An Automated System for Achieving Steady-State Transpiration in Potted Plants

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ABSTRACT

Here we describe a datalogger-controlled, gravimetrically-driven automated watering system. The system employs electronic output scales in combination with a PC, datalogger, multiplexer, and controller, along with various sensors to continuously and precisely monitor environmental conditions. The scales are housed within two controlled-environment growth chambers. A relatively constant water content is maintained in each pot by automated addition of water once per hour. Transpiration rates of individual potted plants are continuously monitored. Average (whole-plant) stomatal conductances are calculated using transpiration rate values in combination with concurrent relative humidity and leaf and air temperature measurements. A method for normalizing transpiration data to account for differences in plant size is described.

MATERIALS AND METHODS

Growth Chamber Conditions

Studies were conducted in two separate controlled-environment growth chambers at the USU Crop Physiology Laboratory in Logan, Utah. Growth chamber temperatures varied among crops and treatments but were typically 25 to 30 ±1^{o}C during the day and 20 to 25 ±1^{o}C at night. High pressure sodium lamps provided a photosynthetic photon flux (PPF) of 800 to 1200 µmol m^{-2} s^{-1}, with a 16-hour photoperiod. Relative humidity in the growth chambers was dependent on plant size, transpiration rate, and supplemental humidification and ranged from 15% to 90%. Light level, temperature and humidity were sometimes manipulated to study the effects of abrupt environmental changes on transpiration rate. Each of the two growth chambers houses five scales.

The System: Scales, Monitoring, and Control

Our system consists of digital-output scales (Acculab, Edgewood, NY) in combination with a monitoring and control system comprised of Campbell Scientific (Logan, UT) components. Initial studies were carried out using five Acculab Vicon® scales with 3-kg capacity (~$350 each). Later studies also employed five Acculab ALC® scales with 4-kg capacity (~$700 each). All scales have 0.1g digital resolution.

Scales were wired to an AM416 multiplexer connected to a CR1000 datalogger (Campbell Scientific, Logan, UT) for continuous monitoring of their output (Figures 1 and 2). The scales have a digital serial output as opposed to an analog voltage output, so the multiplexer must be connected to a serial COM port on the CR1000. We used COM4, which corresponds to control ports C7 and C8. The serial cable must be modified in order to wire the scales directly to a logger or multiplexer. Specifically, female end of the cable must be removed to expose the individual wires. The TxD, RxD, and GND wires (pins 2, 3 and 5 on the 9-pin interface for Vicon scales; pins 2, 3 and 4 on the 25-pin interface for ALC scales) are connected to the multiplexer according to Figure 3.

Scale readings were recorded at 10-minute intervals. Once per hour, each scale reading was cross-referenced with a target weight. When an individual scale reading dropped below the target weight, the datalogger triggered an SDM-CD16 controller (Campbell Scientific, Logan, UT) which opened a solenoid to add tap water to bring the plant back to within 10% of the target weight (Figure 4).

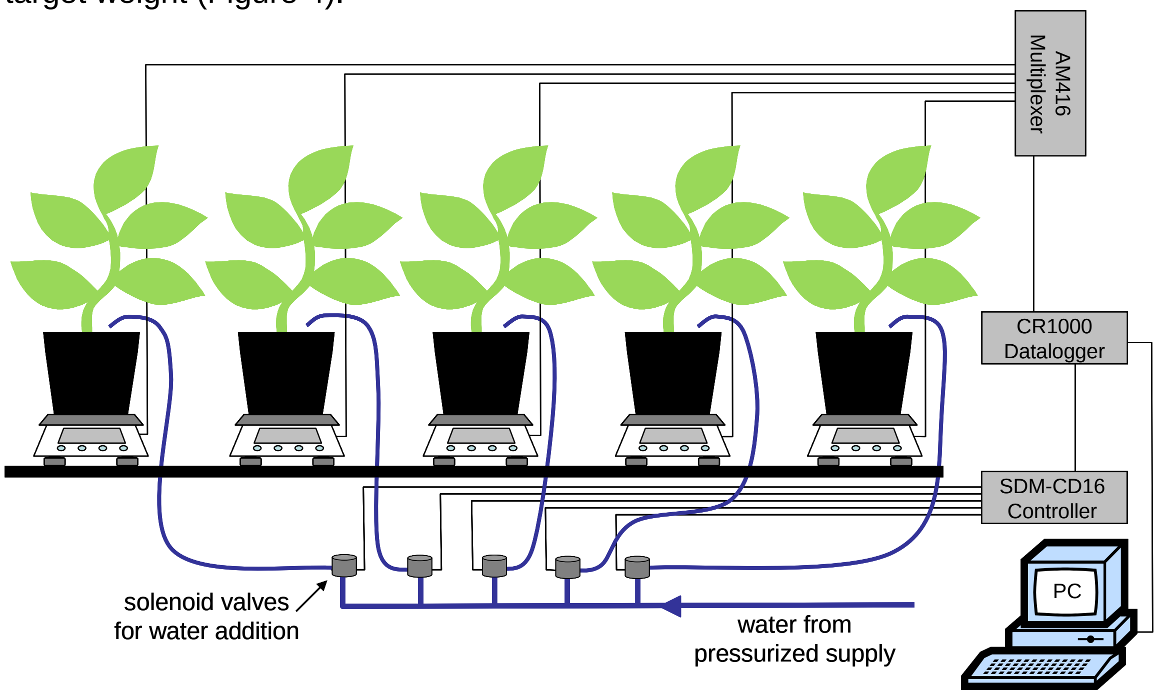

Figure 1. System overview.

