U.S. Department of Energy (DOE)
Bioenergy Technologies Office (BETO)
2017 Project Peer Review

Project Name: Integrated Pest Management for Early Detection Algal Crop Production
W.B.S. Number: 1.3.2.310 - Ongoing

March 9, 2017
Advanced Algal Systems

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Overarching Goal

- Develop simple, automated, affordable, and robust technologies for the **early detection and identification** of pathogens, predators, and non-productive competitors in an algal production pond.

Desired Outcomes

- Identify and characterize **molecular signatures** of infestation or infection of a production pond.
- Develop an **automated, mass spectrometry (MS)**-based early detection system that monitors the headspace and culture of production ponds for these molecular signatures to trigger an automated identification system utilizing qPCR augmented with high resolution melt analysis **(qPCR-HRMA)** in order to inform a grower of pond health problems and potential contaminant-specific interventions.
- Demonstrate **improved detection sensitivity and timing** compared to existing technologies across a range of predator and crop organisms.

Relevance

- *In response to the DOE BETO TABB area 2 :* development projects focused on developing crop protection and CO_2 utilization technologies to increase yields
- This technology will inform growers of pond health problems **before** they become a problem, enabling contaminant-specific interventions at an earlier stage than currently available.
- Early interventions will result in reduced biomass losses, smaller interventions to remove contaminants, faster recovery times following interventions, and reduced sterilization and re-inoculation costs.
- Overall, this will increase the stability of algal biomass production and reduce costs and losses due to pond crashes or reduced yield due to contamination, enabling commercial viability of algal biofuels.

Quad Chart Overview

Timeline

- Project start date 10/01/2015
- Project end date 03/31/2019
- Percent complete 32% as of 12/31/16

Budget

	Total Costs FY 12 –FY 14	FY 15 Costs	FY 16 Costs	Total Planned Funding (FY 17-Project End Date)
DOE Funded	-0-	-0-	$90K	$730K
Project Cost Share (Comp.)*	-0-	-0-	$29.7K	$180K

*If there are multiple cost-share partners, separate rows should be used.

Barriers

- Multiyear BETO barrier - increased biomass productivity leading to higher yields by developing crop protection
- Biofilm sampling in MiniMART and ponds
- Molecular identification of novel unknown signatures
- Will the biology be consistent?

Partners

- 90%Thermo Scientific
- 7%Qiagen
- 3%Sandia Laboratory

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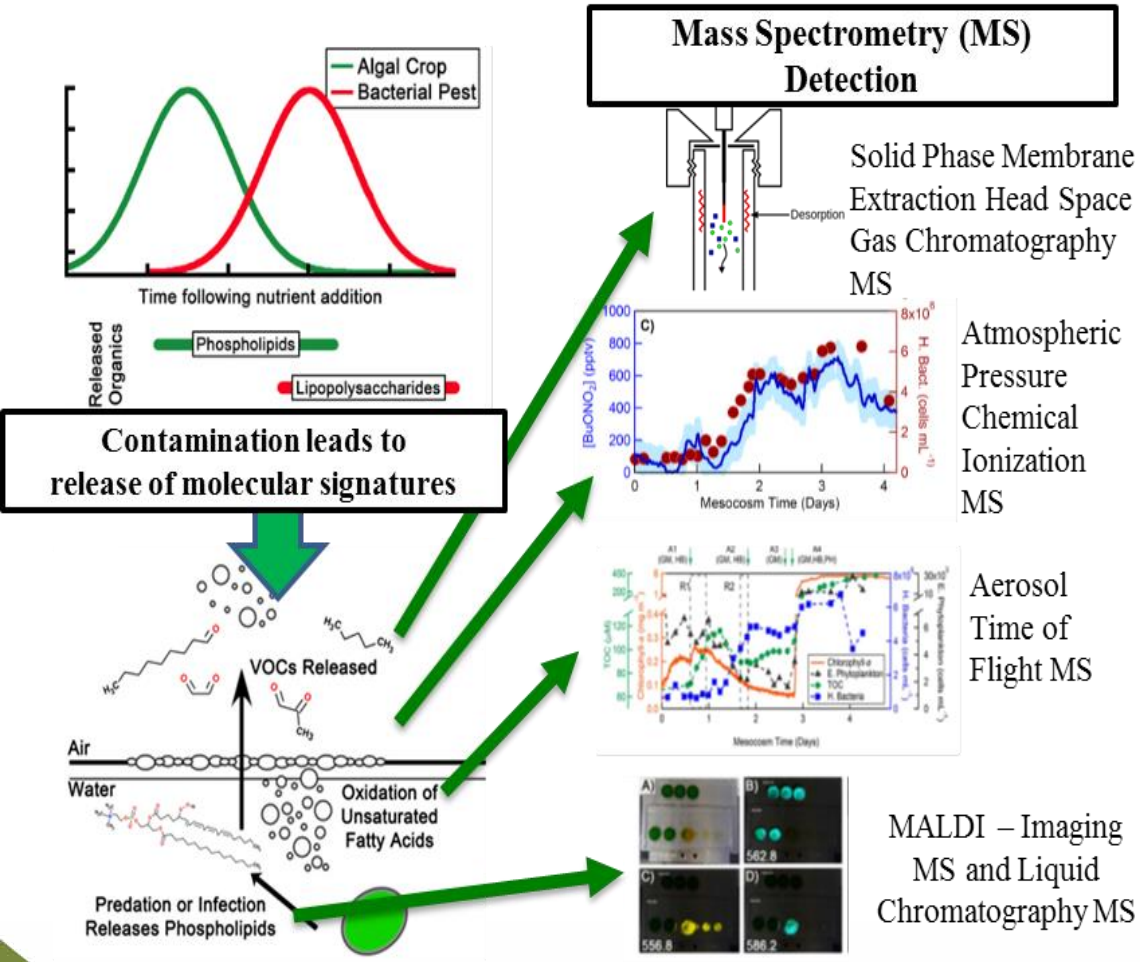
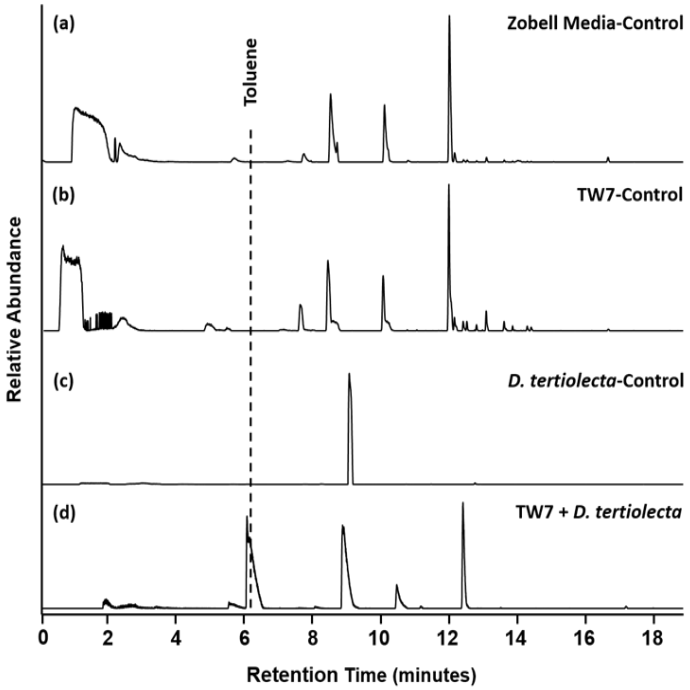
1 - Project Overview





Toluene is detected in cultures within 24 hrs only when algicidal bacteria (*Alteromonas* sp. 114 TW7) is added to alga (*Dunaliella tertiolecta*) culture.

