Investigating the Potential for Vitamin D3 to serve as a Therapeutic Against the Degenerative Effects of Diabetes using Drosophila melanogaster as a Model Organism

Analyzing the Access to Quality Nutrition of Students

Metaheuristic Analysis of Transcriptional Control to Capture Leukemia Cell Line Characteristics

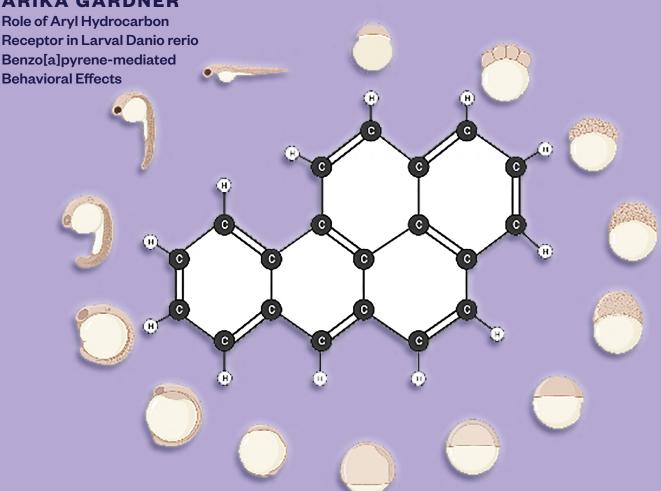
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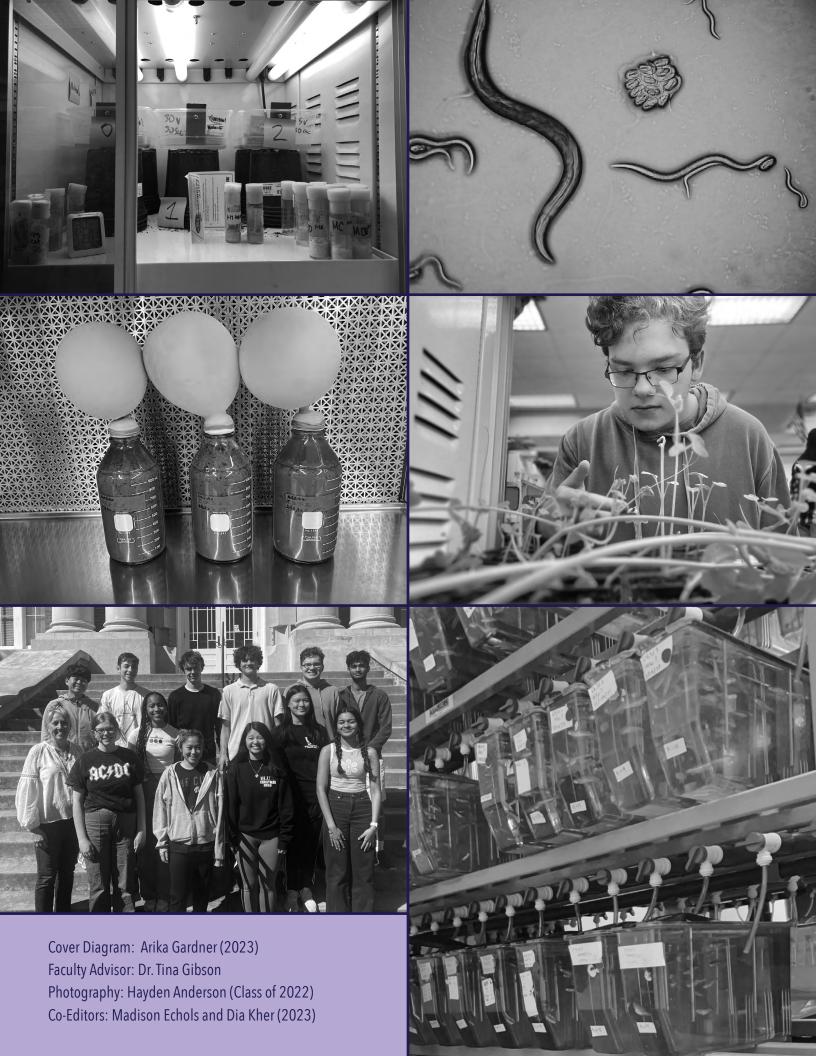
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Investigating the Potential for Vitamin D3 to serve as a Therapeutic Against the Degenerative Effects of Diabetes using *Drosophila melanogaster* as a Model Organism

by Madison Echols

Type two diabetes is an epidemic that is especially prevalent in America's deep south, known as the diabetes belt. Because there is no cure for diabetes and the current treatments can be expensive and inaccessible, exploration for more affordable management tools for the disease is needed. Found in many places like food, sunlight, and supplements, with more conclusive research, Vitamin D3 could serve this purpose. This experiment tests the potential for Vitamin D3 to serve as a potential management tool for T2 diabetes using the model organism *Drosophila melanogaster*. Wild-Type *Drosophila melanogaster* were sorted into three experimental medium groups per sex, Control (15% sucrose), High Sugar Diet (18% sucrose), and High Sugar Diet with Vitamin D3 supplementation (18% sucrose and a VD3 concentration of 0.50 mcg/mL) The flies were tested for locomotor, retinal, and survival degeneration through negative geotaxis, positive phototaxis, and survivorship assays at the beginning, middle, and end of five days of feeding. Based on the testing, the effects of VD3 supplementation on the diabetic flies caused them to mirror the survivorship, light responsiveness, and locomotor ability of the control flies, while the purely HSD flies suffered degenerative effects. The significance of the assay results from a one-way ANOVA test forced the nulls for male negative geotaxis and both sexes' positive phototaxis data to be rejected, and alternatives were accepted. However, neither results for longevity nor female negative geotaxis assays were significant, and their nulls were accepted. More conclusive research is necessary to determine the application of Vitamin supplementation in cheaper and more accessible diabetes medication, but these results show potential for VD3 to serve as a more accessible tool to manage type two diabetes.

Introduction

Type two diabetes is a epidemic (WHO, 2022). This problem is especially prevalent in the deep south, known as the diabetes belt; Mississippi is the only state that is entirely covered by the belt (CDC, 2015). This issue extends farther than just the US. It is even estimated that the number of people affected by diabetes will rise to 700 million by 2045 (IDF, 2021). The high amounts of glucose in the blood without insulin to signal for the breakdown and storage of glucose, causes degeneration. This can lead to the severe side effects of diabetes like retinal degeneration, loss of motor function, neurodegeneration, which can lead to dementia-related diseases like Alzheimer's, and a shortening of lifespan (CDC, 2021).

Despite the devastating effect diabetes has on millions, the treatments for diabetes complications like diabetic neuropathy, diabetic retinopathy, and other side effects are costly, and some without specific medical treatments at all. Given the socioeconomic status and access of the regions and ethnicities that type two diabetes affects, a cheaper alternative or protectionary measure is vital to make management treatments as accessible as possible.

Vitamin D3 could serve this purpose as it is found in many places like food, sunlight, and supplements, with more conclusive research (McMaster University, 2018). With 75% of their disease-causing genes aligning with that of humans and the induction diabetes-like symptoms like insulin resistance of on a high-sugar diet (HSD) the *Drosophila melanogaster* is a suitable model for testing the effectiveness for Vitamin D3 supplementation to manage the developmental, locomotive, and retinal degeneration of diabetes. This experiment could lend to future endeavors like potentially researching Vitamin D3 as a credible management tool for diabetes.

Hypotheses

Null(s):

- There is no change in motor function of the Drosophila after Vitamin D supplementation.
- There is no change in the amount of retinal degeneration of the Drosophila after Vitamin D supplementation.
- There is no change in the lifespan of the Drosophila after Vitamin D supplementation.

Alternative(s):

- There is a positive change in motor function of the Drosophila after Vitamin D supplementation.
- There is a positive change in Drosophila's retinal degeneration after Vitamin D supplementation.
- There is an increase in the lifespan of the Drosophila after Vitamin D supplementation.

Experimental Design

The wild-type strain Canton-S (Carolina Biological Supply) was used in all experiments. Flies were maintained in climate chamber with a constant temperature of around 25°C a humidity of around 60% on instant fly medium (modifications were made for each experimental group).

To ensure age-synchronicity, the eggs obtained were agematched. Newly hatched flies were separated into males and females within one day of eclosion to ensure a lack of mating. The flies were kept in a freezer for 20 minutes to slow their movements in order for sorting to take place. Twenty sorted male and female flies were then transferred into their respective culture vials containing their experimental diets. Flies were kept on a control, high-sugar (HSD) or high-sugar diet with .5 mcg/mL Vitamin D3 supplementation for each sex. Each experimental group had three trials.

Assays:

Survivorship:

Survivorship of the wild-type flies was documented over the study period of five days. The number of dead flies was recorded each day of expetiementation, excluding weekends and holidays. Climbing:

To measure the flies' motor ability, a climbing assay, taking advantage of Drosophila's natural tendency to climb upwards against gravity, which is known as negative geotaxis, as used. The flies from the different food and sex vials were transferred into their respective climbing test tubes. The flies were observed climbing up the tube and the number of flies reaching the marked

> orange ring at 10 cm height within 10 To

seconds was counted.

Retinal Degeneration:

study the impact of VD3supplementation on retinal degeneration from diabetes, a positive phototaxis experiment, which takes advantage of drosophila's natural tendency to gravitate towards perceivable

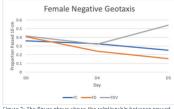
light, was run. Each group was placed in the same apparatus used in the negative

Figure 1: The figure above shows the a annoratus used for the negative geotaxis assay

geotaxis assay laid on its side. Before the UV light is lit on one end, flies are evenly distributed. After 10 seconds, the flies on the side of the UV light were counted.

Results

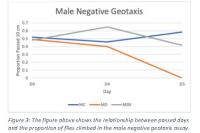
Negative Geotaxis Assays:



s and the proportion of flies climbed in the female negative

For both the male and female fly groups, the results of the negative geotaxis assay for the diabetic flies reared on purely sugar water medium had the lowest climbing

proportion overall. The climbing ability of the VD3supplemented diabetic flies had the highest. However, these results were not significant for both sexes. For the female



flies, the f-ratio value was 2.01062, and the p-value was .155843. The result was not significant at p > .05. For the male flies, the f-ratio value was 4.72267, and the p-value was .018643. The result was significant at p < .05.

Positive Phototaxis:

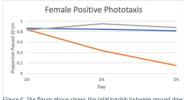


Figure 4: The figure above shows the relationship between passed day and the proportion of flies that responded to the light side in the male positive phototaxis assay.

For both the male and female fly groups overall, the results of the positive phototaxis of the diabetic flies reared on purely sugar water medium had the lowest

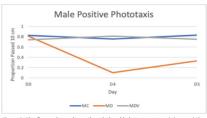
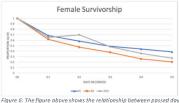


Figure 5: The figure above shows the relationship between passed days and the

response to light overall, and the light responsiveness of the VD3supplemented diabetic flies mirrored that of the control flies. For the

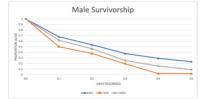
female flies, the f-ratio value was 9.11096, and the p-value was .001219. The result was significant at p \leq .05. For the male flies, the f-ratio value was 5.24739, and the p-value was .012867. The result was significant at p < .05.

Survivorship:



For both the male and female fly groups, the survival rates of diabetic flies reared on purely sugar water medium had the lowest survivorship; the survivorship of the

VD3 supplementied diabetic flies was in the middle, and the control flies had the highest survivorship rates. For the female flies, the f-



ratio value was 0.99761, and the p-value was .375839. The result was not

significant at p < .05.

Figure 7: The figure above shows the relationship between passed days and the proportion of flies alive in the female survivorship assay.

For the male flies, The f-ratio value was 1.84183, and the pvalue was .168916. The result was not significant at p < .05. Discussion

Over the duration of the experiment, the effects of the VD3 supplementation on diabetic flies caused them to mirror the survivorship, response to light, and locomotor ability of the control flies. Given the significance of the results, only the nulls for male negative geotaxis and both positive phototaxis data for both sexes were rejected, and the alternatives were accepted. Though there was a graphical trend, the results of the survivorship assay and the longevity and female negative geotaxis assays were not significant, and the nulls were accepted. Further research is necessary to determine the application of Vitamin supplementation in cheaper and more accessible diabetes medication and treatments, but these results show potential for more effective and accessible tools to manage T2 diabetes, supporting the UN Sustainable Goal number three: Good Health and Well-Being for all.