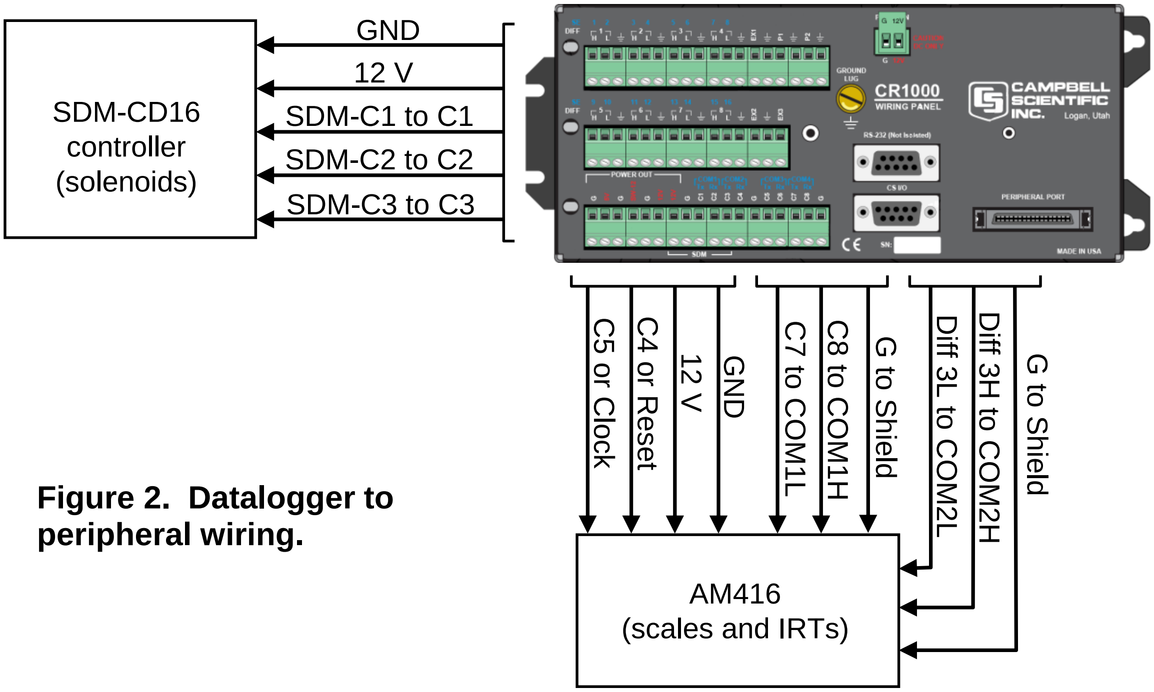

Figure 2. Datalogger to peripheral wiring.

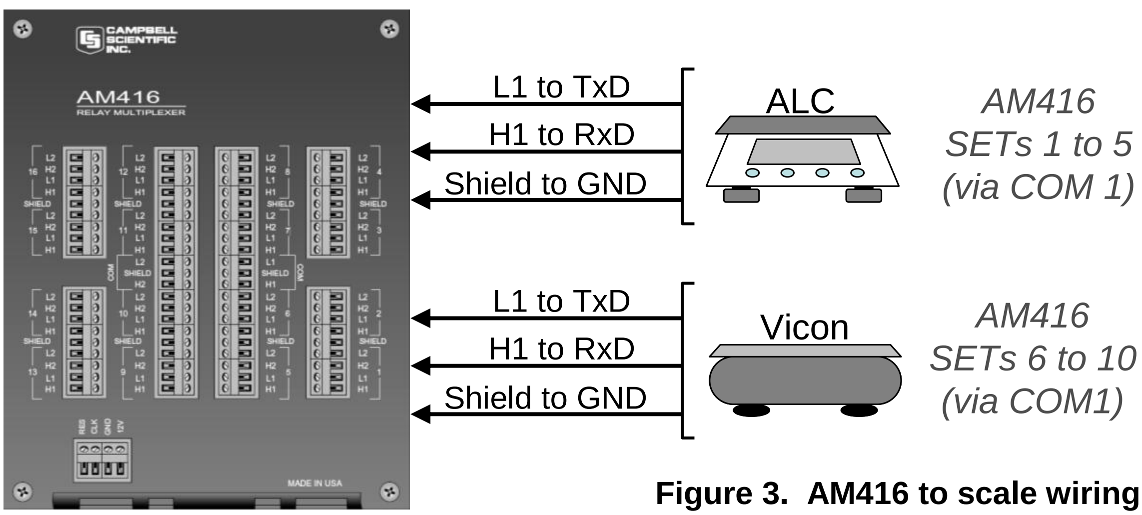

Figure 3. AM416 to scale wiring.

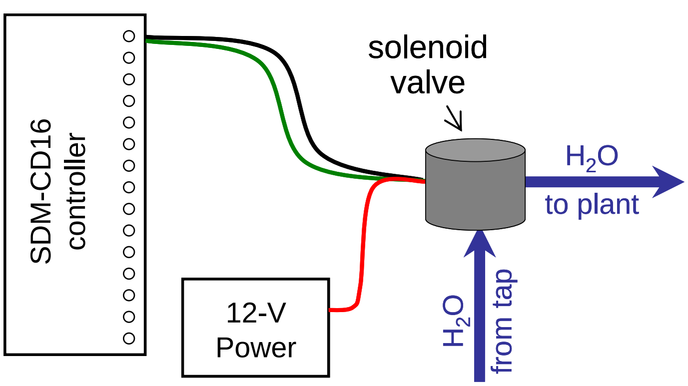

Figure 4. SDM-CD16 to solenoid wiring.

