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Effects of Elevated CO2 Concentrations and Elevated Temperatures on Isoprene Emissions of Rhamnus cathartica (European buckthorn), Quercus rubra (red oak) and Quercus michauxii (swamp chestnut oak)

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ABSTRACT Tropospheric ozone is a ground-level pollutant and is produced from primary air pollutants like nitrogen oxides and volatile organic compounds (VOCs). While many VOCs are anthropogenic, isoprene is emitted from certain species of plants and reacts with nitrogen oxides to form tropospheric ozone. Previous studies have found that isoprene can be suppressed by elevated CO_2, but such suppression can be reduced under conditions of increased temperature. However, this pattern is not seen in all isoprene-emitting plants. The focus of this study is to determine if similar patterns are present in the invasive tree species of buckthorn (*Rhamnus cathartica*) and an oak species, swamp chestnut oak (*Quercus michauxii*), to red oak (*Quercus rubra*), where both oak species are native in Chicago, Illinois. Two of the tested hypotheses are that patterns of isoprene suppression by CO_2 at ambient temperature in *Q. michauxii* will be the same as the patterns seen in *Q. rubra* (H1) and that patterns of isoprene suppression by CO_2 at ambient temperature in *R. cathartica* will be the same as the patterns seen in *Q. rubra* (H2). Regarding the invasive species, the hypothesis that isoprene suppression by CO_2 at an elevated temperature will be reduced in *R. cathartica* (H3) was also tested. To test these hypotheses, individuals of the native species were grown in a greenhouse and the *R. cathartica* species was sampled from a field and brought into the greenhouse. CO_2 concentrations (400 ppm and 800 ppm) and temperatures (30°C and 35°C) were adjusted for leaves of all trees using a leaf gas exchange system and isoprene was collected and measured concurrently with a gas chromatograph. Differences in isoprene emissions from low to high temperatures were not significant for any of the species studied, thus rejecting H1 and H2. Similarly, significant isoprene suppression was not observed in *R. cathartica*, thus rejecting H3. These findings will help contribute to our understanding of how isoprene-emitting plants could respond to changes in temperature and CO_2 concentration in the context of climate change.

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INTRODUCTION

Global climate change has been a widely discussed topic among the public and it has been appearing more often in both news and social media outlets. Among these discussions, one central idea is that human activities are one of the main contributors to increased CO_2 concentrations in the atmosphere. Increased CO_2 is predicted to lead to several consequences, one of the most notable being the elevation of global temperatures. In their 2014 Synthesis Report, the IPCC states that CO_2 concentrations in the atmosphere, along with other greenhouse gases, have been increasing rapidly as a result from the growing population and economy, thus making such greenhouse gases "extremely likely" to be the main driver of global warming (IPCC, 2014). Some plants, however, seem to have evolved a mechanism that helps them survive in high temperature conditions. One of the most abundant biogenic volatile organic compounds (BVOCs) emitted into the atmosphere by plants is isoprene (Jiang et al., 2018). Isoprene is a hydrocarbon gas emitted by fully developed leaves and its emission is largely based on isoprene synthesis and MEP pathways in plastids, the latter of which provides the essential component of dimethylallyl diphosphate (DMADP) (Sharkey et al., 2008). These mechanisms, according to Sharkey et al. (2008) are key components to isoprene emissions affected by conditions of temperature and elevated CO_2. It has been hypothesized that plants with the ability to produce isoprene do so to resist the detrimental effects of heat stress (Sharkey et al., 2008). However, isoprene emissions bring another concern when it comes to air quality. Isoprene reacts with nitrogen oxides (NO_x) present in the atmosphere to produce ozone (O_3) (Sharkey et al., 2008). These nitrogen oxides can come from fossil fuel combustion, usually used to power vehicles for transportation and factories like coal-fired power plants (UCAR, 2014). Although ozone has been known to protect the biosphere from the sun's harmful UV radiation, this function takes place in the stratosphere. The ozone referenced in relation to isoprene emissions is ozone in the troposphere, the layer where the biosphere is located. Trophospheric ozone, also known as ground-level ozone, is harmful to humans. High concentrations of this pollutant can cause respiratory issues, chest pain, eye irritation and other health risks (UCAR, 2014). Not only does it harm humans, but it can also severely damage plants, making them more vulnerable and less likely to survive (National Park Service, n.d.). Thus, as CO_2 increases partly due to the combustion of fossil fuels and consequently contributes to the rise of global temperatures, then isoprene-emitting plants, in response to heat stress, can potentially contribute to the creation of toxic tropospheric ozone.

