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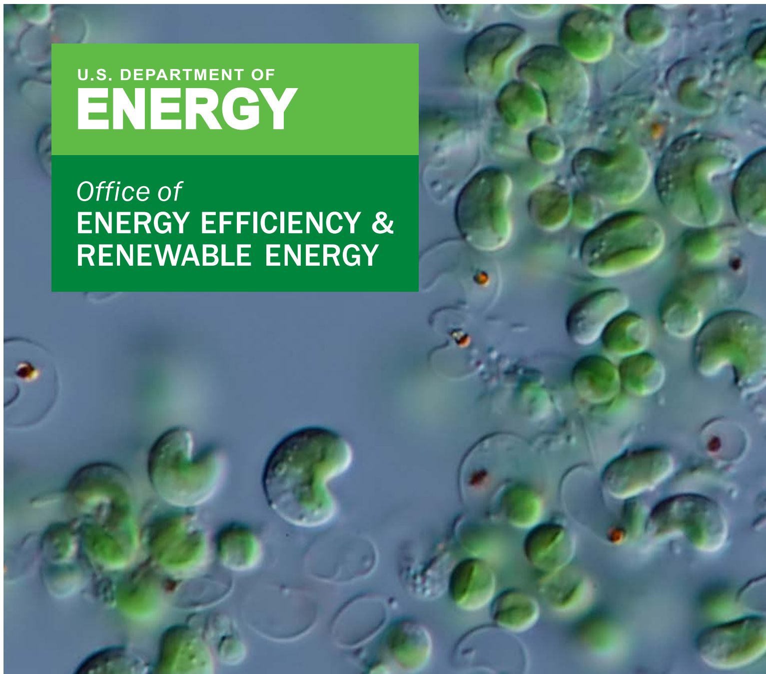
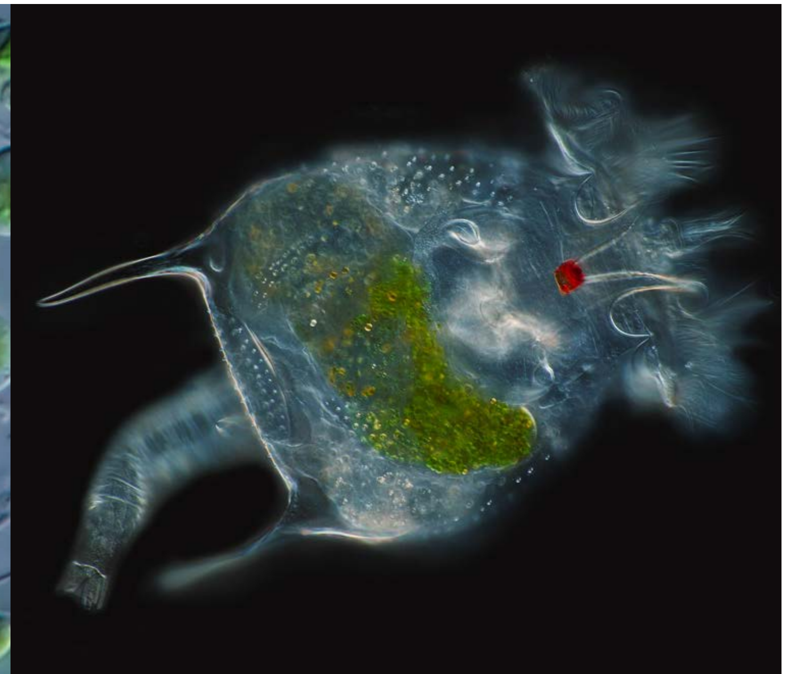
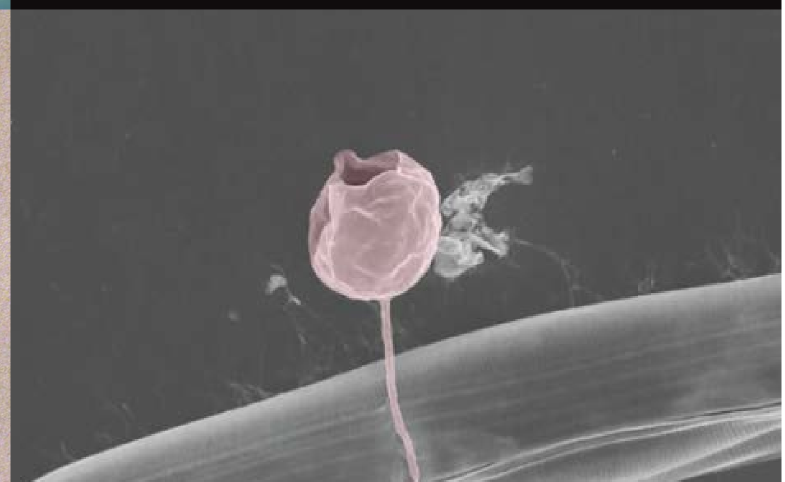
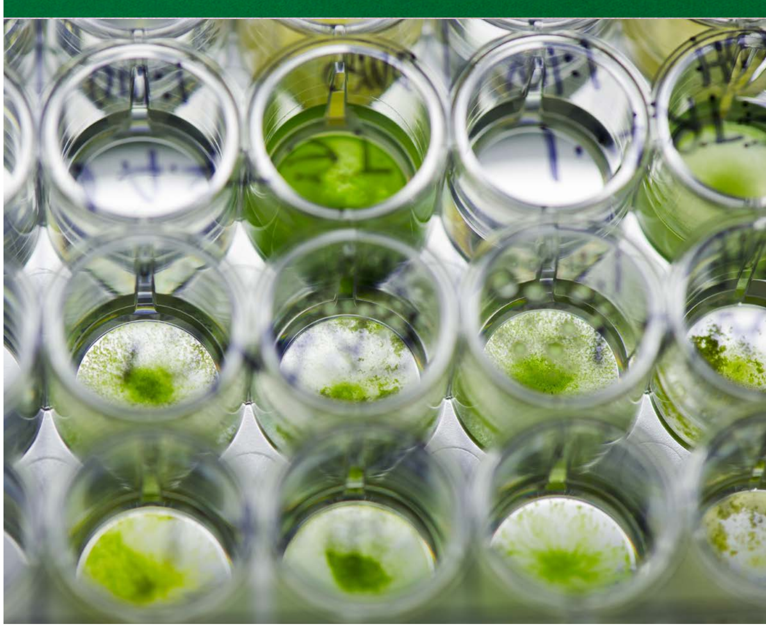


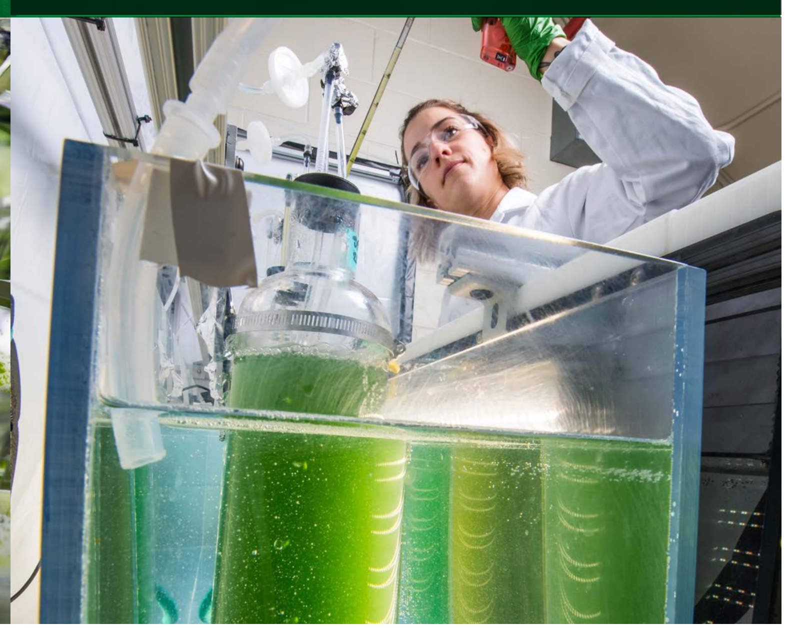




Barriers to Scale: Algae Crop Protection

Workshop Summary Report | April 20-21, 2021





Foreword

The U.S. Department of Energy (DOE) Office of Energy Efficiency and Renewable Energy (EERE) invests in a diverse portfolio of technologies to ensure domestic energy security, continued economic competitiveness, environmental sustainability, and the availability of cleaner fuels and power. The mission of the DOE EERE Bioenergy Technologies Office (BETO) is to develop transformative and revolutionary sustainable bioenergy technologies for a prosperous nation. BETO develops technologies that convert domestic biomass and waste resources into fuels, products, and power to enable affordable energy, economic growth, and innovation in renewable energy and chemicals production. This report summarizes the input received from attendees of the public workshop sponsored by BETO on April 20–21, 2021.