Growth chamber temperature and relative humidity were continuously measured using a Vaisala HMP50-L probe (Campbell Scientific, Logan, UT). Real-time saturated vapor pressure was calculated directly from these measurements using the built-in "SatVP" datalogger instruction. The datalogger was also instructed to calculate chamber vapor pressure (VP) by multiplying the saturated VP by the measured relative humidity (expressed as a fraction). Vapor pressure gradient (VPG, the difference between the vapor pressure inside the leaf and the vapor pressure of the air) was then calculated by subtracting chamber VP from saturated VP. The calculation of VPG assumes that the RH inside the leaf is 100% and that leaf and air temperature are the same. A more accurate estimate of VPG may be made if leaf temperature is measured (see Stomatal Conductance Calculation section, below).

Temperature Effects on Scale Output

The output of electronic scales is affected by temperature. The more expensive ALC scales have built-in temperature compensation but the less expensive Vicon scales do not. Temperature compensation is not an issue when experiments are conducted at constant temperature. In plant growth experiments, however, diurnal swings of up to 15^{o}C are not uncommon. We characterized the effects of temperature on scale output by recording the weight readings for clean and dry rocks over the course of one week with diurnal temperature swings of 5 to 10^{o}C.

Plant Materials

Plants were grown from seed in the greenhouse. Seeds were sown directly or young plants were transplanted into round, 4-L plastic pots with drainage holes. Pots were filled with a 50/50% (v/v) mixture of peat and vermiculite. Each pot was amended with 10 g of slow-release fertilizer (Osmocote Plus® 15-9-12, 3 to 4 month release, Scotts Company, Marysville, OH).

Transpiration Rate Measurements

Transpiration rate was determined gravimetrically by continuous monitoring of the weights of individual potted plants. The pot of each plant was enclosed in a plastic bag that was sealed with a twist tie at the base of the plant stem to minimize water loss by evaporation. Previous measurements indicate that under these conditions less than 1% of evapotranspirative weight loss during the light period is due to evaporation. Each plant was placed on a scale, and the scales were connected to a datalogger for continuous monitoring of pot weight. Transpiration rates were calculated on 10-minute intervals. Transpiration rates ranged from 0.1 g/min for small corn plants to 2.0 g/min for large cotton plants. Plants were automatically watered once per hour as needed to maintain a target weight in order to minimize the effect of fluctuating soil water content on transpiration rate. Transpiration calculations assume that changes in plant weight due to growth are negligible.

Stomatal Conductance Calculation

Stomatal conductance is a more sensitive measure of stomatal regulation than transpiration because it corrects for the effects of changes in temperature and relative humidity during the measurement period. Transpiration rate, chamber air temperature and relative humidity measurements were used to calculate average whole-plant stomatal conductance using Equation 1,

$$g_s = T / \Delta H_2O \qquad \textit{Equation 1}$$

Where g_s is stomatal conductance in mmol H_2O plant^{-1} s^{-1}, T is the transpiration rate in mmol H_2O plant^{-1} s^{-1}, and ΔH_2O is the vapor pressure gradient (VPG, the dimensionless (mol/mol) driving gradient for water vapor diffusion out of the stomates). The VPG is the difference in the partial pressures of water vapor inside and outside the leaf, which are determined from measurements of temperature and relative humidity. The partial pressure of water vapor outside the leaf is assumed to be equal to that of the chamber air. Barometric pressure varies by less than 2% over the course of a year and is assumed to be constant at 86 kPa for our elevation. The air on the inside of the leaf is assumed to be at 100% RH. We have used infrared thermometers (IRTs) for direct measurement of leaf temperature of individual leaves. It should be noted that these measurements do not take into account the variability in leaf temperature among leaf layers and therefore do not provide a representative temperature for the whole plant. For the data presented here, the leaf temperature was assumed to be the same as the chamber air temperature.

Drought Stress

The automated watering system was used to impose steady-state drought stress by lowering the target weights of individual plants. The desired reduction in stomatal conductance of drought-stressed plants was 30 to 50% of the conductance of a well-watered plant. In a typical study with five plants, one plant was well-watered as a control and the other four plants were drought-stressed. Two of the four drought-stressed plants received an experimental treatment (in our case the treatment was application of the ethylene inhibitor 1-MCP) and the remaining two served as drought-stress controls.

RESULTS

Temperature Effects on Scale Output

We recorded the weight readings for clean and dry rocks over the course of one week with diurnal temperature swings of 5 to 10^{o}C. The apparent change in weight of the rocks with temperature at 75% of scale capacity is large for four out of five of the Vicon scales, but small (less than 0.25 g per ^{o}C) for all of the ALC scales (Figure 5). However the error with the ALC scales increases when smaller (<100 g) weights are being measured (Figure 6). The two types of scales were tested in different growth chambers with different temperature set-points. The rate of temperature change may also play a role in the effect of temperature on scale performance. The transition between day and night temperature in the growth chamber with the Vicon scales was rapid. The transition between day and night temperature in the growth chamber with the ALC scales was more gradual.