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Role of Aryl Hydrocarbon Receptor in Larval *Danio rerio* Benzo[a]pyrene-mediated Behavioral Effects

by Arika Gardner

Benzo[a]pyrene (BaP), an aromatic polycyclic hydrocarbon, is an environmental contaminant and carcinogen that causes neurological and developmental effects to children when exposed in pregnant women. The aryl hydrocarbon receptor (Ahr) is known to mediate the effects of BaP, but its behavioral effects have yet to be explained. This study focuses on examining the role of Ahr2 to mediate BaP behavioral effects. Zebrafish $(Ahr2^{+/+}, Ahr2^{+/-}, Ahr2^{-/-})$ were either exposed to DMSO (0.01%), the control, or 100 µg/L BaP, the experimental group, from 6 to 120 hours post-fertilization (hpf). The larvae were accessed in a photometer response assay where they were tested in two phases, dark and light. Results show that the BaP treatment caused a decrease in activity compared DMSO (0.01%) treatment. To identify the genotype, full larvae were digested. The outcome of this study proposes that Ahr2 shields the larvae from the behavior effects of Benzo[a]pyrene.

Introduction

Benzo[a]pyrene (BaP), an polycyclic aromatic hydrocarbon (PAH), is an environmental contaminant that is the result of incomplete combustion of organic compounds (NCBI 2022). Benzo[a]pyrene is found nearly everywhere in our everyday environment. From tobacco smoke, car exhaust to charred meat, almost everyone within the world has been introduced or exposed to this toxin. PAHs are known risks to human health, especially developing fetus and infants which can lead to inutero mortality, cardiovascular effects, and lower intelligence. (Knecht, A. L. 2017). Preterm births are also a cause for concern, and prolonged exposure to fetuses in the womb to BaP can increase morbidity (Padula A.M. 2014). It has lasting developmental and neurological effects on children exposed inutero. The aryl hydrocarbon recptor (Ahr) is an important nuclear transcription factor best known for mediating toxic responses (Yin. J 2016This suggests that Ahr could protect or reduce the behavioral effects of benzo[a]pyrene. Because zebrafish have biologically similar organs and share about 70% of human genes, they are a great organism to test the aryl hydrocarbon receptor in mediating benzo[a]pyrene's behavioral effects (Howe K 2013).

Hypotheses

Null(s):

- The aryl hydrocarbon receptor gene is not correlated to the effects of benzo[a]pyrene in Danio rerio larvae
- 100 μg/L of BaP treated Danio rerio will not show significant changes in behavior

Alternates(s):

- The aryl hydrocarbon receptor is gene directly correlated to benzo[a]pyrene effects in Danio rerio larvae.
- 100 μg/L of BaP treated Danio rerio will show significant negative changes in behavior

Methodology

I. Zebrafish Husbandry

Embryos were collected from aryl hydrocarbon receptor heterozygotes $(Ahr^{+/-})$ adults and housed in a 28°C incubator until exsposure began at 6 hours post fertilization (hpf). Offspring consisted of $Ahr^{+/+}$, $Ahr^{+/-}$, and $Ahr^{-/-}$, that were genotyped following the photometer response at 120 hpf.

II. Benzo[a]pyrene Exsposure

Benzo[a]pyrene, that was acquired from Sigma Aldrich, was dissolved in DMSO (Dimethyl Sulfoxide) to obtain the 100 μg/L of BaP in zebrafish water. For exposure, a subset of zebrafish was treated with the 100 μg/L of BaP in zebrafish water (60 ppm Instant Ocean, pH 7.6, 28°C) or DMSO (0.01%) in zebrafish water beginning 6 hpf and ending at 120 hpf. water was changed and redosed every 48 hours. The larvae were checked for mortality and hatch daily.

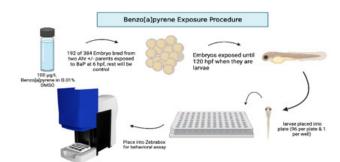


Figure 1: Diagram of BaP exposure procedure made in Biorender

III. Larval Photomotor Response (LPR)

At 120 hpf, larvae were transferred 96 well plate with A01-D12 the control larvae and E01-H12 the BaP-treated larvae. The Viewpoint Zebrabox was used to run a 50 minute behavioral assay on the unidentified Ahr2 Danio rerio larvae. The first ten minutes will be the acclimation phase, which will be in normal lighting, the next 40 minutes will consist of two phases, dark (lights off) and light (lghts on), that will alternate every 10 minutes.

IV. PCR & Genotyping

After the LPR, whole larvae were digested whole using sodium hydroxide Tris-HCL. It was put in a PCR machine, and while processing, the gel was being made for the gel electrophoresis. The PCR products were loaded into the gel wells. After gel electrophoresis, the gel was inserted into a gel imaging system were the genotypes will be assessed.

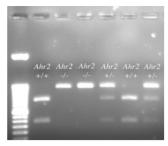


Figure 2: Agarose gel electrophoresis of PCR products from Plate III

Results

Behavioral Assay:

There were three phases recorded during the larval photomotor response behavioral assay: Acclimation, Dark and Light. The graph below shows the total distance travelled in millimeters by each treatment group and genotype per every two minutes over a span of 50 minutes. The shaded areas indicate periods of the dark phase.

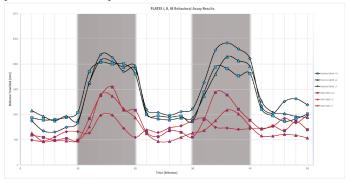


Figure 3: Graph created in excel spreadshee

T-Test Results:

* indicates data was significant different from a p < .05

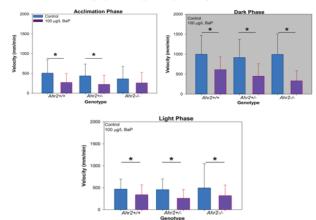


Figure 4: Graphs made from sigma plots

Conclusion

While zebrafish are normally active in the dark, the BaP treatment group showed significant hypoactivity during the dark phase. The BaP-treated larvae that are Ahr2-/- showed the most hypoactivity with their total distance not exceeding 1500 millimeters (mm) anytime throughout the behavioral assay. Among the genotypes in controls, behavior was not significantly different. Therefore, presence or absence of Ahr2 does not affect their behavior. Within each genotype, BaP significantly reduced velocity in the light and dark phases. This shows that there is a relative difference in zebrafish behaviors when exposed to BaP. The results suggest that the aryl hydrocarbon receptor not only mediates the effect of toxins but possibly provides protection against polycyclic aromatic hydrocarbons.

Discussion

Understanding how Ahr2 mediates BaP effects can assure the well-being and health of everyone. As previously stated, benzo[a]pyrene is all around us and threatens the well-being of us all, especially fetuses and the women that bear them. Since it is known that BaP is metabolized by Ahr2, if a person is Ahr2 deficient, they are likely to have unfavorable Ahr2 - mediated behavioral effects. Its is also important to learn if early life behavioral deficits are persistent. Essentially, the sustainability goal of this project is to ensure healthy lives and promote wellbeing for all at all ages. With this information we could potentially find ways to help all persons combat the effects of toxins and protect ourselves for other eviromental contminants.

Implications and Recommendations

For a possible future study or a continuation of this project, I would suggest Danio rerio be exposed at an early stage and are raised into adulthood. This will allow us to learn if the behavioral effects from early life exposure to BaP is consistent throughout the growing stages of the zebrafish's life. Environmentalists could use this data and create ways of decreasing the BaP concentrations within our everyday environment, especially starting in major cities, and metropolitan areas. These behavioral assays can also be used to identify fetal behavior within the womb that can correlate to early detection of BaP exposure in pregnant women.

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Analyzing the Access to Quality Nutrition of Students

by Dia Kher

Severe distances between grocery stores and a lack of transportation restrict residents from making trips for nutritious food, discriminating against poverty-ridden areas. Often trade-offs between traveling long distances for nutritious food and traveling short distances for unhealthy food take place where families choose the option in closer proximity. With about 12% of the children in the U.S. being food insecure since 2010, acts such as the Healthy Hunger-Free Kids Act (HHFKA) and the National School Lunch Program (NSLP) seem to have no effect on this number despite lowering the percent of obese students. Food Intelligence and Child Health are measured in a survey intended for parents of students enrolled in school. I test the relationship between household structure, sociodemographics, and food-oriented beliefs with eating behaviour among children. It was found that household structure, sociodemographics, and food-oriented beliefs are valuable predictors in analysing the access to nutrition in students. Parental food-oriented beliefs along with food availability and financial success are determining factors in quality nutritional intake in children. It is presented that food availability is not the only hinderance that parental figures face when providing nutritional meals.

Introduction

Food insecurity has plagued the world for centuries. Countries such as India and China face overpopulation and poverty, causing a struggle to find and consume nourishing food. The United States has not escaped the grasp of food insecurity (Swann 2017). Mississippi, specifically, is considered to be a food desert. Severe distances between grocery stores and a lack of transportation restrict residents from making trips for nutritious food, discriminating against poverty-ridden areas. About 12% of children in the United States are food insecure. In 2010, the Healthy Hunger-Free Kids Act (HHKA) was enacted. HHKA funds school meals and child nutrition programs to increase access to nutritional, healthy food. The act was implemented in about 4,000 schools, reducing obesity risk for children in poverty; however, it is estimated that about 9 million children in the U.S. are food insecure / live in a food insecure household. In fact, these conditions drive individuals to consume unhealthier options from stores in closer proximity, such as dollar stores, leading to widespread nutrition deficiencies. A continuous cycle of constant tradeoffs is known as a nutrition poverty trap, which further entrenches future generations in this struggle (Ford, Beaumier 2011). Additionally, if given the opportunity to choose entertainment or nutrition, impoverished families would rather put their money towards entertainment (Lagerlöf, Kvernes 2021). Decision making directly influences the economic wellbeing of the family in turn affecting the household's accessibility to quality nutrtion, thus, affecting the children's nutritonal intake. The trade-offs families are willing to make over a healthy meal lead to the question of what value families have placed on food and the health of their children.

Methodology

Twenty-two original questions were sent out in a survey through various media, including Facebook and email. The questions were designed to measure household structure. sociodemographics, and food-oriented beliefs and/or circumstances. The sample size consisted of 68 parents or guardians with dependent children from kindergarten through 12th grade. The majority of the responses were from Mississippi and accurately resembled the state's demographics. All responses from the survey were compiled together, processed, and analyzed.

Question: Why are students still malnourished despite acts, such as the Healthy Hunger-Free Kids Act (HHKA) and the National School Lunch Program (NSLP)?

The objective of the study is to discover the barriers that cause students to be malnourished despite access to food in school whether it be school lunch or packed lunch.

Null Hypothesis: Parental knowledge of nutrition does not influence nutrition in children.

Alternate Hypothesis: Parental knowledge of nutriton does influence nutriton in children.

Results

Each regression is held at the significance level of p < 0.10.

$$egin{aligned} Y_i &= lpha + eta_i Health_i + eta_i Dinner_i + eta_i \ Health_i &= Health \ Variable \ Dinner_i &= Dinner \ Binary \ Y_i &= McDonald's \ Indicative \end{aligned}$$

\mathbf{X}_{i}	β	p-Value
Health Variable	-0.350	0.005
Dinner Binary	+0.110	0.366

*McDonald's Indicative measures whether or not the parental figures find McDonald's unhealthy.

$$Y_i = \alpha + \beta_i Eating_i + \beta_i Income_i + \varepsilon_i$$

Eating : = Eating Out Binary

Income; = Income Variable

 Y_{i} = Asian or Pacific Islander or Hispanic or Latino

$\mathbf{X}_{\mathbf{i}}$	β	p-Value
Eating Out Binary	-0.294	0.020
Income Variable	+0.051	0.681

$\mathbf{X}_{\mathbf{i}}$	β	p-Value
Eating Out Binary	-0.294	0.020
Income Variable	+0.051	0.681

$$Y_i = \alpha + \beta_i X_i + \beta_i Income_i + \epsilon_i$$

 $X_i = Sole \ or \ Joint \ Custody \ Binary$

 $Income_i = Income\ Variable$

 $Y_i = Home \ or \ School \ Breakf \ ast \ Binary$

\mathbf{X}_{i}	β	p-Value
Joint Custody Binary	+0.399	0.002
Income Variable	+0.043	0.722

X_{i}	β	p-Value
Sole Custody Binary	+0.212	0.096
Income Variable	-0.122	0.335

Interpretation of Results

Parental food-oriented beliefs and financial success determine whether household children will have access to quality nutrition outside of school. There is a negative correlation between the impact of a diet on health and the extent of McDonald's unhealthiness. Parents and/or guardians who believe that a diet affects the health are less likely to consume McDonald's or food in a similar

Minority families, specifically Asians/Pacific Islanders or Hispanics/Latinos families, tend to eat out less than a White/Caucasian families despite having the financial ability to eat out. Culture tends to influence a family's beliefs as to what is classified as healthy or unhealthy. Black/African American families did not have a significant relation with eating out. This may be due to the consumption of similar foods.