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Project Overview
Our Research Questions

- **Q1** - Can MS be used to detect VOCs, particulate matter (PM), or aquatic molecules that signal the early onset of algal degradation?
- **Q2** - If so, can a smaller, simplified, less expensive MS system be developed to make these measurements in the future?
- **Q3** - Can quantitative PCR (qPCR) with High Resolution Melt Analysis (HRMA) be used to identify pathogens or contaminants individually or as classes?
- **Q4** - Can qPCR with HRMA be developed into a simple, inexpensive, automated, single tube technique that is robust and field deployable?
- **Q5** - Will early detection and identification of pathogens and predators in algal production ponds yield enhanced productivity and reduced biomass production costs?

2 - Approach (Management)
Project Team

Dr. Robert Pomeroy, PI

- Assoc. Prof., Dept of Chemistry & Biochemistry
- Member, Cal-CAB and CAICE
- Downstream processing of algae conversion for fuel, fuel additives and polyols, and characterization of molecules at the sea surface microlayer and those directly emitted to gas phase during algal blooms
- Lead investigator, manager
- Responsible for flow of Mass Spectrometry and qPCR HRMA work

Dr. Ryan Simkovsky

- Project Scientist
- Member of Cal-CAB, CAB-Comm, and CEC CILMSF
- Crop protection, mechanisms of biofilm formation, and phage in cyanobacteria
- Responsible for the IMS and LC-MS/MS experiments and the day-to-day operations of the project's objectives

2 - Approach (Management)

Project Team

Dr. Kimberly Prather - UCSD & SIO

- Distinguished Chair in Atmospheric Chemistry
- Director of CAICE – ATOFMS, CIMS

Dr. Pieter Dorrestein - UCSD

- Associate Professor, SPPS, Pharmacology, Chemistry and Biochemistry
- Director of CMBB & CMSIC - IMS

Dr. Stephanie Fraley - UCSD

- Asst. Prof. Bioengineering
- Developing artificial learning algorithms for qPCR-HRMA identification of crops and contaminants

Dr. Susan Golden - UCSD

- Chancellor's Associates Chair in Molecular Biology
- Member of Cal-CAB, CAB-Comm, CEC CILMSF
- Providing organisms and biological support/infrastructure

Dr. Alissa Kendall - UC Davis

- Assoc. Prof. Civil and Environmental Engineering
- Member of Cal-CAB & CAB-Comm
- Life cycle modeling and Technoeconomic analysis

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2 – Approach (Technical)

- **Task A – Validation**: Project Validation
- **Task B – Baselines**: Demonstrate ability to grow and monitor crop health with or without contamination using current methods to establish baselines of detection & values for TEA/LCA.
- **Task C – Gas Phase**: Develop & evaluate mass spectrometry detection of crop health & death signatures in gas phase – volatiles & aerosol particles
- **Task D – Liquid Phase**: Develop & evaluate mass spectrometry detection of crop health & death signatures in liquid phase – IMS & LC-MS/MS
- **Task E – qPCR+HRMA**: Establish primers & methodology for crop & contaminant identification for automation and rapid analysis.
- **Task F – Integration/Prototype**: Integrate best detection & identification methodologies into a single, automated prototype detection system.

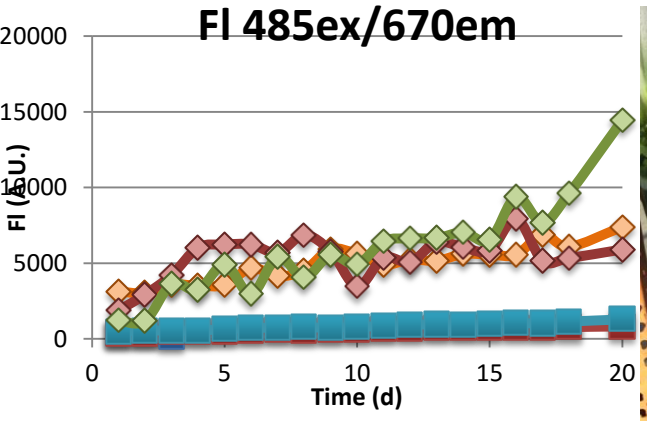
- Evaluate methods at **increasing scales** (flask, mini-MART, polybag, and ponds) on a **diversity** of crops and predators.

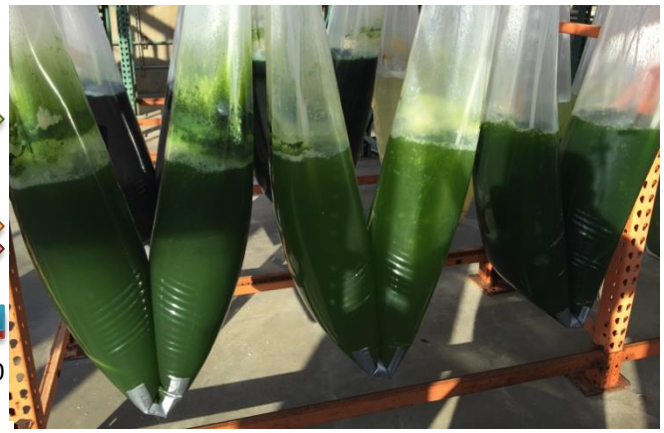
2 – Approach (Technical)

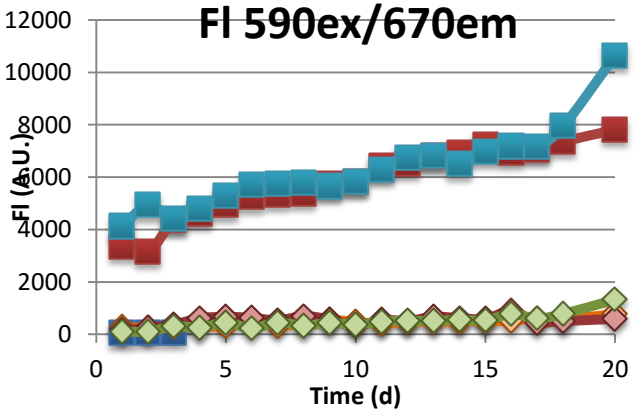
- **Potential Challenges:**
  - Sampling film states for accurate baselines
  - Representative & voracious contaminants
  - Identifying molecular signatures
  - Sensitivity of qPCR-HRMA at early time points from diverse cultures
- **Critical Success Factors & Measures of Success:**
  - Catalog of molecular signatures in gas or liquid phases indicative of contamination or crop health; Associated time of detections/sensitivities relative to current methods.
  - Catalogs of primers and associated HRM curves for diverse crops & contaminants
  - Functioning prototype demonstrated at pond scale
- **Project-wide Go/No-Go at FY17 Q4 (M24) checks for key capabilities necessary to build a functioning prototype:**
  - Demonstration of scaled growth & analysis, TEA/LCA analysis
  - Molecular candidates in gas or liquid phases
  - Primer sets distinguishing predators & crops via qPCR-HRMA

