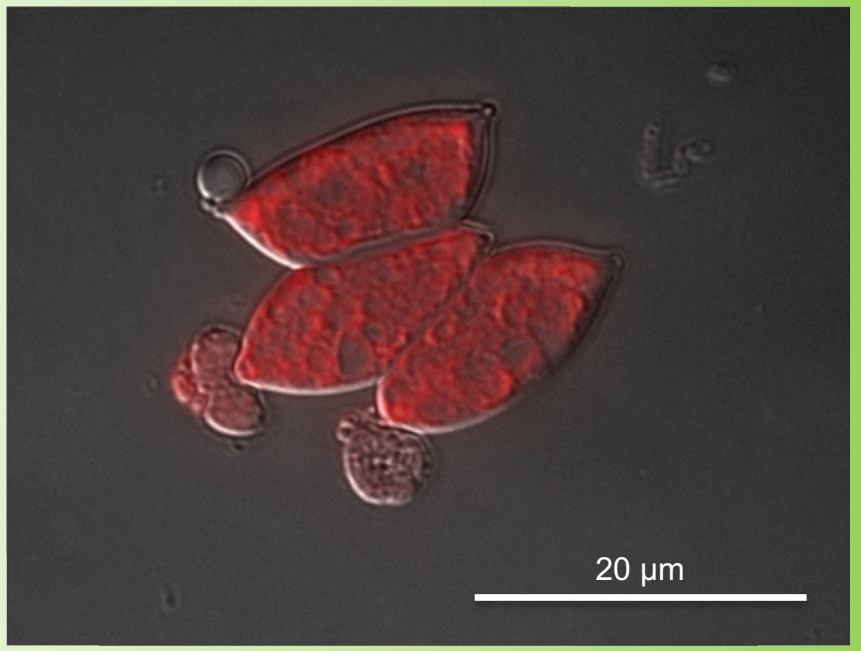
# Chytrid Control Advancing Algal Targets (ChytCAAT)




March 23, 2021

Advanced Algal Systems
PI: **Ty Samo,** LLNL



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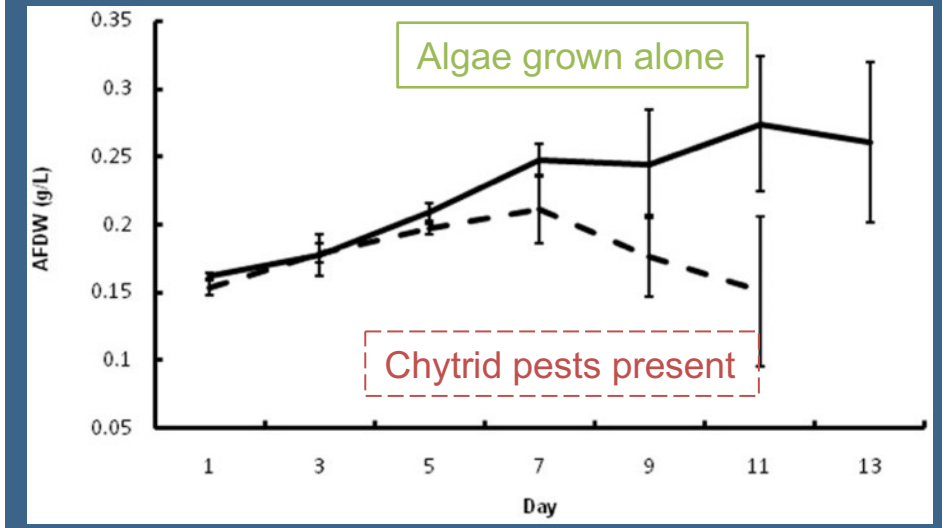
# Chytrids significantly impact algal crops

- Chytrid infections can result in **rapid and complete loss** of algal cultures, reducing annual crop returns
- **Current strategies** include **harmful chemical applications and/or premature biomass harvest**

- Our overarching **goal is to develop treatments of chytrid infections informed by chytrid biology** that are **TARGETED** to eliminate unintended outcomes, **RATIONALLY DESIGNED** to avoid excessive costs and maintain system balance, and **SUSTAINABLE** to ensure long-term pest mitigation