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Acknowledgments

The U.S. Department of Energy (DOE) Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office (BETO) thanks all the participants who contributed invaluable experience and insights to making the Barriers to Scale: Algae Crop Protection Workshop a success.

This summary report was prepared by Daniel Fishman (BETO), Philip Lee (AST), Colleen Tomaino (BCS, LLC), and Lauren Illing (BCS, LLC). The authors sincerely thank the workshop participants for their contributions, which provided input for this publication. The full list of individuals who registered for the workshop is provided in Appendix A.

Workshop conceptualization and planning was led by Philip Lee with support from Daniel Fishman, Colleen Tomaino, and Lauren Illing. Lauren Illing led the stakeholder input session facilitation planning, including group discussion process, virtual format, and training the team of session leaders. Workshop scribes, who recorded the workshop presentation and discussion highlights, were Colleen Tomaino and Ty Robinson (The Building People).

We gratefully acknowledge the keynote speakers who framed the workshop topics for attendees: Barry Goldman (Pluton Biosciences) and Claire Gachon (Scottish Association for Marine Science). Additionally, we appreciate the panel participants who provided their expertise and insight to kick off each stakeholder input session (listed in Table 1). Finally, we thank the session rapporteurs who quickly created and shared session summary presentations with their peers (Table 1).

Table 1. Workshop Panelists and Rapporteurs

Stakeholder Input Sessions	Session Panelists	Session Rapporteurs
Current State of Crop Protection	Charles O'Kelly (Cyanotech), John McGowen (AzCATI), Jason Quinn (Colorado State University)	Zackary Johnson (Duke University)
Alternative Crop Protection Approaches to Chemicals and Pesticides	Rhona Stuart (Lawrence Livermore National Laboratory), Kimberly Ogden (University of Arizona), Jeremy Guest (University of Illinois Urbana-Champaign)	Amanda Barry (Sandia National Laboratories)
Pest Models: Understanding Pest Life Cycles and Infection Mechanisms	Todd Lane (Sandia National Laboratories), Shawn Starkenburg (Los Alamos National Laboratory), Timothy James (University of Michigan)	Blake Hovde (Los Alamos National Laboratory)
Current and Future Pest-Monitoring Practices	Jerilyn Timlin (Sandia National Laboratories), Natalie Cookson (Quantitative BioSciences, Inc.), Ryan Simkovsky (University of California San Diego)	Scott Edmundson (Pacific Northwest National Laboratory)

List of Acronyms

AzCATI	Arizona Center for Algae Technology and Innovation
BETO	Bioenergy Technologies Office
DOE	U.S. Department of Energy
EERE	Office of Energy Efficiency and Renewable Energy
QBI	Quantitative Biosciences
qPCR	quantitative polymerase chain reaction
R&D	research and development

Executive Summary

The U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office (BETO) hosted a virtual workshop on April 20–21, 2021, focused on algae crop protection. A major barrier to the scaling and intensification of algae cultivation is the partial or complete loss of valuable biomass caused by pests, as in terrestrial plant biomass production. Developing effective strategies to protect algal biomass production is essential to meeting BETO's long-term goals of promoting cost-competitive algal-derived biofuels and bioproducts through increasing annual average harvested yield of algal biomass.

The workshop convened 124 interdisciplinary experts from fields including algae research and production, agriculture, and aquaculture. The workshop kicked off with plenary presentations from BETO on the goals of the event and an overview of the office's mission and interest in this topic area. Additionally, both days began with a keynote plenary presentation from outside the algae industry on relevant crop protection strategies in agriculture and aquaculture, respectively. The main technical content of the workshop was presented via panel presentations, followed by group discussions on prepared topics.

The first panel discussion and stakeholder input session focused on the current state of algae crop protection, what tools are in use right now, and where gaps in technology exist. The second session focused on crop protection approaches that are alternatives to chemicals and agricultural pesticides, such as biological and mechanical controls. The third session focused on pest models, specifically understanding pest life cycles and infection mechanisms. The final session covered current and future pest-monitoring practices that are used to detect pest populations in the field. The sessions covered input from academia and industry and highlighted the importance of crop protection in maintaining algal productivity and scaling the industry.

Key takeaways from these sessions are summarized as follows:

- Crop protection is a significant issue for all algae growers—when algae is cultivated at scale, there will be pests.
- Not enough pest-mitigation solutions are available now to facilitate the growth of the industry.
- Public data on pests are limited, and the algal community is disincentivized from sharing pest-related data.
- No single pest is the most problematic, and new pests are continually discovered.
- Pest-mitigation lessons can be learned from agriculture and aquaculture, including potentially overlapping methods and strategies.
- The best way to determine the efficacy of crop protection is harvested productivity. Mean time to failure was also highlighted as a valuable metric.

Barriers to research:

- Selecting which host and pest to research to be most impactful is a significant barrier to conducting applied research and development.
- Pesticide use might be undesirable or untenable, and alternative solutions are required.
- It is difficult to replicate field conditions in the laboratory.
- Developing solutions to a field-deployable state is expensive.
- It is difficult to understand the complex interactions between both biotic and abiotic variables, especially within the microbiome.

Potential solutions and paths forward:

- The algae industry would benefit from both applied and fundamental pest research.
- Because crashes are not always apparent in short and small-scale experiments, experiments/demonstrations must be done at a larger scale and for longer time frames.
- Future research should focus on developing alternatives to pesticides, including altering the culture media; biological mechanisms, such as designed consortia or microbiome manipulation; pest-resistant strains; and mechanical/operational strategies.
- Additional research is needed on developing cheaper and more effective engineering solutions for mechanical crop protection solutions.
- A better mechanistic understanding of pest-host interactions is needed to accelerate the development of applied solutions.
- Better strategies for storing and sharing information about pests are needed, particularly between academia and industry, while protecting intellectual property.
- Generating foundational knowledge of pests available now will facilitate quicker results in the future, even if different pests are the focus at that time.
- Improved real-time, *in situ*, multifunctional monitoring tools are needed to assess pest populations in the field.

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Introduction

On April 20–21, 2021, the U.S. Department of Energy (DOE) Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office (BETO) hosted the Barriers to Scale: Algae Crop Protection online workshop to discuss strategies to overcome the significant technical challenge of pests to the scaling and process intensification of the algae industry. Increasing the scale of algal cultivation is necessary to meet DOE goals for supporting the development of cost-competitive biofuels and bioproducts. Like traditional agriculture, algae farming is prone to pest damage. Pests compete with, consume, or otherwise have a detrimental effect on productivity or biomass quality. Crop protection research seeks to better understand these problems and to develop solutions. This is currently an underreported area of research, especially relative to the impact pests can have on an algal pond system, where in some circumstances a complete loss of productivity can be observed in a matter of days. Resolving crop protection challenges is essential to scaling the algae industry, and unique strategies are required. The traditional approach of chemical (e.g., pesticide or herbicide) application might not be viable in the long term because of high costs, regulatory impediments, and low consumer acceptance.

This workshop connected scientists from across disciplines to identify elements of a research strategy necessary to overcome these challenges and to reduce the impact of pests on culture stability, biomass quality, and realized productivity. The workshop sought to achieve the following goals:

- Identify the gaps in current knowledge, research, and technical capabilities.
- Foster collaboration among basic, applied, and commercial researchers.
- Determine the regulatory factors for crop protection at scale.
- Establish strategies to overcome technical barriers.

A keynote presentation was given each day to discuss the state of crop protection in the related fields of agriculture and aquaculture to identify where comparable issues have been solved or solutions are under development and where areas of synergy might exist. After these framing presentations, the workshop focused on technical sessions on the following topics:

- Current state of crop protection
- Alternative crop protection approaches to chemicals and pesticides
- Pest models: understanding pest life cycles and infection mechanisms
- Current and future pest-monitoring practices.

Each session began with presentations from invited panelists on the topic of interest; followed by a moderated panel question-and-answer period; and then facilitated open discussion with opportunity for direct participant input via a web-based collaboration software, XLeap by MeetingSphere.