3 – Technical Results – Task B: Growth

- Established baselines yields and kinetics for healthy growth of 5 – 6 strains at flask, polybag, and pond scales.
- Established methods of monitoring for contamination & ongoing establishment of baselines for contamination detection
- Isolating new voracious predators from pond cultures

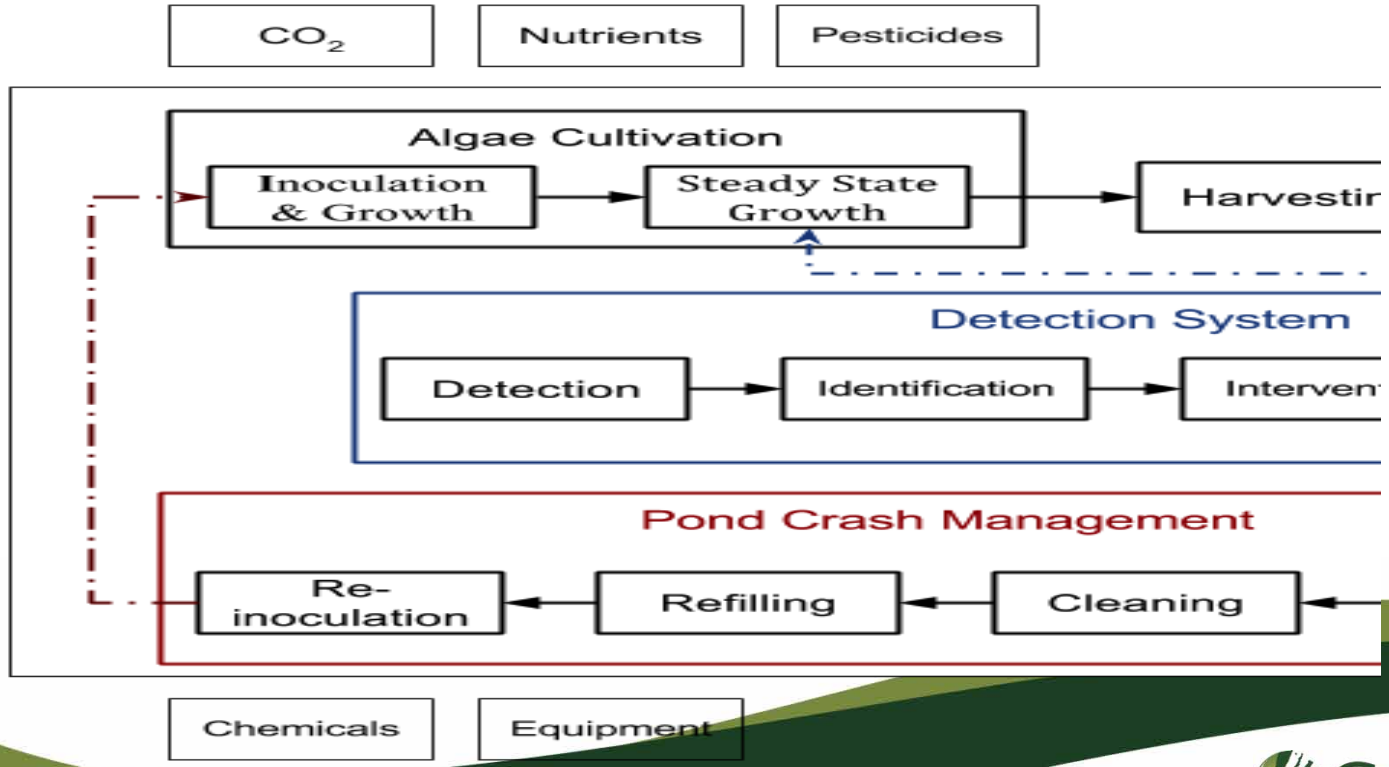






3 – Technical Results – Task B: TEA/LCA

- A mass balance model established including all the parameters and assumptions used in the ideal case, where no crashes or contaminations occur, based on previously work from UC Davis group.
- Yield losses, cleaning costs, intervention costs, and monitoring costs added to baseline model to account for oscillations in biomass accumulation and interruptions in harvesting for four cases on next slide.



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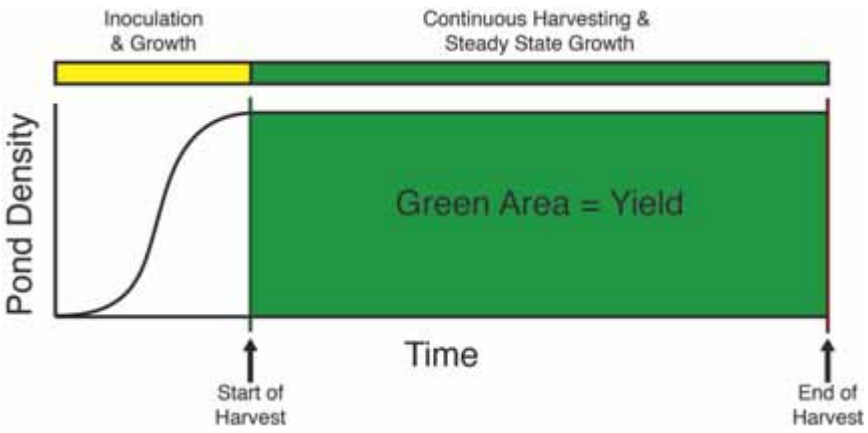
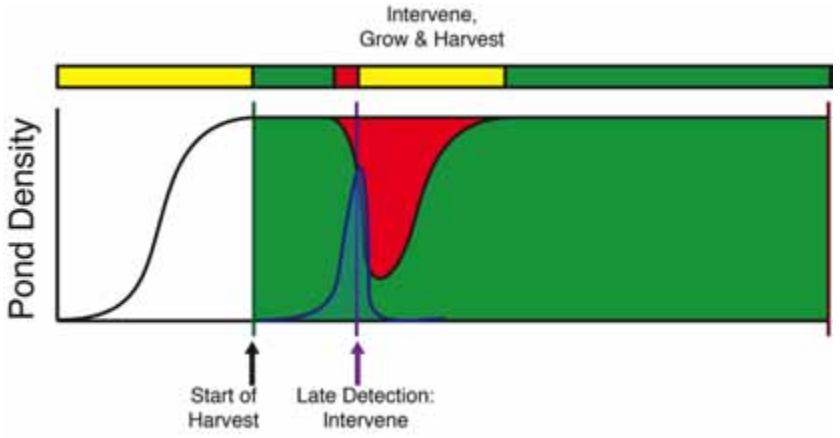
3 – Technical Results – Task B: TEA/LCA

