to find suitable 'C'-shaped hydraulic clamps that were compact enough and not too heavy to carry. This also involved the design and machining of the upper and bottom adaptors. The third stage was the acquisition of a light hydraulic pump system controlled by a precise pressure gauge. The whole machine was fixed on a wooden plate with two handles and weighed less than 20 kg.

Trial of the new sap press at the field site

The xylem sap in the trees was extracted by cores with a 5-mm inside-diameter increment borer, just under the first branches, in a poplar close to the piezometers. This sampling method was destructive and was carried out using an increment borer. It was conducted twice, a few days after a moderate flood (rainfall far upstream), which provided good soil moisture conditions without the input of local rain. Working with suberized tissues such as wood cores allowed the elimination of bark from the sapwood, so that only xylem sap was obtained. However, the study was limited to larger trees, where the drilling to extract the wood cores could be carried out without critically damaging the trunk. The water table during the study (June–July 99) was between 0.5 and 1.5 m deep, similar to that for the pressure chamber.

Analytical techniques

The water and sap samples were analyzed for pH, electrical conductivity, and ^{18}O stable isotope content. The pH and conductivity were measured with a portable Ionometer (Consort C531, Belgium) equipped with a temperature probe and standard cells. Conductivity results are expressed in mS m^{-1}. To accommodate the small sap volumes, a narrow combined pH glass electrode (diameter=4 mm) or a thin conductivity cell (2×2 mm) from Tacussel (France) was used. All the samples for isotope measurements were purified on 13 mm, 0.45 μm filters (Whatman, PVDF/PP) and collected in appropriate small glass vials with tight caps (Brosse packaging, Paris, France) on the field site and kept in a well-insulated box. The samples were than sent quickly to the Biogeochemical Isotopic laboratory in Paris, France, using an Optima spectrometer from Micromass, equipped with a Isoprep off-line gas production. Results for ^{18}O are expressed as:

$$\delta^{18}O(‰) = (\text{R sample}/\text{R standard} - 1) * 1000,$$

where R sample is the ratio $^{18}O/^{16}O$ of the sampled water and the R standard is the ratio of $^{18}O/^{16}O$ from the ocean water ($2.005*10^{-3}$) (Standard Mean Ocean Water: SMOW). This technique has an analytical performance with a precision of 0.04‰.

As the xylem sap obtained with the press could be mixed with water coming from damaged cells, the sugar content was measured. Sucrose concentrations were determined through conversion with the invertase enzyme (EC 3.2.1.26) (Sigma-Aldrich). The glucose was quantified after the reaction with glucose oxidase and peroxidase.

Results

Pressure chamber technique

The values for groundwater collected from the piezometers along the three transects show the mixing of the water table and the river water in the gravel bar.

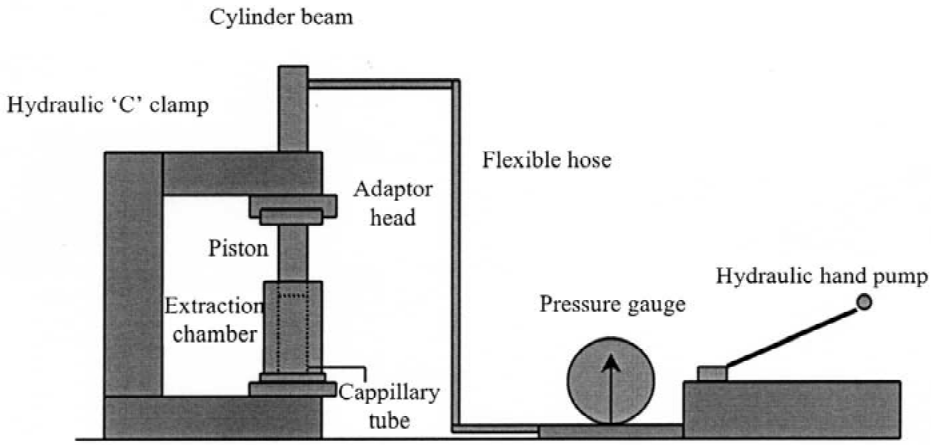

Figure 2. Schematic representation of the new sap press.

