PHYSIOLOGY AND ISOPRENE EMISSIONS OF DROUGHT-STRESSED AND OZONE EXPOSED PLANTS IN A LABORATORY CHAMBER

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ABSTRACT

Physiology and Isoprene Emissions of Drought-Stressed and Ozone Exposed Plants in a Laboratory Chamber. (May 2015)

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Studying the response of trees in urban areas to environmental stresses, such as drought stress and high ozone exposure, may be an important proxy for the effects of future climate change on tree physiology and trace gas emissions. However, such field experiments lack the element of reproducibility; variables such as ozone concentration, light exposure of the plant, ambient temperature, and available soil moisture cannot be controlled in the field. In this experiment, we use an established laboratory-based Teflon foil chamber to study the effect of a changing environment on the emissions and physiology of several isoprene-emitting tree species. With the laboratory setup, variables such as light levels and gas composition can be manipulated. Measurements of temperature, humidity, and gas concentrations can be constantly recorded with a data logger, and soil moisture can be regulated to simulate drought stress with the use of potted plants. These experiments will allow for the analysis of trace gas exchange and plant physiology while also allowing for the manipulation of variables normally left to nature.

DEDICATION

This thesis is dedicated to the ones who have always been there to support me. They pick me up when I am down, make me happy when I am sad, and work their hardest to help me work towards my dreams. I am doing what I love thanks to their love and support.

Thank you, mom and dad. This is for you.

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NOMENCLATURE

CO_2 – carbon dioxide

HO_2 – hydroperoxyl radicals

IR – infrared

MACR – methacrolein

MVK – methyl vinyl ketone

NMOC – non-methane organic compound

NO – nitrogen oxide

NO_2 – nitrogen dioxide

NO_3 – nitrate radical

NO_x – term for the sum of NO and NO_2

O_3 – ozone

OH – hydroxyl radical

RO_2 – generic term for organic peroxy radicals

VOC – volatile organic compound

CHAPTER I

INTRODUCTION

Isoprene and its Role in the Troposphere

Isoprene Production in Plants

Isoprene is the dominant volatile organic compound (VOC) emitted to the atmosphere by vegetation (Guenther et al., 1995). Produced by many plant species, it is not stored in the leaf and released directly into the atmosphere after its production (Harley et al., 1996; Dani et al., 2014). The rate of isoprene production in plants is primarily controlled by temperature and light intensity (Fuentes et al., 2000). Since the worldwide emission of VOCs exceeds emissions of anthropogenic non-methane organic compounds (NMOCs) by a factor of ~10 (Harley et al., 1999; Atkinson and Arey, 2003), isoprene emission from vegetation and other VOCs play a dominant role in air chemistry in the lower troposphere and surface boundary layer (Fuentes et al., 2000).

While a definitive answer as to why some plants have evolved to create isoprene while others have not has not been reached yet (Dani et al., 2014), three theories exist that suggest the advantages of isoprene production by the plant. The first theory posits isoprene's role in plant thermotolerance (Sharkey et al., 2008). Sunlight causes leaves in the tree canopy to experience rapid, significant fluctuations in temperature known as heat flecks. Studies of the distribution of isoprene emission through the canopy show that isoprene emission is as much as four times higher at the top of the canopy, where leaves are much more likely to experience heat flecks (Harley et al., 1996; Singsaas et al., 1999). The second theory suggests isoprene's role as an

antioxidant for the plant (Loreto and Velikova, 2001; Loreto et al., 2001). Experiments conducted by Loreto have shown that concurrent ozone (O_3) and isoprene exposure to leaves that do not endogenously produce isoprene causes less damage than only ozone exposure. His results posit that isoprene stabilizes the thylakoid membranes of leaves and quenches ozone to a non-toxic concentration within the leaves or in a humid atmosphere (Loreto and Velikova, 2001). The third theory postulates isoprene's role in drought tolerance (Dani et al., 2014). Isoprene is not affected by drought stress as strongly as the plants' photosynthesis and stomatal conductance rates (Fang et al., 1996; Pegoraro et al., 2004a) and may reduce membrane damage during moderate desiccation (Beckett et al., 2012).

As stated, isoprene production in plants is primarily controlled by temperature and light intensity (Fuentes et al., 2000). Fuentes et al. (2000) provides a thorough description of the environmental controls on isoprene and other VOC emissions. Temperature is the most dominant control on emission rates from plants at any given time (Dement et al., 1975); increased temperatures are correlated with increased vapor pressure of isoprene and other VOCs. Isoprene emissions, unlike many other VOCs, are also strictly light dependent. Greater light intensity correlates with higher isoprene emissions. Isoprene emissions are subject to short- and long-term light and temperature responses. The light and temperature environment over several days can explain some of the variability in current basal emission rates.

Isoprene emissions are also a function of ambient carbon dioxide (CO_2) concentration. In several experiments, it has been shown that isoprene emissions decrease with elevated CO_2 concentration (Guenther et al., 1991; Sharkey et al., 1991; Pegoraro et al., 2004b). Conversely,

isoprene emissions increase with lowered CO_2 concentration. Isoprene emissions are inhibited at ambient [CO_2] compared to lower levels of CO_2 (Sharkey et al., 1991). However, the CO_2 suppression of isoprene emissions can be reduced at high leaf temperatures (Potosnak et al., 2014b)

Changes in plant physiology may affect its isoprene emissions. An increase in vapor pressure deficit causes stomatal closure, resulting in decreased stomatal conductance and a related increase in isoprene emission if leaf temperatures increase (Pegoraro et al., 2004b; Potosnak et al., 2014a). However, isoprene emission functions independently of stomatal dynamics (Monson and Fall, 1989). For example, stomatal closure during drought stress does not affect a plants' isoprene emissions (Fang et al., 1996). Additionally, isoprene emission is inhibited by elevated CO_2 even though stomatal conductance remains constant (Monson and Fall, 1989).

Isoprene Reactivity in the Troposphere

Atkinson and Arey (2003) provided a comprehensive review of tropospheric air chemistry of VOCs. The following summary of isoprene reactivity and relevant chemical reactions in the lower troposphere originates from this source, unless stated otherwise.

VOCs are highly reactive in the atmosphere, with atmospheric lifetimes (the average time that it takes for a VOC to decay to 1/*e* of its initial concentration in the atmosphere) ranging from several minutes to several days. Isoprene is a highly reactive VOC, especially with respect to the hydroxyl (OH) radical. Fuentes et al. (2000) list calculated tropospheric lifetimes for several VOCs; isoprene has a calculated atmospheric lifetime of 1.4 hours for OH reaction, 1.6 hours for

nitrate (NO_3) reaction, and 1.3 days for O_3 reaction with oxidant concentrations (molecules cm^{-3}) for OH of 2.0×10^6 (12-hour daytime average), NO_3 of 5×10^8 (12-hour nighttime average), and O_3 of 7×10^{11} (24-hour average). Isoprene reacts in the atmosphere primarily through the addition of OH radicals, NO_3 radicals, and O_3 to the double carbon bonds (Fuentes et al., 2000). Since OH and NO_3 radicals have pronounced diurnal cycles in the troposphere (Tanner et al., 1997; Fuentes et al., 2000) and tropospheric ozone concentrations vary regionally, the dominating oxidative agent varies locally as well (Dreyfus et al., 2002). Generally, photooxidation of isoprene is dominated by its reaction with the OH radical (Claeys et al., 2004).

Isoprene is oxidized into a variety of organic peroxy (RO_2) radicals. The primary reaction products of the oxidation of isoprene by OH are formaldehyde, methacrolein (MACR), and methyl vinyl ketone (MVK); isoprene oxidation under conditions of sufficient NO_x (the sum of NO and NO_2) also contributes to tropospheric ozone production (Tuazon and Atkinson, 1990; Dreyfus et al., 2002). Dreyfus et al. (2002) utilized the observed MVK/MACR ratio to calculate the fraction of total ozone production due to isoprene oxidation. Their results indicated that isoprene oxidation can be a dominant source of ozone production in the troposphere. The amount of ozone produced through isoprene oxidation during atmospheric transport is highly variable, with factors such as temperature, [NO_x], and other chemical processes in the atmosphere influencing the variability.

The most important driver of the effect of isoprene oxidation on tropospheric ozone is likely the regional composition of the atmosphere into which it is emitted. In areas with low concentrations of NO_x, RO_2 radicals preferentially react with each other or with O_3 (Harley et al., 1999). The

result can be net O_3 destruction in areas with low NO_x, such as remote areas. In polluted and rural areas with medium to high NO_x concentrations, RO_2 molecules promote NO_2 formation (Atkinson and Arey, 2003), which subsequently creates O_3 through photolysis (see below). The formation of additional NO_2 molecules by RO_2 radicals thus causes net O_3 production.

Important Tropospheric Chemical Reactions

In the lower troposphere, the dominant source of O_3 is the photolysis of nitrogen dioxide (NO_2):

(1) $NO_2 + h\nu \rightarrow O(^3P) + NO$ ($O(^3P)$ = ground state atomic oxygen)

(2) $O(^3P) + O_2 + M \rightarrow O_3 + M$ (M = air molecule involved in a termolecular reaction)

Excess NO_2 is formed in the lower troposphere by the reaction of RO_2 radicals and hydroperoxyl (HO_2) radicals with NO, when bypassing the *Null-cycle* (reactions 3-5):

(3) $O_3 + NO \rightarrow O_2 + NO_2$

(4) $RO_2 + NO \rightarrow RO + NO_2$

(5) $HO_2 + NO \rightarrow OH + NO_2$

The photolysis of NO_2 through (1) and (2) leads to net O_3 formation in the lower troposphere when either (4) or (5) bypass the Null-cycle. NO_2 photolysis is the primary source of tropospheric ozone, and isoprene photochemical oxidation is a major source of NO to NO_2 conversion leading to this ozone production (Dreyfus et al., 2002).

Relatively low levels of O_3 occur naturally in the troposphere due to stratospheric transport and NO_2 photolysis but are essential in the production of the OH radical. The photolysis of O_3 at

wavelengths ≤ 320 nm forms excited oxygen atoms, which generate OH radicals in the troposphere:

(6) $O_3 + h\nu \rightarrow O_2 + O(^1D)$ $\lambda \leq 320$ nm

(7) $O(^1D) + M \rightarrow O(^3P) + M$

(8) $O(^1D) + H_2O \rightarrow 2\ OH$

The NO_3 radical is formed in the troposphere due to the presence of ozone and NO_2:

(9) $NO + O_3 \rightarrow NO_2 + O_2$

(10) $NO_2 + O_3 \rightarrow NO_3 + O_2$

NO_3 concentrations remain low during the day due to its extremely rapid photolysis in sunlight, with an atmospheric lifetime of approximately 5 seconds for overhead sun. However, NO_3 concentrations can increase to measurable levels at night.

Isoprene Analysis in a Laboratory-Based Chamber

Understanding the expected changes to plant physiology and emissions in a warming environment, and thus a plant's role in future changes in atmospheric chemistry, is important to assess and predict the impact warming will have. In our changing climate, higher average global temperatures, greater air pollution and tropospheric ozone concentrations (Ainsworth et al., 2012), and drier soil could have a significant effect on plant growth and VOC emissions. That is why this research project utilizes a laboratory-based Teflon foil chamber designed for live plant trace gas analysis to analyze the relationship between drought stress, ozone exposure, and isoprene emissions in a controlled setting. The analysis of the relationship of these environmental

stresses to isoprene emissions will illuminate the effect that our changing climate could pose to tree physiology and emissions.

The objectives of this research project are as follows: (1) To create a controlled setting for live plant analysis: the first step of this experiment is the design and construction of a PFA Teflon foil chamber and light assembly. Several different tree species will be introduced to the Teflon chamber for the analysis of physiology and isoprene emissions. The chamber will be designed in such a way that variables such as the flow rate of air introduced to the chamber, volume of the chamber, and turbulent mixing in the chamber can be manipulated. (2) To manipulate plant growth variables: soil moisture and ozone exposure will be controlled and manipulated through the chamber setup. By using potted trees, a water regime can be implemented to create a drought-stressed environment. Ozone will be produced and introduced to the chamber to create an O_3-rich environment for the plant to be exposed to. (3) To sample, record, and analyze gas composition: CO_2, H_2O, CO, and O_3 concentrations will be recorded with a data logger. The chamber temperature and humidity, soil moisture of the potted plant, and leaf temperature of the plant are also constantly recorded. Isoprene samples will be periodically collected from the chamber for analysis. By relating the calculated isoprene emissions, the plant's calculated stomatal conductance and photosynthesis, the ozone uptake by the plant, and the soil moisture of the plant, I will be able to determine if and how much isoprene emissions are affected by the variables controlled in this experiment: ozone exposure and soil moisture.

CHAPTER II

METHODS

Chamber Assembly

The Teflon foil chamber used for the experiments in this research project is constructed using a ½" aluminum skeleton and a pre-sealed, transparent PFA Teflon curtain (Figure 1). The top of the chamber is constructed of Plexiglas and is covered by a second piece of PFA Teflon foil. Four holes are drilled through the top of the chamber: one to insert a fan while the motor is affixed to the top of the Plexiglas board, two to admit tubing to pump air in and out of the chamber, and one to insert a temperature and humidity sensor and other sensor cables. The fourth hole for sensors is not airtight and allows some gas to escape the chamber, which protects it from becoming over-pressurized. The Teflon cover for the top of the chamber and the Teflon curtain that forms the sides of the chamber are affixed using a silicon O-ring, which is fitted to a groove in the Plexiglas board. At the bottom of the chamber, the curtain is held taut by Teflon tubing that is flexible enough to manipulate into the groove built into the aluminum ring, but is strong enough to hold the curtain once it is in place. Once a plant is inserted into the chamber, the end of the Teflon curtain is closed around the stem using zip ties. This is a second location where air may escape the chamber, preventing over-pressurization; however, the two holes in the chamber are small enough to prevent significant mixing between the air in the laboratory and the air in the chamber. The bottom aluminum ring is attached to the vertical skeleton using set screws. This means that the volume of the chamber is adjustable, and can be increased or decreased to accommodate differently sized trees.

The air provided to the chamber originates from the building's compressed air supply. Before this air is introduced to the chamber, it goes through several modification steps (Figure 2). It is routed through a separator to remove particulates and any condensable water vapor, an activated charcoal filter to attempt to "clean" the air through adsorption of low volatility compounds, and a humidifier that provides a steady stream of water to the air. The air-flow is controlled through a pressure controller and a flow meter; the pressure controller is installed before the air modification begins and prevents high pressure from occurring in the air modification panel, and the flow meter is installed at the end of the panel to set the flow between 20 and 60 L/min. After the air is conditioned, it enters and leaves the chamber through Teflon PFA tubing.