- Modeling 4 cases of single contamination events with regard to contamination and timing of interventions

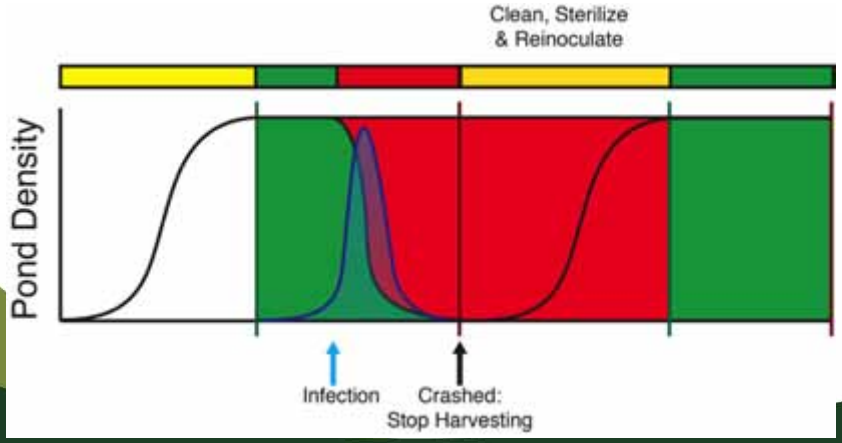
Ideal



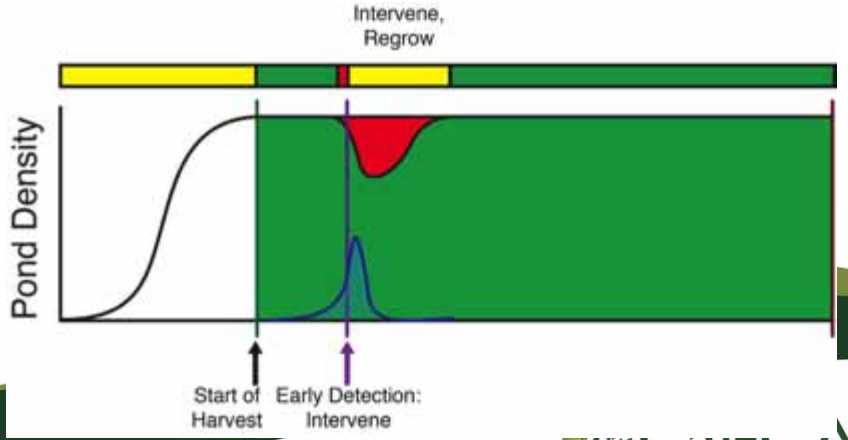
Late Detection & Intervention



No IPM or IPM Failure

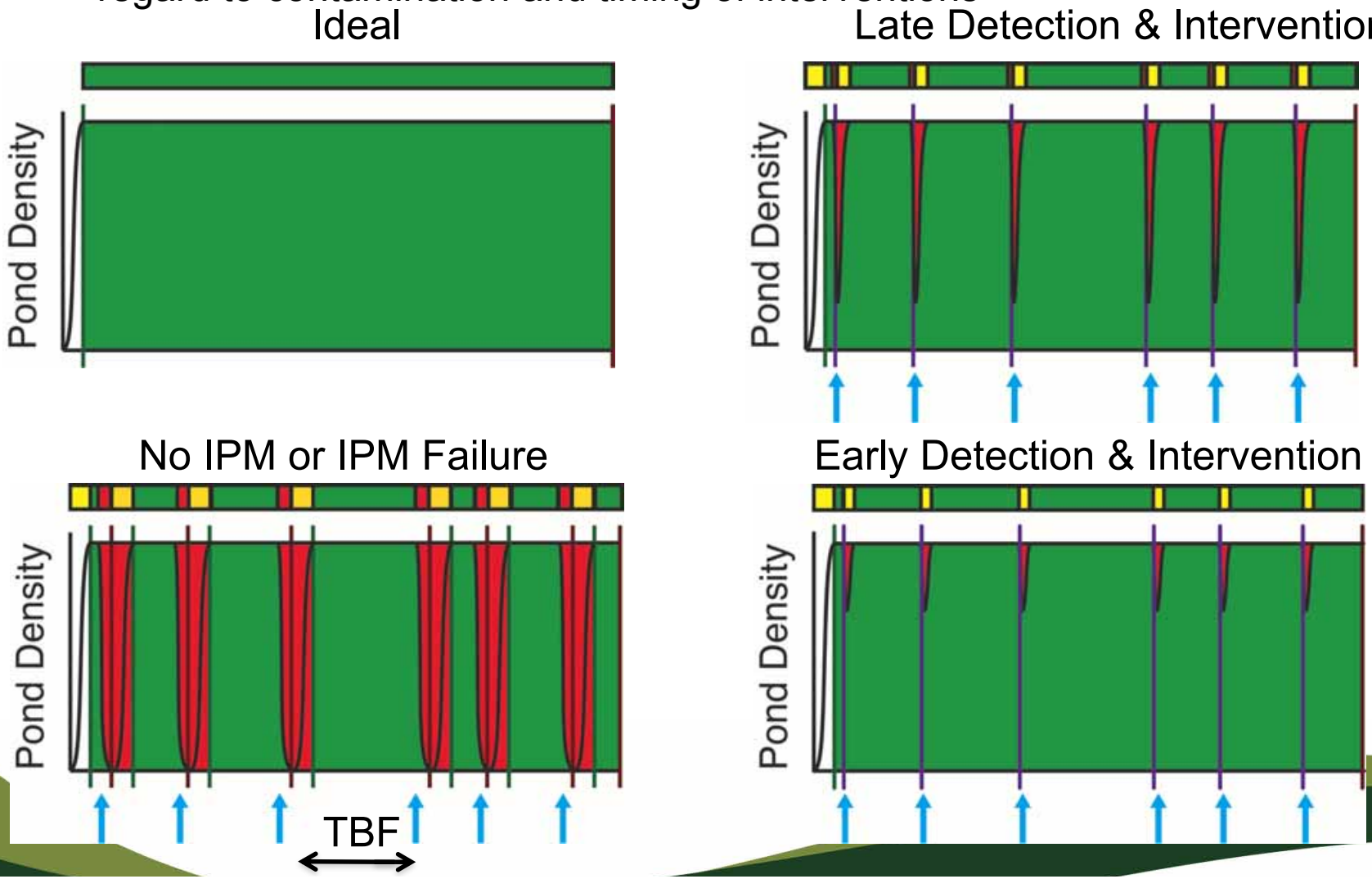


Early Detection & Intervention



3 – Technical Results – Task B: TEA/LCA

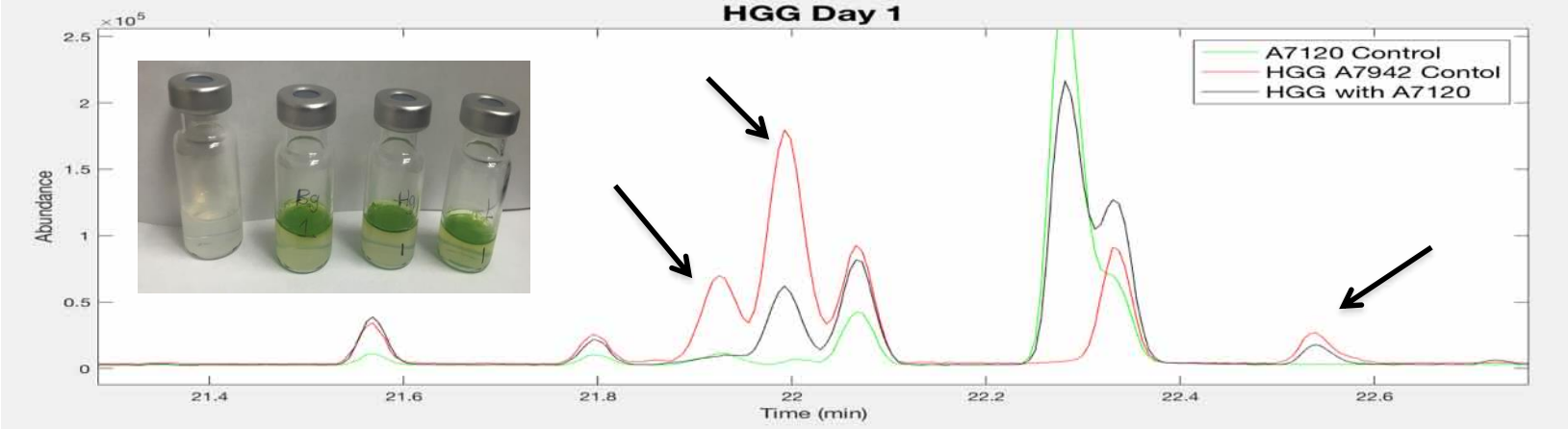
