Trees (2012) 26:1669–1676
DOI 10.1007/s00468-012-0731-6

SHORT COMMUNICATION

Measuring whole-plant transpiration gravimetrically: a scalable automated system built from components

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Received: 3 January 2012 / Revised: 12 April 2012 / Accepted: 21 April 2012 / Published online: 11 May 2012

Abstract Measuring whole-plant transpiration is highly relevant considering the increasing interest in understanding and improving plant water use at the whole-plant level. We present an original software package (Amalthea) and a design to create a system for measuring transpiration using laboratory balances based on the readily available commodity hardware. The system is modular, capable of multiple-balance synchronisation and is highly scalable from one to one hundred units. The software runs under GNU/Linux and it requires little computer resources. Reporting of transpiration rates is based on the linear regressions of data from pre-set intervals which yields high resolution and provides some level of noise filtering. Using a scale with 0.01-g precision we have measured mean rates as low as 0.01 mmol s^{-1} over intervals of 220 s. Its flexibility accommodates varied applications, such as monitoring nighttime transpiration or long-term drought treatments and would be suitable for measuring a range of plants, from small herbs to potted trees.

Keywords Transpiration · Gravimetric · Software

Introduction

Transpiration (E) is one of the most fundamental processes in vascular plants and its inextricable nature governs many aspects of their physiology. Transpiration is linked to carbon gain as both water loss and CO_2 uptake (and consequently assimilation, A) occur through stomata and the water-use efficiency (WUE) is often expressed as the ratio E/A (Stanhill 1986). High rates of E may foster higher A, but may also come at a peril for the plant and some authors regard E as a negative side-effect (Kramer and Boyer 1995; Raven et al. 2005; Niklas 1997). Other authors have attributed specific functions to E, from nutrient delivery to leaf cooling (Cramer et al. 2009; Mahan and Upchurch 1988; Dawson et al. 2007).

Plant transpiration is of interest not only in the context of whole-plant physiology, but also in a more applied sense since it is tied to production-sensitive factors like drought tolerance and WUE. In addition, the discussion of plant water-use patterns has become of central importance since water availability and distribution are predicted to change around the globe (Naik et al. 2003; Rees and Ali 2007; Ridgwell et al. 2009; Oliveira et al. 2011). Several recent publications highlight the current interest in understanding and improving crop WUE (e.g. Yoo et al. 2009) and concerted efforts are underway to uncover its genetic and mechanistic basis (Karaba et al. 2007; Masle et al. 2005; Davies et al. 2002). It is therefore still relevant to develop and improve upon tools to measure whole-plant E accurately and reliably as it will be essential to integrate sub-organismal mechanisms into a whole-organism level.

Many techniques are available to measure or estimate E, from instantaneous leaf level to landscape level, each having its own scope and limitations. Arguably, the most popular techniques to measure E are based on the porometry or sap-flow meters (Wullschleger et al. 1998; Pearcy et al. 1988). Sap-flow-based metrics can be used on very large trees in the field and in many cases they constitute the only option for whole-tree measurements, but they make several assumptions, such as constant heat dissipation

Communicated by A. C. Franco.

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across the sapwood and uniform cross-sectional flow, although proper calibration can significantly enhance accuracy (Sun et al. 2011). Furthermore, the flow of sap can differ from transpiration due to stem capacitance, particularly at night. These techniques are also unsuitable for most herbaceous plants. Porometric techniques are convenient for instantaneous readings of stomatal conductance (g) in a wide variety of plants. These leaf-level measurements may not be representative of whole-plant responses and water use partly because the porometer strips the boundary layer from the leaf surface with rapid air movement which is more appropriate for measuring water diffusion through stomata and calculating g. When measuring water use at the whole-plant level, the leaf boundary layer (consider e.g. tomentose versus glabrous leaves) may well significantly affect WUE. Even when the boundary layer is properly taken into account by porometric methods, the architecture of the entire plant is inexorably overlooked by leaf-level measurements. Although porometry is an invaluable tool, estimations of E from leaf-level g seldom agree with in situ whole-plant transpiration measurements (McDermitt 1990).

