A laser-diode-based system for measuring sap flow by the heat-pulse method

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Abstract

Transpiration, the movement of water through plants from the soil to the atmosphere, is an important process in plant physiology, the hydrologic cycle, and the global energy balance. Transpiration at the scale of individual plants can be measured with weighing lysimetry, but this technique is limited to small plants and is generally impractical for trees and many field crops. Heat-pulse velocity methods offer an alternative, and several plant sap flow gauges have been marketed. Because these gauges use electrical resistance heaters to heat the stem, they present several problems: they are invasive, typically bulky, provide poor temperature control (killing the cambium), and have lengthy response times, so they cannot measure short-term transients.

In this report, we describe a system for measuring sap flow in real-time and without the need to puncture the stem. Instead of a resistance heater, it uses a laser beam as a heat source, and instead of contact thermometers, it uses non-contact infrared thermometers. Used with a precision-mounting unit that insures constant alignment, it determines whole-plant transpiration. The laser has the added advantage of delivering a precisely controlled amount of heat for a discrete time period. The end product is a more accurate, less invasive way to gauge water flow through herbaceous plant stems. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Accurate measurement of water use by plants is central to understanding the water and energy balance of orchards, agronomic crops, forest plantations, and natural stands in response to environmental change. Direct measurement of transpiration by individual plants or small stands is possible via weighing lysimetry, but this technique is limited to relatively small plants in containers and is impractical for mature trees and many field crops. Sap flow gauges are a practical alternative and are especially useful tools for scaling between the leaf and whole plant as well as between the individual and the entire community (Hatton and Wu, 1995; Wullschleger et al., 1998). Sap flow gauges have research applications in forestry (Marshall, 1958; Miller et al., 1980; Olbrich, 1991; Smith, 1992; Hatton et al., 1995; Wullschleger et al., 1998).
et al., 1998), hydrology (Hatton and Wu, 1995; Dye et al., 1996; Vertessy et al., 1997; Wullschleger et al., 1998), plant physiology (Hinckley and Ritchie, 1970; Wullschleger et al., 1998; Naderzahina, 1999), and agriculture (Stone and Shirazi, 1975; Cohen et al., 1990; Lott et al., 1996; Wullschleger et al., 1998).

Practically, sap flow gauges can be used to detect the onset of water stress to trigger irrigation (Cohen et al., 1990), to evaluate recovery from root pruning and transplant shock (Lott et al., 1996), and to evaluate tree hydraulic architecture to aid in pruning decisions (Moreshet et al., 1990).

Four thermal methods for sap flow measurement are well-documented and originated as follows: (1) heat-pulse (Huber, 1932), (2) tissue-heat balance (Cermák et al., 1973, 1976), (3) stem-heat balance (Sakuratani, 1981), and (4) heat-dissipation (Granier, 1985). At the current time, all of the methods are commercially available with their own unique set of advantages and disadvantages. The fifth method described herein, the laser heat-pulse gauge (LHPG), describes a system for measuring sap flow in real-time and without the need to puncture the stem. A brief chronological synopsis of each method follows; however, a complete description of heat-pulse, tissue-dissipation, heat-dissipation, and stem heat balance techniques are beyond the scope of this paper and the reader should consult reviews by Swanson (1994), Smith and Allen (1996), and Wullschleger et al. (1998) for in depth descriptions of instrumentation, theory of operation, and practical considerations.

1.1. Heat-pulse method

The heat-pulse methodology is based on measuring the rate of sap flow by applying a heat-pulse and measuring a rise in temperature downstream of the heat application. In order to compensate for conduction, an additional temperature sensor is placed upstream of heat application (Huber and Schmidt, 1937). Heat has been used to trace sap flow in plant stems since 1932 (Huber, 1932; Huber and Schmidt, 1936, 1937; Dixon, 1937). Current methods use resistance cables to supply a heat-pulse and either thermocouple or thermistor probes to detect the heat downstream from the point of application (Smith and Allen, 1996). For the past decade, several heat-pulse flow gauges of this design have been marketed commercially (Smith and Allen, 1996). Their acceptance and utility beyond the research community has been virtually nil due to three technical problems. First, holes must be drilled into the stem to accommodate the heater and temperature probes (Smith and Allen, 1996). This disrupts the normal flow of water through the xylem. Although this can be compensated for arithmetically, plants react to wounding in different ways over unknown periods of time, so the stem sections containing the probes cannot be assumed to represent water flow in undisturbed stems. Wound width measurements are required to correct for flow interruption (Swanson and Whitfield, 1981; Green and Clothier, 1988).