- Modeling 4 cases of a growing season's worth of contamination events with regard to contamination and timing of interventions

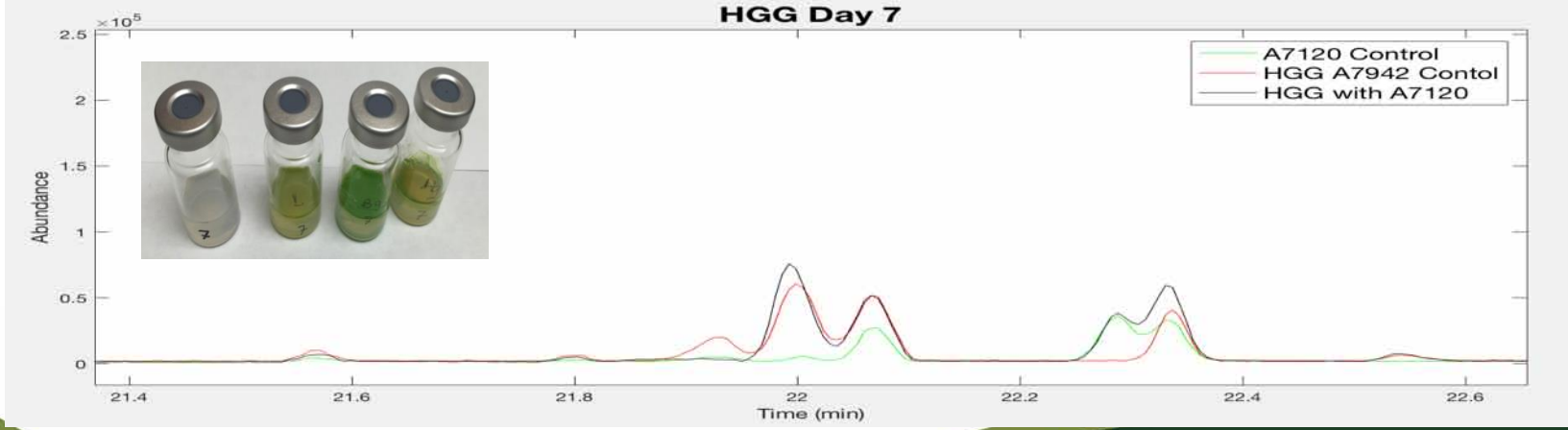


TBF = Time Between Failures; TTR = Time to Recover (Yellow Bars)

3 – Technical Results – Task C: Gas Phase

- Detected volatile organic compounds (VOCs) specific to predation as predicted by results of Task D & tracked changes in intensity over time
- MS detection on Day 1, two days prior to visual observation of grazing