Household structure determines the ability for children to consume breakfast at either home or school. Parents with joint custody tend to provide breakfast at home while parents with sole custody tend to drop their children off at school without breakfast.

Conclusion

Household structure, sociodemographics, and food-oriented beliefs are valuable predictors in analyzing the access to nutrition in students.

The regressions between each measure demonstrated the impact that income, knowledge, and accessibility have on not only parents, but their children as well. The NSLP and the HHFKA only provides children with one meal out of the three recommended. It is presented that food availability is not the only hinderance that parental figures face when providing nutritional meals.

The study portrays the discrepancies of univariate relationships between child eating behaviors and food-oriented beliefs and/or circumstances.

Implications and Recommendations

The purpose of this research is for social utility. As parents become more aware of foods that are beneficial and the foods that are harmful, the overall, nutritional health of children will increase. To implement ways to improve nutritional intake, educational seminars pertaining to healthy diets will be made available to the public. With this, food intelligence within the parents will significantly improve. This study helps work towards Sustainable Development Goal 2: Zero Hunger.

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Metaheuristic Analysis of Transcriptional Control to Capture Leukemia Cell Line Characteristics

by Nicholas Popescu

Cancer is an extremely variant and personalized disease that is characterized by the disruption of regular cell functions. These disruptions can be analyzed by examining regulatory network changes between normal and cancer cell lines. There are two main characteristics that identify a cancer cell: the cancer cell's dysregulation from an otherwise highly structured hierarchical regulatory network and the increase in cancer promoting transcription factor (TF) bindings with a loss of tumor suppressing TF bindings. Using TF binding data from ENCODEc, a cancer-focused database derived from the ENCODE project, an initial regulatory network rewiring was created to display TF binding changes between GM12878, a control healthy blood cell line, and K562, a Chronic Myeloid Leukemia (CML) cell line. To further analyze this network, a Genetic Algorithm (GA) method was designed and implemented to model the 'evolution' from a healthy to a diseased cell. This method selects the most significant network rewirings in CML as it searches for the most disruptive TF binding changes through thousands of different network evolutions. A fitness function that measures network centralization penalized for the amount of network changes over each evolution. This method models a healthy cell line's evolution towards its cancerous counterpart through TF rewiring crossover and mutation, resulting in a dynamic view of regulatory network changes in CML. Finally, the GA search identifies key regulatory network targets of the driving oncogenes in CML, MYC, JUND, BHLHE40 and STAT5A.

Introduction

Chronic Myeloid leukemia (CML) is a life-threatening disease derived from malfunctioning stem cell development in bone marrow. The JAK/STAT pathway and JUND gene have been identified as major components in the development of CML, however, the specifics behind the TF regulatory network changes that drive progression from healthy to cancer are still to be discovered. Two key differences encompass the distinct alterations in a cancer regulatory network versus a healthy one. First, cancer causes the disruption of an otherwise hierarchical and highly organized transcription regulation network. Second, there is a general loss of binding sites for tumor suppressing TFs and an increase in bindings for cancer promoting TFs. To understand cancer dysregulation, a new method to analyze transcription network changes in cancer cells studied in the ENCODE project is proposed (Encode, n.d.). The ENCODEc, a cancer-focused database derived from the ENCODE project, was used to obtain data for two cell lines: GM12878, a control healthy blood cell line, and K562, a chronic myeloid leukemia (CML) (Zhang et al., 2020). The project analyzes the regulatory network differences using a GA, a metaheuristic search method that searches for the optimum solution using crossover and mutations (Zhang et al., 2020). The method identifies the most significant network rewirings in CML as it searches for the most disruptive TF binding changes through thousands of different network evolutions (Goldberg, 2013).

Research Question: Can GA identify key TF rewiring patterns in CML?

Methodology

TF network data: TIP networks quantitatively measuring the regulatory relationships between TFs and target genes merged with enhancer target-based linkages were used; this data was used in calculation a differential network.

Genetic Algorithm: The goal of this research is to find the most important TF - gene interactions that define how the cancer cell line differs from the healthy control. A GA was designed to search for the most significant changes between the regulatory network structures of the two cell lines. To build a GA, an encoding of the problem, a Fitness function, Evaluation, Crossover, and Mutation methods were created. A binary encoding of the network change solution applies to the pre-computed difference network of edge gains and losses. A pool of network change solutions is maintained, with low fitness solutions being discarded.

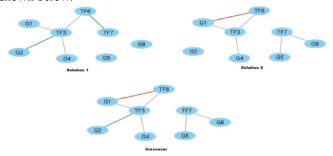
Differential Network: The *difference network* (rewiring) is defined as the set of edges that are gained or lost between the cancer and control cell line. The *difference network* is a signed network with +1 meaning a gained edge and -1 meaning a lost edge and 0 meaning a preserved edge. The objective of GA method is to search for the minimum number of *network changes* that reduces the organization hierarchy of the normal cell line to resemble that of the cancer cell line, while preferentially selecting oncogene gains and tumor suppressor gene losses.



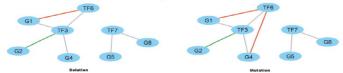
Fitness: The *Fitness* function was designed to measure the loss of hierarchy of the regulatory network structure. A graph-level centralization, a centrality measure from network theory implemented using *igraph*, is used. Fundamentally, the stem-like cancer cell line has lower centrality in comparison to the control cell line. The GA is programmed to evolve a high centrality control cell line towards the decentralized cancer cell line. To select the key changes required to decentralized the regulatory network, the fitness function includes a term to penalize edge addition/removal from the normal cell line. where w is the vertex with the maximum degree and e(G) signifies the edge set of a graph G.

$$Fitness = -a * \frac{\sum_{v} \deg(w) - \deg(v)}{H} - \frac{\|xor(e(ga_{sol}), e(GM))\|_{1}}{\|xor(e(K), e(GM))\|_{1}}$$

Crossover: The crossover between two encoding strings uses a single crossing site. A new network change solution is obtained by joining two subnetworks from the parent solutions, as shown below.



Mutation: Mutations are implemented by selecting or removing at random non-zero positions in the *difference network*. In the figure below, edge (TF6, G4) is added to the solution from the previous step.

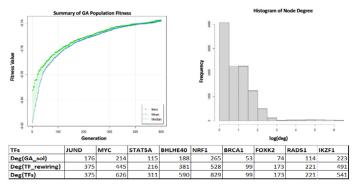


Results

Regulatory Network: ENCODEc data was processed to generate the input for the metaheuristic search method. *Cytoscape* software was used for network visualization and packages *NetworkAnalyzer* and *DyNet* were used for the analysis of network structure. The differences in regulatory network architecture of GM12878 (Blue) and K562 (Red) are highlighted in the figures.



TF Network Rewiring: The TF rewiring analysis method was implemented in R, using the *GA* and *igraph* packages. The crossover rate was set to 0.8, the mutation rate was 0.4 and the population size 300. The evolution of the solution was analyzed over 500 iterations. The best, mean and median *fitness values* of the solution population are shown in the figure below. The final network solution has a graph-level normalized centralization of 0.0264, close to that of the K562 TF network (0.0289) and smaller than that of the GM12878 network (0.0486), while the node degree distribution is similar to that of K562 TF network.



Among the most rewired TFs in CML are JUND, STAT5A, MYC and BHLHE40, shown in the figure below. The GA selected edges are highlighted in red, the GM12878 nodes are green, the K562 nodes are red and common nodes are gray.



Conclusion/Future Research

This project has analyzed the rewiring between a CML and a normal cell through a GA method that identified the most significant regulatory network changes by evolving the high centrality normal cell to a decentralized cancer cell for drugtargeted therapy. The findings of this research is in line with the overarching goal of promoting health and wellbeing for all.

This paper can increase the scope of analysis on certain aspects of cancer. Namely, the disorganization of cancer regulatory network has similarities to a transformation into a stem-like state of the cell. Discovering which TF bindings are critical in the "stemness" transformation of a cell line will allow for a wider understanding of the critical components of cancer.

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What's in That Crack? Exploring Urban Micro Environments for Novel Amoeboid Diversity Using Next-Gen Sequencing

by Danielle McConnell

Protists are eukaryotes that are predominantly unicellular. These organisms are found in most terrestrial and aquatic environments. Amoeboid protists are represented across the Eukaryotic Tree of Life and have diverse morphologies, behaviours, ecologies, and evolutionary histories. Despite the overwhelming diversity of amoeboid protists, there is limited laboratory research dedicated to discovery and characterization of novel species from unique terrestrial and urban environments. This project aims to document the morphology, identify the species, and sequence the 18S rRNA gene of an amoeba isolated from the biofilm on a sidewalk crack. A clean culture of the environmental isolate was prepared so the organism could be observed without the influence of outside factors. Using a microscope, data regarding the structure and behaviour was documented to conduct morphological analysis. Along with descriptive data, extraction, and amplification of gene sequences via PCR and Sanger Sequencing were utilized to produce DNA sequences for species identification. Later, mRNA was extracted and amplified to produce transcriptomic data for phylogenetic analysis. Piecing together observational and experimental data, this project sheds light on the unexplored diverse ecology of amoeboid protists and the potential to find novel species in unexpected places.

Introduction

Due to the unique nature and difficulties of studying these eukaryotic microbes, the application of molecular and phylogenetic study has been greatly delayed. Projects utilizing protists with modern-day phylogenomics has emerged but may struggle to confidently place unique protists due to a substantial lack of information. Recently, scientists ceased declaring six supra-kingdom-level groups within the evolutionary tree and, in turn, began to represent robust apomorphies that fall deep within eukaryotic monophylies (Burki, 2019). Therefore, the need for protistology research remains greater than ever. To confidently identify and understand evolutionary relationships, the use of metagenomics and phylogenomics on protists must become regularly performed in the scientific community (Singer, 2020). Computer programs, such as those used in this project, need to be preformed on these organisms (Stamatakis, 2014). Scientists need to take samples from understudied environments, such as infastructure in the middle of cities. Thus, this project aims to observe the morphology, identify the species, and sequence the 18s gene of an amoeba. With the absence of in-depth sampling and research regarding protists, this project centered around understanding an amoeba discovered in an unusual, urban environment.

Methodology

With a sample found on the sidewalk, a clean culture was curated of an unique amoeba, coined I2AT22-3. Using microscopy, several pictures were taken of the amoeba to document the distinct physical features of the organism. The program, ImageJ, was used to measure the length and width of

the amoebae in the pictures. In tandem with the collection of descriptive data, a polymerase chain reaction (PCR) was used to amplify the 18s gene extracted from the amoeba. Forward primers, reverse primers, GoTag and the DNA solution were the reagents in the process used to amass the desired sequences. Sanger Sequencing extracted the gene sequences from the PCR product. Utilizing an SSH session through the MobaXTerm application, the nucleotides generated from sequencing underwent phylogenetic analysis. After the sequenced data was returned, a BLASTn search on the NCBI database identified several known Amoebozoan 18s sequences. The genetic sequences of the comparable protists were gathered and used to construct an alignment of the nucleotides. MAFFT was used to create the nucleotide alignment of all sequences and useful sequences were selected and trimmed using BMGE. After running these applications, the 18s sequence was aligned, trimmed, and complied with other sequences of protists. The program, RAxML, was used to construct a maximum likelihood phylogenetic tree. The tree was built with all of the sequences, including those gathered from NCBI and I2AT22-3.

Results

The results show that I2AT22-3 is typically 15.650 μm long and 10.212 μm wide when moving across an agar plate. The standard deviation for the length and width of the amoeba is 2.9627 μm and 2.3030 μm , respectively. The average length of a cyst was determined to be 11.727 while the average width is 11.077. This information warranted the size of the amoeba which can be used to compare the organism to hose closely related to it throughout the building of the phylogenetic tree.