The 124 registered participants who attended the discussion sessions included professionals from industry, academia, DOE national laboratories, federal agencies, and other affiliations. The largest single group of participants self-identified as being affiliated with industry, followed be academia (Figure 1). Most participants reported having direct experience with algae cultivation and pest management (Figure 2).

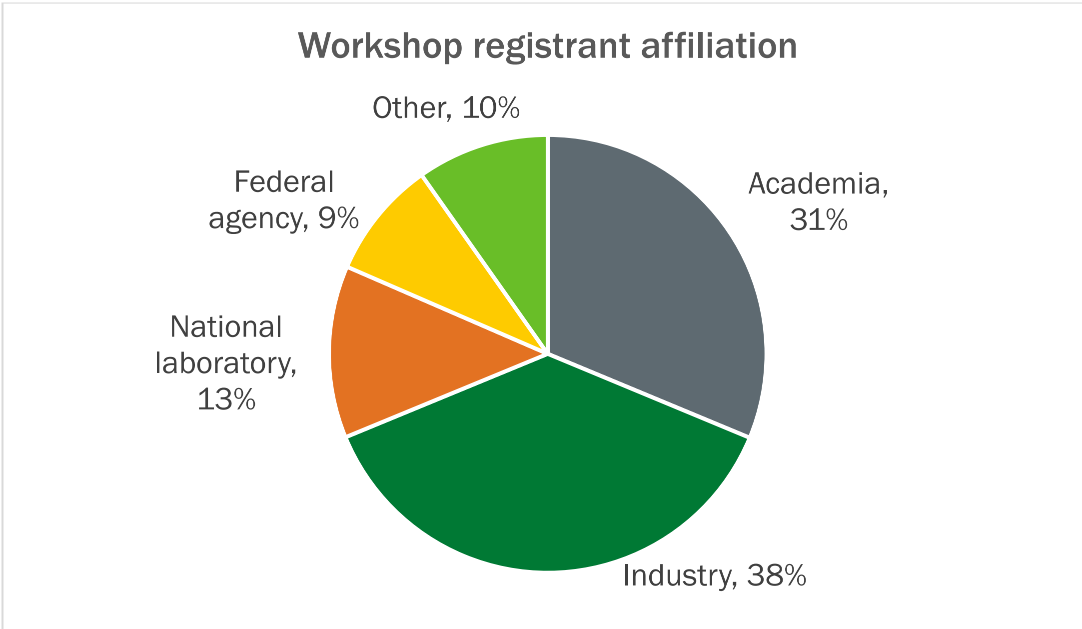


Figure 1. Registrant affiliation categories

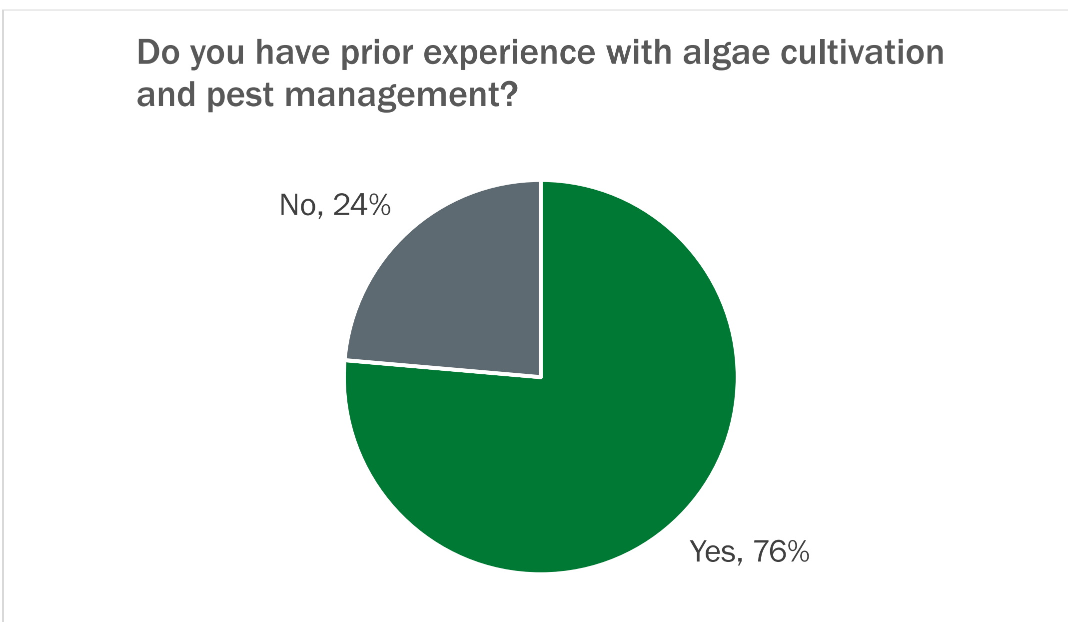


Figure 2. Registrant direct professional experience with algae cultivation and pest management

Keynote Presentations Summary

Both days of the workshop began with a keynote presentation from a scientist outside of the algae industry to provide a unique perspective on how the agriculture and aquaculture industries are addressing crop protection and how those strategies are relevant to protecting algal crops.

Pluton Biosciences, Barry Goldman (Pluton Biosciences)

The day one keynote presentation was given by Dr. Barry Goldman of Pluton Biosciences. Dr. Goldman shared his experiences garnered during a career at Monsanto and Indigo Ag and how he is leveraging that knowledge at Pluton Biosciences with their "micromining" platform. Micromining identifies candidate microbes quickly by using a machine learning approach on screens of subpopulations of native microbiome samples. Dr. Goldman emphasized that the single-gene/product solution approach to crop protection is difficult because of the cost and time it takes to get a product to market. This traditional approach also suffers from the development of pest resistance, which has been observed in both insect and weed systems. He suggested that a holistic systems approach is a more practical solution and that natural products and probiotics have a faster and cheaper product development cycle.

Physiology and Ecoanthropology of Algae Pathogen Interactions, Claire Gachon (Scottish Association for Marine Science and Muséum National d'Histoire Naturelle)

The second day of the workshop began with a keynote presentation from Dr. Claire Gachon from the Scottish Association for Marine Science and the National Museum of Natural History in Paris. Dr. Gachon presented research on pests of macroalgae (seaweeds). She highlighted that there is an unknown diversity of pests, new pests are continually being discovered, and reservoirs of pests exist globally. For one type of seaweed, when farming was intensified at different global locations, there was a grace period of 5–10 years prior to culture collapse as a result of the establishment of a local pest population. This highlights the risk of farming new species without understanding the pests or developing mitigation strategies as well as the need for a global biosecurity framework for seaweed aquaculture. She presented work on possible solutions—including using probiotics, breeding for disease resistance, and mining for genetic markers of pest resilience—to better understand how seaweeds defend themselves and how to apply that to crop protection.

Discussion Session Overview

The workshop featured four technical discussion sessions. Each session was framed by a panel of presentations to review the current work in each research area, followed by high-level discussion questions designed to provide participants an opportunity to directly contribute their experience and insights. A facilitator guided the group through session activities, including short answer prompts and quantitative assessments to prioritize input. All input was visible in real time for participant review and comment via the XLeap collaboration software tool. Key takeaways from these discussions are summarized in the following sections.

Current State of Crop Protection

Topic Introduction

Crop protection is the science and practice of managing algal diseases, weeds, predators, and other pests that negatively impact the strain (or strains) of interest. Pests are any organism that reduces overall yield, including pathogenic organisms (e.g., bacteria, viruses, fungi), grazers (e.g., rotifers, daphnia, amoeba), resource competitors (i.e., weeds), or even larger animals that could damage equipment. New pests are still being discovered as new strains are being cultivated. Because the algae industry is small and relatively young compared to terrestrial agriculture, it has fewer resources and a smaller knowledge base to address these issues. Additionally, there are disincentives to sharing pest information because of desires to maintain a competitive advantage and to mitigate negative press about infections. Because of this lack of shared knowledge, predictive techno-economic analysis models for developing companies or approaches do not always adequately address the cost implications of pests, including mitigation approaches, pond downtime, and annual crop loss.

Meeting participants were polled on a scale from 0–10 on the significance of crop protection to the algae industry, with a 0 indicating not at all significant, 5 indicating somewhat significant, and 10 indicating very significant. From 63 respondents, the average rating was 8.7. Participants commented that controlling crop yield and quality is critical to building a sustainable algae industry, and without ways to maintain crop integrity, end products will be neither consistent nor marketable. One participant noted that there are currently no good solutions, and the problems are getting worse.