Sapphire Energy Test Facility



Letcher et al. 2013

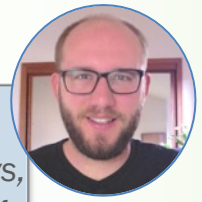
# Project Overview

| Need | Solution |
|---|---|
| **Tools and resources to study chytrid biology** | Address the scarcity of research methods and resources related to studying chytrid biology and ecology through design and implementation approaches to rapidly characterize and quantify chytrid growth and infection within a subset of chytrid strains |
| **Understand chytrid interactions with *Scenedesmus* spp. production strains** | Measure effects of chytrid infectivity on Scenedesmus using tools developed in Aim 1 to generate baseline understanding of impact on algal productivity in laboratory conditions |
| **Identify controls of chytrid activity** | Perform multiple lab-scale experiments to examine the role of physicochemical variables on chytrid metabolism, growth, and lifestyle |
| **Apply chytrid treatments at laboratory-scale under conditions that mimic outdoors** | Assess efficacy of substrate additions and environmental controls on chytrid infectivities at lab-scale featuring simulated, accurate outdoor perturbations |

# A multi-institutional effort featuring unique expertise

**Ty Samo (PI)** — Lawrence Livermore National Laboratory

*Metabolic assays, cultivation, tracking and reporting, data synthesis, treatment design*



**Chris Ward (Co-I)** — BGSU Bowling Green State University

*Flow cytometry and infection assays, cultivation, treatment design*

**Tim James** — University of Michigan

*Chytrid isolations & identification*

**Rhona Stuart** — Lawrence Livermore National Laboratory

*Technical & logistical advisement*

- Work is conducted independently and simultaneously at partner institutions and coordinated at LLNL via daily-weekly emails and weekly phone calls
- Samo and Ward regularly discuss results, design experiments, and provide advice to and accept input from all project members
- Monthly meetings are conducted with BETO TM and PM to discuss results and progress

| Risks | Mitigation |
|---|---|
| Delay in obtaining new chytrids from crashed ponds due to COVID-19 | 1. Study existing chytrid isolates known to impact *Scenedesmus* spp. ponds: *Paraphysoderma sedebokerense* FD61 and *Amoeboaphelidium occidentale* KS120<br>2. Rely on long-term viability of chytrids for future isolation efforts |
| High cost of treatments | 1. Wholesale purchasing of reagents<br>2. Utilize “waste” substrates from algal spent media, brewery wastewater, and invasive seaweed |

# Technical Approach

## Background and history of the project

- Builds upon a component of Dr. Stuart's Targeted Algal Biofuels and Bioproducts (TABB) work that characterized chytrid infections of *Haematococcus pluvialis*
- *Key observation:* reduced infection in early algal growth stage and in the presence of spent media from *H. pluvialis*
- *Proposed mechanism:* Elicitation of saprotrophic/osmotrophic lifestyle of chytrids through provisioning of algal exudates

**Overarching goal**

**Develop tools to evaluate manipulation of chytrid ecophysiology that mitigates their negative effects on algal growth**

