Determining the Water Dynamics of Flowering Using Miniature Sap Flow Sensors

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Abstract

Angiosperms dominate almost every ecosystem globally, and for the vast majority, flowering is vital to successful reproduction, yet little work has characterized the dynamics of water use during flowering and its impacts on the reproductive biology of wild plants. Here we demonstrate a new implementation of the heat ratio method to measure sap flow dynamics of leaves and flowers. We employed this new method to estimate the water requirements of flowering in four tropical species: the understory tree, *Hybanthus prunifolius* (*Violaceae*), the liana *Clitoria javitensis* (*Fabaceae*), the canopy tree *Cordia alliodora* (*Boraginaceae*), the shrub *Annona acuminata* (*Annonaceae*). Our results suggest that there is substantial diversity in the timing and magnitude of water transport to flowers. Some species show almost no water flow to flowers, while flowers of other species show sap velocities approximately 30-50% that of nearby leaves. This variability is likely related to the pathway and mechanism of water transport to flowers, to floral morphology, and to evaporative demand in the floral microenvironment.

INTRODUCTION

Flowering requires the allocation of significant resources and for many species can be the single largest aspect of reproductive investment (Bazzaz et al., 1987). Many species flower under suboptimal conditions when resources are limiting. For example, many tropical trees flower in the dry season, when water is scarce (van Schaik et al., 1993). Because many flowers and fruits are maintained at higher, less negative water potentials than subtending stems and leaves (Trolinder et al., 1993; Chapotin et al., 2003), the direction of water flow to and from reproductive organs may vary with time of day, plant water status, and reproductive development. In mango, for example, inflorescences exhibit unidirectional sap flux to the developing inflorescences (Higuchi and Sakuratani, 2005), while developing fruits exhibit sap flux towards the fruit at night, and sap flux away from the fruit into the stem during the day (Higuchi and Sakuratani, 2006).

Here we report on a new implementation of the heat pulse method (Clearwater et al., 2009; Marshall, 1958; Burgess et al., 2001) to measure sap flow dynamics of individual leaves, flowers, and fruits in four tropical plants common in the moist, lowland forests of central Panama. Our objectives were: (1) to test these miniature, external sap flow sensors under natural field conditions, (2) to determine whether species differ in the temporal dynamics of sap flow to reproductive organs and (3) to use sap flow measurements to estimate the relative water costs of reproduction in wild, tropical plants.

MATERIALS AND METHODS

Plant Material

From January to April 2011, we studied four species growing in the forests and clearing of Barro Colorado Island, Panama, at the Smithsonian Tropical Research Institute. The liana *Clitoria javitensis* (*Fabaceae*) flowers towards the beginning of the dry season, in early February. The shrub *Annona acuminata* (*Annonaceae*) flowers

throughout the year, but was measured in March and April. The understory tree *Hybanthus prunifolius* (*Violaceae*) flowers towards the end of the dry season when the first rains appear toward the end of March. The canopy tree *Cordia alliodora* (*Boraginaceae*) flowers during the dry season. On flowers, we installed 3 sensors on *C. javitensis*, 6 sensors on *A. acuminata*, 8 sensors on *H. prunifolius*, and 2 sensors on *C. alliodora*. On leaves, we installed 7 sensors on *C. javitensis*, 5 sensors on *A. acuminata*, 8 sensors on *H. prunifolius*, and 3 sensors on *C. alliodora*. All data shown are representative traces from one sensor in each group. Petioles, pedicels, peduncles, and branchlets on which we installed sensors ranged in diameter from approximately 1.5 mm to 8 mm.

Sensor Design

We modified the design of Clearwater et al. (2009) to use a nonconductive silicone backing instead of cork in order to minimize the effects of gauge material on thermal diffusivity. Sensors were connected to 10 cm leads with Molex quick-connectors that were then connected to 10 m long leads to an AM16/32 multiplexer and CR23X datalogger (Campbell Scientific Inc., Logan, UT). Sensors were held in place with parafilm, and sensors and connections were insulated with bubble wrap and aluminum foil at least 1 cm above and below the sensor. Our implementation of the heat ratio method was consistent with previous uses (Burgess et al., 2001; Clearwater et al., 2009), except that we used a four second heat pulse, measured thermocouples every 2 seconds for 200 seconds, and made measurements every 15 minutes.

