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# Dynamic Classification of Moisture Stress Using Canopy and Leaf Temperature Responses to a Step Changes of Incident Radiation

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Dynamic Classification of Moisture Stress Using
Canopy and Leaf Temperature Responses to a
Step Changes of Incident Radiation

An Undergraduate Honors Thesis
Submitted in Partial Fulfillment of
University Honors Program Requirements
University of Nebraska-Lincoln

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April 16, 2018

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# Abstract

Environmental conditions affect plant productivity and understanding how plants respond to drought stress can be measured in different ways. This study focused on measuring leaf response time to induced water stress. Leaf response time to a step increase and step decrease in radiation was computed for four species of well-watered and water-stressed plants in a controlled environment. The canopy temperature was measured with an infrared thermometer and a thermal imaging camera. Thermal images were analyzed to determine the average temperature of a selected single, unobstructed leaf at the top of the canopy. Both the canopy response time and the single leaf response time were computed for this study. The response times to a step change of radiation for well-watered plants were generally longer than the response times of water stressed plants. These results show that response time may be used as an indicator of plant water stress.

Keywords: Response time, moisture stress, canopy temperature, thermal imaging, leaf temperature

## Background

Plants are dynamic control systems that constantly react to the environment that surrounds them. Understanding how plants respond to various environmental factors is crucial for increasing crop quality, while decreasing the amount of resources required to grow the crop. In order to feed the growing world population, it is crucial to maximize yield while minimizing the resources used in the agriculture industry. Plants respond to environmental stimuli, and the "speaking plant" approach suggests that optimal cultivation conditions should be determined by the physiological response of a plant (Hashimoto et al., 1985). Studying how a plant responds to different conditions could lead to more efficient irrigation systems, which would reduce water usage and increase crop quality, both in greenhouses and in the field (Bajwa et al., 2007).

One way that plants act as a dynamic control system is through transpiration. Leaves are able to evaporatively cool themselves through moisture exchange through tiny pores on leaf surfaces called stomates. When the stomates are open, water can exit the leaf, and therefore lower the temperature of the leaf as the water evaporates. However, when a plant is not receiving enough water, the roots will produce chemical signals telling the plant to conserve water by closing the stomates. These signals are sent from the roots, through the xylem to the shoots (Grant et al., 2007). Stomatal closing results in the loss of temperature control through transpiration, and causes the overall temperature of the plant to increase. This higher canopy temperature leads to more water use, which is inefficient (Al-Faraj et al., 1994).

Monitoring how stomatal conductance changes with time would be a very effective system for determining plant stress. However, measuring stomatal conductance is generally an invasive procedure, and only provides a single data point (Grant et al., 2007). Therefore, leaf or canopy temperature is often used as an indirect measurement of stomatal conductance, and therefore an indicator of the stress level of the plant (Bajwa et al., 2007, Prenger et al., 2005, Grant et al., 2007, Boonen, et al., 2000, Al-Faraj et al., 1994, 2000, 2001). However, it must be noted that canopy resistance is not a simple sum of all of the leaf resistances, and therefore studying canopy responses always has some degree of uncertainty associated with it (Pitacco et al., 1996). Despite this uncertainty, canopy temperature is widely used to determine the stress level of plants, and is a major factor in the design of irrigation systems (Prenger 2003).

An important index used in irrigation control systems is the Crop Water Stress Index (CWSI). The CWSI measures the crop stress level at a given point in time using baselines, developed from transpiring, well-watered, and non-transpiring plants (Prenger 2003). The CWSI can be determined from the canopy temperature either empirically or theoretically (Idso et al., 1981, Jackson et al., 1981). There are limitations to using the CWSI as an irrigation scheduling tool since the empirical calculation requires assumptions that are not always true in the field, for example, clear sky conditions, and constant wind speed (Payero et al., 2005). It has been shown that solar radiation levels and wind speed can both affect the transpiration rates, therefore affecting the CWSI. For this reason, the CWSI may not be the most accurate measurement of the plant stress. A system that takes into account the dynamic nature of plant water relationships should be developed in order to provide the most accurate information about the plant at a given time.

Before infrared thermometry and thermal imaging became widely available, invasive methods of measuring stomatal resistances and leaf temperature were used to try to determine plant stress. These methods relied on a simple leaf energy balance and psychrometrics model to compute a stomatal resistance value.  The device called the steady-state porometer is available commercially. However, both infrared thermometry and thermal imaging provide non-invasive methods for collecting accurate temperature data from a leaf or a canopy, without interfering with the plant growth (Prenger et al., 2005, Grant et al., 2007). Infrared themocouples (IRT/c) use Planck's radiation equation to detect the amount of radiation that a surface emits or absorbs, which is proportional to the fourth power of the absolute temperature of the surface (Cengel & Boles, 2002). Infrared thermometers must be calibrated using a standard black body to ensure that they are giving correct readings. One limitation to using an infrared thermometer or an IRT/c is the field of view, or what surface the sensor "sees". These sensors simply take a weighted average of the temperatures of the objects in the field of view (Woebbeck et al., 1994). In order to get an accurate temperature reading, it is crucial to eliminate unwanted objects from the field of view. However, this is difficult when measuring the canopy temperature, especially if the leaves are spaced apart because the infrared sensor will inevitably measure some of the soil temperatures. Because of these limitations, thermal imaging is being used in combination with infrared thermometers to try to eliminate some of the background noise in the temperature readings. Thermal images can be analyzed in such a way that the areas of interest (i.e. the canopy or a single leaf), can be selected and isolated from the image. Therefore, the average temperature of only the areas of interest can be calculated. Many studies have looked into the possibility of using thermal imaging to gather canopy temperature data because it is easy to gather information in real time and it does not interfere with the normal plant growth and development functions (Grant et al., 2007). Thermal imaging has been used for a wide range of applications when studying plant response and plant stress. One study utilized thermal images to show how a water deficit develops in each individual leaf. They found that the edges of the leaves closed their stomates before the central sector, which means that the water status of a plant determined by a porometer could be very different depending on the location on the leaf that a sample is taken from (Hashimoto et al., 1984). Another study used this information along with thermal imaging to create calibration curves to help estimate the stomatal conductance's of different parts of the leaf (Jones et al., 1999). The combination of thermal imaging and visible light imaging was used to detect water stress in both drought and flood conditions for Sunagoke moss (Ondimu et al.,2008). Thermal imaging has had a wide array of applications and has already been used extensively in the field as a tool to help determine canopy temperature. Thermal imaging in combination with infrared thermometry means that accurate data can be taken rapidly to monitor plant water status in real time.

Solar radiation is a very important factor that affects canopy temperature. In the field, a passing cloud can cause a step change in radiation received by the plant (Ondimu et al., 2008, Al-Faraj et al., 2000, Payero et al., 2005). Al-Faraj's study looked into the effect that step changes in irradiation had on well-watered, moderately stressed, and severely stressed plants, using both a mathematical model and comparisons with actual measurements. They found that well-watered plants showed a second order, underdamped response, but as water stress increased, the damping response decreased, and ultimately disappeared in the severely stressed plants, which tended to show a first order temperature response, as opposed to the second order response seen in well-watered plants. This study also showed that as the plant water stress increased, the transpiration rate decreased, and the severely stressed plants had a higher ending temperature than the well-watered plants (Al-Faraj et al., 2000). Another study showed that different plant physiological response processes occur in a “lights on” versus a “lights off” situation; suggesting that the “lights on” situation required three dynamic processes, while the “lights off” situation only relied on two processes (Boonen et al., 2002). Ultimately, incident solar radiation is a factor that must be considered in canopy temperature measurements.

There is considerable evidence that plants are dynamic control systems that are constantly responding to their environment (Boonen, 2005, Prenger et al., 2005) There are studies that show the effect that radiation has on the canopy temperature of a plant, but these studies have not considered the time it takes for a plant to begin to regulate its temperature, also known as the plant response time. The response time of plants has the potential to be used as an indicator of a stressed plant. Well-watered plants are assumed to transpire at maximum rates, thus cooling the plant faster than a stressed plant that is not transpiring at a maximum rate (Schymanski et al., 2013). Monitoring temperature changes over time is also a way to observe the dynamic stomatal control of a leaf in action. While the leaf approaches the steady state temperature, small temperature deviations that look similar to a sine wave may be seen, which would indicate that the leaf is actively opening and closing the stomates to maintain its temperature. However, in a stressed plant, this sine wave form may not be visible since the plant is not able to transpire to regulate its temperature. The steady state canopy temperature of a well-watered plant is generally lower than that of a water-stressed plant due to the lack of transpiration of the water-stressed plant (Al-Faraj, et al., 2000). This study focuses on measuring leaf and canopy temperatures in response to step changes in radiation, as a potential indicator of water stress levels. If there is indeed such a correlation, a simple test that only takes a few minutes could be done to show a plant’s stress level. This has the potential to have an impact on irrigation scheduling both in greenhouses and in the field.

## Hypothesis

Plants subjected to different levels of water stress respond differently to changes in their environments. Acquiring the ability to determine when a plant is stressed is crucial for designing irrigation systems and selection of cultivars developed using genomic technology. Due to the evaporative cooling process, the well-watered plants may be expected to have longer temperature response times than the stressed plants. The well-watered plants may also be expected to have lower steady state temperatures than the stressed plants because well-watered plants should be able to better regulate their leaf temperatures. These hypotheses were tested using a variety of plants in a special, instrumented, growth chamber. Temperature versus time graphs were acquired and plotted in real time using a LabVIEW® program with instrumentation to be described in the Materials and Methods section. Response times using thermal images of the plants were also analyzed.

## Study Objectives

In this study, the temperature response to a step change in radiation was investigated for both well-watered and water-stressed plants. The specific objectives were:

1. To measure first-order temperature response times of well-watered and water-stressed plant canopies to a step radiation increase and decrease
2. To compare response times of different plant species
3. To statistically compare whole canopy response times and individual leaf response times

# Materials and Methods

## Response Time Mathematics

The response time of the leaf is an important factor for many processes, particularly photosynthesis, respiration, and transpiration of a green plant. To study leaf thermal response, a LabVIEW State Machine program was used to determine both rise time and decay time constants for different air temperatures, soil water condition, and short-wave radiation. More details about how the program was developed and used to determine these constants can be found in the LabVIEW Programs section of Materials and Methods. The rise time constant method described below assumes that the air temperature is constant. Leaf temperature will rise to a steady state value when subjected to a sudden increase in short wave radiation. Similarly, the fall or decay time constant method described below assumes that the function decays to a constant air temperature. In this study, the initial value in the light-on test, and the steady state value in the light-off tests was assumed to be the air temperature, which has been incorporated into a differential equation for temperature change. The first law of thermodynamics provides an energy balance statement for a leaf, which is simply given as:

$$Q_{net} = \Delta H \qquad (1)$$

where:

$Q_{net}$ = net leaf heat absorption rate from solar or artificial lighting sources and heat losses, kJ $s^{-1}$.

$\Delta H = m \cdot cp \cdot \frac{dT_{leaf}}{dt}$ is the intrinsic change in leaf internal energy plus a small amount of boundary work expansion or contraction, or leaf enthalpy, kJ $s^{-1}$.

The leaf's dynamic temperature change $\frac{dT_{leaf}}{dt}$ may be approximated as a first order differential equation, given as:

$$\frac{dT_{leaf}}{dt} + \frac{T_{leaf}}{\tau} = \frac{T_m}{\tau} \qquad (2)$$

where:

$T_{leaf}$ is the current leaf temperature at time t, °C,

$T_m$ is the maximum steady-state leaf temperature, close to air temperature $T_{air}$, °C.

A is the exposed surface area of the leaf to the LED radiation, $m^2$.

h is a leaf to air, latent-sensible convective heat transfer coefficient, kJ/ sec·m$^2$·$^o$C.

m is an approximate mass of the leaf, kg.

$c_p$ is the specific heat of the leaf, kJ /kg $^o$C.

t is the elapsed time, sec.

and, four of the above factors can be combined into a single time constant τ.

$\tau = \frac{m \cdot c_p}{h \cdot A}$ is the response time coefficient for rise or decline in leaf temperature, sec. τ is a relationship for the thermal mass of a leaf or canopy, along with a sensible and latent heat exchange coefficient h between the plant and its surroundings.

## Rise Time Constant

The 63% method of determining the rise time constant was used to determine the response time of the leaf in the light-on tests. Integrating Equation 1, a general form of a step rise response process can be written as:

$$T(t) = (T_{leaf} - T_{air}) \cdot (1 - e^{-t/\tau}) + T_{air} \qquad (3)$$

where T(t) = temperature at which the response time is determined; $T_{leaf}$ is the steady state leaf temperature; $T_{air}$ = surrounding air temperature (constant); t = elapsed time (s); and τ = time constant. When the time constant (τ) is equal to the elapsed time (t), the exponent becomes -1, and the overall equation becomes:

$$T(t) = (T_{leaf} - T_{air}) \cdot (0.632) + T_{air} \qquad (4)$$

Based on Equation 4, the time constant for a rise response is equivalent to the time it takes for the output response to reach 63.2% of the steady state value. The light on tests conducted in this study follow this general equation form, therefore the response time could be calculated using the 63% method.