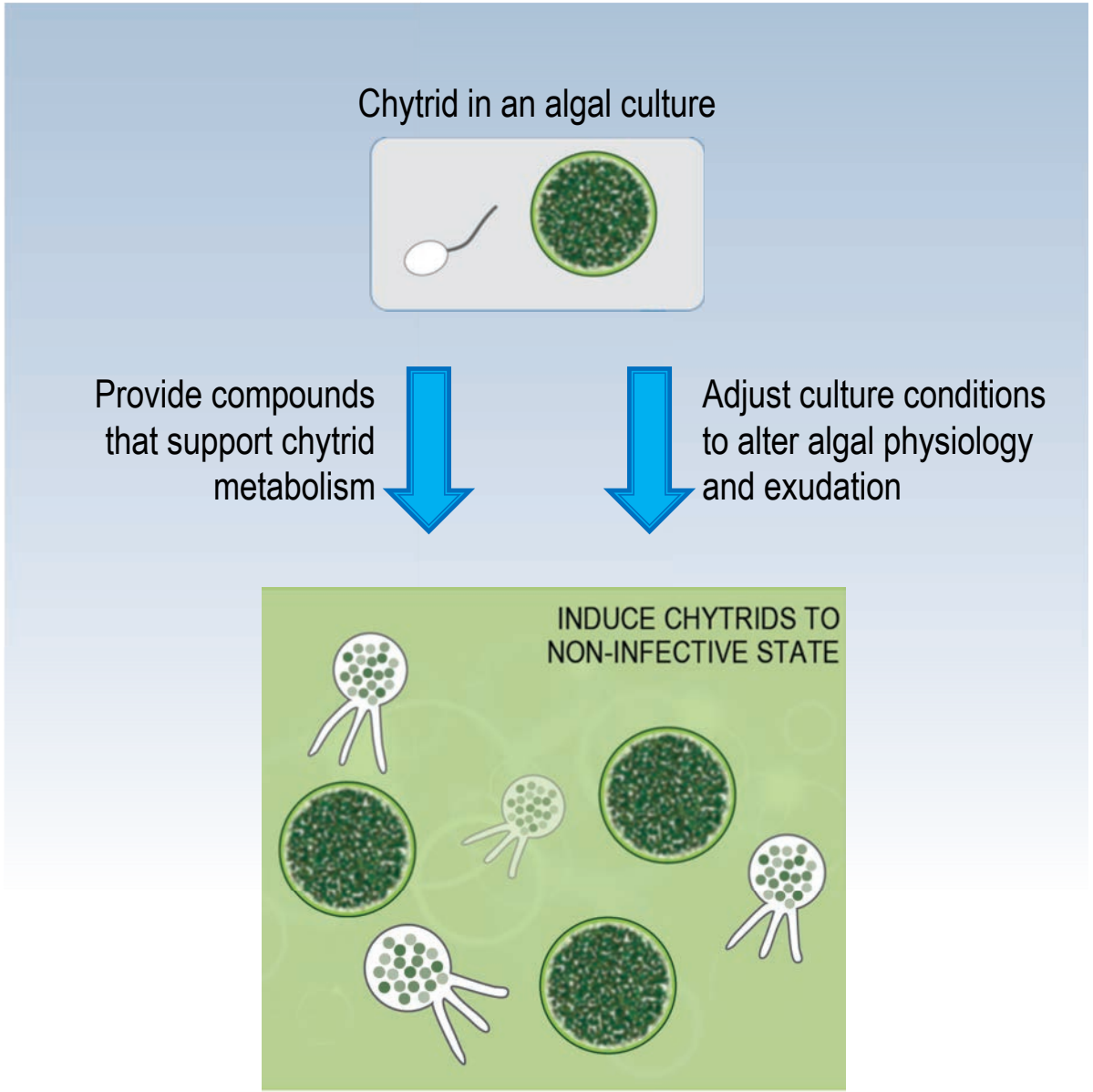
# Technical Approach

- Develop tools and leverage expertise to:

1. Sensitively quantify preferences for individual substrates that support chytrid growth using high throughput well-plate assays
2. Quickly measure infection prevalence across time and in response to manipulations using flow cytometry
3. Expand the pool of chytrid strains available for experimental studies by isolating new chytrids from infected pond samples

- Evaluate approaches that convert chytrids from parasite to commensal by:

1. Adding substrates that "sate" chytrid appetites or enhance algal defense
2. Manipulating culture conditions or culturing schema to maintain adequate organic levels
3. Assessing efficacy of treatments by measuring onset and intensity of infections