Arrows = Predator or grazing-specific peaks

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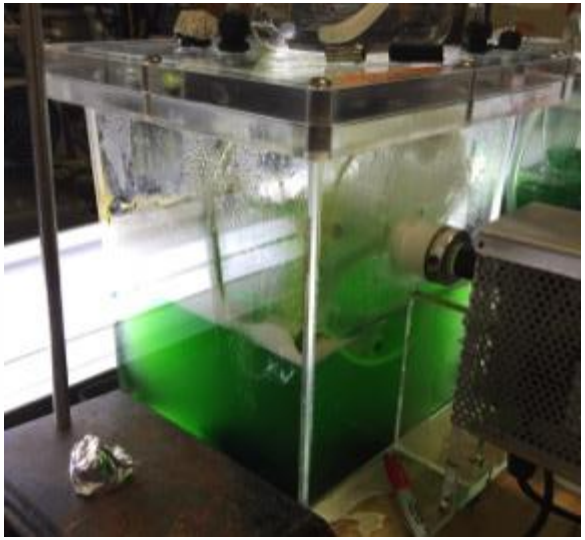
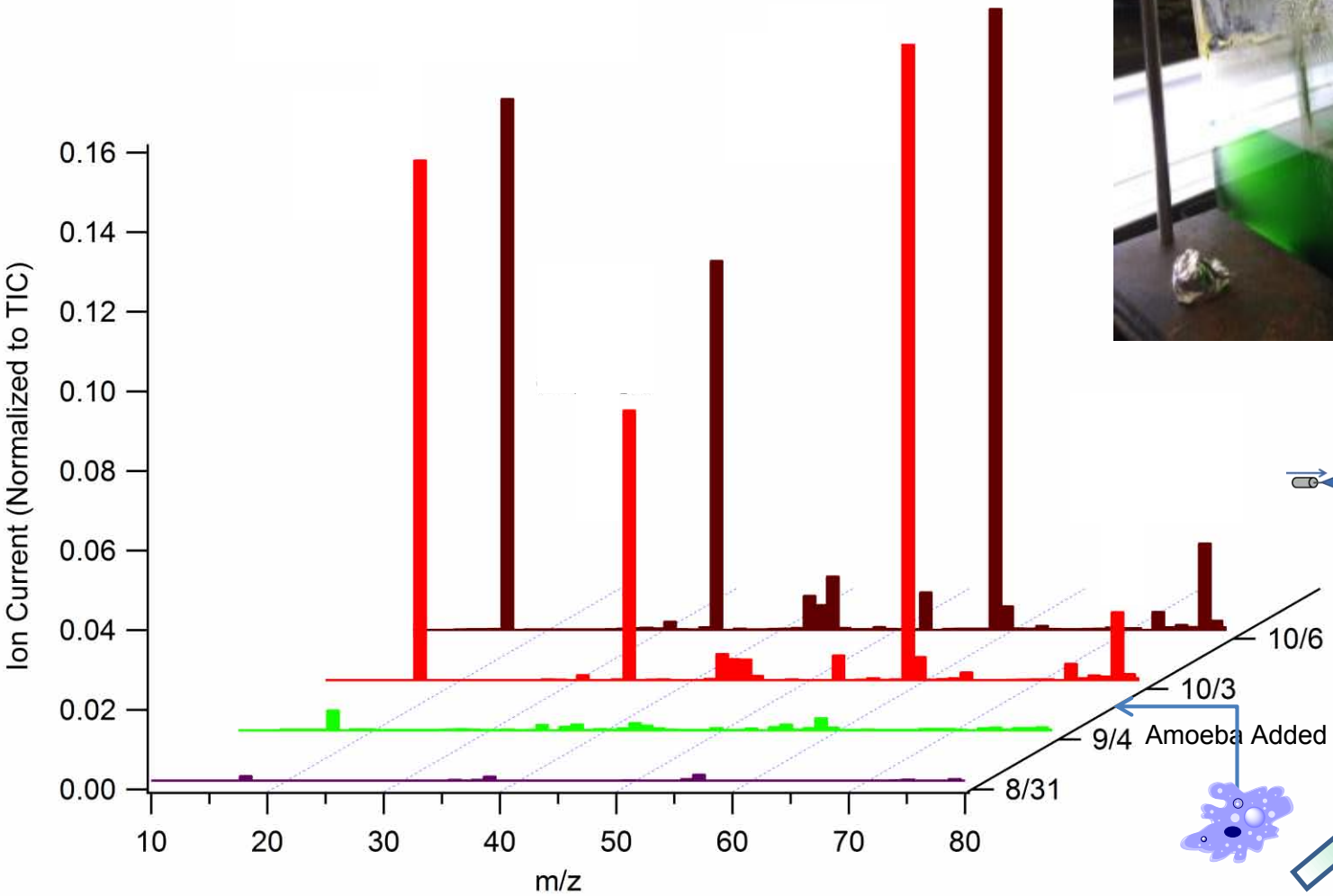
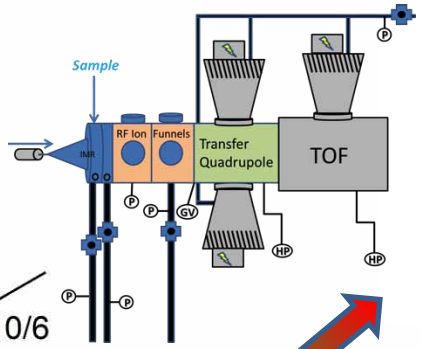
3 – Technical Results – Task C: Gas Phase

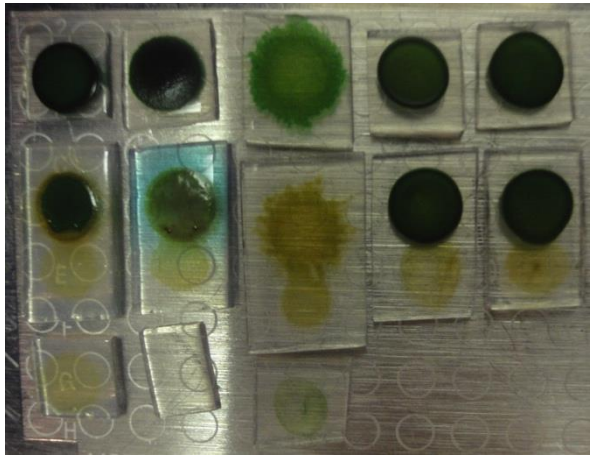
- Detected changes in gas phase above 7 L mini-MART using chemical ionization MS (CIMS) upon addition of predator.
- Standard metrics could not detect predator activity.



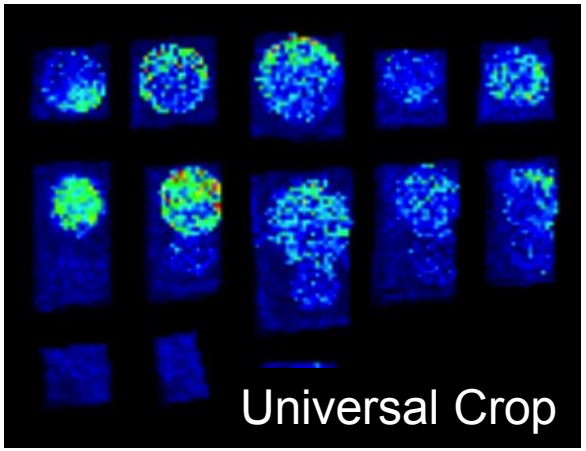


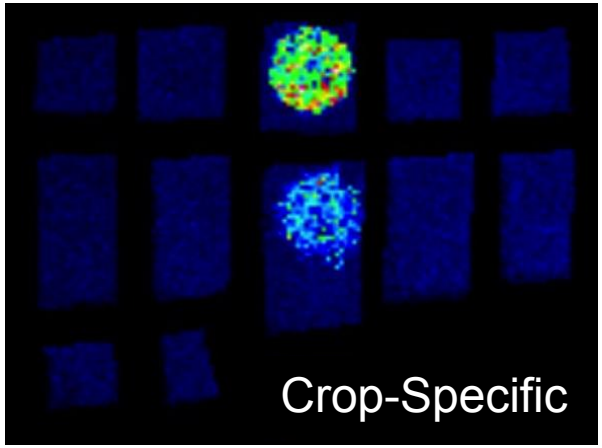


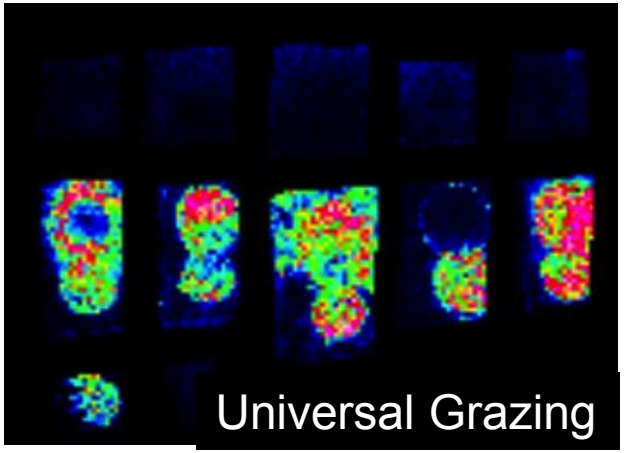
3 – Technical Results – Task D: Liquid/Solid Phase