Chamber air is pumped to several analyzers: these record the concentration of CO, CO_2, H_2O, and O_3 that are present in the chamber air. A three-way valve connects these analyzers either to the air exiting the chamber or the reference air that exits the conditioning panel before entering the chamber. By intermittently switching between the two connections using the valve, the properties of the chamber air and the reference air are recorded. Calculating the difference between the reference air and chamber air provides the whole tree flux according to

$$\textit{Flux = flow (through chamber)} \times \textit{concentration difference [moles per time]}$$

and is typically referenced to the emitting leaf area, if known. Additional data that is collected from the chamber includes the moisture of the soil the tree is growing in, the temperature and humidity of the chamber air, and the temperature of a leaf on the tree. All data are recorded using a Campbell Scientific CR-23X micrologger.

An aluminum ring that hangs separately from the chamber assembly holds the light sources used in this experiment: twelve 16 W, 1075 lumens, dimmable 5000 K light temperature LED light bulbs (Duracell Procell model 10845A). These bulbs provide visible light to the chamber with minimal infrared (IR) radiation, which means that they do not add heat to the chamber. All twelve bulbs are connected in two sets to dimmer switches that allow for the tuning of light to a desired brightness. The circular arrangement of the bulbs creates an evenly distributed cone of light in the chamber; the dimmer switches are set in such a way that a maximum brightness of 1000 to 1250 µmol m^{-2} s^{-1} is achieved in the center of the chamber, with light levels decreasing towards the edges of the chamber. This maximum brightness corresponds to approximately 50-62.5% of the brightness of full sunlight (2000 µmol m^{-2} s^{-1}). Additionally, two IR lamps can be installed on the light bulb ring to provide additional heat to the chamber as needed.

Environmental Stress and Isoprene Sampling Procedures

Data Collection Procedures

For each experiment conducted in the chamber, all data besides the isoprene flux was calculated using a thirty-minute average of one-minute data. During each thirty-minute period, twenty-five minutes of chamber air samples and five minutes of reference air samples were collected. The first and last minute of each sample period were omitted and the difference between the twenty-three minute chamber period and three-minute reference period was calculated. The difference between the reference and chamber air samples provided a whole tree flux calculation for the time period. These thirty-minute averages constitute the plotted points for each environmental stress series.

Isoprene Sampling Procedures

Isoprene concentrations in the chamber are not recorded by the micrologger; instead, isoprene samples are collected from the chamber using standard glass cartridges filled with Tenax adsorbent, and analyzed using a thermal desorber and gas chromatography with flame ionization detection (TD-GC-FID). Gas samples of 200 mL per cartridge are collected from the chamber air and the reference air; the concentration calculated from the chamber air samples is corrected by the concentration calculated from the reference air samples. The accuracy of the calculated concentration of the samples is determined by comparing them to samples collected from a gas mixture with a known concentration of isoprene. An isoprene response curve was recorded twice during the experiments reported here, and a single response factor was used for most isoprene samples due to the GC-FID's high sensitivity and stability. Isoprene concentrations are given in parts per billion, ppb (by volume), and typically have an associated random error of ≤5%. Figure 3 depicts one of the isoprene response curves and the line of best fit that describes the response factor.

Drought Stress Procedures

Drought stress was simulated in this experiment using potted trees. When a drought stress experiment was begun, the plant was watered to saturation before watering was suspended. Data from the gas analyzers and soil moisture sensor were collected, and daily isoprene samples were taken. The experiment continued until the whole tree gas flux became neutral or near neutral, or until the isoprene flux from the plant was severely reduced. When that point was reached, regular watering was resumed. Sampling typically continued for several days to measure the plants' response to the rewatering cycle.

Ozone Exposure Procedures

Ozone is introduced to the chamber using a UV-ozone generator and zero air. By manipulating the flow rate of air through the chamber and the position of the shield that protects the UV-C bulb in the ozone generator, a chamber concentration as high as 130 ppb can be recorded without a plant in the chamber. Ozone losses in the chamber are calculated by comparing the O_3 concentration recorded in the chamber and reference air. The [O_3] of the chamber air was corrected for chamber flux (loss of O_3 to the Teflon PFA curtain and other surfaces inside the chamber). This value of ozone loss was determined prior to the seedling tests by comparing the reference and chamber air when the chamber was empty, and calculating the difference (the chamber flux). The chamber [O_3] reported is corrected by this value for all calculations. In these experiments, the chamber flux was approximately 6% of total [O_3]. By accounting for ozone losses to the chamber, the difference between the reference air and chamber air will represent O_3 uptake by the plant.

The O_3 concentration and the amount of time a tree is exposed to ozone depends on the relationship that is being represented by the experiment. In an O_3 curve, for example, ozone concentrations will be raised incrementally and isoprene samples will be taken at certain concentrations. The resulting data will represent the relationship between ozone flux and isoprene flux for the tree in the chamber. A long-term ozone exposure test was conducted by leaving the UV-ozone generator on for several days. The plant was exposed to high ozone concentrations during the day and low ozone concentrations at night. The isoprene samples taken during this time as well as the data collected by the micrologger represent any changes in the tree's emissions during this time period.

CHAPTER III

RESULTS

Drought Stress Series

Quercus alba

The *Quercus alba* sample used for drought stress was purchased from a local nursery and was not repotted in new soil; instead, the plant remained in the potting soil that it was planted in at the nursery. The specimen was potted in a 13 L pot. After being transported to the laboratory, it was given several days to acclimate to its new environment before experimentation began. Additionally, the tree was placed next to the chamber during chamber testing, then inside without closing the chamber for several days in order to further acclimate it. This procedure was repeated with all potted species.

On the first day of the experiment, the *Q. alba* sample was watered to saturation (a soil moisture value of approximately 20%). The soil sensor was calibrated to the type of soil the tree was planted in using a comparison conducted on a *Q. muehlenbergii* specimen, which was planted in the same soil as this seedling (see *Q. muehlenbergii* section). Figures 4 through 7 graph the response of *Q. alba* to drought stress.

Soil moisture decayed logarithmically throughout the drought period of the experiment; a steep drop on day 302 was probably caused by a loss of contact of the sensor with the soil as it dried and contracted. During the first ten days of drought, the H_2O flux from this seedling decreased nearly linearly. CO_2 fluxes remained unaffected during the first four days of the experiment, and

then began to decrease linearly. By day 309, CO_2 and H_2O flux had decreased by 75 percent since the start of the experiment. The isoprene flux showed a weaker response to drought. Isoprene fluxes did not significantly vary during the first nine days of the experiment. On day 309, isoprene flux had decreased by 40 percent. Isoprene flux, CO_2 flux, and H_2O flux decreased by nearly 100 percent by day 314.

Rewatering of the seedling began on day 314, as is evident from the increase in soil moisture. Gas sampling continued for several days after rewatering. Despite the saturated soil, tree fluxes did not begin to recover by day 317. This was apparently due to early senescence of the plant. Leaf senescence was observed during the drought experiment; by the end of the experiment, the sample had stopped photosynthesizing and did not exert a response to rewatering. An attempt can be made to correct the data by estimating the amount of photosynthesizing leaf area at a given time in the experiment; the data currently presented has not been corrected for leaf senescence. This results in significant uncertainty of the effect of drought stress on the sample, since the decrease in tree gas fluxes is due to both drought and loss of photosynthesizing leaf area.

Heat flecks were evident on several occurrences during *Q. alba* drought stress testing (Figure 6). Many mornings and afternoons saw a sharp increase of several degrees in leaf temperature. This was possibly caused by an external source: the sun shining through uncovered windows in the morning and afternoon hours. When sunlight came through the windows of the laboratory at the right angle, it may have caused additional radiation loading on the temperature sensor that raised the recorded leaf temperature. Since the laboratory chamber was not completely isolated from

sunlight, the leaf temperature sensor was susceptible to radiation loading by the sun during every experiment conducted in this research project; as a result, heat flecks were occasionally recorded in every experiment. Additionally, the recorded leaf temperatures of 30° C and greater on day 301 were caused by temporarily turning on the IR lamps that provide additional heat to the chamber. This effect was also recorded in the chamber temperature (not graphed).

Quercus virginiana

The *Quercus virginiana* sample used for drought stress was donated by a faculty member in Ecosystem Sciences and re-potted into a 2:1 volume to volume (v/v) mix of commercially available topsoil (locally purchased pasture topsoil) to sand (Quikrete washed play sand). This specimen was potted in a 4.5 L pot. After replanting, the *Q. virginiana* sample grew for several months in a Texas A&M greenhouse. When the sample was ready for experimentation, it was transported from the greenhouse to the laboratory and given several weeks to acclimate to its new environment before experimentation began (see above).

On the first day of the experiment, the *Q. virginiana* sample was watered to saturation (a soil moisture value of approximately 19%) before watering was suspended. Soil moisture data for this sample are representative of the real value of soil moisture. Figures 8 through 11 graph the response of *Q. virginiana* to drought stress.

CO_2 and H_2O fluxes from this sample were more resilient than *Q. alba* in the first days of the experiment. After six days without watering, CO_2 and H_2O fluxes had not significantly decreased. However, this specimen shut down rapidly, though, with fluxes plummeting within

two days to nearly neutral chamber flux values. The plant was rewatered on day 358 after nine days of drought stress. CO_2 and H_2O fluxes responded just as rapidly to rewatering as they did to the drought stress, with significant increases in flux after the first day of rewatering. CO_2 and H_2O fluxes did not return to pre-drought stress values by the end of the sampling period; however, the graphed fluxes have not been corrected for a significant amount of leaf area loss that occurred after rewatering, when many of the specimen's leaves died.

The isoprene flux of this seedling was less responsive to the effect of drought stress than the CO_2 and H_2O fluxes were. During the first several days of drought, the isoprene flux from the plant did not vary significantly. A sudden increase in isoprene flux on day 356 occurred simultaneously with the sudden decrease in H_2O flux from the sample; lowered transpiration from the leaves caused slight leaf temperature increases during this time, which likely contributed to the isoprene flux increase in response (Figure 10). After this spike in isoprene, the flux decreased at a linear rate, even during the rewatering cycle. Isoprene flux had only just begun to increase when sampling stopped on day 363.

Quercus muehlenbergii

The *Quercus muehlenbergii* specimen used for drought stress was purchased from the same nursery as the *Q. alba* sample. It was potted in an 18 L pot in the same potting soil as *Q. alba*, and was not re-potted into the soil and sand mixture. The seedling was kept in the laboratory for several months before experimentation began, and as a result was well acclimated to laboratory conditions when it was placed in the chamber for experimentation.

On the first day of the experiment, the *Q. muehlenbergii* sample was watered to saturation (a soil moisture value of approximately 28%) before watering was suspended. A soil sensor calibration was conducted for this type of soil using a second pot filled with the same soil as the *Q. muehlenbergii* sample and fitted with the same type of soil sensor. The precision of the soil sensor was assessed by suspending watering and weighing the pot over several weeks as the soil dried. A comparison of water mass loss and the corresponding soil moisture sensor reading indicated that the soil sensor measured the water loss precisely. When drought testing was completed for the *Q. muehlenbergii* sample, the soil sensor used in the drought experiment was placed in the second pot. Then, the soil was well-watered and the values of the two soil sensors were compared. In the well-watered soil, the soil sensor used in the drought experiment recorded values approximately 5% lower than the soil sensor used for comparison. Therefore, the soil moisture values from this experiment were corrected by uniformly increasing the data by 5%. Figures 12 through 18 graph the response of *Q. muehlenbergii* to drought stress.

The *Q. muehlenbergii* sample proved to be the most resilient out of the three seedlings subjected to drought stress. Soil moisture decayed logarithmically throughout the 36-day drought period. During the first 18 days of drought, H_2O flux and CO_2 flux remained relatively unaffected except during periods of elevated ambient [CO_2] (see below). After day 46, H_2O and CO_2 flux from the sample decrease linearly, except during high [CO_2] episodes. By day 60, H_2O and CO_2 flux had decreased to nearly neutral chamber flux values. However, isoprene flux remained unaffected, except during a period of elevated ambient [CO_2], until approximately day 57. From day 57 to the day 63, isoprene decreased on a relatively linear scale. On day 64, a sharp decrease in isoprene flux occurred.

Rewatering of the sample began on day 64, as is evident from the increase in soil moisture. Gas sampling was continued for several days after rewatering. CO_2 flux and H_2O flux began to show recovery after two days, and by the end of the sampling period had recovered to approximately 50% of their starting values. Isoprene flux generally increased during the rewatering period, but significant uncertainty resides in the isoprene flux during the rewatering period due to technical issues with the TD-GC-FID used to analyze the isoprene samples.

Due to the longevity of this drought experiment and the use of compressed air for the chamber, an additional observation throughout the experiment was the effect of elevated ambient [CO_2] on the specimen's gas exchange. In particular, days 33-36, 47-48, and 54-56 (Figure 12) illustrate the effect of elevated [CO_2] on plant gas exchange during the drought test period. The first two [CO_2] episodes were marked by a decrease in isoprene flux, an increase in CO_2 flux, and a decrease in H_2O flux. The third [CO_2] episode was only marked by a decrease in isoprene flux; the effect was not observed in CO_2 flux or H_2O flux.

For this drought period, the isoprene flux and elevated ambient [CO_2] were compared (Figure 16). For this comparison, only [CO_2] above 450 ppm was considered "elevated". Additionally, isoprene flux and elevated [CO_2] values from the period where isoprene flux was significantly impacted by drought stress were omitted. Figure 16 shows a weak statistically significant ($p < .01$) correlation between higher CO_2 values and lower isoprene flux.

Since there were three periods of significantly higher chamber [CO_2] during the *Q. muehlenbergii* drought stress series, the CO_2 was normalized (Figures 17 and 18). For this normalization, CO_2 flux and chamber [CO_2] values were taken from the chamber data at a constant light level (maximum light during the afternoon) and a relative humidity level between 60% and 70%. Correlating the CO_2 flux (y) and chamber [CO_2] values (x) in this controlled scenario and drawing a line of best fit through the data creates a normalization equation that reduces the effect of variations of chamber [CO_2]. Figure 18 depicts the actual CO_2 flux and the normalized CO_2 flux (to 400 ppm chamber CO_2) from 12 pm to 4 pm on days 29 through 50; the normalization eliminates much of the variation in CO_2 flux during this period.

Ozone Exposure Series

Quercus virginiana

The *Q. virginiana* sample used for drought sampling was also subjected to an ozone exposure series. In this experiment, the seedling was exposed to chamber ozone concentrations of 40, 60, and 80 ppb. Ozone exposure was limited to the period when the sample was under maximum light intensity from the light bulbs. The experiment's duration was seven days. Figures 19 through 23 graph the responses of *Q. virginiana* to ozone exposure.

CO_2 and H_2O fluxes from this sample did not vary significantly during the ozone exposure period. Isoprene flux was also not significantly affected by this level of ozone exposure. Figures 20 and 21 graph the isoprene flux from *Q. virginiana* during ozone exposure. During the seven-day experiment, isoprene flux reached a maximum of 11.3 nmol m^{-2} s^{-1} and a minimum of 5.6 nmol m^{-2} s^{-1}, with a standard deviation of 1.0 nmol m^{-2} s^{-1}. Isoprene samples collected on a given

day varied between 0.7 and 3.2 nmol m^{-2} s^{-1}; thus the standard deviation of isoprene flux observed during this period was within the daily range of isoprene variability.

