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Michael Cole
DePaul University, mcole81@gmail.com

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Combinatory Effect of Changing CO_2, Temperature, and Long-term Growth Temperature on Isoprene Emissions

Michael Cole

Department of Environmental Science and Studies

Mark Potosnak, PhD; Faculty Advisor

Department of Environmental Science and Studies

ABSTRACT Isoprene, the most abundant hydrocarbon in the atmosphere, plays a significant role in atmospheric chemistry. Its reactions with NO_x lead to the formation of ozone in the lower troposphere, which is harmful to plants and detrimental to human health. As air temperatures and CO_2 concentrations increase with climate change, it is uncertain how isoprene emissions from plants will respond. We hypothesized that isoprene emissions will increase with the combination of increasing temperature and CO_2 concentrations. We predict that oaks grown at a higher temperature will exhibit an increase in isoprene emissions with combined short-term increases in temperature and CO_2 concentration. Five post oaks (*Quercus stellata)* were placed in two growth chambers set at 25°C and 30°C. Isoprene emissions were measured at varying temperature and CO_2 conditions with two different instruments. Results indicate that in the presence of a combinatory increase in temperature and CO_2 concentration, isoprene emission is suppressed, contrary to results from a short-term experiment.

INTRODUCTION

Isoprene (2-methyl-1,3-butadiene) is the most abundant biogenic volatile hydrocarbon emitted into the atmosphere by vegetation per year (Guenther *et al.* 1993). Isoprene is thought to play an important role as a thermoprotective agent, protecting plants against oxidizing agents (Sharkey *et al.* 2001; Loreto *et al.* 2001), and an important role in the chemistry of the lower troposphere (Fuentes *et al*. 2000). In the presence of high levels of NO_x, isoprene contributes to the production of atmospheric nitrate oxidants and ozone (Sharkey *et al*. 2014). Ozone in the troposphere is destructive to plants (Heagle *et al.* 1973) and human health (Bell *et al.* 2007). Reactions of isoprene and the hydroxyl radical (OH) increase the lifetime of methane (CH_4), an important greenhouse gas (Poisson et *al.* 2000). Additionally, the oxidation of isoprene has a significant effect on regional air quality and formation of secondary organic aerosols (Andreae and Crutzen 1997).

Numerous studies such as those performed by Sharkey *et al.* 2014, Potosnak *et al.* 2014, Fiore *et al.* 2011, and Petron *et al.* 2001, have reported

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* *mcole81@gmail.com*

Research Completed in Summer 2015

that isoprene emissions are highly dependent on temperature. As stated by Sharkey *et al.* 2001, the thermotolerance of plants increases with the presence of isoprene. While the isolated effect of temperature on isoprene emissions is relatively well understood, the effect of CO_2 concentration on isoprene emission is uncertain (Potosnak et al 2014). Additionally, little information is available on the combined effects of temperature and CO_2 on isoprene emissions, especially during long-term exposure experiments. At low temperatures, an increase in CO_2 can be found to suppress isoprene emissions (Sharkey *et al.* 2014). By increasing the temperature, it has been observed that the suppression effect caused by increased CO_2 is eliminated in the short term (Sharkey *et al.* 2014). It is not well known how isoprene-emitting vegetation will respond to long-term growth in a hotter climate and air more concentrated with CO_2. Long-term, conditions oaks are grown in, exposure experiments are relevant in estimating how isoprene emissions will increase due to a combinatory increase of global temperature and CO_2 concentration.

The objective of this study is to understand how the combined increase of temperature and CO_2 concentrations will affect isoprene emissions in mid-latitude plants, specifically focusing on one species (see figure 1). We hypothesize that isoprene emissions from mid-latitude oaks (*Quercus stellata)* will increase as temperature and CO_2 concentrations rise for short-term, 5-15 minutes during measurments, changes; the increasing temperature will offset the suppression affect caused by CO_2 (H1). We also predicted (H2) that plants grown at an increased temperature would not exhibit short-term CO_2 suppression of isoprene at any leaf measurement temperature.