## Decay Time Constant

The 37% method of determining the decay time constant was used to determine the cooling response time of the leaf with the light-off tests. The general form of a step decay response process can be written as:

$$T(t) = (T_{leaf} - T_{air}) \cdot (e^{-t/\tau}) + T_{air} \quad (5)$$

where T(t) = temperature at which the response time is determined; $T_{leaf}$ = initial leaf temperature; $T_{air}$ = surrounding air temperature (constant); t = elapsed time (s); and τ = time constant.

When the time constant (τ) is equal to the elapsed time (t), the exponent becomes -1, and the overall equation becomes:

$$T(t) = \left(T_{leaf} - T_{air}\right) \cdot (0.368) + T_{air} \quad (6)$$

Based on Equation 6, the time constant for a decay response is equivalent to the time it takes for the output response to reach 36.8% of the initial value. The light off tests conducted in this study follow this general equation form, therefore the response time could be calculated using the 37% method.

## Study Plants

This study used four types of plants, which included common house plants and basil. Some of the plants were bought from a local garden center, and other plants were grown in the east campus greenhouses at the University of Nebraska – Lincoln. Two plants of the same species were tested at a time, and plants were chosen to be as similar in structure as possible to minimize differences in temperature and response time due to other variables. Species used in this study included green basil, golden pothos, prayer plant, and green oxalis, all grown in pots. Table 1 lists and shows images of the plants used in this study. All healthy plants were allowed to dry down.  To prepare for tests, half of dried down pots were watered (or irrigated) and allowed to sit for two hours.

Table 1. Common Name, Scientific Name and Photos of Four Plants Presented in the Study

| Common Name | Scientific Name | Image |
|---|---|---|
| Basil | *Ocimum basilicum* |  (Dyer, 2018) |
| Golden Pothos | *Epipremnum aureum* |  (Guide-to-Houseplants.com, 2018) |
| Maranta Prayer Plant | *Maranta leuconeura* |  (Costa Farms, 2018) |
| Green Oxalis | *Oxalis triangularis* |  (Plant Identification, 2018) |

## Growth Chamber Tests

All tests were conducted in a CONVIRON E15 Reach-in environmental chamber (Controlled Environments Ltd, Winnipeg, Canada), located in Chase Hall, University of Nebraska, Lincoln. Air temperature was set to a constant 22ºC. Lighting was provided by a Light Emitting Diode (LED) bank (S720 Advance Spectrum MAX LED Grow Light Panel, GrowAce.com, division of Niche Webstores, Inc, CA), designed for growing plants. The LED full spectrum lights consisted of 240 diodes and had a footprint of illumination of 0.9 x 1.7 m, with a High Intensity Discharge (HID) equivalent of 960 W. All tests during this study were run for a period of 10 minutes. Samples of air and leaf temperatures, humidity, soil moisture, and radiation (photosynthetically active radiation –PAR) along with thermal images of the canopy were acquired at two per minute during each test period. Each plant was subjected to both an "LED lights-on" and an "LED light-off" test. The lights-on test was conducted first, and the lights-off test was conducted immediately after the conclusion of the lights-on test. The lights-off test began with a plant that was already at a high leaf temperature, so that cooling could be observed. After the data acquisition phase of the test, response times were calculated using the LabVIEW state machine. The data was saved to be revisited and analyzed later. The thermal images of the canopy were acquired simultaneously with the data, but analyzed after each different test was run. For the image analysis, one whole leaf was selected from the top of the canopy, outlined with the mouse, and analyzed. The selected leaves generally faced the LED bank, and followed the timed data samples on the same plant. Maximum and minimum target temperature ranges were recorded on each image. A formula was developed and used to map the image pixel intensities to corresponding temperatures. An average temperature and standard deviation was then computed for the outlined leaf. For each different test, each individual thermal image was analyzed to follow changes in leaf temperature. All data from each test was saved together to provide a complete set of average leaf temperatures.

## Light, Plant, and Soil Sensors

The volumetric water content (VWC) of the soil media was measured as a voltage using a Decagon EC-5 capacitance sensor (Decagon Devices Inc., Pullman, WA, USA). This sensor can measure water content with an accuracy of +/- 3% for a variety of soil types (Decagon Devices, Inc., 2010). The background wall temperatures in the chamber were measured by an Omega OS36 IRT/c sensor (Omega Engineering Inc., Stamford, CT, USA), which acts like a type K thermocouple (Omega Engineering, Inc., 1994). The canopy temperature was measured with a Mikron MI-N3000 infrared sensor (Mikron Infrared, Oakland, NJ, USA) placed just above the plant. The MI-N3000 sensor has a field of view of 5:1, meaning that at a distance of 50 cm, the spot size of the sensor would be 10 cm. The MI-N3000 also has a response time of 300ms and can measure temperatures from 0°C to 500°C with an accuracy of 1.5% of that range (Mikron Infrared). The relative humidity of the chamber was measured using a LabJack EI1050 temperature and relative humidity probe (LabJack Corporation, Lakewood, CO, USA) which can measure relative humidity to an accuracy of +/- 3.5% (LabJack, 2018). The photosynthetically active radiation (PAR) was measured by the Li-190SA quantum sensor (LI-COR Inc., Lincoln, NE, USA). Irradiance was also measured using two Eppley Precision Spectral Pyranometers placed on opposite sides of the chamber (Campbell Scientific Inc., Logan, UT, USA). Thermal images (320 x 240 pixels) were acquired using a FLIR E-60 handheld thermal camera (FLIR Systems, Wilsonville, OR, USA) placed on a tripod in the growth chamber and focused on the plant leaves. The FLIR E50 has a thermal sensitivity of less than 0.05 $^{o}$C and an accuracy of $\pm$ 2 $^{o}$C.  The FLIR camera was remotely operated as a webcam from a near-by Windows 10 desktop computer, as part of A LabVIEW program described below. An emissivity value of 0.95 was set on the camera. A labeled schematic showing the key elements of the complete setup is shown in Figure 1, and photographs of the setup are shown in Figure 2.

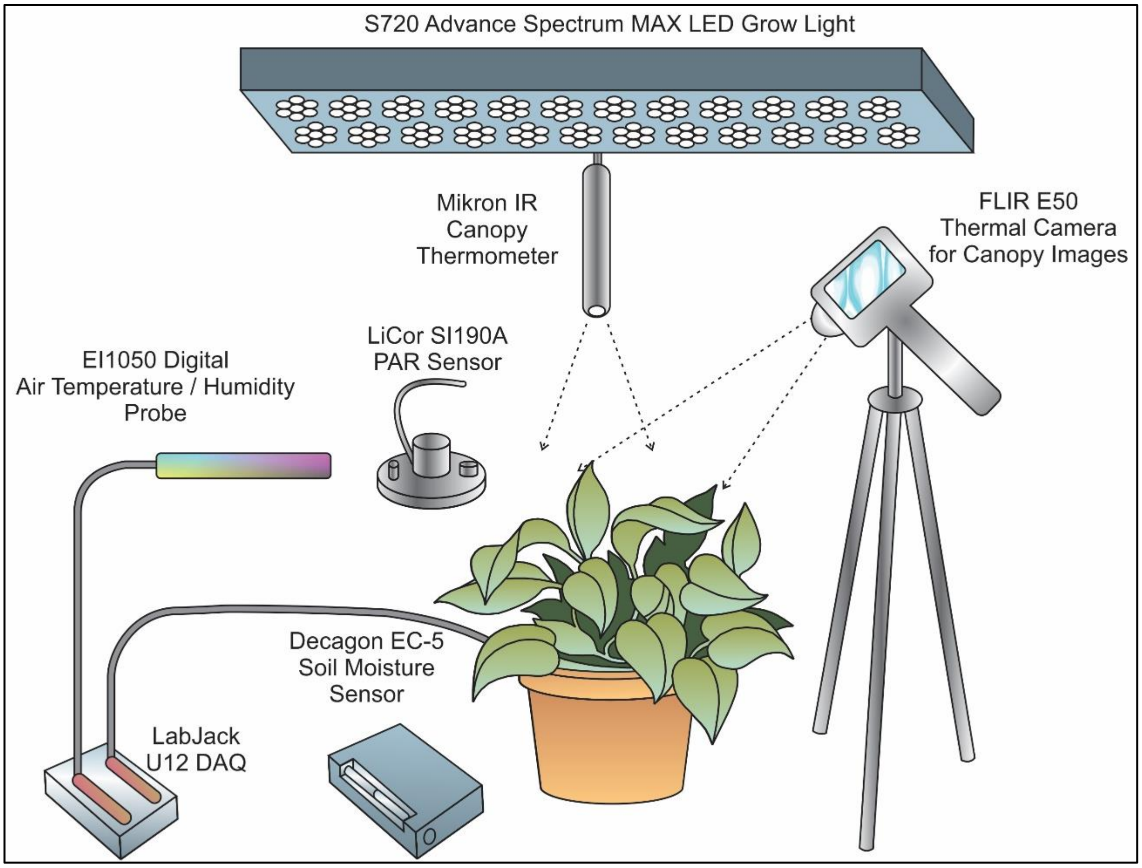

Figure 1. Schematic showing the key elements of the growth chamber setup.

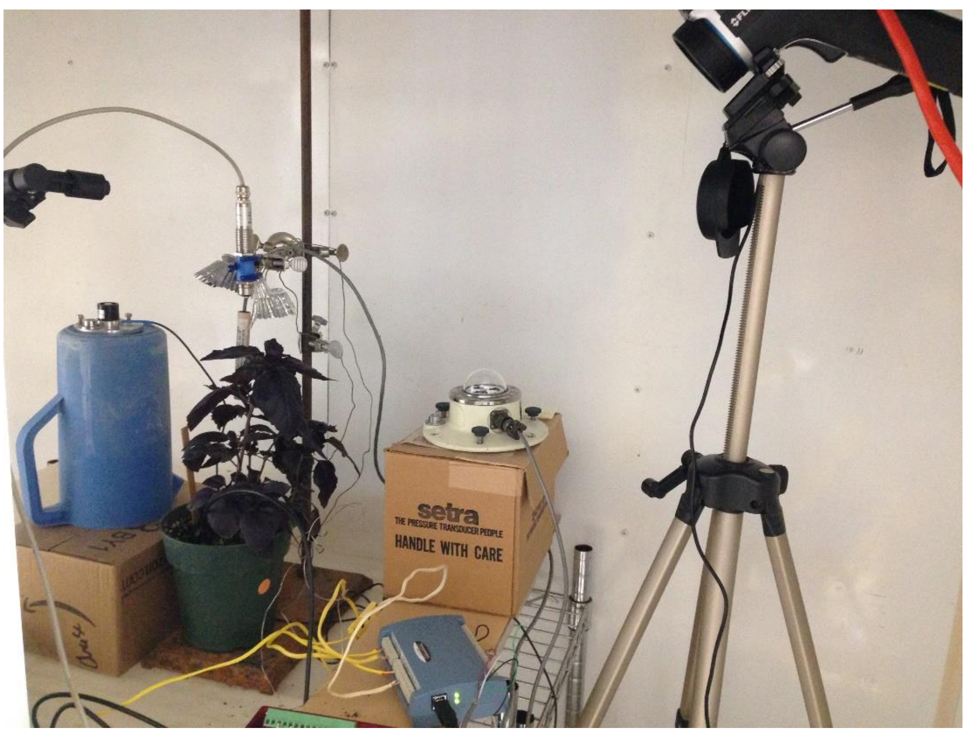

Figure 2. Photograph showing the setup used inside the growth chamber

## Data Acquisition and Data Processing

Two data acquisition devices and sensors were used concurrently in this study. One channel of a two-channel USB ProXR controller (National Control Devices, LLC, Osceola, MO, USA) was used to control the LED bank using LabVIEW. A Measurement Computing 8 Channel Thermocouple and Voltage USB DAQ Module (Measurement Computing Corporation, Norton, MA, USA) was used for the solar irradiance sensors and the type K thermocouple sensors. One thermocouple (Omega OS36 IRT/c) was used for determining the background wall temperature of the chamber, and the other thermocouple was connected to a black body plate as a reference point temperature. A LabJack U12 (LabJack, corp., Denver, CO) multifunction device was used for acquiring air temperature and relative humidity data using the LabJack 1050 Sensiron sensor, as well as the Decagon soil moisture sensor data.