Quercus muehlenbergii

The *Quercus muehlenbergii* seedling used for drought sampling was also subjected to a longer term ozone exposure series prior to water withholding. During the day, chamber ozone concentration was set to 70 ppb; during the evening hours, chamber ozone concentration was set to approximately 20-40 ppb for overnight exposure. The actual nighttime value varied from day to day because this adjustment was made by hand by various experimenters rather than electronically, introducing the element of operator error in creating a consistent nighttime ozone concentration. Figures 24 through 28 graph the response of *Q. muehlenbergii* to long-term ozone exposure. On Figure 25, the daily decrease in chamber ozone concentration before a sharp spike to 70 ppb corresponds to the period between when the chamber lights first turn on and ozone production is increased to the maximum setting.

CO_2 flux and H_2O flux did not vary significantly during the first four days of the experiment (Figure 24). Days 20 through 23 marked a decrease in H_2O flux and an increase in CO_2 flux (decreased transpiration and increased photosynthesis). Coincident to this were observations of a decrease in isoprene and ozone fluxes. These changes in plant fluxes were likely caused by elevated chamber [CO_2]. On days 24 and 25, CO_2 flux and H_2O flux returned to values similar to the start of the experiment. Ozone flux remained approximately constant and isoprene flux began to increase from days five through eight.

CHAPTER IV

CONCLUSIONS

Drought Stress

The drought stress experiments conducted in this laboratory chamber provided results that were consistent with past studies on the effect of drought stress on isoprene emissions. All three drought experiments indicated a stronger relationship of CO_2 and H_2O flux to drought stress than isoprene flux. CO_2 and H_2O flux gradually decreased to neutral chamber values in the *Q. alba* and *Q. muehlenbergii* drought experiments while the isoprene flux decreased rapidly, and later in the experiment than CO_2 and H_2O fluxes. In the *Q. virginiana* drought experiment, shutdown of the CO_2 and H_2O fluxes of the sample happened much more rapidly, and a steady decrease in isoprene flux was observed until the last day of the experiment. The two drastically different drought stress results may have been due in part to the different volumes of the pots used for the samples. *Q. alba* and *Q. muehlenbergii* were planted in larger pots that could hold more water to begin the drought experiment, while *Q. virginiana* was potted in a smaller pot. Additionally, the *Q. virginiana* sample was potted in a mixture of soil and sand that dried out much more rapidly than the soil the other two samples were planted in. This property of the soil may have also contributed to the speedy results of the *Q. virginiana* drought stress experiment.

Ozone Exposure

The results gathered from the ozone exposure experiments conducted in the laboratory chamber are less conclusive than the drought stress experiments. A significant change in isoprene

emissions due to ozone exposure was not observed in the short term or longer term ozone exposure experiments. In both experiments, the chamber [O_3] may simply have been too low to observe an effect on isoprene emissions. In previous studies on the effect of ozone exposure on isoprene emissions, ozone concentrations of 100 to 300 ppb have been used to expose seedlings to (Loreto et al., 2001; Loreto and Velikova, 2001). The highest ozone concentration achieved in this laboratory chamber while a sample was isolated inside was only ~80 ppb. Hence, a peak ozone concentration of 80 ppb may have been too low to observe the effects on isoprene that have been recorded in previous studies.

REFERENCES

Ainsworth, E., C. Yendrek, S. Sitch, W. Collins, and L. Emberson, 2012: The Effects of Tropospheric Ozone on Net Primary Productivity and Implications for Climate Change. *Annual Review of Plant Biology*, **63,** 637-661.

Atkinson, R., and J. Arey, 2003: Gas-phase tropospheric chemistry of biogenic volatile organic compounds: a review. *Atmospheric Environment*, **37,** S197-S219.

Beckett, M., and Coauthors, 2012: Photosynthetic limitations and volatile and non-volatile isoprenoids in the poikilochlorophyllous resurrection plant Xerophyta humilis during dehydration and rehydration. *Plant, Cell, & Environment*, **35,** 2061-2074.

Claeys, M., and Coauthors, 2004: Formation of secondary organic aerosols through photooxidation of isoprene. *Science*, **303,** 1173-1176.

Dani, K. G. S., I. M. Jamie, I. C. Prentice, and B. Atwell, 2014: Evolution of isoprene emission capacity in plants. *Trends in Plant Science*, **19,** 439-446.

Dement, W., B. Tyson, and H. Mooney, 1975: Mechanism of monoterpene volatilization in salvia-mellifera. *Phytochemistry*, **14,** 2555-2557.

Dreyfus, G., G. Schade, and A. Goldstein, 2002: Observational constraints on the contribution of isoprene oxidation to ozone production on the western slope of the Sierra Nevada, California. *Journal of Geophysical Research - Atmospheres*, **107**.

Fang, C., R. Monson, and E. Cowling, 1996: Isoprene emission, photosynthesis, and growth in sweetgum (liquidambar styraciflua) seedlings exposed to short- and long-term drying cycles. *Tree Physiology*, **16,** 441-446.

Fuentes, J. D., and Coauthors, 2000: Biogenic Hydrocarbons in the Atmospheric Boundary Layer: A Review. *BAMS*, **81,** 1537-1575.

Guenther, A., R. Monson, and R. Fall, 1991: Isoprene and monoterpene emission rate variability - observations with eucalyptus and emission rate algorithm development. *Journal of Geophysical Research - Atmospheres*, **96,** 10799-10808.

Guenther, A., and Coauthors, 1995: A global model of natural volatile organic compound emissions. *Journal of Geophysical Research*, **100,** 8873-8892.

Harley, P., A. Guenther, and P. Zimmerman, 1996: Effects of light, temperature and canopy position on net photosynthesis and isoprene emission from sweetgum (Liquidambar styraciflua) leaves. *Tree Physiology*, **16,** 25-32.

Harley, P., R. Monson, and M. Lerdau, 1999: Ecological and evolutionary aspects of isoprene emission from plants. *Oecologia*, **118,** 109-123.

Loreto, F., M. Mannozzi, C. Maris, P. Nascetti, F. Ferranti, and S. Pasqualini, 2001: Ozone Quenching Properties of Isoprene and Its Antioxidant Role in Leaves. *Plant Physiology*, **126,** 993-1000.

Loreto, F., and V. Velikova, 2001: Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiology*, **127,** 1781-1787.

Monson, R., and R. Fall, 1989: Isoprene emission from aspen leaves - influence of environment and relation to photosynthesis and photorespiration. *Plant Physiology*, **90,** 267-274.

Pegoraro, E., A. Rey, J. Greenburg, P. Harley, J. Grace, Y. Malhi, and A. Guenther, 2004a: Effect of drought on isoprene emission rates from leaves of Quercus virginiana Mill. *Atmospheric Environment*, **38,** 6149-6156.

Pegoraro, E., A. Rey, E. Bobich, G. Barron-Gafford, K. Grieve, Y. Malhi, and R. Murthy, 2004b: Effect of elevated CO2 concentration and vapor pressure deficit on isoprene emission from leaves of Populus deltoides during drought. *Functional Plant Biology*, **31,** 1137-1147.

Potosnak, M., and Coauthors, 2014a: Observed and modeled ecosystem isoprene fluxes from an oakdominated temperate forest and the influence of drought stress. *Atmospheric Environment*, **84,** 314-322.

Potosnak, M., L. LeStourgeon, and O. Nunez, 2014b: Increasing leaf temperature reduces the suppression of isoprene emission by elevated CO2 concentration. *Science of the Total Environment*, **481,** 352-359.

Sharkey, T., F. Loreto, and C. Delwiche, 1991: High-carbon dioxide and sun shade effects on isoprene emission from oak and aspen tree leaves. *Plant Cell and Environment*, **14,** 333-338.

Sharkey, T., A. Wiberly, and A. Donohue, 2008: Isoprene Emission from Plants: Why and How. *Annals of Botany*, **101,** 5-18.

Singsaas, E., and Coauthors, 1999: Kinetics of leaf temperature fluctuation affect isoprene emission from red oak (Quercus rubra) leaves. *Tree Physiology*, **19,** 917-924.

Tanner, D., A. Jefferson, and F. Eisele, 1997: Selected ion chemical ionization mass spectrometric measurement of OH. *Journal of Geophysical Research - Atmospheres*, **102,** 6415-6425.

Tuazon, E., and R. Atkinson, 1990: A product study of the gas-phase reaction of isoprene with the OH radical in the presence of NOx. *International Journal of Chemical Kinetics,* **22,** 1221-1236.

APPENDIX

Figure 1. A photo of the completed chamber assembly. The light ring and chamber are suspended by separate pulley systems; note the fan motor above the Plexiglas plate, and the fan blade within the chamber.

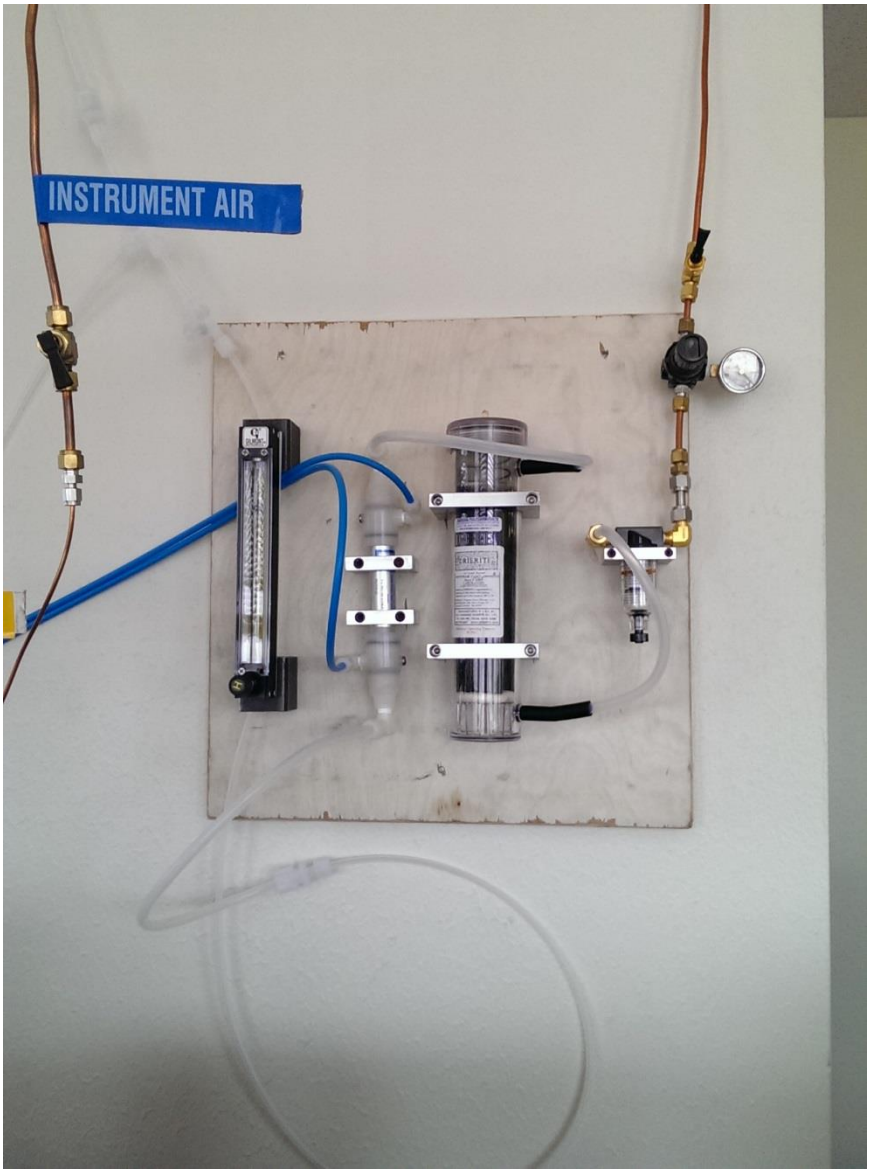

Figure 2. Pictured above is the air modification panel. From right to left: the separator, the activated charcoal filter, the humidifier, and the second flow meter.

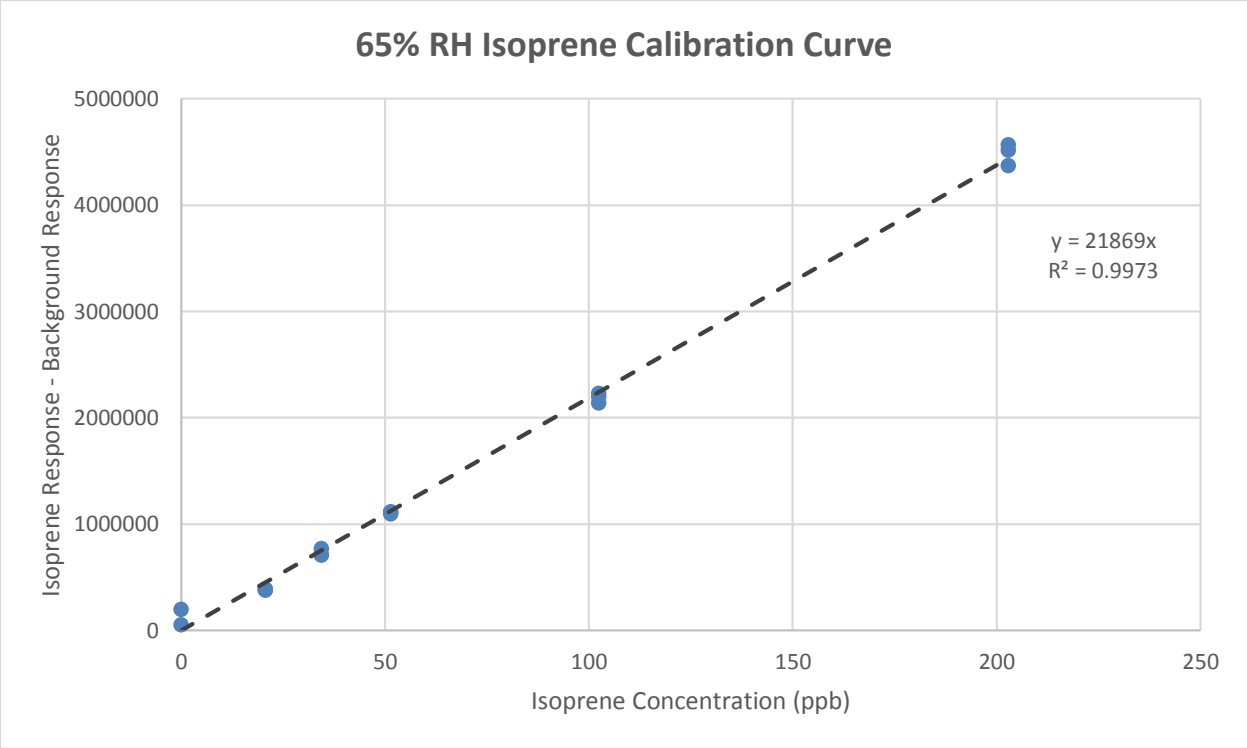

Figure 3. Results from one of two isoprene response curves conducted during the experiments reported in this thesis. Both response curves gave a response factor of ~22000 per ppb.