The sap collected from the poplar trees close to the piezometers displayed less negative isotopic values, reflecting a high increase in the heavy isotope. The difference of value between the free underground water (from piezometer) and the sap ranged from about 2.9 for 6 points (i.e. p1, p2, p4, p6, p16, p18) and 1.6 for the 5 points almost closed to the back channel (i.e. p5, p9, p10, p11, and p15). The second set were in wetter with certainly a smaller isotopic gradient in the soil. When sap extractions were done again later, the δ-values continued to increase (p.e. $-5.91‰$ became $-5.41‰$ for the tree near p15), before becoming more negative ($-6.44‰$ for the same poplar and $-6.39‰$ for a nearby willow) before about noon to be dry.

In the second experiment, we wanted to test the influence of pressure on the sap isotopic composition for a possible discrimination between xylem sap and phloem sap. Previously, all the extractions were done about 2.7 Mpa. Here the extraction were done on a willow, starting at 1.5 MP (the minimum value to extract sap) and ending at 3.3 Mpa with a increment of 0.3 MPa. From 1.5 to 1.8 MPa the average δ value was $-6.65\pm0.07‰$ and from 2.4 to 3.3 MPa $-7.04\pm010‰$. There was effectively a little difference, but relatively small as the reference ground water had a δ-value of $-9.3‰$.

Sap press performances

The yield of the sap press was calculated by measuring the weight of the fresh wood cores against the volume of the extracted sap (see Figure 3), the density of the sap being very close to unity. The total water content of the poplar wood cores was determined by weighing the cores before and after drying overnight at 105 °C, and was found to be about 50%. As the maximum extraction yield is 40%, at least 10% of the water or sap remains in the wood samples. After a few hours, the pressed wood cores recovered their original cylindrical shape. Other than a small fitting correction with the piston, there were no problems with the wood fibres as had been found with the French press. As the sap volume obtained in Figure 3 required pressures as high as 35 kN, we followed the volume of sap as a function of the applied pressure on the wood cores. This test also showed the influence of the core orientation in the extraction chamber. When the cores are all standing up the volume of sap increases more rapidly than when the cores are lying down (see Figure 4), but the sap yield at high pressures were largely independent of core orientation.

An additional concern was the possible rupture of parenchyma cells, linked with the phloem sap, under high pressure and the release of their intracellular water. To assess the origin of the sap extracted, we measured the sugar concentration of sap extracted at both low and high pressures. Two extractions each were conducted on three wood samples, one at low pressure (from 0 to 7 kN) and the second at high pressure (from 7 to 30 kN). The results of Table 1 are the mean values of the three series. The pH of the sap extracted under both pressures ranged from 6.5 to 8.0. As the sucrose determination used an enzyme that worked better in acidic conditions, we divided again the sap tubes into two groups: one with the original pH and the other adjust for the enzyme (pH 4). The yield of the enzyme was high and ranged from 85 to 90%, showing the absence of inhibitors coming from the