## LabVIEW Processing Programs

Two separate LabVIEW 2015 (National Instruments, Austin, TX, USA) programs or Virtual Instruments (VI) were programmed and used for this study. Computer programming was assisted by Dr. Meyer. Each program was developed in the form of a LabVIEW state machine for user selection of various operations such as; to acquire data and thermal images, compute response times, analyze thermal images, and save the results to file. The state machine uses a combination of event, case, and loop structures (all within a single while loop for continuous operation, until the user stops the program). Each state is dependent either on a previous state, or on a user selected function. For the data and thermal image acquisition state machine, the user was able to select the length of time they wished to acquire data, and the number of samples that are taken each minute. After selecting these parameters, data could be acquired with the LED lights on or off. The program collected and temporarily stored data from all of the sensors using shift registers, as well as capturing and saving images from the FLIR E50. All data was then saved to a selected Windows folder.  After the leaf temperature data was collected for the desired length of time, the user was also able to go to a different tab on the front panel to immediately conduct a leaf response time analysis. The leaf response time subVI utilized three different user cursors to determine the response time constants from the temperature –time graph. A near virtual cursor was placed on the initial data point in the graph, and the far virtual cursor was lined up to the steady state temperature value. Next, a target virtual cursor was moved along the graph until it reached either 63% for a rising graph or 37% for a decaying graph. Once all cursors were placed properly, the response time was immediately calculated and displayed. The user had the option to save the data collected for each sample data point over time from all the sensors in the chamber into a convenient displayed table which could be easily transferred into Microsoft Excel for further analysis, if desired. A flow chart showing the basic operation of the data acquisition LabVIEW program is given in Figure 3. A screenshot of the LabVIEW program Front Panel shown in action in Figure 4.

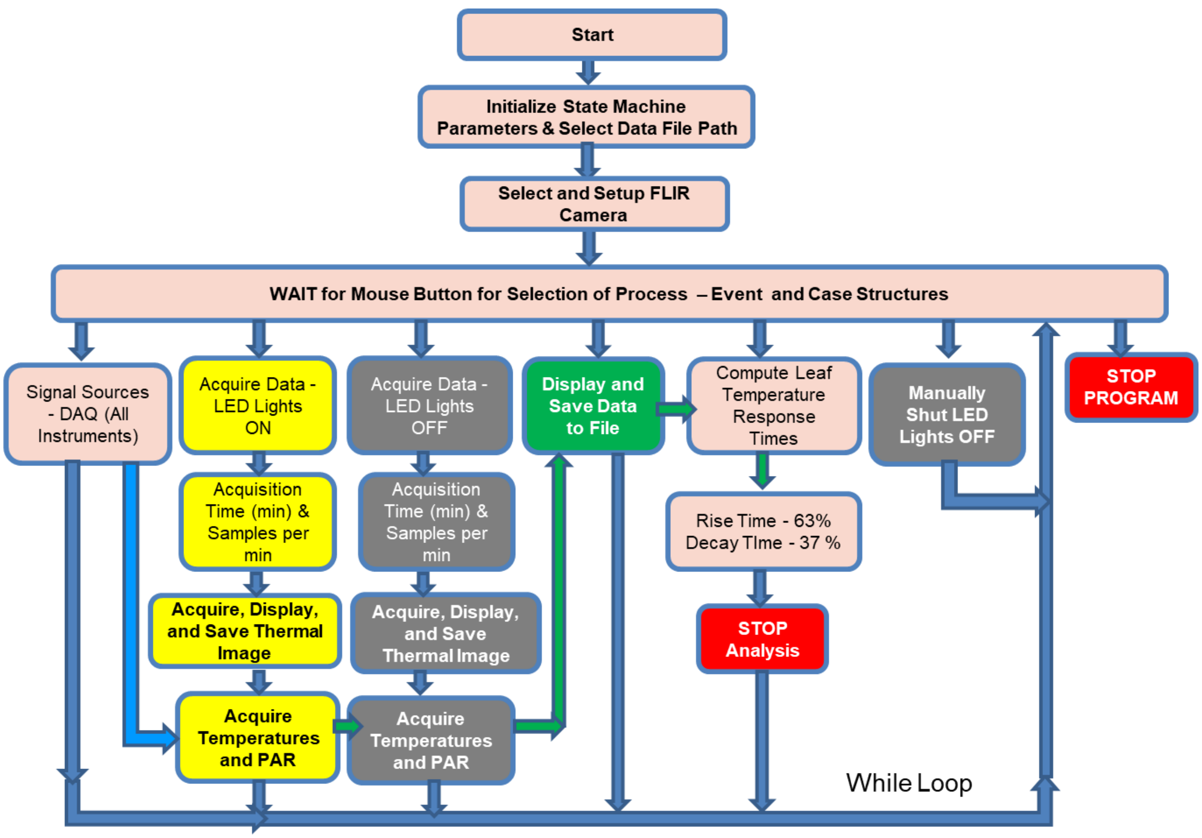


Figure 3. Flow chart showing the operations of the LabVIEW Data Acquisition State Machine program

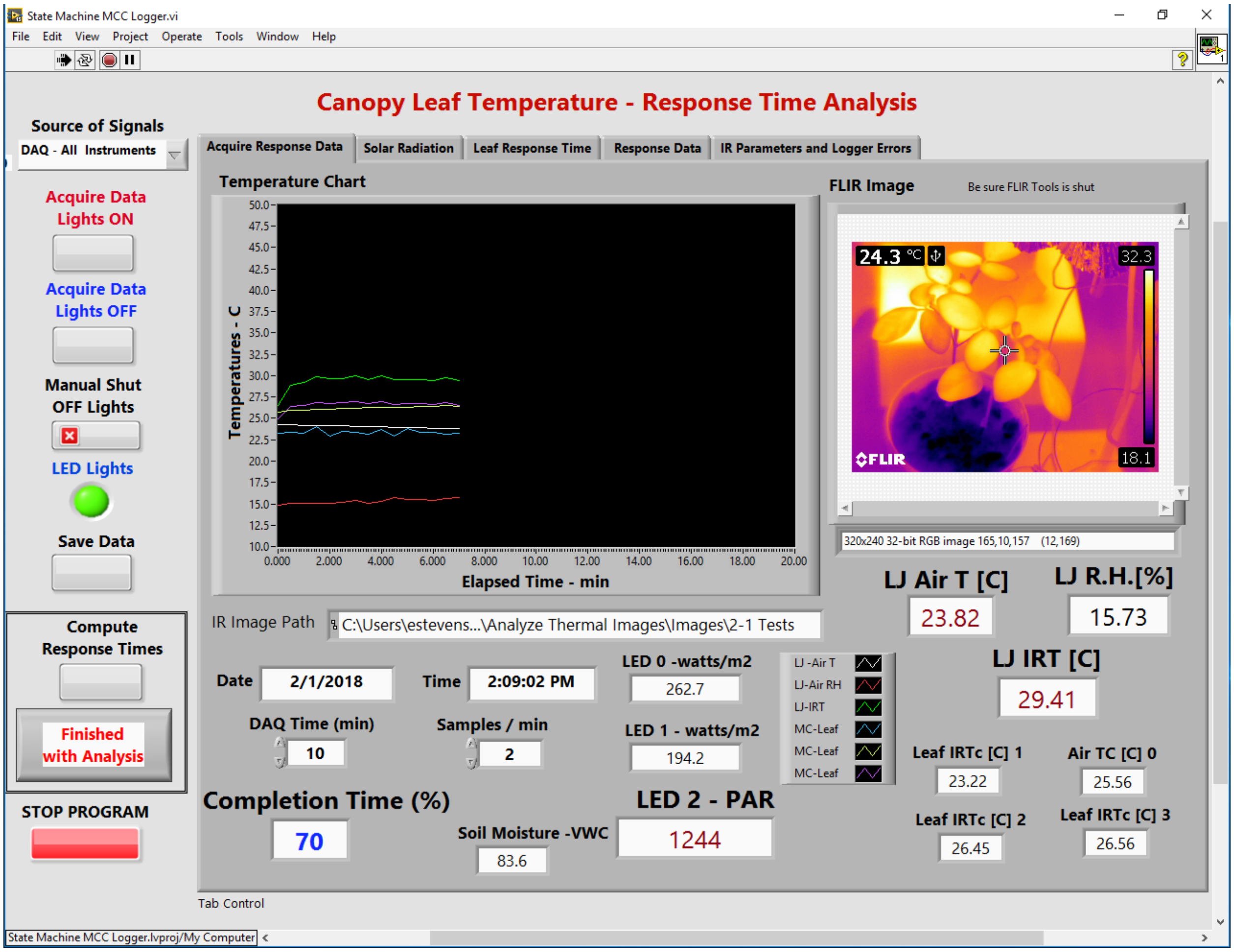


Figure 4. Front Panel of the LabVIEW data acquisition program.

The second LabVIEW program designated as the “Thermal Image Analyzer” was also used in this study. The Thermal Image Analyzer allowed the user to import a previously saved thermal image, as a .jpeg. These images are tonal grayscale images, with the brightness integers or pixels corresponding to a leaf temperature. Each image must be calibrated for leaf temperatures. To calibrate the image, the user sets the minimum and maximum temperature values, recorded and present on each FLIR image. She/he then selected the corresponding locations of minimum and maximum temperatures on the image scale represented by the darkness and brightness pixel regions, using the mouse. Next, the user outlined with the mouse a specific whole leaf edge contour on the thermal image (in this case, the shape of a specific leaf). The program then calculated the average leaf temperature and standard deviation of the selected leaf. When the contour was closed, the user was able to do this analysis for a sequence of thermal images and then save final data. When the user chose to save the final data, the average leaf temperatures and corresponding standard deviation since the last save was compiled into a table. A flow chart showing the basic structure of the thermal leaf image analysis program is shown in Figure 5. A screenshot of the LabVIEW program Front Panel in action is shown in Figure 6. More information on each of these LabVIEW programs may be obtained from the Biological Systems Engineering Department at the University of Nebraska – Lincoln.

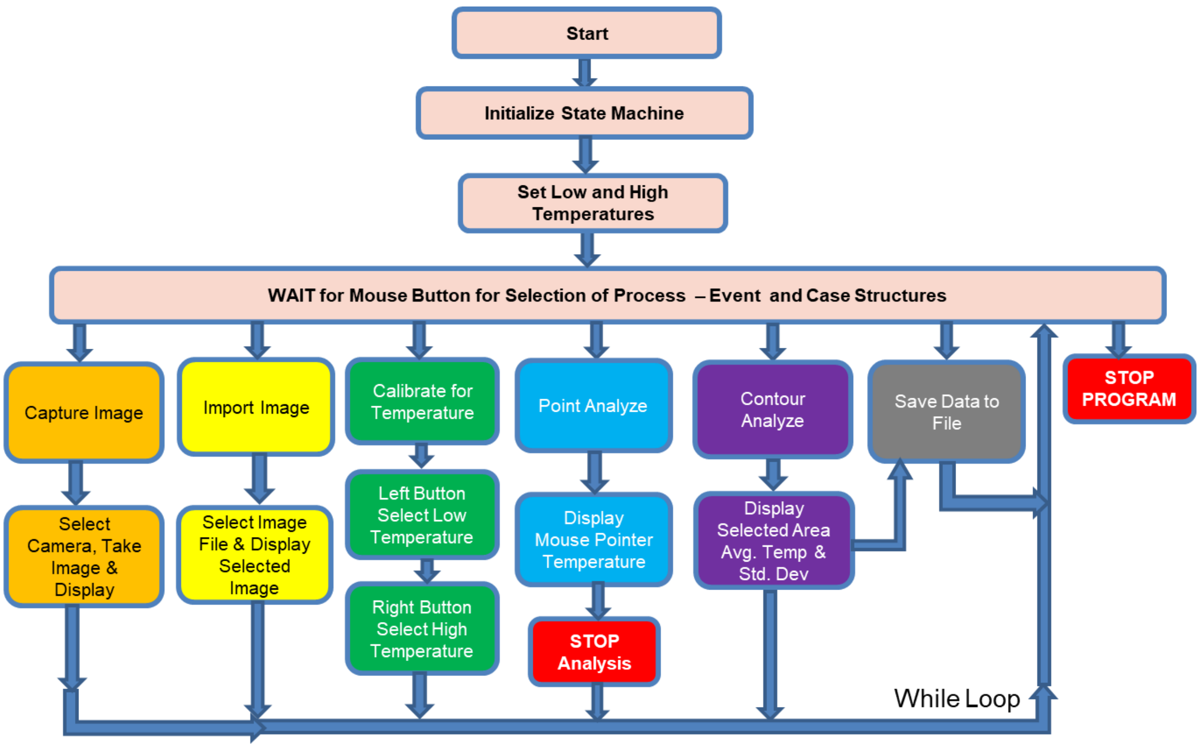

Figure 5. Flow chart showing the operations of the Thermal Image Analysis program.

