Assessing water use in plants: an introduction and guide to methods of measurement

by Dr Victoria Stokes

Forest Research, Alice Holt Lodge, Farnham, Surrey

Summary

The value of many experiments carried out in forest science could be increased with knowledge of water availability or water use of the plants involved. This article introduces the options available for the assessment of plant water status and is aimed at people who have little, or no, previous experience of this type of study. Each method is considered in terms of its advantages and disadvantages, practicality, cost and contribution to the objective of the project being undertaken.

Why measure water use?

Biomass production is directly proportional to plant water status and the supply and use of water, particularly its effect on root and leaf extension. Stomatal pores control the amount of water lost through the leaves of a plant. These openings on the leaf surface are sensitive to environmental conditions and close to prevent damaging amounts of water loss. However, this is a compromise, as stomatal closure also prevents carbon dioxide from entering the leaf, stopping photosynthesis.

The stomata therefore have an important role in balancing photosynthetic gain and water loss. Although only a minute amount of water is used directly in the chemical reactions of photosynthesis, the movement of water from the roots through the plant and out of the leaves (transpiration) is essential for survival; the evaporation of the water from the leaves provides the plant's cooling mechanism and is essential in maintaining temperatures suitable for photosynthesis.

Foresters may be interested in plant water use to determine the likelihood of survival and growth at a site, to compare different water requirements of a range of species or to investigate competition from weeds or from other trees. The impact and implications of drought, climate change or management strategies may also be assessed.

Unfortunately, the lack of understanding of plant water status and confusion over methods by which it can be measured often lead to poor experimental practices or inappropriate measurements being taken. An example of this is given in a paper by Foggo and Speight (1993) that considered the effects of root damage and water stress on the exploitation of ash buds by ash bud moth larvae. In the experiment water stress was supposedly induced in trees by placing a circular polythene sheet of one metre diameter around the base of the tree and sealing it to the trunk. However, no quantitative measure of soil moisture beneath the mulch was made and no evidence that the trees were actually water stressed is presented. However, based on the work of Davies (1987) it would not be unreasonable to assume that the mulched trees were less water stressed than the others; this questions the validity of some of the results presented. Another example is in Stokes (2003) where it was initially assumed that transpiration in branches of mature oak and sycamore trees stopped at night. However, this was proven incorrect by a chance measurement and this had a significant effect on calculated daily water loss values.

There is also often a tendency to over-do measurements, using sophisticated equipment, when a simple technique may suffice. For example, if a site is to be characterised using Ecological Site Classification (ESC) (Pyatt *et al* 2001) there is no need to install dataloggers and soil moisture probes to determine site moisture level, when a simple manual rain gauge or data from a nearby weather station would give sufficient information. It is therefore important to assess exactly what questions are to be answered before commencing fieldwork and to have a clear idea of how the data gathered will be used in the final conclusions of the work.

Direct sampling of plant water content is a destructive process, but there are two broad non-destructive ways to consider plant water relations in the field. The first is to consider the availability of water at the site and the second is to measure water movement through or loss from the plant. Appendices 1a and 1b present the advantages, disadvantages and approximate costs of each of the methods discussed in the following sections.

1. Measurement of water availability and water content

There are various types of meteorological equipment designed to measure availability of water at a site such as rain gauges, wetness sensors, humidity sensors and soil moisture probes. Sensors can be also be deployed to measure other variables not directly related to water availability, such as solar radiation, temperature, atmospheric pressure, wind speed and direction, and sunshine duration. The advantages of these techniques are their relatively low cost compared with measurement of water loss from a plant and once set up and connected to a datalogger the measurement can be remote and continuous throughout the growing season, requiring very little maintenance.

Downloading the datalogger and checking the equipment should be carried out once a fortnight. As there is unlikely to be large variation in rainfall, surface wetness or humidity across a small experimental site, the measurements made will apply to all the plants on the site. The data therefore tend to be coarse-grained and although useful for contrasting between sites, are less suitable for within-site comparisons.

Alternatively, information from a nearby Meteorological Office automatic weather station (AWS) could be used. For many variables this usually provides a reasonable estimate of the conditions on the experimental site. However, some variables, particularly rainfall and temperature, can vary over small distances. If the soil type, aspect, slope and vegetation are different from that at the AWS and the distance is significant, installing an AWS on site may be a better option.