Second, accurate positioning of the heater and sensor probes is critical (Olbrich, 1991; Smith and Allen, 1996). In conventional sap flow gauges, the plane of the heater and thermocouple sensors can easily be misaligned during insertion into the plant, resulting in errors that are impossible to detect.

Third, the current models can raise the temperature of the cambium sufficiently to kill the living tissue. Accuracy from a single probe placement may vary over time, and laboratory measurements have found that heater surface temperatures reached 44 ± 2°C (Miller et al., 1980).

1.2. Tissue-heat balance

The tissue-heat balance methodology is based on the application of heat to a discreet portion of stem tissue. Heat dissipated in the vertical, radial, and lateral direction is accounted for and the mass flow of sap is determined from the heat loss by convection in the moving sap stream (Cermák et al., 1973). Briefly, five stainless steel electrode plates are inserted into the wood in order to create four segments of sapwood. The segments are then bounded by electrodes and eight thermocouples are inserted into the sapwood: two located midway across the central segments, two placed 60 mm laterally from the outer electrode, and four 100 mm below and outside of the heated zone (Cermák and Kucera, 1981; Cermák et al., 1984). Although, the measurement system can be purchased commercially, the hardware installation requires careful alignment. Like the heat-pulse method, substantial variation in sap flow around tree trunks would require...
installations of several gauges on the circumference of the trunk (Cermák et al., 1995).

In addition to measuring periods of sap flow, it is critical that data are collected under zero sap flow in order to estimate the thermal conductivity of the tissue and insulation materials surrounding the heated area. It is assumed zero sap flow occurs prior to dawn or after a rain event; however, this is only an assumption and large trees may require substantial time to equilibrate. The estimates of thermal conductivity, therefore, may be difficult to obtain in certain environments and more importantly, could be subject to error if zero flow does not occur.

1.3. Stem-heat balance

Similar to tissue-heat balance, a uniform amount of heat is applied to an estimated stem area, and zero flow must be assumed at some period during measurement. Unlike the tissue-balance method, measurement of sap flow is possible in both woody (Steinberg et al., 1989) and herbaceous (Baker and van Bavel, 1987) stems. Heat application occurs around the entire circumference of the stem with a flexible heater and the mass flow of sap is calculated from the balance of fluxes into and out of the heated stem section (Sakuratani, 1981; Baker and van Bavel, 1987). The commercially available gauges are limited with respect to the variation in individual stem diameter they can encompass. Furthermore, continuous heating of the entire stem can demand large amounts of power and may not be suitable for remote locations.

1.4. Heat-dissipation

Heat-dissipation uses cylindrical probes that are inserted into the stem, similar to the heat-pulse equipment used primarily prior to this report. The technique uses two probes vertically placed 100 mm apart. The upper probe provides continuous heat and a measurement of temperature. The heated probe is then referenced to another temperature sensor in the lower probe and the rate of sap flow is calculated in relation to the difference in temperature between the two probes. The calculations for sap flow are relatively simple with the heat-dissipation technique, but Smith and Allen (1996) suggest calibration take place on species that have no prior heat-dissipation validation.