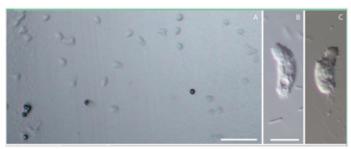


Figure 2: Light Micrographs of Isolate I2AT22-3. A: Picture of trophic amoeba and cysts on agar (scale bar = 100 uM) B-C: Tropic amoeba on agar (scale bar = 10 uM)

With the sequences from NCBI and the sequence of I2AT22-3, a maximum likelihood tree was built to demostrate the evolutionary relationships of all of the gathered amoeba. Upon studying the constructed tree, the amoeba was found to be a new genus, the taxa above species.

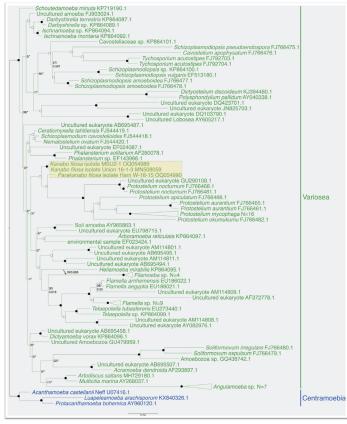


Figure 1: Phylogenetic Tree Including the Isolate. The highlighted region is where the sequence of I2AT22-3 falls in the tree. The sequence is named Kanabo filosa isolate Union 16-1-3 on this chart.

This discovery aided in the mission to contribute to the growing emphasis on studying unicellular eukaryotes.

Discussion

With morphological and genetic analysis, an amoeba was successfully isolated and identified from an unusual, urban micro environment in Mississippi. Due to the position of I2AT22-3 on the tree configured, the amoeba represents, not only a new species, but an entirely undiscovered genus. Consequently, this study takes an important and necessary step

toward extensive knowledge of protozoa by uncovering a novel genus within protozoa. The data gleaned from this project will be vital in replicating results for unique amoeba isolated from similar environments in the world in order to continue strengthening scientific knowledge regarding protists. Samples from unique, urban microenvironments are incrediably understudyied. The increase of infastracture does not necessarily coorelate to a decrease in organisms in the urban microcosms, therefore studys must take place in these areas.

Implications and Recommendations

This experiment focuses on the seventeenth sustainable development goal, life on land. By researching protists, scientists may better understand and, therefore, effectively preserve microorganism biodiversity. Without the influence of microorganisms in nature, many essential species would experience a drastic drop in numbers. To protect the natural world and pursue the seventeenth sustainable development goal, scinetists must analyze unique microenvironments, such as the one is this study.

Future Research

Since the microcosm remains a positive direction for stimulating scientific discoveries, this study will continue using different cultures extracted from the surrounding environment to culture interesting amoebae. The future direction of this study will also include inserting the findings into the NCBI database and observing the internal structures of I2AT22-3.

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Effects of Metabolic Changes on Pneumococcal Sensitivity to Fosfomycin

by Jeremy Dawe

Antibiotic resistance is an ever-growing problem. Combating it is essential to Sustainable Development Goal 3, ensure healthy lives, and promote well-being for all at all ages. *Streptococcus pneumoniae*, the main etiological agent in Pneumonia, has the ability to undergo genetic transformation, acquiring DNA from other streptococci in the nasopharyngeal carriage. This allows for the development of high levels of antibiotic resistance. By investigating two-component systems (TCS), one of the mechanisms involved in the uptake of molecules from the environment, novel treatments can be explored. This research examines how the growth medium of mutant strains of *S. pneumoniae*, with modified TCS, respond to different environmental stimuli and how they affect sensitivity to fosfomycin. The study tests the null hypothesis that genetic deletion of various two-component systems linked to sensing of specific carbon sources will not impact susceptibility to antibiotics. By understanding the role of TCS, this research has the potential to identify new targets for combatting the growing problem of antibiotic resistance.

Introduction

Streptococcus pneumoniae causes many diseases ranging from non-invasive and localized infections, such as sinusitis, to aggressive and life-threatening ones such as pneumonia or meningitis. S. pneumoniae has been declared by the WHO as one of 12 pathogens that require new treatments. S. pneumoniae undergoes genetic transformation and can acquire DNA from other streptococci in the nasopharyngeal carriage, allowing the bacteria to spread and develop resistance easily (Cillóniz 2018). Two-component regulatory systems (TCS) are made of a histidine kinase and cognate response regulator and work through the phosphorylation of the response regulator. TCS are heavily used for environmental awareness of microorganisms (Gomez-Meija 2017). Antibiotic resistance in these bacteria can be countered by the inclusion of carbon sources that facilitate the uptake of the antibiotic. This idea has been effective in Staphylococcus aureus where saturating the hexose phosphate transport system with G6P, a carbon source, facilitated an uptake of fosfomycin (Park 2015).

Question and Hypothesis

Are two-component systems involved in susceptibility to different antibiotics based on potential sensing of carbon sources?

H0: Genetic deletion of various two-component systems linked to sensing of specific carbon sources will not impact susceptibility to antibiotics.

H1: Genetic deletion of various two-component systems linked to sensing of specific carbon sources will impact susceptibility to antibiotics, allowing for research into ways to make S. pneumoniae more susceptible or reverse resistance.

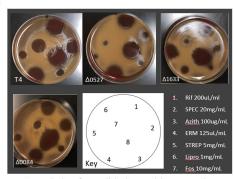
Methodology

Spot plating **for antibiotic sensitivity screening.** Wild-type *Streptococcus pneumoniae* strain TIGR4 (T4) and isogenic

mutants lacking individual two-component systems (Δ0084, Δ0527, Δ1633) were grown to mid log phase and frozen in 100uL aliquots at -80°C. Individual aliquots were thawed on ice and spread onto tryptic soy agar (TSA) blood agar plates. Individual antibiotics (rifampin, spectinomycin, azithromycin, erythromycin, streptomycin, ciprofloxacin, or fosfomycin) of various concentrations were pipetted in 5uL volumes onto specific locations on the plates and incubated overnight at 37°C. Plates were imaged the next day and qualitative susceptibilities to antibiotics were determined.

Growth curve analysis. Frozen aliquots of *S. pneumoniae* T4 or TCS isogenic mutants were diluted to a final concentration of $5x10^5$ cfu/mL in media lacking or supplemented with various individual antibiotics or antibiotics plus carbon sources (maltose or cellobiose, 2% final concentration). Samples (200uL) were added to three separate wells of a 96-well plate and incubated at 37° C with optical density (600nm) readings taken every 30 minutes for 24 hours. Data from triplicate wells were averaged, curves plotted, and unpaired t-test analysis performed using GraphPad Prism software. A two-tailed p-value of less than .05 was considered significant.

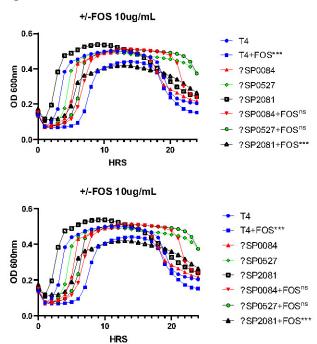
Results Figure 1:



Spot plating for antibiotic sensitivy screening

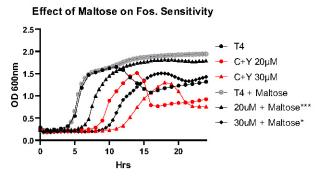
S. pneumoniae T4 or isogenic two-component system mutants were plated and various antibiotics were spotted onto plates to qualitatively determine susceptibility. Focus was primarily on fosfomycin resistance to limit the scope of the study. $\Delta0084$ and $\Delta0527$ were chosen as the strains most resistant to fosfomycin.

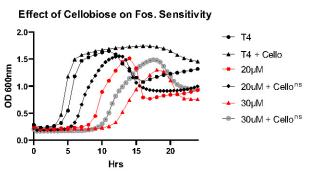
Figure 2:



Growth Curve analysis in the presence of fosfomycin Key: *** p<0.001, * p<0.05, ns (not significant).

Figure 3:





Growth curve analysis in the presence of fosfomycin and maltose or cellobiose

Figure 2:

S. pneumoniae T4 or isogenic two-component system mutants were grown for 24hrs in the presence or absence of fosfomycin (10 or 20ug/mL). Relative contribution to fosfomycin resistance was determined by unpaired t-test analysis. While all three TCS mutants were more resistant than T4, SP0084, and SP0527 showed the most significant resistance.

Figure 3:

S. pneumoniae T4 or isogenic two-component system mutants were grown for 24hrs in the presence or absence of fosfomycin (20 or 30ug/mL) with or without supplementation with 2% maltose or cellobiose. Relative contribution to fosfomycin resistance was determined by unpaired t-test analysis. While both carbon sources increased resistance to fosfomycin, only maltose had a significant increase.

Discussion and Implications

The results from this study demonstrate that certain two-component systems impact susceptibility to antibiotics including fosfomycin. Additionally, some of the substrates sensed by these two-component systems can alter susceptibility to fosfomycin. These results provide insight into systems involved in the uptake of antibiotics. It is possible that in the presence of carbon sources such as maltose or cellobiose, the transport pathways involved in the uptake of fosfomycin could be saturated with these substrates, thereby increasing resistance.

Future Research

Future directions include determining if these transport pathways could be induced in the absence of the metabolizable substrate to enhance antibiotic uptake. Furthermore, investigation of other antibiotics and their effect on TCS would work towards SDG 3 as well.

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Modeling Lattice Fins as Flight Control Surfaces for a Precision Guided Landing of Reusable Rockets

by Rushyendranath Reddy Nalamalapu

The frequency of rocket launches has intensified in the recent decade. Reusability is ideal to reduce the cost of launches and give rise to a new era of space exploration. Landing a rocket booster requires steering the booster at high speeds to a landing pad. Lattice fins are being utilized as a flight control surface due to their flight authority. Three novel designs are modeled along with a Control using Computer-Aided-Design. The fins are tested using Computational Fluid Dynamics (CFD) in an online wind tunnel simulation system to compare the normal force coefficients. Furthermore, data will be collected from a physical wind tunnel at varying angles of attack for each model on the same fuselage. The data collected would be analyzed and compared with the data retrieved from the CFD simulation. From the results, an optimal novel design can be determined. Implementing the novel design will increase efficiency and allow for more development in reusable rockets. My novel design can be implemented in other precision guidance systems. Furthermore, this innovation supports the United Nations Sustainable Development Goal 9 for industry, innovation, and infrastructure.

Introduction

Since the turn of the century, the space industry has rapidly advanced methods of space transportation. An important aspect of this modernization is the ability to reuse rockets. This is possible by performing carefully choreographed positional maneuvers to land a booster on the Earth from a location originating in the upper stratosphere. This is analogous to dropping a toothpick from the top of the Empire State Building and landing it vertically on a postcard on the ground. Such aerodynamic operations require the usage of heightened reaction control surfaces (RCS). Previous research highlights the effectiveness of lattice fins for usage as RCS in contrast to traditional planar delta or trapezoidal fins.[1] Lattice fins have seen measurable success in private and public space agencies and even missilery.

Objectives

Four lattice fin configurations are tested. Control authority will be used to determine the ideal grid configuration. The control authority is essentially how effective a fin is at maneuvering a vessel. This can be quantitatively measured by comparing the normal force coefficient. Such a metric is the ratio of the force of lift to the force of drag. Data retrieved from the CFD software will be analyzed alongside statistics that will be collected from the physical wind tunnel testing results.

Methodology

The models were created using Computer Aided Design (CAD) software. Figure 1 shows the models in the CAD design software. After the model was imported into the CFD software a mesh was designed to begin computational experimentation. From this, a solution field was generated and is displayed in Figure 2. The software will be instructed to record the normal force coefficients.

The CFD software produces a quantitative predictive model by differentiating the Navier-Stokes energy equations in Equation 1

at each point on the mesh. Using this, a solution field is generated with visualizations of fluid flow for the model.

The second phase of this research is testing the same models in a physical wind tunnel at similar or higher speeds. The data from the physical wind tunnel will be analyzed alongside data from the simulation once collected.

Results

The models are displayed in the CAD software that was used to design the models. Each model has the same dimensions for the frame. The following are renders of each of the four models.