However, one fairly serious consideration with all dataloggers and meteorological equipment is that of site suitability. Dataloggers can be powered by nearby mains electricity (stepped down to 12 volts via a transformer), by car batteries, or by small windmills and solar panels. If batteries are used vehicle access may be needed to within reasonable proximity to allow regular changing. Some sites also have a high risk of theft and vandalism; for example, sites with public access may not be suitable, particularly if loggers are powered by car batteries, which are likely to be stolen. Storage of loggers and batteries in strong lockable boxes may deter vandalism, but can also draw attention to the equipment and increase interest.

Soil moisture and water potential sensors

Soil moisture probes measure volumetric soil moisture content and can be used to make instantaneous spot readings or left *in-situ* for long-term logging (Fig. 1). The probes work by generating a 100MHz signal, which is reflected back to the probe in proportion to the water content of the soil. Profile probes have the advantage that they simultaneously measure at up to six different depths in the soil although measurements can only be made in a previously installed access tube. Soil moisture probes are simple to use, relatively cheap and are available with a hand-held meter to record measurements in the field and download later. However, soil moisture content can vary hugely over very small distances and depths, so many measurements may be required to obtain a reasonable average value for soil moisture at a site.

Water potential is a measure of the 'suction pressure' in the soil or plant, and therefore measures water-stress directly. Soil water potential may be measured using a tensiometer. This is an airtight water-filled tube with a porous ceramic tip at the base and a vacuum gauge at the top. When inserted into the soil, water moves out of the tube leaving a vacuum which is measured by the gauge as soil water potential. However, tensiometers can only be used between 0 and —0.08 Mega-Pascals (MPa), and most plants are not adversely affected until levels as low as —0.15 MPa are reached.

Leaf or shoot water potential can also be measured in the field using a pressure chamber. A leaf or shoot cut from the plant is inserted into the chamber with the cut end projecting through a hole in the sealing bung. Pressure is applied in the chamber until water appears at the cut surface. The amount of pressure applied is then equal and opposite to the tension in the xylem sap. Pressure chambers have been used extensively in the field and, although they do require some destructive harvesting, are a quick and simple method of characterising plant water status. However, the result obtained is simply a 'snap-shot' of the leaf or shoot water status at that moment, as for a growing plant in the field, both uptake of water from the roots and loss through the leaves are continually varying processes. As plants adapt to water stress through changes in properties of their water relations, such as the relative water content that prompts stomatal closure or the diurnal pattern of stomatal conductance, pre-dawn water potential is usually measured, when it is assumed that there is no potential gradient through the plant.

Alternatively, leaf water potential can be measured by psychrometry. This method is similar to that of pressure chambers; however, the detached leaf is placed in the chamber and the water potential is allowed to gradually come into equilibrium with the chamber air. The vapour pressure of the chamber air is then measured by a wet-bulb psychrometry. The equipment is expensive, complex and requires regular calibration and measurements are time consuming, so this technique is better suited to laboratory conditions.

2. Measurement of water loss from a plant

The difference between transpiration rate and stomatal conductance

Transpiration is the rate of water vapour loss (mmol m^{-2} s^{-1}) from the leaf mesophyll surface to the free air. For water vapour to cross this route it must first pass from the mesophyll surface through the stomatal pores into the leaf boundary layer (referred to as stomatal conductance, g_s) and then from the boundary layer into the free air (referred to as boundary layer conductance, g_b). Therefore:

$E = (w_i - w_a)/R_{tot}$ (Eqn 1)

Where E is transpiration rate, w_a and w_i are the water vapour concentrations in free air and air at the mesophyll surface respectively, and R_{tot} is total resistance, consisting of stomatal resistance (the dominant component) and boundary layer resistance. Therefore, under most field conditions, stomatal conductance (the inverse of resistance) is directly proportional to transpiration rate.

