Cantaurus, Vol. 16, 2-5, May 2008 © McPherson College Division of Science and Technology

Aerobic Methane Production of Tropical Plants

Adan Ghaffarian

ABSTRACT

Since 1990 global methane emissions have been carefully observed as they increase. From recent research the common known methane producers include: landfills, coal mining, natural wetlands, ruminate animals, energy production, rice agriculture land, biomass burning and natural gas systems. Methane contributes only a fraction of pollution compared to carbon dioxide but the atmospheric half-life is much longer. Observed in 2003 by Frankenberg and his crew, the SCIMACHY (Scanning Imaging Absorption Spectrometer for Atmospheric Chartography) showed an unexpected high methane source in the tropical regions (Frankenberg 2005). Following these results, in 2006, Keppler and his colleagues conducted tests to show that plants are a source of aerobic methane producers. As a positive result, he found a correlation in manipulating the variables, sunlight and temperature. A follow-up of Keppler's study was done by Rodriguez in 2007. She examined the aerobic methane production of the banana plant (*Musa acuminata*); as a result, she detected methane production by the light-incubated samples. I first studied methane emissions from the leaf of a banana tree, a plant prevalent in the tropics, using gas chromatography with a flame-ionizing detector. Banana leaf samples were incubated in 22 mL glass vials, in the dark at 30C. Banana leaf samples were also incubated in 22 mL glass vials in the presence of 300 µmol/s?m^2 of light at 30C. This process was then repeated by increasing temperature to 35C. Methane concentration in the vials were measured at T= 0 and 24 hours. Second, I used dominating variables of my banana plant experiment to conduct methane observations for leaves of Orchid (*Dendrobium*), Monstera (*Monstera deliciosa*) and leaves of Dumb Cane (*Dieffenbachia amoena*). The results indicated a statistically significant increase in aerobic methane production by the banana leaf samples kept in the dark along with the change in light with constant temperature at 30C and at 35C. In conducting methane observation for my second experiment, I observed no statistical production in the leaves of Orchid, Monstera and Dumb cane. My results suggest that aerobic methane production by the banana plant is more active with an increase in temperature and incoming source of light.

Keywords: *methane, aerobic, anthropogenic, methanogenesis, biomethanation, greenhouse gas*

INTRODUCTION

In the past two centuries, atmospheric methane has more than doubled and now constitutes 20% of the anthropogenic climate forced by greenhouse gases (Frankenberg 2005). With an atmospheric half-life of seven years, and its strong absorption of infrared radiation, methane contributes to the greenhouse effect just as much as carbon dioxide. Methane plays a central role in atmospheric oxidation chemistry and affects stratospheric ozone and water vapor levels (Keppler 2006). It was thought that the significant sources of methane emissions were known, which include: natural wetlands, rice agriculture lands, ruminant animals, energy production and biomass burning.

In 2005, Ferretti and his colleagues studied global methane accumulation in the past 2000 years. He reported a 2000-year Antarctic ice-core record of stable carbon isotope measurements in atmospheric methane. In this study his results stated that levels of methane varied by period of the earth's life, but overall identified that CH_4 emissions have been previously underestimated in methane budget research (Ferretti 2005). Frankenberg and his associates supported this concept by analyzing a higher than normal methane concentration during the dry season above a specific tropical region. For their results, they used the SCIAMACHY (Scanning Imaging Absorption Spectrometer for Atmospheric Chartography) to measure the emissions of CH_4. This spectrometer was onboard a research satellite that recorded the intensity of solar radiation, reflected from the earth's surface. They found a significant relationship between areas of usually higher methane concentrations and the presents of evergreen forests (Frankenberg 2005). It was earlier assumed, from lack of evidence, that biomethanation was strictly an anaerobic, bacterial process. In January 2006, Frank Keppler and his colleagues at the Max-Planck Institute for Nuclear Physics in Heidelberg, Germany produced evidence of aerobic methanogenesis by certain plants, including leaves of ash (*Faxinus excelsior*), leaves of beech (*Fagus sylvatica*), sweet vernal grass (*Anthoxanthum odoratum*), maize (*Zea mays*), and wheat (*Triticum aestivum*) (Keppler 2006). As a positive result, he found a correlation by manipulating the variables sunlight and temperature. A follow-up of Keppler's study was done by Rodriguez in 2007. She examined the aerobic methane production of the banana plant (*Musa acuminata*). In this study she used samples of the

banana plant followed by incubation in either a dark or light environment for a period of 24 hours. Using gas chromatography, she examined the amount of methane produced and detected methane production by the light incubated samples (P= 0.00473) mean (low/high) 2.08 (-3.35/7.77) ng per g (dry weight* h) which readily correlated with the emission rates found by Keppler 0.2 to 3 ng per g (dry weight* h).