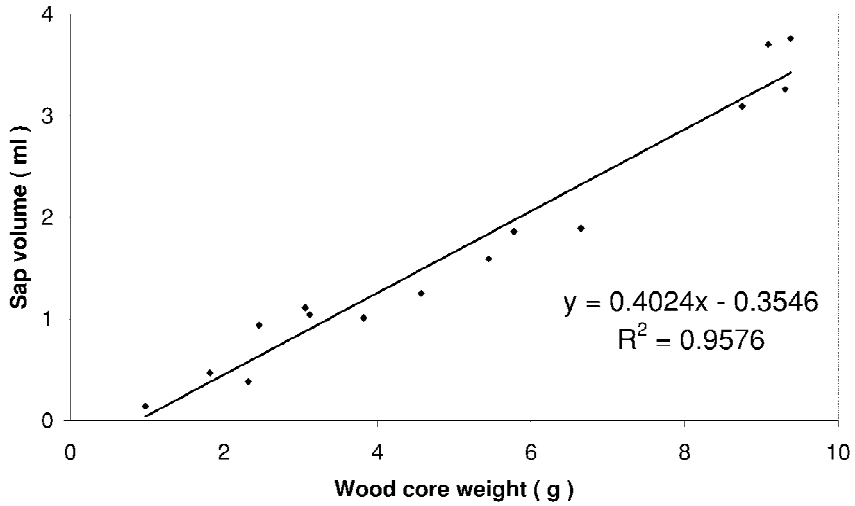

Figure 3. Volume of sap extracted by the sap press for different sample weights of poplar sapwood cores.

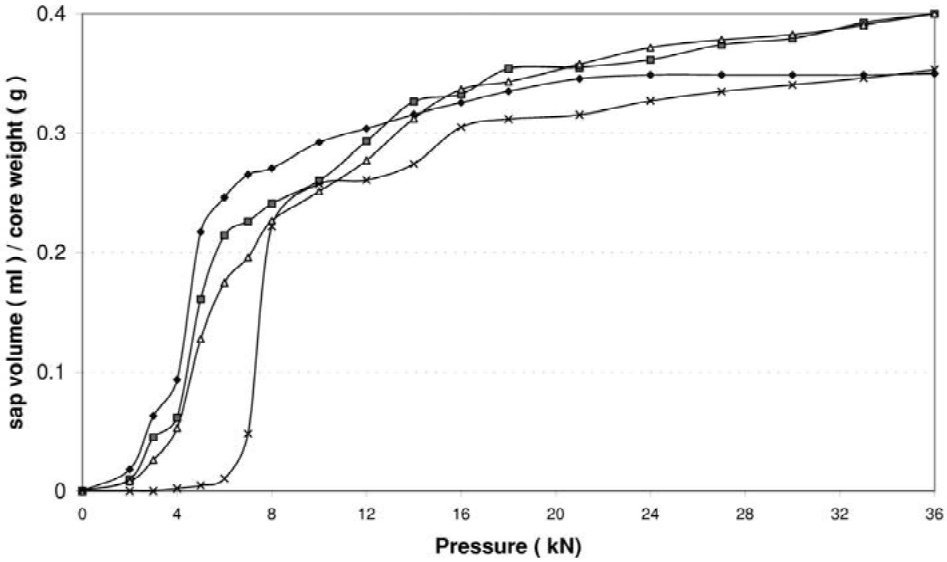

Figure 4. Volume of sap extracted by the sap press at different pressures for two kinds of cores storage: standing up with three samples (♦, □ and ♦) and lying down with one sample (X). The wood core weight ranged from 8.75 to 9.38 g.

extraction. The overall results showed very few differences between the high and low pressure extractions at the adjusted pH, i.e., 0.16 and 0.17 g/l for glucose and 0.17 and 0.16 g/l for sucrose, respectively. Even a sample of sap obtained without the press (in some rare cases a few drops of sap flow out when the wood cores are drilled) gave similar sugar concentrations (0.20 g/l of sucrose). The total sugar concentration was about 0.33 g/l. In the existing literature the xylem sap of plants is between 0.2 and 0.5 g/l, whereas the phloem sap is between 250 and 400 times higher (Heller et al., 1995; Pate and Jeschke, 1993; Pate and Dawson 1999). This suggests that the sap obtained with the sap press is xylem sap, and that the relative high pressure used had no effect on the sap composition.