Forest Research Photo Library

Fig. 1: soil moisture content being measured using a Delta-T Devices Theta probe and moisture meter

Measurement of these parameters is usually carried out on individual plants and the techniques are more intensive, time consuming and require the operation of technical field equipment. However, detailed information, such as diurnal trends, the volume of water lost by a plant, and the relationships with meteorological conditions may be determined, enabling prediction of future water loss without repeating the measurements.

a) Lysimetry

Water loss can be measured at several scales, and the coarsest of these (measuring whole-plant transpiration rate) is lysimetry. Although this technique has been carried out on mature trees, it is only really practical for small pot grown plants in experimentally controlled environments, such as greenhouses. The pot and soil are sealed in a plastic bag and made airtight around the root collar of the tree and a record of the volume of water provided is kept. The change in weight of the plant, soil and pot over time gives a measure of the water loss through the leaves of the plant. However, this method is only suitable for greenhouse experiments where there will be no interference from browsing animals or rainwater on the leaves.

b) Sap flow

Sap flow is the movement of sap up the xylem vessels of the tree to replace the water lost through transpiration. In the short-term, sap flow rate may differ from transpiration rate. For example, in the early morning transpiration rates rise, but sap flow at a point upstream (downstem) measured by a gauge does not start instantly, due to the hydraulic resistance of the leaf and stem tissues between the leaves and sap flow gauge. Conversely when stomata close, stopping transpiration, sap flow continues for a while, increasing stem water content, until the tissues downstream are rehydrated. However, over a sufficiently long period the two are equal, as the water used in biochemical processes is negligible. Therefore sap flow rate can be measured and used to determine the long-term transpiration rate of a stem, branch or whole tree.

Flow rate is measured as it passes the gauge which usually consists of a heat source and temperature-measuring device placed either on the surface of the stem or inserted into it, depending on the method used. The sap is heated using constant, variable or pulsed power, and the moving sap transports the heat up the stem. The rate of sap flow may be determined either by the time taken for heat to travel between thermometers, by the temperature differential between points above and below the heat source, or by the amount of heat input required to maintain a constant temperature gradient up the stem. The most commonly used sap flow gauge techniques are the stem heat balance method (SHB) and the Granier method or thermal dissipation probe (TDP).

SHB methods can be intrusive, such as the trunk sector method which uses electrodes inserted into the wood to supply heat, and thermocouples in metal probes to record it (Daum 1967; Cermák, Kucera, & Penka 1976) or non-intrusive, using surface heating and recording methods. Baker & van Bavel (1987) modified the SHB method by the use of a flat resistance heater wrapped around the stem, with thermocouples contacting the stem at the heater and above and below it. Heat input is constant and fluxes of heat out of the system are calculated from measured vertical and radial temperature gradients, so that mass flow rate of sap in the stem can be calculated.

The TDP method consists of two needle-like thermocouple probes. These are inserted horizontally into the sapwood one above the other, with the upper one containing an electric heater. The thermocouples measure the temperature difference between the heated probe and the sapwood ambient temperature below. The temperature difference and the maximum temperature difference under zero flow conditions provide a calibrated conversion to

sap velocity. These methods are discussed in greater depth and evaluated by Smith & Allen (1996).

Sap flow gauges are powered, controlled and measured by dataloggers allowing continuous measurement. This is usually combined with some meteorological equipment, such as a net radiometer, temperature sensor, anemometer, humidity sensor and rain gauge, to facilitate correlation with environmental variables. Although continuous measurement is possible with little intervention, gauges do need to be moved every 6 to ten weeks to avoid damage to the stem, and loggers will need to be downloaded at fortnightly intervals. Sap flow rates are also more useful if converted to a leaf area basis (from g h^{-1} to g h^{-1} m^{-2}) allowing comparison between trees and species. This requires the measurement or estimation of total leaf area above the gauge.

Forest Resarch Photo Library

Fig 2: PP-Systems CIRAS-1 infra-red gas analyser and leaf chamber

A major advantage of sap flow gauges over the following single-leaf techniques is that the value of water loss obtained is a total for a whole branch or tree, which if the leaf area is known, can be converted to an average rate per unit leaf area. This reduces the incorporation of errors when scaling up to canopy level. However, the cost of installing a datalogger and gauges can be high, each gauge with a projected life of three years costing between £300-£600, depending on size.

c) Infra-red gas analysis

The techniques of IR gas analysis and porometry both measure leaf-level stomatal conductance (g_s). Measurement of stomatal conductance can be a complicated process as stomata respond quickly to many different variables (light quality and quantity, temperature, humidity, plant water status). Species, age and health of the plant also influence these responses. It is therefore important to standardise and control all possible variables during measurements and to sample extensively over long periods of time. In both IR gas analysis and porometry the boundary layer resistance is minimised by mixing well the air in the chamber with a fan to prevent pockets of still air developing, simulating field conditions and ensuring a direct proportionality between stomatal conductance and transpiration rate.