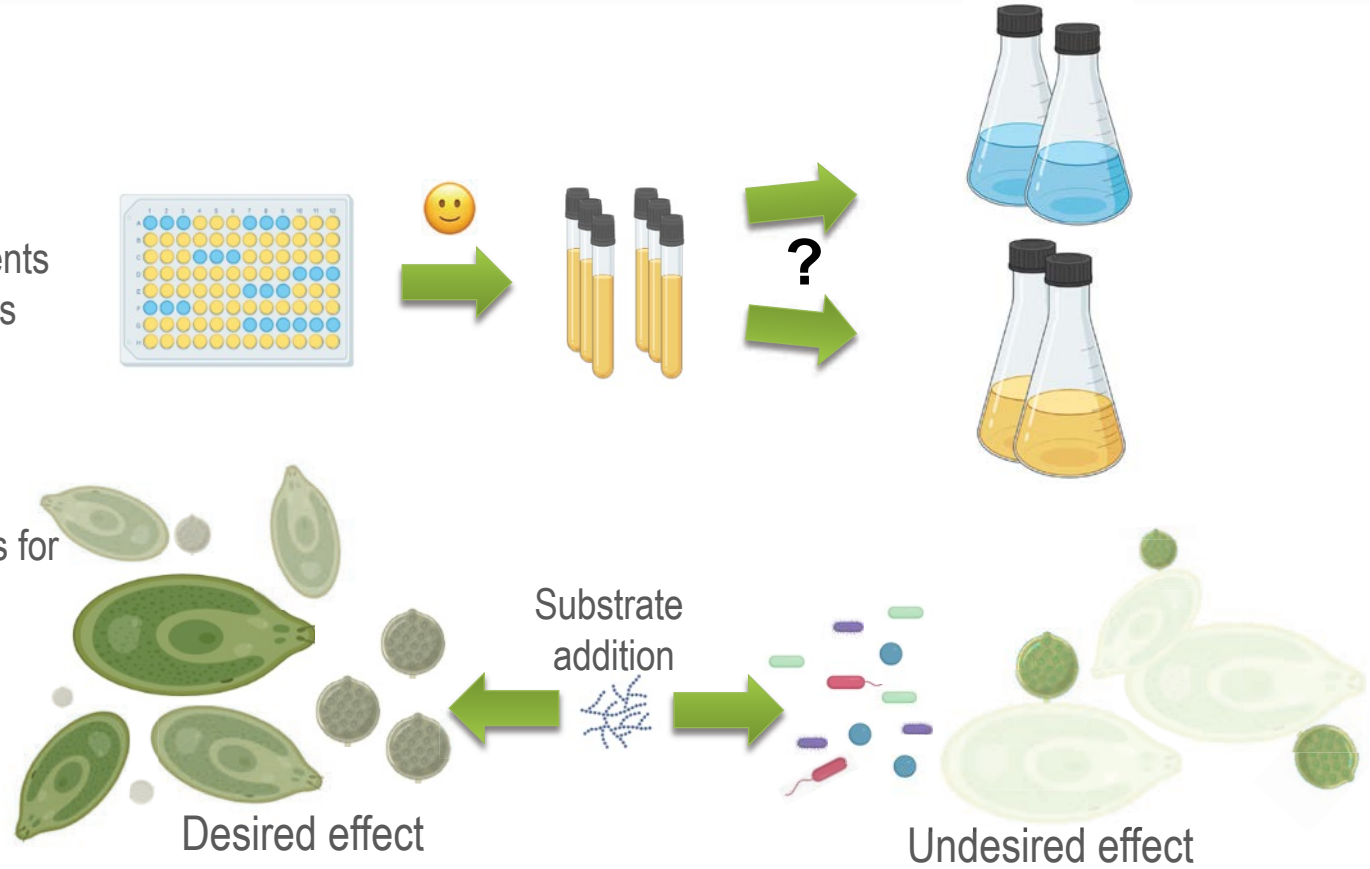


# Technical Approach

**Potential challenges– the impact of scale**

1. Achieving meaningful results during scale-up of conditions and treatments
   - Manipulations of algal cultures in the lab generate different responses when observed at microliter vs. milliliter vs. liter scales
   - Changes in algal physiology due to scale-up may have unintended consequences for treatment options

2. Treatments affect or benefit microbiomes, with unknown consequences for *Scenedesmus* spp. and chytrids
   - Heterotrophic bacteria can effectively outcompete chytrids for some substrates
   - Addition of compounds may instead stimulate bacteria with indirect impacts on algae, *e.g.* metabolite production, increasing pathogen abundance, etc.




**One Go/No-go at project mid-point**

Identification of of at least 4 treatment options to boost algal defenses against chytrid infection and/or revert chytrid to non-infective state via provisioning of carbon substrate

This decision will illustrate efficacy of approaches developed in the first half of the project

# Technical Approach

**Technical metrics – guided by statistics**

Project seeks significant statistical differences compared to realistic experimental controls and traditional measurement methods of chytrid infection