Use of the sap press on the field site

After the laboratory trials, the sap press was ready to be tested at the field site. The advantage of directly pressing the sap cores after their removal from the tree eliminated the problem of conserving of the hydric

Table 1. Experimental results for glucose and sucrose determination in black poplar xylem sap obtained with the sap press

	Original neutral pH		pH adjust for enzyme	
	HP[a]	LP[b]	HP	LP
Glucose (g/l)	0.11±0.06	0.13±0.05	0.16±0.04	0.17±0.09
Sucrose (g/l)	0.23±0.02	0.13±0.06	0.17±0.04	0.16±0.08
Yield of enzyme	85.3%	87.9%	90.6%	86.5%

[a] HP=High pressure extraction.
[b] LP=Low pressure extraction.

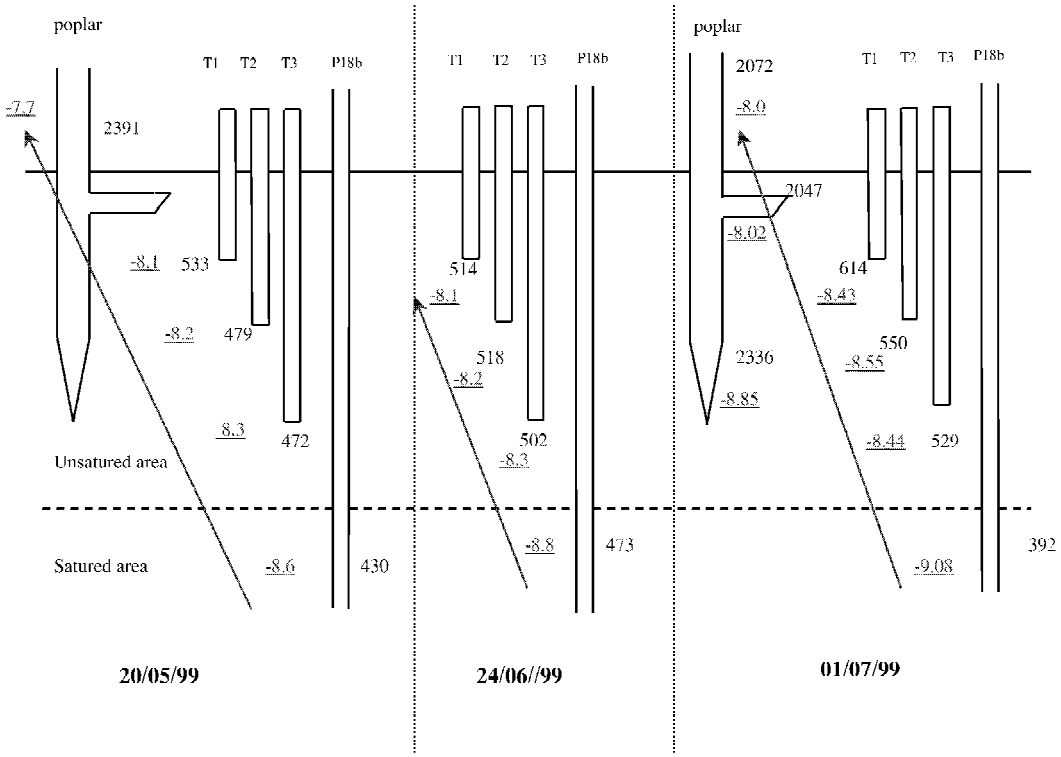

Figure 5. Vertical water profiles obtained from the piezometer, the tensionics set (T1 at 30 cm, T2 at 60 cm and T3 at 90 cm) and sap data obtained from the sap-press at the p18 area of the downstream transect. The negative underlined numbers correspond to the isotopic content, whereas the positive numbers give the conductivity values.

