Novel Patterns of Hysteresis in the Response of Leaf-Level Sap Flow to Vapor Pressure Deficit

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Abstract
        Recent methodological advances toward measuring sap flow on small-diameter stems allow for near continuous monitoring of leaf-level water flux in response to naturally varying environmental conditions. Elucidating the response functions of sap flow to environmental variables is necessary for accurately understanding and modeling the processes underlying leaf transpiration. In main stems, hysteresis often occurs in the response of sap velocity to vapor pressure deficit (VPD). Hysteresis occurs when, for a given VPD that occurs in both morning and evening, morning sap velocity is higher than it is in the evening, producing a clockwise rotation in the hysteresis curve. Here we ask whether the relationship between sap velocity and VPD for leaf petioles is similar to that for main stems. We measured sap velocities using the heat ratio method on leaf petioles of the tropical tree *Cordia alliodora* (*Boraginaceae*) and the tropical liana *Clitoria javitensis* (*Fabaceae*). In most cases, we found clockwise patterns of hysteresis in the sap velocity-VPD relationship similar to those previously published for main stems. However, on some days, we observed a figure-8 pattern of hysteresis in the relationship of sap velocity to VPD. Furthermore, we examined what may cause variation in the amount of hysteresis on a given day. We found a significant positive relationship between the magnitude of hysteresis and both the maximum daily VPD and the integrated daily VPD. These results suggest that the type and magnitude of hysteresis may result from an interplay between environmental variables and plant hydraulic architecture.

INTRODUCTION

        Sap flow measurements are commonly used to monitor plant water use and transpiration in response to environmental variables, such as water availability, light, and vapor pressure deficit (VPD). Understanding the response functions of sap flux to these variables is important for modeling how plant water use changes under daily and seasonally varying conditions. VPD, which varies substantially over daily and seasonal timescales, is an important driver of plant transpiration. However, daily increases and decreases in VPD often do not produce the same results in sap velocity, causing hysteresis in the daily relationship between sap velocity and VPD. For a given VPD that occurs in both the morning and the evening, sap velocity is higher in the morning than in the evening, producing a clockwise rotation in the hysteresis curve. Moreover, daily responses of sap velocity to VPD vary seasonally. In the summer, there is greater hysteresis in the relationship between sap velocity through main stems and VPD than there is in the winter, and the magnitude of hysteresis is positively related to maximum daily VPD (O'Grady et al., 1999, 2008; Zeppel et al., 2004). Hysteresis may indicate a decoupling of physiological processes from their environmental drivers.
        Recently, Clearwater et al. (2009) applied the heat ratio method (HRM; Marshall, 1958; Burgess et al., 2001) to the development of external sap flow sensors that can be installed on small-diameter stems. In the present study, we used this method to measure sap flow rates through leaf petioles of the tropical tree *Cordia alliodora* (*Boraginaceae*) and the tropical liana *Clitoria javitensis* (*Fabaceae*). We asked whether sap flow rates

through leaf petioles showed similar response functions to those commonly measured through main stems. Leaves have small microclimates and may experience relatively large variation in light throughout the day. As a result, sap flux through petioles may be more variable than through main stems and may also show different patterns of hysteresis in response to VPD. We also asked whether the magnitude of leaf-level hysteresis is related to VPD, as it is for main stems (Zeppel et al., 2004). In the current study, we reanalyze data published in Roddy and Dawson (2012) and include previously unpublished data to understand patterns of sap flow hysteresis in tropical plants.

MATERIALS AND METHODS

Plant Material

In February 2011, we measured sap flow rates to individual leaves of the tropical liana *Clitoria javitensis* (*Fabaceae*) and the canopy tree *Cordia alliodora* (*Boraginaceae*) growing in the laboratory clearing of Barro Colorado Island, Panama, at the Smithsonian Tropical Research Institute. Seven leaves of *C. javitensis* and three leaves of *C. alliodora* were chosen for full sun exposure throughout most of the day. All data shown are representative traces from one leaf of each species.