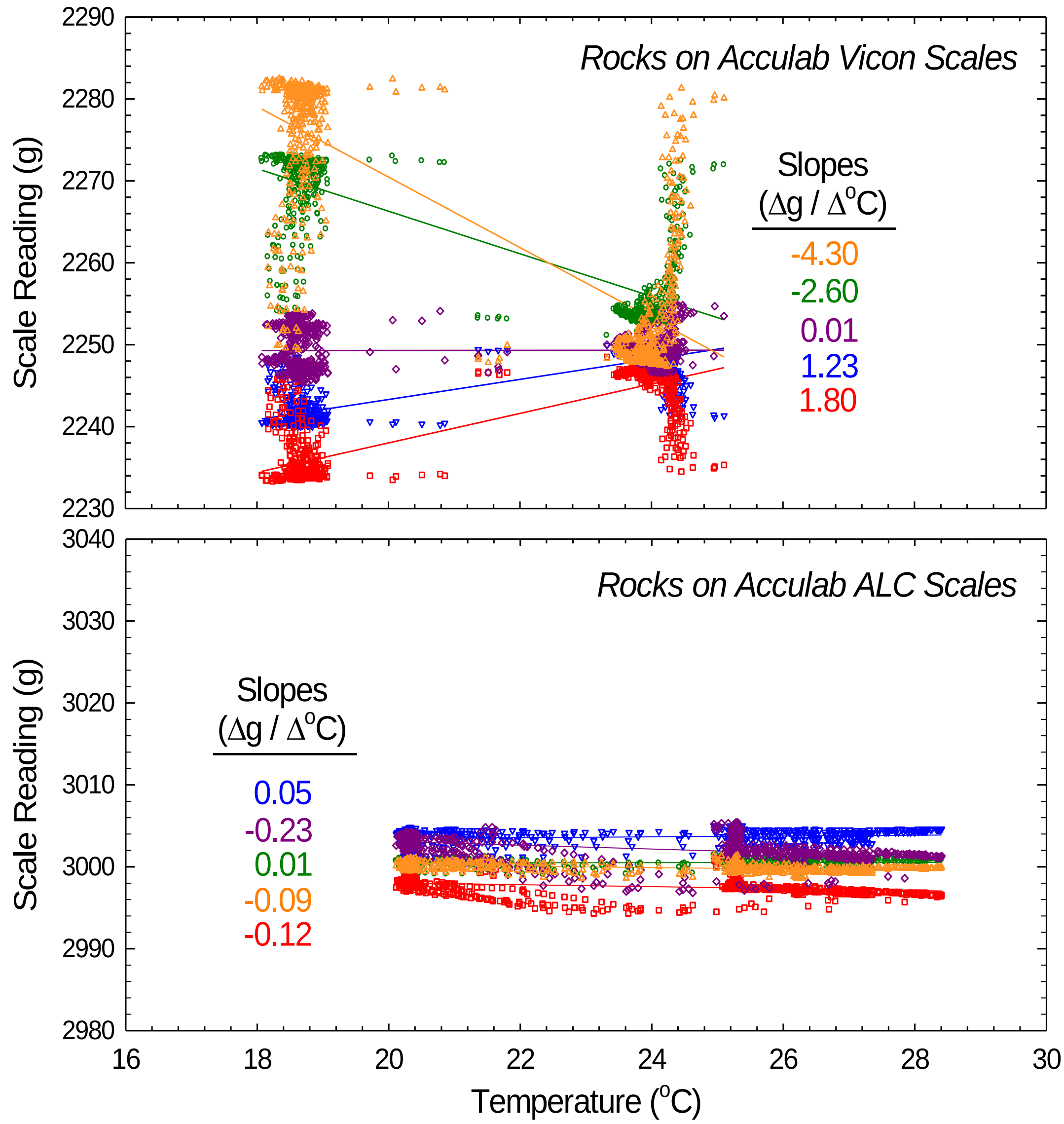

Figure 5. Effect of temperature on scale readings at 75% of scale capacity. The temperature sensitivity of the Vicon scales was highly variable from scale to scale. The Vicon scales (~$350. each) were much more temperature-sensitive than the ALC scales (~$700 each) as indicated by the slopes of the lines.