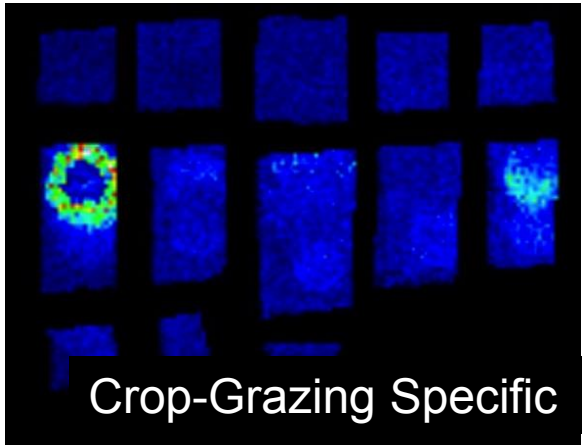


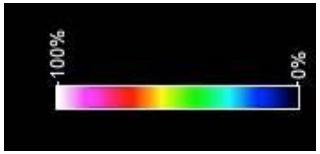
Imaging mass spectrometry (IMS) allowed cataloging of molecular masses associated with diversity of crops, diversity of grazers, or specific interactions of crop and grazer.











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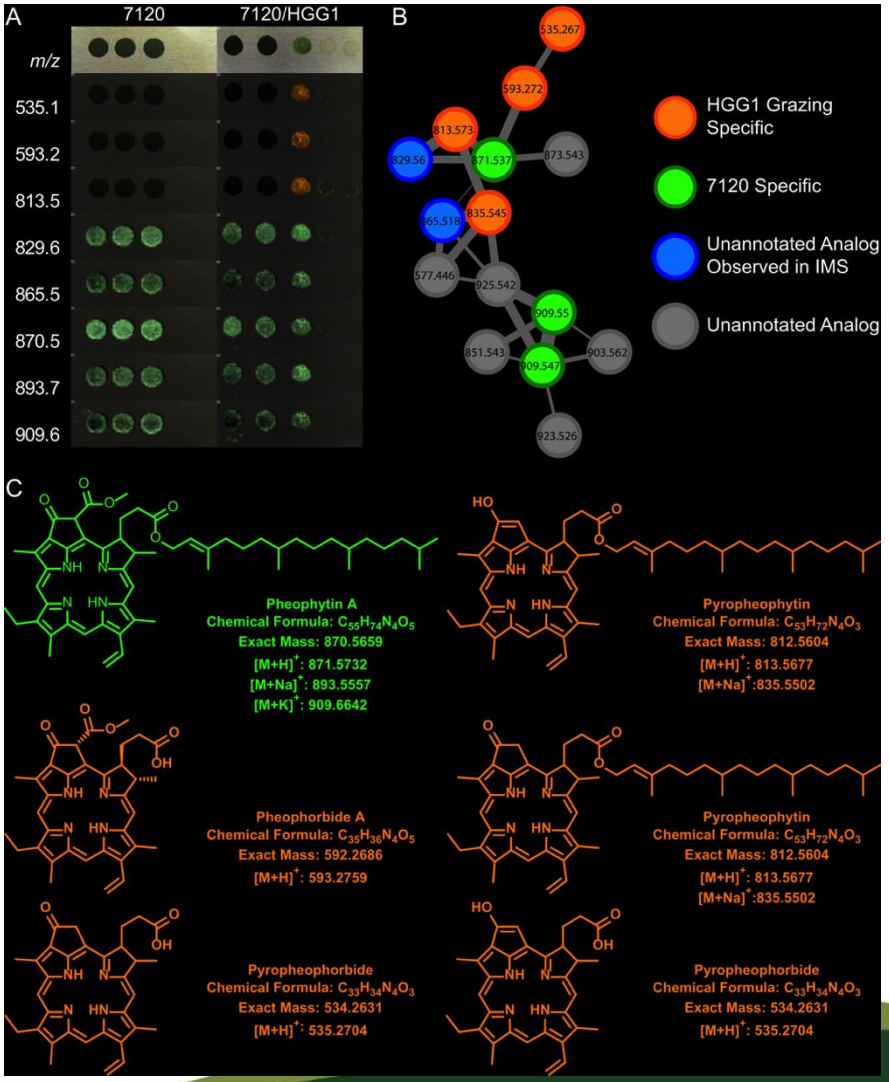
3 – Technical Results – Task D: Liquid/Solid Phase

- IMS cataloging of molecules, combined with LC-MS/MS fragmentation analysis on liquid cultures and molecular networking identified a cluster of molecules related to chlorophyll break-down products as being grazing indicators.
- These results predicted VOCs detected in Task C.



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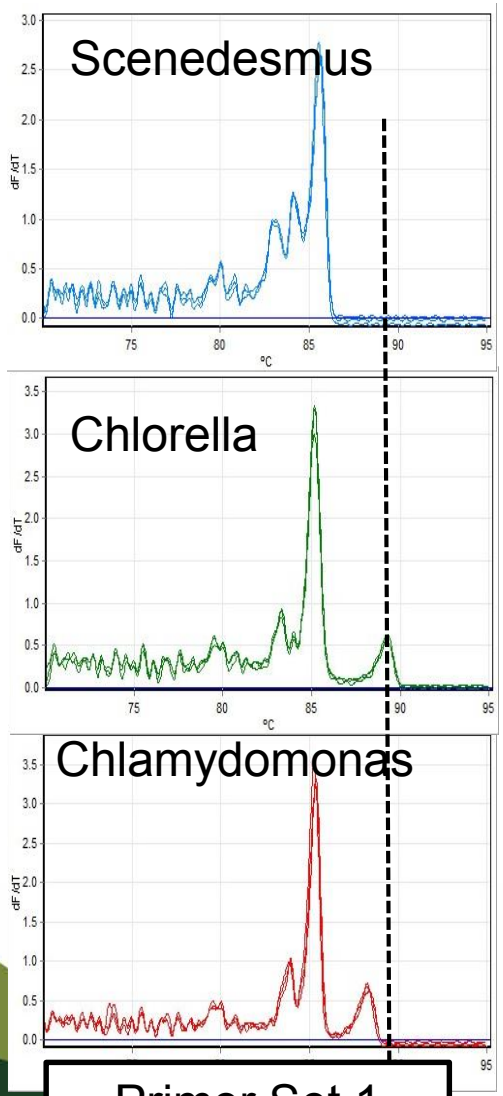
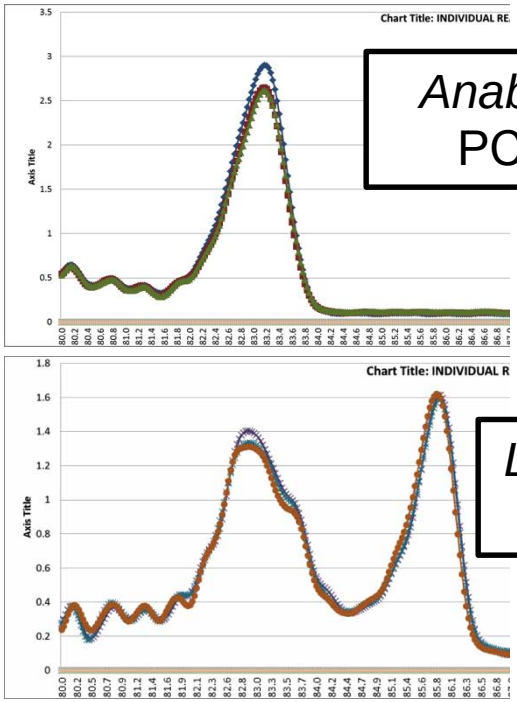
3 – Technical Results – Task E: qPCR-HRMA