Sensor Design and Heat Ratio Theory

Sensors and methods were identical to those used by Roddy and Dawson (2012). Sensors were based on the design of Clearwater et al. (2009) and modified to use a non-conductive silicone backing instead of cork. Briefly, sensors were connected to 10-cm leads that were then connected to 10-m long leads by quick-connectors and then to an AM16/32 multiplexer and CR23X data logger (Campbell Scientific Inc., Logan, UT). Sensors were held in place with parafilm, and sensors and connections were insulated with multiple layers of bubblewrap and aluminium foil at least 1 cm above and below the sensor.

Our implementation of the heat ratio method was identical to that of Roddy and Dawson (2012) and consistent with previous uses (Burgess et al., 2001; Clearwater et al., 2009). Briefly, every 15 min, the datalogger averaged temperatures on each thermocouple for 10 s prior to firing a 4-second heat pulse. Thermocouple temperatures were measured every 2 s for 200 s after the heat pulse. The heat pulse velocity, v_h (cm s^{-1}) was calculated from the temperature ratio based on the following equation by Marshall (1958) and Clearwater et al. (2009):

$$v_h = \frac{k}{x}\ln\left(\frac{\delta T_1}{\delta T_2}\right)$$

(1)

where v_h is the heat pulse velocity in cm s^{-1}, k is the thermal diffusivity (cm^2 s^{-1}), x is the distance from the heater to each of the thermocouples (cm), and δT_1 and δT_2 are the temperature rises (°C) above and below the heater, respectively. We estimated the thermal diffusivity as:

$$k = \frac{x^2}{4t_m}$$

(2)

where t_m is the time (seconds) between the heat pulse and the maximum temperature rise recorded x cm above or below the heater under conditions of zero sap flow (Clearwater et al., 2009). We measured t_m every morning before dawn when atmospheric vapor pressures were lowest (between 0500 and 0630 h). At this time of day, the vapor pressure deficit was almost always below 0.3 kPa. Our estimates of zero-flow on days when

predawn VPD was less than 0.3 kPa were consistent with our estimates of zero-flow based on excising the petiole above and below the sensor at the end of the experiment. Therefore, we assumed that when VPD was less than 0.3 kPa, sap flow was very near zero. From these daily zero-flow estimates, thermal diffusivity, k, was calculated separately for the upstream and downstream thermocouples and averaged. This daily k was used to calculate v_h from the heat ratios for the subsequent 24 hours. Measurements of k on nights with VPD always above 0.3 kPa were discarded and replaced with the most recently measured k during conditions of VPD <0.3 kPa. VPD was estimated from temperature and relative humidity measurements made every 15 min with a HOBO U23 datalogger (Onset Computer Corp., Bourne, MA).

To account for misaligned probes, at the end of each series of measurements, we excised the stem above and below the sensor at predawn and greased the cut ends. The sensor and stem segment were then placed in a cooler kept at outside ambient temperatures for 2-6 hours, during which time heat ratio measurements were made. The average of these measurements made at zero flow was subtracted from all calculated heat ratios. This corrected heat ratio was then used to calculate v_h. We tested whether temperature ratio measurements were influenced by ambient temperature variation by removing the heat pulse for at least 24 hours, then reconnecting the heaters, and ensuring that there were no obvious, diurnal trends in the temperature ratio (Roddy and Dawson, 2012).

Data Analysis

All analyses and figures were made using the R software (R Development Core Team 2011). We smoothed the sap flow measurements using the 'loess' function in R. A loess smooth creates a locally-weighted polynomial regression for moving windows of points throughout the dataset and plots the midpoint of that window. For each smoothed point, we used less than 1% of all data, which corresponded to less than 25 measurements. We visually compared the relationships between smoothed velocities and VPD and unsmoothed velocities and VPD. Although smoothing changed the absolute values of points in the plots, it did not change the overall shape of the relationship between sap velocity and VPD.

We calculated hysteresis area by manually measuring the area circumscribed by the points in the sap velocity versus VPD plot for each 24-hour day using ImageJ (v. 1.44o; Rasband, 2012). The units for hysteresis area are therefore cm kPa h^{-1}. We used linear regression to test for a relationship between hysteresis area and both maximum daily VPD and integrated daily VPD.