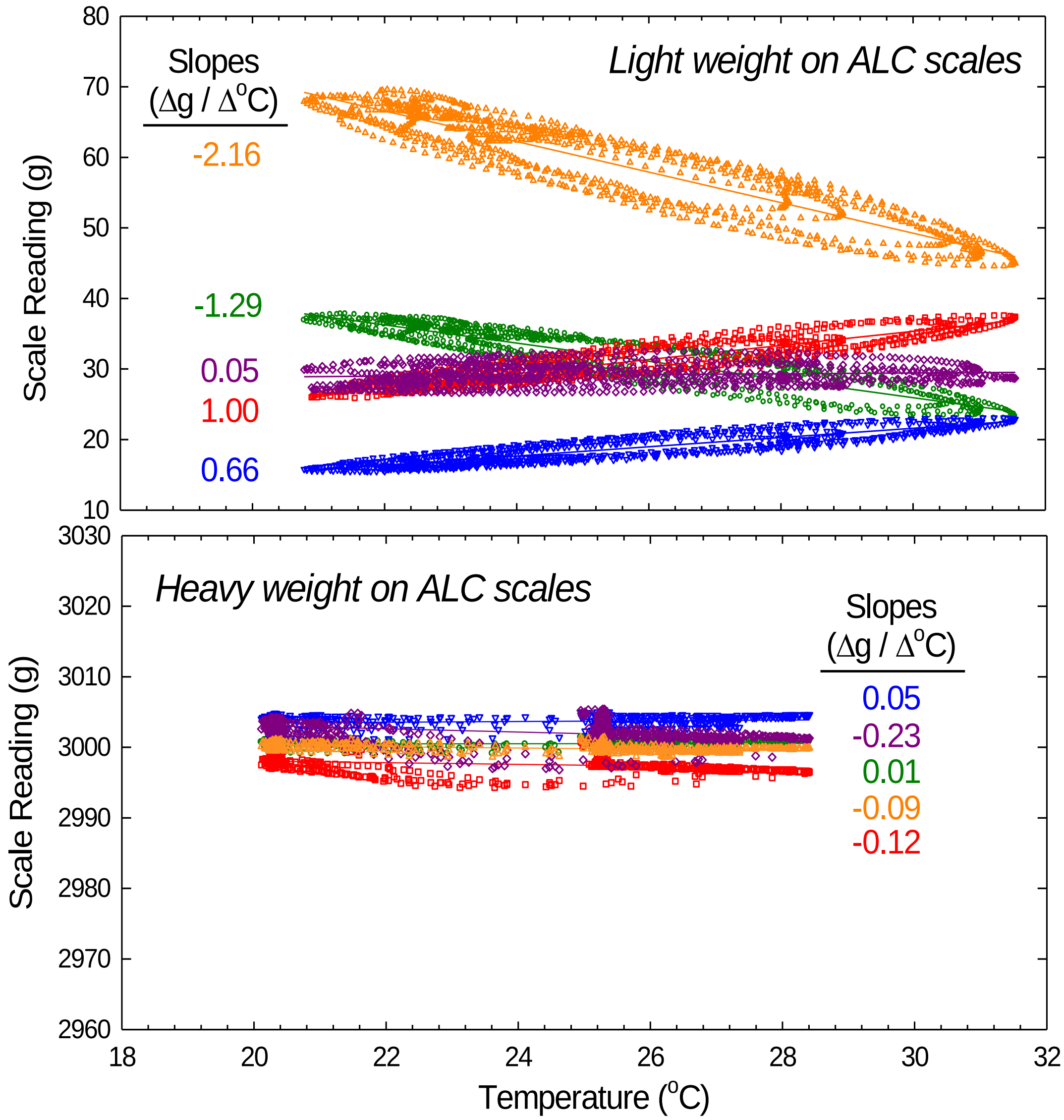

Figure 6. Effect of weight on temperature sensitivity of ALC scales. Temperature sensitivity was greater with lightweight rocks than with heavier rocks as indicated by the slopes of the lines.

Stomatal Conductance Graphs

Stomatal conductance data are presented graphically, with the number of hours that the plants were in the growth chamber on the x-axis, and stomatal conductance on the y-axis. Data for individual plants are shown, with each plant represented by a different color. MCP applications are shown as vertical, dashed, green lines. In cases where drought stress was applied, the times at which programmed weight setpoints were lowered are shown as vertical, dotted, green lines.

Integrated Normalized Daily Stomatal Conductance Graphs

In April of 2007 we began analyzing stomatal conductance data in a way that more clearly shows the differences in stomatal conductance between treatments. First, the stomatal conductance data for each individual plant were normalized to the well-watered control. This was done by dividing the peak stomatal conductance of the well-watered control plant on the day before treatment by each individual plant's peak stomatal conductance. This generated individual multipliers for each plant by which the stomatal conductance data for each plant were multiplied. This procedure normalizes the data to account for differences in transpiration rate due to plant size. An example of how this changes the way the data look is shown in Figures 7a (before normalization) and 7b (after normalization), below. Normalization may also be accomplished by dividing the stomatal conductance of a particular plant by its average stomatal conductance on the day before the treatments were started.

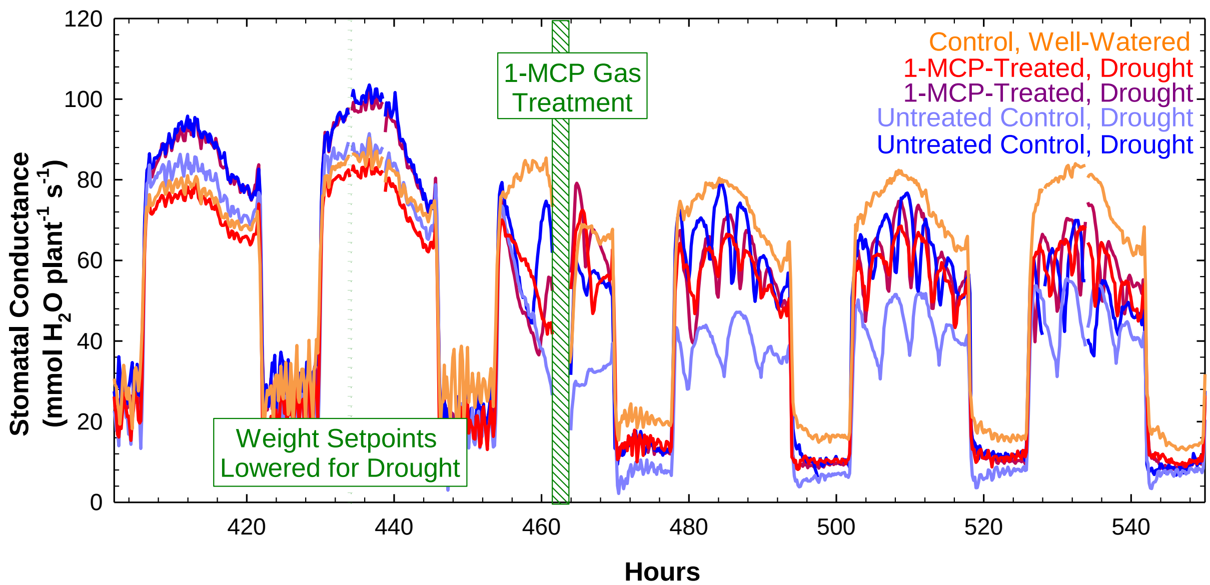

Figure 7a: Sample stomatal conductance data before normalization.