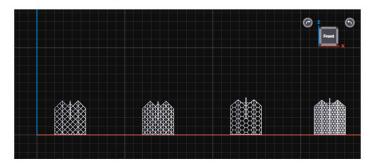


Figure 1: Models in CAD editing software. From left to right: Control, Model 1, Model 2, Model 3.

$$\begin{aligned} \nabla \cdot \vec{u} &= 0 \\ \rho \left(\frac{\partial \vec{u}}{\partial t} + (\vec{u} \cdot \nabla) \vec{u} \right) &= -\nabla p + \mu \nabla^2 \vec{u} + \rho \vec{F} \end{aligned}$$

Models were exported into the Sim Scale simulation software to test their aerodynamic performance. This equation is how all CFD software functions. Equation 1 is the Navier-Stokes Equation.

Equation 1: The Navier-Stokes Equations are a series of equations that model and help predict the behavior and nature of flow for a viscous Newtonian fluid. The simulation works by solving this equation at every point on the mesh.

In the SimScale software, a wind tunnel mesh was created around each lattice fin model. The solution fields are displayed below.

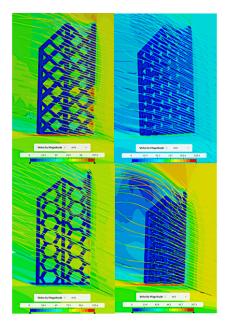


Figure 2: The solution fields are displayed with false coloring and particle traces to model the behavior of the fluid. From left to right the models are Control, Model 1, Model 2, and Model 3.

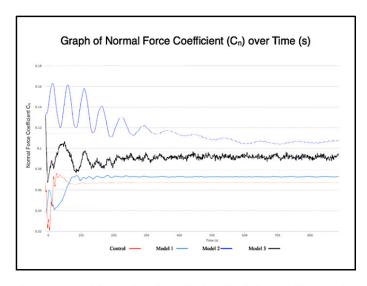


Figure 2 provides a visual qualitative depiction of the solution fields. The graphs for the normal force coefficient are plotted in Figure 3 to show comparative flight authorities.

Figure 3: This is the data produced from the SimScale software modeling the normal force coefficient for each fin as a function of time.

Implications

The CFD software provided data to support that Model 2 has the largest force moment coefficient. This hypothesis validated that the lattice structure for Model 1 had the highest normal force coefficient. It was found that Model 3 had a relatively large drag coefficient with a normal force coefficient. Essentially, Model 3 has a substantially higher drag but less maneuverability.

Overall, the results show that Model 2 has the highest normal force coefficient (C_n) . The second highest Cn is Model 3 with the third highest Cn being Model 1. The control has the lowest Cn of all the models tested. Model 3 has some instability as shown in Figure 3. Therefore, the ideal grid fin is Model 2. This research coincides with the United Nations Sustainable Development Goal 9 of Industry, Innovation, and Infrastructure.

Future Research

Space transportation has reached a significant milestone, Earlier the realm of space transportation was seen as a feat only accomplished by large governmental administrations. In the future, the private space industry will make major headway. A major issue with space exploration is the financial burden of launching payloads to orbit. Reusable rockets provide are essential and they use lattice RCS. By creating the optimal model, the cost of space transportation can be further reduced allowing for more advancements to be made in that field.

Using a novel design, such as Model 2, the success of aerospace engineering can be further improved. This engineering experimental design is most accurate due to the usage of both CFD and wind tunnel testing. Both data sets will provide a full view of the nature of lattice fins and their significance in this field.

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The Effect of Low-Voltage Electricity on the Plant Health and Germination of *Raphanus sativus* and *Pisum sativum*

by Ashton Taylor

In the next 25-30 years, the human population is expected to grow by over two billion people. To combat the increase in food consumption that accompanies this population growth, food production must increase. An increase in inorganic fertilizer usage to aid food production will not be feasible as an excessive amount of fertilizer used on crops leads to a release of greenhouse gases which contribute negatively to climate change. To find an alternative to inorganic fertilizer usage, low-voltage electricity was applied to sub-irrigated plots of *Raphanus sativus* and *Pisum sativum* seeds. Electricity was applied to two plots of each seed: fifteen voltage treatment and thirty voltage treatment, through electrodes placed in the watering vessel. The plots were treated for thirty seconds each weekday with germination results recorded each day. After three weeks of growth, the plots were harvested, and height and mass were measured for each germinated plant. Chlorophyll from the leaves of each plot were also extracted and placed in a spectrophotometer with absorbance values measured. Using statistical analysis, including T-Tests, of the data, an increase in mass from electrically treated plots compared to control plots can be seen to be statistically significant. This research can be used to cement low-voltage electricity as a viable alternative to inorganic fertilizers.

Introduction

In the future, an increase in human population will lead to an increase in food consumption (Roser et al., 2013). This increase would result in increased land usage and increased fertilizer usage. Increased fertilizer usage is especially concerning due to the release of greenhouse gases when fertilizer is used in excess on crops (Aydnialp and Cresser, 2018). To combat the increased usage of resources, a change in farming practices by introducing electricity to plants can increase germination rate as well as plant mass. Extensive studies have been conducted on the effect of high-voltage electricity on plant health and germination. Results from these studies have concluded that the application of certain electrical fields has the possibility to become an abiotic stress elicitor and affect the germination rate as well as affecting the plant health of the tested plant species (Dannehl, 2018). The issue with the studies lies with the use of deadly, high-voltage electricity. This would make the implementation of the research into modern farming techniques very unappealing due to the dangerous voltage involved as well as an increase in power consumption. It was hypothesized that low-voltage electricity will have an effect on plant health and germination on Raphanus sativus (Radish) and Pisum sativus (Pea) plants and could potentially serve as an alternative to high-voltage electricity.

Methodology

Planting the Seeds: The seeds were planted in 36 celled self-watering seed starter trays. The trays were filled with Miracle-Gro Potting Mix. Radish seeds were planted half of an inch into the soil. Pea seeds were soaked in water for 12 hours before being left overnight to dry to help induce germination. The seeds were planted an inch into the soil. Copper wire was bent into a rectangular shape to form two electrodes. The electrodes were placed into the opposite sides of the watering reservoir. The seed trays were lowered into the water reservoir and the water reservoir was filled with tap water.

Voltage Treatment: Three seed trays were used to gather data on plants exposed to variable levels of low-voltage electricity. The control tray was exposed to zero volts of electricity and was divided equally between pea and radish seeds. The fifteen-volt tray was exposed to fifteen volts of electricity for thirty seconds every weekday for two and a half weeks and was equally divided between radish and pea seeds. The thirty-volt tray was exposed to thirty volts of electricity for thirty seconds every weekday for two and a half weeks and was equally divided between radish and pea seeds. Water reservoirs were replenished daily.

Extracting Plants: The plants were extracted from the seed starter trays after two and a half weeks. The soil was carefully scooped out of each cell and the plant was removed carefully to avoid breaking off any root material. The plants were dried off and placed on paper towel. Each plant was labelled (ex. Plant 1, Plant 2) for each data set (ex. Radish 15 Volt, Pea 30 Volt). The mass and height of each plant was recorded.

Spectrophotometer: Leaves were gathered plants in each data set and placed into separate petri dishes. Ethyl alcohol was added to each petri dish until the leaves were fully submerged. The petri dishes were left refrigerated overnight, and liquid chlorophyll was extracted from each dish via a pipette. This liquid was placed inside of a cuvette. The cuvette was placed inside of a spectrophotometer and absorbance values were recorded from the wavelengths of 320-720 nm, with a measurement at every 20 nm.

Results

An increase in average mass can be seen among the datasets that were electrically treated compared to the control sample.



Dataset	Percentage of Increased Mass Compared to Control Sample
R ¹⁵	55.23%
R ³⁰	52.56%
P ¹⁵	26.95%
P ³⁰	20.05%

Figure 1 and Table 1: Average Mass and Comparison of Mass

Data collected from the spectrophotometer shows an increased spike in absorbance rate for the dataset of radish that were treated at 15 volts. This trend in increased absorbance rate can also be seen in the dataset of peas treated at 15 volts. This increase in absorbance rate correlates to an increase in photosynthetic activity among the plants which led to an increase in mass among the affected datasets.

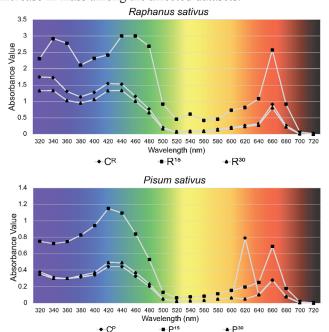


Figure 2: Spectrophotometer Readings from Radish Datasets.

A series of T-Tests were conducted to evaluate the significance of the low-voltage electricity on the datasets. Using the p-values from the T-Tests, it can be seen that the data comparing the control dataset of the radish compared to the datasets of the radish that were electrically treated are statistically significant. The p-values regarding the pea datasets show that the comparison between the control dataset and the electrically treated dataset were not significant.

Datasets	T-Value	P-Value
CR vs. R ¹⁵	-4.47557	0.00013
CR vs. R ³⁰	-2.06425	0.03170
CP vs. P15	-1.29796	0.11524
CP vs. P ³⁰	-0.80943	0.22752

Table 2: T-Test Results Comparing Different Datasets

Discussion

Using the p-value data from the results, the hypothesis can be accepted with the matter of affecting the plant health of *Raphanus sativus*, but the hypothesis cannot be accepted with the matter of affecting the plant health of *Pisum sativus*. It is notable that there is an increase in mass among the radish plants that received the 15-volt treatment. It can be inferred that this is due to the increase in absorbance value of the 15-volt treated radish plants compared to the control group. This increase in absorbance value can show an increase in photosynthetic activity among the voltage treated plants that affected the mass in the Radish plants. This trend can also be seen with the pea plants,

but p-values indicate that there is not a statistical significance among the pea plant data and its control group.

Implications and Recommendations

Using the data from this research, developments can be made for alternatives to conventional methods of promoting plant growth such as fertilizer. This research shows that there is promise in low-voltage electricity being used as an alternative to conventional methods, and asserts that the use of low-voltage electricity on plants can cause an increase in photosynthetic activity. Using electricity as an alternative to conventional methods such as fertilizer will be essential in the future due to fertilizer's release of greenhouse gases and its effects on climate change. This new way of promoting growth in plants like radish can fall in line with the UN Sustainability Development Goal #2, which discusses and promotes sustainable agriculture (United Nations, 2018). This is due to the sustainability of replacing toxic fertilizers with more sustainable methods of electricity. This method of promoting plant growth would most likely be implemented into indoor, vertical farming as well as greenhouses due to the ease of controling soil moisture which is vital for electrical current to pass through.

Future Research

In the future, more extensive research can be conducted on different variables of this research, such as plant species, duration of electrical treatment periods, and amount of voltage exposed to the plants. With an increase in dataset size, a more accurate assessment can be conducted on the effects of low-voltage electricity on radish and pea seeds as well as measuring different datapoints among the plants such as protein yield. Protein yields in plants correlate to nutritional value for humans. By increasing protein yields as well as growth among plants, crops in the future will be able to keep up with population growth through, not only crop yields, but also through nutritional values among crops.

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Antibacterial Effects of Culinary Spices on Serratia marcescens and Bacillus cereus

by Lisa Seid

According to the World Health Organization, there are 600 million cases of food borne diseases and 420,000 deaths each year. This disparity can be especially harmful and prevalent in developing countries with poor sanitation and food handling. Current antibacterial agents used to preserve and keep food viable are limited in their ability to resist bacterial toxicity. However, many cultures do include spices in their preparation and cooking. Spices are a natural bioingredient that could mitigate the side effects and concerns that accompany using synthetic chemical compounds. The objective of this study is to investigate antibacterial potential of four common household spices – cinnamon, clove, oregano, and thyme – to prevent or reduce the effect of food borne pathogens. The quality of inhibition was tested on bacteria samples of *Serratia marcescens* and *Bacillus cereus*, a gram-negative and gram-positive bacterium respectively. Blank discs soaked in essential oil extracts of these spices were placed on each culture of bacteria, and the results were measured by the growth of the zone of inhibition (ZOI). Oregano had the largest radius measured from the ZOI indicating that it has the greatest antibacterial effect, and thyme had the smallest ZOI indicating that it has the least antibacterial effect. Based on these results, both null hypotheses were rejected. The alternates were accepted: spices have an antibacterial effect and a greater effect on gram-positive bacteria.