status of the vegetative tissues until in the field, i.e. we avoided condensation in the bag, which leads to an isotopic fractionation of the sap water. We waited until the large spring flood (and the related heavy rain) was over before sampling in the field. Only 15.4 mm of rain fell between 20/5/99 and 01/07/99, allowing us to focus on the soil moisture coming from the groundwater. Figure 5 gives examples of the vertical water profile at our field site around the p18 piezometer for three successive dates. The first date shows a well establish vertical gradient, both for the isotope and conductivity measurements, due to the homogeneous hydration of the soil profile 3 days after a moderate flood. The sap was extracted with the new machine from wood cores taken from the trunk just below the first branches to avoid any evaporation from the leaves. As there is no isotopic fractionation of the absorbed water by the roots, the soil layer depth in which the tree obtains the water can be determined by identifying the xylem sap value with the corresponding soil water layer-value. A vertical gradient is observed, from the saturated water (δ^{18}O=−8.6‰) at 1 m in depth to the unsaturated at 30 cm in depth (−8.1‰). However, the obtained sap value, −7.7‰, is quite high compared to this last value, suggesting water absorption closer to the surface. For the second trial, 1 month later,

Table 2. Variation of the isotopic content, (δ^{18}O), between the water taken from the piezometers between May and July 99 and the water absorbed by the tree (xylem sap). The notation of the piezometer (p14 for instance) corresponded to that of the field site map (Figure 1). The sampled tree are black poplar (*Populus nigra*) except for p1 and p16b which are white willow (S=*Salix alba*). The river and ground water (G.W.) values are added as the two major water origins. The lines quoted difference reflects the isotopic difference between the water available under the tree and the water moving in the trunk

Date	Source	Isotopic content (δ^{18}O)									
		River	p1 (S)	p82	p55	p18b	p17b	p16b(S)	p16	p14	G.W
20/05/99	Water	−8.6	−8.3	−8.9	−8.0	−8.6	Flood	Flood	Flood	Flood	−6.6
20/05/99	*Sap*		*−7.2*	*−7.0*	*−5.5*	*−7.7*					
	Difference		1.1	1.9	2.5	0.9					
24/06/99	Water	−9.6				−8.8	−7.3	−8.3	−7.3	−7.0	−6.9
01/07/99	Water	−9.03		−9.66	−9.03	−9.08	−8.86	−8.37	−8.37	−8.04	−7.07
01/07/99	*Sap*			*−7.92*	*−6.2*	*−8.0*	*−8.12*	*−7.33*	*−7.44*	*−7.28*	
	Difference			1.74	2.83	1.08	0.74	1.04	0.93	0.76	

sap data was missing due to technical problems with the sap press: the piston was clogged with sand. The soil profile in the unsaturated soil remained similar, although the saturated water began to change (−8.8‰). For the last date, 1 week later, the saturated water continued to change (−9.08‰) and the distribution in the unsaturated parts was also affected. This indicated that during this time the influence of the river water (−9.0 to −9.6‰ over the week) was greater. For a better understanding of the water absorption by the tree, a hole was dug at the foot of the tree to access the superficial roots and the deeper vertical roots. It was interesting to note that the value of the deep roots presented an isotopic content of −8.85‰, whereas in the nearby piezometer (p18) the value was −9.08‰. On the same day, a similar result was found for the piezometer p16 (−8.37‰) in a nearby tree (deep root −7.83‰, and trunk −7.33‰). This deep water does not seem to be used by these trees since the sap value is more charged in ^{18}O, less negative value, and is more similar to the value obtained from the superficial roots. This suggests that for these trees, the main quantity of water taken up lies between a depth of 30 cm and the surface. Only the first centimetres of the soil profile in these riparian zones contain loam and soil nutrients; the rest is mainly composed of raw sand and gravel. The roots of these riparian trees are present in all layers, but the surface ones are the densest.

Table 2 summarises the difference of the isotopic content of local table water and the sampled tree sap for 20 June and 1 July. Although there was less useful isotope sap data on the 24/06 due to technical problems, the δ values of the water were maintained, because it explained well the water distribution in the gravel for the next week sampling. For instance, this explained why on 1 July, the water δ values in p82 (−9.66‰) is more negative that the Garonne value (−9.03‰) because it is older water from the previous weeks high value (−9.6‰). For the tree along the transect (p1, p14–p18), the difference between the water from the saturated soil and the sap was about one unit, whereas for the older and more isolated tree at p82 and p55 the difference is much higher. What is also interesting is that for trees where we have data for both dates (p18b, p55 and p82), although the water and sap values have changed, the difference remained of the same order.