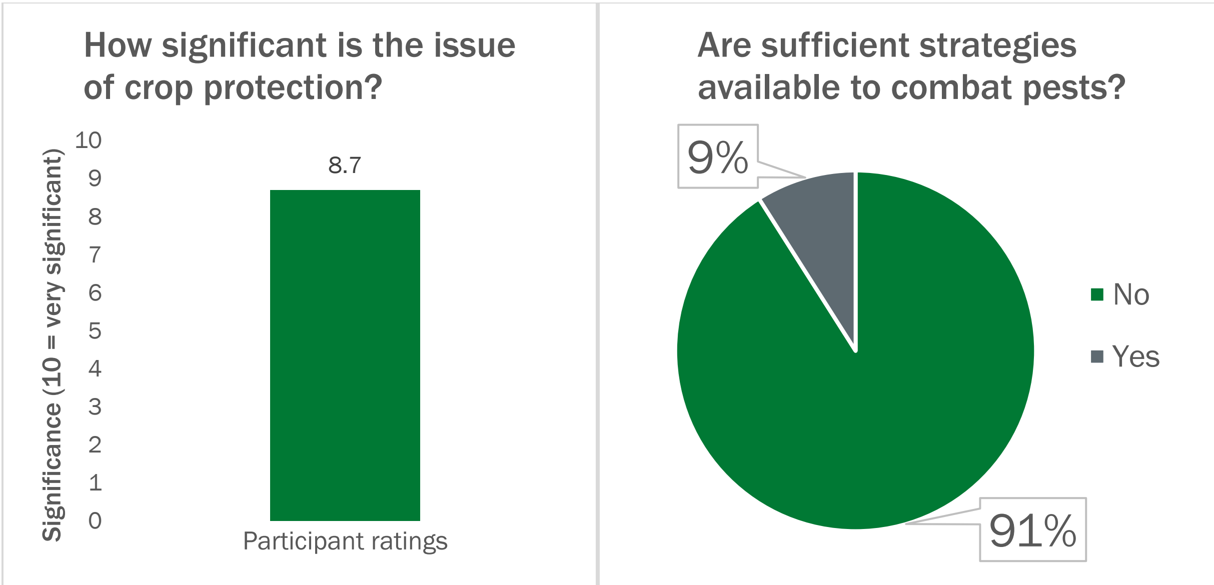


Figure 3. (Left) Participants were asked, "How significant is the issue of crop protection to the algae industry?" Responses were scored on a scale from 0–10, with 0 = not at all significant, 5 = somewhat significant, and 10 = very significant. (Right) Participants were asked, "Are sufficient strategies available to combat pests?" Possible responses were yes or no.

Panelist Presentations

Charles O'Kelly, from Cyanotech; John McGowen, from the Arizona Center for Algae Technology and Innovation (AzCATI) at Arizona State University; and Jason Quinn, from Colorado State University, delivered panel presentations that highlighted the limited understanding and public data on algal pests, both in production systems and in their natural ecosystems. The panelists emphasized that growing algae grows pests, and the cultivation of algae outdoors requires active pest management approaches.

Algal Crop Protection When It's Your Stock at Stake, Charles O'Kelly (Cyanotech)

Charles O'Kelly, from Cyanotech, gave the first panelist presentation to frame the discussion on the current state of algae crop protection. In his experience growing algae, he has found that the field requires more information on pests in their natural environment. In his experience, the field is far from having made sufficient strides in the basic science necessary to deliver practical generalities on pests; therefore, more data are necessary. It is important to start in the environment where the pests are found—getting outdoors with field work and then going back to the lab to examine pest data, and then to work in iterative field/lab cycles to accomplish crop protection strategies. When gathering pest data, researchers must incorporate depth of data, including determining the pest's vector (how it enters the system), reservoir (how it stays in the system), and latency (how long it is in the system before it is noticed). Pest observations can be affected by geography, environmental factors, and operational management of the pond. One main difficulty of crop protection research and development (R&D) is that there are many pest targets to study, but at some point, the field must narrow its focus on the key deleterious species. For example, one might find a protozoon with algal cells in it that grows very slowly or consumes only a small number of algal cells per unit of time, and therefore it has little impact on productivity and can "safely" be ignored, allowing the unit to focus on a shorter list of truly bad actors. The ultimate goal of crop protection strategies must be increasing and stable algae productivity, therefore increasing how much money can be made from the system.

Perspectives from an Academic Algae Test Bed, John McGowen (AzCATI)

John McGowen, from AzCATI, described the facilities and services of the center and the pests they have encountered in their cultivation experiments. AzCATI has conducted outdoor cultivation on-site since 2009, at first using primarily photobioreactors, then increasingly open ponds since 2012. Their main species of interest through 2015 were *Nannochloropsis sp*., *Chlorella sp*., and *Scenedesmus sp*., but they have increased strain diversity since that time. They have used a diverse range of media types and cultivation conditions, so they have had many opportunities for pest observations and mitigation strategies. Each algal strain has its own pests. During most of the early cultivation years, AzCATI primarily saw grazers, but more recently, other pests have emerged as the dominant threat, including fungal parasitoids and predatory bacteria. There remains much to explore for the effective mitigation of fungal parasitoids, but AzCATI has had marginal success with changes to pH and adding fungicides, though success is strain dependent. Cultivation of algae outdoors requires active pest management approaches because pest pressures change from one season to another and from one year to another. Once a pest is in a system, it is very difficult to rid. Understanding appropriate pest protection measures per a specific crop takes time, patience, and significant effort. Replication and longevity of algae experiments are important, but it is difficult to accomplish pest data collection when a team is focused on other research questions, and it is likely that a lot of useful and relevant data on crop protection goes uncollected.

Impact of Reliability Techno-Economics, Jason Quinn (Colorado State University)

Jason Quinn, from Colorado State University, has developed techno-economic models capturing standard algal growth, validated with AzCATI cultivation data, then integrated with Algae Testbed Public-Private Partnership pond reliability data from across four different growing seasons. Anything that impacts algae productivity also impacts the economics of the system. The data were limited, but the reliability data integration proved effective in capturing key pond crash events to improve the efficacy of the models in conveying real-world growing conditions. Ideally, these models can be used in the future to compare mitigation strategies and their impact on the mean-time-to-failure curves.

Challenging Pests

The discussion session began with a quick question to survey participants on their experience with pests and to frame the basis of the conversation that followed. Participants were asked to list algal pests and then to indicate their severity to algal cultivation on a scale from 0–10 (Figure 4). The most highly ranked set of pests were "unknowns." Researchers are still working to better understand the microbiome and to identify deleterious species, so the most worrisome pests are the least well-understood pests. As highlighted in this and other sessions, new pests are still being discovered, underscoring the perceived risk of unknown organisms. Fungal infections, bacteria, and viruses were the next highest-ranking pests because they are too small to remove via filtering, and they can be very difficult to treat. Flagellates and rotifers are also commonly seen algae grazers.

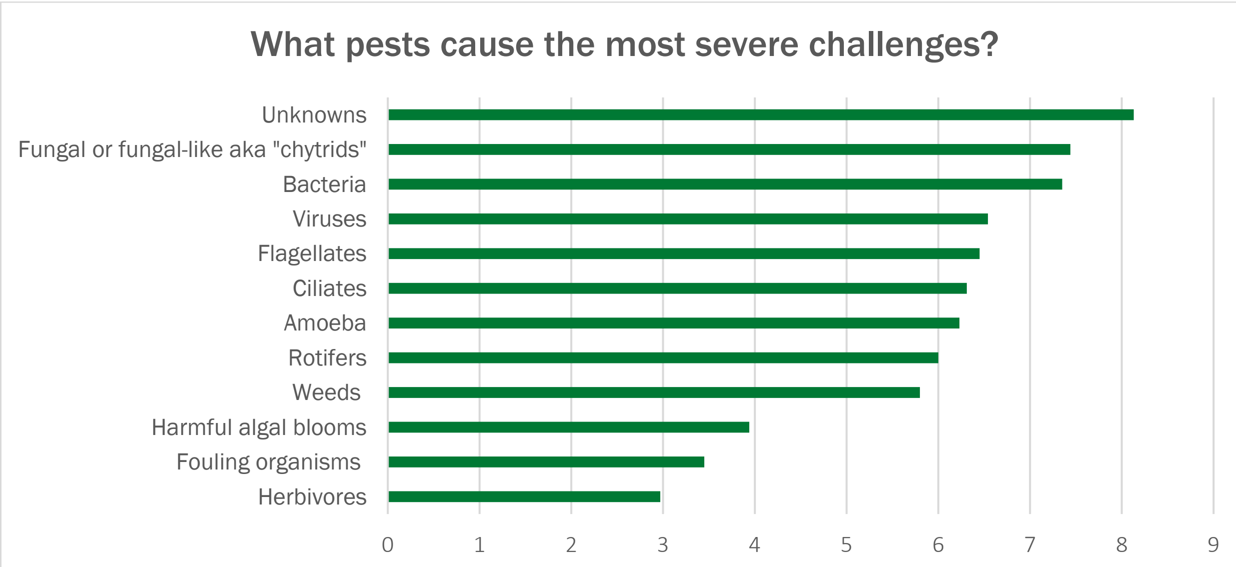


Figure 4. Participants were asked, "What pests cause the most severe challenges?"