Introduction

Many cultures include spices in their preparation and cooking as a nutritional addition. The flavorful spices known around the world come from plants that produce phytochemicals (Lu et al., 2017), chemical compounds that are used to protect themselves in natural environments. All the spices used in this experiment contain phytochemicals.

Bacterial strains *B. cereus* and *S. marcescens* were chosen for this experiment because of their role in causing food borne diseases. They may affect food quality and shelf life (Tajkarimi et al., 2010). *B. cereus* was recognized as a major cause of gastrointestinal disease (Macwan et al., 2016), and *S. marcescens* can infect intestinal epithelial cells (Ochieng et al., 2014). *S. marcescens* is a gram-negative bacteria, and *B. cereus* is a gram-positive bacteria. This aspect is very important because it can affect the spice's ability to deteriorate bacterial cell membranes. Gram-negative species have a complex double membrane cellular envelope: the outer membrane, the peptidoglycan cell wall, and the inner membrane. Gram-positive bacteria lack an outer membrane, making the cell more susceptible to penetration and destruction (Macwan et al., 2016).

This research tests which spice yields the least growth of pathogenic bacteria in-vitro conditions. According to the World Health Organization, there are 600 million cases of food bome diseases and 420,000 deaths each year. It is imperative for the future to start looking into naturally acquired materials to prevent foodbome illnesses before they spread. Spices are non-synthetic and prevent the pursual of other antibacterial agents, alongside being eco-friendly and biodegradable (Ahmed et al., 2021). With this knowledge, incorporating certain spices into meals may prevent foodborne illnesses caused by bacteria.

Questions and Hypotheses

Questions

- Do household culinary spices Cinnamomum zeylanicum (cinnamon), Eugenia caryophyllus (clove), Origanum vulgare (oregano), and Thymus vulgaris (thyme) have an antibacterial effect on bacteria?
- Do spices have a greater effect on gram-positive or gram-negative bacteria?

Hypotheses

H₀: The culinary spices used do not have any antibacterial effect on bacteria.

H₁: The culinary spices used have antibacterial effect on bacteria.

H₀: The culinary spices do not perform significantly different between gram-positive and gram-negative bacteria species.

H₁: The culinary spices have a greater effect on gram-positive species than gram-negative species.

Methodology

Preparation of Essential Oils

The spices were received in extracted EO form. Blank antibiotic sensitivity discs were soaked in the EO solution for 24 hours and used as the source of spices on the petri dishes.



Figure 1. Preparation of Essential Oils. Blank discs soaking in each essential

Preparation of Bacterial Samples

Each bacteria culture (B. cereus and S. marcescens) was inoculated on 15 prepared plates of nutrient agar to create a

bacteria colony. Then, they were treated with the EO-soaked discs. Each bacteria has 12 samples to measure (3 petri dishes x 4 discs on each plate). The control in this experiment are blank discs placed on the bacterial culture to ensure that it is not the disc affecting the resistance.

Agar Disc Diffusion Assay

A microbial assay technique was used to test disc diffusion susceptibility. After treating the targeted bacterium with the various culinary spice extracts, a ruler was used to measure the radius of each bacteria's zone of inhibition.

Results





Figure 2. Agar Disc Diffusion Assay. Zones of inhibition are present from each spice on *S. marcescens*.

Figure 3. Agar Disc Diffusion Assay. Zones of inhibition are present from each spice on *B. cereus*.

To summarize the data measured from each dish from each spice, the values from each group were averaged together. From these resulting means, a t-test was performed to determine the significance of the data. The t-test compared the mean ZOI of *B. cereus* to *S. marcescens* for each spice.

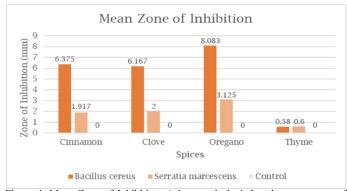


Figure 4. Mean Zone of Inhibition. A bar graph depicting the mean zone of inhibition of each bacterium.

Spice	t-value	p-value	Significance
Cinnamon	10.46	< .00001	Significant at p < .05
Clove	17.289	< .00001	Significant at p < .05
Oregano	19.011	< .00001	Significant at p < .05
Thyme	-0.141	0.445	Not Significant at p < .05

Figure 5. Table displaying significance values, resulting from a t-test, of Mean Zone of Inhibition between *B. cereus* and *S. marcescens*.

The data for cinnamon, clove, and oregano were significant at a 5% significance level compared to the control. However, overall, thyme had very little effect on both bacterium, resulting in a non-significant value.

Discussion

The results of this research suggests that there is a correlation between the culinary spices tested in this experiment and antibacterial resistance. Since the control petri dish without spices present did not display any growth at all, the first null hypothesis was rejected, and the alternate was accepted. The culinary spices used in this study do have antibacterial effects.

Oregano displayed the greatest antibacterial resistance, and thyme displayed the least.

Figure 5 displays the statistical t-test that proved that there is a significant difference the effect on *B. cereus* and *S. marcescens* for cinnamon, clove and oregano. The null hypothesis can be rejected, and the alternate hypothesis can be partially accepted. Out of the two species of bacteria tested in this experiment, *B. cereus*, the gram-positive bacteria was affected the greatest.

Implications and Recommendations

Foodborne illnesses are too common globally and are also an economic burden. Current agents are increasing in ineffectiveness and are limited by their toxicity, creating health concerns. Thus, it is imperative to meet Sustainable Development Goals (SDG) established by the United Nations. This study could improve nutrition with safer food preparation precautions (SDG 2). Eating is a necessity and using spices as an antibacterial agent is a sustainable method to reduce the consequences of foodborne illnesses for all ages (SDG 3).

Future Research

There are many ways this project could be expanded because the use of natural bioingredients in safety is a new and expanding field.

- Expanded Testing: The use of more bacterial species, spices, or combinations of spices could broaden the range of knowledge about antibacterial resistance of different spices on different bacteria.
- Chemical Constituents: Finding a correlation between the chemical constituents in spice plants and their potency would be very beneficial to identify which spices have the greatest antibacterial effect (Gottardi et al., 2016).
- ➤ Biomedical Application: The study of spices may expand into natural antimicrobial bandages (Ahmed et al., 2021), sterilizers, or other biomedical treatments.

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Statistical Analysis Between Vegetation Indices Derived from UAV-Based RGB Images and Crop Yield for the Growth of Maize

by Max Feng

Unmanned aerial vehicles (UAVs) are becoming more prevalent in agriculture as they can autonomously and quickly gather data about plant stresses in agroecosystems. This data can then be analyzed and interpreted to help farmers understand exactly where to implement field operations, maximizing yield and profit while minimizing the environmental footprint. However, current well-researched and widely used remote sensing techniques require expensive accessories, hindering the adoption of UAVs in the farming community. This study explored an inexpensive and entry-level remote sensing method by comparing 18 red-blue-green (RGB) derived vegetation indices (VIs). To achieve this, a DJI Phantom 3 Standard equipped with the stock RGB camera was flown over a maize experiment field in May, June, and July. VIs were then extracted and compared. The results indicate that using RGB-deprived VI to predict maize grain yield is an acceptable entry-level remote sensing option.

Introduction

In recent years, unmanned aerial vehicles (UAVs) have become a new tool in agriculture to monitor crop growth on commercial farms. However, current widely used methods have an extensive learning curve, due to the complicated data manipulation techniques used, and require expensive sensors that hinder the adoption of the new technology onto farms (Skevas et al., 2020). On the contrary, a consumer-grade UAV made for aerial photography and filmmaking is affordable and accessible to ordinary farmers (Huang et al., 2016). These UAVs carry a digital camera that captures red-blue-green (RGB) images over crop fields. From these RGB images, the red (R), blue (B), and green (G) band data can be extracted into quantitative values and fed through the equations to calculate and obtain various RGB vegetation indices (VIs). Due to the availability of RGB cameras, this is a more inexpensive approach compared to current methods. Previous studies have identified and compared VIs that use expensive sensors and have seen a high correlation between the VIs and crop yield. Limited studies have explored the application of RGB VIs and consumergrade UAVs to monitor maize growth. This research is aimed to discover if RGB VIs derived from consumer-grade UAVs can accurately monitor maize growth.

Methodology

This experiment was conducted at the Pontotoc Ridge-Flatwoods Branch Station, Mississippi Agricultural and Forestry Experiment Station. The experiment field comprises of seven fertilizer treatments, including no fertilizer (control); broiler litter (BL); broiler litter + flue gas desulfurization (BL+FGD); one-ton flue gas desulfurization + lignite (2T FGD+L); two-ton flue gas desulfurization + lignite (2T FGD+L); three-ton flue gas

desulfurization + lignite (3T FGD+L); urea ammonium nitrate (FRT), all of which were replicated six times with complete randomized block design. Maize was planted on April 7, 2022. A DJI Phantom 3 Standard took a series of images using Pix4DCapture, and by using Pix4DCloud, it stitched the images together to create an orthomosaic image. Images were taken at an altitude of 20 m with corresponding days after planting and growth stages in 2022: 34 (May 11: V6-V8), 50 (May 27: V9-V11), 73 (June 19: R1-R2), 82 (June 28: R3), 90 (July 6: R4), 104 (July 27: R5-R6). Eighteen well-known RGB VIs were compared in this study (Fig 1), and Quantum Geographic Information System (a user-friendly open-source geographic information system) was employed to extract each VIs from each experiment field from each day. R2 values were then calculated by correlating the VIs of each fertilizer treatment plot to its grain yield from each day. For each fertilizer treatment, the days with the most VIs with an $R^2 > 0.5$ were chosen to then find the VI with the highest R². For the VI with the highest R² on that day, a two-tailed t-test for the regression slope was done.

VI	Equation	VI
GRRI	G/R	GRVI
B/R	B/R	IKAW
B/G	B/G	VARI
G-B	G - B	GLI
R-B	R - B	NGRD:
2G-R-B	$2 \times G - R - B$	FO
INT	(R+G+B)/3	ExG
m	R/(R+G+B)	ExGR
gn	G/(R+G+B)	CIVE
bn	B/(R+G+B)	

011	$2 \times G + R + B$
NGRDI	gn – rn gn + rn
ExG	$2 \times gn - rn - bn$
ExGR	$ExG - 1.4 \times rn$ - gn
CIVE	$0.441 \times rn \times 0.9$ $\times gn + 0.4 \times bn$ $+ 19$

Equation (G – R)/(G + R)

(R-B)/(R+B)

(G-B)/(G+B)

 $2 \times G - R - B$

Figure 1. A list of VIs compared in this study.

Results

With 12 RGB VIs with an $R^2 > 0.5$ on May 27, the VIs favored the control treatment the most out of any other fertilizer treatment. On May 27 (V11), GRVI had the greatest correlation with an $R^2 = 0.8604$ (Fig 2).

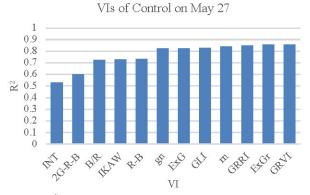


Figure 2. R² values of the control fertilizer treatment on May 27.

Similarly, other fertilizer treatments, except FRT, had a high correlation between the VI and the crop yield (Fig 3).

VI	Treatment	Day	\mathbb{R}^2
2G-R-B	BL+FGD	May 11, 2022	0.6517
G-B	BL	June 19, 2022	0.6483
IKAW	1T FGD + L	June 28, 2022	0.7672
R-B	2T FGD + L	May 27, 2022	0.6035
ExGR	3T FGD + L	July 27, 2022	0.6479.

Figure 3. The highest R² values of other fertilizer treatments.

These R² indicate that the values derived from the VIs correlated well with the grain yield. This implies that the VIs can accurately monitor maize growth.

To investigate if this correlation is due by chance or is statistically significant, a two-tailed t-test for the regression slope on the highest correlated VIs for each treatment was conducted. The results of the tests show that only two VIs had a P-value that satisfies α =0.05. However, if the significance level is raised to α =0.06, many of the tests are significant (Fig 4). On the other hand, this increases the Type 1 error (when the true null hypothesis is incorrectly rejected) and decreases the confidence level to 94%. This could lead to false conclusions about the maize growth.

VI	Treatment	Day	P-value
GRVI	Control	May 27, 2022	0.0077
2G-R-B	BL+FGD	May 11, 2022	0.0521
G-B	BL	June 19, 2022	0.0532
IKAW	1T FGD + L	June 28, 2022	0.0221
R-B	2T FGD + L	May 27, 2022	0.0691
ExGR	3T FGD + L	July 27, 2022	0.0534

Figure 4. The results of a two-tailed t-test for the regression slope on the highest correlated VIs for each treatment with an $R^2 > 0.5$.