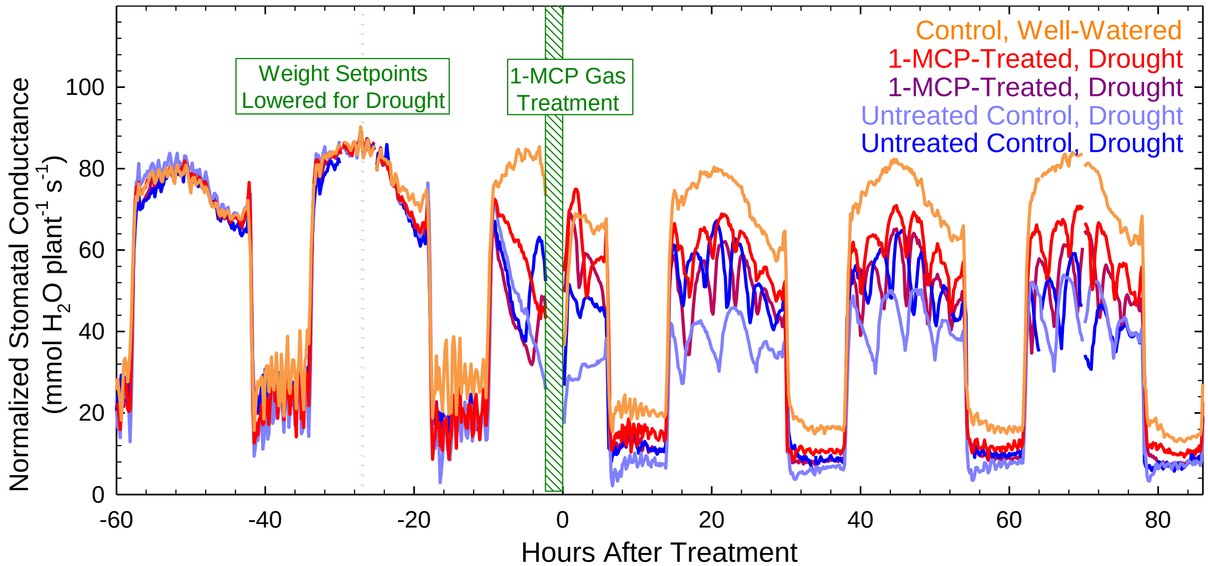

Figure 7b: Sample stomatal conductance data after normalization.

Once the data are normalized, the stomatal conductance during the first half of the light period of each day (the AM period) is “integrated” by summing all values. The same is done for the second half of the light period (the PM period). The summed data (two values per day: one for AM and one for PM) are divided by the corresponding summed data for the control to give a ratio, expressed as “% of Well-Watered Control”. The ratios are plotted against time, and the result is a graph like the one in Figure 7c.

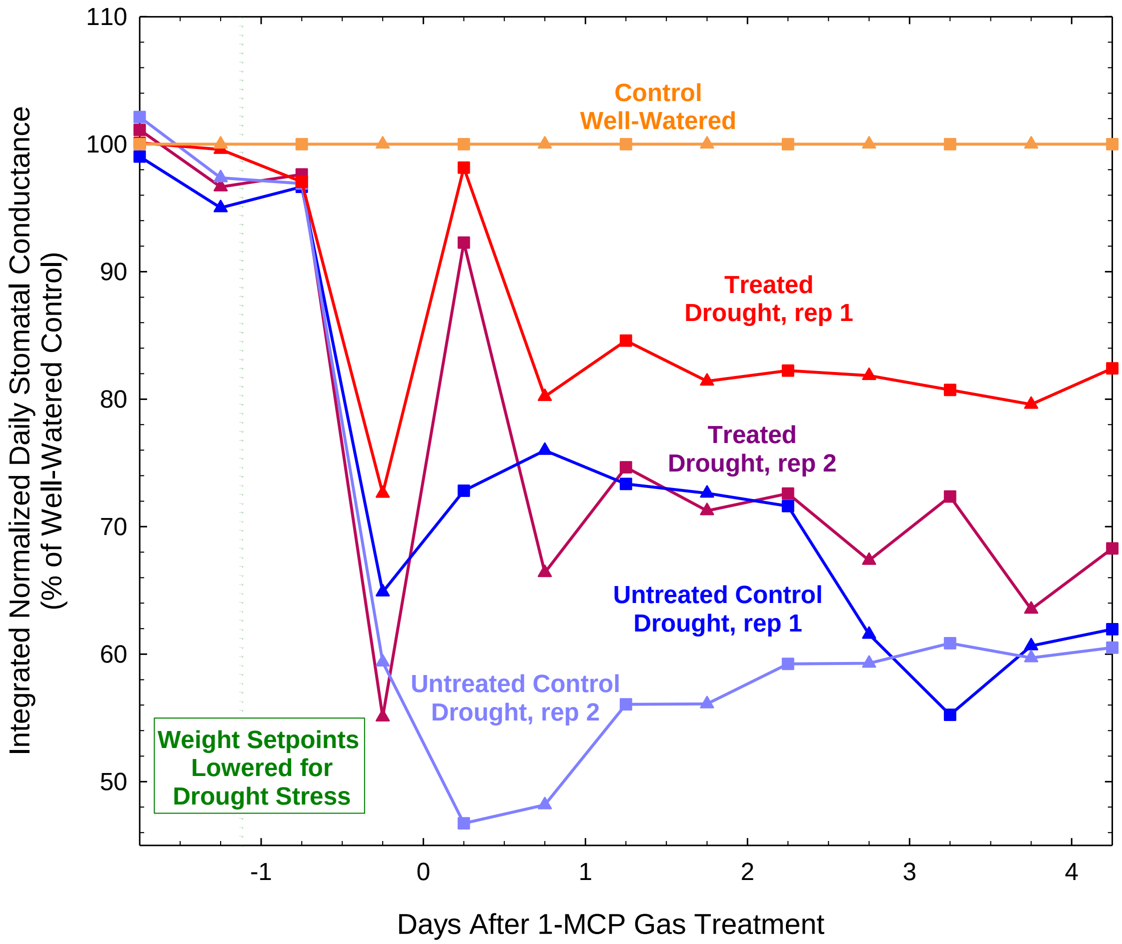

Figure 7c: Sample integrated normalized daily stomatal conductance data. Triangles represent AM data. Squares represent PM data. The raw dataset used to generate this graph is shown in Figure 7a.