Gravimetric instruments, such as weighing lysimeters can provide reliable, accurate measurements and a resolution-to-scale ratio at the whole-plant level if properly constructed (Yoo et al. 2009; Edwards 1986). The nature of lysimeters makes replication cumbersome, due not only to the cost and the work involved in their installation, but also because they are field-only stationary instruments by design. Laboratory balances are well suited to function as small-scale weighing lysimeters, are relatively inexpensive, provide remarkable precision and accuracy, and have been used in a number of studies to measure E, sometimes with a fine degree of time resolution (Edwards 1986). Unlike the case of lysimeters, a system with balances can easily incorporate replicates if carefully designed. Despite this, to date no specific software and technical reports are available to help researchers build such a system.

Measuring E by means of weighing can be considered a sort of "gold-standard" for whole-plant E since a balance is used to account for the loss in (water) mass. Three simple assumptions can be identified: (i)—the water is all lost through the plant (zero soil evaporation), (ii)—the plant gains no mass, and (iii)—the plant loses no mass other than that of water. The first of these assumptions can be controlled, to some extent, by minimising soil exposure and by measuring it (e.g. a pot with no plant). Plant gain or loss in dry weight can be ignored since their contribution to balance mass can only be detected after long periods of time (much longer than those needed to measure E).

It is clear that measuring E with a high degree of resolution on several plants simultaneously using balances would be a very impractical task unless automated. Most electronic (digital) balances can be easily interfaced to a computer. Likewise, the task of converting regular balances to weighing lysimeters is conceptually simple. However, the details of wiring multiple balances and coordinating and logging their readings are not trivial. First, installing a multiple-balance system controlled by a computer running a Windows operating system will need customised hardware. Secondly, an off-the-shelf approach using the universal serial bus (USB) as the basis of serial port expansion is problematic since port numbering is not standardised, making the production of the software a technical challenge and highly inefficient. In contrast, this is more feasible under a Unix-like system. Thirdly, there is no available software to coordinate this system and compute E from mass loss.

We have taken advantage of the current availability of "user-friendly" Linux distributions to build a flexible and reliable multiple-balance system capable of over 100 concurrent balances. Here, we present an original Free and Open-Source Software (FOSS) called *Amalthea*[1] (Cirelli 2010) and a highly scalable system designed to measure whole-plant transpiration using laboratory balances. The strengths of the system are that it allows multiple balances to be used simultaneously, can be run for long periods with dependable stability and obtains high-resolution measurements with good noise filtering, suitable to many types of studies and many scales of plants from small herbs to large potted trees. Moreover, the system can be built entirely with readily available components.

Methods

System description, materials and installation

The system was designed to be modular so balances could be added or removed and the software quickly reconfigured. We also focused on using easily available and economical parts. Essentially, the system consists of a computer that runs the software, a laboratory scale (balance) and a connection between them. This would comprise a "minimal unit" to which more balances can be added. Below, we provide the general specifications to be met by the hardware and a thorough description of the software.

Hardware requirements

The most minimal installation requires a computer with an available serial port, a balance with serial bi-directional communications capability and a serial communications

[1] Named after one of Jupiter's moons, a small satellite subjected to large gravitational forces.

cable. Multiple balances require an equal number of serial ports. Universal Serial Bus (USB) to RS-232 (RS232) converter cables are used for this purpose and can be connected to a USB hub. The USB approach allows for easy expansion of the system and this was how our final test system was installed. The configuration we used for testing consisted of one computer to which nine balances were connected through two USB hubs. Figure 1 shows the connection diagram of a complete system.

Since nine serial ports were required to connect the nine balances, we used two 7-port self-powered "industrial" USB hubs (StarTech ST7200USBM, StarTech.com Ltd., Canada) connected independently (one hub per built-in system port), each with its own DC power supply. The hubs were attached to the computer with 28/24AWG gold-plated type A/B USB cables (Monoprice Inc., USA). Although we chose a star topology, a daisy-chain arrangement of the hubs could have been used and it would be our recommended wiring scheme. The nine serial ports for balance connection were provided by nine USB-to-RS232 converter cables (StarTech ICUSB232, StarTech.com Ltd., Canada).