METHODS

GROWTH STAGE

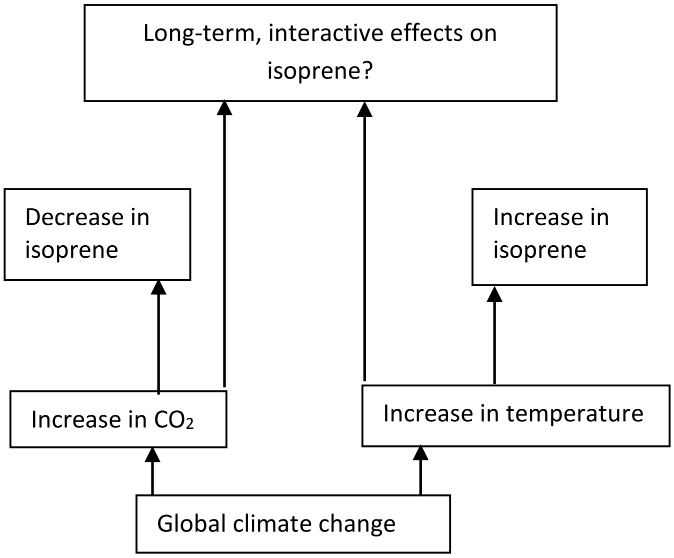


Figure 1: Conceptual model of proposed hypotheses on the combinatory effect of temperature and CO_2 on isoprene emission.

Ten post oak seedlings of the species *Quercus stellata* were used in the testing of the hypotheses. Two growth chambers (Conviron, Winnipeg, Manitoba, Canada) were used to house the seedlings. In each chamber, each seedling was labeled 1-5. One chamber was set at 25°C and the other at 30°C during the day. At night, the temperature decreased by 6°C. The 25°C chamber dropped to 19°C and the 30°C chamber to 24°C. The oaks experienced a period of 16 hours of day (light) and eight hours of night (dark). The light slowly turned on for the day period over the period of an hour to best simulate the sun rising. The soil moisture was monitored with a soil moisture probe to ensure the plants did not dry out in the warmer temperatures. This prevented the experiment turning into a drought study rather than a growth temperature study. The volumetric soil moisture level was kept above 0.25 to keep the soil moist. The high temperature chamber was kept at 70% relative humidity and the low chamber was kept at 60% relative humidity. CO_2 levels were controlled during the day and kept at 450 ppm in both chambers. Soil and fertilizer were added halfway through the growing phase. After the first set of measurements, the chambers conditions switched for replication: the high temperature chamber became the low temperature chamber, and the low became the high. Oaks numbered 2 and 4 from each chamber were moved to opposite chambers. Because chamber conditions were swapped, these oaks remained in their previous conditions. All measurements were repeated six weeks after the conditions were swapped.

LEAF MEASUREMENTS

Leaf-level isoprene emissions were first measured using a portable photosynthesis system (LI-6400, LI-COR Biosciences, Lincoln, NE) attached with PTFE tubing to a gas chromatograph with a flame ionization detector (GC/FID, model 8610, SRI Inc., Torrance, CA). A Fast Isoprene Sensor (Hills Scientific, Boulder, Colorado) was then used to make similar measurements. Isoprene emission was measured from the leaves from each oak at different measurement temperatures and CO_2 concentrations.

Both procedures were used to measure leaf level isoprene response to changing CO_2 and temperature. Conditions for each procedure were set using the LI-6400.

Gas Chromatograph Procedure:

Four different sets of data are reported. Set 1: measurement took place at a temperature of 25°C and a CO_2 concentration of 400 ppm (ambient). Set 2: measurement was set at 25°C and 800 ppm of CO_2 (elevated). Set 3: measurement was set at a temperature of 30°C and a CO_2 concentration of 500 ppm. Set 4: measurement was set at 30°C and 1000 ppm of CO_2. Measurement CO_2 varied between each chamber due to measurement temperature differences. At higher temperatures, the internal CO_2 concentration in the leaves is lower than that of leaves at lower temperatures. To ensure an equal measurement CO_2 concentration, more concentrated CO_2 is used at higher temperatures. Each measurement took approximately 20 minutes to complete. The first 15-17 minutes allowed for the plant to equilibrate to the conditions. The remaining time was allotted to measuring isoprene emission. Once the measurements were complete, ratios were reported. The first ratio was reported as Step 2: Step 1. The second ratio was reported as Step 3: Step 4. These ratios are of elevated to ambient CO_2 concentrations at the two different measurement temperatures. Using the program PeakSimple, the concentration of isoprene emitted from the leaf is measured by the area under the curve.