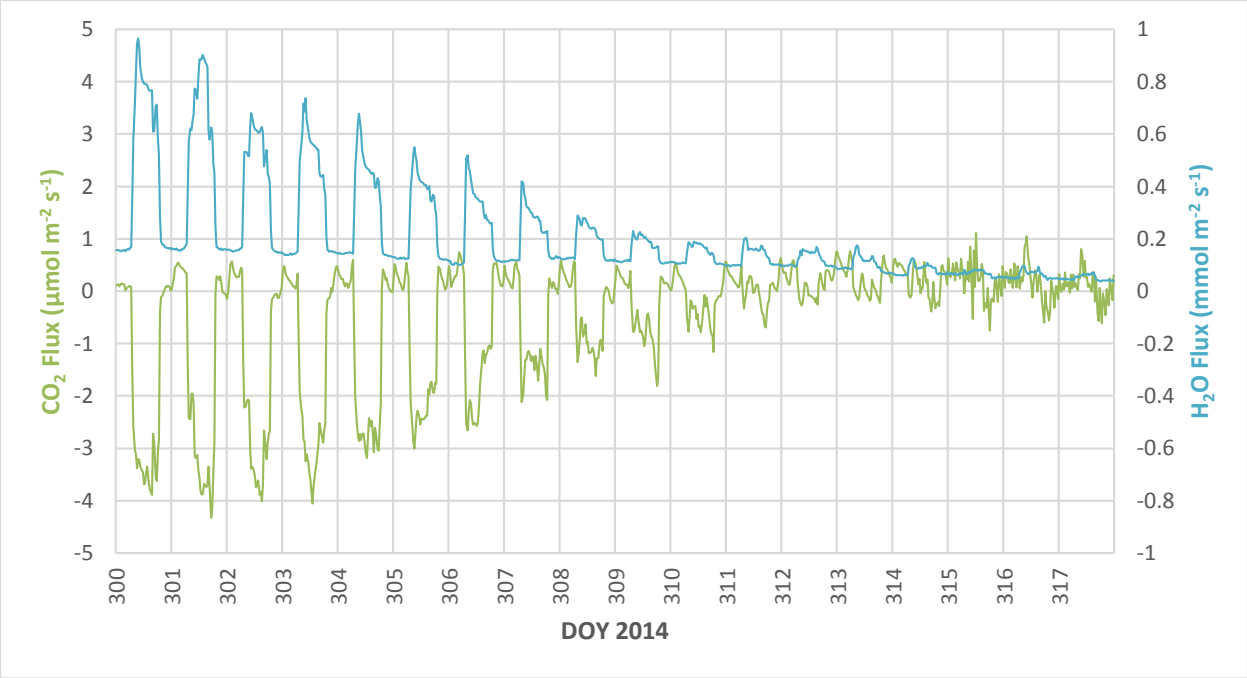

Figure 4. CO_2 flux and H_2O flux values from *Q. alba* during drought stress.

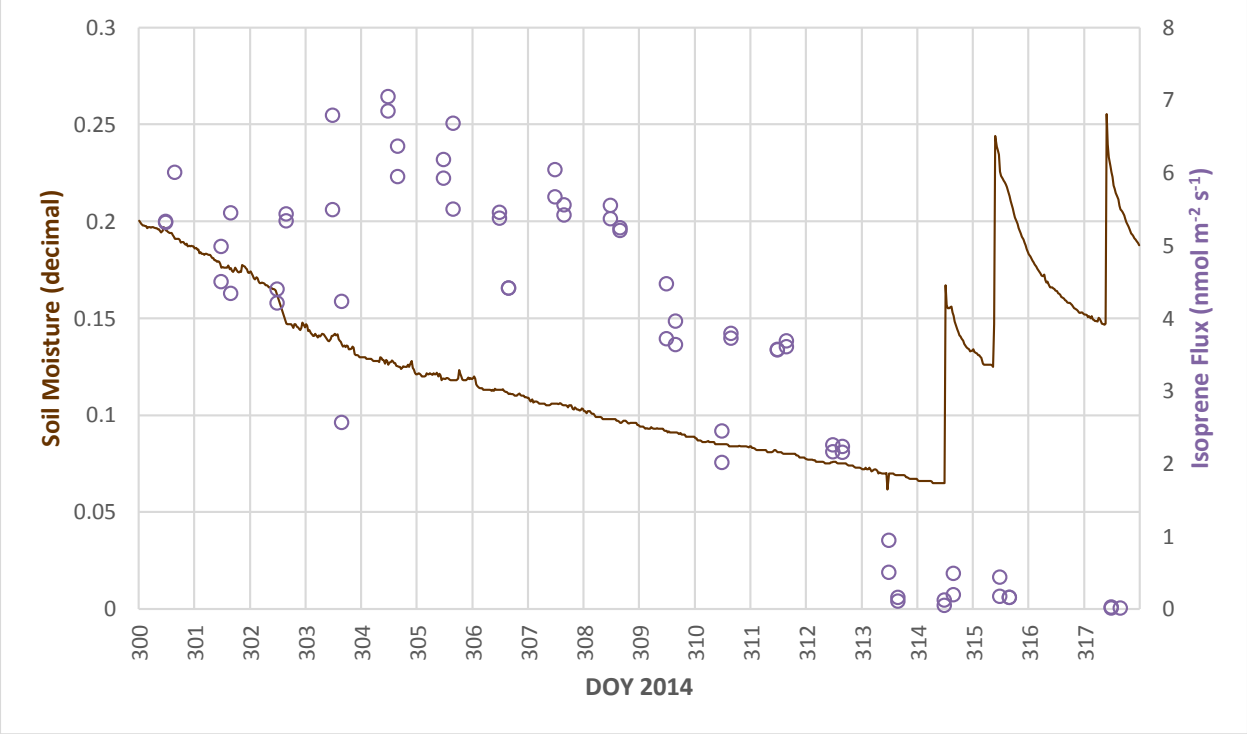

Figure 5. Soil moisture and isoprene flux values from *Q. alba* during drought stress.

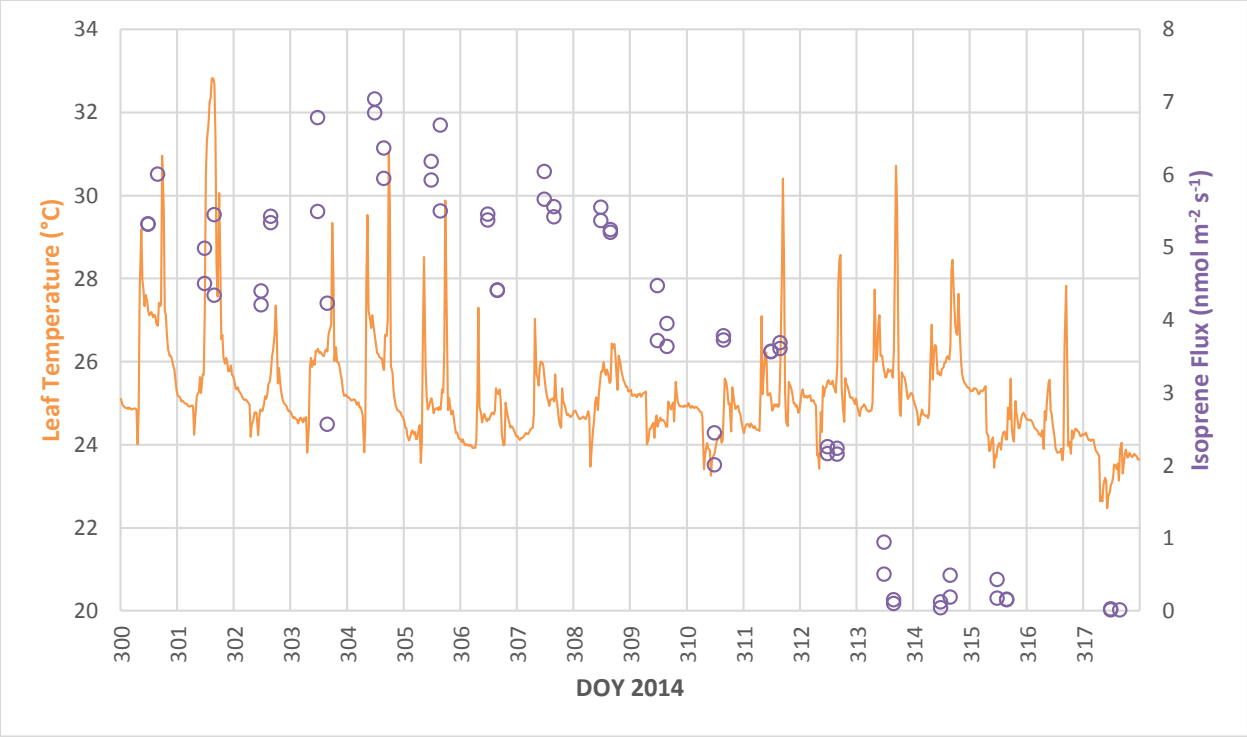

Figure 6. Leaf temperature and isoprene flux values from *Q. alba* during drought stress.

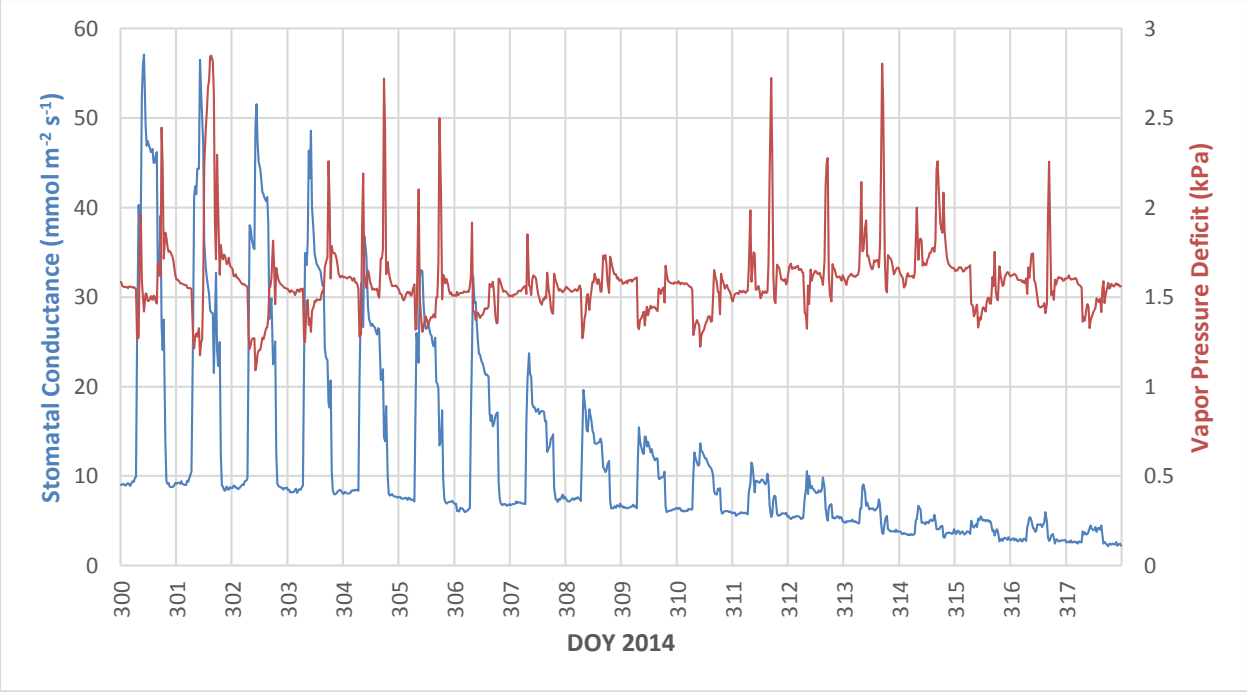

Figure 7. Stomatal conductance and vapor pressure deficit values from *Q. alba* during drought stress.

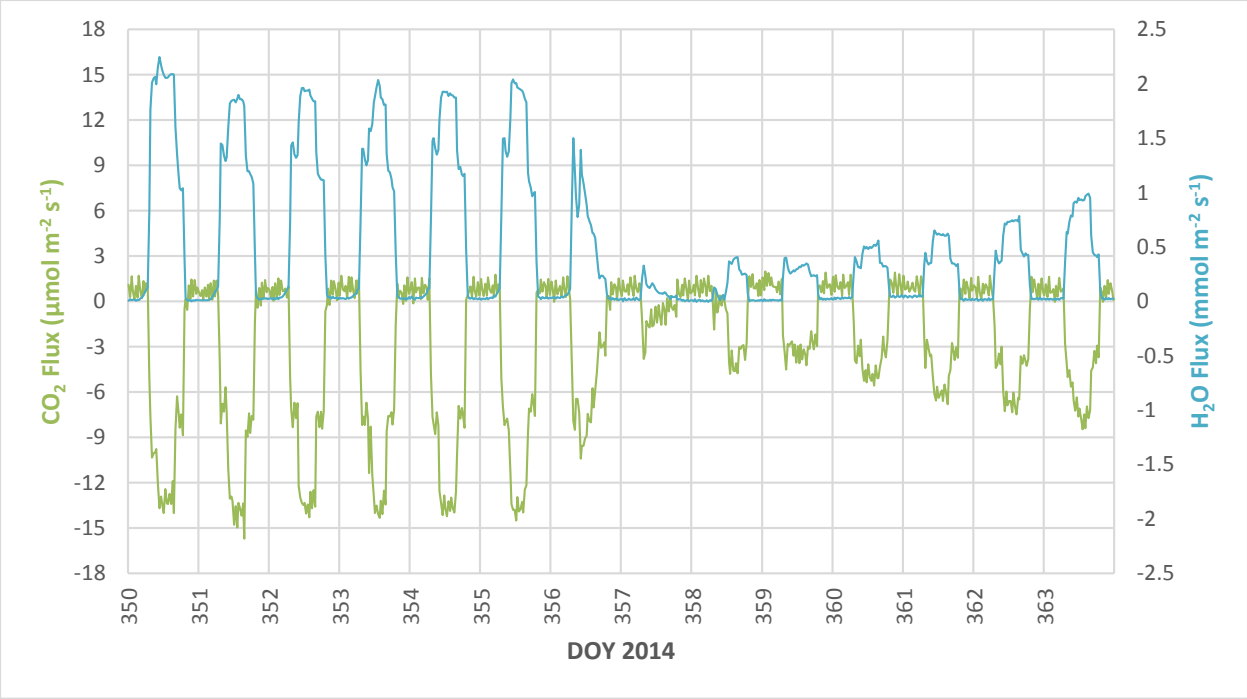

Figure 8. CO_2 flux and H_2O flux values from *Q. virginiana* during drought stress.