The second interesting point is the ion content of the sap. Some data are given in Table 3, in comparison with the available groundwater from the 20 June to the 1 July. The trees outside the back channel (p17, p55 and p82) which are also older, displayed the higher sap ions content (about 400 mS m^{-1}). The sap from the other trees, including at p18, are less charged and the conductivity value is about the half (around 200 mS m^{-1}). These values seems to be independent from the available water. For instance at p82, the water in the piezometer range in the time from 31.2 mS m^{-1} (elution by the flood on 20 May), to 38.3 mS m^{-1} (middle value on 10 June) and finally 55.4 mS m^{-1} (normal value on 1 July) whereas the sap concentration remain about the same value: 440.3, 452.3 and 438.6 mS m^{-1}, respectively, so less than 3% variation. The roots of the trees seem to be able to compensate for this lack of ions by concentrating the absorbed water, a physiological mechanism known as 'epictese' (Heller et al., 1995). We can also notice than the two willows at p1 and p16b, growing in places where the

Table 3. Variation of the conductivity values, expressed in milli Siemens per meter, between the water absorbed by the tree (xylem sap) and the water taken from the piezometers. The sampled trees are black poplar (*Populus nigra*) except where (S) followed the sap value (S=*Salix alba*). The last line refers to the vertical ratio, which reflects the ability of the roots to concentrate the ions

Date	Location	Piezometer (mS m^{-1})	Sap (mS m^{-1})	Ratio
20/05	p1	51.4	215.0 (S)	4.2
	p82	31.2	440.3	14.1
	p55	83.3	409.4	4.9
	p18b	23.0	239.1	10.4
10/06	p82	38.3	452.3	11.8
	p18b	45.7	180.6	3.95
17/06	p16b	41.9	233.9	5.6
24/06	p1	75.3	177.2	2.4
	p15	79.6	256.3 (S)	3.2
	p14	74.5	204.7	2.7
01/07	p1	82.6	215.0	2.6
	p82	55.4	438.6	7.9
	p55	87.6	383.6	4.4
	p18b	39.2	287.2	7.3
	p17b	50.5	411.1	8.1
	p16b	63.7	211.6 (S)	3.3
	p16	79.3	209.8	2.6
	p14	84.7	237.4	2.8

humidity is more constant due to groundwater output, displayed the lower ratio.

Discussion

Water origin

Stable isotopes can be applied to water-source studies (Ehleringer and Osmond, 1999). The origin of the water can be separated into three main categories: river water, groundwater and rain water. Many authors studying riparian vegetation have use Deuterium and/or Oxygen18 to characterize these water sources (Busch et al., 1992; Smith et al., 1991; White et al., 1985). In some case, other sources are possible, as mixing of two rivers (Hardegree et al., 1995) or water input through coastal fog precipitation (Ehleringer and Dawson, 1992). In our case, river water coming from high elevations in the Pyrénées Mountains, is the main source for the Garonne water. A typical $\delta^{18}O$ value is $-9.6‰$ for snow at 3000 m, which shows a high depletion in heavy isotopes due to high altitude and low temperatures. The Garonne river maintains this characteristic of mountain water until downstream of Toulouse, where the mean value is still at $-9.1‰$. This means that the proportion of low altitude river water (about $-7.4‰$) and groundwater (mean value $-6.7‰$) in the river water remains low. On the other hand, during some high floods with heavy local rain (from -7.6 to $-5.9‰$), the value of the river water can go up to -8.6, showing the elution by low altitude rainfalls. The variation in ion content is more pronounced and the value can range between 17 (during flood) and 26 mS m^{-1}. The gravel bar received both river and ground water depending at different proportion in function of the local water table level (Lambs, 2000).