We used nine top-loading digital balances with a 0.01 g resolution and a maximum capacity of 4,500 g (Adam Equipment model PGW 4502e, Adam Equipment, South Africa). The balances were placed on levelled metal greenhouse benches with ad hoc medium-density fibreboard platforms for stability (1.25 cm thickness). Each balance was numbered and connected to the respective USB-serial port with a null-modem (cross-linked) serial cable (StarTech SCNM9FF, StarTech.com Ltd., Canada). We performed weekly calibrations of the balances to maintain linearity and offset any drift.

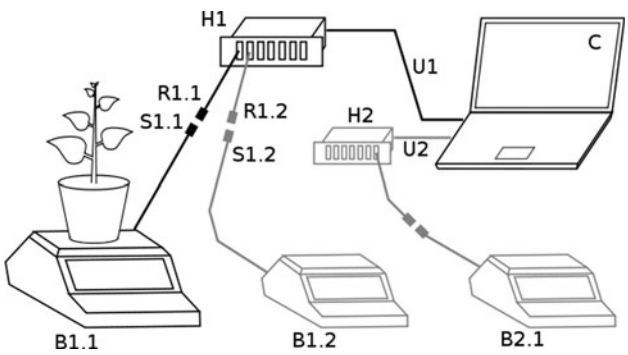

Fig. 1 (C) Computer running Amalthea; (U[*i*]) standard USB cable; (H[*i*]) USB hub; (R[*i.j*]) USB-to-RS232 cable; (S[*i.j*]) serial communications cable (bi-directional); (B[*i.j*]) weighing balance. The chain formed by (U1)-(H1)-(R1.1)-(S1.1)-(B1.1) is a complete system unit. Repeats of (R)-(S)-(B) can be added to (H1) to create more units until all ports are exhausted; addition of a [(U)-(H)] unit to the computer allows further expansion (depicted here by (U2)-(H2)). A single-unit system is possible by connecting (R1.1)-(S1.1)-(B1.1) directly to (C)

All electronic equipment was connected to an uninterrupted power supply (UPS) (APC Back-UPS CS 350, Schneider Electric SA, France).

Software overview

Amalthea was programmed mainly in the Python language (http://www.python.org) with a programme wrapper (amalthea-wrapper) written as a bash shell script (http://www.gnu.org/software/bash/bash.html) which makes use of standard GNU/Linux utilities. For simplicity, Amalthea runs in traditional linear fashion and relies on the 'cron' dæmon (a time-based scheduling programme) for periodic execution. Upon installation, the configuration script adds an entry to the crontab, a file read periodically by cron to execute scheduled tasks.

Once called by cron, Amalthea will run for the configured period taking mass readings at regular intervals (default is every 10 s for 4 min). Every reading is sent to the *standard error*[2] *stream* (*stderr*) and at the end of the run, Amalthea compiles all the readings of the period and performs a linear regression thus obtaining a rate of mass loss. Although this rate integrates the mass loss over the entire period, the time values (seconds) comprising the abscissa are averaged and the result is taken as the point value of time for the calculated rate, which allows said rate to be presented as an approximation of the tangent for that time point. The rate obtained is in units of g s^{-1} and it is further converted to mmol s^{-1}. This information is then sent to the *standard output stream* (*stdout*) as a single line of comma-separated values (CSV).

The wrapper script takes both *stderr* and *stdout* and respectively appends the raw values to a CSV file with extension ".raw" and the processed values to a CSV file with extension ".csv". Both files have the same base name which corresponds to the date in the format YYYY-MM-DD (e.g., data collected on July 5, 2011 would be written to the files '2011-07-05.raw' and '2011-07-05.csv').

Environmental monitoring

Measurements of temperature, relative humidity, and light intensity were collected using a custom-built interface to connect the required sensors to the serial port of the same computer running Amalthea. The hardware design and software to read the sensors are publicly available as Creative Commons and open source projects respectively (Cirelli 2011). The designed interface and software allowed the use of the same logging facility as the balances, thus both measurements coincided in time (see "Results and discussion").