Controls: media blanks, *Scenedesmus* sp. cultures without chytrids

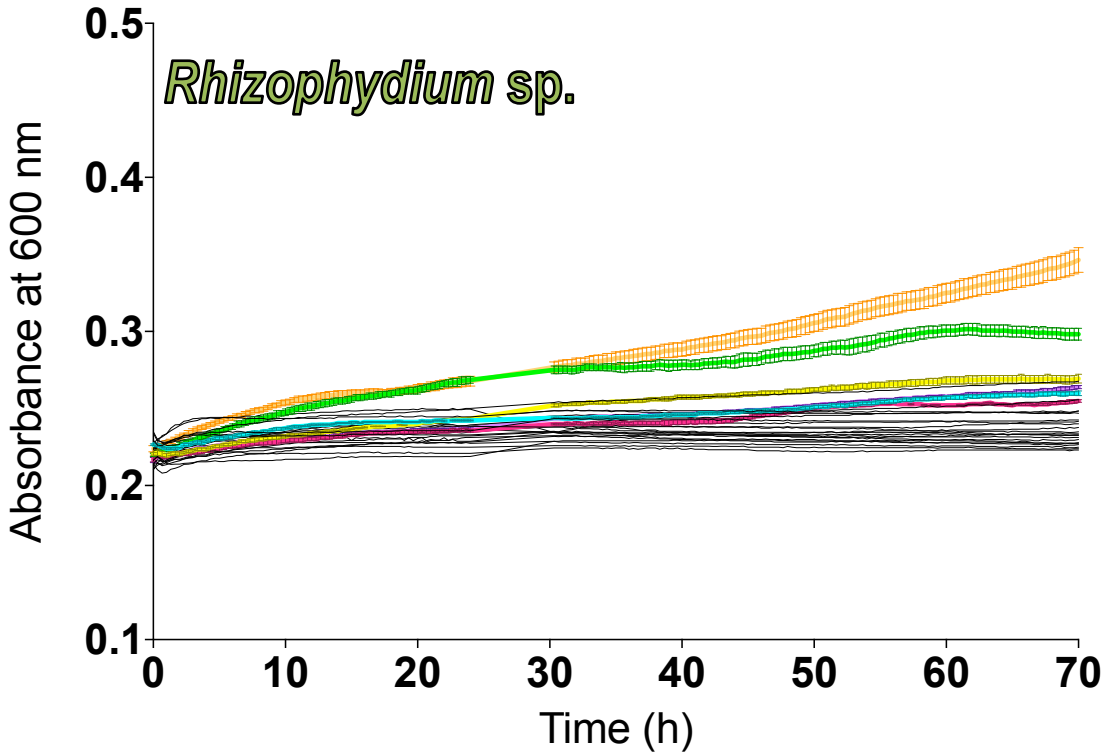
Traditional methods: growth assays, microscopy

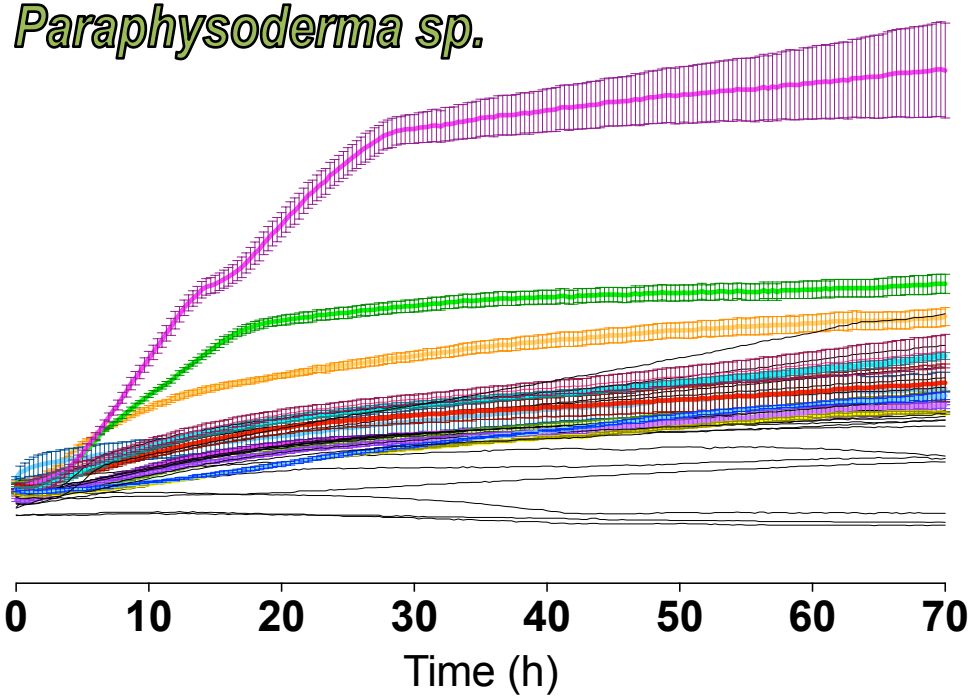
# Impact

- Project goal: Advance our understanding of chytrid biology and ecology for improved pest management
  1. Establish effective approaches that reduce impact of chytrid infections on algal growth and biomass production through addition of organic substances for chytrid uptake, modifying culture conditions to change algal physiology, or both
  2. Identify new infective chytrids to enable eventual treatment development
- Results dissemination:
  1. Currently drafting manuscript on chytrid toolkit development
  2. Pending results, submit record of invention paperwork for patent application

**This undertaking will benefit stakeholders in chytrid, algal, and microbiome research space, as well as pond operators and laboratories in the commercial sectors**

# Progress and Outcomes – substrates that support chytrid growth are candidates to induce non-infective state (Milestone 1)