Strategies to Combat Pests

When asked whether sufficient strategies are available to combat pests, participants overwhelmingly answered no; however, participants described several existing successful approaches currently used in the field.

The participants grouped crop protection strategies into several categories: pond additives (such as fungicides and bleach), pond operations (e.g., changing media composition, reducing time in culture and harvesting before fouling, and mechanical shearing), and selecting for robust strains. The group voted that the most promising strategy was community engineering—trying to create diverse and stable communities with complementary morphological or chemical traits that reduce the severity of grazing and parasitism. Designing consortia with an understanding of native ecology and relationships found in nature could improve the success of this strategy. Community engineering strategies that augment a "natural" community with members that possess protection traits could be particularly impactful. For example, slow-growing, highly defended (e.g., chemically or morphologically) strains could protect highly productive, poorly defended strains (e.g., Chlorella), ideally without reducing overall productivity. Other related strategies could be introducing known predators that feed on the algal pest but not the algae or adding probiotic bacteria to a culture.

Temporary changes in media or cultivation conditions also ranked high in the prioritization of strategies. These include adding ammonia to mitigate rotifers and amoebas or reducing the pH for *Vampirovibrio*. A similar strategy is maintaining a permanent media or cultivation condition inhospitable to pests (e.g., high pH, salinity, alkalinity, specific salts) that could be used with algal species that can tolerate these more extreme environments.

The strategy of choice will depend on the characteristics of the algae crop, the desired end product, the cultivation system, and the operational parameters. Every alga has its own pests, and each pest has its own most effective treatment method. Additionally, the willingness to accept the cost of the crop protection strategy hinges on the expected biomass value. When producing high-value end products, the algae farmer might be more willing to invest in high-cost crop protection strategies, such as media additives or specialized equipment.

Crop Protection Metrics

Participants were asked how they would measure crop protection success. The dominant response, as indicated via participant vote, was algae productivity and product yield (Figure 5). Annual productivity improvement under a crop protection regime compared to a baseline and an untreated control is a general measure of pond health. Measuring annual productivity (in $g/m^2/day$) captures biomass loss from crashes, time to recovery, reduced algae health that does not necessarily result in a full crash, and other related metrics. Other culture health metrics can be used to refine this approach, including quality of the biomass (i.e., concentration of the desired constituent, such as a lipid) or end product. Sequencing or microscopy could be leveraged for quality control to show the dominance of desired species to measure pond health or the percentage of contamination with competing algae or pests. In the case of invasion with a phenotypically similar species, however, microscopy might not be sufficient. Additionally, the time to recovery once the pest has been remediated is a useful indicator of culture health.

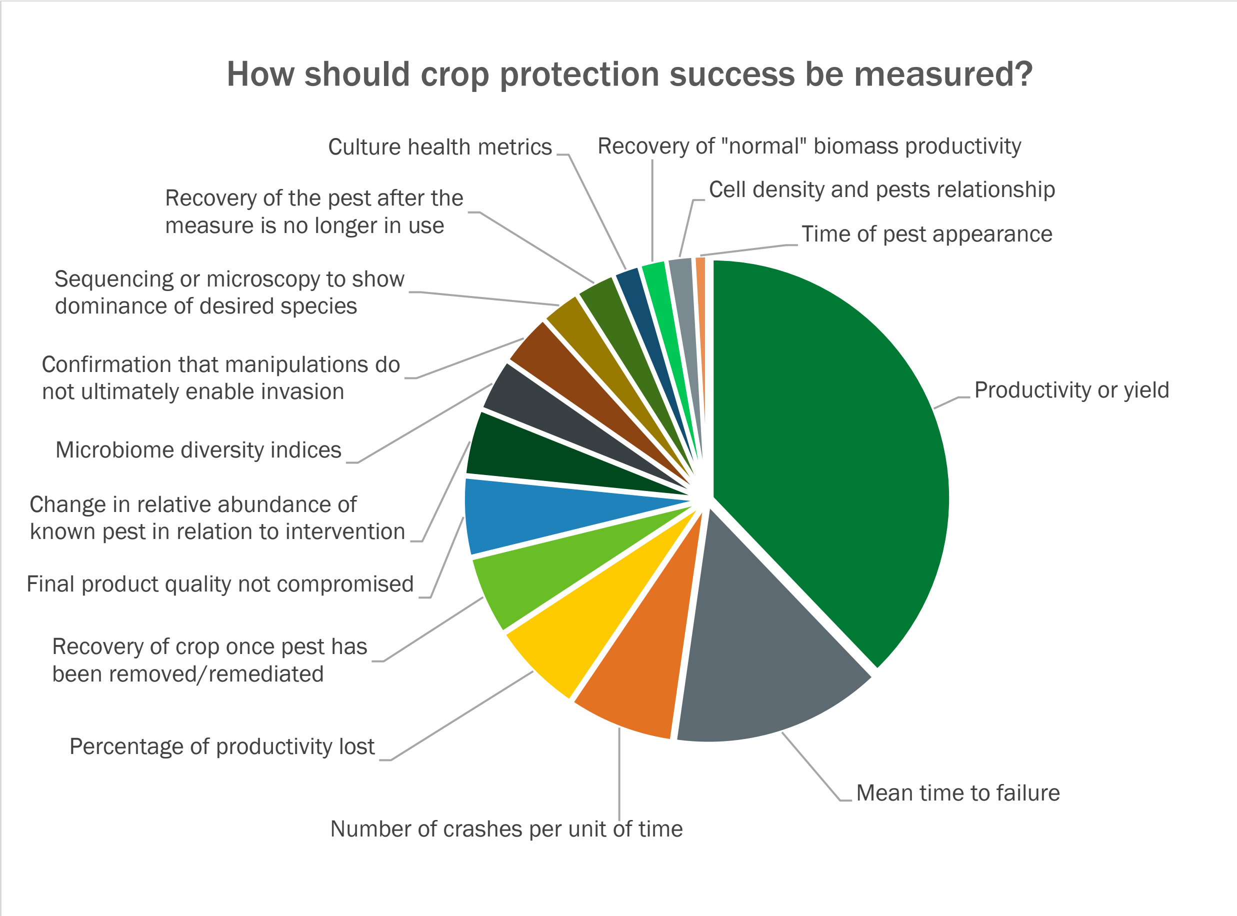


Figure 5. Participants were asked, "How should crop protection success be measured?" Then participants were asked to cast votes for the most impactful responses.

After annual productivity, the next most popular crop protection metric was mean time to failure. Failure would need to be specifically defined in this case, such as a measurable percentage of contamination or a full culture crash/complete loss of productivity. A similar measurement is the number of crashes per unit of time. For a polyculture, a failure might be more difficult to measure than in a monoculture, when it is clear that the strain of interest is no longer dominant. Additionally, a culture crash might not occur for all pest examples. Pests that compete for resources or simply reduce productivity are still problematic even if a total crash is not observed.

Instead of measuring the algae species of interest, a measurement of pest populations could be another means to determine the success of a crop protection strategy. Monitoring the change in the relative abundance of a known pest in relation to the intervention or monitoring the recovery of the pest after the crop protection measure is no longer in use could be accomplished via quantitative polymerase chain reaction (qPCR) of total DNA samples from ponds. In this way, the algae grower can confirm that the crop protection measure prevented the invasion and dominance of pest species. Success could be measured as the difference in deleterious pests before and after the mitigation strategy or as the change in the growth rate of the pests.