Fast Isoprene Sensor Procedure:

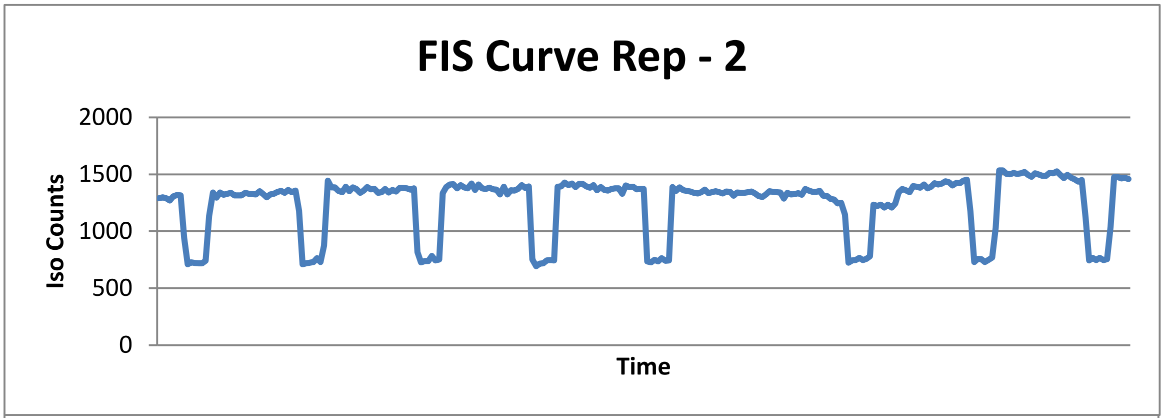
Measurements taken at leaf level were ordered into two sets. The first set, at 25°C, included the following CO_2 concentrations: 400 ppm, 300 ppm, 200 ppm, 100 ppm, 50 ppm, 400 ppm, 400 ppm, 600 ppm. The leaf experienced each CO_2 concentration for 5 minutes. The second set of measurements, at 30°C, used the same CO_2 concentrations. The Fast Isoprene Sensor yields a curve of isoprene emission response due to the changing CO_2 concentration.

Once the ratios were established, we looked at standard error to determine significance. If the ratio was one standard error above 1, there was stimulation of isoprene. If the ratio was one standard error below 1, there was suppression. Means and standard errors were calculated for the FIS, but values were normalized to values observed at 400 ppm CO_2. That is, each value observed was divided by the value observed at 400 ppm CO_2.