Sample CR1000 Datalogger Program

The following program was written for a system that includes a CR1000 datalogger, AM416 multiplexer, and SDM-CD16 controller (Campbell Scientific, Logan, UT).

```
'CR1000 Series Datalogger
'Program to monitor temperature and RH in Units 5&6 and
' to read up to 16 Acculab scales through an AM416 multiplexer.
'Program uses temperature and RH to calculate VPG,
' and uses scale readouts to calculate transpiration rates.
'Assistance in programming provided by Kevin Rhodes at Campbell Scientific
' (krhodes@campbellsci.com, 435-750-9595)
'Potted plants are automatically watered based on balance readings.
'Watering is controlled by opening and closing individual solenoids
' for each scale using an SDMCD16 controller with
' CRBasic address = 8 (Loggernet address = 20).
'Versions starting with this one (070718) have Vicon scales in U5 & ALC scales in U6.

'ENTER STUDY/PLANT-SPECIFIC VARIABLE VALUES HERE
Const WtChk= 60  'Enter the number of minutes between weight checks
Const U5Water1= 2400 'Enter the weight on U5 scale #1 that will trigger watering
Const U5Water2= 2400 'Enter the weight on U5 scale #2 that will trigger watering
Const U5Water3= 2400 'Enter the weight on U5 scale #3 that will trigger watering
Const U5Water4= 2400 'Enter the weight on U5 scale #4 that will trigger watering
Const U5Water5= 2400 'Enter the weight on U5 scale #5 that will trigger watering
Const U6Water1= 2400 'Enter the weight on U6 scale #1 that will trigger watering
Const U6Water2= 2400 'Enter the weight on U6 scale #2 that will trigger watering
Const U6Water3= 2400 'Enter the weight on U6 scale #3 that will trigger watering
Const U6Water4= 2400 'Enter the weight on U6 scale #4 that will trigger watering
Const U6Water5= 2400 'Enter the weight on U6 scale #5 that will trigger watering
Const Duration= 3  'Enter the number of scans for the watering solenoid to stay open
Const SI= 15   'Enter the number of seconds per Scan Interval

'Declare variables for reading scales and sensors and calculating VPG and transpiration
Public PTemp, batt_volt
Public OutString as string * 16
Public InString as string * 16
Public U5Weight(5), U5Wdiff(5), U5TR(5) '***Change with # of scales***
Public U6Weight(5), U6Wdiff(5), U6TR(5) '***Change with # of scales***
Public HMP35aRH
Public RH5, RH_T5, RH6, RH_T6
Public TC
Public bytes
Public SatVP5, SatVP6, VP5, VP6, VPG5, VPG6
Dim LCount5, LCount6  'Increments the scale number when reading the scales

'Declare public variables for automated watering
Public Sol(16)                 'Must have an array of 16 to work with SDMCD16
Dim Wcount(10)                 'Counts the number of scans while watering
Dim i,j,k                                            'Variables for running loops

'Define Data Tables
DataTable (RHtest,1,-1)
        DataInterval (0,600,sec,10)
        Sample (5,U5Weight(),IEEE4)  '***Change with # of scales***
        Sample (5,U5TR(),IEEE4)  '***Change with # of scales***
        Average (1,RH5,IEEE4,False)
        Average (1,RH_T5,IEEE4,False)
        Average (1,VPG5,IEEE4,False)
        Sample (5,U6Weight(),IEEE4)  '***Change with # of scales***
        Sample (5,U6TR(),IEEE4)  '***Change with # of scales***
        Average (1,RH6,IEEE4,False)
        Average (1,RH_T6,IEEE4,False)
        Average (1,VPG6,IEEE4,False)
EndTable
```

```
SequentialMode

'MAIN PROGRAM

BeginProg

        Scan (SI,Sec,0,0)

                'Turn AM416 Multiplexer on and read scale weights
                PortSet(4,1)

                'Open serial port with parameters for Vicon scales in Unit 5
                SerialOpen (Com4,19200,11,0,10000)
                OutString=chr(27)+"P"
                LCount5=1
                SubScan(0,uSec,5) '***Change with # of scales***
                        'Switch to next AM416 Multiplexer channel
                        PulsePort(5,20000)
                        'Read weights on Vicon scales
                        SerialFlush (Com4)
                        SerialOut (Com4,OutString,"",0,100)
                        SerialIn (InString,Com4,100,chr(13),100)
                        SplitStr (U5Weight(LCount5),InString,"",1,10)
                        bytes=SerialInChk (Com4)
                        LCount5=LCount5+1
                NextSubScan
                SerialClose (Com4)

                'Open serial port with parameters for ALC scales in Unit 6
                SerialOpen (Com4,1200,9,0,10000)
                OutString=chr(27)+"P"
                LCount6=1
                SubScan(0,uSec,5) '***Change with # of scales***
                        'Switch to next AM416 Multiplexer channel
                        PulsePort(5,20000)
                        'Read weights on Acculab scales
                        SerialFlush (Com4)