Discussion

This study reveals that a consumer-grade UAV can gather RGB VIs with a high correlation to maize growth. However, after conducting a two-tailed t-test for the regression slope, the P-values ranged from 0.0077 to 0.0691. This suggests that while the RGB VIs obtained from the UAV provides valuable information about maize growth, caution should be exercised when interpreting the data. This is because some degree of error may be present.

Implications and Recommendations

These results indicate that using a consumer-grade UAV with RGB VIs is only a good entry point into this new technology for maize growers. Using a consumer-grade UAV alongside RGB VIs for monitoring maize growth does not replace current methods that use expensive sensors and complicated orthomosaic image manipulation. However, using a consumergrade UAV with RGB VIs can allow farmers to learn the basic practices of this new technology at a low cost. This promotes modern agriculture practices, also more commonly known as precision agriculture, to be implemented on more farms. And with precision agriculture, less fertilizer, pesticides, water, and fuel will be used, leading to more sustainable agricultural practices. Fertilizers and pesticides are also scarce in some countries due to poorly developed markets, and because of this, maize production is much lower in these areas. Using a consumer-grade UAV with RGB VIs can severely benefit, not only countries with poor markets, but on a global scale. This research promotes the UN Sustainable Goal 2 of Zero Hunger: promoting sustainable agriculture.

Future Research

If more orthomosaic images of maize treatment plots were examined using these RGB VIs, it would provide a more robust understanding of the application and behavior of these VIs on maize. Variables (like cover crops and soil pixels) can also be manipulated to observe if the RGB VIs performance decreases or increases. These RGB VIs could also be statistically compared to current widely used UAV methods to analyze their differences.

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Rapid Screening of Paddy Field Soil Microbiomes with High Methane Sequestration Activities

by Vivian Peng

The UN Environment Program and the Climate and Clean Air Coalition have recently assessed that reducing methane emissions from agriculture, particularly rice paddies, is crucial in combatting climate change. Rice paddies are a significant source of methane due to anaerobic decomposition. Although research on methanogenic microorganisms in these environments has received extensive attention, there has been little exploration of methanotrophic activities and their potential for mitigating methane emissions because efficient assessment methods are lacking. A technique to quickly evaluate methane sequestration in paddy soil microbiomes was created. Microbiomes with high methane sequestration capabilities were identified and their transcriptome and s were examined. These microbiomes displayed decreased methane emissions when compared to other samples, indicating that the genetic resources of soil microbiomes could be used for farming purposes.

Introduction

Methane is a potent greenhouse gas that significantly contributes to the formation of tropospheric ozone (Koffi, et al., 2020). It can lead to approximately one million premature deaths every year (Malley, et al., 2017). The cultivation of rice has been identified as a significant human-induced source of methane emissions, with both methanogenic and methanotrophic microorganisms found in paddy fields (Jiang, et al., 2019). Despite widespread concerns about methane emissions, there remains limited research on the role of microbiome's methanotrophic activities in these fields due to challenges associated with accurately assessing this phenomenon. Additionally, efficient monitoring techniques are still lacking because rapid diffusion occurs within fields. As such, it remains unknown how much soil microorganisms sequester through their activity. To address this knowledge gap and reduce farming-associated methane emissions, the study focuses on developing an accurate method for efficiently evaluating the methane sequestration activities carried out by paddy soil microbiomes while testing its application under field conditions.

Project Goals

Develop an efficient method for accurate assessment of the methane sequestration activities in rice paddy soils. Screen for the soils harboring microorganisms that possess high methane sequestration activities using the method Test if the soils with high methane sequestration activities emits less methane in farming practices.

Null Hypothesis: There is no significant difference in methane sequestration activities among the microorganisms present in the soils. The microorganisms with high methane sequestration activities can not be identified if an efficient assay is available. Alternate Hypothesis: There is a significant difference in methane sequestration activities among the microorganisms present in the soils, and some of them exhibit high methane sequestration activities. The microorganisms with high methane sequestration activities can be identified if an efficient assay is available, and these microorganisms can reduce farming related methane emissions.

Methodology

Soil and water collection: The soil and water were collected from rice paddy fields in Mississippi, Louisiana, and Arkansas for over 82 locations.

Assembling of Mini-paddy: Mini-paddy was assembled using the soil and water collected from rice paddies as shown in Figure 1. Three repeats were assembled for the sample from each location (Soil and water from various rice paddy fields; Pyrex bottles; balloons)

DNA and RNA extraction: RNA and DNA were extracted following the procedures provided by the kits (RNeasy PowerSoil Total RNA kit and DNA elution). After quality check with Nanodrop, the samples were shipped to a company for transcriptome and 16S rDNA (V4,V5) sequencing analysis.

Methane detection: The presence and concentration of methane was detected using gas chromatography by an analytical company. The analytical instrument was calibrated with a NIST traceable gas standard Samples were diluted with Helium to be in the calibration range.

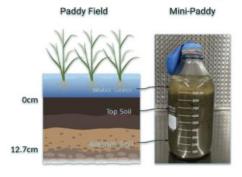


Figure 1. Image of the Mini rice paddy design. About 13 centimeters of soil was collected from rice paddies and was placed in the bottle in the same order as in the rice paddy. The water level is about 4 centimeters on the surface.

Results

Assembled for accurate measurement of methane emission and sequestration in soil microbiomes. Mini-paddies assembled from rice paddy components offer a solution to the challenge posed by gas diffusion, allowing precise study of this process.

Inclusion of methanogens and methanotrophs within the system ensures that these microbes are accurately represented during experimentation (Lee, et al., 2015). Careful control over environmental factors is essential to ensure reliable results when studying methane emission and sequestration in soil microbiomes using mini-paddies. The mini-paddy mimics the conditions of a rice paddy, allowing for microbial growth and interaction. A balloon connected to the bottle captures all emitted gas and its size reflects gas quantity. By connecting a pre-filled methane balloon, changes in size can indicate methane sequestration progress. This system effectively addresses challenges in field studies on methane sequestration.





Figure 2. RPS0 is a sample with high sequestration activities, RP 46 is a sample with medium activities, RP81 is a sample with low activities. Balloons prefilled with methane (-800 cm) at the same size were connected to the minipaddies and tightened with rubber bands. Balloon integrity and result reproducibility were checked to ensure no leakage issues in the experiments. A: Image of the minipaddies connected with the prefilled balloons. B: Image of the minipaddies after incubating with methane filled balloons for three weeks.

The methane sequestration activities of the soils from different locations were compared using the mini-paddy system. The results showed that there were substantial variations in methane sequestration among the samples (Figure 2). Based on the average sequestration capability, the collected samples were grouped into three categories, low, medium, and high (Table 1).

Table 1: Number of Rice Paddy Soil Samples in the Three Methane Sequestration Categories

Methane Sequestration Activities	Low	Intermediate	High
Sample Number	23	50	9

Methane Sequestration Assay: The results were assessed following two weeks of incubation at 37°C after connected with a balloon prefilled with ~800 cm³ methane. Low: less than 150 cm³ methane was sequestrated; Intermediate: about 150 to 500 cm³ methane sequestrated; High: over 500 cm³ sequestrated. The experiments had three repeats for each soil sample and the averages were used. The balloons were checked to ensure no leakage before measurements.

Methane emission was reduced in mini-paddies with high methane sequestration activities when crop residue and manure were applied to the mini-paddies compared with other groups (Figure 3.) Crop residues and manure are frequently applied to paddies as fertilizers, leading to methane emissions due to anaerobic decomposition.

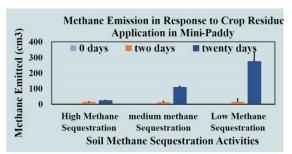


Figure 3. Methane emission is reduced in the soils with high methane sequestration activities compared to other solid in response to crop residue application in the mini-paddy system.

Discussion

Accurately assessing methane emissions is vital for identifying effective climate change mitigation strategies. However, measuring these diffuse emissions and quantifying sources like livestock or natural wetlands poses challenges. Inadequate measurements may underestimate emissions and miss opportunities to reduce them. Improved assessment methods can also reveal novel ways to cut methane emissions by uncovering methanotrophic behaviors. This study demonstrates the potential of naturally reducing methane through such microorganisms, while highlighting respiration chambers as precise tools for continuous long-term measurement of emission levels.

Implications and Recommendations

A novel method has been developed. The method can rapidly assess soil methane sequestration and emission. The results demonstrated that there are substantial variations in the capability of methane sequestration of the soils (with their microbiomes) collected from different locations. Some soils (with the inhabited microbiomes) possess high methane sequestration capability. They have the potential to be used to reduce methane emission and sequestrate emitted methane.

Future Research

The present study corresponds with the thirteenth goal of the United Nations' sustainable development goals, which emphasizes expeditious measures to counteract climate change and its ramifications. In pursuit of this goal, prospective research initiatives encompass uncovering the DNA sequence of methanotrophic microorganisms and comprehending their modus operandi for assimilating methane. Furthermore, exploring techniques for integrating these genomes into other microbial entities at a molecular level and scrutinizing if such genes induce analogous conduct in alternative bacterial strains or plant roots would be crucial avenues for exploration.

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Dietary-Supplementation of Copper to evaluate physiological defects within *Caenorhabditis elegans*

by Julia Nguyen

Caenorhabditis elegans (C. elegans) have been used as model organisms for neurological disorders such as Autism, Alzheimer disease, and Parkinson's disease— despite year of research regarding these disorders, there has been little breakthrough with discovering the validity of metal-induced neurotoxicity and how it alters a C. elegans' neurology and physiology. Copper metal will be dietarily supplemented in the C. elegans' original food source, Escherichia coli (E. coli), to monitor and analyze the behaviors associated with its consumption and exposure. Four petri plates were used in the experiment— two control groups and two experimental groups. All four plates had Nematode Growth Medium (NGM), Escherichia coli (E. coli), and C. elegans, but two of the four had an additional 0.375 grams of copper sprinkled on half of the plates (A mechanism to measure whether or not copper exposure could result in the same effects as if the C. elegans consumed them.) Because the nervous system controls physical movements and behavior, as also seen in humans, the physical impairments will mirror the effects of copper on the C. elegans' nervous system. The physiological effects, fertility, and behavior of the C. elegans were observed under a digital microscope and the qualitative results were recorded. In comparison to the behavior of the C. elegans' control groups, it was found that the copper induced neurodegeneration due to their elaborate physiological defects. Their motor skills were impaired, their fertility became low, and the frequency of their aggregations dropped— all contributing to the idea that the heavy metal copper did induce neurodegeneration over the C. elegans.

Introduction

Copper is an essential trace mineral for the human body due to its diverse presence in crucial, bodily functions—two of which being copper's role in things such as the production of collagen and hemoglobin (Mason et al., 2020). However, the benefits of copper within the human body are yet to be uncovered—making it unknown whether copper induces neurodegeneration within humans and ultimately playing a role in things such as Alzheimer's and Menke's disease. Due to the nature of neurodegenerative diseases, it is difficult to determine its root causes and variables of which stimulate its progression. Approximately 100 million Americans are affected by at least one of more than 1,000 neurological disorders (Gooch et al., To test the hypothesis that copper induces neurodegeneration, this project will utilize the model organism Caenorhabditis elegans (C. elegans) to replicate this controversial trend in humans. Taking advantage of the simplicity of the C. elegans and its similarities between humans, supplementing copper into their diets to examine the physiological defects associated with neurological impairments will be the most viable way to propose a hypothesis.

Hypotheses

<u>Null hypothesis:</u> The dietary-supplementation of copper into the *C. elegans'* diets will not induce neurodegeneration.

<u>Alternate hypothesis:</u> The dietary-supplementation of copper the into *C. elegans*' diets will induce neurodegeneration.

Methodology

Preparing the plates:



A bottle of Nematode Growth Medium was heated in a hot water bath at 35°C until it became stringy when swirled. Once it was warm to the touch, it was aseptically poured under a laminar flow

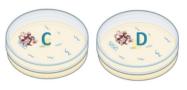
hood onto four petri plates and later transferred to an incubator to cool at 25°C for 24 hours. *Escherichia coli (E. coli)* was aseptically poured onto the plates until they covered the Nematode Growth Medium, and too were cooled at 25°C for 24

hours. Two of four of the prepared plates would servee as Control groups A and B. The other two plates would





serve as the experimental groups: one half of their plates would be sprinkled with pieces of E. coli. These groups would serve as the Experimental Groups C and D. The copper bits were



sprinkled to only half of the plates in order to consider the possibility of metal-induced neurodegeneration through its simple exposure.