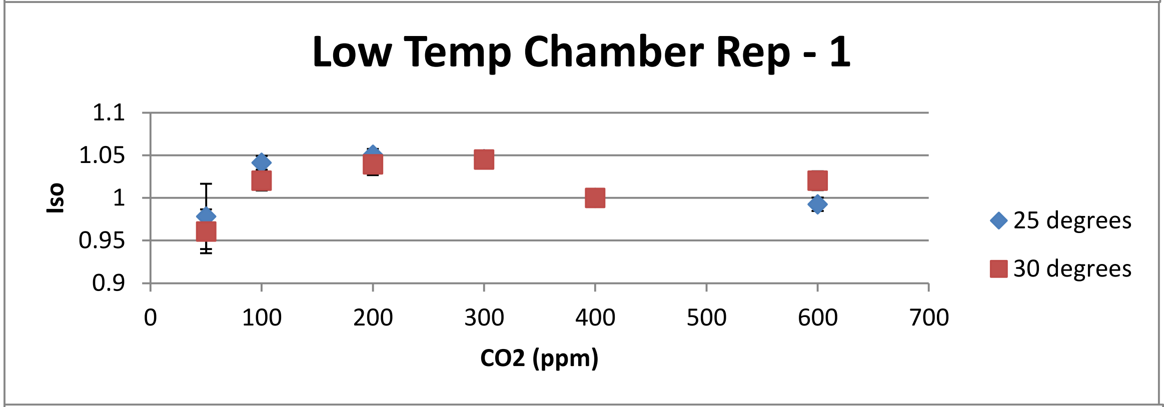
RESULTS

At low measurement CO_2 ($CO_2 < 100$ ppm), which is only observed in the FIS experiments, CO_2 correlates with isoprene response (that is, as CO_2 increases from 50 ppm to 100 ppm CO_2, an increase in isoprene is similarly observed) in all experiments for replication 1 (Figure 2). This correlation is only seen at the 30°C measurement temperature for the high and low chambers for replication 2 (Figure 2). Isoprene response is generally insensitive to CO_2 concentration from 100 ppm to 400 ppm CO_2 (Figure 2), with the exception of the low chamber measurements from replication 1 (Figure 2).

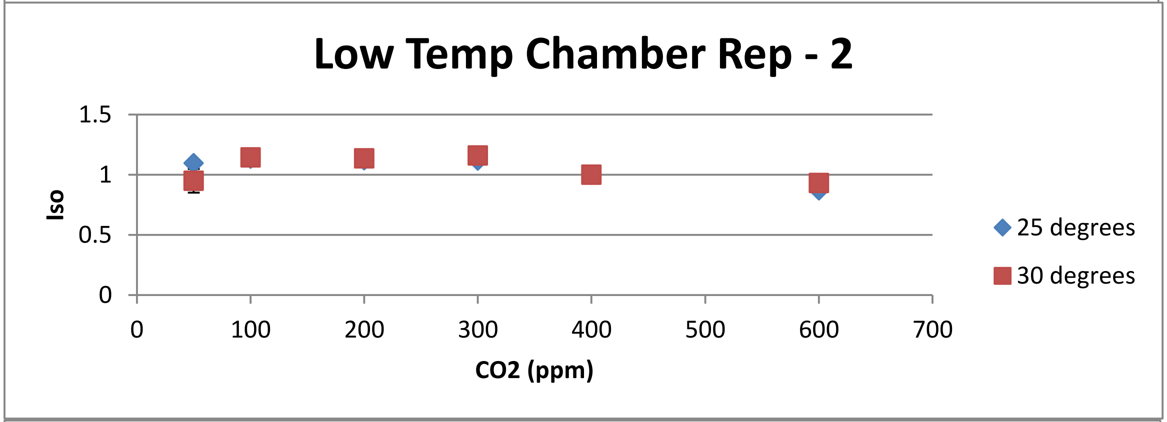
Replication 2 had methodology issues. The low chamber experienced power issues, only remaining powered on for a few hours a day, which presented a problem for how the trees equilibrated to their new conditions and produced inconsistent results. Replications 1 and 2 for the low chamber GC measurements produced contradictory data. Replication 1 produced results agreeing with our first hypothesis (H1)