Major advances in the study of leaf photosynthetic activity and transpiration rate have been made using IR gas analysis, usually in conjunction with a leaf chamber (Fig. 2). The technique allows measurement of net photosynthesis, stomatal conductance and transpiration rate under ambient growth conditions and determination of the response to light, temperature, humidity and carbon dioxide concentration (von Caemmerer & Farquhar 1981, Field, Ball & Berry 1989, Parsons *et al.* 1997). This has the advantage that as well as information on water loss, photosynthetic rates are calculated simultaneously, allowing determination of water use efficiency (or carbon assimilation per unit of water lost), a useful variable for comparison between crops. However, the equipment is very expensive, time consuming to use and requires a trained operator both to take the measurements and to interpret them.

While still attached to the plant, the leaf is clamped in a chamber of known area and air of known humidity and CO_2 concentration is passed over it at a constant rate. As the leaf is exposed to light through the window of the chamber, photosynthesis and transpiration take place, altering the composition of the chamber air. The air is mixed by a fan and leaves the chamber where it passes through the IR gas analyser (IRGA). As heteroatomic gas molecules, such as CO_2 and H_2O, absorb radiation at specific IR wave bands, their presence in the gas cell decreases the IR radiation reaching the detector in proportion with the gas concentration. The change in water vapour content and carbon dioxide are determined, representing the transpiration rate and net photosynthetic rate. Temperature of the air, photosynthetic photon flux density (PPFD), vapour pressure deficit (D) and CO_2 concentration are manipulated to make the chamber conditions as similar to the ambient conditions as possible or to make changes to environmental conditions while monitoring the response of the plant.

One of the most widely available field portable IRGA systems is the CIRAS-1 (PP Systems, Hitchin, Herts, UK). This functions by having two flows of air: the analysis flow, passing through the chamber and over the leaf, and the reference flow that does not. This results in four channels that are measured simultaneously: the absolute CO_2 concentration and humidity of the reference gas, and the difference between the CO_2 concentration and humidity of the analysis gas and that of the reference gas. The air is equilibrated to the temperature and pressure of the absorption cells so that no correction factors are needed. Reference air is regularly passed through all the cells to adjust for slight differences in the filters and reflection characteristics of the cells, enabling true

Appendix 1a: Equipment for measurement of water availability and water content						
Method	**Equipment**	**Approximate cost**	**Time**	**Advantages**	**Disadvantages**	**Best for**
Soil moisture probes	Hand held probe or permanent installation with logger	£300-600 per probe. £1500 for logger	Fortnightly measurements or installation plus fortnightly download	Not very technical or time consuming	Environmental only — must infer plant water loss	Plant survival rates, quick surveying of differences between many plots
Tensiometer	Permanent installation with logger	£150-200 per tensiometer, £1500 for logger	Installation plus fortnightly download	Cheaper than soil moisture probes	Doesn't work below —0.08 MPa. Refill and de-gassing required after dry periods. Susceptible to frost damage	Monitoring irrigation requirements
Automatic Weather Station	Logger plus meteorological equipment	£3000	Installation plus fortnightly download	Low maintenance, continuous recording	Environmental only — must infer plant water loss	General monitoring, long term experiments
Pressure chamber	Pressure chamber	£600-£2000	Few minutes per measurement	Quick and simple to use	Destructive sampling of leaves	Assessing plant water status
Dew-point psychrometry	Dew-point psychrometer	£400-£4000	Can take several hours for each measurement		Destructive sampling of leaves, time consuming, expensive, regular calibration, technical	Assessing plant water status

differentials to be determined. More detailed description of the principles, design and uses of IRGAs can be found in Long & Hällgren (1993) Long, Farage & Garcia (1996) and Parsons *et al.* (1997). The equations used by the CIRAS-1 to calculate assimilation rate, transpiration rate, leaf temperature, stomatal conductance and intercellular CO_2 concentration are described by Postl & Bolhàr-Nordenkampf (1993).