RESULTS AND DISCUSSION

We found similar patterns of hysteresis in the relationship between sap velocity and VPD to those seen previously for sap flow through main stems of canopy trees. On many days, sap flow hysteresis for leaves of both species showed the typical clockwise hysteresis patterns seen previously on main stems (Fig. 1). In general, sap velocities were higher on days with higher VPD. However, we also observed another pattern of hysteresis not previously reported for main stems. On some days, the plot of sap velocity versus VPD had a figure-8 pattern of hysteresis (Fig. 2). This figure-8 pattern of hysteresis appeared in both species, though on different days.

Although the hysteresis plots O'Grady et al. (1999, 2008) seemed to show similar figure-8 hysteresis, the magnitude of the upper loop is much smaller than what we report, and they make no mention of why this unusual pattern of hysteresis may occur. The causes of such figure-8 hysteresis in our dataset are unclear. On days that had the figure-8 hysteresis shape, early morning sap flow was similar to that of days with the clockwise sap flow. After midday, however, sap velocity continued to increase while VPD decreased. In the evenings, when VPD was low sap velocity was below its morning values at the same VPD. On all days, sap velocity was higher in the morning than in the evening, which corresponds to the times just after sunrise and sunset. The major

difference between days with the clockwise pattern of hysteresis and days with the figure-8 pattern of hysteresis seems to be the midday response of sap velocity. On these days, sap velocity increased despite a decrease in VPD. For *C. alliodora*, this occurs when VPD reaches its maximum early in the day, at approximately 1000 h, before sap velocity peaks around 1400 h. On days that have clockwise hysteresis, sap velocity peaks before or at approximately the same time as VPD. Clockwise hysteresis in the response of gas exchange (stomatal conductance or transpiration) to VPD have been shown for a variety of species at the leaf (Meinzer et al., 1997; Takagi et al., 1998) and canopy levels (Katsoulas et al., 2001).

We propose two additional conditions that could cause a figure-8 pattern to sap flow hysteresis. First, midday depression of gas exchange and sap flow could cause an increase in sap flow in the afternoon over midday values, perhaps when VPD is decreasing. Midday depression of leaf gas exchange is thought to result from either hydraulic limitation that causes leaf water potentials to drop below a critical level and induce stomatal closure (Weber and Gates, 1990) or by regulation of transpiration that causes stomatal closure when leaf-to-air vapor pressure differences are high (Schulze et al., 1974). Midday depression of transpiration could depress midday sap velocity such that when transpiration increases later in the afternoon when VPD is lower, sap velocity also would increase. Second, stomatal responses to varying light conditions could influence gas exchange and thus sap velocity through the petiole. Cloudy mornings followed by sunny afternoons could cause afternoon sap velocities to be higher than morning sap velocities, leading possibly to this figure-8 pattern. This figure-8 pattern of hysteresis may be more readily apparent in sap flow measurements on petioles than on main stems because sap flow through main stems represents an integrated response to variation in transpiration throughout plant canopy. Indeed, the responses of stomatal conductance and transpiration to VPD (e.g., Meinzer et al., 1997; Takagi et al., 1998; Katsoulas et al., 2001) are undoubtedly important factors influencing the response of sap flow to VPD. However, sap flow measurements may additionally be influenced by constraints on water balance and may be decoupled from gas exchange, particularly at night during vessel refilling.

We were also interested in understanding what factors may affect the magnitude of sap flow hysteresis with VPD. We found that hysteresis area measured on days showing the traditional clockwise pattern of hysteresis was positively related to maximum daily VPD (R^2=0.42, P<0.05). Higher VPD caused greater hysteresis in the relationship between sap velocity and VPD (Fig. 3; Zeppel et al., 2004; O'Grady et al., 2008). We also tested whether integrated daily VPD was related to hysteresis area and found a similarly significant, positive relationship (R^2=0.35, P<0.05; data not shown). Although these relationships may be inherently autocorrelated, transpiration is probably higher on days with higher VPD, leading to more negative leaf water potentials and greater cavitation. Greater decline in leaf functioning on hot, dry days would likely be reflected in petiole sap flow by higher sap velocity rates in the morning accompanied by a greater drop in sap flow in the afternoon, producing large hysteresis. Interestingly, the magnitude of VPD seemed not to influence the pattern of hysteresis (clockwise vs. figure-8).

