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### Acknowledgements

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## Do Oaks with a Provenance Related to Warmer Climates Emit More Isoprene?

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**ABSTRACT** The hydrocarbon isoprene plays an important role in atmospheric chemistry, particularly in regards to air pollution and climate change. It is important to know why certain plant species emit isoprene and what factors affect its production in order to predict future air quality. Past research has indicated that isoprene aids in coping with heat stress, so it was hypothesized that source latitude (a proxy for climate) would significantly impact isoprene production by oaks grown in a common location. Twelve bur oaks, *Quercus macrocarpa*, collected from a latitudinal range (30-45°) and cultivated at the Morton Arboretum were assayed for their isoprene emission rate in the summer of 2014. There was no significant effect of source latitude on isoprene emission rate. As an alternative explanation, the influence of average daily temperature on isoprene emission rate was also tested, but again there was no significant effect. However, slight trends in the anticipated direction suggest that stronger relationships could be revealed if more data are collected.

### INTRODUCTION

Isoprene, a hydrocarbon that many plant species produce, has a significant impact on atmospheric chemistry. Isoprene is not classified as a greenhouse gas, but it can increase the residence times of existing greenhouse gases by changing the composition of airborne

compounds (Sharkey and Yeh 2001). As a biogenic volatile organic compound (BVOC) with two double bonds, isoprene reacts quickly with other compounds in the air, and the products of its reactions can contribute to the creation of ground-level ozone and secondary organic aerosols (Sharkey et al. 2008). Both ground-level ozone and secondary organic aerosols lower air quality and can cause a variety of respiratory health issues such as difficult or painful breathing and coughing, and they can also aggravate existing conditions like asthma, emphysema, and chronic bronchitis or it can even scar lung tissue if exposure is

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prolonged (United States Environmental Protection Agency 2014a, 2014b). Since isoprene can impact air quality and health, it is important to understand the factors that influence isoprene emission rates.

This study investigates the questions of why plants produce isoprene and how temperature impacts isoprene emission rates. The cost of isoprene production for the plant is relatively high, in both energy and carbon, so there must be some benefit for plants that outweighs the costs of production. One possible explanation is the thermotolerance hypothesis, which suggests that plants experiencing frequent but brief periods of high temperature emit isoprene to help combat heat stress (Sharkey and Yeh 2001). Since isoprene has been found to stabilize membranes at high temperatures and suppress reactive oxygen species in previous studies, a literature review has suggested that plants produce isoprene as a defense against heat stress (Sharkey and Yeh 2001).

Other studies have also found support for the thermotolerance hypothesis based on the relationship between temperature and isoprene emissions. According to Guenther et al. (1993), there is an exponential short-term relationship between isoprene emission and temperature up to 30°C with a rapid decrease in emissions at temperatures greater than 40°C. The exponential relationship explains the vast majority of short-term emissions data over seconds to minutes and some of the longer-term data spanning days to weeks (Guenther et al. 1993). A study further investigating the long-term effect of temperature on isoprene emission levels found that emission capacity of bur oaks doubled after raising the temperature from 25°C to 30°C and fell to roughly half the peak emission when the temperature was lowered to 20°C (Pétron et al. 2001).

Since temperature has been shown to play an important role in isoprene emission, it is possible that isoprene evolved over time in species associated with warmer temperatures rather than in species that are closely related in the evolutionary tree. An evolutionary review of isoprene emission focusing on plants concluded that isoprene has separately evolved in multiple

plant lineages, while some lineages have lost the ability to produce isoprene. Among the angiosperm families investigated, no major patterns of isoprene production were found despite taxonomic relationships between isoprene producers and non-producers (Harley et al. 1999). This may imply that trees originating from warmer climates emit more isoprene.

The focus of this study is to determine whether isoprene emission is genetically associated with climate based on provenance. If isoprene emission evolved as a form of heat tolerance for plants genetically adapted to warmer climates, then plants sourced from warmer climates should emit more isoprene when grown under common garden conditions. For plants at the Morton Arboretum, I hypothesize that those wildy collected as seeds from warmer climates will have higher isoprene emission rates. For this study, latitude was used a proxy for climate, with lower latitudes indicating higher temperature, and it was assumed that trees sourced from different latitudes were genetically different from each other since specimens were wildy collected from seed.