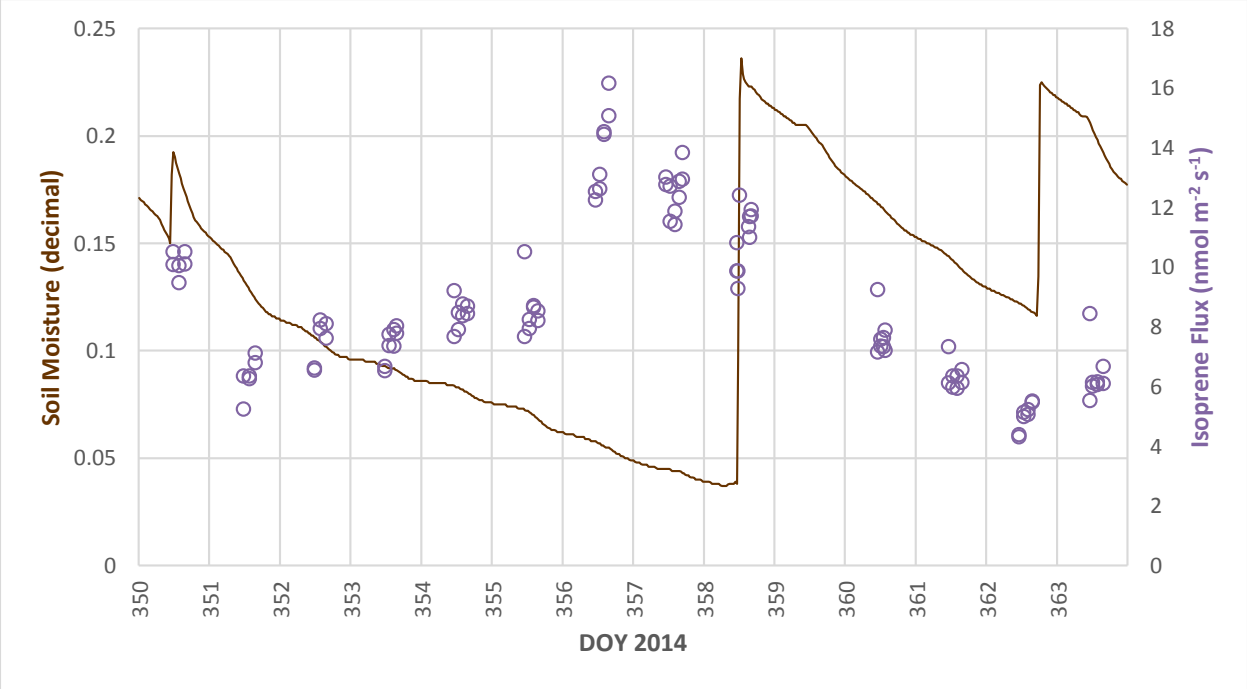

Figure 9. Soil moisture and isoprene flux values from *Q. virginiana* during drought stress.

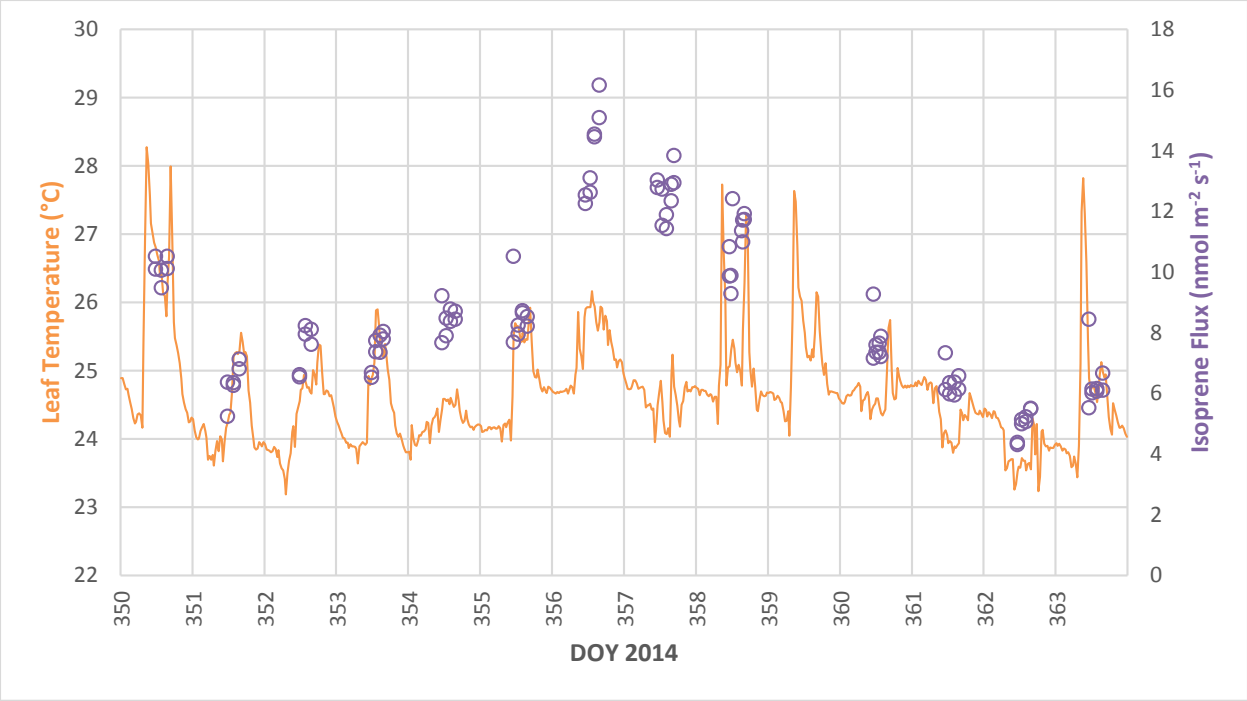

Figure 10. Leaf temperature and isoprene flux values of *Q. virginiana* during drought stress.

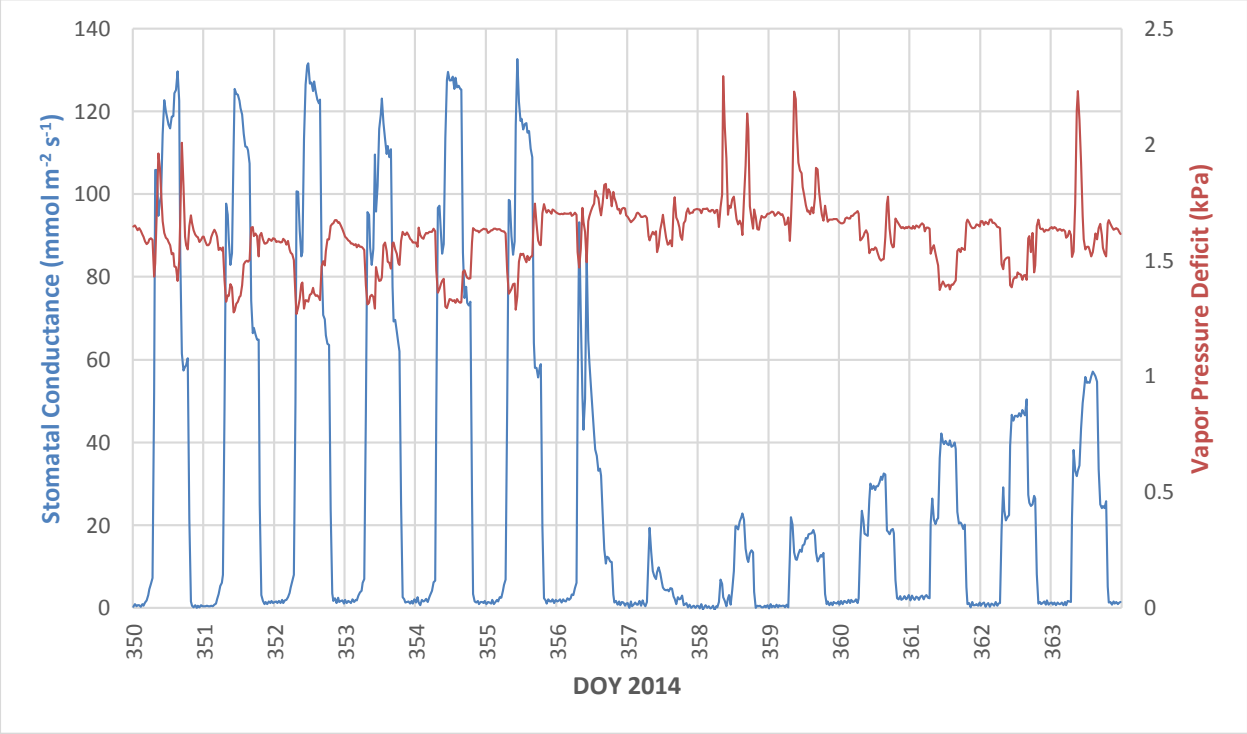

Figure 11. Stomatal conductance and vapor pressure deficit values from *Q. virginiana* during drought stress.

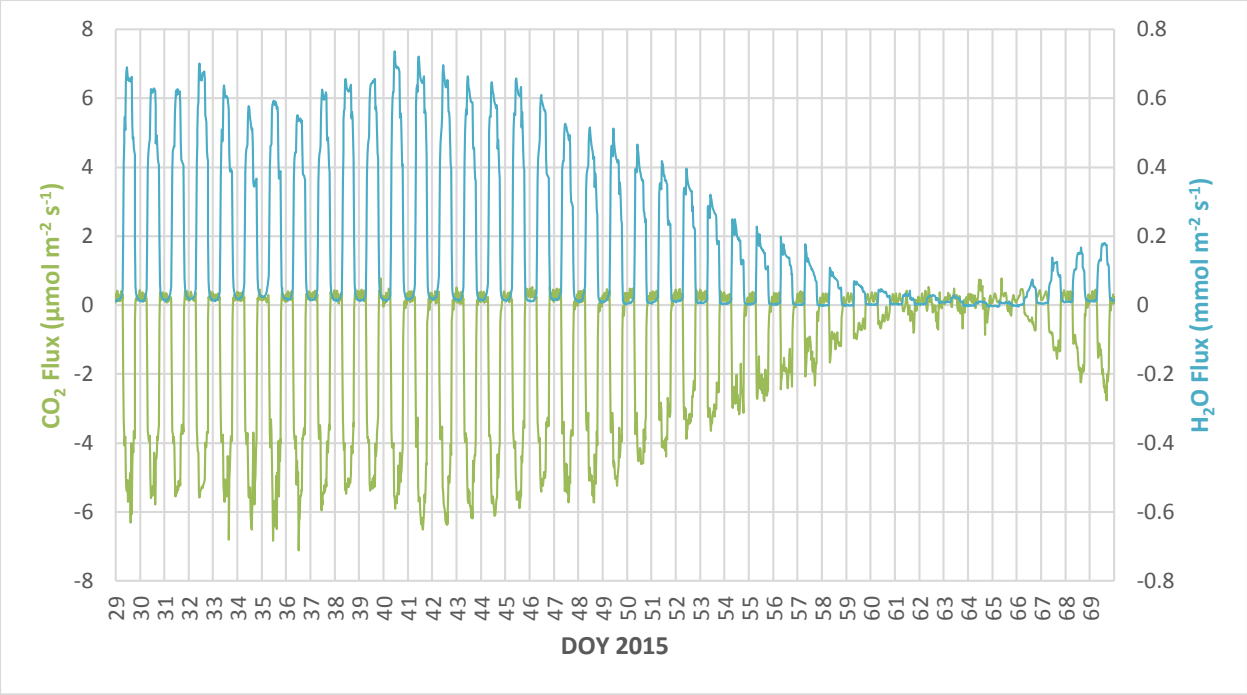

Figure 12. CO_2 and H_2O flux values from *Q. muehlenbergii* during drought stress.

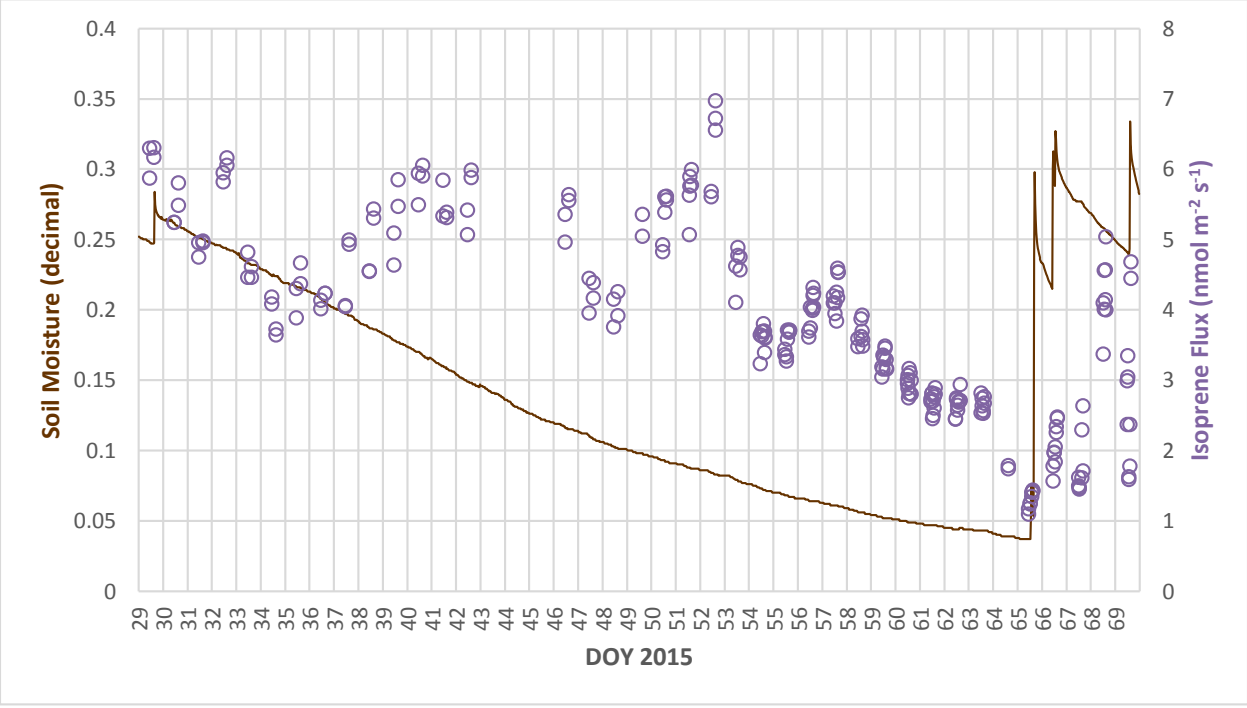

Figure 13. Soil moisture and isoprene flux values from *Q. muehlenbergii* during drought stress.

