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The Effects of Temperature Increase and Presence of Light on Methane Production and Emission of *Alocasia x amazonica*

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ABSTRACT

Previous research has been inconclusive about the sources of methane in our atmosphere. Recent studies have indicated that plants may play a larger role than expected as a source of methane emissions due to aerobic methanogenesis. Studies done by Frank Keppler and his colleagues introduced this idea when they discovered that certain plants are a source of aerobic methane production. This was followed by a study by Z.P. Wang, who discovered that there may be many variables that affect each plant's ability to produce methane aerobically. Recently, Jamie Rodriguez discovered that light has a significant effect on the production of methane by *Musa acuminate*. Although the predicted annual methane emissions due to plants is now thought to be much lower than previously, methane is still an important greenhouse gas due to its long half-life and its effects on global warming. Using a flame-ionizing gas chromatography detector, I studied methane emission from the leaves of *Alocasia x amazonica*, a common household tropical plant known as an Elephant-ear, and one that is prevalent in tropical regions of southeast Asia. Leaf samples were incubated in 30 air-tight glass vials in darkness, at temperatures of 25°C, 35°C, and 45°C, with 10 samples at each temperature. Leaf samples were also incubated in 30 air-tight glass vials in the presence of 300µEinsteins, with 10 samples at each of the previously mentioned temperatures. Methane readings were taken at times of T=0 and T=24 hours. Using the paired t-test, the data from the experiments were analyzed. The results indicated that the *Alocasia x amazonica* plant is a significant source of aerobic methane production, but the variable affecting its methane production could not be determined. It was found that incubation in 300µEinsteins showed no direct effect on the aerobic methane production of the tested samples, where only one set of samples, those incubated at 35°C, showed a statistically significant increase in methane concentration. While the other two sets of samples, analyzed in 300µEinsteins, at 25°C and 45°C, showed that the change that occurred with the incubation could not exclude the possibility that it was due to chance. This was also the case for the set of samples incubated in darkness at 45°C. However both sets of samples tested in darkness at 25°C and 35°C showed a statistically significant change in methane concentrations. These results, along with ones from other scientists, give more reason to find the mechanism by which plants produce methane and the issue of eliminating the large output of global greenhouse gases.

Keywords: *methane, aerobic methanogenesis, Alocasia x amazonica*

INTRODUCTION

Recently, scientists discovered than many plants contribute methane to our environment. Methane is a very strong greenhouse gas and is the most abundant trace gas in the atmosphere (Keppler, 2006). Recent studies indicate that not only is methane produced anaerobically by decomposition of organic material, but may also be produced by vegetation's stems and leaves. Keppler performed experiments in the presence of oxygen rather than anaerobic conditions and still found a significant amount of methane emitted from the plants. Many studies have also indicated that regions of wetland vegetation and tropical areas are associated with the global methane emissions in these areas (Butenhoff, 2007). Other studies have indicated that methane emissions may strongly be linked to seasonal variations by comparing the recorded methane emissions to the mean temperatures in a specific area for an extended period of time. During warm, summer seasons many of the tested regions show a significant increase in their methane production and emission into the environment (Wang, 2005). Recently, scientists released a study where ultraviolet radiation caused plant pectins to produce methane (McLeod, 2008). This is a possible indication that a variable such as temperature may have a noticeable effect on the methane production and emission of certain plants. However, it could also be that methanogenic bacteria found in wetland vegetation are affected by temperature more than the plants themselves. By testing the variables of temperature and light and their effects on methane emissions, I hope to prove that increased temperature and the presence of light do have significant effects on the plants.

Another recent experiment indicated that light played a noticeable role in the methane production of a common tropical plant, *Musa acuminate*, or the banana plant (Rodriguez, 2007). In this experiment, Rodriguez used similar sized samples from the leaf of the banana plant and incubated them in air-tight glass vials at a constant temperature for 24 hours, using light as her variable. The samples were then tested for methane emissions by a flame-ionizing gas chromatography detector. By using methods similar to Rodriguez's, I will test for the emission of methane due to increased temperature and light as my variables. In my experiment, a plant from the genus *Alocasia* will be used to test the variable of increased temperature on the amount of methane emitted by a sample of these plant's leaves. The plant, *Alocasia x amazonica*, commonly known as an Elephant-eared plant, can be found in many tropical areas throughout the world and is also a common household or garden plant.

MATERIALS AND METHODS

For my standard I used a known concentration of 50 ppm CH_4 in air (Scott's Specialty Gas, Plumsteadville, PA). From the *Alocasia* plant, I collected leaf matter and prepared it in a method similar to Rodriguez's, where the plant leaf sample was submerged in a 5% bleach solution to surface sterilize the leaf sample. I collected 30 samples of approximately 0.3 g per piece. I then placed the samples into 30 gas-tight vials with rubber septa tops. I then placed 10 samples in an incubator with light at 25°C, another 10 samples in light at 35°C, and the last 10 in light at 45°C. The light intensity used was 300µEinsteins. I then took methane concentration readings at T=0 and 24 using a 500µL gas-tight syringe and a flame-ionizing gas chromatography detector with an oven temperature of 40°C and a run time of 3 minutes. Finally, I dried each sample at 105°C until I found a constant mass. I then repeated this method using darkness as my variable rather than light.

