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Pond Crash Prevention by Terpene Production for *Nannochloropsis oceanica*

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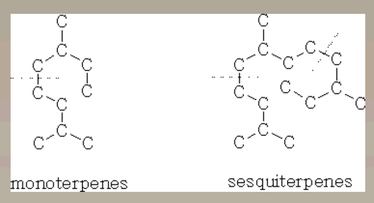
Background & Introduction to the Project

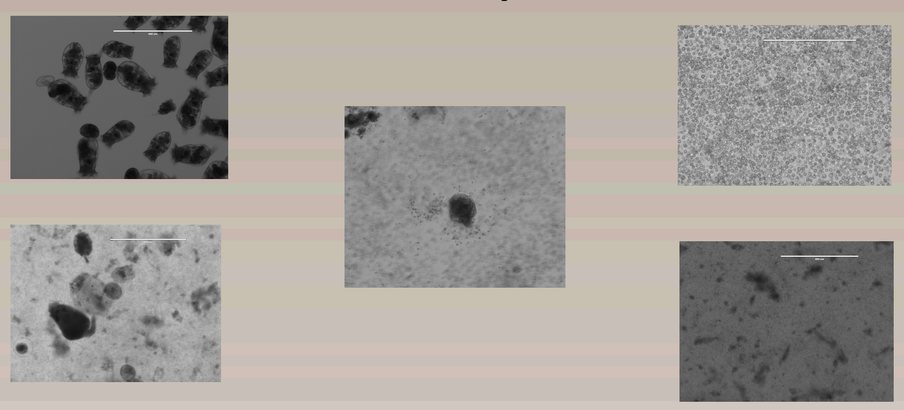
The cultivation of microalgae is gaining interest due to its potential to provide a variety of more environmentally sustainable products ranging from fuel and plastics to pharmaceuticals and nutritional supplements. The main obstacle these new technologies are facing is the efficient cultivation on a large industrial scale. Pond crashes due to predation, competition and disease are a common failure in the industry, especially in open pond systems, which would be the least energy intensive method for growing microalgae cultures.

This project focused on developing a method for protecting microalgae crops from grazing predators, specifically rotifers, in hopes of preventing pond crashes without the use if expensive and harmful synthetic pesticides. The species of microalgae used in the was *Nannochloropsis oceanica*, strain CCMP1779, genetically modified with a gene that allows for the production of terpenes. Terpenes are volatile, aromatic hydrocarbons usually produced by plants to deter herbivores.



The concept of natural terpenes as crop protection has already been proven by multiple studies, including a successful gene discovery in terpene producing plants and their improved response to environmental stressors. However, little research has been done regarding this next generation biocide in algae cultivation.

Predator Populations



Conclusions & Future Research

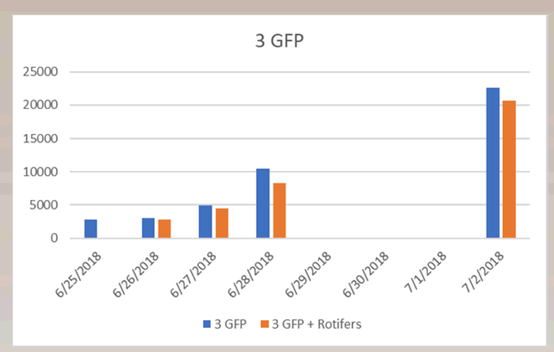
The current data provides a general idea of the effect that terpene production by algae has on its resistance to grazing predators, but is inconclusive in regards to the amount of stress in which it can still survive. When subjected to only 10 rotifers per mL, the terpene producing algae still grew even with the presence of grazers, though it grew slower compared to the cultures that did not have predators added. From the micrograph figures above, a visual observation concludes that the terpenes successfully killed the rotifers. However, the control strain that was proven by GCMS to not be producing terpenes also survive in the presence of a low concentration of rotifers. When the samples were subjected to a much higher predator concentration, the terpene production was not enough to kill the rotifers before the biomass was consumed to below a critical density. Future research would address a quantity of predation that the amount of terpene production could defend against, especially on a larger scale like a raceway testbed system.

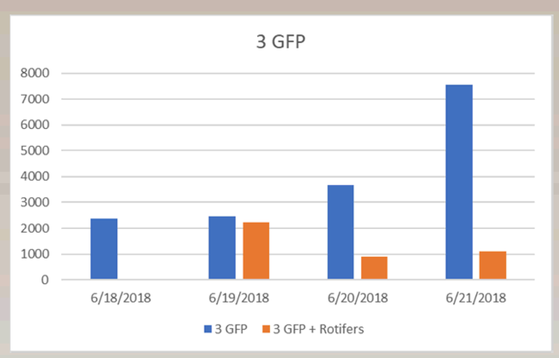
Terpene Production Results

Left: Multiple cultures of the Nannocloropsis species were tested for volatile compounds via the manual SPME injection method for gas chromatography. Culture 24N, as pictured, produced the highest concentration of terpenes. Though it is possible that all genetically modified cultures are producing the compounds to some extent, it may not be in quantities large enough to be detected by the GCMS. The terpene produced by this particular strain is Aristolochene, whose chemical structure is provided by the program.

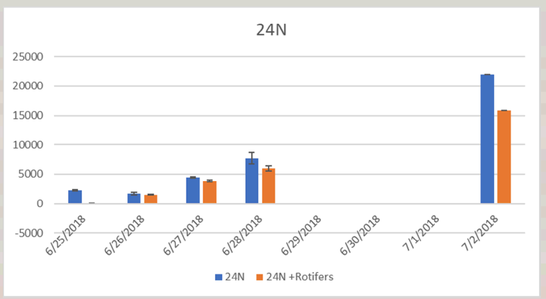
Bottom Left: The genetically modified algae was compared to the control samples which did not produce any significant volatile compounds that would have a biological impact. For these algae samples, a GFP was used as the control instead of inserting the terpene producing gene.

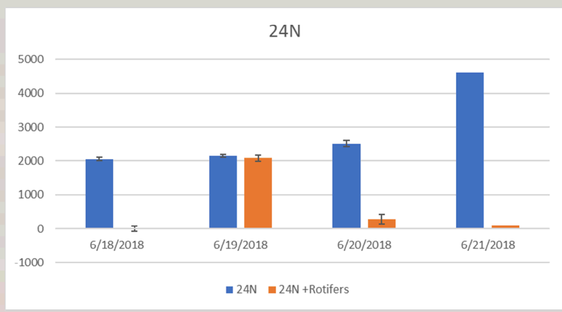
Fluorescence Results





The fluorescence reading of algae cultures were taken using TECAN to monitor the growth and decline of algae biomass density as a result of predation by rotifers. The figures presented on the left represent a GFP control and a terpene producing culture subjected to a predator density of 10 rotifers per mL while the cultures presented on the right were subjected to 50 rotifers per mL.





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