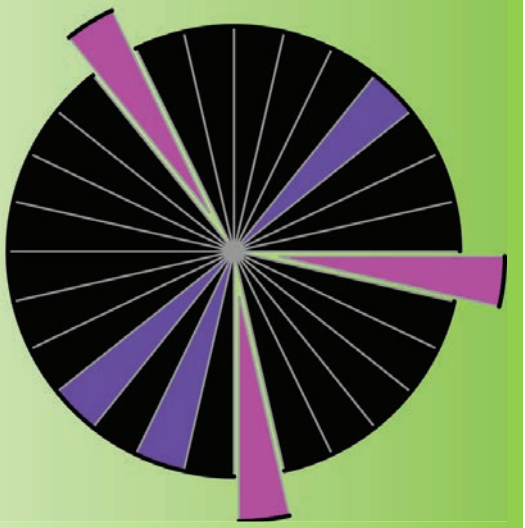




**Significant growth determined by comparing maximum growth rates to the media control using the extra-sum-of-squares F test, $p < 0.05$**

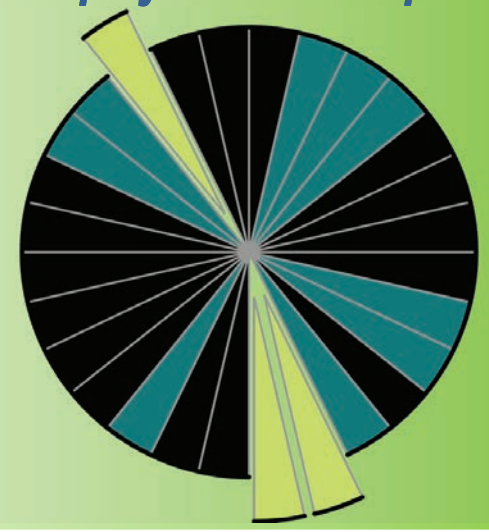
# Progress and Outcomes – chytrid preferences for 'substrate pie' overlap but are also distinct

- A total of 28 ecologically relevant substrates were tested
- ***Rhizophydium*** exhibited significant growth on **six** substrates, and high growth on **three**
- ***Paraphysoderma*** exhibited significant growth on **twelve** substrates, and high growth on **three**
- Two substrates enabled high growth of both strains → good candidates for general treatment
- Conversely, these substances may be 'too much of a good thing', causing chytrid biomass to overtake algal biomass even at lower concentrations