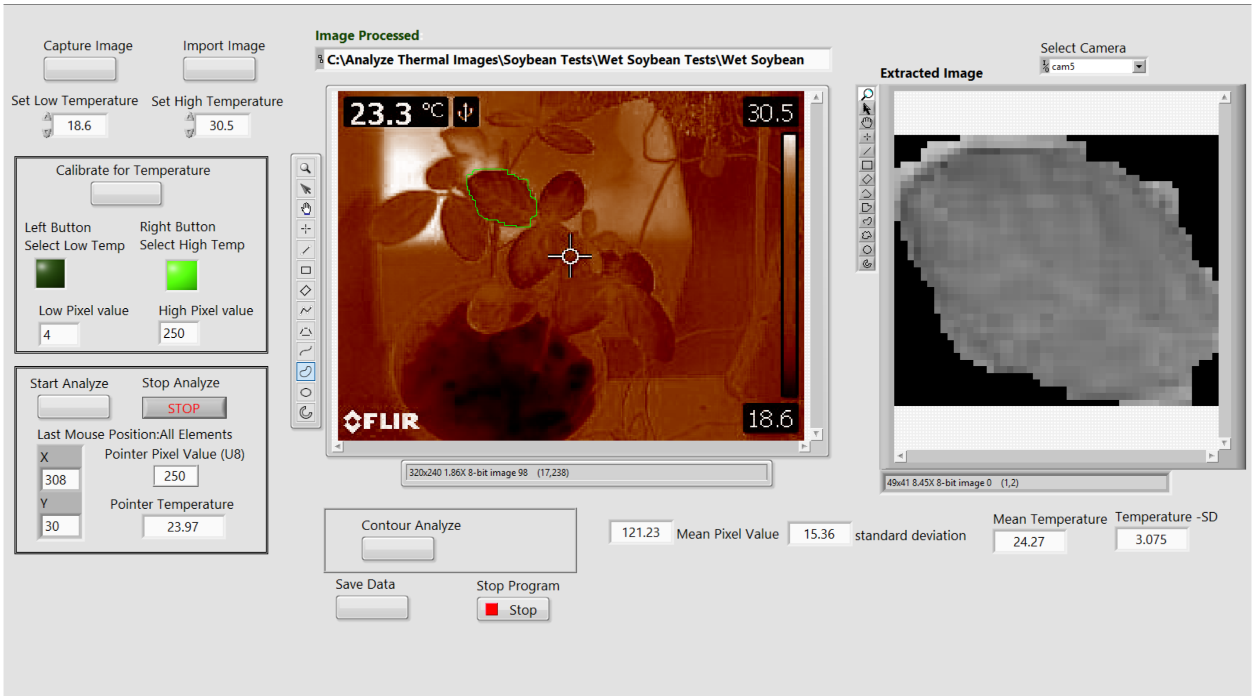

Figure 6. Front Panel of the LabVIEW Thermal Image Analyzer.

## One Way ANOVA Tests

MATLAB script (Mathworks, inc., Natick, MA) was written to conduct a one-way Analysis of Variance (ANOVA) test to statistically compare the single leaf and canopy response times for each plant. The MATLAB function ANOVA1 was used. A one-way ANOVA test was also conducted to statistically compare the well-watered and water-stressed response times for each plant.

# Results and Discussion

This section presents the results of canopy and leaf temperature response times computed for four plants: green basil, pothos, prayer plant, and green oxalis. The response curves generally show that the IRTc consistently measured a higher temperature than the average temperature for the single leaf determined by the thermal image analysis program. This may be due to other objects in the field of view of the IRTc.

## Green Basil Tests

Two green basil plants were used in this study. Plant 1 was water-stressed, and plant 2 was well-watered. Plant 1 was tested twice in a light-on test, and twice in a light-off test. Plant 2 was also tested twice in a light-on test, and twice in a light-off test. For each of these tests, the Thermal Image Analyzer program was used to find the average temperature of a fully exposed, single leaf at the top of the canopy. Both the canopy temperature data and the single leaf temperature data were graphed with respect to time. Figures 7 and 8 show the results for plant 1, and figures 9 and 10 show results for plant 2. The canopy response times were calculated for all tests using the LabVIEW state machine program, the single leaf response times were calculated by hand, and these times are shown in Table 2. The response times support the hypothesis since the well-watered basil plant had longer response times in both the light-on and light-off tests. A high degree of variability was observed in the well-watered times compared to the water-stressed times.

Table 2. Average Response Times Computed for Well-Watered and Water-Stressed Green Basil Plants

| **Average Green Basil Response Times** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Canopy | | | | Single Leaf | | | |
| **Radiation** | Light On Test | | Light Off Test | | Light On Test | | Light Off Test | |
| **Moisture Class** | Time (s) | Std Dev | Time (s) | Std Dev | Time (s) | Std Dev | Time (s) | Std Dev |
| **Water-Stressed** | 41.05 | 2.57 | 22.90 | 2.57 | 39.38 | 2.65 | 24.53 | 2.44 |
| **Well-Watered** | 54.96 | 5.13 | 31.97 | 10.27 | 50.88 | 12.20 | 35.53 | 10.37 |

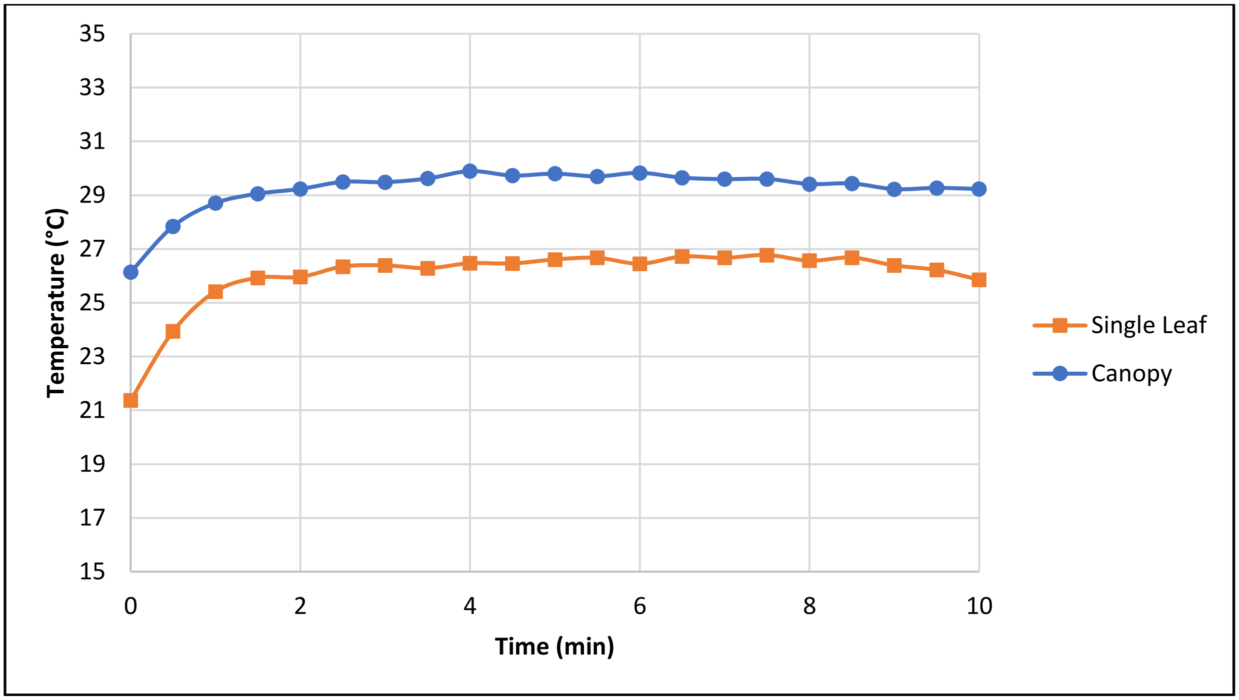


Figure 7. Average green basil temperatures versus time response from the Mikron MI-N3000 infrared canopy measurements (blue line) and for a single thermal image extracted leaf (orange line) for water-stressed green basil plants (plant 1), subjected to a step increase of 1200 $\mu\text{Einsteins}/\text{m}^2 \cdot \text{s}$ of LED light.

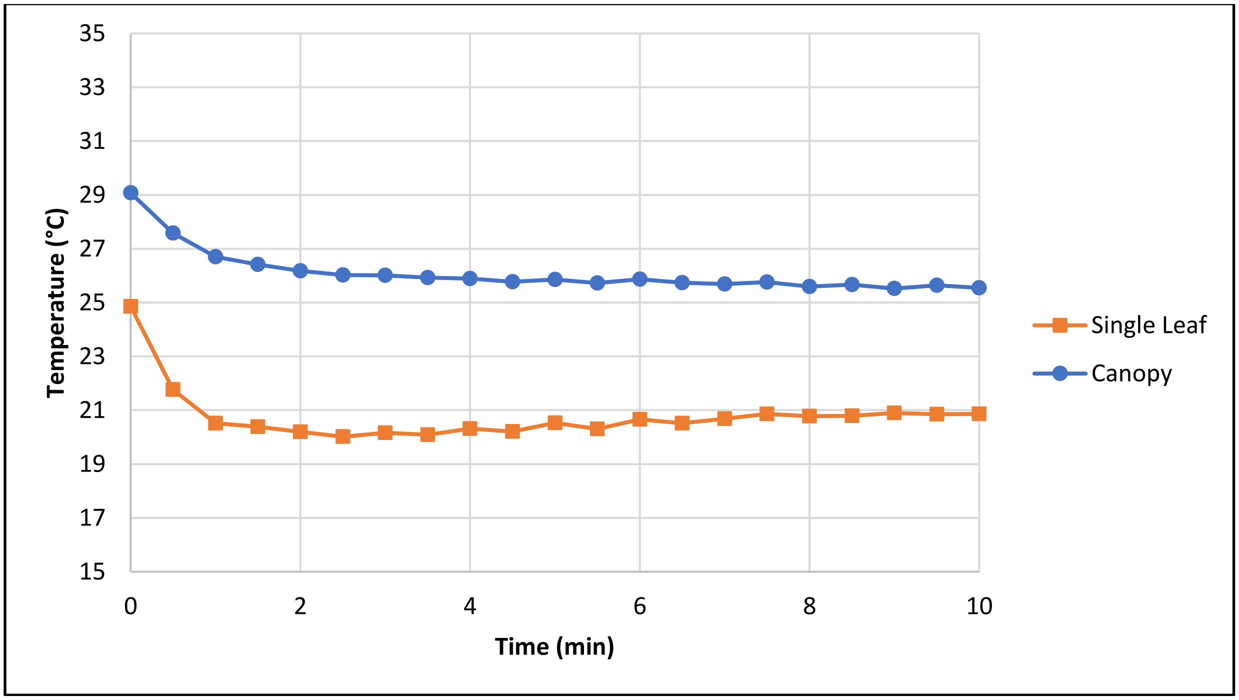


Figure 8. Average green basil temperatures versus time response from the Mikron MI-N3000 infrared canopy measurements (blue line) and for a single thermal image extracted leaf (orange line) for water-stressed green basil plants (plant 1), subjected to a step decrease of 1200 $\mu\text{Einsteins}/\text{m}^2 \cdot \text{s}$ of LED light.

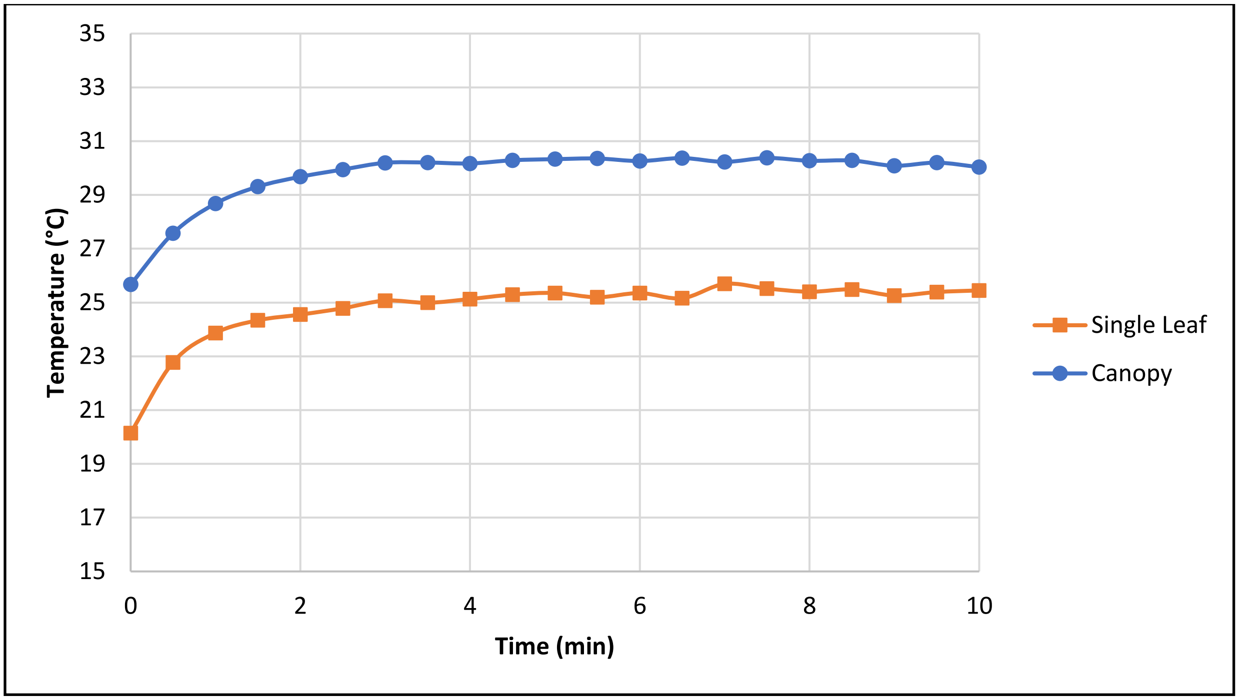

Figure 9. Average green basil temperatures versus time response from the Mikron MI-N3000 infrared canopy measurements (blue line) and for a single thermal image extracted leaf (orange line) for well-watered green basil plants (plant 2), subjected to a step increase of 1200 $\mu\text{Einsteins}/\text{m}^2 \cdot \text{s}$ of LED light.