[2] Not to be confused with the statistical term. For clarification see Rosen et al. (2006).

Plant material and growing conditions

We tested the system with aspen (*Populus tremuloides*) and hybrid poplar (*Populus* sp.) seedlings grown at the University of Alberta. All plants were cultivated in 2.4-l pots containing Sunshine® Mix #4/LA4 potting mix (Sun Gro Horticulture Ltd., Vancouver, Canada) and fed with Osmocote® slow-release fertiliser (ScottsMiracle-Gro, Marysville, USA). Air mixing in the greenhouse was achieved by the use of a low-speed ceiling fan. Supplemental light was provided with Gro-Light fluorescent tubes with a photoperiod of 18 h, which is representative of midsummer conditions at this latitude.

During measurement, the tops of the pots were covered with aluminium foil to minimise evaporation. Drain holes were not covered so as to allow air exchange in the pots. To avoid water percolating through the pots during the experiment, each pot was watered to excess and allowed to fully drain before placing them on the balances. Subsequent additions of water during the experiment were done with the pots on the balances while administering the water slowly with a large nozzle[3] wash bottle until the mass was about 100 g less than the initial weigh-in. This was done to avoid percolation.

Quantification of evaporation from pots

The Amalthea system was used to quantify the contribution of water evaporating directly from the pots. Six pots containing live root systems of decapitated plants were watered and allowed to drain completely. There were four 2.5 × 2 cm drain holes on each pot placed on the sides, where the side meets the bottom. The top of each pot was covered with a square of aluminium foil wrapped around the rim and containing a slit from the edge to the middle as would be the case when accommodating the stem of an intact plant. Drain holes were not covered. The pots were placed on the balances and their mass loss monitored over a period of 4 days. No exudate from root pressure was present after cutting.

Sensitivity analysis

To assess the adequacy of both the balance resolution and the chosen length of one run, a sensitivity analysis was conducted. A slope of 0.01 g period^{-1} was chosen as the starting point, which, with the default period of 220 s, it represents a rate of 4.54×10^{-5} g s^{-1}. From this slope, a set of data were constructed containing 23 points, one every 10 s for 220 s starting at 0 s. Each point i was then subjected to the operation $floor\ (10^n \times i) \cdot 10^{-n}$ where n is the number of decimals available and the function floor takes only the integer part of the number discarding the decimals. This was done to simulate a conservative scenario in which for a balance to display an increment of one unit of resolution (0.01 in the case of two-decimal resolution), the full mass of this unit has to be added before the balance registers the new mass. For example, under such condition, adding 0.007 g to a balance reading "0.01" will not cause a reading of "0.02"; only adding a full 0.01 g will change the display to "0.02".

A linear regression was fitted to the new "rounded down" data set, and the resulting slope (*rrwl*) as compared to the known true slope (*trwl*). The percentage of variation between the slopes was calculated as $(100 \cdot rrwl \cdot trwl^{-1}) - 100$ and this process was repeated for slopes of 0.03–0.93 g period^{-1} in 0.09-g increments. A series of slope variations was obtained for $n = 1$ and $n = 2$. In addition, the entire process was also repeated with a period of one half of the original period and another of double length, but maintaining the same reference rates in the series as grams per second, thus varying the rate in grams per period to evaluate the effect of period length.

Results and discussion

Figure 2 shows five consecutive intervals of a transpiring plant during the transition from lights-on to lights-off. Each group of points displays the linear regression fitted to that particular group, the slope of which is the rate of water loss in g s^{-1} unit^{-1}. The differences among the slopes indicate that lengthening the runs would result in a loss of temporal resolution. Some runs may display a degree of non-linearity and in these cases a quadratic function is a better fit than a linear regression. The reported abscissa corresponding to each slope is the average of the time period, thus the linear regression slope can be taken as a very close approximation to the first derivative of a quadratic function for the same midpoint in time.