***Rhizophydium* sp.**

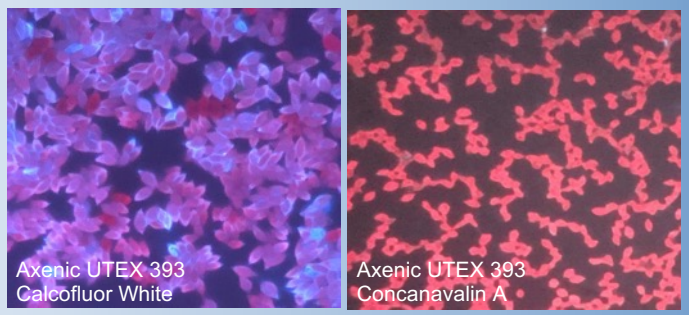


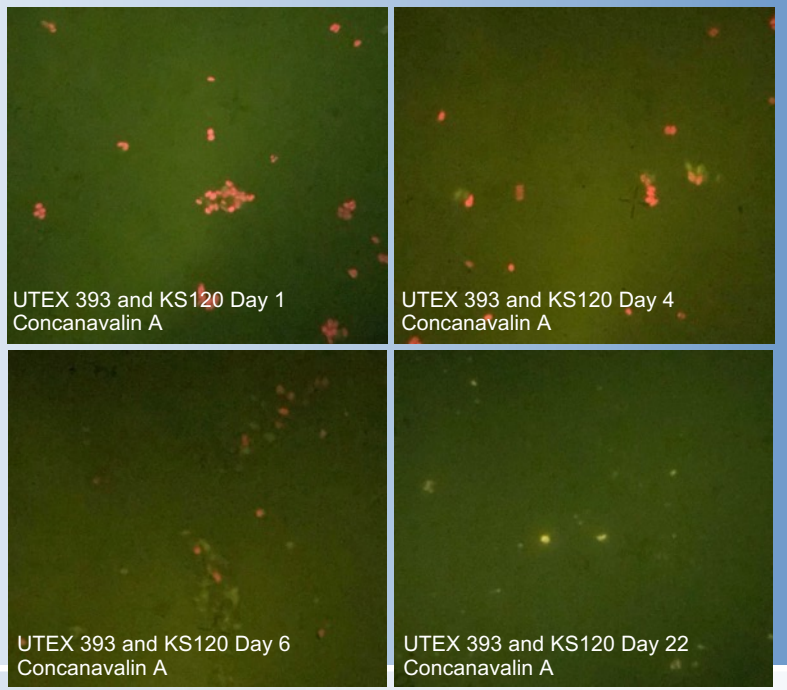
***Paraphysoderma* sp.**



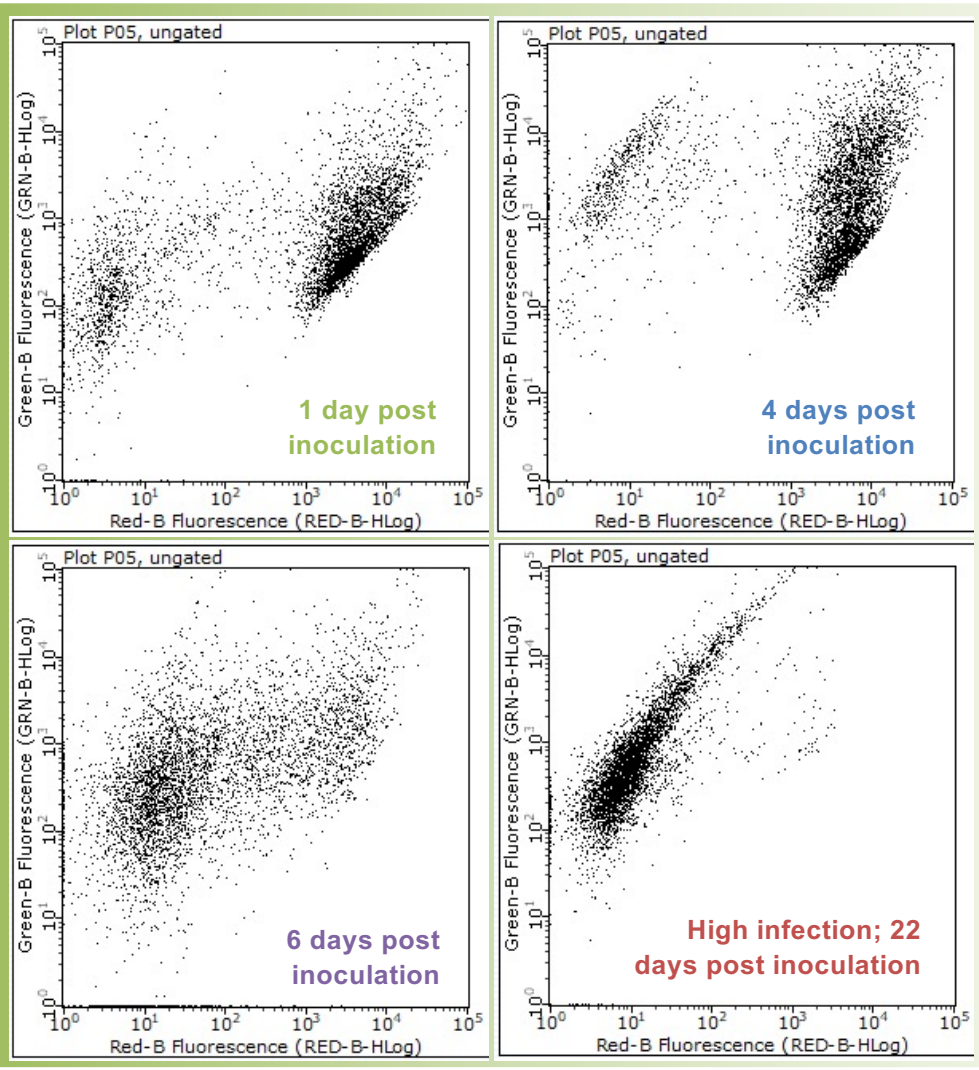
# Progress and Outcomes – illuminating chytrids with probes for improved chytrid infection quantification

- Calcofluor White, commonly used for environmental chytrids, **nonspecifically stains** *Scenedesmus* and other algae
- A variety of probes were identified to investigate **selective staining of chytrids**
- Concanavalin A-AlexaFluor 488 provides bright signal from *Scenedesmus*-infecting *Amoeboaphelidium occidentale* cells, with little to no staining of axenic *S. dimorphus*
- Infection prevalence was calculated as the percent of *S. dimorphus* cells with colocalized ConA signal