1.5. LHPG

Our method overcomes all three problems associated with heat-pulse techniques. Instead of using heated wire, we used a laser diode capable of applying a controlled amount of energy that is spatially discrete, instantaneous, and eliminates heating the stem to lethal temperatures. And instead of a conventional thermistor, we use an infrared thermometer that senses temperature non-invasively. This design either eliminates or controls for artifacts resulting from damaged xylem. Alignment is assured with a small "Pelon" housing that holds both the heater and sensor, aids installation, and requires substantially less set-up time than other commercial units. The unit may be operated either singly or in multiples to allow comparison of experimental treatments or flow through different portions of the canopy.

In this report, we test the LHPG on stems of Phaeolus vulgaris L. (kidney bean). Validation and calibration was achieved by comparing transpiration measurement from the LHPG to an independent measurement based on weight loss (WL) (Green and Clothier, 1988). Specifically, we examine whether the LHPG accurately measures water loss in the following situations: (1) among a group of plants over a 5-min period, (2) among a group of plants exposed to low versus high light conditions over a 5-min period, and (3) among individual plants. In addition, we report on the precision of the LHPG in relation to calibration accuracy.

2. Materials and methods

2.1. Plant material

Kidney bean (P. vulgaris L.) was sown in flats containing a 1:2:1 mixture of sand, peat moss, and silt loam soil (1:2:1, v/v/v) on 1 July 2000, and germinated on a propagation bench. Seedlings were transplanted into 1.5-l plastic pots containing a mixture of sand, peat moss, and silt loam soil (1:2:1, v/v/v), placed in an evaporation-cooled greenhouse, and fertilized twice a week with 5 g/l 5-11-26 NPK (Hydrol Sol, Scotts Co., Marysville, OH). Seedlings were grown for 29 days at 25 °C in day time and
18 °C at night temperatures. On day 29, all pots were watered to saturation and permitted to drain for 18 h.

The next morning, day 30, seven plants were randomly selected, the pots were covered in plastic to prevent evaporation from the potting medium, and the plants were moved to a walk-in growth chamber (Environmental Growth Chamber Co., Chagrin Falls, OH). The procedure was repeated on days 31 and 32 until 20 total plants were measured. The growth chamber temperature was maintained at 25 °C and relative humidity conditions ranging 60–70% during the experiment.

2.2. Velocity calculation

Heat-pulse velocity is calculated by:

\[ V_h = \frac{X_d - X_u}{2t_e} \]  

(1)

where \( V_h \) is the velocity (mm s\(^{-1}\)), \( X_d \) is the distance between the heater and the downstream thermometer (mm), \( X_u \) is the distance between the heater and the upstream thermometer (mm), and \( t_e \) is the time elapsed until both sensors equilibrate to the first recurrence of the initial temperature difference (s\(^{-1}\)) (Swanson and Whitfield, 1981; Cohen et al., 1988; for a historical review, see Swanson, 1994).

2.3. Instrumentation

The instrumentation consists of two infrared thermocouples (OS36, Omega Co., Stamford, CT) and a fiber coupled diode laser heat source (OPC-DO15-965-FCTS, Spectra-Physics, Mountain View, CA) connected to a Campbell CR10X data logger powered by a 12-V lead-acid battery and accessed by the user from a laptop computer.

A 15-W fiber array laser diode bar was used to generate variable power in the form of coherent light. The coherent light was sent to an optical fiber jumper of 600 \( \mu \)m core diameter. The fiber jumper permitted precise application of a specific wavelength of high-power laser light to a small, discrete area of the stem. A preset control feature of the diode driver permitted precise setting of the power level before the output to the laser diode was turned on. Power resolution and variation capabilities were accurate and quantifiable to 0.01 W.

After the laser energy was applied to the stem, changes in sensible heat were detected above and below the point of application by infrared thermocouple thermometers. The thermal electromotive force (emf) produced by the bimetallic junction is proportional to temperature. The emf was read by the data logger and \( t_e \) stored on the laptop computer for later analysis.

The laser jumper cable and the two thermometers were mounted in a stem housing consisting of a 10-cm length of Teflon\textsuperscript{®} pipe (0.5 cm wall thickness), sliced lengthwise and rejoined with a piano hinge and two spring loaded clamps to form a clamshell assembly (Fig. 1). The stem housing was supported on the stem by two sets of three nylon screws located near the ends of the pipe. The screws were positioned at equal distances around the perimeter of the pipe and were equipped with soft rubber grommets to protect the stem from damage.