Primer sets designed & tested to produce reproducible, species-specific melt profiles.

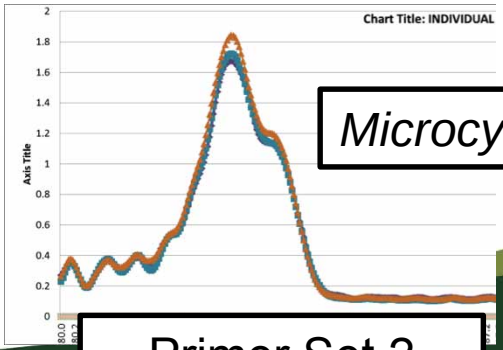


Primer Set 1



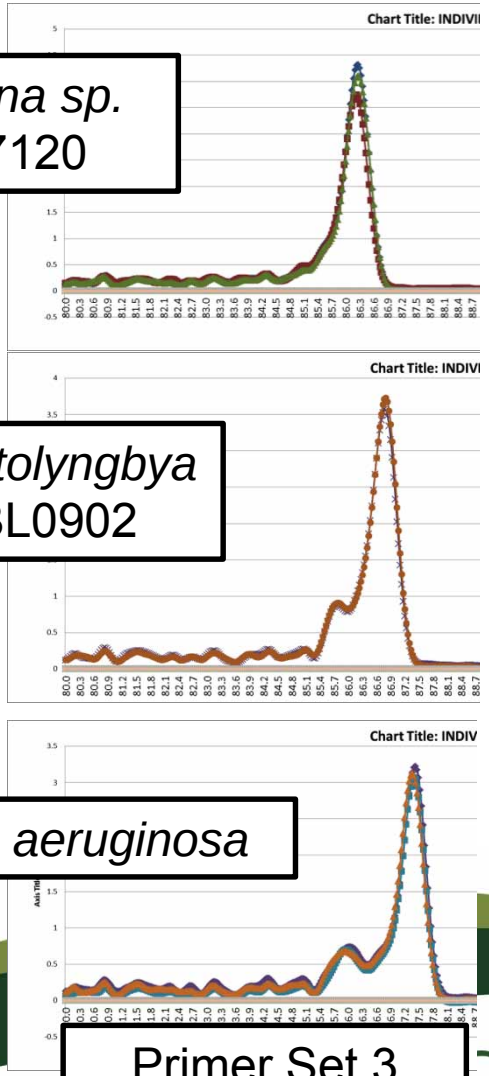
Anabaena sp. PCC 7120

Leptolyngbya BL0902



Microcystis aeruginosa

Primer Set 2



Primer Set 3

4 – Relevance

Overarching Goal

- Develop simple, automated, affordable, and robust technologies for the **early detection and identification** of pathogens, predators, and non-productive competitors in an algal production pond.

Importance

- Early detection systems enable a **more robust** production system with **reduced losses and costs** from catastrophic pond failures and grazing-reduced yields, ultimately allowing **commercial viability**.

Relevance of Project Accomplishments

- Task B: Demonstration of growth & baseline establishments enable comparison with current technology and data for TEA/LCA models.
- Task C: Ability to detect & monitor gas phase molecules indicative of contamination prove principle of detection & sensitivity of system.
- Task D: Catalog of molecular signatures according to crop or contaminant provides IP for technology and enables broad detection capacities.
- Task E: Catalog of qPCR-HRMA compatible primers & resulting curves proves principle of identification in rapid, automated manner.

5 – Future Work

- **Task B – Baselines**: Complete baseline measurements for newly isolated/acquired predators; input data into TEA/LCA model.
- **Task C – Gas Phase**: Apply methodologies to diverse predator-crop systems in vials and MART tanks to catalog detectable molecules, determine sensitivity to contaminant abundances & demonstrate at scale.
- **Task D – Liquid Phase**: Catalog further predator-crop pairs, test monitoring of a contaminated culture, determine sensitivity to contaminant abundances & demonstrate at scale.
- **Task E – qPCR+HRMA**: Catalog primers & HRM curves for diversity of crops & contaminants, test sensitivity in mixed samples, feed data into artificial learning algorithms for automated detection.
- **Task F – Integration/Prototype**: Integrate best detection & identification methodologies into a single, automated prototype detection system and test system at scale.

Summary

- We have been investigating mass spectrometry as a platform for an early detection system
- We are cataloging molecules specific to prey-predator interactions, both in the head space & bulk liquid.
- We are refining a Chemical Ionization MS, CIMS, capable of continuous monitoring with out the need for the Gas Chromatography front end.
- We are developing new primer sets to incorporate qPCR HRM as a simple closed tube method of class/species identification.
- These advances are being modeled for the techno economic impact of this form of Integrated Pest Management.
- We are developing IP in the form of chemical marker identification, primer sequences, and new application specific instrumentation.
- Potential link to other industries: brewing, Fermentation (Pharma), Aquaculture, Hydroponics, Field crops (Corn ?)

Conclusion

- Predated samples produced detectable differences that increased over time starting on day 2 following inoculation
- Predation-specific peak changes appear to be different for LPG1 and HGG1
- These results indicate that the GC-MS is a viable early detection source and that, for S7942 predated by amoeba, the earliest detection of infection was two days following infection, a maximum of 3 days prior to visual observation of predation

In Summary

- Developing a mass spectrometry based early detection system

- Catalog of molecules specific to prey-predator interactions
  - Head space volatiles & liquid bulk molecules

Future Directions

- Identify more signatures in diverse predator-prey systems
- Scale the detection system from flasks to open ponds

Publications, Patents, Presentations, Awards, and Commercialization

- List any publications, patents, awards, and presentations that have resulted from work on this project
- Use at least 12 point font
- Describe the status of any technology transfer or commercialization efforts

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