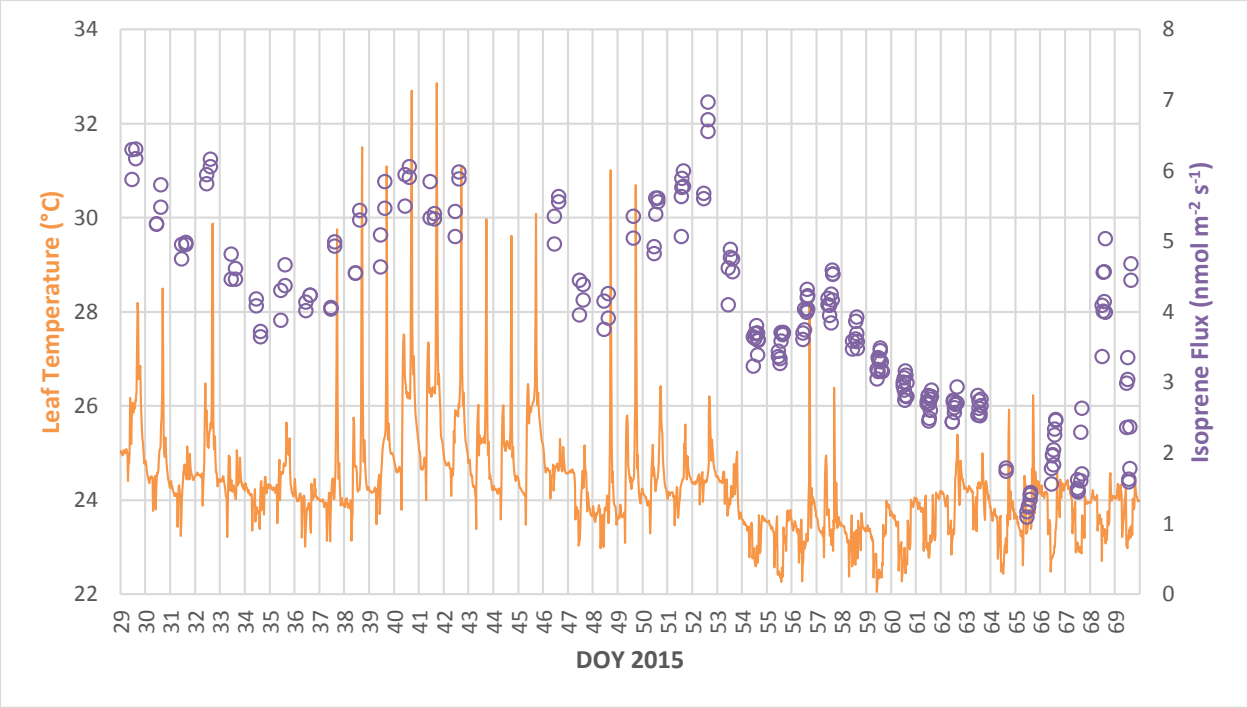

Figure 14. Leaf temperature and isoprene flux values from *Q. muehlenbergii* during drought stress.

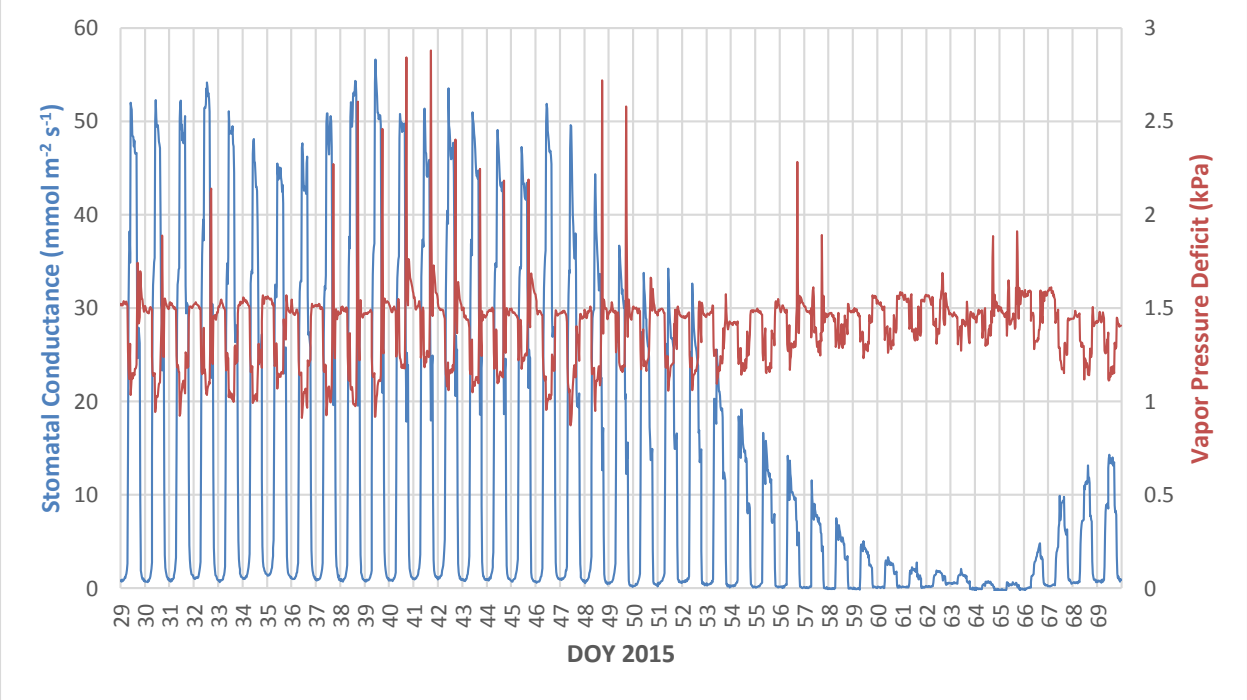

Figure 15. Stomatal conductance and vapor pressure deficit values from *Q. muehlenbergii* during drought stress.

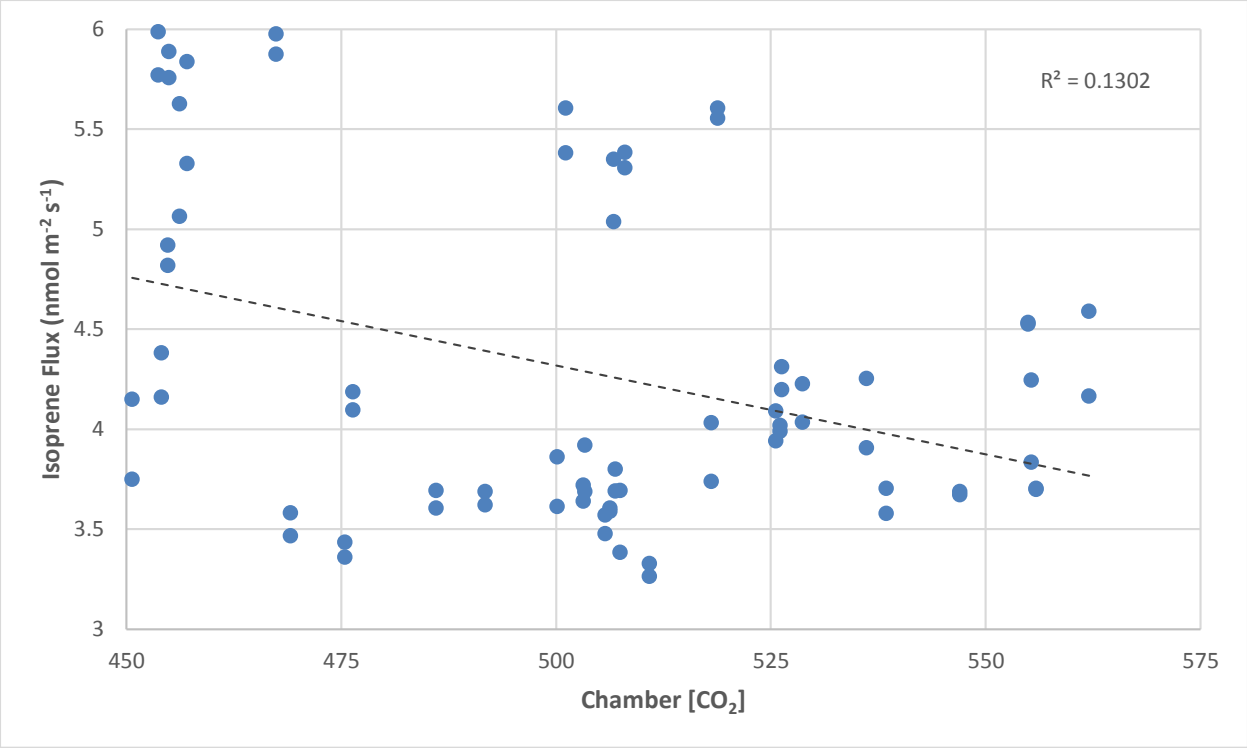

Figure 16. Isoprene emission suppression at [CO_2] > 450 ppm from *Q. muehlenbergii* ($p < .01$).

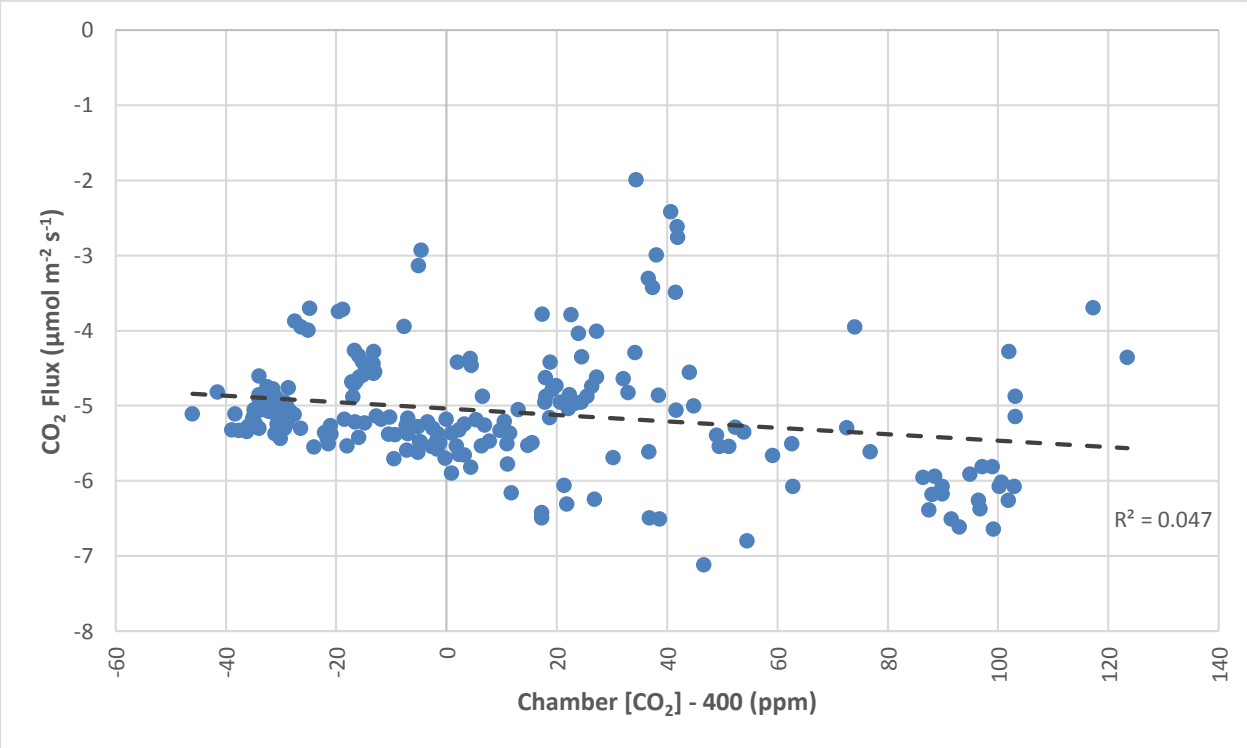

Figure 17. CO_2 flux and chamber [CO_2] for *Q. muehlenbergii*. The trendline represents the CO_2 normalization flux at a [CO_2] of 400 ppm.

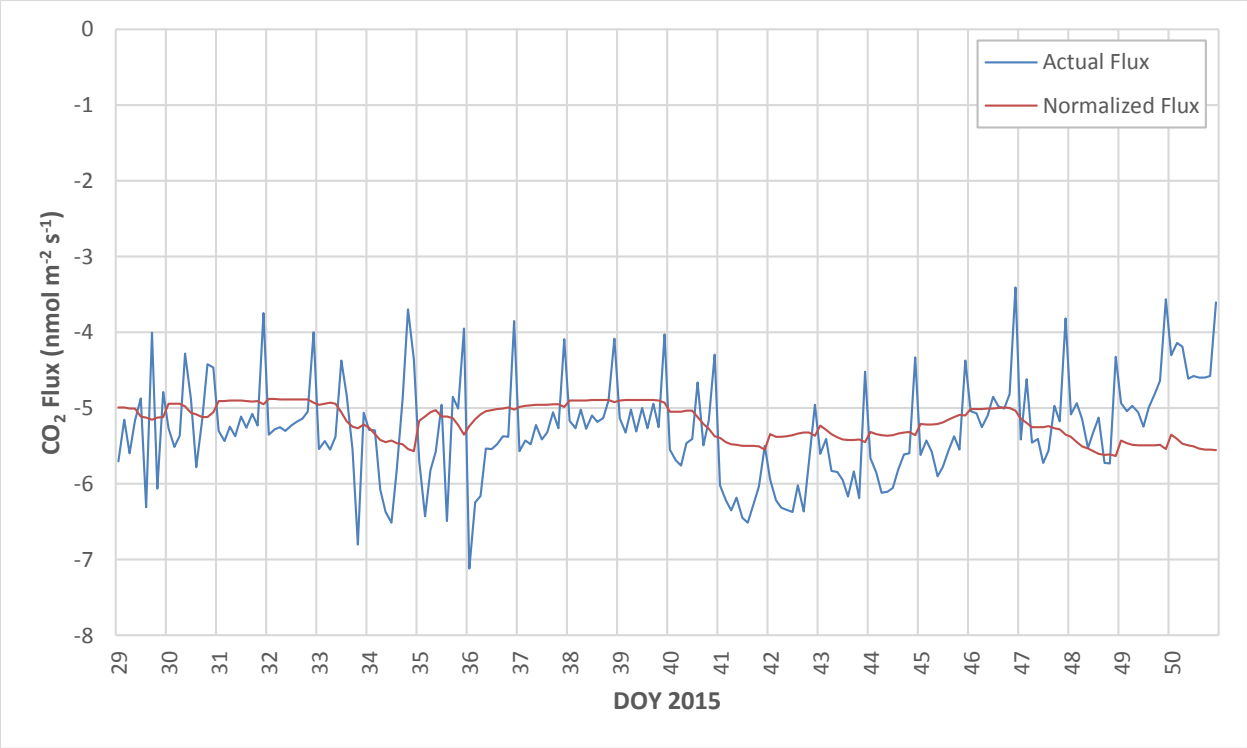

Figure 18. Actual CO_2 flux and normalized CO_2 flux values from *Q. muehlenbergii* during the early stages of drought stress.