I propose to duplicate Rodriguez's study to verify the results given, and furthermore to study the methane production, with the ideal variable, of a variety of tropical plants using gas chromatography with a flame-ionizing detector (FID).

MATERIALS AND METHODS

For my standard curve in figure 1, I used a known concentration of 50 ppm methane in air (Scott Specialty Gas, Plumsteadville, PA) that I used for injections ranging from 0.02 mL to 0.2 mL of methane. With all the collected samples acquired, an aseptic technique was used that resembles Rodriguez. The aseptic technique consists of a 10% bleach solution along with sterile tweezers and scissors for handling. After collecting 20 samples for each plant approximately 0.2 g in weight into 22 mL glass vials with appropriate gastight Teflon-lined tops, I used a gastight syringe to take samples exactly 0.5 mL before and after incubation. These measurements and methods were consistent for each sample I collected.

Once the sample is prepared and collected, an injection was made into the Clarus 500 Gas Chromatography with flame-ionizing detector (FID). The set method started its cycle at 40C for 3 minutes with a rate= 30, then increasing temperature to 200C for 1 minute with a rate= 30, this remained constant for each sample. Readings of methane concentration took place at T= 0 and 24 hours.

Incubation took place immediately after T=0 injections were made. Between the 24 hour incubation periods, different variables were applied such as light (300 µmol/s?m^2) or dark, along with temperatures at 30C or 35C. Finally, I dried the plant sample matter at 105C for a constant mass.

RESULTS

Methane Standard Curve

As seen in figure 1, a linear regression for my standard curve shows peak areas produced given ng of CH_4. Given the R_2 value of 0.999, my points confirm my standard curve to minimum percent error by user along with equipment. The Y value, 4.626e-5 is used to calculate ?ngCH_4.

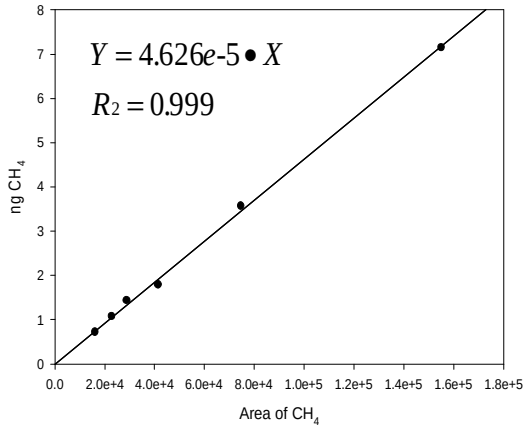

Figure 1. Methane standard curve. Samples of CH_4 in 50 ppm constant fell between .2 and .02 mL.

Banana Plant (*Musa acuminata*)

While observing my results, methane amounts increase with temperatures at 35C compared to 30C. Light also correlated with temperature, raising methane amounts. Viewed in figure 2, banana samples incubated in the dark with the temperature at 30C has a mean (low/high) value of -1.045 (-3.56/0.61) ng per g (dry weight* h). Adding light under the same temperature increased samples to 0.523 (-1.03/1.90) ng per g (dry weight* h). Increasing temperature to 35C incubated in dark improved methane production 80% to mean (low/high) values of -0.21 (-1.62/0.768) ng per g (dry weight* h). Adding light correlates results in an increased methane production of 0.539 (-0.34/1.58) ng per g (dry weight* h).

Using a paired t-test, the results from the banana plant samples were analyzed. In comparison, leaves of banana incubated at 30C in dark and light environments did show a significant aerobic methane production ($P < 0.001$). Banana plant samples developed at 35C did as well, showing a significant increase in methane concentrations (P= 0.002).

Other Plant Species

Orchid (*Dendrobium*) samples had similar results from the banana plant incubated at 30C in light with a mean (low/high) value of 0.246 (-1.03/1.90) ng per g (dry weight* h). Figure 3 illustrates the change in methane production of the Monstera (*Monstera delicious*) plant with values of 0.227 (-.008/1.16) ng per g (dry weight* h). The Dumb cane (*Dieffenbachia amoena*) displayed the most methane produced within a 24 hour incubation period with mean values reaching 1.54 (-0.44/3.99) ng per g (dry weight* h).