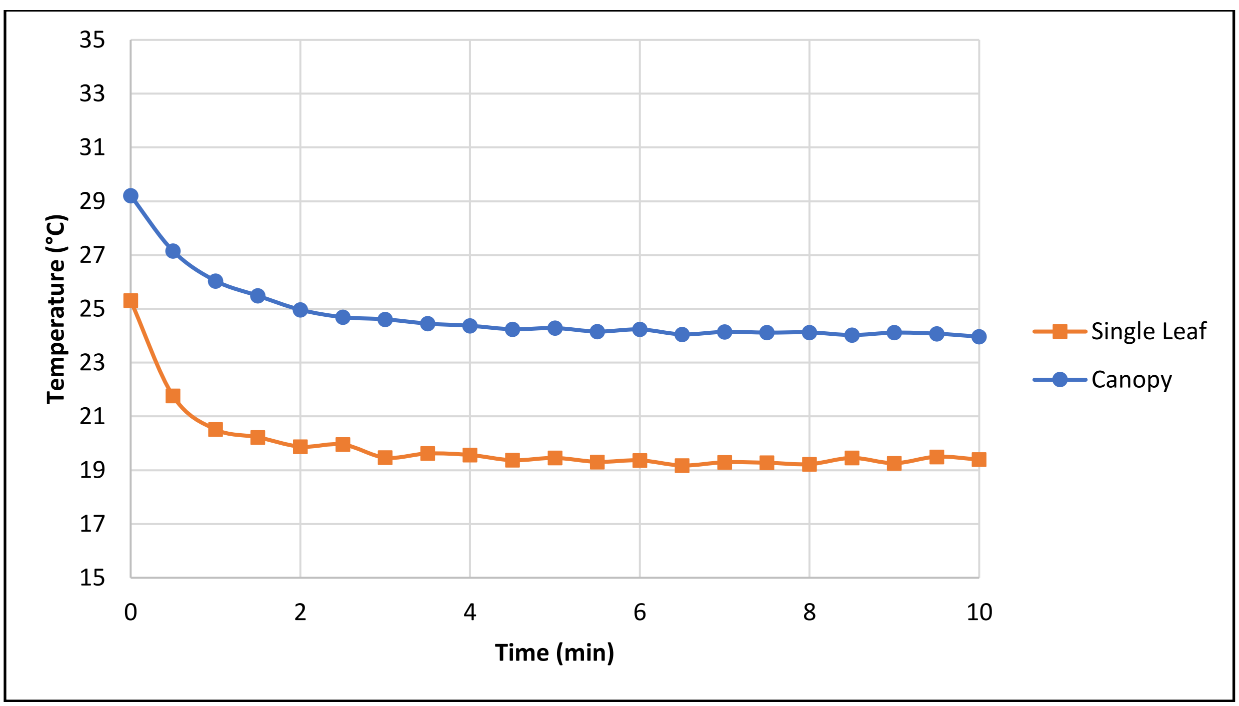

Figure 10. Average green basil temperatures versus time response from the Mikron MI-N3000 infrared canopy measurements (blue line) and for a single thermal image extracted leaf (orange line) for well-watered green basil plants (plant 2), subjected to a step decrease of 1200 $\mu\text{Einsteins}/\text{m}^2 \cdot \text{s}$ of LED light.

## Pothos Plant Tests

Two pothos plants were used in this study. Both plants started out water-stressed, and each plant was tested three times in a light-on test, and three times in a light-off test. After these tests were finished, both plants were watered, and these well-watered plants were tested three times in a light-on test and three times in a light-off test. For each of these tests, the Thermal Image Analyzer program was used to find the average temperature of a fully exposed single leaf at the top of the canopy. Both the canopy temperature data and the single leaf temperature data were averaged for both plants and were graphed with respect to time, and are shown in Figures 11, 12, 13, and 14. The canopy response times were calculated for all tests using the LabVIEW state machine program, the single leaf response times were calculated by hand, and these times are shown in Table 3. The variability data is not consistent when comparing the well-watered tests and the water-stressed tests. The canopy response times support the hypothesis since the well-watered plants had longer response times in both the light-on and light-off tests. However, the single leaf response times had mixed results, with the well-watered plant having a longer response time in the light-on test, but a shorter response time in the light-off test.

Table 3. Average Response Times Computed for Well-Watered and Water-Stressed Pothos Plants

| Average Pothos Response Times | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Canopy | | | | Single Leaf | | | |
| **Radiation** | Light On Test | | Light Off Test | | Light On Test | | Light Off Test | |
| **Moisture Class** | Time (s) | Std Dev | Time (s) | Std Dev | Time (s) | Std Dev | Time (s) | Std Dev |
| **Water-Stressed** | 51.73 | 9.70 | 30.16 | 3.13 | 57.32 | 7.29 | 76.97 | 10.98 |
| **Well-Watered** | 73.31 | 8.94 | 36.00 | 4.63 | 63.91 | 12.75 | 61.25 | 5.40 |

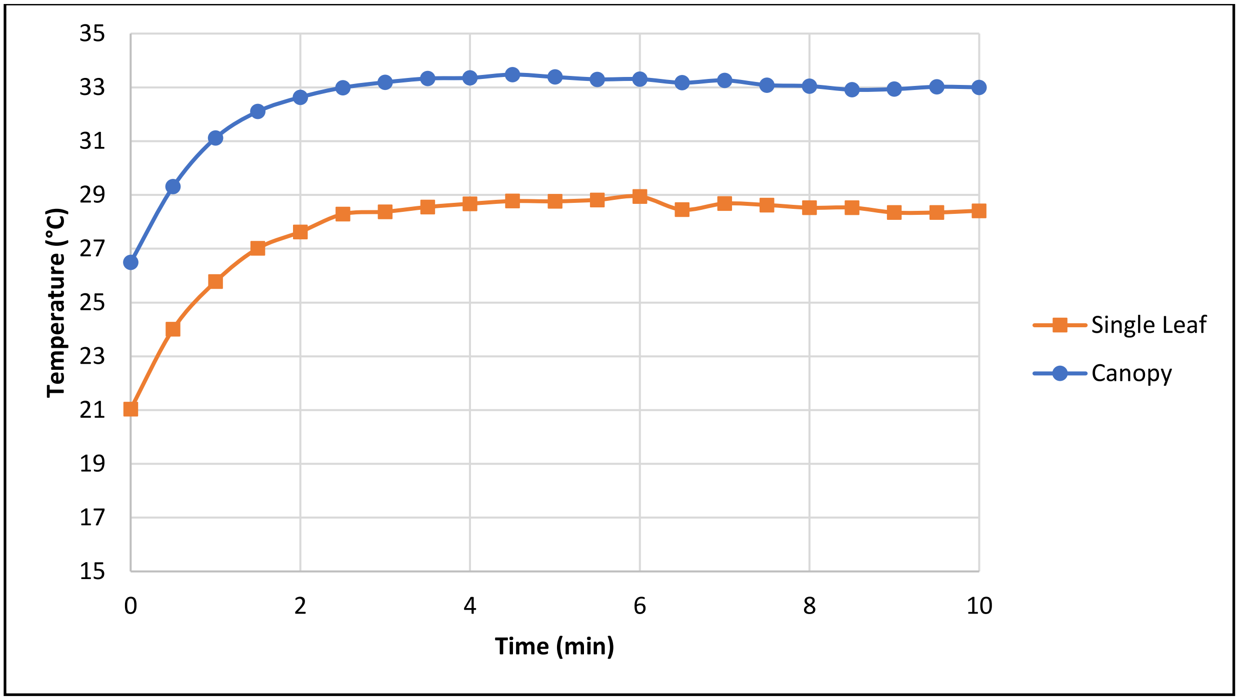


Figure 11. Average pothos temperatures versus time response from the Mikron MI-N3000 infrared canopy measurements (blue line) and for a single thermal image extracted leaf (orange line) for water-stressed pothos plants, subjected to a step increase of 1200 $\mu Einsteins/m^2 \cdot s$ of LED light.

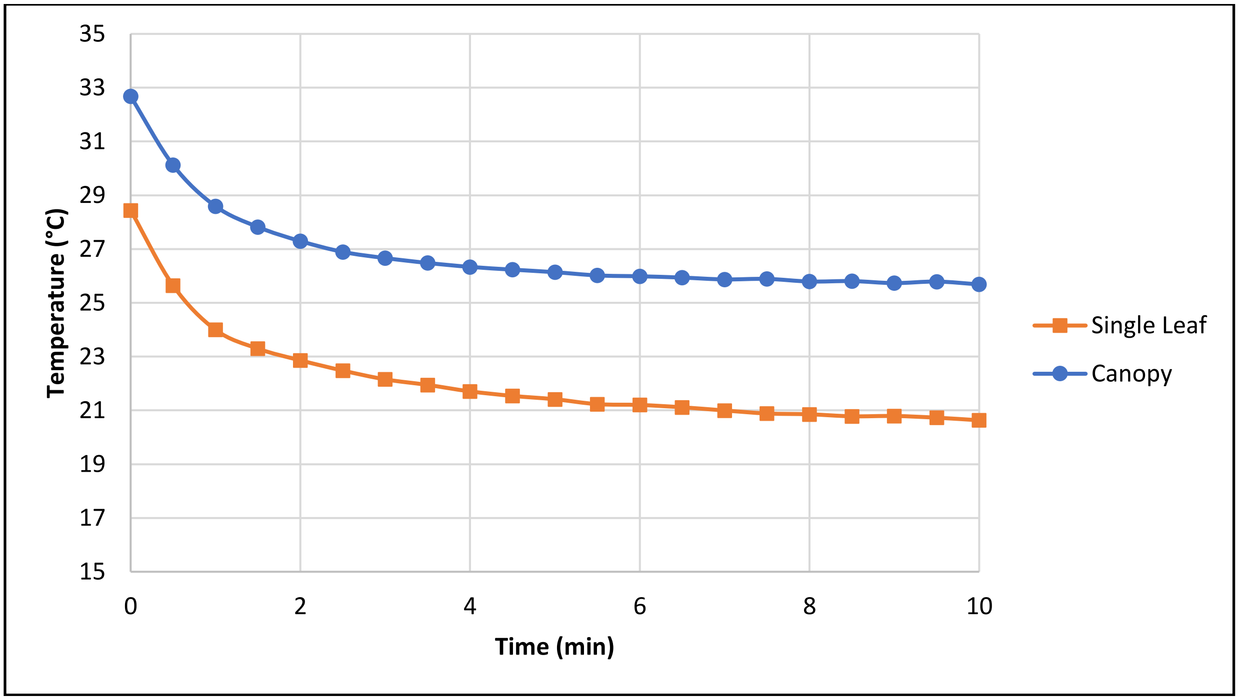


Figure 12. Average pothos temperatures versus time response from the Mikron MI-N3000 infrared canopy measurements (blue line) and for a single thermal image extracted leaf (orange line) for water-stressed pothos plants, subjected to a step decrease of 1200 $\mu Einsteins/m^2 \cdot s$ of LED light.