One such species of plants is buckthorn, *Rhamnus cathartica* - an invasive tree species that is abundant in Chicago, Illinois. The presence and rising population of invasive species is another major environmental issue. Invasive species present ecological concerns as they tend to take over resources in their new environments, increasing their population while diminishing populations of native species. Buckthorn, in particular, tends to take over spaces that young native oaks need to grow and survive (Garcia, 2016). It is also the most abundant invasive tree species in Chicago (Chicago Botanic Garden, n.d. and Nowak et al., 2013). Invasive species are also better able to adapt to environmental changes. One study found three invasive plants - *Mikania micrantha*, *Wedelia trilobata*, and *Ipomoea cairica* - had fared better than native species - *Paederia scandens*, *Wedelia chinensis*, *Ipomoea pescaprae* - under conditions of elevated CO_2, as they had a higher rate of photosynthesis and biomass (Song et al., 2009). So, if CO_2 concentrations continue to rise, invasive species may continue to thrive and displace native species.

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In a different study done by a DePaul University student, isoprene emissions were compared between native and invasive species of trees in Chicago (Mistry et al., 2021). Although there was not a significant difference in isoprene emissions between native and invasive tree species, it was found that buckthorn had significant contributions to forest isoprene emissions when considering individuals growing at forest edges (Mistry et al., 2021). Thus, the presence and continued growth of invasive species can have a contribution to the creation of tropospheric ozone via isoprene emission. Considering the findings from Mistry et al. and an abundant population of buckthorn in Chicago, one objective of this study is to see the potential impact that *R. cathartica* has on air quality in terms of its isoprene emissions. However, some factors may mitigate plant isoprene emissions. In another DePaul study, isoprene emissions were found to be suppressed in red oak, *Quercus rubra,* when exposed to CO_2 concentrations rising from 400 ppm to 800 ppm from leaf temperatures of 20°C to 25°C (Potosnak et al., 2014). However, this suppression by CO_2 was reduced when temperature increased to 25°C, and there was no suppression when temperatures went from 25°C to 30°C, nor from 30°C to 35°C (Potosnak et al., 2014). Still, this pattern is not seen with all species. Cole (2016) found that isoprene suppression by CO_2 was observed in long-term growth, for *Quercus stellata*, at slightly higher temperatures of 25°C to 30°C. From these studies, patterns of isoprene suppression can differ among different species, even if they are of the same genus. Thus, this study can provide more information on which species may or may not experience isoprene suppression.

Given that future climate changes predict both an increase of CO_2 concentrations and high temperatures, the effects that these changes can have on isoprene emissions can be difficult to determine in the long term for different kinds of plant species. The relationship between these conditions in terms of isoprene emissions in native and invasive species of trees can be seen in Figure 1. Given the patterns found in *Q. rubra* in the study by Potosnak et al. (2014), it would be valuable to see if such patterns can also be observed, or not observed, in both an invasive and a native tree species. This is especially considering how some invasive plant species seem to have better adaptability, as noted earlier from the study by Song et al. (2009). Thus, this study aims to compare any isoprene suppression patterns from a native and invasive tree species from Chicago, IL to *Q. rubra*. One proposed hypothesis is that patterns of isoprene suppression by CO_2 at ambient temperature in *Q. michauxii* will be the same as patterns seen in *Q. rubra* (H1). Similarly, it is also hypothesized that patterns of isoprene suppression by CO_2 at ambient temperature in *R. cathartica* will be the same from the patterns seen in *Q. rubra (H2)*. And to focus on the invasive tree species being studied, it is hypothesized that isoprene suppression by CO_2 at an elevated temperature will be reduced in *R. cathartica* (H3). Investigating these hypotheses will contribute to the knowledge of isoprene-emitting plants by finding what other species do or do not also exhibit patterns of isoprene suppression. Although the tree species being studied are exposed to conditions of both elevated CO_2 concentrations and temperature at a smaller scale, seeing how isoprene emissions may be affected in these circumstances is still worth knowing when considering future global climate change in the context of both rising CO_2 concentrations and temperature. Additionally, it will provide insight on how air quality is influenced by trees.