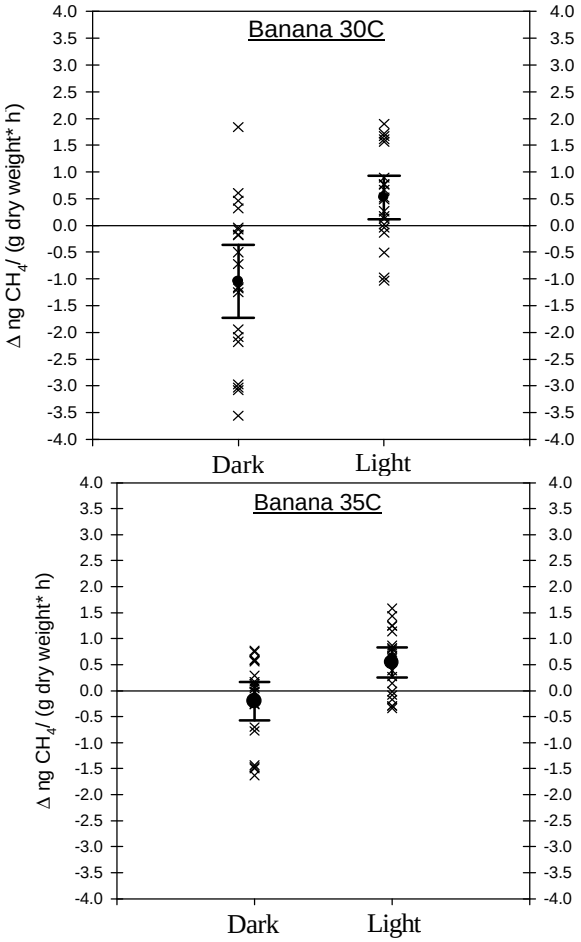

Figure 2. Change of CH_4 concentrations per gram of leaf per hour for the plant samples incubated in the dark and in 300 µmol/s?m^2 of light at 30C or 35C. The mean of both sets of data is indicated by the bold dots and the 95% confidence levels are indicated by the error bars.

Using a single t-test, the data from the Orchid, Monstera, and Dumb cane samples were examined. With a mean value of 0.246 ng per g (dry weight* h), the orchid samples did not show a statistically significant aerobic methane outcome ($P > 0.25$). Samples of Monstera incubated at 35C in light still did not show significant methane increase ($P > 0.25$). Even though the Dumb cane had the highest mean value of 1.538 ng per g (dry weight* h), the t-test still showed no significant production of methane ($P= 0.15 > P > 0.10$).

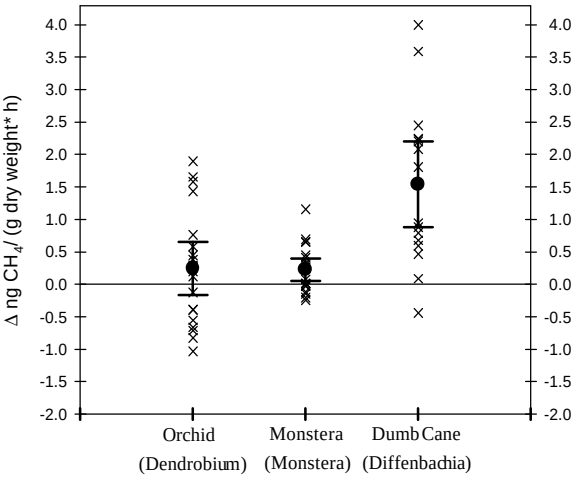

Figure 3. Changes of CH_4 concentrations per gram of leaf per hour for the plants samples incubated in 300 µmol/s?m^2 of light at 35C. The mean of both sets of data is indicated by the bold dots and the 95% confidence levels are indicated by the error bars.

DISCUSSION

The initial objective of this study was to recreate results done in Rodriguez study of Banana plants as well as manipulate variables to increase methane production in a given incubation period. To clarify Rodriguez study, I found significantly different figures but still confirming methane production in Banana plants at 30C and while introducing light (300 µmol/s?m^2).

My further studies let me use 3 other tropical plants to observe methane production. Tropical plants used in the experiment were picked mostly due to availability. The Dumb cane (*Dieffenbachia amoena*) samples seemed to have a high methane production rate but given insufficient sample size, my t-test proved to be insignificant in my results.

Further studies could be done with this project. Comparing methane growth with a considerable increase in temperature would correlate with Keppler's findings, reporting results of increasing methane production from temperatures up to 70C. These results more than tripled in his research.

ACKNOWLEDGEMENTS

I would like to thank Dr. van Asselt for his assistance during my research. I would also like to thank Dr. Frye, my research advisor at McPherson College.

LITERATURE CITED

Frankenberg, C., Meirink, J.F., van Weele, M., Platt, U. and Wagner, T. Assesing Methane Emissions From Global Space-borne Observations. Science 308, 1010-1014 (2005).

Ferretti, D. et al. Unexpected changes to the global Methane budget over the past 2000 years. Science 309, 1714-1717 (2005).

Keppler, F., Hamilton J.T.G., Brass, M., and Rockmann, T. Methane emissions from terrestrial Plants under aerobic conditions. Nature 439, 187-191 (2006).

Rodriguez, J. Aerobic methane production by Banana plant. Cantaurus, Vol. 15, 21-23 (2007).