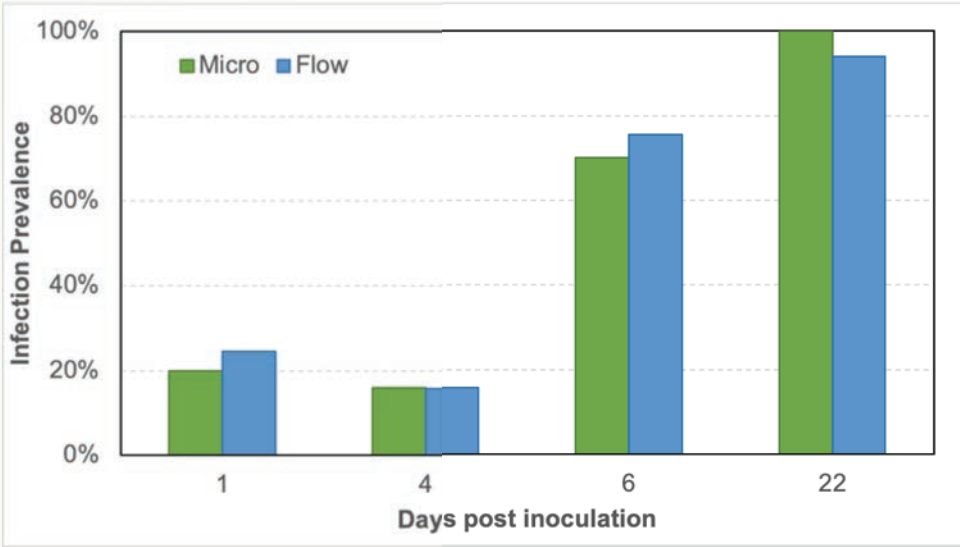


# Progress and Outcomes – a flow cytometric assay to quickly and sensitively quantify chytrid infection (Milestone 2)




- Application of ConA followed by flow cytometric detection of algal chlorophyll autofluorescence and ConA fluorescence
- Cytograms showed decreasing algal biomass and increasing chytrid signal over the time course
- Data were comparable to microscopy-based assessment



# Summary

- Laboratory and field work confirms that **chytrids are highly detrimental to algal productivity**
- **Characterizing the metabolism and ecology of chytrid pests** will likely offers insights into effective ways to combat their detrimental impacts
- Simple alterations to culture conditions may **reduce chytrid infectivity or boost algal defenses**
- **Rapid quantification of chytrid infection** onset and progression will facilitate timely applications of pest mitigation strategies to save algal crops
- This work establishes a **biologically and ecologically inspired framework to develop novel treatments for algal pests** by considering how chytrid lifestyles and responses to algal physiology direct their behavior

# Quad Chart Overview

## Timeline

- Start date: 1/1/2020
- End date: 12/31/2021

| | FY20 | Active Project |
|---|---|---|
| **DOE Funding** | (10/01/2019 – 9/30/2021) | $400k |

## Project Partners

- Bowling Green State University
- University of Michigan

## Barriers addressed

Aft-B – Sustainable Algae Production

Aft-C – Biomass Genetics and Development

## Project Goal

Develop tools to study chytrid metabolism and ecology and implement them to design improved pest management strategies that use targeted, rational, and sustainable principles to reduce costs associated with algal crop loss

## End of Project Milestone

Assess efficacy of novel chytrid treatments/cultivation strategies at lab scale, determining at least one treatment that changes infectivity from the standard condition

## Funding Mechanism

Seed AOP, 2019

# Credits

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

BIOENERGY TECHNOLOGIES OFFICE

## LLNL

- Kristina Rolison, research technician

## BGSU

- Fiona Harrigian, graduate student

## UM

- Kensuke Seto, postdoctoral scholar

## BETO project management support

- Dan Fishman
- Phil Lee

# Milestones

1. Demonstration of metabolic phenotyping assay by determining chytrid growth on various carbon substrates - **completed 9/30/2020**
2. Demonstration of flow cytometry protocol to quantify chytrid infection as compared to microscopy protocol - **completed 12/31/2020**
3. Determination of chytrid infectivities under various culture conditions (i.e., temperature, light, nutrients) to examine condition-dependent infectivity of model
4. Determination of carbon substrate utilization by saprotrophic chytrid to identify at least one carbon substrate that can be used as an energy source
5. Assess efficacy of novel treatments/cultivation strategies at lab scale, determining at least one treatment that changes infectivity from the standard condition
6. Establishment of 3 novel chytrid isolates from Scenedesmus ponds