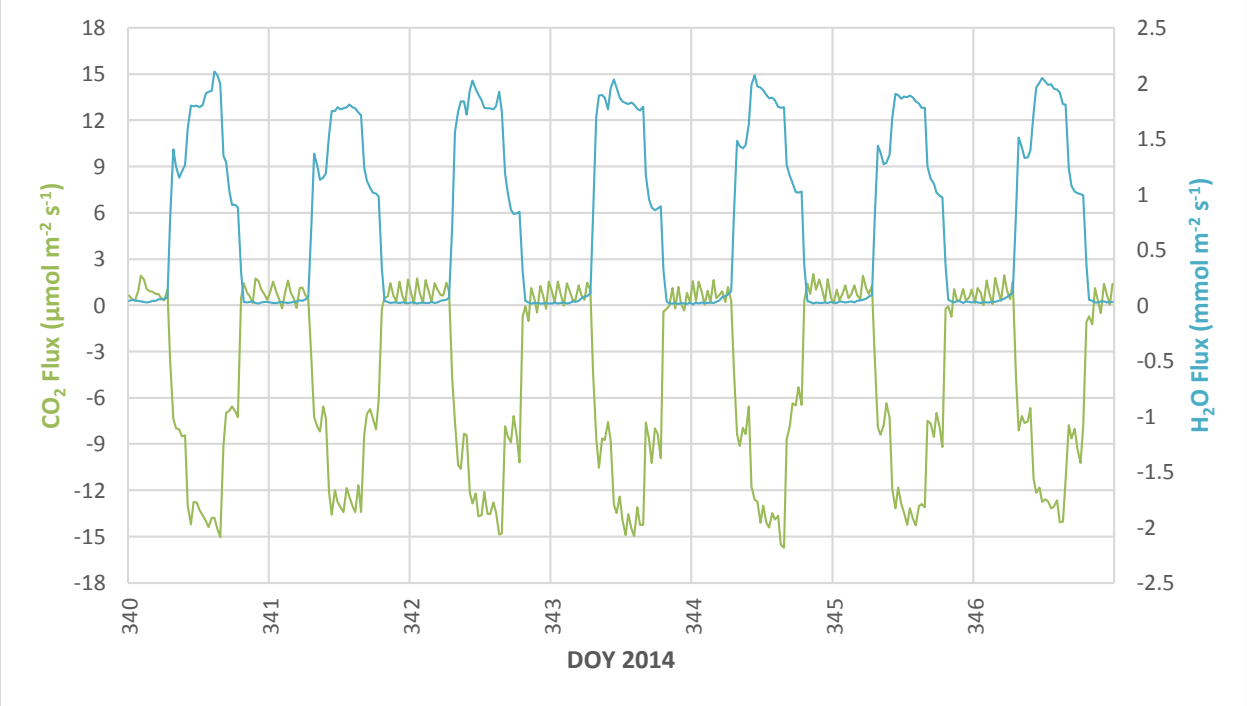

Figure 19. CO_2 flux and H_2O flux values from *Q. virginiana* during ozone exposure.

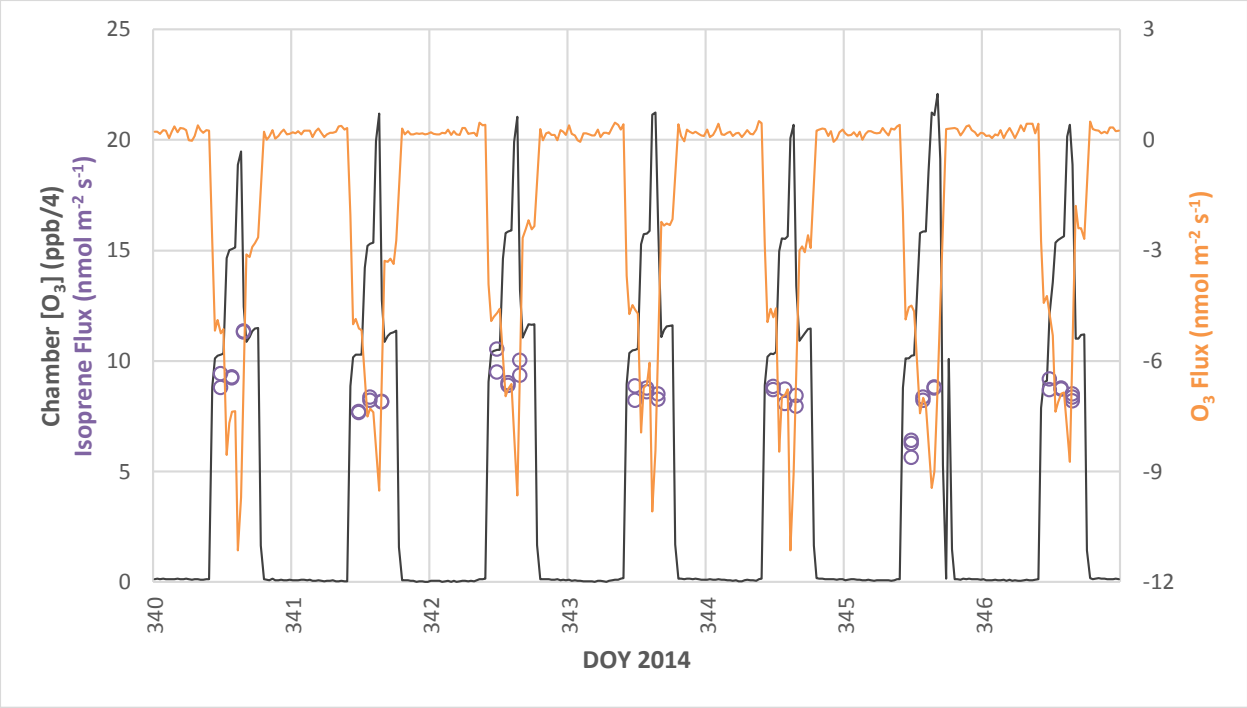

Figure 20. Ozone flux, chamber ozone concentration, and isoprene flux values from *Q. virginiana* during ozone exposure.

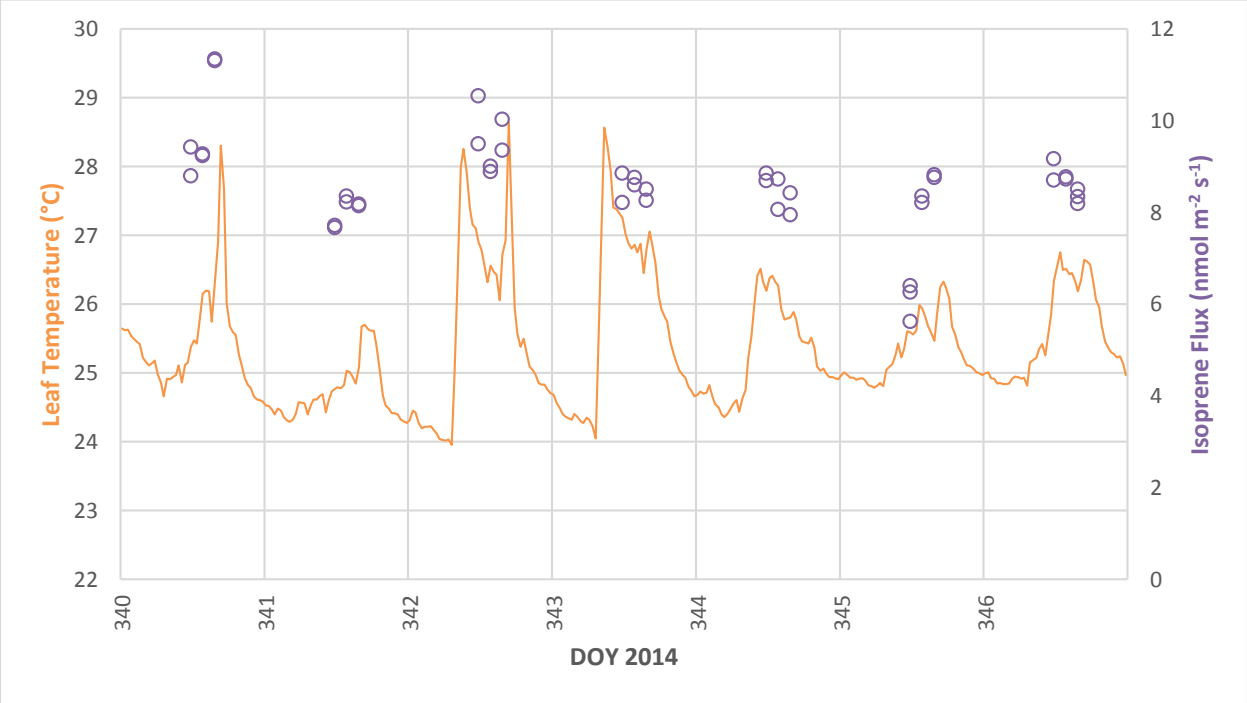

Figure 21. Leaf temperature and isoprene flux values from *Q. virginiana* during ozone exposure.

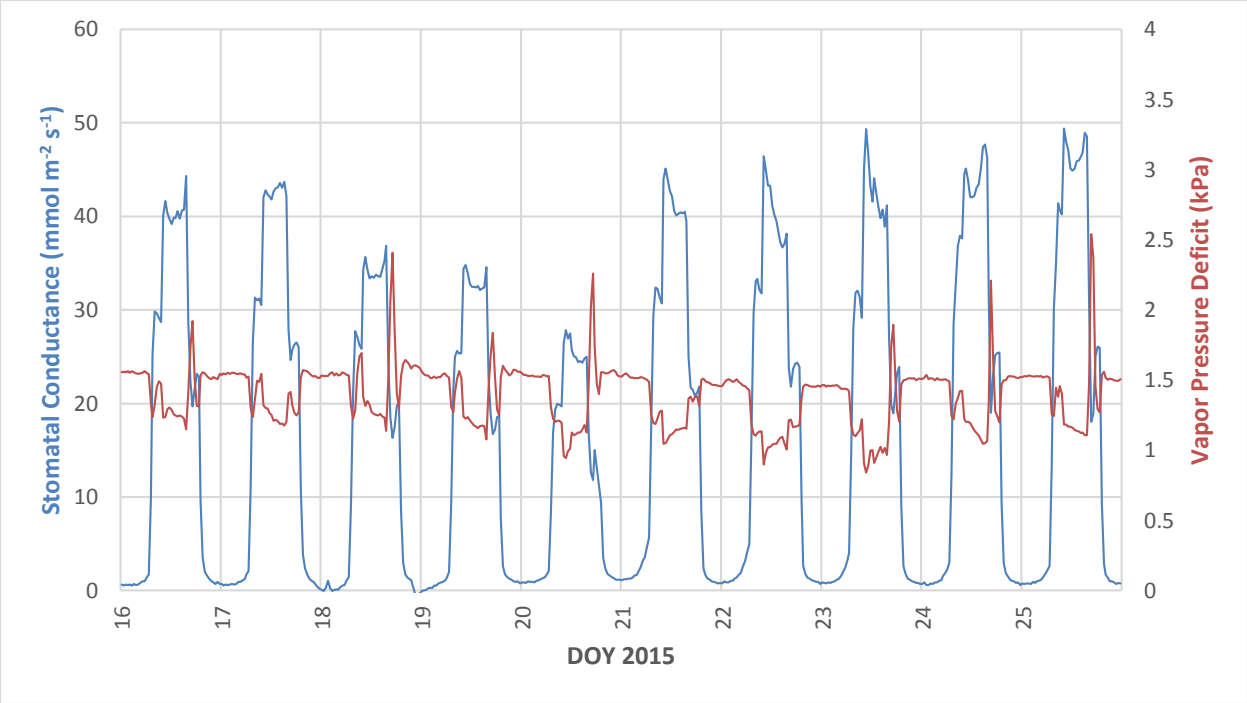

Figure 22. Stomatal conductance and vapor pressure deficit from *Q. virginiana* during ozone exposure.

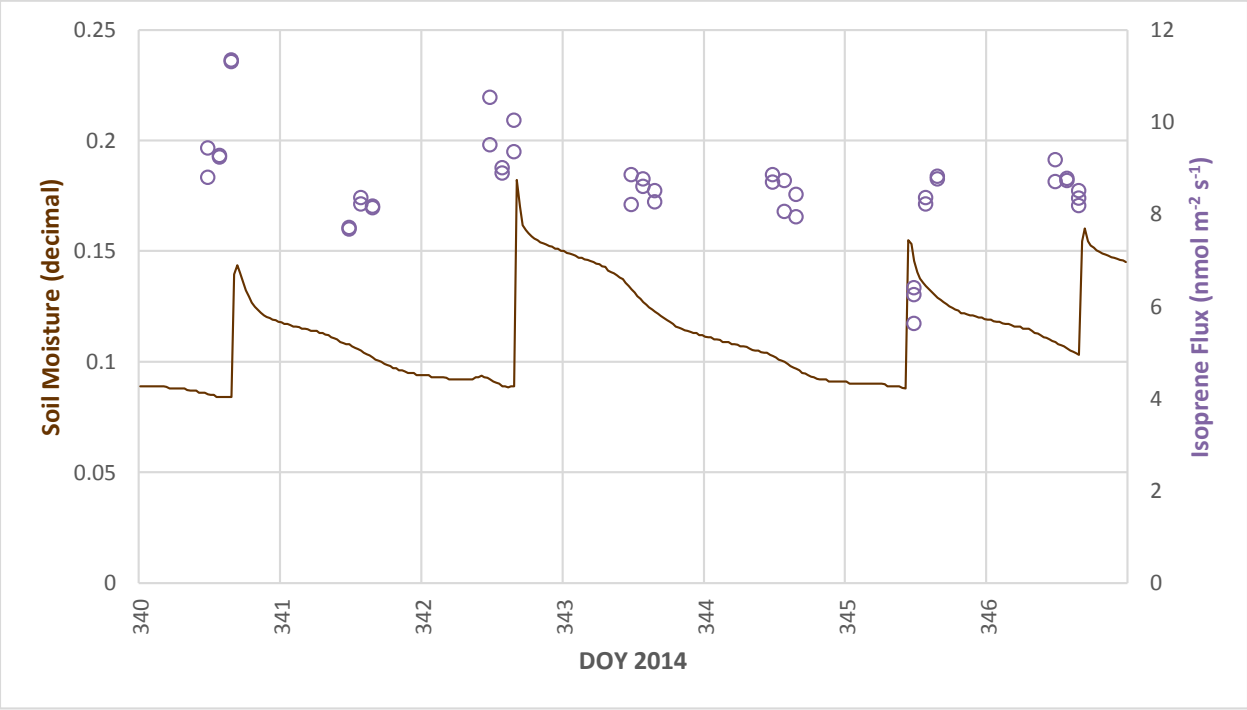

Figure 23. Soil moisture and isoprene flux values from *Q. virginiana* during ozone exposure.