Monitoring potted plants for long periods denies the option of sealing the pots as it would create an anoxic environment in the roots. For this reason, we deemed it necessary to quantify the water loss from pots with leaky top covers (foil) and open drain holes. Water evaporation from the pots was linearly correlated with the evaporative demand of the air, expressed as vapour pressure deficit (VPD, Fig. 3a). Figure 3b shows a 4-day span of evaporation measurements from "empty" pots and a second trace of evaporation modelled from the VPD data. Compared with a transpiring plant, the evaporation rate from the pots is negligible, amounting to no more than 1 % of the total measured water loss (see Fig. 4).

[3] This can be achieved by snipping off the tip of the original nozzle to the desired size.

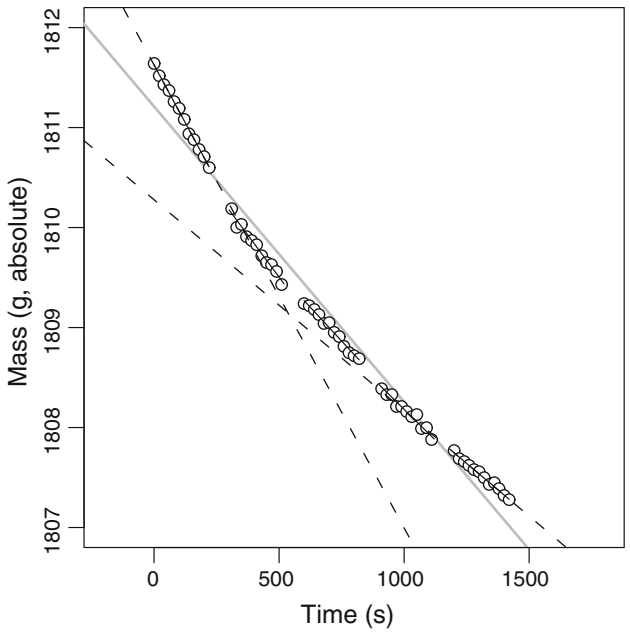

Fig. 2 Five runs of "raw" mass data (25 min) in the transition between light (first two runs) and dark (last three runs). Individual linear regressions are fitted to each run by Amalthea (*thin black lines*) and rate information derived from them. An overall linear regression was fitted here for comparison (*thick grey line*), and to show that periods longer than 5 min are unsuitable if fine granularity is desired. *Dashed lines* show extended regression lines of the first and last runs

The transpiration over a 2-day continuous run of an ~40-cm aspen seedling can be seen in Fig. 4. This seedling was representative of the six plants measured. This figure also shows a measure of evaporative demand experienced by the plants during the same period. It is evident from these data that, the effect of light on stomatal opening notwithstanding, VPD was the principal driver of transpiration in these plants. It is also noteworthy that transpiration and atmospheric conditions were each measured independently, yet there is great parallelism between both traces with some segments clearly recognisable in both data (arrows in Fig. 4). This emphasises the close relationship between VPD and *E*, not only as the main driver, but also acting on smaller scales, which Amalthea is able to detect (the particular drop in VPD—first arrow—can be explained by the opening of a ventilation window in the greenhouse). Comparatively high-amplitude oscillations are visible from midday onward lasting about two hours. These are partly in response to variations in VPD and natural light intensity, but may also exhibit an intrinsic nature (i.e. an oscillatory component is still present after normalising). Regardless of the cause, these oscillations are not an artifact of the system which is capable of reliable measurements and high accuracy, as seen for example after the first arrow in Fig. 4.