Any of these measurements would need to incorporate a baseline or before/after control intervention framework of analysis to compare an untreated control to a treated pond over time; however, outdoor algae cultivation baselines can be difficult to establish because of inherent weather and other unmitigable environmental conditions. Also, the question arises as to how long an operator needs to establish a system to get a meaningful baseline for a specific strain and growing season. When first growing a novel strain, there is often a period where pest issues are not observed, presumably prior to a significant local pest population being established; thus, determining the baseline from the first day of cultivation might not be realistic, and using a mature pond might be more effective. AzCATI is trying to run strains as long as possible, past their optimized season, but they have limited resources and limited pond space, and often data and information are left without being collected. It is difficult to replicate outdoor growing conditions in the laboratory, especially where pest experiments are concerned. There is limited prior art in the algae field, and there is very limited information on algal/microbial community dynamics in the natural environment. Even if more ponds were run concurrently for long periods of time, it would be difficult to pinpoint root causes without a better understanding of population dynamics.

Research and Technology Gaps

Participants were asked to list and rank the key gaps in algae crop protection research or technologies. The top answers were closely ranked, and many answers were provided, emphasizing the key takeaway that there are many gaps and a general lack of fundamental knowledge in this research area (Figure 6). An underlying theme that was common to many of the responses was of a lack of understanding system diversity and how organisms interact with each other. The highest ranked gap was the lack of understanding of the interaction effects of cultivation variables (e.g., temperature, light, salinity, nutrients). The next most popular response was the lack of communication and collaboration among scientists who are focused on basic science research and among those who are working on applied technologies and on growing algae outdoors. The ability to rapidly identify new pests as well as beneficial microbial actors in algal ponds is also a key research need. This could take time because pond systems are very dynamic and their populations change rapidly, and many pests are discovered only after a culture crash. The environmental sources and the broader ecological roles of pests are also not well understood. A difficulty for all crop protection research in a relevant outdoor environment is that it might not be possible to establish a pest-free baseline for algae productivity measurement.

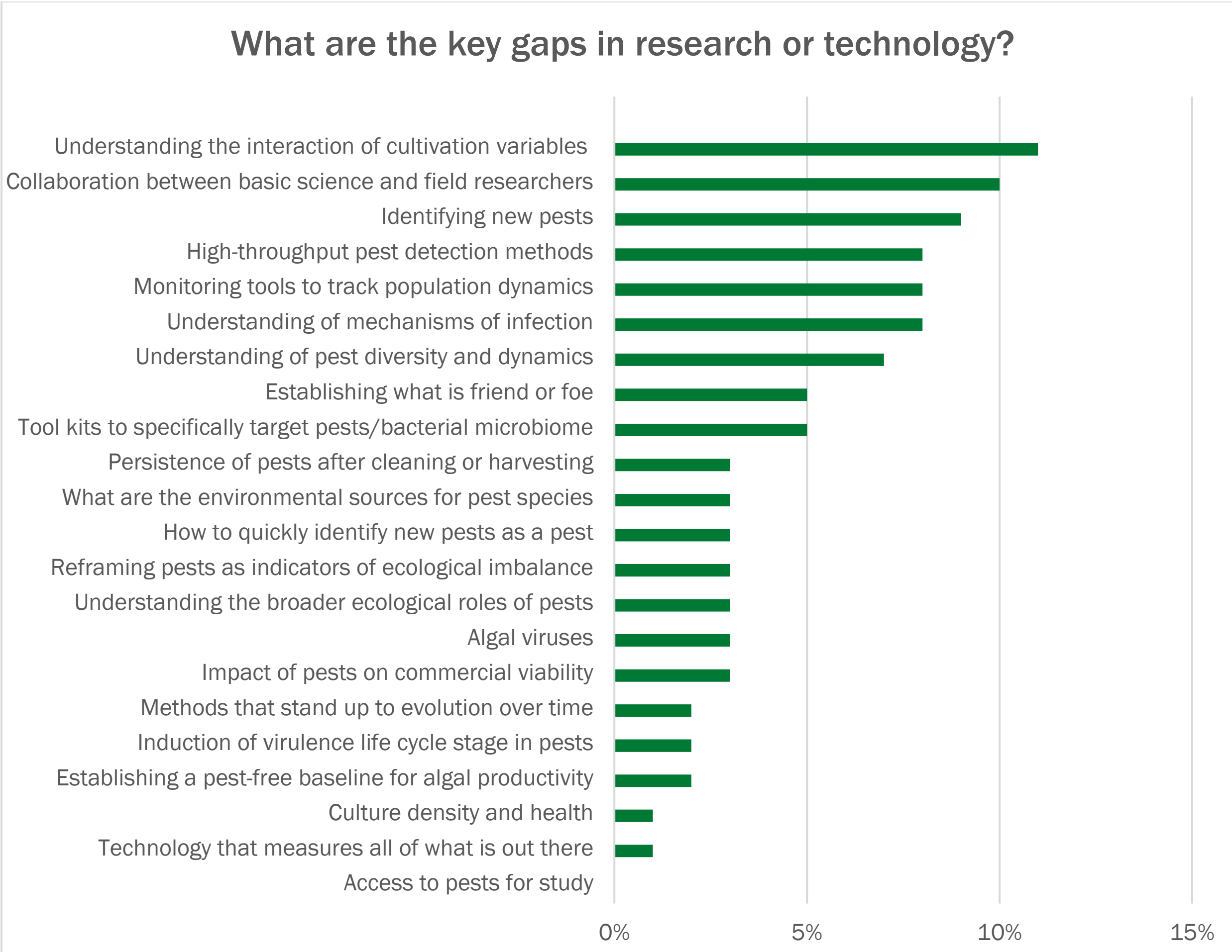


Figure 6. Participants were asked, "What are the key gaps in research or technology?" Then participants were asked to cast votes for the most impactful responses.

To improve pest discovery time, novel methods are needed, perhaps including machine learning. A better understanding of the mechanism of infection for key pests could aid the development of pest detection and mitigation methods. Key technology needs are high-throughput pest detection methods, particularly improved monitoring tools that can track culture population dynamics during a crash as well as genetic tool kits that are capable of specifically targeting pests and the bacterial microbiome. Potentially, methods focused on discovering population correlations rather than those focused explicitly on the identification of individuals could improve detection rates, reframing pests as indicators of ecological imbalance. Crop protection methods need to stand up to the power of evolution over time, as is often a problem in traditional agriculture, for example, where pests have evolved resistance to pesticides.

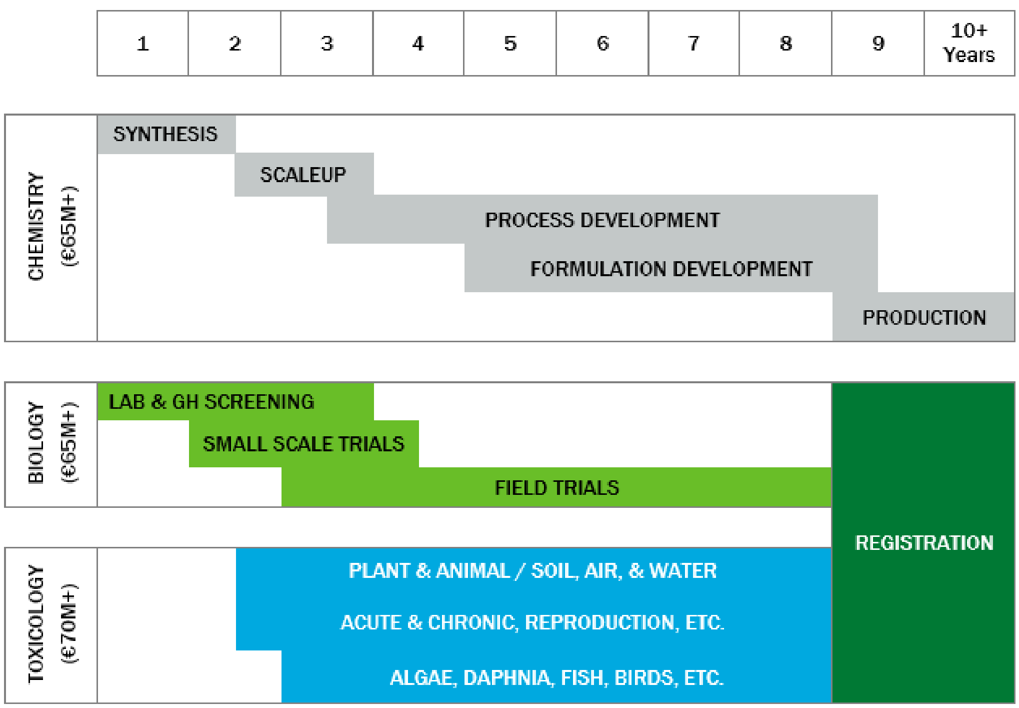
Session Summary

The key messages from this discussion session are that pests are a significant issue for all algal ponds, and we do not currently have sufficient strategies to combat them. Measuring a pest's impact on productivity is the best metric of success of a crop protection strategy, but mean team to failure and the number of crashes over time are also helpful in describing pond health conditions. Key barriers to developing successful crop protection strategies are replicating field conditions in the lab, understanding the native microbiome, and the high costs of interventions. Because of the complexity and diversity of algae and pests, one solution might not be widely applicable. The current gaps in crop protection research are numerous. Better pest-monitoring and identification methods, deeper understanding of pond ecology and the interaction of environmental variables, and improved communication among researchers are all needed to realize commercial-scale, advanced algal systems.