RESULTS

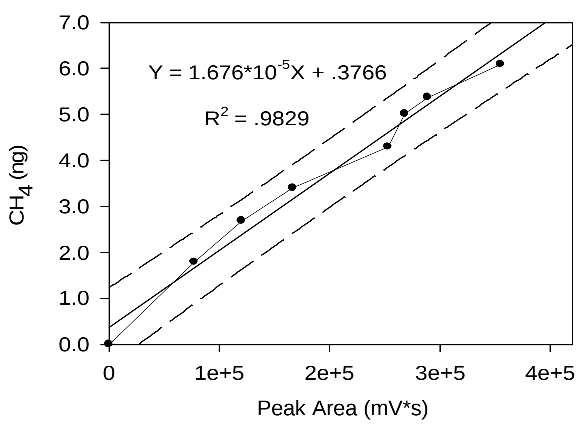

Figure 1. Standard methane curve. CH_4 samples taken were between 50 and 170 µL. Samples are plotted in mV*s vs. ng of CH_4.

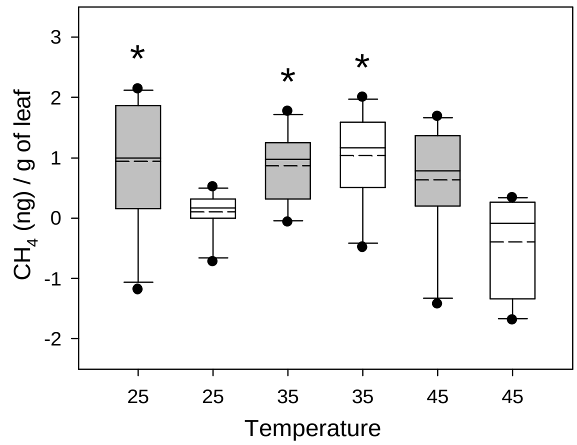

Figure 2. Change of CH_4 concentrations per gram of leaf for the samples incubated in the dark and in 300µEinsteins of light at 25°C, 35°C, 45°C. The boxes shaded in grey indicate the sets of samples incubated in darkness, while the un-shaded boxes indicate the sample incubated in light. The median of each set of data is indicated with a solid line and the mean is marked with a dotted line. The limits of the boxes indicate the 25th and 75th percentiles. The bars indicate the 5% and the 95% confidence intervals. Each outlier is shown by a black dot. The * indicates the three sets of data which showed statistically significant increases in methane.

The data from the experiments were analyzed using the paired t-test. It showed that *Alocasia* leaf samples incubated at 45°C in darkness and in 300µEinsteins of light did not show significant aerobic methane production (P=0.060, P=0.148). A signed-rank test verified these results indicating that the change that occurred with the treatment was not enough to exclude the possibility that it occurred by chance (P=0.064, P=0.375). It also showed that samples incubated at 25°C in 300µEinsteins of light did not show significant aerobic methane production either (P=0.339), and a signed-rank test concluded that it could have occurred by random chance (P=0.131). However, the set of samples incubated at 25°C in darkness did show a statistically significant increase in methane production (P=0.020). Also, both sets of samples incubated at 35°C, in darkness and in 300µEinsteins of light, showed a statistically significant increase in methane concentrations (P=0.001, P=0.002).

DISCUSSION

The data from my procedure indicates that the *Alocasia x amazonica* plant seems to be a significant source of aerobic methane production, however the variables by which it produces methane can not be determined by my results. Judging by the outcome of my experiment, light did not seem to have any affect on the aerobic methane production of the *Alocasia* plant. It cannot be determined by my results alone if my second variable, increased temperature, had any affect on the plant's methane production. It is apparent that 45°C may be too high of a temperature to accurately test the methane production of this particular plant. The data set at 45°C in darkness contained one outlier which did not allow it to be considered statistically significant. This could be a possible indication that if more tests were run with larger groups of samples, that darkness could be the key variable in which the *Alocasia x amazonica* produces methane aerobically. There is also a possibility that there could be an optimum temperature range correlating with this plant's methane production. This could be tested by using a lower and smaller temperature range with more samples. I can be sure due to my results, that the *Alocasia x amazonica* plant aerobically produces methane.

It is still not certain if all plants produce methane aerobically, however the discovery of plants possessing the ability to do so is occurring more frequently. It was only recently discovered that plants emitted atmospheric methane into the air. The mechanism by which aerobic methane production occurs is still unknown, but is hypothesized to involve pectin. A recent study indicated that ultraviolet radiation caused plant pectins to produce methane (McLeod, 2008). Originally, estimated emissions from plants was 10-30% of the total annual atmospheric methane, however it is recently believed that the actual methane emission from plants is much lower (Lowe, 2007). It is also still not known what variables may cause certain plants in different regions to produce methane aerobically, where the same plants in different conditions may not. There are many variables that could have different effects on plants in different regions.

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