Transferring the *C. elegans:*



To ensure that there are no trace contaminants found in the plates, the inoculating loops used for chunking the *C. elegans* was soaked in Ethanol for 5 minutes before use. Under a laminar flow

and using an inoculating loop, a 1x1 cm square was cut into the C. elegans plate (pre-purchased from the Carolina Biological



Supply Company) and transferred *C. elegans*-side downward onto the four petri plates in order to allow the *C. elegans* easier access to their food, *E. coli*. Following a 25°C, 24-hour inoculation period in an incubator, the four plates were analyzed under a digital microscope and the results were recorded.

Results

As expected, the A & B control groups displayed a *C. elegans'* normal behavior. Their social nature makes them prone to aggregate together when feeding, and their plentiful food source, *E. coli*, causes a high egg count among the plate—both well exemplified in <u>Figures I and II</u>, respectively.



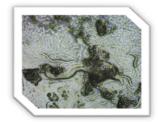


Figure I

Figure II

It was found under the digital microscope that the copper half of the two experimental plates showed no signs of *C. elegans*, as seen in Figure III.

Due to the lack of activity seen on the copper halves, the half without copper was analyzed instead.

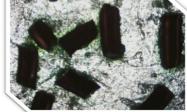


Figure III

C. elegans are known to be very social while feeding (Avery, et al., 2002), but in contrast to Figure I, the C. elegans in Figure IV were visibly less social after the addition of copper into their environment-- suggesting that there was a shift in their motor skills. In Figure V, the C. elegans' eggs are widely dispersed and lower in numbers versus the close vicinity of eggs and its high numbers earlier discussed and seen in Figure II. This observation from both plates solidifies the concept that traces of copper affect the way of which C. elegans reproduce.





Figure IV

Figure V

Discussion

When *C. elegans* were in an environment of heavy copper, it was discovered that copper does play a role in neurodegeneration due to its physiologic and behavioral changes. This result rejects the null hypothesis and instead, accepts the alternate hypothesis of this experiment in which states that the dietary-supplementation of copper the into *C. elegans*' diets will induce neurodegeneration. With this knowledge, it offers a step closer to answering whether the trace mineral copper could stimulate neurodegenerative diseases seen in humans.

Implications and Future Research

The experimentation was conducted with limited experimental groups and materials; the results found at the conclusion of the research would be further solidifed had the experiment utilized multiple trials and generations of *C. elegans*.

Aligning with the United Nations' Sustainable Development Goal 3 of Good Health and Well-being, the project's goal was to address the controversial correlation between the induction of neurodegeneration and the heavy-metal copper. Because this project only analyzes the physicality aspect of the *C. elegans'* behavior and defects, future research is needed to solidify any genealogical impact of copper. The neurology effects of the copper were seen in a qualitative perspective rather than faceting all aspects of perspectives. In addition, utilizing quantitative data would further solidify the accepted, null hypothesis: the dietary-supplementation of copper the into *C. elegans'* diets will induce neurodegeneration.

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Analyzing the causes of the Jackson Water Crisis using the Community Capital Framework

by Sebastian Harvey

In late August of 2022, Jackson, Mississippi suffered one of the worst environmental crises in its history. After historic flooding in Pearl River, the O.B Curtis Water Plant's equipment failures caused significant damages, cutting 150,000 Jackson residents' water. However, this was not Jackson's first water problem. Jackson residents are accustomed to water failures and boil water notices. In the winter of 2021, burst pipes after severe winter storms also left much of the city without water. The systemic nature of these issues highlighted historic racial, financial, and political inequalities within Jackson. This research used a data-driven, Community Capital Framework to examine Jackson and the water crisis. Jackson's unique natural location, skilled labour, governmental support, and financial situation were all analyzed using census and FRED data. Population, GDP, government GDP, migration, income, and bachelor's education census data were used in comparison to surrounding counties and states. Strong positive correlations were found between population and GDP. Only 24% of Mississippi residents have a bachelor's degree or higher, a lower figure than neighbouring states. Over 1,000 Hinds County residents left the county last year alone. Governmental spending was found to not be significantly correlated when compared with overall GDP and individual prosperity. This research lays the groundwork for using the Community Capital Framework to analyze crises in a community, provides insight into the Jackson Water Crisis, and how to sustainably develop a community to combat such issues.

Introduction

In late August 2022, severe flooding in the Pearl River devastated Jackson's infrastructure and left Jackson's population without water for several weeks. Many factors attributed to the crisis, and six main ones were identified through researching articles on the crisis:

- 1. Jackson's proximity to the Pearl River and lack of available groundwater.
- 2. The mass exodus of first the white population, and then the educated black population.
- 3. A decrease in federal and local taxes.
- 4. The loss of educated labor from Jackson's economy.
- 5. Generational racism (connected to all other causes).
- 6. A lack of community representation in state and local government.

The objective of this paper is to understand the causes of the Jackson Water Crisis.

Methodology

The Community Capital Framework (CCF) is a comprehensive way of looking at a community and the relationship between different aspects of the community. The community capital framework has seven capitals: social, political, built, human, cultural, natural, and financial. **Social:** groups, organizations, networks in the community, sense of belonging, bonds between people. **Political:** connections to people in power, access to resources, leverage, and influence to achieve goals. **Built:**

buildings and infrastructure in a community, schools, roads, water and sewer systems, and main streets. Financial: Money, charitable giving, grants, access to funding and wealth. Human: The skills and abilities of the people, leadership, knowledge, and the ability to access resources. Cultural: ethnicity, stories and traditions, spirituality, habits, and heritage. Natural: the environment, lakes, rivers and streams, wildlife, soil, forests, the local landscape (Community Capitals framework, 2015).

Modification: Social Capital was removed. There are several issues with trying to quantify the effects of connections to a disaster. In-depth interviews are preferred when ascertaining organization, and are very difficult to analyze with data. Community Capital faces many of the same problems, but looks at a difference between race and background instead of social networking (Hinton, 2015.). Because of the complicated and interconnected nature of the Jackson Water Crisis, it is impossible to draw a complete conclusion from one individual factor without placing it in context. Using the Community Capital Framework, the initial question can be modified as, "How was Jackson's built capital affected by the other capitals?"

Six additional hypotheses were created to narrow down the individual capitals and focus the "lens." Each hypothesis was chosen based articles and available data. A significance test of α =.05 was used.

Political: Null- Government GDP had no impact on the Jackson Water Crisis. Alternate- Government GDP had an impact on the Jackson Water Crisis.

Financial: Null- Real GDP and the migration of people outside of Hinds County had no impact on the Jackson Water Crisis. Alternate- Real GDP and the migration of people outside of Hinds County had an impact on the Jackson Water Crisis.

Human: Null- The migration of skilled labor had no impact on the Jackson Water Crisis. Alternate- The migration of skilled labor had an impact on the Jackson Water Crisis.

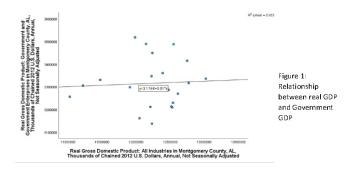
Cultural: Null- Jackson's unequal distribution of races had no impact on the Jackson Water Crisis. Alternate- Jackson's unequal distribution of races had an impact on the Jackson Water Crisis.

Natural: Jackson's dependance on the Pearl River had no impact on the Jackson Water Crisis. Jackson's dependance on the Pearl River had an impact on the Jackson Water Crisis.

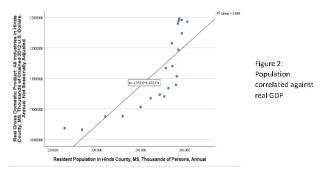
A two-tailed significance test was used with Pearson Correlation. Conclusions can be drawn through comparing Hinds County externally (with counties outside of itself) and internally (through relationships within the county).

Results

Political Capital: There is no statistical difference as p=0.055 and r=0.003, so fail to reject null. Jackson's overall GDP doesn't reflect the government GDP. This demonstrates the disconnect between Jackson's government and economic state.



Financial Capital: p=.001, significance .805. There is a strong positive relationship between population and real GDP. There is a bidirectional relationship with real GDP and the population of Hinds County.

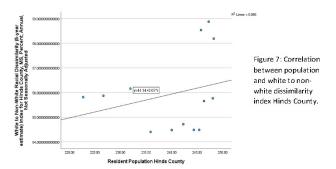


Human Capital: Hinds County and Mississippi have a lower proportion of residents with a Bachelor's degree and higher when compared to surrounding counties and states.

Jackson	Hinds County	Rankin	Madison
County		County	County
Bachelor's	30.9%	31.5%	50.2%
degree and			
higher			

State	Mississippi	Alabama	California
Bachelor's	24.8%	27.4%	36.2%
degree and			
higher			

Community Capital: Sig: .397. Fail to reject Community Capital null Hypothesis, as p is > .05 and r=.006. Hinds County's resident population doesn't reflect the white to non-white dissimilarity index. As residents left, the spatial distribution of people within the county didn't change.



Conclusions:

Out of the five null and alternate hypotheses, three alternate hypotheses can be accepted. We can accept the alternate hypothesis for the Financial, Cultural, and Natural capitals. Hinds County had the highest government spend per-person out of Rankin and Madison Counties, so overall government investment had no relation to the crisis. The population correlated to a drop in GDP which impacted the Jackson water crisis.

Future Implications:

The Community Capital Framework has never been used for a crisis before, and this project sets a trend for analyzing crises in the context of a community. It addresses Goal 5 of NATO's sustainability goal, which is to provide clean water and sanitation. This can help identify and combat water crises.

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NOTES

SCIENCE JOURNAL ENTRIES

Name/County	Competition and Awards		
Jeremy Dawe/Okitbbeha	Region VI 1st Place Microbiology; 4th Place Microbiology (State)		
Madison Echols/Lamar	Region VI 1st Place Biomedical and Health Science; Best of Fair ISEF Alternate; 1st Place Biomedical and Health Sciences (State); Best of State ISEF Recipient; 1st Place Junior Science and Humanities Symposium		
Max Feng/Okitbbeha	Region V 1st Place Mathematics and Systems Software; US Metric Association Award; US Air Force Award; Yale STEM Award; STEM Achievement Award Excelling in Math Award; Best of Fair ISEF Alternate; 1st Place Mathematics and Systems Software (State)		
Arika Gardner/Sunflower	Region III Best of Fair Alternate; 1st Place Animal Sciences, National Geographic Award (State); Best of State ISEF Recipient		
Sebastian Harvey/Okitbbeha	Region V Stockholm Junior Water Prize; Global Environmental Impact Award		
Dia Kher/Harrison	Region VI 2nd Place Behavioral and Social Sciences; Best of Fair ISEF Recipient; 4th Place Behavioral and Social Sciences (State); 2nd Place Junior Science and Humanities Symposium		
Danielle McConnell/DeSoto	Region VII 1st Place Microbiology; Best of Fair ISEF Recipient; 1st Place Microbiology, Society for InVitro Biology Award (State)		
Rushyendranath Reddy Nalamalapu/ Bolivar	Region III Best of Fair Recipient; Yale STEM Award; US Metric Association Award; 1st Place Engineering Mechanics (State)		
Julia Nguyen/Harrison	Region VI 3rd Place Biomedical and Health Sciences; 5th Place Biomedical and Health Science (State)		
Vivian Peng/Okitbbeha	Region V 1st Place Earth and Environmental Sciences; 3rd Place Earth and Environmental Science, Association for Geoscientists Award (State)		
Nicholas Popescu/Okitbbeha	Region V 1st Place Biomedical and Health Sciences; Regeneron Biomedical Science Award; Office of Naval Research Award; US Air Force Award; Best of Fair Recipient ISEF; 2nd Place Biomedical and Health Sciences (State)		
Lisa Seid/Washington	Region III Best of Fair ISEF Recipient; Society for InVitro Biology Award; 2nd Place Microbiology, Yale STEM Award (State)		
Ashton Taylor/Lowndes	Region V 1st Place Plant Sciences; National Geographic Award; Office of Naval Research Award; US Air Force Award; Best of Fair Recipient ISEF; 1st Place Plant Sciences (State)		



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