Alternative Crop Protection Approaches to Chemicals and Pesticides

Topic Introduction

The next session focused on alternative crop protection approaches to chemicals and agricultural pesticides. Agricultural pesticides, such as fungicides and herbicides, are obvious solutions for a farmer looking to protect their crops. It is relatively easy to react to a potential short-term loss by adding these to an algal pond; however, there are multiple benefits to alternative crop protection approaches that do not require the application of chemicals. Developing crop protection chemicals can be prohibitively costly. In traditional agriculture, it can take more than 10 years and cost between $100–350 million to develop and market a new agrochemical (Figure 7) (Nishimoto 2019, D19–101). In addition to costs, regulations, operator safety, consumer acceptance, ecological and environmental concerns, and eventual pest resistance can make the use of pesticides an unattractive option for the industry. Alternatives to chemicals can include mechanical (e.g., paddle-wheel speed, pumping, cavitation), operational (e.g., pond alkalinity, harvest timing, nutrients), and biological (e.g., releasing rotifers to consume weed algae, breeding for resilient strains) approaches.



Adapted from: Innovation: the secret of our success (BASF, 2018)

Figure 7. Timeline for the production of agrochemicals.

Panelist Presentations

Rhona Stuart, from Lawrence Livermore National Laboratory; Kimberly Ogden, from the University of Arizona; and Jeremy Guest, from the University of Illinois Urbana-Champaign discussed their crop protection strategies, ranging from biological to mechanical controls. The panel discussed that changes to aspects of the cultivation system might create selective pressures that could be managed for successful outcomes and that the microbiome might be manipulated for crop protection. The panelists noted that alternative approaches present financial costs as well as regulatory implications for commercial algae cultivation.

Biological Control of Pests: Consider the Microbiome, Rhona Stuart (Lawrence Livermore National Laboratory)

Rhona Stuart's research focuses on applying biological controls to manage algal pests, particularly grazers and, more recently, parasitic fungi. Her project at Lawrence Livermore National Laboratory is developing a protective bacterial application to deter rotifers that could be appliable to other grazers. Her team found that the protective bacterium introduced to the system does not negatively affect the microbiome, and because it is a soil bacterium in an aquatic setting, it does not persist for longer than it is useful. She emphasized that the algae field is behind the curve on the discovery of biological control solutions, and there could be many ways to proactively manage the microbiome to protect species of interest. For example, by examining the needs of parasite organisms and simply providing them—such as exuding a source of nutrients—the pest could be induced into a nonpathogenic life cycle. More modeling is needed to investigate the impacts of these biological crop protection applications.

Alternative Crop Protection Approaches to Chemicals and Pesticides, Kimberly Ogden (University of Arizona)

Kimberly Ogden reviewed pond crash data from the Regional Algal Feedstock Testbed final report (Ogden et al. 2019). A total of 272 cultivation experiments were completed at the three outdoor test bed locations during a 3-year period. The team isolated *Vampirovibrio chlorellavorus*, which they found to be a pest specific to *Chlorella*, prevalent in the summer seasons, and capable of bringing down cultures. The close relationship with the *Chlorella* strain made it easy to study. In addition to biocide application, the team used their pond design to manage the pest. By creating a reservoir at the end of a paddle-wheel pond, they were able to control temperature as well as oxygen levels. During the light period, the culture was propagated in the raceway; and in the dark period, it was stored in a canal reservoir to oxygenate and mix, which reduced the pest population to low enough levels that would no longer impact the algae culture. While targeting the manipulation of the culture temperature, the team discovered that the dissolved oxygen level was an important factor in pest control as well. This highlights that there are many unknowns that might be discovered by initiating research on a pest system.

Selective Pressure as a Resilient Approach for Algae Crop Protection, Jeremy Guest (University of Illinois Urbana-Champaign)

Jeremy Guest's research on photobioreactors for treating wastewater focuses on creating a competitive advantage for mixed populations of naturally occurring microorganisms with a desired function rather than focusing on specific strains of interest. For wastewater treatment, this means targeting nitrification, denitrification, and phosphorus accumulation through selective pressures, such as biomass age, retention time, dissolved oxygen, pH, and temperature. The system results in mixed communities that have natural variability over time. The local conditions (wastewater composition, weather, etc.) will influence how the microbial community develops. Developing an understanding of the biological system in a highly controlled system was important to this work, and hopefully it will enable future understanding of more complex and less controllable systems.

Barriers to Pest-Resistant Strain Research and Development

During the discussion portion of the session, participants were asked to identify and then rank the key barriers researchers face in conducting R&D focused on developing pest-resistant algal strains or communities, specifically the reasons the R&D community is not developing pest-resistant lines through genetic modification, evolution, or breeding. The most popular response was the inherent lack of understanding of the native microbiome and the roles and functions community members have in keeping the culture stable. Natural community assemblages are varied at different scales, making predictions in the laboratory difficult to apply to field conditions, and there is a lack of long-term community dynamics data. The next responses were closely ranked. Participants identified the need for better genetic tool development (e.g., for transformations, breeding, and directed evolution). The long-term use of genetic modification work is hindered not only by regulators but also by community groups and consumers of the end products. Another key issue is that there are numerous algal strains in the field. Strains of interest vary among researchers and are changing over time as strain selection projects mature, so there is limited long-term investment in crop protection research for those strains. Similarly, the community has also not decided on a pest to target. It is not well known what pests are prevalent geographically and per growth condition/season, and it is not clear that solutions that mitigate one pest would be applicable to others. Additionally, targeting algal strain resistance to one pest or mitigating a single targeted pest might make a strain susceptible to another. Selective pressures might also cause pests to adapt and overcome any mitigation efforts or strain-resistance traits in algae. Participants noted that they believe there are currently no reliable pest models to accurately and predictably measure algae resistance. The ranging conversation emphasized that a number of variables have prevented a sustained research program focused on improving strain pest resistance; however, these were predominantly technical issues that could be overcome, and the concept of developing pest-resistant strains remains a viable research direction and crop protection solution.

Nonchemical Strategies

Participants were asked what nonchemical crop protection strategies are viable for commercially relevant algae cultivation operations. Participants focused on three key categories of alternative approaches to chemicals (biocides or pesticides): biological mechanisms, such as designed consortia and selecting for or developing resilient strains; mechanical/operational strategies; and altering the culture media. As in the prior session, the participants prioritized the biological approach involving a consortium of organisms with collectively robust and resilient characteristics to achieve a target function (e.g., high growth rates and desirable biochemical compositions). Algae cultivators could leverage ideas from niche theory/coexistence theory to design communities that are *a priori* likely to coexist over time; have complementary traits that induce overyielding; and confer resistance to grazers, pathogens, and invasion by algal competitors. Mechanical/operational strategies include filters to remove larger pests, physical disruption to destroy weaker pests, and moving ponds with pumps to mitigate rotifer infections. Changes to media composition can include altering pH or adding inorganic compounds (e.g., calcium, salt). Breeding, directed evolution, or genetic modification of pest-resistant strains would help to reduce the need for crop protection interventions.

Overcoming Barriers

Participants were then asked what would be needed to overcome the technical barriers related to the three categories of alternative approaches: biological, mechanical/operational, and media design.