The fiber optic jumper cable was connected and threaded into the stem housing with an SMA 905 bulkhead connector (Metrotek Industries Inc., St. Petersburg, FL). Two holes drilled into the housing held the infrared thermometers 20 mm above and 10 mm below the laser heat source. The thermometers were optically isolated by a neoprene gasket, wired differentially, and connected to the data logger. The data logger was programmed to sample the outputs of the infrared thermocouples and to control the laser heat-pulse. The thermocouples were measured before the heat-pulse was applied, when the temperature difference was zero. The laser energy was applied for 1 s at a power of 15 W, and the temperature differential decayed to zero, the elapsed time for this to occur, \( t_e \), was recorded. Heat-pulse velocity was then calculated using Eq. (1).

Sap flow was measured on 20 plants during normal photoperiods, simultaneously, using the LHPG and WL techniques. A heat-pulse was discharged every 5 min and sap flow was computed using the following relationship:

\[ Q = V_h a \]  

(2)

where \( Q \) is the sap flow (mm\(^3\) s\(^{-1}\)), \( V_h \) the heat-pulse velocity, as previously defined and \( a \) is the cross...
sectional area of the stem at the point where heat was applied (mm$^2$). Sap velocity units and nomenclature follow the convention of Edwards et al. (1996).

Total transpiration by each plant was monitored independently using a top loading balance with 0.1 g resolution. Sample weights were made at 1-min intervals and then averaged over the 5-min interval between pulses. During measurements, each bean plant was placed in a three-sided Mylar® reflective chamber to avoid the effect of air movement on the stability of the balance. A total of 12 paired measurements were taken per plant. Photosynthetic photon flux density (PPFD) was varied to induce variation in transpiration rates. The initial six measurements were at a PPFD = 375 μmol m$^{-2}$ s$^{-1}$ ± 15 and the final six were taken at 75 μmol m$^{-2}$ s$^{-1}$ ± 10.

After measurements, a random sample of plants ($n = 5$) were cut off 5 mm above the pot soil surface. The cut end was immersed in a xylem-mobile stain (1% safranin in 95% ethylalcohol) to determine the cross sectional area of the stem actively conducting sap. The stem was cut into three 10-mm segments and the stained cross sectional area was estimated. The remaining plants were visually monitored for the next 3 weeks for cambium damage to the stem surface.
3. Results

Eq. (1) was used to estimate flow rates for all measurements. Eq. (2) was then used to compute actual sap flow (transpiration). Fig. 2 depicts the mean of 20 different kidney bean plants at both the 375 and 75 μmol m⁻² s⁻¹ light levels. Heat velocity in a bean stem multiplied by its stem cross-sectional area (Vₚₛ) was compared with transpirational WL (Fig. 2). Calculated sap flow and the WL determined from the balance were linear and highly correlated (WL = −0.439 + 1.664 LHPG, \( R^2 = 0.99 \)), both WL and LHPG in units of cm³ h⁻¹ (Fig. 2). The standard deviation of the residuals was 0.0264 and the 95% confidence interval for the slope was between 1.568 and 1.759.

Transpiration rates were nearly constant for the level of light application in both methods (Fig. 3). At low light levels, transpiration was underestimated by 4% with the laser heat-pulse technique; at high light levels, transpiration was underestimated by 20%. Although transpiration measured by the LHPG was underestimated at each light level, a linear calibration compensated for the underestimation within the imposed light levels (Fig. 2). The calibration allows us to adjust the laser heat-pulse estimate to match whole plant transpiration determined gravimetrically. To further understand the calibration curve, Fig. 4 represents the relationship of the 20 kidney bean plants measured with the two independent techniques at both low and high light levels (\( R^2 = 0.80 \)). The position of the points relative to the 1:1 relationship suggests that calibration is required over a range of light intensities.