## METHODS

This study took place at the Morton Arboretum in Lisle, Illinois during the summer of 2014 and focused on oak trees due to their naturally high emission rates. The Morton Arboretum *Quercus* database was used to determine the genetic origins of the oaks, and the species *Quercus macrocarpa*, bur oak, was chosen based on the range of individuals that were collected as seeds in the wild from different latitudes. In total, twelve bur oaks were chosen with collection sites ranging from Texas to Minnesota. The collection sites represent the source latitude or provenance of the sample bur oaks as opposed to the Morton Arboretum's latitude where they were grown from seed in a common garden setting.

Between 6 and 9 air samples were collected in air sample bags (SamplePro, model 236-001, SKC Inc., Eighty Four, PA) from the leaves of each tree over the course of this study. Either three or four trees were sampled on a rotating basis each day depending on time and equipment

constraints and two or three different leaves were measured each time. Low hanging leaves were measured because of their accessibility and limited height of the equipment. An effort was made to choose healthy leaves in full sunlight when possible to ensure the leaves would be at peak productivity. A portable photosynthesis system (LI-6400, Li-COR Biosciences, Lincoln, NE) was used to collect air samples and to control the CO<sub>2</sub>, leaf temperature, and light levels for each leaf.

The isoprene concentration of the air in the sample bags was later measured using a gas chromatograph with a flame ionization detector (GC/FID, model 8610, SRI Inc., Torrance CA) and the program PeakSimple (see Potosnak et al. 2014 for additional details). I used an algorithm developed by Guenther et al. 1993 to correct for slight temperature variation and to obtain the modeled rate of isoprene emission at 30°C. Isoprene emissions were measured in relative units, which are the normalized gas chromatograph response area units for the area under the isoprene peaks in the measurement program. The average modeled rate of trees measured on each day was graphed by date (Fig. 1). The average isoprene emission rate for each tree was also graphed as a function of latitude based on the location where each tree was collected (Figure 2).

Latitude data were collected from Google Maps. The county recorded as the collection location for each tree was input into Google Maps and the latitude of the county's approximate center was chosen to represent the respective tree's source latitude. In some cases, a more specific location was provided and a center point for that location was used instead of the county listed. The average daily isoprene emission rate was graphed as a function of average daily temperature at the Morton Arboretum during the period of data collection (Figure 4). Average daily temperature data were obtained from the Naper Blvd. station of Weather Underground based on averages of hourly readings for the days when samples were collected.

Relative isoprene emission rates were obtained from the peak area output of each

sample measured with the gas chromatograph. Emissions were plotted by date, latitude, and daily temperature. Standard errors, regression coefficients of the linear regression models investigated, and the r-squared values of the linear models were calculated. The significance of the regression coefficients were tested, the average emissions by tree were plotted and a pairwise t-test with a Bonferroni correction was performed to determine whether there are significant differences in individual average isoprene emission rate between trees.

## RESULTS/DISCUSSION

The average isoprene emissions of bur oaks measured each day graphed by date of sampling demonstrate a significant difference between days (Figure 1). Error bars indicate that the variations seen between different dates are statistically significant. The average isoprene emissions for each tree sampled also demonstrate significant differences between some of the trees but not many of them (Figure 2). Trees with isoprene emissions that were not significantly different from each other tended to be located near each other within the Morton Arboretum.

Though this was a common garden setting since all of the trees were grown at the same location, the sites within the arboretum were different so some environmental factors could not be controlled. At one site, trees were located in an open field with limited shading while trees at two other sites were in a more forest-like area and experienced shading from nearby trees, and the final site included trees near a small pond that could affect moisture levels. Differences in factors such as sunlight levels, soil nutrients and moisture, and microhabitats at these sites could be investigated and measured in future studies to determine the extent of site variability and if these variations affect isoprene emissions. The statistically significant differences seen between dates and between some trees were investigated by testing whether these differences were due to differences in source latitude or daily temperature using linear regression modeling.

Though there appears to be a slight negative correlation between average isoprene emission rate and source latitude with  $R^2 = 0.09$  (Figure

3), statistical analysis indicated that the coefficient of the slope is not statistically different from zero. Average isoprene emission rate appears to demonstrate a slight positive correlation with average daily temperature with  $R^2 = 0.13$  (Figure 4). Again, further analysis indicated that this slope is also not statistically different from zero. A p-value of  $<0.05$  was used to indicate significance in this study but isoprene emission rate as a function of source latitude and as a function of average daily temperature had a p-value greater than 0.05 and neither relationship was statistically significant.