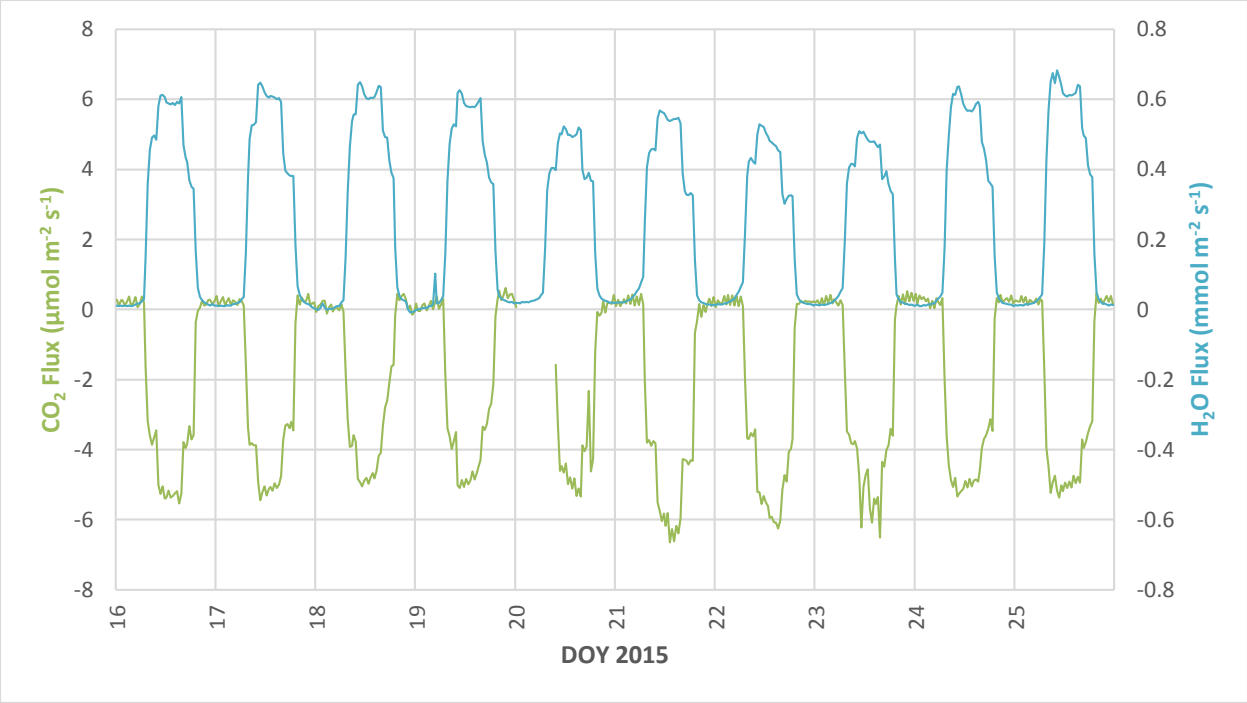

Figure 24. CO_2 and H_2O flux values from *Q. muehlenbergii* during ozone exposure.

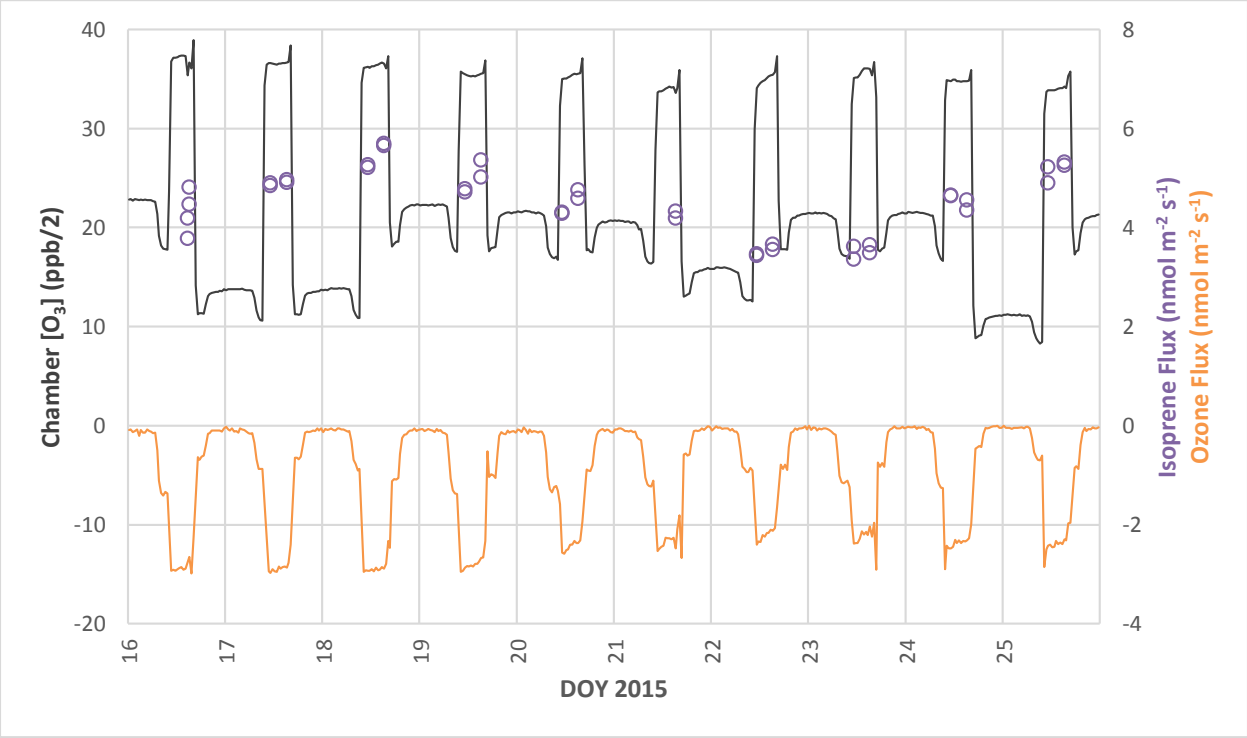

Figure 25. Ozone flux, chamber ozone concentration, and isoprene flux values from *Q. muehlenbergii* during ozone exposure.

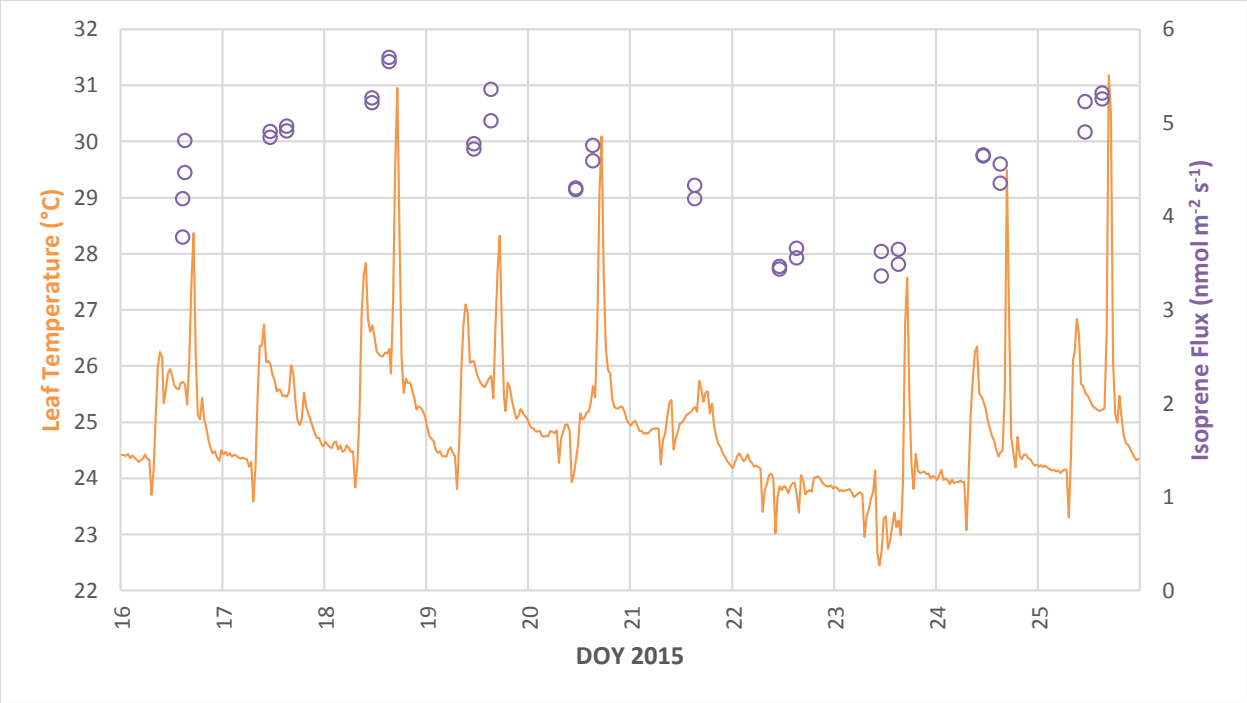

Figure 26. Leaf temperature and isoprene flux values from *Q. muehlenbergii* during ozone exposure.

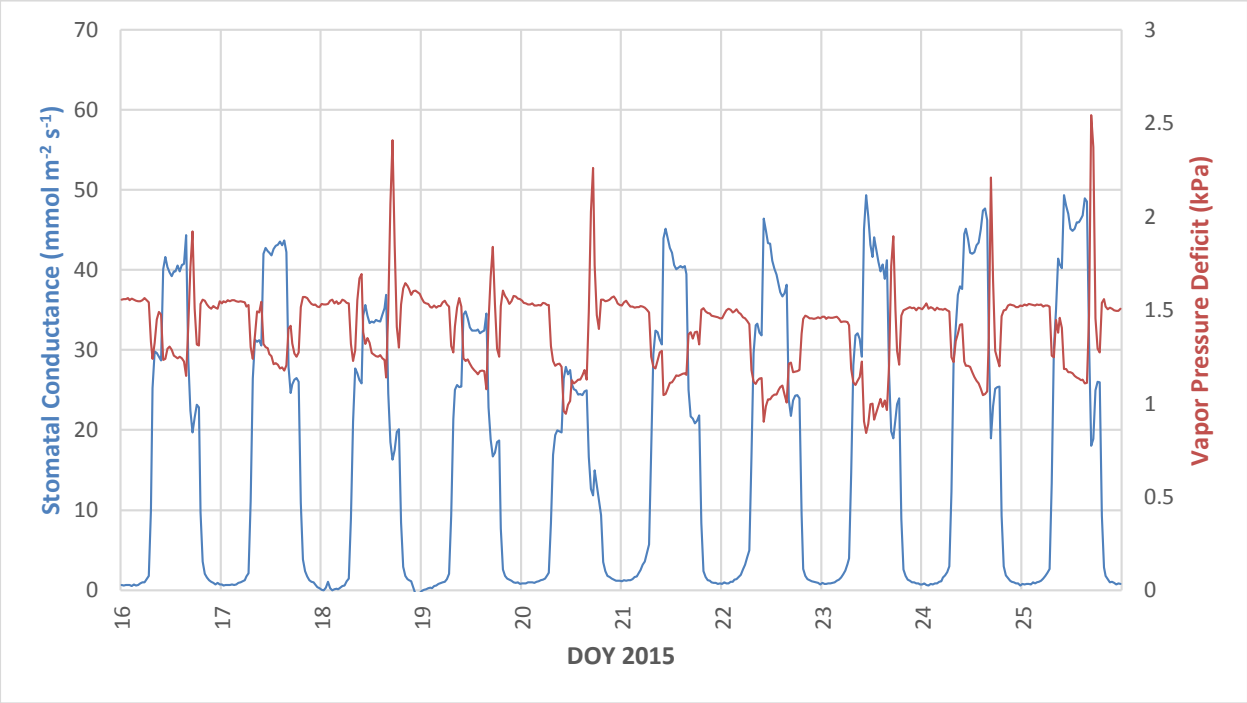

Figure 27. Stomatal conductance and vapor pressure deficit values from *Q. muehlenbergii* during ozone exposure.

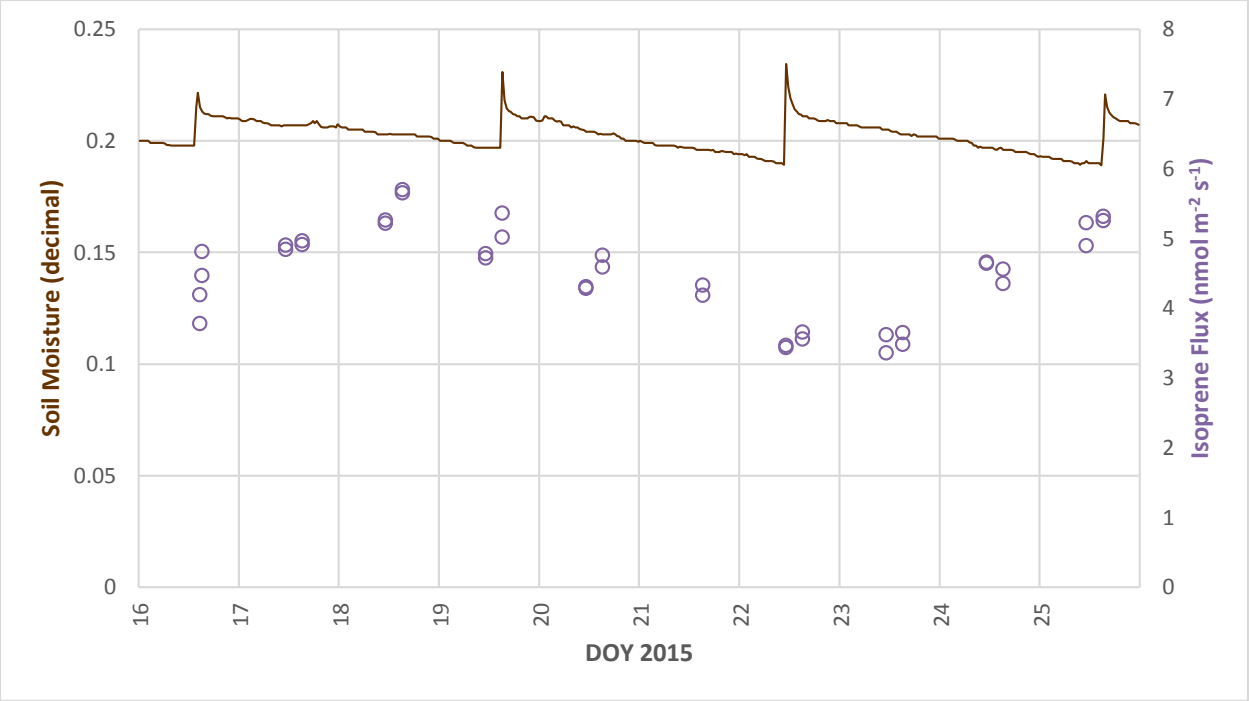

Figure 28. Soil moisture and isoprene flux values from *Q. muehlenbergii* during ozone exposure.