CONCLUSIONS

New methods for measuring sap flow of individual petioles using the HRM are allowing us to ask novel questions about what controls sap flow to leaves. In the present study, we found novel patterns of hysteresis in the relationship between sap velocity and VPD that may be related to variation in the leaf microenvironment and physiology. Examining patterns of hysteresis may be a useful tool in disentangling the complexity of leaf responses to multiple environmental drivers. Future work will examine how other microenvironmental parameters, such as light, may interact with VPD to cause different patterns of hysteresis and examine how hysteresis may be related to hydraulic architecture.

Literature Cited
Burgess, S.S.O., Adams, M.A., Turner, N.C., Beverly, C.R., Ong, C.K., Khan, A.A.H. and Bleby, T.M. 2001. An improved heat pulse method to measure low and reverse rates of sap flow in woody plants. Tree Physiol. 21:589-598.
Clearwater, M.J., Luo, Z., Mazzeo, M. and Dichio, B. 2009. An external heat pulse method for measurement of sap flow through fruit pedicels, leaf petioles and other small-diameter stems. Plant Cell Environ. 32:1652-1663.
Katsoulas, N., Baille, A. and Kittas, C. 2001. Effect of misting on transpiration and canopy conductances of a greenhouse rose canopy. Ag. For. Met. 106:233-247.
Marshall, D.C. 1958. Measurement of sap flow in conifers by heat transport. Plant Physiol. 33:385-396.
Meinzer, F.C., Hinckley, T.M. and Ceulemans, R. 1997. Apparent responses of stomata to transpiration and humidity in a hybrid poplar canopy. Plant Cell Environ. 20:1301-1308.
O'Grady, A.P., Eamus, D. and Hutley, L.B. 1999. Transpiration increases during the dry season: patterns of tree water use in eucalypt open-forests of northern Australia. Tree Physiol. 19:591-597.
O'Grady, A.P., Worledge, D. and Battaglia, M. 2008. Constraints on transpiration of *Ecualyptus globulus* in southern Tasmania, Australia. Ag. For. Met. 148:453-465.
Rasband, W.S. 1997-2012. ImageJ, US National Institutes of Health, Bethesda, Maryland, USA. http://imagej.nih.gov/ij.
Roddy, A.B. and Dawson, T.E. 2012. Determining the water dynamics of flowering using miniature sap flow sensors. Acta Hort. 951:47-53.
Schulze, E.-D., Lange, O.L., Evenari, M., Kappen, L. and Buschbom, U. 1974. The role of air humidity and leaf temperature in controlling stomatal resistance of *Prunus armeniaca* L. Under desert conditions. I. A simulation of daily course of stomatal resistance. Oecologia 17:159-170.
Takagi, K., Tsuboya, T. and Takahashi, H. 1998. Diurnal hysteresis of stomatal and bulk surface conductances in relation to vapor pressure deficit in a cool-temperate wetland. Ag. For. Met. 91:177-191.
Weber, J.A. and Gates, D.M. 1990. Gas exchange in *Quercus rubra* (northern red oak) during a drought: analysis of relations among photosynthesis, transpiration, and leaf conductance. Tree Physiol. 7:215-225.
Zeppel, M.J.B., Murray, B.R., Barton, C. and Eamus, D. 2004. Seasonal responses of xylem sap velocity to VPD and solar radiation during drought in a stand of native trees in temperate Australia. Funct. Plant Biol. 31:561-470.
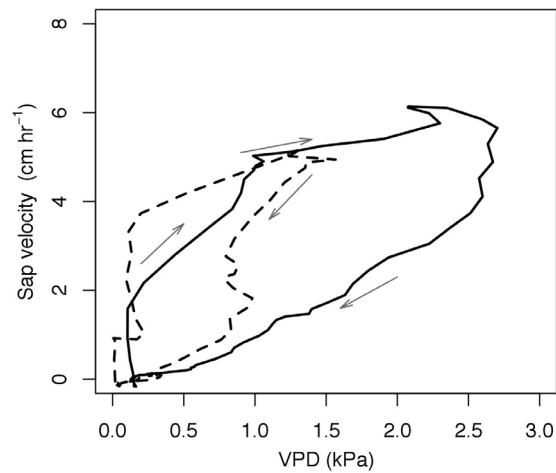
Fig. 1. Hysteresis plot of petiole sap velocity versus VPD for two days for *C. javitensis*. One day was warm and sunny (solid line), while the other day was cool and cloudy (dashed line). On both days, sap velocity shows the clockwise pattern of hysteresis characteristic of the relationship between VPD and sap velocity measured on main stems. Arrows indicate temporal progression through the two days.
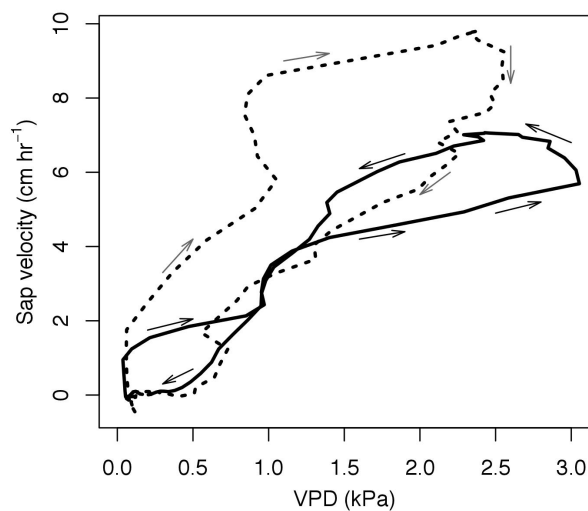