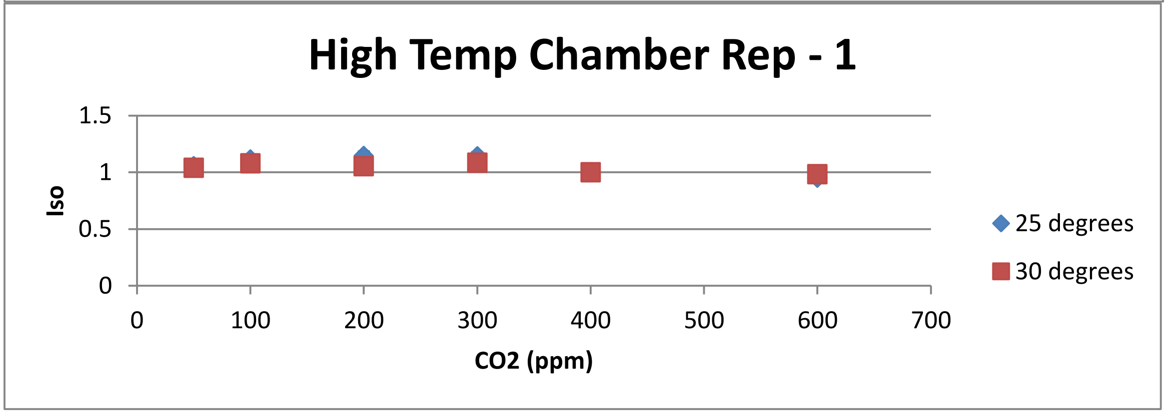
FIS Curve Rep - 2



Low Temp Chamber Rep - 1



Low Temp Chamber Rep - 2



High Temp Chamber Rep - 1

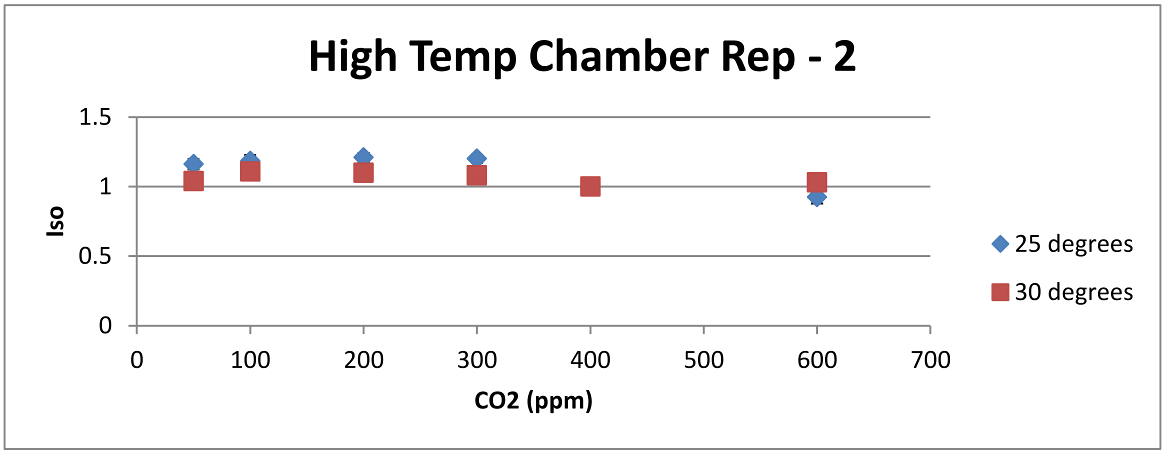


Figure 2: Above shows an example FIS curve from replication 2, and the oaks from the low temperature chamber and high temperature chamber measured at 25°C and 30°C for replication 1 and 2 for the FIS methodology. Isoprene values are all normalized to measurements taken at 400 ppm CO_2 and units on the Y-axis are relative to that. Values of 1 represent measurments taken at 400 ppm CO_2. Any varience from 1 represents different isoprene emission values. For replication 1 low chamber, measurements taken at 600 ppm CO_2 were significantly different from 1. At 600 ppm, 30°C temperature stimulated isoprene emission. Replication 2 low chamber exhibited a suppression of isoprene at the higher CO_2 concentrations. For replication 1 high chamber, no CO_2 effect was observed at 30°C. Replication 2 for the high chamber showed stimulation of isoprene at 600 ppm CO_2 and 30°C.

Table 1: Comparison of data from replication 1 and replication 2 summarizing the effects of elevated CO2 on relative isoprene emission. Measurement temperatures were 25°C and 30°C. Data from 400 ppm CO_2 vs 600 ppm CO_2 were used from the FIS methodology.

		Replication 1		**Replication 2**	
		FIS	GC	FIS	GC
High Chamber	30°C	No CO_2 effect	CO_2 suppresses isoprene	Stimulation of isoprene	No CO_2 effect
	25°C	CO_2 suppresses isoprene	CO_2 suppresses isoprene	CO_2 suppresses isoprene	No CO_2 effect
Low Chamber	30°C	Stimulation of isoprene	No CO_2 effect	CO_2 suppresses isoprene *	No CO_2 effect *
	25°C	CO_2 suppresses isoprene	CO_2 suppresses isoprene	CO_2 suppresses isoprene *	Stimulation of isoprene *

*Indicates low temperature chamber malfunctions during the second growth stage.