Heat Ratio Theory

The heat pulse velocity, v_h (cm s^{-1}), is calculated from the temperature ratio based on the following equation by Marshall (1958) and Clearwater et al. (2009):

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

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 (^oC) above and below the heater, respectively. We estimated the thermal diffusivity as:

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

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 are lowest (between 0500 and 0630 hrs). At this time, the vapor pressure deficit was almost always below 0.3 kPa, and therefore we assumed there was no sap flow. From t_m, we calculated a thermal diffusivity, k, which was used to calculate v_h from the heat ratios for the subsequent 24 hours. Measurements of k on nights with vapor pressure deficit (vpd) above 0.3 kPa were discarded and replaced with the most recently measured k during conditions of vpd < 0.3 kPa. Vapor pressure deficit was estimated from temperature and relative humidity measurements made every 15 minutes with a HOBO U23 datalogger (Onset Computer Corp., Bourne, MA).

The calculation of v_h depends on equidistant spacing of thermocouples above and below the heater. To account for misaligned probes, we excised the stem above and below the sensor at predawn and greased the cut ends at the end of each series of measurements. The sensor and stem segment were then placed in a cooler next to the sampled plant 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. Additionally, we covered one leaf of *C. javitensis* with plastic wrap and aluminum foil for approximately 36 hours to test whether

this method would yield an in situ zero-flow measurement comparable to excision and zeroing based on vpd.

Because of the small diameter of stems being measured, sun exposure or ambient temperature gradients could induce artifacts into the measurement of the temperature ratio. For all sensors installed, we tested whether ambient temperature influenced heat ratio measurements for at least 24 hours. After installing sensors, we removed the heat pulse to observe whether there was a pattern to measured heat ratios and then reconnected the heaters.

Data Analysis

All analyses and figures were made using the R software (R Development Core Team 2011). Because of the relatively high level of variation in the measurements, we smoothed the 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.

RESULTS AND DISCUSSION

Sensor Design and Analysis

All heat-based sap flow measurements are prone to errors caused by external thermal gradients that can have significant impacts on measured temperature ratios. We found that with sufficient insulation (at least 1 cm above and below the sensor of multiple layers of bubble wrap), we could essentially eliminate the effects of external thermal gradients. By unplugging the heaters from the power source, we tested whether background thermal gradients affected temperature ratios. When heaters were unplugged, we saw no obvious trends in the measured temperature ratios (Fig. 1). The increased noise of measured temperature ratios is due to the calculation of the ratio of temperature change based on measurements before and after a heat pulse. When the heaters were reattached to the power supply, measured temperature ratios were consistent with expected sap flow dynamics. Because the magnitude of thermal gradients may depend on the microenvironment around the sensor, we performed this test on all sensors a few days after installation. Our data from these sensors installed in the understory and exposed environments of a tropical forest suggest that there are no obvious influences of background temperature on measurements of the temperature ratio. Figure 1 shows the recorded heat ratios of one representative *C. alliodora* branchlet with and without the heat pulse.

Interpreting sap flow data requires an accurate estimation of zero-flow, which can be difficult. We tested three methods for generating zero-flow estimates: (1) zeroing based on low vpd conditions (removing the demand for water and its movement), (2) zeroing after excising around the sensor at predawn (severing the pathway for sap flow), and (3) covering the leaf with plastic wrap and aluminum foil (also removing the demand at the leaf). Zeroing during low vpd conditions was the most reliable method, although it assumes that there is no flow at low vpd. Excising around the sensor sometimes produced zero-flow estimates higher or lower than the low-vpd method. One possible reason for the discrepancy between these two methods is that excising may have disturbed thermocouple spacing and affected measured temperature ratios. It was impossible for us to test whether this is the case. Additionally, on one leaf of *C. javitensis*, we saw that covering the leaf in plastic wrap and foil estimated a zero-flow comparable to the other two methods. In our figures, we present zero-flow estimates based on excision.

Despite effective insulation, we still saw substantial noise in measurements made during the middle of the day (Fig. 2). The causes for such variance are unclear. There seemed to be no obvious effects of ambient temperature (Fig. 1), suggesting that such variable measurements may result from rapid changes in sap flux at the leaf level. We applied a loess smooth to the data, which creates a locally weighted polynomial fit in a

moving window throughout the dataset. This smoothing procedure seems to effectively extract and highlight the overall pattern in sap velocity without removing important differences between days and between samples. Figure 2 shows that the smoothed values capture day-to-day variation in sap velocity in response to differences in evaporative demand. Subsequent figures show only the smoothed velocities.