The null hypotheses that source latitude and average daily temperature have no effect on isoprene emission cannot be rejected based on the statistical analysis. However, a negative correlation between source latitude and isoprene emission was expected so the apparent slight negative trend could indicate the potential for a statistically significant relationship. The slight positive correlation of average daily temperature and isoprene emission may also indicate a potentially significant relationship since a positive trend was expected. The collection of more data from a greater number of trees could reveal whether significant relationships exist or if these factors do not impact the emission of isoprene.

Statistical differences in isoprene emissions between days and between trees were not explained by source latitude or daily temperature in this study. However, something must be driving these differences, and several other factors could be investigated. Microclimate of the leaves is one of the possible drivers. The number of hours of sunlight a leaf receives could differ greatly among the trees based on self-shading and location relative to other trees that may shade them, which could influence the temperature of the leaf. In terms of light levels, the sampled trees clustered near each other

would likely have a similar number of sunlight hours due to their similar location (for example, open savannah versus closed canopy). Location could also impact light and wind depending on the aspect of the sampled leaves. A canopy leaf would also experience a different microclimate in terms of wind, and the height of the leaves sampled was limited to branches within reach. Another potential factor influencing isoprene is health. Several of the oaks sampled showed signs of insect herbivory and parasitism, and soil nutrients might also play a role in determining isoprene emission rates.

## CONCLUSION

In this study, neither source latitude nor average daily temperature was found to significantly affect isoprene emission rate. The slight correlations with source latitude and temperature may indicate that stronger correlations may be possible if more data were collected. The observed significant differences between daily isoprene emission rates suggest that there may be other factors that affect isoprene emission rates. Future iterations of this study will explore the potential significant relationships between isoprene emission and source latitude and average daily temperature in more depth by increasing the number of trees sampled and by incorporating other factors. Other uncontrolled environmental factors including tree herbivory, sunlight levels, soil nutrient and water composition, and microclimate effects will be quantified to determine whether variability in site parameters affects isoprene emission rates. If possible, all trees sampled should be approximately the same age and grown at the same site on the arboretum grounds to minimize the influence of variability between trees in forthcoming studies.