Two poplar clones with contrasting characteristics (primarily in growth rate) were simultaneously monitored

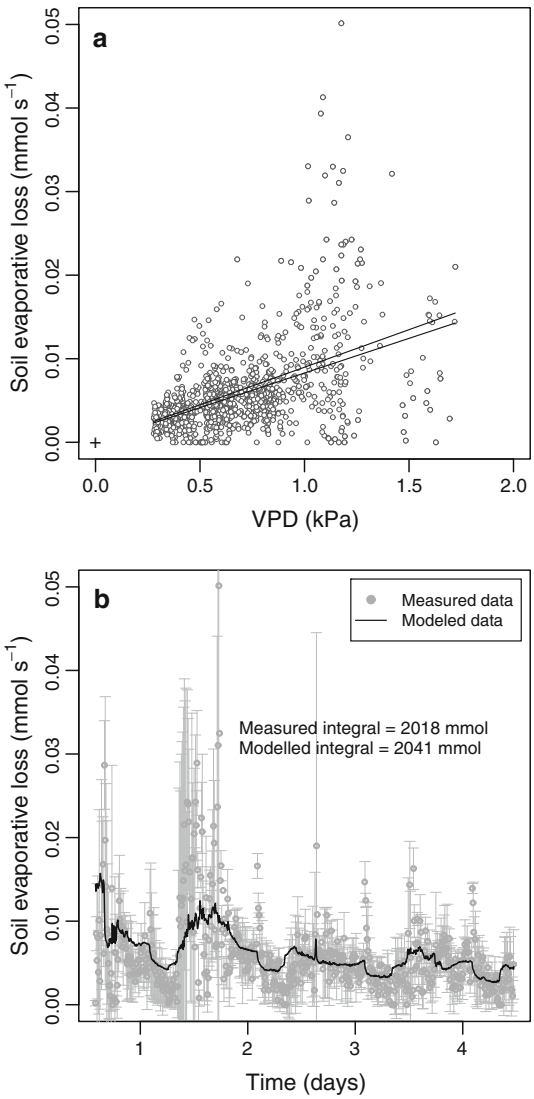

Fig. 3 **a** Linear regression of soil water evaporation in response to evaporative demand. The fitted regression line is not shown; instead the lines are the 95 % confidence interval of the regression. "+" marks the origin ($x = 0$, $y = 0$) through which the function was constrained since there is no evaporative driving force at VPD = 0. Regression function is $y = 8.637 \times 10^{-3}x$. **b** Monitoring evaporation from covered pots for 4 days. *Open circles* are the means of three pots collected simultaneously every 5 min; *bars* indicate standard deviations. The *black line* shows the expected evaporation based on the regression in **a**

for five consecutive days (and nights) to test the system and its handling of multi-species configuration while obtaining data to compare their transpiration responses. For clarity and brevity, Fig. 5 shows two of the five days and the traces were expressed on a leaf-area basis area to make direct comparison possible.

Area correction is a necessary post-processing step to obtain a fine level of resolution, especially when measuring for long periods. The system assumes a total leaf area equal to 1; however, it is possible to override the default value in

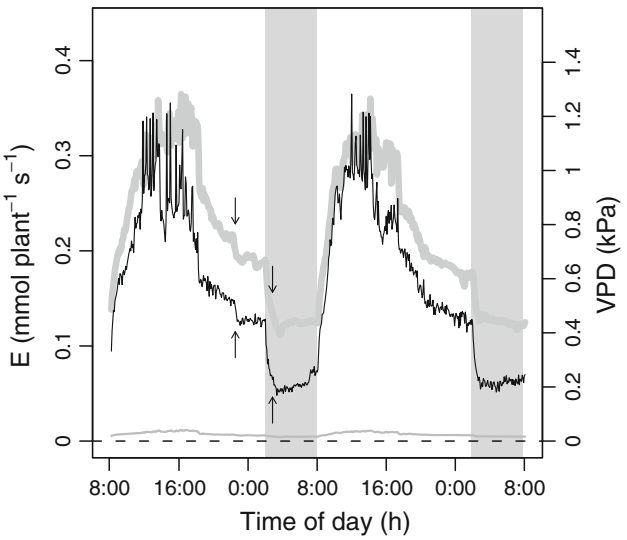

Fig. 4 Two-day traces of a transpiring 30-cm aspen seedling (*thin black line*, *left axis*) and the evaporative demand of the air measured close to the plant canopy (*thick grey line*, *right axis*). *Arrows* mark sections in both traces that parallel each other; each trace was measured simultaneously but independently. The *thin grey line* above the zero line (*dashed*) shows the estimated contribution to *E* of direct water evaporation from the pot. Nighttime is indicated by *grey vertical bars*