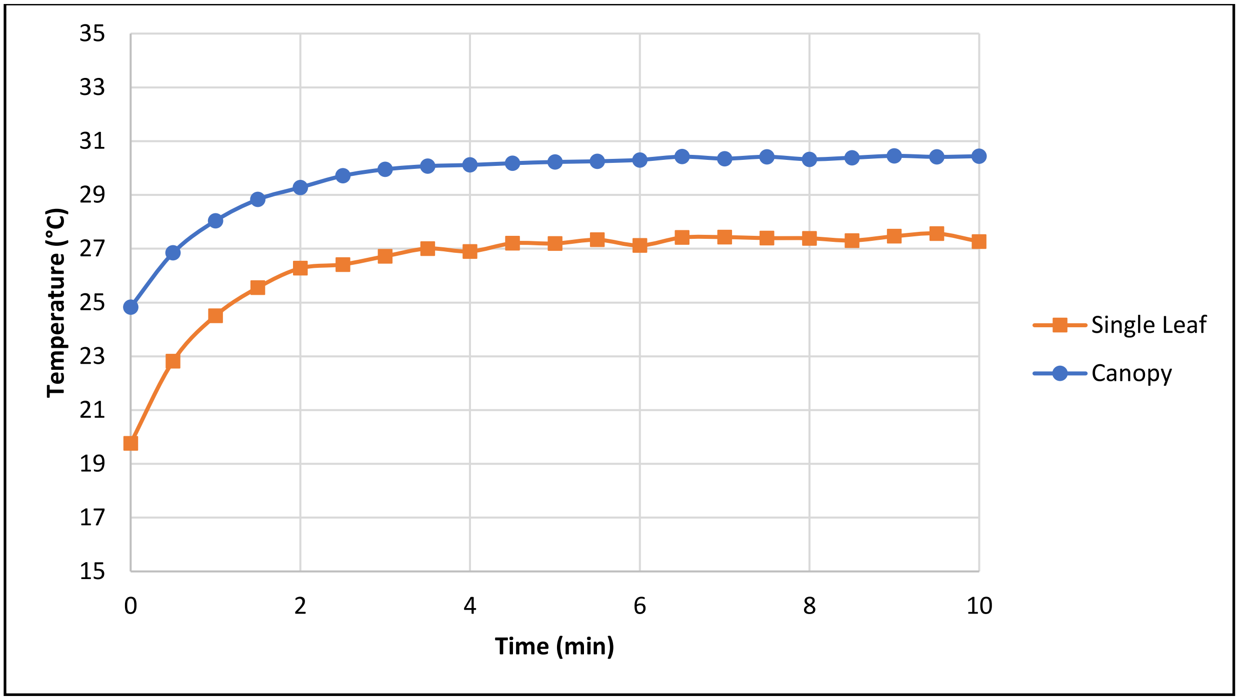


Figure 13. Average pothos temperatures versus time response from the Mikron MI-N3000 infrared canopy measurements (blue line) and for a single thermal image extracted leaf (orange line) for well-watered pothos plants, subjected to a step increase of 1200 μEinsteins/$m^2 \cdot s$ of LED light.

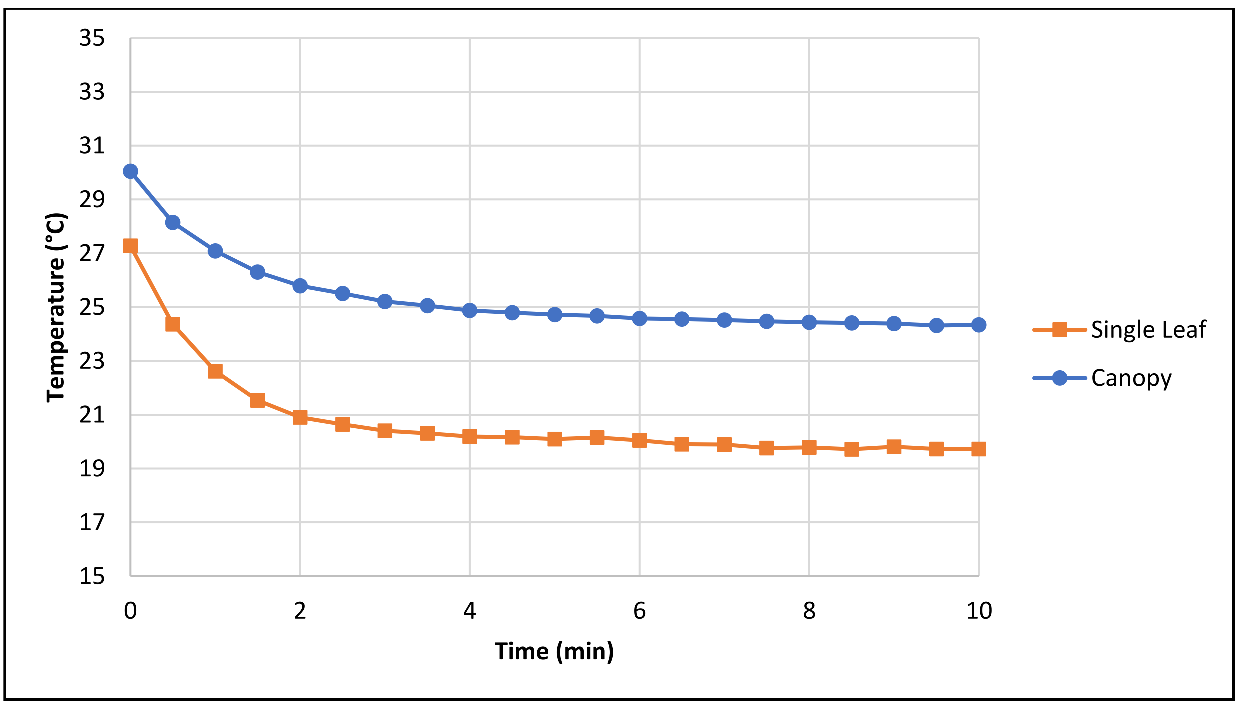


Figure 14. Average pothos temperatures versus time response from the Mikron MI-N3000 infrared canopy measurements (blue line) and for a single thermal image extracted leaf (orange line) for well-watered pothos plants, subjected to a step decrease of 1200 μEinsteins/$m^2 \cdot s$ of LED light.

## Prayer Plant Tests

Two prayer plants were used in this study. Plant 1 was water-stressed, and plant 2 was well-watered. The water-stressed plant was tested three times in a light-on test, and three times in a light-off test. The well-watered plant was also tested three times in a light-on test, and three times in a light-off test. For each of these tests, the Thermal Image Analyzer program was used to find the average temperature of a fully exposed single leaf at the top of the canopy. Both the canopy temperature data and the single leaf temperature data were graphed with respect to time. Figures 15 and 16 show the results for plant 1, and figures 17 and 18 show results for plant 2. The canopy response times were calculated for all tests using the LabVIEW state machine program, the single leaf response times were calculated by hand, and these times are shown in Table 4. There is a high degree of variability in the well-watered response times compared to the water-stressed response times. The response times support the hypothesis since the well-watered prayer plant had longer response times in both the light-on and light-off tests.

Table 4. Average Response Times Computed for Well-Watered and Water-Stressed Prayer Plants

| Average Prayer Plant Response Times | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Canopy | | | | Single Leaf | | | |
| **Radiation** | Light On Test | | Light Off Test | | Light On Test | | Light Off Test | |
| **Moisture Class** | Time (s) | Std Dev | Time (s) | Std Dev | Time (s) | Std Dev | Time (s) | Std Dev |
| **Water-Stressed** | 42.86 | 6.29 | 26.32 | 2.79 | 35.00 | 3.27 | 56.07 | 1.64 |
| **Well-Watered** | 94.90 | 20.96 | 33.18 | 5.54 | 52.14 | 15.20 | 72.50 | 28.39 |

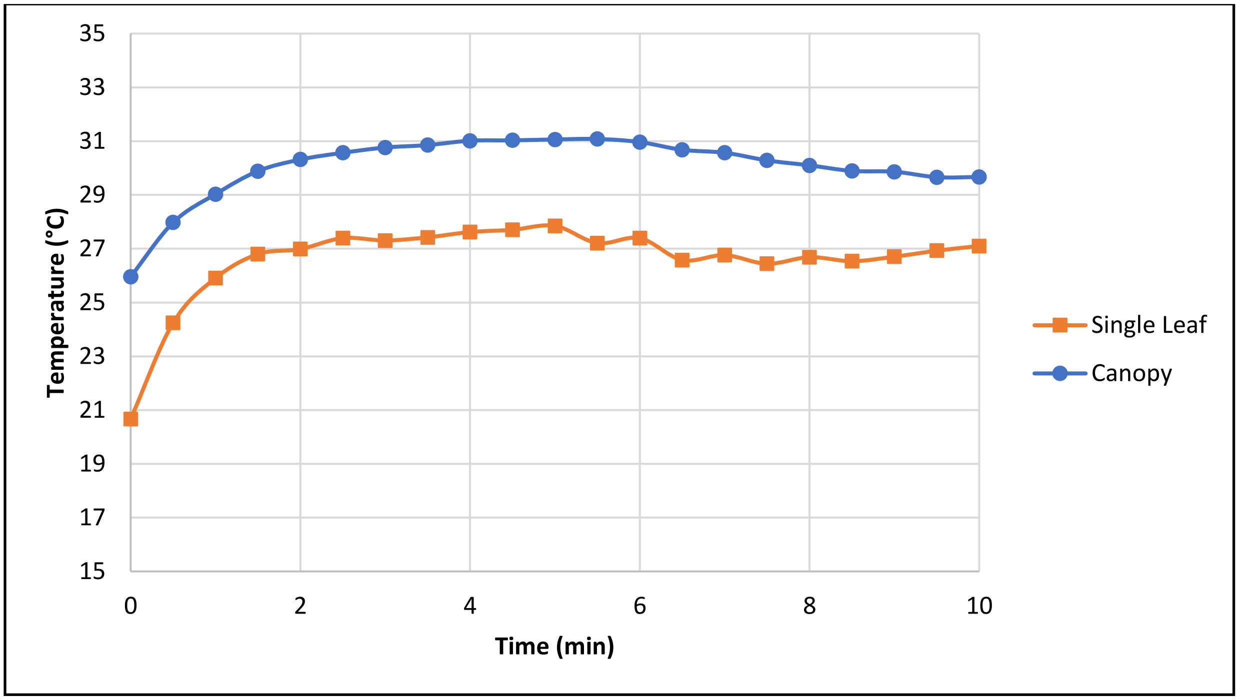


Figure 15. Average prayer plant temperatures versus time response from the Mikron MI-N3000 infrared canopy measurements (blue line) and for a single thermal image extracted leaf (orange line) for water-stressed prayer plants (plant 1), subjected to a step increase of 1200 $\mu\text{Einsteins}/\text{m}^2 \cdot \text{s}$ of LED light.

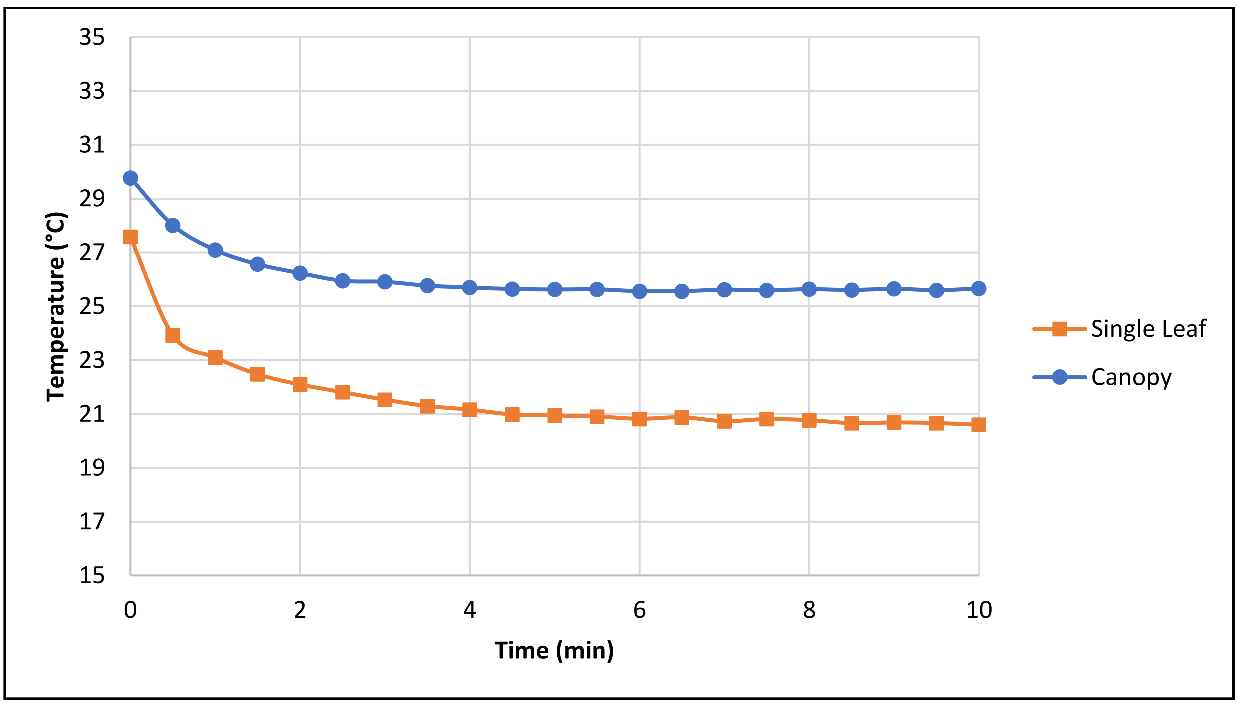


Figure 16. Average prayer plant temperatures versus time response from the Mikron MI-N3000 infrared canopy measurements (blue line) and for a single thermal image extracted leaf (orange line) for water-stressed prayer plants (plant 1), subjected to a step decrease of 1200 $\mu\text{Einsteins}/\text{m}^2 \cdot \text{s}$ of LED light.