Drawbacks of the method are that accurate determination of boundary layer conductance (g_b) is difficult as it is affected by the anatomical characteristics of the leaf surface. Measurement of water loss from a piece of wet filter paper is normally used, as suggested by the CIRAS-1 manual, however, any error will be incorporated into the already sensitive leaf temperature calculations and affect the g_s and intercellular CO_2 concentration. Variations in microclimate and leaf characteristics make the scaling-up of measurements made on a leaf or small number of leaves very difficult, and care must be taken. The use of large ventilated chambers or 'branch bags' in the place of a leaf chamber reduces the problem of scaling from single leaf measurements by allowing larger parts of the canopy or small trees to be measured at once. Measurements can only be made on days when the leaf surfaces are completely dry, otherwise evaporation of leaf surface-water is recorded as transpiration water. IR gas analysis is the most expensive technique described here, both in equipment and time, however, it also provides far more detailed information than the other techniques.

Porometry

Porometry is a similar technique to IR gas analysis in that it involves leaf-scale measurements made in the field with specialist equipment. However, unlike gas analysis it measures stomatal conductance only, and so provides no extra information on photosynthesis. This has the advantage that the equipment and measurements are both simpler and quicker and the interpretation is less complex.

Appendix 1b: Equipment for measurement of water loss

Method	Equipment	Approximate cost	Time	Advantages	Disadvantages	Best for
Lysimetry	Sensitive balance	Low	Intensive measurements, e.g several times daily for duration of experiment	No technical equipment	Only suitable for pot grown greenhouse plants. May need to harvest plant	Short term experiments. e.g. immediate effects of chemicals on plant functioning
Sap Flow	Logger and sap flow gauges. Possibly include meteorological equipment	£300-600 per gauge. £1500 for logger plus any other meteorological equipment	Continuous measurement, fortnightly download and 6-10 weekly checking	Detailed continuous info relating to immediate meteorological conditions. Suitable for mature trees with no need to access canopy	Expensive, further calculation and data handling required. Leaf area measurement beneficial	Mature trees, scaling up to canopy water losses, relationships with meteorological variables
IR gas analysis	IRGA and leaf chamber	£8000 for a basic model	Many measurements to assess all leaf types under weather conditions through the season	Photosynthesis data as well as water loss	Need canopy access. Expensive, highly technical and time consuming. Need dry leaf surfaces	Detailed plant physiology studies. Information on water use efficiency and carbon assimilation

Diffusion porometry is a field-portable technique based on the measurement of the rate of water vapour loss from a leaf or part of a leaf enclosed in a chamber, while still attached to the plant. The rate of water loss may be determined by the rate of increase in humidity of the chamber due to transpiration (transit-time porometer) or the rate at which dry air must be added to offset the increase in humidity (null-balance porometer). Porometers measure stomatal resistance (s m^{-1}) which is then converted to stomatal conductance (m s^{-1}).

The relationship between g_S m s^{-1} (as measured by a porometer) and g_S $molm^{-2}$ s^{-1} (as measured by a gas analyser) is shown in the equation below. This allows comparisons to be made between measurements made on porometers and gas analysers.

$$g_s\ (ms^{-1}) = g_s\ (molm^{-2}\ s^{-1}) \times \frac{R(T+273)}{P \times 1000} \qquad \text{(Eqn 2)}$$

Where g_S is stomatal conductance (in either m s^{-1} or mol m^{-2} s^{-1}), R is the universal gas constant (8.314), T is temperature (°C) and P is pressure (kPa).

Transit-time porometers must be aspirated (with the chamber mixed by a fan) to measure stomatal resistance of needle leaves as this minimises the boundary layer resistance, increasing the sensitivity to measure stomatal resistance. As porometers are affected by temperature the difference between the leaf and sensor temperature must be kept to less than 1°C. If the leaf temperature is higher than the chamber temperature the resistance will be underestimated and *vice versa*. Calibration of transit-time porometers is complicated and time consuming. As with IR gas analysis measurements, leaf surfaces must be dry for porometers to work.