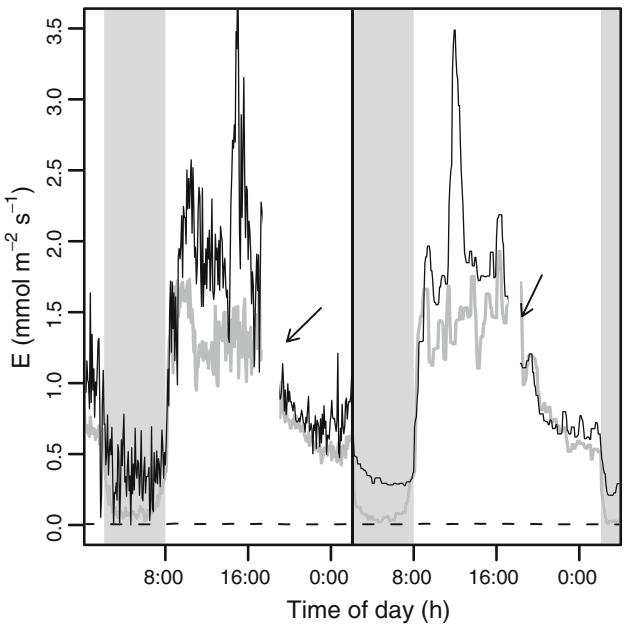

Fig. 5 Monitoring *E* of clones ap9 (*thin black line*) and ap2403 (*thick grey line*) over 2 days. Transpiration rates have been corrected by leaf area. *Arrows* point to gaps in the data when the system was paused to water the plants. In the second day shown, the traces have been smoothed for visual clarity (*right panel*). Nighttime is indicated by *grey vertical bars*. Traces represent individual plants

the configuration. This is advisable only when measuring times are short (1 or 2 days) or when measuring plants that have negligible expansion rates in the desired time frame. During long periods of monitoring fast-growing plants, leaf area increments can be appreciable as was the case with the clones showed in Fig. 5. The expansion rates in this case were $\sim$38 cm^2 day^{-1} for ap2403 and $\sim$12 cm^2 day^{-1} for ap9. Thus, we configured Amalthea to collect data assuming a leaf area of 1 and transpiration was corrected afterwards with individual leaf-area functions.

It can be seen that, despite oscillations in the data, Amalthea consistently measured higher *E* on a leaf-area basis in the slower growing ap9 clone. The traces in the right panel of the figure have been subjected to a line-smoothing algorithm (Tukey's running-median smoothing) for visual clarity only, while the left panel shows the original data. Overall, oscillations in *E* appear larger than those in Fig. 4 due to the fact that the absolute whole-plant rates measured were much smaller, but became amplified when standardised to the per-square-meter scale. Naturally, direct "signal" (*E* in this case) amplification will also amplify noise, although these data are a time series more specialised analyses can be applied (e.g. frequency domain analysis, highly relevant to circadian rhythm studies).

Since all the individual data points collected by Amalthea (both "raw" and "compiled") are synchronised within 3 (typically 1) s of each other among balances, and since all the files contain the same number of points, averages of the entire transpiration patterns of two or more plants are simple to produce. Thus, the synchronised replicates of which Amalthea is capable and the response envelopes they allow are an important advantage of this system. Comparing two or more envelopes that are one-standard-deviation wide incorporates a robustness not attainable otherwise, but indispensable when working with natural genetic variability or when accounting for every independent variable is not feasible. The average trace of an envelope is also a more reliable signal to use for analysis when cyclic or circadian fluctuations are of interest.

As explained in the "Methods" section, the main programme in our software only communicates with the balances and performs the regressions. A helper programme that is part of the package (*amalthea-config*) configures files and coordinates how the data logging is to be done. However, the actual recursion essential to any logging software is carried out by *cron*. This is a key design choice; by outsourcing the repetitive execution to another, standard programme, we can take advantage of the resulting coordination not only with multiple instances[4] of Amalthea but also with other components. We chose to build a custom environmental hardware package and software (both available as open source) in order to obtain coordinated measurements of transpiration *and* evaporative demand.