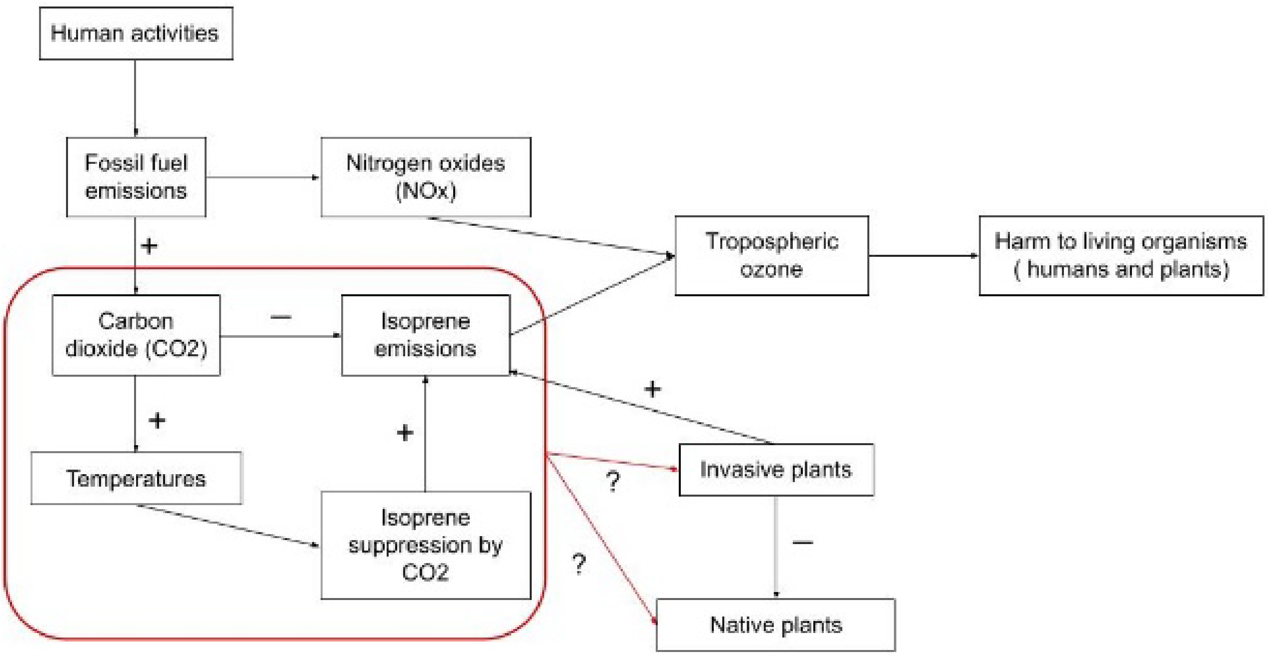

Figure 1. A conceptual model summarizing the overall cause and effects that human activities have on air quality and health to living organisms. The part enclosed by the red outline is the focus of this study, where the relationship between CO_2 emissions, temperature, and isoprene is summarized.

METHODS

Laboratory Preparation

Three tree species were analyzed for this study. These species were buckthorn (*Rhamnus cathartica*), red oak (*Quercus rubra*) and swamp chestnut oak (*Quercus michauxii*). Five individual trees of each species were used for the experiment. Buckthorn trees that were about 30 cm in height were collected from an area near North Park Village Nature Center, located in the North Park community area of Chicago, IL. The buckthorn was placed in 1 L plastic pots with soil from the area of which they were dug out of and quickly transferred over to the rooftop greenhouse at DePaul University. The red oak and swamp chestnut oaks used for this study were ones already growing in the greenhouse.

Individuals of both oak species were about 1 – 1.5 m in height. The red oaks were obtained from The Possible Place Nursery (Monee, IL) and the swamp chestnut oaks were from the Morton Arboretum (Lisle, IL). Trees from all species were watered at a minimum of 3 times per week. The buckthorn remained in the greenhouse for about 6 weeks before the experiment was conducted. Within this time, the leaves of the buckthorn were cleaned with a solution of soap and water, as the leaves were infested with what was believed to be spider mites. Removal of the insects and soap residue was ensured before laboratory analysis was conducted.

Laboratory Analysis

To measure isoprene, an LI-6400 leaf-gas exchange system (LI-6400, LI-COR Biosciences, Lincoln, NE) was used. The device was connected to a gas chromatograph (GC/FID, model 8610, SRI Inc., Torrance, CA) via a thin Teflon tube. Before running any measurements,

an "empty" run was conducted so that any impurities in the tube would be removed.
An "empty" run refers to a measurement that was conducted with no leaf inside the cuvette of the LI-6400 leaf-gas exchange system. A single, random leaf that was large enough to cover the area inside the cuvette was selected from each individual tree. A run began immediately after the leaf was enclosed in the cuvette. The leaf was left to acclimate for 10 minutes. Gas, including isoprene from the leaf, flowing out of the gas exchange system would run through the Teflon tube and reach the gas chromatograph at a steady concentration. After these 10 minutes, the gas chromatograph was started and allowed to measure the isoprene. Seven minutes after the gas chromatograph began to take measurements, conditions of CO_2 concentration and leaf temperature were changed for a new run.