Estimating sap flow requires accurate measurements of *k*. Across all our measurements, *k* ranged from 0.0014 to 0.0020 cm^2 s^{-1}. There were no differences between species, and within individual samples, *k* did not vary as a function of duration into the dry season.

Sap Flow Dynamics of Leaves and Flowers

We measured sap flow on four tropical species varying in growth habit and habitat in the forest. Two species, *H. prunifolius* and *A. acuminata*, occur in the shaded understory and produce flowers born on leafing shoots. Sensors were installed basal to both leaves and flowers, and we defoliated these shoots to determine the sap flow due to flowers. In both *H. prunifolius* (Fig. 3) and *A. acuminata* (data not shown), sap flow was consistently near zero after defoliation. During fruiting in *H. prunifolius*, there were slight diurnal increases in sap velocity, suggesting that stomata on the fruit may be controlling transpiration. In contrast to previous sap flow measurements on fruits (Higuchi and Sakuratani, 2006), we found no substantial sap flow reversals from these fruits, probably because these fruits store little water.

In contrast to these understory species, we saw clear sap flow patterns driven by flowering in the two species with sun-exposed flowers. In the liana *C. javitensis*, we measured increases in velocity due to flowers as compared to leaves (Fig. 4). Interestingly, flowers not only increased daily maximum velocity but also changed the pattern of sap flow. During the day prior to anthesis and on the first day of anthesis, flowers showed no midday depression in sap flow, unlike adjacent leaves. However, after petals had senesced and only the green, photosynthetic sepals remained, flowering shoots showed similar midday depressions to those of leaves. This pattern suggests that petals may require a constant supply of water, the magnitude of which may overwhelm the midday depression in water use by subtending sepals.

Throughout its canopy, the canopy tree *C. alliodora* produces inflorescences, each with flowers in various stages of development. We installed sensors on individual inflorescences and nearby leaves on a fully sun- and wind-exposed tree in a clearing (Fig. 5). Inflorescences underwent similar daily variation in sap flow as observed in leaves, increasing to daily maxima around midday and then declining to zero flow overnight. However, inflorescences had maximum velocities only about 30-50% that of nearby leaves. Furthermore, the day-to-day variation in vpd affected sap velocities to leaves but had little effect on inflorescence sap flow.

Here we have reported on the application of miniature, external sap flow sensors in a tropical forest to understand leaf- and flower-level sap flow dynamics. We show that these sap flow sensors work well under field conditions and are minimally influenced by thermal gradients when properly installed and insulated. Our results also indicate that the sap flow dynamics of flowering is highly species- and habitat-specific; flowers of some species show little measurable sap flow, while flowers of other species have substantial sap flow rates. Overall, we estimate that maximum sap velocities to an inflorescence can be as high 50% that of leaves, suggesting that for some species the water requirements of flowering may be substantial.