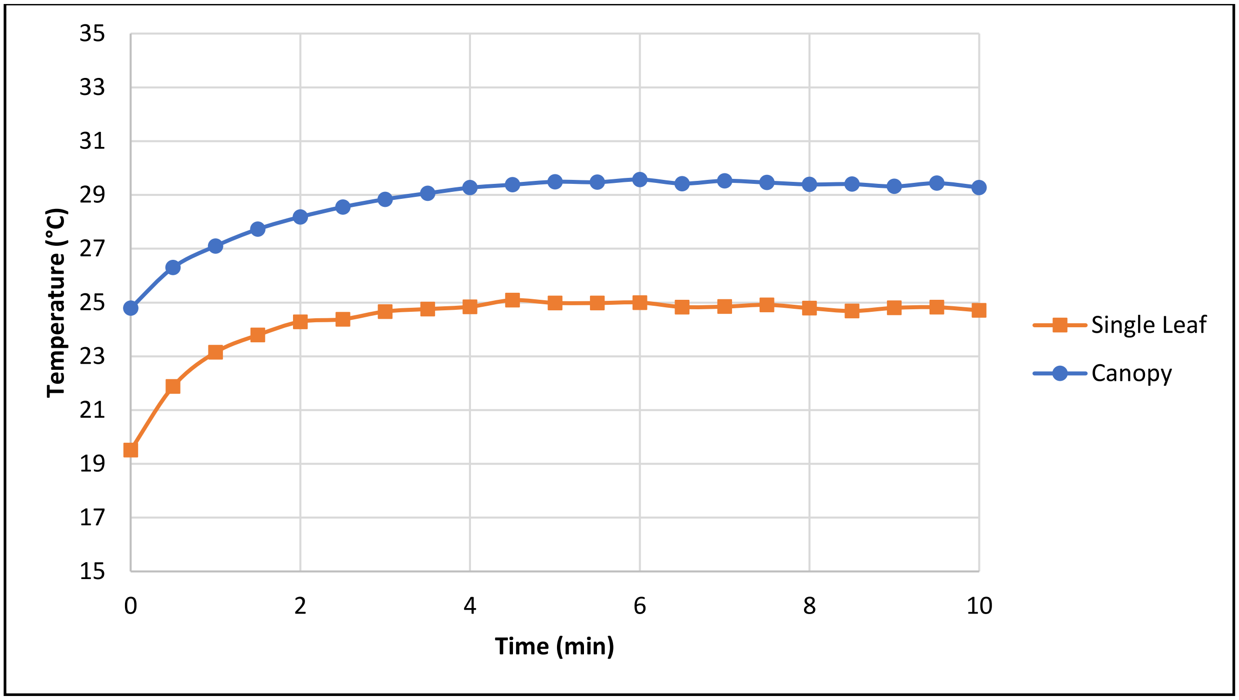

Figure 17. Average prayer plant temperatures versus time response from the Mikron MI-N3000 infrared canopy measurements (blue line) and for a single thermal image extracted leaf (orange line) for well-watered prayer plants (plant 2), subjected to a step increase of 1200 $\mu Einsteins/m^2 \cdot s$ of LED light.

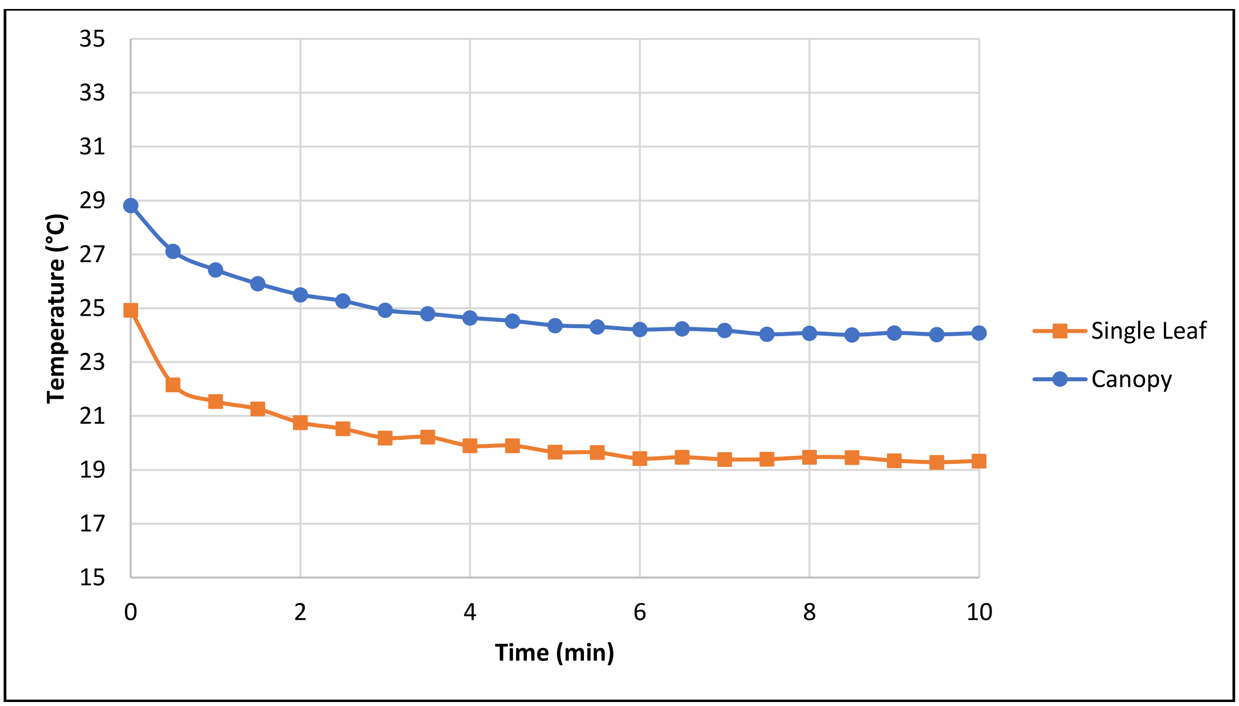

Figure 18. Average temperatures versus time response from the Mikron MI-N3000 infrared canopy measurements (blue line) and for a single thermal image extracted leaf (orange line) for well-watered prayer plants (plant 2), subjected to a step decrease of 1200 $\mu Einsteins/m^2 \cdot s$ of LED light.

## Green Oxalis Tests

Two green oxalis plants were used in this study. Plant 1 was water-stressed, and plant 2 was well-watered. The water-stressed plant was tested three times in a light-on test, and three times in a light-off test. The well-watered plant was also tested three times in a light-on test, and three times in a light-off test. For each of these tests, the Thermal Image Analyzer program was used to find the average temperature of a fully exposed single leaf at the top of the canopy. Both the canopy temperature data and the single leaf temperature data were graphed with respect to time. Figures 19 and 20 show the results for plant 1, and figures 21 and 22 show results for plant 2. The canopy response times were calculated for all tests using the LabVIEW state machine program, the single leaf response times were calculated by hand, and these times are shown in Table 5. The variability data is inconsistent between the well-watered and water-stressed tests. The canopy response times support the hypothesis since the well-watered oxalis plant had longer response times in both the light-on and light-off tests. However, the single leaf response times do not support the hypothesis since the well-watered plant had shorter response times in both the light-on and light-off tests. This difference may be due to the location of the leaf selected for analysis.

Table 5. Average Response Times Computed for Well-Watered and Water-Stressed Green Oxalis Plants

| **Average Green Oxalis Response Times** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Canopy | | | | Single Leaf | | | |
| **Radiation** | Light On Test | | Light Off Test | | Light On Test | | Light Off Test | |
| **Moisture Class** | Time (s) | Std Dev | Time (s) | Std Dev | Time (s) | Std Dev | Time (s) | Std Dev |
| **Water-Stressed** | 99.75 | 7.38 | 52.54 | 5.54 | 49.64 | 6.28 | 74.29 | 28.39 |
| **Well-Watered** | 107.40 | 4.85 | 52.94 | 16.43 | 36.78 | 4.06 | 43.45 | 5.70 |

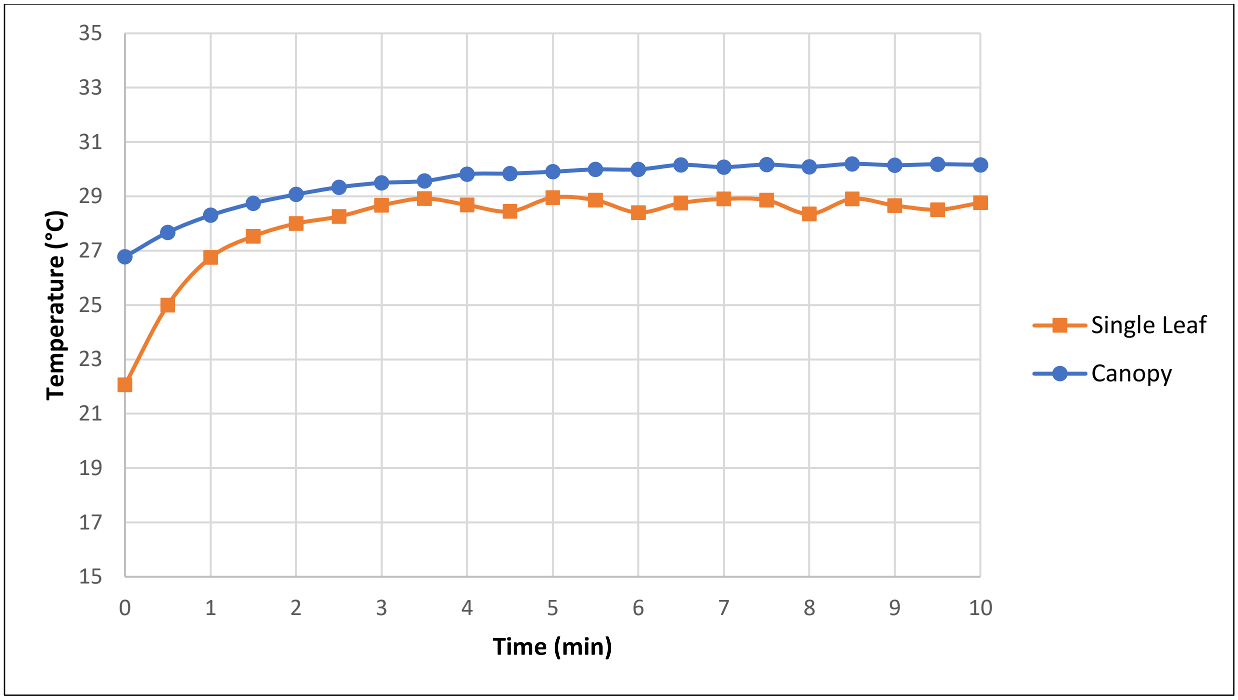


Figure 19. Average temperatures versus time response from the Mikron MI-N3000 infrared canopy measurements (blue line) and for a single thermal image extracted leaf (orange line) for water-stressed green oxalis plants (plant 1), subjected to a step increase of 1200 $\mu\text{Einsteins}/\text{m}^2 \cdot \text{s}$ of LED light.