Four runs were conducted on the same, single leaf of each tree, with the addition of a fifth "empty" run. In each run, conditions of light, leaf temperature and CO_2 concentration were adjusted. For all runs, light was set at a PAR value of 1000 µmol m^{-2} s^{-1}. For the first two runs, leaf temperatures were set at 30°C, serving as a low temperature. The second two runs were set to 35°C, serving as a high temperature. At both the low and high temperature conditions, concentrations of CO_2 were first set at 400 ppm (low CO_2 concentration) for one run, then raised to 800 ppm (high CO_2 concentration) in the next run. This ensured that each low and high CO_2 concentration would be paired with the low and high leaf temperatures.

Although measurements were taken for five trees of each tree species, only four measurements from *Q. michauxii* were considered. Results from a fifth swamp chestnut oak tree yielded very low emissions of isoprene, so these outliers were not taken into account for statistical analysis.

Statistical Analyis

To begin finding any significant changes in isoprene emissions among changing conditions of leaf temperature and CO_2 concentrations, the isoprene peak area measured at 800 ppm was divided by the isoprene peak area measured at 400 ppm (Potosnak et al., 2014). This ratio was determined for each leaf temperature condition of 30°C and 35°C for each individual tree. An average and standard error of these ratios was then calculated for each species of trees.

To determine any significant differences in these isoprene emission ratios between low and high temperatures for all species, a paired, two-tailed *t*-test was conducted through Excel using the T.TEST function. Isoprene was considered to be suppressed by elevated CO_2 if the average isoprene emission ratio was less than a value of 1.0. If the ratio was more than or equal to a value of 1.0, then suppression of isoprene by elevated CO_2 was not observed. To see if there was a significant difference between the isoprene emission ratio and a value of 1.0, a one-sample *t*-test was conducted through Excel. The p-value for the one sample *t*-test result was found using the T.DIST function in Excel. Statistical significance for all tests was based on comparison of p-values to a significance level of $p = 0.05$.

RESULTS

For *R. cathartica*, the isoprene peak area ratio of high to low CO_2 concentrations at a leaf temperature of 35°C was greater than the average isoprene ratio at 30°C (Figure 2). However, this difference was not significant ($p = 0.22$). The average ratio at 30°C was not significantly different from 1.0 ($p = 0.93$). At 35°C, the isoprene ratio is greater than 1.0, but this difference is also not significant ($p = 0.22$).

Similarly, for *Q. rubra*, the average isoprene peak area ratio was greater at 35°C than the average ratio at 30°C (Figure 3). This difference, however, is not significant ($p = 0.16$). At both low and high temperatures, average ratios were less than a value of 1.0, but neither were significantly different from 1.0 ($p = 0.16$, $p = 0.23$, respectively).

For *Q. michauxii*, the average isoprene peak area ratio at 35°C was less than the ratio at 30°C (Figure 4). Again, there was not a significant difference between these two ratios (p = 0.58). At both low and high temperatures, the average ratios are both greater than 1.0 (Figure 4), but they were not significantly different than 1.0 (p = 0.26, p = 0.24, respectively).

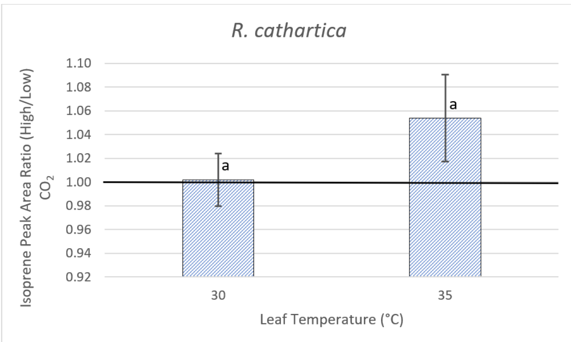

Figure 2. Average ratio of isoprene suppression of high to low concentrations of CO_2 under different leaf temperatures for *R. cathartica* grown in a greenhouse. Differences between average isoprene peak area ratios at low and high temperatures were not statistically significant from each other. Ratios greater than 1.0 indicate isoprene suppression by CO_2. Neither ratio from either temperature conditions are significantly greater than 1.0, indicating that there was no isoprene emission suppression at elevated CO_2 concentrations at ambient temperature for *R. cathartica*.