The average imprecision of both the LHPG and WL was estimated using one-way analysis of variance. Imprecision here refers to the variability among plants at any of the 5-min intervals, corresponding to the analysis presented in Fig. 2. Expressed as standard deviations, the imprecision of LHPG was 0.173 cm³ h⁻¹ and of WL was 0.222 cm³ h⁻¹. Although this imprecision is small for both techniques, the imprecision of the LHPG, which is the predictor variable for the
analysis presented in Fig. 2, results in a slight reduction of the estimated slope—the estimated slope is 96% of the true slope. A simple adjustment yields an estimate of 1.738 rather than 1.664 (Fuller, 1987). Multiplication of 1.738 by 0.96 yields 1.664, meaning the estimated slope is 96% of the true slope.

Analysis of the imprecision is important to determine how many plants are needed to obtain an accurate calibration curve. In this study, 20 plants were used; whereas, in practical applications, it may often be preferable to use fewer. Had only 10 plants been used, the estimated slope would have been 91% of the true slope. The effect on the predicted transpiration values with 10 plants would have been very small, with the extremes of the range of observed transpiration rates amounting to an over “or under” estimation of 0.03 cm$^3$ h$^{-1}$ at most. Therefore, although the calibration slope could easily be adjusted for the attenuation, the practical effect of attenuation is negligible if at least 10 plants are measured.

The staining of xylem elements indicated that the entire cross-section of xylem is active in the kidney beans measured. Visual inspections of the surface stem area where heat was applied revealed no cambium death or damage up through three weeks after the heat-pulses were applied.

4. Discussion

This study demonstrates that the LHPG system can be used to measure transpiration in a herbaceous annual plant over short time intervals. This demonstration shows that the instrumentation and methodology used in sap velocity estimates can be improved considerably. Such measurements are useful in the investigations of the plant response to environmental stress or climate change. Our study clearly indicates that more work needs to be done in this area, and we envision that a range of plants could be periodically sampled by this technique.

Sap flow measurements are ideally suited to applications where plant water use is routinely required (Smith and Allen, 1996). For example, conventional irrigation practice is based on a critical threshold of available soil water (Cohen et al., 1990). The LHPG described here could measure transpiration rate and estimate water requirements continuously and over short intervals. The heat-pulse technique has been used successfully in field cotton studies (Fuchs et al., 1987; Cohen et al., 1995), controlled environments of soybean and corn plants (Cohen et al., 1990), and field-grown sunflower and corn (Cohen and Li, 1996). The use of deep cell batteries and weather resistant shielding could easily adapt the LHPG system for field use.

In a monoculture production system, it is often necessary to extrapolate water use by sampled plants to an entire greenhouse range or field section. Mass flow rates for individual plants must be used to estimate water loss for a known area to address hydrological problems (Hatton and Wu, 1995). Scaling-up from the area of occupied ground (Hatton and Vertessy, 1990) and calculations from plant density (Ham et al., 1990; Dugas and Mayeux, 1991) have succeeded at estimating transpiration for entire stands. The LHPG system could function as heat-pulse gauges before it and eliminate some of the problems associated with previous heat-pulse systems.

Underestimation of sap flow is characteristic of all heat-pulse methods, and compensation requires calibration under a range of relevant environmental conditions. Additionally, variations in stem anatomy among herbaceous species require a calibration coefficient for each species (Cohen, 1994). In herbaceous plants with a high concentration of sap-conducting elements near the surface, the infrared temperature detection would immediately sense a wave of heat carried by the sap. The calibration coefficient, therefore, would be different than it would be for a plant with conducting bundles distributed throughout the stem (Cohen and Li, 1996). An empirical calibration can be used to estimate transpiration, and the calibration factor is a function of stem properties that vary from species to species (Cohen and Fuchs, 1989).

The relationship between actual water loss and that measured with the LHPG was linear. Linear relationships have been found in woody (Smith, 1995) and herbaceous species (Cohen and Li, 1996). Moreover, Cohen and Li (1996) found WL to be linear and highly correlated with the product of stem cross-sectional area and apparent heat-pulse velocity in the stems of two herbaceous species, irrespective of various sensor depths.