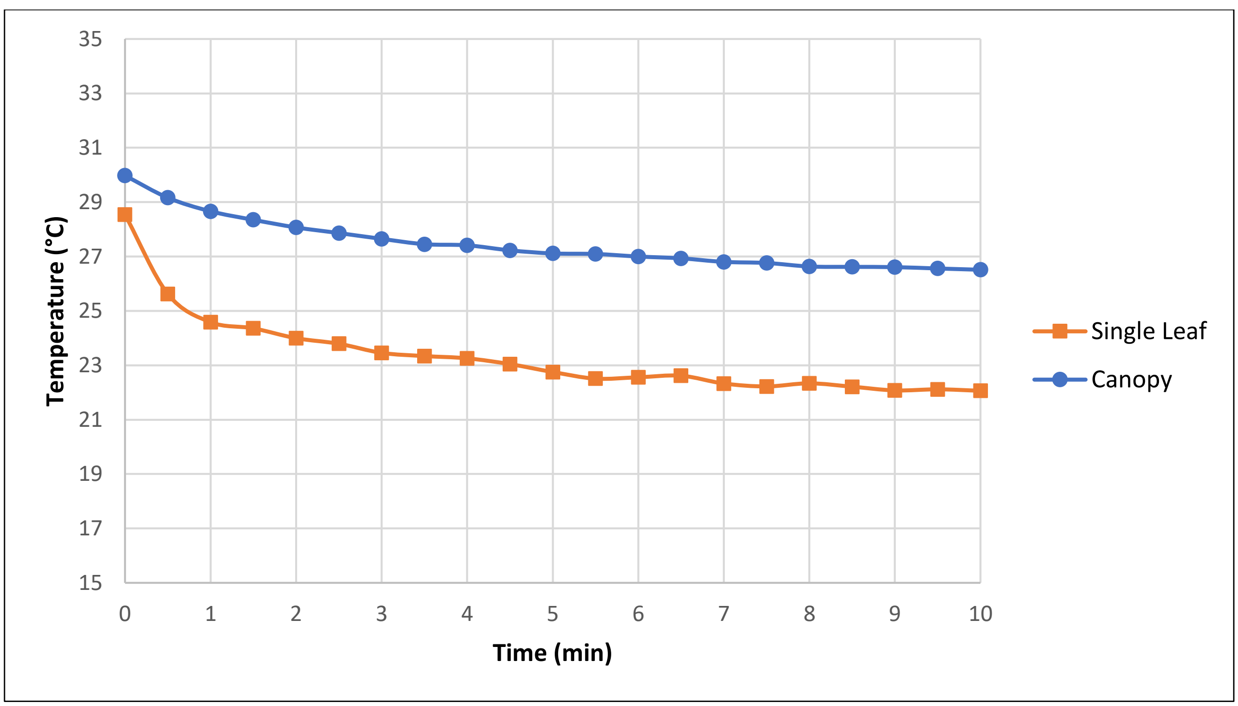


Figure 20. Average temperatures versus time response from the Mikron MI-N3000 infrared canopy measurements (blue line) and for a single thermal image extracted leaf (orange line) for water-stressed green oxalis plants (plant 1), subjected to a step decrease of 1200 $\mu\text{Einsteins}/\text{m}^2 \cdot \text{s}$ of LED light.

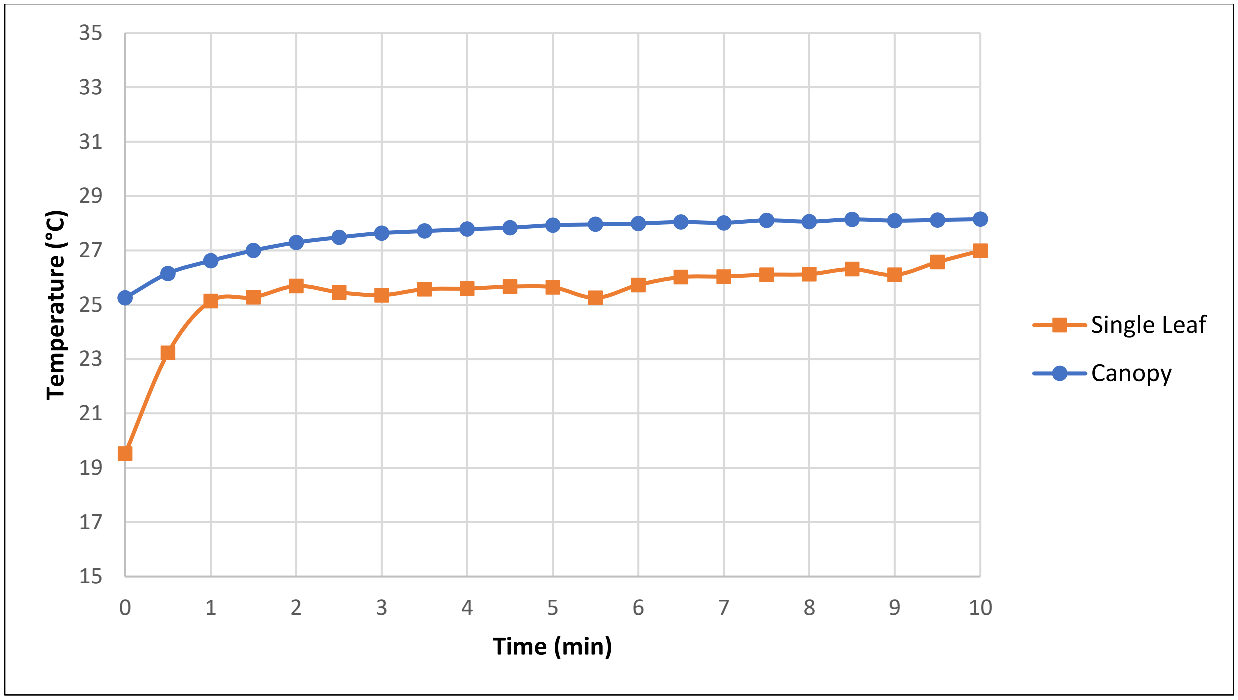


Figure 21. Average temperatures versus time response from the Mikron MI-N3000 infrared canopy measurements (blue line) and for a single thermal image extracted leaf (orange line) for well-watered green oxalis plants (plant 2), subjected to a step increase of 1200 $\mu Einsteins/m^2 \cdot s$ of LED light.

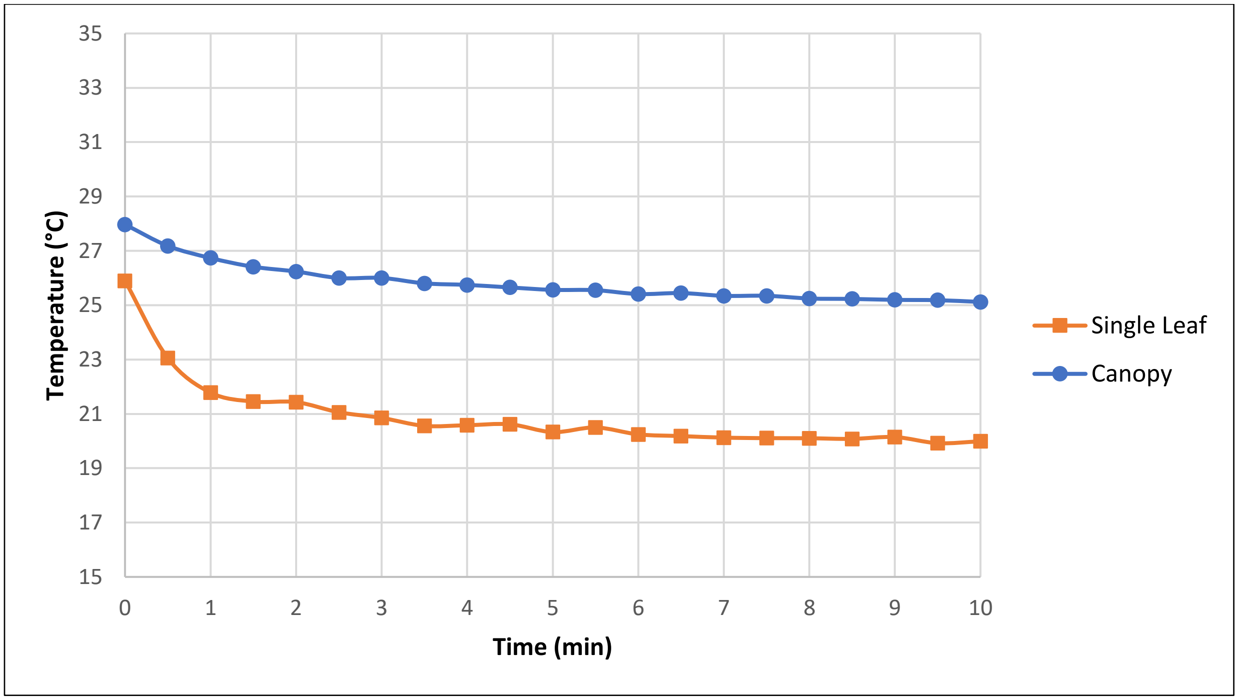


Figure 22. Average temperatures versus time response from the Mikron MI-N3000 infrared canopy measurements (blue line) and for a single thermal image extracted leaf (orange line) for well-watered green oxalis plants (plant 2), subjected to a step decrease of 1200 $\mu Einsteins/m^2 \cdot s$ of LED light.

## Statistical Results

One-way ANOVA tests were conducted to compare the canopy and single leaf response times values, and to compare well-watered versus water-stressed plant response times values. Table 6 summarizes the statistical comparisons for the well-watered and water-stressed response times. The basil plants had no significant results. Others are inconclusive. Table 7 summarizes the statistical results for the canopy and single leaf response times. There were a few statistically significant results for both comparisons, but results are variable. It appears that more tests need to be conducted providing a larger sample size for statistical validation.

Table 6. Summary of One Way Analysis of Variance (MATLAB ANOVA1) Statistical Tests for Response Time Means for Well-Watered and Water-Stressed Plants.

| Plant Type | LED Lights | Temperature Acquisition Method | Difference In Response Time Mean Values[1] |
|---|---|---|---|
| Green Basil | On | Canopy | ns |
| Green Basil | On | Single Leaf | ns |
| Green Basil | Off | Canopy | ns |
| Green Basil | Off | Single Leaf | ns |
| Pothos Plant | On | Canopy | * |
| Pothos Plant | On | Single Leaf | ns |
| Pothos Plant | Off | Canopy | * |
| Pothos Plant | Off | Single Leaf | * |
| Prayer Plant | On | Canopy | * |
| Prayer Plant | On | Single Leaf | ns |
| Prayer Plant | Off | Canopy | ns |
| Prayer Plant | Off | Single Leaf | ns |
| Green Oxalis | On | Canopy | ns |
| Green Oxalis | On | Single Leaf | * |
| Green Oxalis | Off | Canopy | ns |
| Green Oxalis | Off | Single Leaf | ns |

[1] * – statistically significant
ns – not significant

Table 7. Summary of One Way Analysis of Variance (MATLAB ANOVA1) Statistical Tests for Response Time Means for Canopy and Single Leaf Temperature Measurement Methods.

| Plant Type | LED Lights | Water Treatment | Difference In Response Time Mean Values[1] |
|---|---|---|---|
| Green Basil | On | Well-Watered | ns |
| Green Basil | On | Water-Stressed | ns |
| Green Basil | Off | Well-Watered | ns |
| Green Basil | Off | Water-Stressed | ns |
| Pothos Plant | On | Well-Watered | ns |
| Pothos Plant | On | Water-Stressed | ns |
| Pothos Plant | Off | Well-Watered | * |
| Pothos Plant | Off | Water-Stressed | * |
| Prayer Plant | On | Well-Watered | * |
| Prayer Plant | On | Water-Stressed | ns |
| Prayer Plant | Off | Well-Watered | ns |
| Prayer Plant | Off | Water-Stressed | * |
| Green Oxalis | On | Well-Watered | * |
| Green Oxalis | On | Water-Stressed | * |
| Green Oxalis | Off | Well-Watered | ns |
| Green Oxalis | Off | Water-Stressed | ns |

[1] * – statistically significant
ns – not significant

# Conclusions

The first project objective was to determine if canopy and/or leaf temperature response times could be used as an indicator of water status in a plant. After conducting tests on four different plant species, it was concluded that well-watered plants generally have a longer response time to a step change in radiation than water-stressed plants, therefore leaf response time may be used as an indicator of plant water stress. Measuring leaf thermal response times can be done quickly and easily, but must be done under controlled conditions. Thermal response times may be a more appropriate representation of the moisture stress level of a plant than a simple incidental or static measure of the canopy temperature because of the dynamic process of a plant responding to its environment. Such results could have a major impact in the design of irrigation control systems for greenhouses as well as in the field. Although this project only used four plant species, tests could be easily replicated for other species in future studies. In future studies, response times could be measured in a greenhouse setting, as opposed to a growth chamber to see if response time still indicates the water stress level of the plant. Future studies could also investigate using response time in an irrigation control system.

The second project objective was to compare response times of different species. The response times of each species was similar within the species, but had a large variance between species. The leaf anatomy and size of the leaves may have an impact on the variance between species. The basil and prayer plant have leaves of similar thickness, and they responded similarly in terms of the variance of the well-watered versus water-stressed tests. The pothos plant, which has larger, variegated leaves does not follow the same variance pattern as the basil and prayer plants. The oxalis plant, which has much smaller, clover-shaped leaves, also had a different variance pattern. In general, the response times of the well-watered plants were longer than the response times of water-stressed plants within the same species, but the same correlation was not necessarily true when comparing all species. It is important to get baseline data for a species of interest in order for response times to be used as an indicator of water status since response times are not consistent across all species.

The third project objective was to statistically compare whole canopy response times with single leaf response times. The basil plant showed no statistically significantly different results for the canopy versus single leaf response times. However, the other three plants did have some statistically significant results, but they did not seem to follow any specific pattern. This data suggests that the canopy and single leaf response times may be different depending on the species and water treatment.

# Acknowledgements

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