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Figures

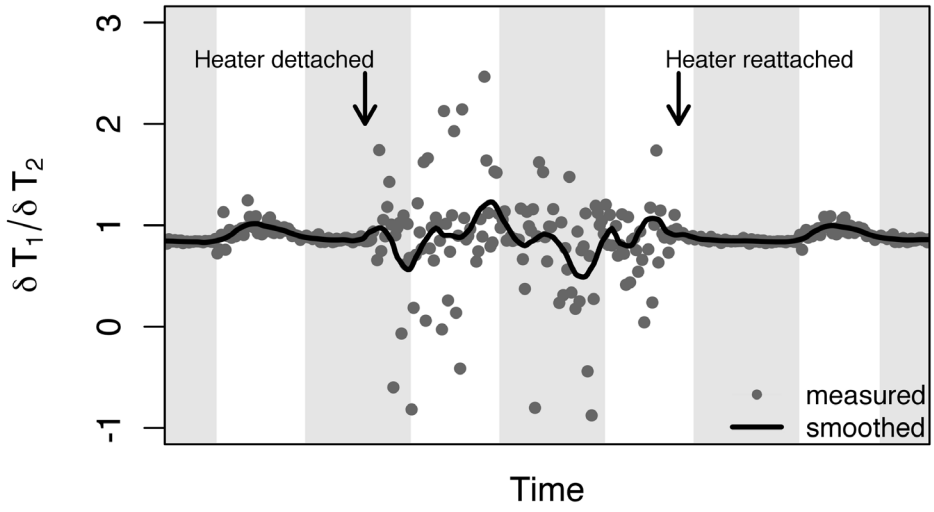

Fig. 1. Time series (approximately 96 hours) of one sensor subtending two leaves of *C. alliodora* during testing for thermal gradients. The heater on the sensor was originally plugged in to a power supply, then it was detached from power, and finally reattached when indicated. Points represent the measured temperature ratios, and the solid line is the loess-smoothed values. Shading represents nighttime.

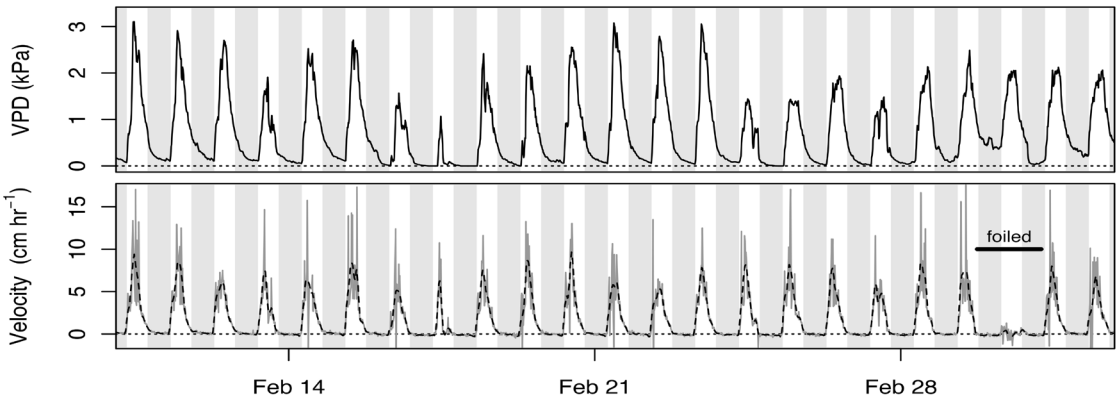

Fig. 2. Time series of sap flow velocities recorded on one representative fully sun-exposed leaf of *C. javitensis* for 23 days during the dry season. The grey line represents the raw velocities calculated from temperature ratios and measurements of thermal diffusivity, k. The dashed, black line represents the velocities after loess smoothing. For about 36 hours, the leaf was covered in plastic wrap and aluminum foil to stop transpiration, as indicated in the figure.

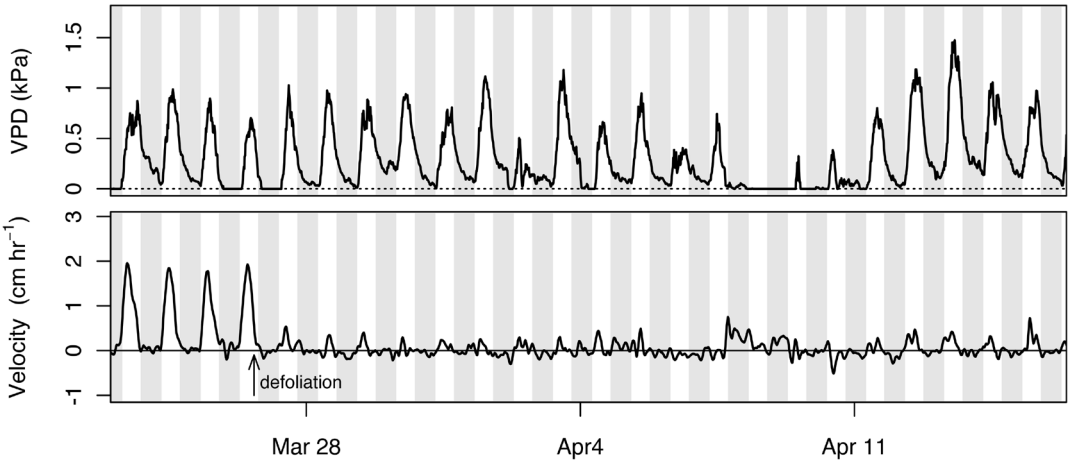

Fig. 3. Sap velocities for a flowering shoot of the understory tree *H. prunifolius*. The 95% confidence intervals around the zero-flow estimate are virtually indistinguishable from the zero-flow line shown in the figure and are thus not presented.