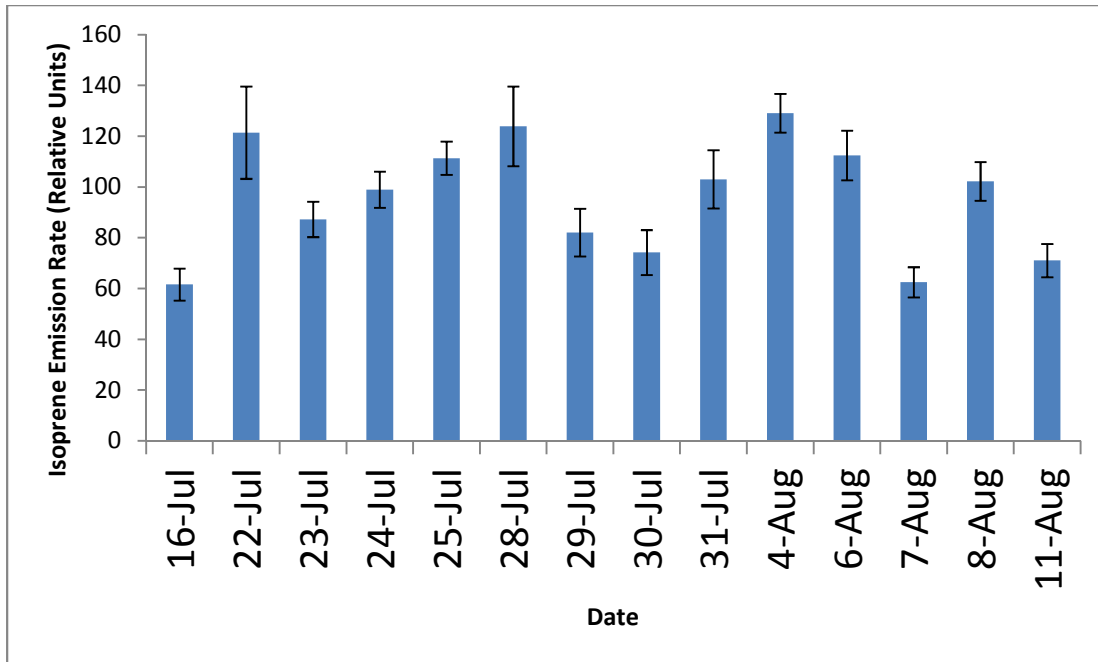


Figure 1. Average isoprene emissions of the three or four trees measured per day by date (bars representing  $\pm 1$  standard error). All twelve trees were measured three times over this time period.

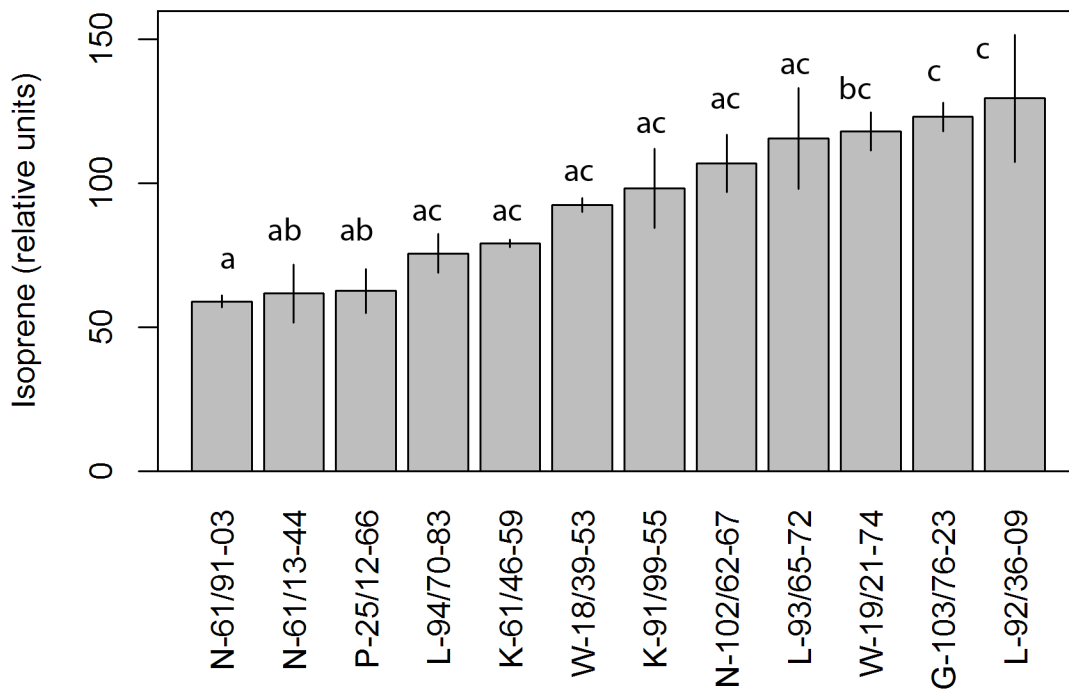


Figure 2. Isoprene emission rate per tree averaged over the time period shown in figure 1 ( $\pm 1$  standard error). A pairwise t-test with a Bonferroni correction determined grouping.