Water gradient in soil

Due to evaporative losses to the atmosphere, water in the soils unsaturated zone tends towards isotopic enrichment relative to ground water. Soil water shows decreasing evaporative enrichment in deuterium and

^{18}O from the surface to the zone where liquid transport is dominant (Allison and Hughes, 1983). The obtained values depend on the relative amount of each water reservoir and the variation of the rainwater. Many studies of such vertical profiles have been studied under arid climates (Allison and Hughes, 1983; Busch et al., 1992; Le Roux et al., 1995). Only more recently, have similar studies been performed in other climates. In Scandinavia, which has a cold oceanic climate (Plamboeck et al., 1999), the forest soil water ranged from δ ^{18}O $-13‰$ at 55 cm deep to $-9‰$ close to the surface. Bariac et al. (1990) have studied the soil water isotopic profile from 0 to 50 cm for a maize field in France. They found δ^{18}O value of $-6.5‰$ at 50 cm deep, $-6.4‰$ at 30 cm, $-5.5‰$ at 15 cm, $-4‰$ at 7 cm and $-2‰$ at 2 cm. This shows the rapid evaporation in the last 15 cm of soil even under temperate climate. In our study, the gradient between 90 ($-8.3‰$) and 30 cm ($-8.1‰$) is small, but could continue in the upper part of soil as observed by Bariac et al. During this 7-month period (from 21/01/99 to 22/07/99), the ions charge of the soil water content at 90, 60 and 30 cm followed the variability of the local water table and the input of rain seemed to remain low. However, during the winter, the gradient for p18 was also found to be reversed, showing the complex equilibrium under the different water sources and the episodic floods. During the same period, a discontinuity in gradients was found; no water could be extracted at the 60 cm, whereas the two others at 30 and 90 cm remained wet.

Absorption by roots

Measurements of the natural abundance of oxygen or hydrogen isotopes in plant water are increasingly used to infer the source of plant water in natural vegetation (Takahashi, 1998). Since water moves from the soil into the roots, and then to the stems of terrestrial plant without fractionation of isotopes, and the water can be sampled from soils and plant tissues without fractionation or isotopic contamination (Dawson and Ehleringer, 1991, 1993; Dawson and Pate, 1996; Pate and Dawson, 1999; Thorburn and Mensforth, 1993; White et al., 1985). It is then possible to identify the soil layer where the tree takes its water. In the Colorado banks, Busch et al. (1992) have shown that *Populus fremontii* and *Salix gooddingii* display a phreatophytic uptake pattern, whereas for the allochtone species *Tamaris ramossisima*, this pattern is only facultative, which can explain its rapid extension. However, they only measured at 50 and 100 cm depths. Dawson and Pate (1996) reported that *Banksia prionotes* used its lateral roots (0–0.5 m) in wet period and the sinker roots (2–3 m) in dry period. Plamboeck et al. (1999) found that Scots pines are able to draw proportionally more water from deeper horizons when water becomes less abundant. Breda et al. (1995) arrived at the same conclusion with different techniques. Under our more temperate climatic conditions, the riparian trees in our study seem to take soil water predominately from the first 30 cm. Poplars have been reported to used soil moisture even when groundwater was available (McQueen and Miller, 1972). In our results (Figure 5) the sap value ($-8.0‰$) is very similar to the upper root sap ($-8.02‰$), suggesting a similar water extraction. The fact that the water at -30 cm is more depleted in heavy isotope ($-8.43‰$) could be explained by the enrichment of this water by the fractionation in the last decimeter, as reported by Bariac et al. (1990) continues to fractionate. Since during this period the rain was very scarce, a fourth source of water could be implicated, dew condensation. Heavy morning dew is observed along the river banks during this season, and monitored by comparing the high air moisture content during night and morning. This phenomena has been studied for coastal regions where heavy fog can exist (Cavelier and Goldstein, 1989; Dawson, 1998). In general, the δ^{18}O of fog is depleted in heavier isotopes relative to the water source from which it was formed.