[4] In computer science, *instance* refers to each individual copy of an active programme or process (i.e. executing in memory).

This is an important feature because, following the procedure outlined in the previous paragraph (e.g. point-by-point division), it allows further standardisation of the data according to environmental conditions. Combining transpiration and environmental data point-for-point can be an invaluable tool which allows the comparison of transpirational behaviours of plants measured sequentially in time (e.g. through phenological stages) since there can always be a correspondence with driving factors.

We used balances with two-decimal precision (0.01 g) which, together with the described intervals (220 s and 10 s sub-interval), provided an ideal combination of resolution and noise tolerance for the plant sizes and transpiration rates measured. Sensitivity analyses were performed to investigate the viability of 0.1-g precision and different measuring intervals. These simulations showed that using the system with a balance capable of a 0.01-g resolution, it is possible to measure rates greater than 0.03 g period^{-1} with an accuracy of 5 % of the true rate or better. At the chosen period of 220 s, 0.03 g represents $\sim$0.0075 mmol s^{-1} which is in the range of the measured water loss from the pot. Simulations with 0.1-g precision showed that 220 s is not enough to measure such low rates accurately. However, doubling the period improved the accuracy, making it possible to measure 0.0555 mmol s^{-1} within $<$5 % of the actual rate. The reader should be aware of the trade-offs between period length and accuracy due to round-off errors and between balance precision and signal-to-noise ratio under different environments and transpiration rates. Ultimately, the system is flexible enough to adapt to different situations given proper assessment of the conditions: e.g. it is unlikely that a small *Arabidopsis* plant can be measured with a 0.01-g balance precision in fast-moving air, while conversely, 0.1-g precision would be sufficient for a potted tree with a leaf area of 0.5 m^2.

The system presented in this paper has the advantage, over other methods, of relying on fewer assumptions and giving a precise whole-plant E measurement with a number of true replicates as opposed to estimation. Its applications are wide ranging although clear limitations emerge from the current hardware. Some of these limitations can be circumvented with careful planning and consideration of the experimental requirements, such as weather proofing or load sharing, amongst others. Special attention should be paid in connecting and disconnecting converter cables since a change in port number can create a configuration problem and result in mixed data, but since port numbering is sequential in the Linux kernel, it is easy to predict port assignment (a more in-depth instruction is provided in the 'Readme' file when downloading the software). The number of ports is also limited by the system, to 127 devices per bus. In many cases, the 'different' USB ports on a computer are on the same bus, detracting from the total 127 maximum. For this and other technical reasons, we recognise a limit of 17 daisy-chained hubs to provide a total of 103 ports (i.e. possible number of balances). Larger systems would be possible by adding more buses or by combining computers, the latter afforded by the use of *cron* as the logging control given that all computers are regularly synchronised with a time server. We estimate that delay between balances should not exceed 5 s, even with large systems (the actual figure depends on many factors). This should not be a problem considering the overall time scale of measurement. No single system devised to measure E is suited to every experimental condition or research question and Amalthea is likely to complement other techniques and form part of a repertoire of tools to investigate transpiration and its regulation.

In conclusion, gravimetric techniques have always been reliable, but highly impractical on a larger scale in terms of space (multiple samples) and time, especially without automation. Studies that look at transpiration that have used automated balance measurements (e.g. Medrano et al. 2005; Cavender-Bares et al. 2007; Dodd et al. 2008, 2010) often implement a non-described point-solution[5] software that, although of value to the study, is neither scalable nor universal as evidenced by the presentation of single-plant measurements. Both the open source software and the hardware connection scheme put forth in this paper are designed to be scalable, versatile and simple to implement and use. Since the design is modular, it is entirely possible to "mix-and-match" balances, to combine balances, or to use all or some of the balances as potometers. We encourage researchers interested in transpiration to visit the software website for more information and to obtain help on downloading and running the programme.

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[5] Point-solution or point product refers to a product that is used only for a particular situation but does not address the technical aspects required for a more general implementation.

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