(Figure 3). However, stimulation of isoprene was found at 25°C measurement temperature for replication 2 (Figure 3) during a period when the chamber was experiencing power issues. Replication 2 for the high chamber yielded a suppression of isoprene at both measurement temperatures (Figure 4), contradicting our H2 hypothesis. Replication 2 for GC measurements of the high chamber oaks produced results that suggest there was no CO_2 suppression effect on isoprene. Overall, we see contradictory data for the GC methodology (see Table 1).

FIS results were also different between replications. We observed a suppression of isoprene under all conditions except stimulation at 30°C for the low chamber for replication 1 (Figure 2), which we cannot explain. Suppression of isoprene was seen at the 25°C for the high chamber for both replications 1 and 2 (Figure 2), contradicting hypothesis H1. No CO_2 effect was observed at 30°C, 600 ppm CO_2 for replication 1 and stimulation of isoprene was seen at 30°C, 600 ppm CO_2 for replication 2 (Figure 2). FIS and GC data can be compared by considering changes between 400 ppm and 600 ppm CO_2 from the FIS results (Table 1). The GC and FIS methodology only agree twice in replication 1 at 25°C both chambers. All other experiments differ in results producing contradictory data. However, a general suppression effect is seen at higher CO_2 concentrations.

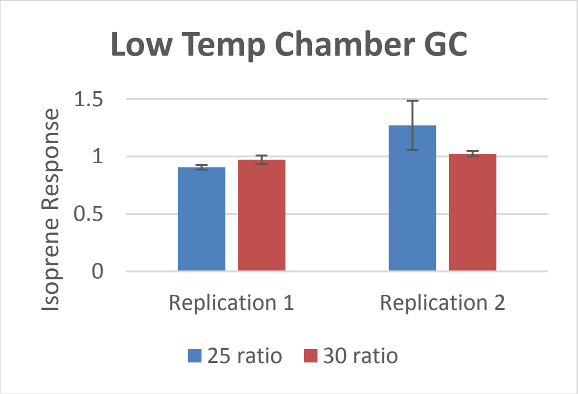


Figure 3: Isoprene concentration ratios from the low temperature chamber from replication 1 and 2 for the GC methodology are compared. Data is shown in ratios. The 25°C ratio is the mean of the isoprene values measured at 800/400 ppm. The 30°C ratio is the mean of the values at 500 ppm/1000 ppm. At 30°C for replication 1, no CO_2 effect is observed, while suppression is observed at 25°C. At 30°C and 25°C for replication 2, stimulation is shown. 25°C ratio is the mean of the isoprene values measured at 800/400 ppm. The 30°C ratio is the mean of the values at 500 ppm/1000 ppm. At 30°C for replication 1, no CO_2 effect is observed, while suppression is observed at 25°C. At 30°C and 25°C for replication 2, stimulation is shown.

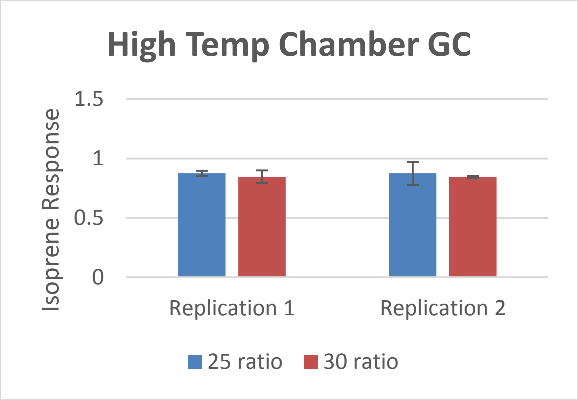


Figure 4: Suppression is observed in all cases, but the two ratios within both replications are not significantly different from each other.