Fig. 2. Hysteresis plot of sap velocity versus VPD for two days for *C. alliodora*. The two days had similar maximum VPD, but sap velocity responses differed between the two days. One day (dotted line with grey arrows) shows the typical clockwise pattern of hysteresis previously published for main stems. The other day (solid line with white arrows) shows a figure-8 pattern of hysteresis that occurred on some days. Arrows indicate temporal progression through the two days.
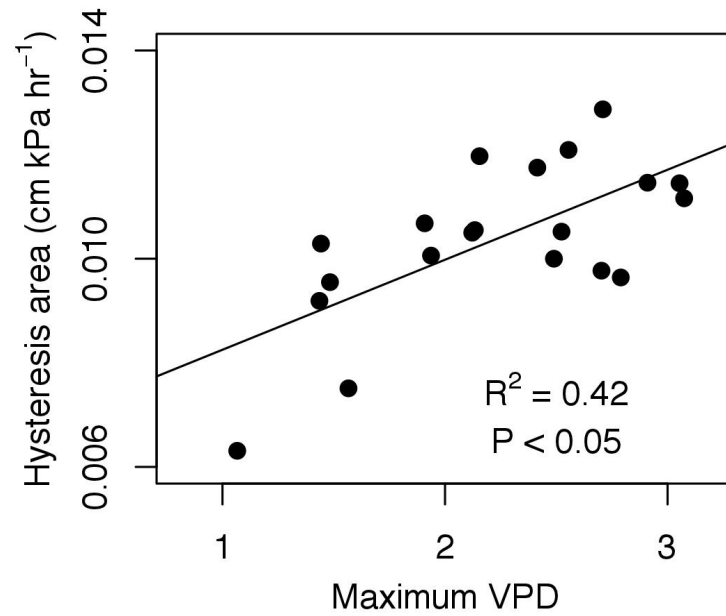
Fig. 3. The relationship between maximum daily VPD and hysteresis area for *C. javitensis*. Hysteresis area was measured as the area circumscribed by the points in the plot of sap velocity versus VPD.