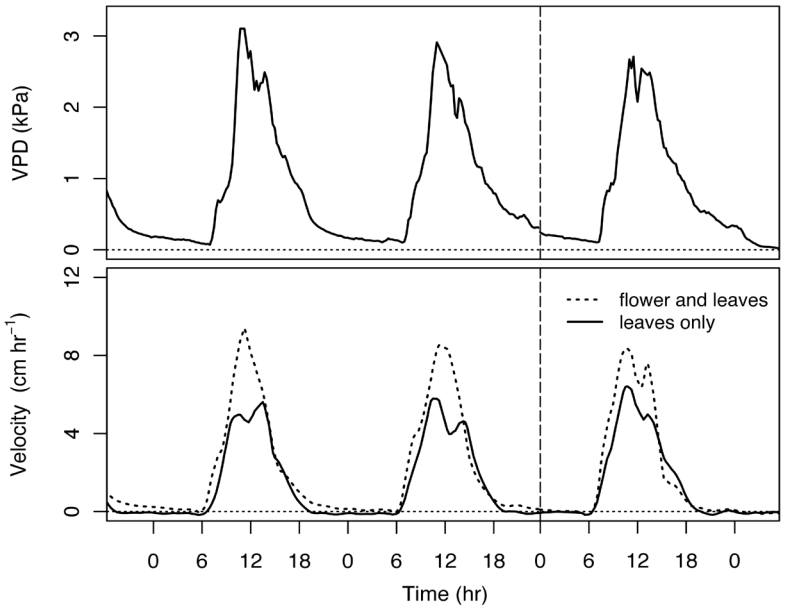

Fig. 4. Three days of sap flow on *C. javitensis*. One branchlet (dotted line) bore both leaves and a flower, while another (solid line) bore only leaves. The first day shown was the day just before floral anthesis. Just before predawn on the second day, the flower opened and remained open throughout the day. On the third day shown, only the sepals of the flower remained, while the petals had fully senesced.

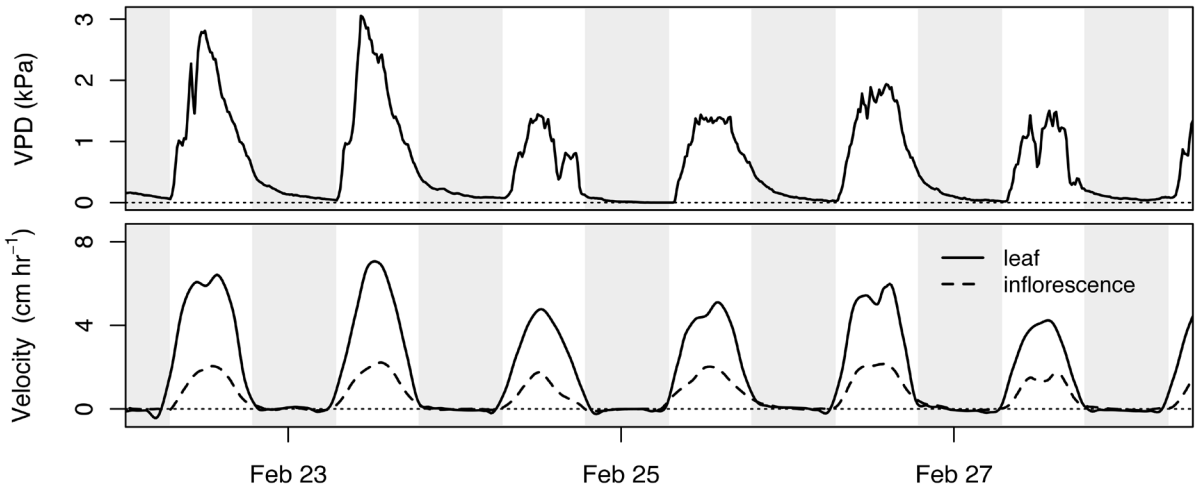

Fig. 5. Sap flow velocities for a leaf and an inflorescence of the canopy tree *C. alliodora*. During the six days shown, individual flowers on the inflorescence were in various stages of development.