Evapotranspiration

Early as 1934, Washburn and Smith had reported the leaf water fractionation in *Salix nigra*. During water transport between the root and the shoot, the isotopic composition (measured as the ratio of heavy to light isotopes) of xylem water remains unaltered from that in the soil, until it reaches tissues undergoing water loss (i.e. leaves or nonsuberized stems), where evaporative enrichment in the heavier isotopes of hydrogen and oxygen takes place. Leaves are the primary site of evaporative enrichment and the magnitude of this enrichment of leaf water is dependent upon humidity gradients, transpiration rate and the isotopic composition of atmospheric water (Ehleringer and Dawson, 1992). To explain this difference in leaf water, Yakir et al. (1990) proposed a model where the leaf water consists of a mixture of several isotopically distinct pools. However, some other authors (Bariac, 1988; Bariac et al., 1990, 1994) have shown that when an isotopic

steady-state is attained in the leaf water, transpiration flux induces an enrichment in the surrounding water vapour by returning to the atmosphere a specific vapour. The isotopic composition of this vapour is the same as that in the soil water of the layers supplying water to the roots. The mixing of the xylem sap with the phloem sap would be the cause of the leaf enrichment. The isotopic composition of the vapour generated by the canopy is different from those of the regional one, coming often from the oceanic area. Using this particularity, Salati et al. (1979) have shown the existence of a recycling of the transpiration vapour water fluxes from the Amazonian forest rain.

Extraction techniques

For the azeotropic and for the freeze-drying distillation, the problem came mainly from the storage of the woody sample (often frozen in liquid N_2) until the laboratory extraction is done. For the pressure chamber, the extraction can be performed directly on the field site, but only on small branches or stems. As these nonsuberized tissues can evaporate, extraction is not valid for the isotope analysis (Allison et al., 1985; Dawson and Ehleringer, 1993). But the pressure chamber is still used for the study of the ions analysis in stems (Berger et al.,1994; Hiltbrunner and Flückiger, 1996). Only the origin of the extracted sap is not well defined: phloem sap and/or xylem sap? The direct extraction of wood-core at the field site (White et al., 1985; present work) seems to have all the advantages: no problem of sample conservation and valid for isotope analysis. In view of patenting our new machine, a research of other possible patents all over the world using the key words 'sap' and 'extraction' was undertaken. A few interesting machines were found for the extraction from soft tissues of plants, but none seems to be available for treating hard or fibrous tissues such as wood cores.

Conclusion

The sap press prototype is an easy and powerful tool in the field, but care has to be taken with sandy samples like roots. The direct extraction of the samples also favoured the use of analytical techniques at the field site on xylem sap, such as conductivity. For complex systems, such as the riparian banks, this flexibility is welcome in order to better understand the multiple interactions between water and vegetation. This first approach revealed the importance of the local climatic conditions, since the formation of dew following colonisation by poplar vegetation allowed the establishment of other plants. The potential use of the sap press is extensive. For example, in an orchard it can be used to follow the absorption of fertilisers or other molecules by the trees. In chemical geology studies, this equipment could also be used to quantify the amount of chemicals elements absorbed from the soil.

Acknowledgements

We are grateful to P. Mordelet (CESBIO Toulouse) for the use of the Scholander chamber and G. Lanéelle (IBCG Toulouse) for the trial with the French press cell. For the sap press, we would like to thank D. Boutaud (CESAC Toulouse) for the conception of the hydraulic part, L. Guiraud (CEMES Toulouse) for the machining map and G. Ferré (FIST Society, Paris) for the patents research. For the sap analysis, we are grateful to G. Marigo (University J. Fourier Grenoble) for his advice on plant physiology, J.-Y. Charcosset (CESAC Toulouse) for the conceiving of the sucrose determination, and the anonymous referees for their valuable comments for improving the manuscript. This study was funded in part by the European Commission, Contract No. ENV4-CY96-0317.

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