DISCUSSION

We hypothesized (H1) that isoprene emissions would increase as temperature and CO_2 concentrations rise for short-term changes, as observed in previous experiments (Potosnak *et al.* 2014). That is, increased leaf temperature would eliminate the suppression of isoprene by elevated CO_2. We also predicted (H2) that this pattern would hold true for long-term increases in growth temperature: plants grown at an increased temperature would not exhibit short-term CO_2 suppression of isoprene at any leaf temperature. We did not see our predicted response for short-term measurements (H1) that was observed in previous experiments. Unexpectedly, as leaf temperature rose, we continued to observe a suppression of isoprene emissions (7 out of the 16 observations). The short-term isoprene response was only clearly seen twice during the first replications for oaks grown in the low temperature chamber, once for each methodology. We observed a stimulation of isoprene between 400 ppm and 600 ppm CO_2 for FIS high and low chamber at 30°C measurement temperature in replication 1, contrary to our predicted no CO_2 effect. Our hypothesis concerning growth temperature (H2) was also not supported in the majority of cases. At high temperature, long-term growth, we see a suppression of isoprene emissions in replication 1 at 25°C for both methodologies and 30°C for the GC. In replication 2 we see suppression at high CO_2 concentrations at 25°C for the FIS methodology. We saw our predicted no CO_2

effect for the GC in replication 2 for both measurement temperatures, and in replication 1 for the FIS at 30°C. We observed an unpredicted stimulation of isoprene at 600 ppm CO_2 for replication 2 FIS experiment at 30°C (2 out of the 16 observations).

The methodology for this experiment was inconsistent. FIS and GC measurements often did not agree on isoprene response to increasing temperature and CO_2 concentrations. FIS measurements from replication 1 between 400-600 ppm CO_2 showed stimulation, however GC measurements did not support FIS measurements. GC measurements for replication 1 showed the opposite effect (CO_2 suppressing isoprene) at both measurement temperatures. In replication 2, stimulation of isoprene was observed at the 25°C measurement temperature for the low chamber, which is inconsistent with our data and generally accepted data for short term isoprene response to rising CO_2 concentrations and low temperature. The difference in isoprene emission response was not significant enough to show a difference in emissions.

For replication 2, the low chamber experienced power issues. Due to a coolant leak, the chamber frequently shut down for long periods of time. Oaks equilibrating in the low chamber only experienced the set conditions for a few hours a day. This upset in equilibrium could explain the inconsistent results from replication 2. Going forward, the coolant leak in the low chamber has been fixed and is operating as normal. The methodology for the FIS was improved from replication 1 to replication 2. A zero procedure was added to remove any background interference during measurements.

CONCLUSIONS

There are a number of improvements that could be made with the experimental procedures that could hypothetically reduce some of the observed inconsistencies. In the future, internal CO_2 concentration in the leaf could be controlled instead of the reference CO_2 so the leaves could experience more precise CO_2 concentrations. When the oaks and chambers were swapped for the start of the second growth stage, the trees did not equilibrate to their new conditions as predicted. Therefore, new seedlings are needed for the start of each new replication. To eliminate leaf-level variation, the same leaves could be tracked and measured for each individual oak; this was not done for this experiment. The difference in the max CO_2 concentrations between the GC and FIS methodology made it difficult to compare data. Thus, extending the isoprene response curve to 800 ppm CO_2 for the FIS methodology will improve the observation of isoprene emission response at higher CO_2 concentrations. The objective for these changes in methodology is to eliminate the variance between the GC and FIS methodologies and to highlight differences intrinsic to how the plants are responding.

The results suggest that as leaf level and long-term temperature are increased, the suppression effect of CO_2 on isoprene emission will not be offset as hypothesized (H2). Long-term isoprene emissions will be lower than suggested by short-term response experiments. As a result, global climate change will not increase total isoprene emissions, rather, emission levels should stay roughly the same. However, caution is needed due to issues with the methodology and malfunctions with a growth chamber during the second growing stage. A new methodology is needed to further investigate this hypothesis. Furthermore, only one species, *Quercus stellata*, was investigated. It may be possible that this species' leaf level interaction with temperature and CO_2 differ from other isoprene emitting species.

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AUTHOR CONTRIBUTIONS

M.C. participated in all aspects of this study under the guidance of faculty advisor M.P.

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