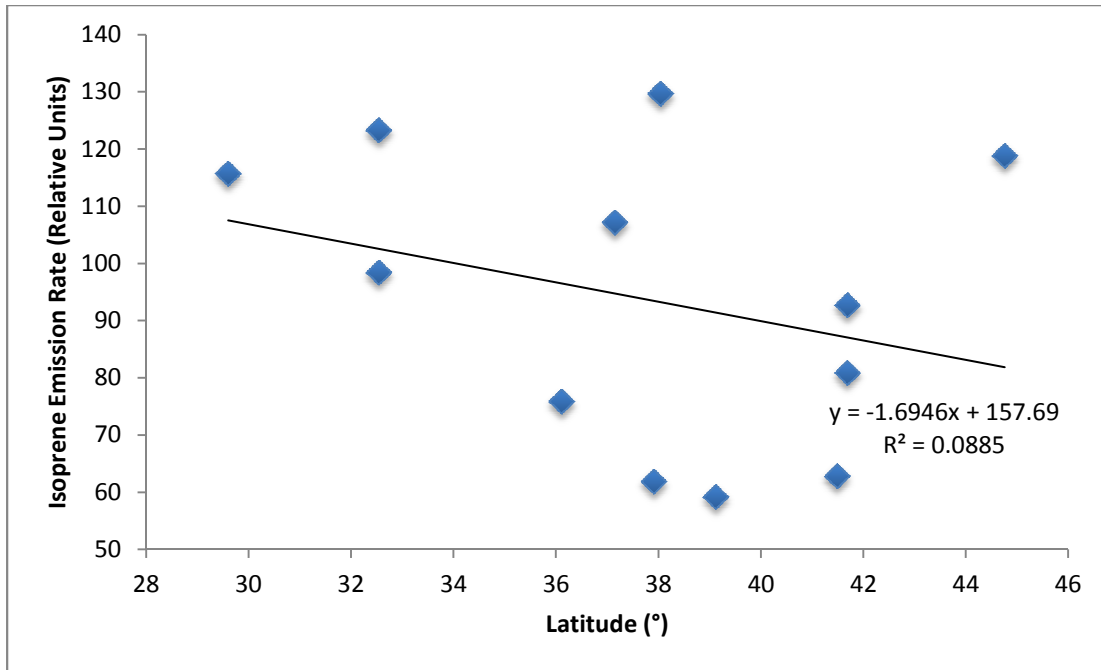


Figure 3. Isoprene emission rates per tree averaged across the respective sample days from 12 bur oaks across a range of latitudes. The p-value of the regression was greater than 0.05 and not statistically significant.

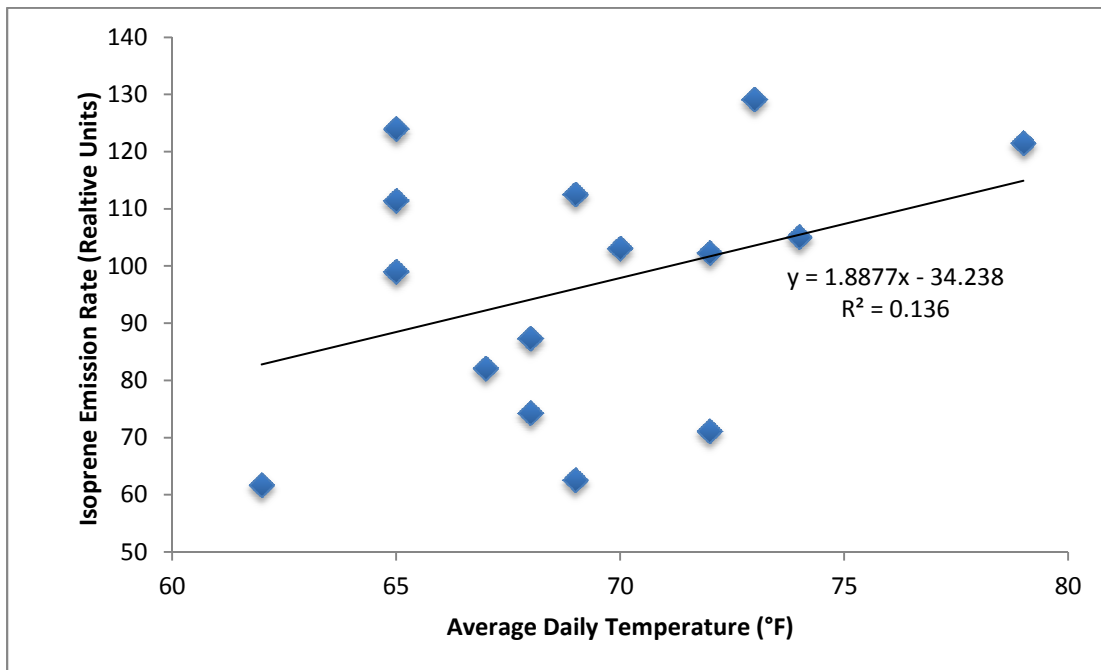


Figure 4. Average isoprene emission rate per day of trees sampled on respective days as a function of average daily temperature. The p-value of the regression was greater than 0.05 and not statistically significant.



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