Sensor depth can influence calibration coefficients within a species (Cohen and Li, 1996). The locality of the thermocouple junction to densely packed
conducting elements resulted in different calibration coefficients within a species (Cohen and Li, 1996). The LHPG design holds depth at the surface of the stem and does not permit measurement at various sensor depths within the stem. The nature of the design, therefore, may reduce variation in calibration coefficients within a species.

Swanson and Whitfield (1981) derived numerical solutions to alleviate the discrepancies between heat-pulse velocity and sap flow. The sap flow interruption was a result of invasive probes; as a result, heat-pulse velocity transpiration correction factors have been established for a number of tree species (Swanson, 1983; Green and Clothier, 1988; Oblrich, 1991). Even surface-mounted sensors, which place pressure on the underlying xylem, cause some callus or wound reaction within the xylem immediately under the sensor (Huber and Schmidt, 1936; Puritch and Mullick, 1975). An examination of thermometric methods of measuring xylem sap flow reveals that no single theory or instrumentation is applicable to all sizes or species of trees (Swanson, 1994). Although our instrumentation was not tested on a woody species, it does not create thermal heterogeneities by implanting sensors; indeed, the heat source and sensors do not contact the stem at all, which eliminates the necessity for numerical solutions that correct for wound tissue.

Various sensor spacing configurations have been tested, e.g. −0.3, 0, 0.8 mm (Gifford, 1968); −0.5, 0, 0.6 mm (Swanson, 1962); −0.5, 0, 0.75 mm (Swanson, 1967); −0.7, 0, 1.0 mm (Morikawa, 1972); −0.5, 0, 1.5 mm and −1.0, 0, 1.5 mm (Swanson, 1994). We used a spacing that has not appeared in any publication (−1.0, 0, 2.0 mm). Swanson (1994) states that the smaller the difference in temperature sensor spacing, the greater the departure of calculated from imposed heat-pulse velocity. The sensor configuration in this study was not intended to deviate from established spacing, but rather was a compromise designed to address miniaturization restraints of available infrared thermocouples, sensitivity limitations of the data logger, and application of non-injurious amounts of thermal energy.

The LHPG was tested in a controlled atmosphere chamber. Although, the primary intention of the chamber was to minimize the effect of wind on balance measurements, convective heat loss to the atmosphere could be negligible under such conditions. Upon modification of the LHPG for outdoor environments, foam insulation and a weather shield surrounding the stem may be critical additions that minimize heat loss to the atmosphere, and at the same time, reduce solar heating of the stem.

Various materials and configurations were tested in developing the mounting unit used in this study which were intended to maintain the alignment of the heat source and temperature sensors. Materials such as Lexan® and polyvinylchloride did not present obvious measurement differences from Teflon® under our application; therefore, fabrication of a mounting unit constructed out of material other than Teflon® may be practical. Furthermore, the stem encompassing mounting unit described herein does not represent a practical solution for stems of large circumference. The results presented in this paper focus primarily on an alternative technique, whereas, future studies could focus on mounting unit specifics and possibly validate different materials and configurations that large stem dimensions necessitate.

Transpiration was compared with two independent techniques: the alternative LHPG instrumentation and WL. Our experimental comparisons indicate that this method can yield accurate sap flow estimates in kidney bean, a herbaceous plant. The majority of published material has focused on the problems and errors associated with heat-pulse instrumentation. However, the significance of whole-plant transpiration measurement that can be scaled to stand flux is critical in hydrology. Therefore, transpiration measurement, an important hydrologic parameter, can be estimated with the alternative technique described here, provided the heat-pulse velocity values are from comparable water-conducting xylem. More research is necessary to scrutinize the method “and at the current time” the laser is an expensive unit. Conversely, the popularity and demand for laser devices has drastically reduced costs of diode lasers. Lastly, the potential for miniaturization of the system warrants further investigation.

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