                        SerialOut (Com4,OutString,"",0,100)
                        SerialIn (InString,Com4,100,chr(13),100)
                        SplitStr (U6Weight(LCount6),InString,"",1,10)
                        bytes=SerialInChk (Com4)
                        LCount6=LCount6+1
                NextSubScan
                SerialClose (Com4)

                'Turn AM416 multiplexer off
                PortSet(4,0)

                'Watering logic:
                'Check weights on scales and trigger solenoids
                If TimeInToInterval(0,WtChk,Min) Then
                        If U5Weight(1) <= U5Water1 Then
                                Sol(6)= 1
                        EndIf
                        If U5Weight(2) <= U5Water2 Then
                                Sol(7)= 1
                        EndIf
                EndIf
                If TimeInToInterval(2,WtChk,Min) Then
                        If U5Weight(3) <= U5Water3 Then
                                Sol(8)= 1
                        EndIf
                        If U5Weight(4) <= U5Water4 Then
                                Sol(9)= 1
                        EndIf
                EndIf
                If TimeInToInterval(4,WtChk,Min) Then
                        If U5Weight(5) <= U5Water5 Then
                                Sol(10)= 1
                        EndIf
                        If U6Weight(1) <= U6Water1 Then
```

```
                Sol(1)= 1
        EndIf
EndIf
If TimeInToInterval(6,WtChk,Min) Then
        If U6Weight(2) <= U6Water2 Then
                Sol(2)= 1
        EndIf
        If U6Weight(3) <= U6Water3 Then
                Sol(3)= 1
        EndIf
EndIf
If TimeInToInterval(8,WtChk,Min) Then
        If U6Weight(4) <= U6Water4 Then
                Sol(4)= 1
        EndIf
        If U6Weight(5) <= U6Water5 Then
                Sol(5)= 1
        EndIf
EndIf
'Close solenoids if weights get to be too far above setpoints.
If U5Weight(1) > U5Water1 Then
        Sol(6)= 0
EndIf
If U5Weight(2) > U5Water2 Then
        Sol(7)= 0
EndIf
If U5Weight(3) > U5Water3 Then
        Sol(8)= 0
EndIf
If U5Weight(4) > U5Water4 Then
        Sol(9)= 0
EndIf
If U5Weight(5) > U5Water5 Then
        Sol(10)= 0
EndIf
If U6Weight(1) > U6Water1 Then
        Sol(1)= 0
EndIf
If U6Weight(2) > U6Water2 Then
        Sol(2)= 0
EndIf
If U6Weight(3) > U6Water3 Then
        Sol(3)= 0
EndIf
If U6Weight(4) > U6Water4 Then
        Sol(4)= 0
EndIf
If U6Weight(5) > U6Water5 Then
        Sol(5)= 0
EndIf
'Increment counter for open solenoids
For i = 1 to 10                '***Change with # of scales***
        If Sol(i)=1 Then
                Wcount(i) = Wcount(i) + 1
        EndIf
        'Close solenoids at end of watering period
        If Wcount(i) >= Duration Then
                Sol(i) = 0
                Wcount(i) = 0
        EndIf
next i

'Read sensors connected to CR1000
TCDiff (TC,1,mV2_5C,1,TypeE,PTemp,True,0,250,1.0,0)
VoltSE (RH5,1,mV2500,6,1,0,250,0.001,0)
VoltSE (RH_T5,1,mV2500,5,1,0,250,0.1,-40)
VoltSE (RH6,1,mV2500,8,1,0,250,0.001,0)
VoltSE (RH_T6,1,mV2500,7,1,0,250,0.1,-40)
VoltSE (HMP35aRH,1,mV2500,9,1,0,0,0.001,0)
PanelTemp (PTemp,250)
Battery (Batt_volt)
```

```
		'Transpiration rate calculations.  Calculate T on 10-minute intervals.
		For k = 1 to 5
			U5Wdiff(k)=RHtest.U5Weight(k,2)-RHtest.U5Weight(k,1)
			U5TR(k)=U5Wdiff(k)/10 'Transpiration rate in g/min
		next k
		For j = 1 to 5
			U6Wdiff(j)=RHtest.U6Weight(j,2)-RHtest.U6Weight(j,1)
			U6TR(j)=U6Wdiff(j)/10 'Transpiration rate in g/min
		next j

		'Vapor pressure gradient calculations.
		'Limit max value of RH (expressed as a fraction) to 1.
		If RH5 >= 1 Then RH5 = 1
		If RH6 >= 1 Then RH6 = 1
		'Compute the saturation vapor pressure in kPa.
		SatVP (SatVP5,RH_T5)
		SatVP (SatVP6,RH_T6)
		'Compute the vapor pressure in kPa (using RH as a fraction).
		VP5 = SatVP5*RH5
		VP6 = SatVP6*RH6
		'Compute the leaf-to-air vapor pressure gradient (kPa).
		VPG5 = SatVP5-VP5
		VPG6 = SatVP6-VP6

		'SDMCD16 Controller instruction
		SDMCD16AC(Sol(),1,1)

		'Call Output Tables
		CallTable RHtest

	NextScan
EndProg

'WIRING OF LOGGER TO MPXR and SDMCD16:
'
'CR1000   AM416       Sensor         SDMCD16
'---------------------------------------------
'C1                                   C1
'C2                                   C2
'C3                                   C3
'G                                    GND
'+12V                                 12V
'C4       Reset
'C5       Clock
'G        GND
'+12V     12V
'C7       COMH1
'C8       COML1
'G        COMH2
'G        COML2
'         1 H1        Rx [Weight(1)]
'         1 L1        Tx
'         1 H2        G
'         1 L2        Shield
'         2 H1        Rx [Weight(2)]
'         2 L1        Tx
'         2 H2        G
'         2 L2        Shield
'         ..... on to Weight(up to)16
```