Null-balance porometers have the advantage that they maintain constant humidity during measurement, reducing any stomatal response during the measurement procedure. Calibration is a much simpler process and they are better suited to measurements on needle leaves and small branches. However, the equipment is more complex, expensive and requires more training before use.

Conclusion

The growth rate of a tree is closely related to its water status and an understanding of this can significantly increase the value of many studies in forest science. It is important to select a method of measurement that will give appropriate information for the nature of the study. Some methods are complicated and time-consuming and these should only be used where there is a clear need for that type of information. The final choice of method is dependent on a range of factors such as the type of measurements required, the type of plants being studied, the site and the training of the people involved. There are often compromises to be made although the best solution is often to use a combination of methods.

References

Baker, J.M. & van Bavel, C.H.M. (1987) Measurement of mass flow of water in the stems of herbaceous plants, *Plant, Cell and Environment*, 10: 777-782.

Cermák, J., Kucera, J. & Penka, M. (1976) Improvement of the method of sap flow rate determination in full-grown trees bases on heat balance and direct heating of xylem, *Biologia Plantarum*, 18: 105-110.

Daum, C.R. (1967) A method for determining water transport in trees, *Ecology*, 48, p425-431.

Davies, R.J. (1987) Trees and weeds. *Forestry Commission Handbook 2*. HMSO, London.

Field, C.B., Ball, J.T. & Berry, J.A. (1989) Photosynthesis: principles and field techniques. In: *Plant physiological ecology, field methods and instrumentation* (Eds. R.W. Pearcy, J. Ehleringer, H.A. Mooney & P.W. Rundell) Chapman and Hall, London.

Foggo, A. & Speight, M.R. (1993) Root damage and water stress: treatments affecting the exploitation of the buds of common ash *Fraxinus excelsior* L., by larvae of the ash bud moth *Prays fraxinella* Bjerk. (Lep., Yponomeutidae), Oecologia, 96: 134-138.

Long, S.P, Farage, P.K. & Garcia, R.L. (1996) Measurement of leaf and canopy photosynthetic CO_2 exchange in the field, *Journal of Experimental Botany*, 47: 1629-1642.

Long, S.P. & Hällgren, J-E, (1993) Measurement of CO_2 assimilation of plants in the field and the laboratory, In: *Photosynthesis and production in a changing environment: a field and lab manual*, (Ed, D.O.Hall, J.M.O. Scurlock, H.R. Bolhar-Nordenkampf, R.C. Leegood and S.P.Long) London, Chapman and Hall, 1993.

Parsons, R., Weyers, J.D.B, Lawson, T. & Godber, I.M. (1997) Rapid and straightforward estimates of photosynthetic characteristics using a portable gas exchange system, *Photosynthetica*, 34: 265-279.

Postl, W.F. & Bolhàr-Nordenkampf, H.R. (1993) "GASEX": a program to study the influence of data variations on calculated rates of photosynthesis and transpiration, In: *Photosynthesis and production in a changing environment: a field and laboratory manual* (eds, D.O.Hall, J.M.O. Scurlock, H.R. Bolhar-Nordenkampf, R.C. Leegood and S.P.Long) London, Chapman and Hall, 1993.

Pyatt, G., Ray, D., & Fletcher, J. (2001) An ecological Site Classification for Forestry in Great Britain, Forestry Commission Bulletin 124, Forestry Commission, Edinburgh, UK.

Smith, D.M., & Allen, S.J. (1996) Measurement of sap flow in plant stems, *Journal of Experimental Botany,* 47: 1183-1844.

Stokes, V. (2003) The impact of microenvironment, leaf development and phenology on annual carbon gain and water loss of two deciduous tree species, PhD Thesis, University of Essex.

von Caemmerer, S. & Farquhar, G.D. (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves, *Planta*, 153: 376-387.

List of suppliers

Delta-T Devices, 128 Low Road, Burwell, Cambridge, CB5 0EJ, UK. www.delta-t.co.uk

Li-Cor, Glen Spectra Ltd, 2 Dalston Gardens, Stanmore, Middlesex, HA7 1BQ, UK. www.licor.com

PP Systems, Unit 2, Glovers Courts, Bury Mead Road, Hitchin, Herts, SG5 1RT, UK. www.ppsystems.com

Dynagauge, Dynamax, Texas.