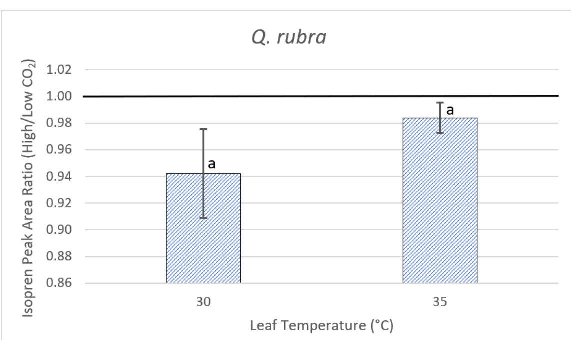

Figure 3. Average ratio of isoprene suppression of high to low concentrations of CO_2 under different leaf temperature for *Q. rubra* grown in a greenhouse. Differences in average isoprene emissions ratios between low and high temperatures were not statistially significant. Although there was an increase in isoprene emission from low to high temperatures, ratios at either temperature are not significantly different from 1.0. Thus, isoprene suppression by elevated CO_2 was not observed in *Q. rubra*.

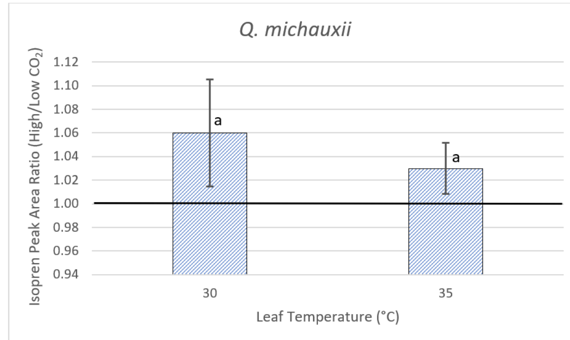

Figure 4. Average ratio of isoprene suppression of high to low concentrations of CO_2 under different leaf temperature for *Q. michauxii* grown in a greenhouse. Differences in isoprene changes between the two leaf temperatures are not significant. Isoprene ratios decreased from low to high temperatures and both ratios are greater than 1.0. However, these differences are also not significant.

DISCUSSION

In comparing isoprene suppression patterns between *R. cathartica* and *Q. michauxii* to *Q. rubra*, results of the experiment do not support hypotheses H1 and H2. Although there was an increase in isoprene emissions from low to high temperatures for both *R. cathartica* and *Q. rubra*, such increases were not significant in either species. Between these two species, it appeared that isoprene suppression was only seen for *Q. rubra*, while isoprene emissions did not seem to be affected in *R. cathartica*. However, isoprene suppression, and lack thereof, was not significant for these species. So, there is no significant evidence to support H1. The same can be said for H2 and H3. For H2, differences between isoprene emissions from low to high temperatures and isoprene suppression (and lack of it), were not significant when comparing isoprene patterns for *Q. michauxii* and *Q. rubra*. For H3, even as there was no observed isoprene suppression at either temperature, it was not significant enough to support H3.

Significant isoprene suppression had been seen in *Q. rubra*, but this occured in lower temperatures of 20°C to 25°C (Potosnak et al., 2014). Notably, in that study, suppression had not been observed when temperatures were increased up to 35°C. This is similar to what was seen in *Q. rubra* in this experiment under temperature conditions of 30°C to 35°C. In another similar experiment with *Q. stellata*, cases of isoprene suppression were seen when temperatures went up from 25°C to 30°C (Cole, 2016). Based on what has been observed in previous experiments, it seems that isoprene suppression by elevated CO_2 is observed at lower temperatures that range from 20°C – 30°C. The likelihood of this happening in temperatures above 30°C seems to decrease, as significant levels of isoprene suppression had not been observed in these higher temperatures in both previous studies and this study.

Based on these conclusions, the effects of increased CO_2 concentrations and temperatures do not have the same effect as they do on other tree species, whether they are invasive or native. Both *Q. rubra* and *Q. michauxii* are native species to Chicago, but they both exhibited different patterns in both isoprene emissions and suppression by CO_2.

Despite not seeing any significant suppression of isoprene by elevated CO_2 in any of the species studied, it is important to note that such observations occurred in conditions that were set at higher temperatures than in previous experiments. It was at lower temperature conditions where significant suppression had been previously observed. Thus, given how temperatures are expected to rise in the future, it will be crucial to continue seeing how far suppression of isoprene persists in higher temperature conditions. If elevated CO_2 is not able to suppress isoprene as temperatures continue to rise, then isoprene-emitting plants can potentially pose a threat to air quality in terms of increasing trophospheric ozone. Such threats should not suggest the removal of isoprene-emitting species, as many are native species. However, it should serve as more reason to continue the efforts to decrease CO_2 and other greenhouse gas emissions that have been increasing global temperatures.

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