To effectively implement biological mechanisms to crop protection, research and tools are needed to better understand the roles, mechanisms, and community interactions in the microbiome and members of the phycosphere, both beneficial and harmful. Sourcing crashed cultures and isolating pests remains difficult, but it could be helpful in this research. It is difficult to draw parallels with terrestrial crops when crafting research plans because turnover in algae cultivation systems is many orders of magnitude faster, and the establishment of community members is fundamentally different. Research focused on how to create the right conditions in the growth environment to allow a competitive advantage for target functions among the population members, yielding pest-resistant and productive communities, would be beneficial in this area. In order to manipulate which species are present in the microbiome, a better understanding of what each community members require is needed. Biological control agents, particularly those that do not persist in the culture, could be leveraged to prey on pests, but this requires significant investigation because some biological control agents have eventually turned into pests themselves in other systems (e.g., becoming invasive species). A better understanding of how pests recognize the host is needed at the molecular level to enable the development of methods to interrupt this step.

Mechanical methods that have proven effective and could be more widely applied include filters to remove larger pests and physical disruption to destroy weaker pests. Using pumps for pond motivation (instead of paddle wheels) has proven effective in mitigating rotifers. Harvesting systems could be improved to remove all organisms in a culture to prevent subpopulations from being returned to the cultivation area when recycling media, which could inadvertently create a selective pressure for pests. All mechanical approaches would benefit from efficiency improvements to limit their costs, and they need to be tested at commercially relevant scales.

Overcoming barriers to implementing media design strategies would require a better understanding and ability to predict the chemical composition of growth media and water quality for large-scale production, with integrated water and nutrient recycling, (i.e., the steady-state water quality of an algae farm). This requires testing the viability of different commercially relevant media compositions and water chemistries over time and under varying conditions, including water/media recycling. This research would benefit from more reproducible pest models for testing different media compositions; advanced sensors and control strategies for key nutrients and additives; as well as a shared and open database of which common pests are sensitive to certain changes in pH, salinity, and other media variables. To best develop media composition strategies, the trade-off between media constituents and cost, their relative effect on pests, as well as productivity and product specification will need to be considered.

Session Summary

The key takeaway from the discussion session is that there is a fundamental lack of understanding of the microbiome and how population dynamics are affected by the introduction of pests. Key strategies to crop protection include changes to media composition, changes to the biological composition of the community, and improvements to mechanical harvesting and operating parameters. To realize these strategies, testing needs to be done under commercially relevant scales and environmental conditions for a meaningful length of time to determine the most economically efficient methods. The costs of these methods need to be weighed against the productivity benefits they can offer.

Pest Models: Understanding Pest Life Cycles and Infection Mechanisms

Topic Introduction

The third session of the workshop focused on pest models to design and inform crop protection strategies. "Pest models" can refer to several things—for example, a model of what happens in the field, a model of an approach to use for similar future infestations, or a model organism that can be used to answer questions about similar organisms. In this workshop, anything that allows research questions to be asked and answered can be considered a model. BETO-funded research has benefited from working with models to develop strategies for addressing pests. Advancing and sharing these models is necessary to further our understanding of pest life cycles and infection mechanisms.

Panelist Presentations

Panelists were Todd Lane, from Sandia National Laboratories; Shawn Starkenburg, from Los Alamos National Laboratory; and Timothy James, from the University of Michigan. The talks included observations by the panelists that strain collections contain few pest species, but better information and ways to store and share pests could be beneficial to advancing the state of the art. They highlighted that genomics tools are becoming increasingly available and, when applied to pests, can greatly accelerate research. Pests can be divided based on broad or narrow host ranges, and techniques of study can be separated into applied and basic. These divisions could help direct future research efforts.

Crash Agent Model Systems for Crop Protection, Todd Lane (Sandia National Laboratories)

Todd Lane described crash agent model systems for crop protection as studied at Sandia National Laboratories and the difference between applied systems, which isolate agents (pests) from pond crashes and are developed for a specific field application (e.g., algal species, environmental and physiochemical parameters) with characterization limited to only what is necessary, and basic systems with a well-defined and characterized host and pathogen used to understand the basic mechanism of their interactions. Strain collections contain very few pest species and are not reflective of the true diversity of pest species present and can limit the applicability of basic model systems. Applied model systems can help to identify a wider diversity of pests; determine their growth conditions; and support the testing of crop protection strategies, such as alternative cultivation regimens, biocides, cocultures, and the development of resistant phenotypes. Translating research in model systems depends on the host range of the threat species (whether narrow, such as *Vampirovibrio chlorellavorus*; or broad, such as rotifers) and the effective range of the countermeasures (narrow, such as viral remedies; or broad, such as the antifungal fluazinam).

Genomics Tools and Challenges, Shawn Starkenburg (Los Alamos National Laboratory)

Shawn Starkenburg described how genomic characterization can help to elucidate pest infection mechanisms. He first studied the *Chlorella* pest *Vampirovibrio* (with the Regional Algal Feedstock Testbed). This work was accelerated because *Vampirovibrio* had been recognized in previous studies in the 1970s and was already well characterized. Later, it was sequenced, and the mechanism of infection was determined—it was driven by the secretion system to consume components of the algal cell. This work highlighted that leveraging prior knowledge can accelerate pest research; thus, generating a knowledge base now for any pests could benefit future research on the same or alternative pests. Next, the team started isolating specific *Vampirovibrio* strains affecting ponds in Arizona. After creating enrichment cultures, they found that the genomic content was more variable than anticipated, and the diversity was greater than was known at the time. There are several varieties of *Vampirovibrio*, and they are not equally deleterious. The team started tracking the genes for the Type IV secretion system and raised the question as to which ones will actually impact algae cultures. Currently, the team is using metagenomic tools as well as ProxiMeta—proximity-guided (Hi-C) assembly of complete genomes—to identify novel pests and to identify pathogens for *Nannochloropsis* using molecular diagnostics tools. The team has assembled the majority of the genome for one pathogen and developed field-deployable sensors to detect genomic signatures in ponds. There are a number of challenges to deploying genomic tools to crop protection. Narrow host ranges limit the extrapolation of knowledge to other production strains and has implications for the type and quantity of model systems. Also, there is a lack of public genomic data for both pest and pond communities to baseline a healthy environment or to distinguish closely related pathogenic and nonpathogenic species. The field could benefit from a common shared knowledge resource to facilitate this work. Field-deployable (cheap) molecular diagnostics tools and more deployed functional omics assays to characterize organisms are also needed.

Developing Resources to Address Chytrid Fungal Pests of Microalgae, Timothy James (University of Michigan)

Timothy James described his work developing resources to address chytrid fungal pests of microalgae. There are challenges to developing a model pest system. It is difficult to culture pests because they can grow weakly in laboratory conditions. Those that can be cultured might not be the most important pests in larger outdoor cultivation systems. Also, taxonomic expertise of fungi and chytrids is waning, and intellectual property rights can impede research; however, opportunities exist in developing partnerships among industry, government, and academics, and several parasites are emerging as clear model systems. The natural ecology of pests has been underexplored, but it could provide greater insights into artificial systems. There is opportunity to expand the research of fungal diversity and biology because zoosporic fungi are major pests of microalgae as well as other important organisms. Systematically collecting parasites and support for culture collections are needed. The Collection of Zoosporic Eufungi at the University of Michigan was supported by the National Science Foundation and includes 1,149 strains cryopreserved in liquid nitrogen, but it contains poor representation of algal pests because they are difficult to culture, and large sections are proprietary and cannot be distributed. The collection is only 3 years old and is still working on defining its niche. Although the collection has a public interface, it is not currently used as an applied R&D resource.

Pest Model Systems

During the discussion session, participants were asked whether they believe there is value in research based on a "pest model" to transfer knowledge of pest interactions and mitigation strategies (e.g., knowledge of a fungal pathogen that only infects *Hematococcus* but is well understood that could be applied to fungal pathogens of lead candidate strains). Participants largely responded that pest models publicly available to researchers are limited and more would be beneficial (Figure 8); however, discussion included caution that some models might have limited applicability to pests that are taxa specific or dependent on environmental conditions (e.g., organic load, light, biodiversity, physiochemical parameters). Pests can also change over time in a culture, so they are difficult to characterize. There are characteristics of culture field deployment that are difficult to replicate in the lab or with models. Very little research on models has been done for micro- or macroalgae because it takes time and resources to cultivate the system and to isolate the pest-alga interaction to collect sufficient data.

Pest models that do exist are not typically in the public domain because companies are hesitant to share information, and there is no central information repository—researchers must piece together literature or contact growers directly. Additionally, there is a lack of funding for pest research, particularly for basic science, and there is a lack of researcher training for cultivation and taxonomy. A basis of understanding of clinical signs of pond health in the field are required, and model organisms and operational approaches can help to develop and refine that basis; however, the algae field requires more tools for organism monitoring and characterization, in real time and/or in high throughput, to develop the data basis for model development.

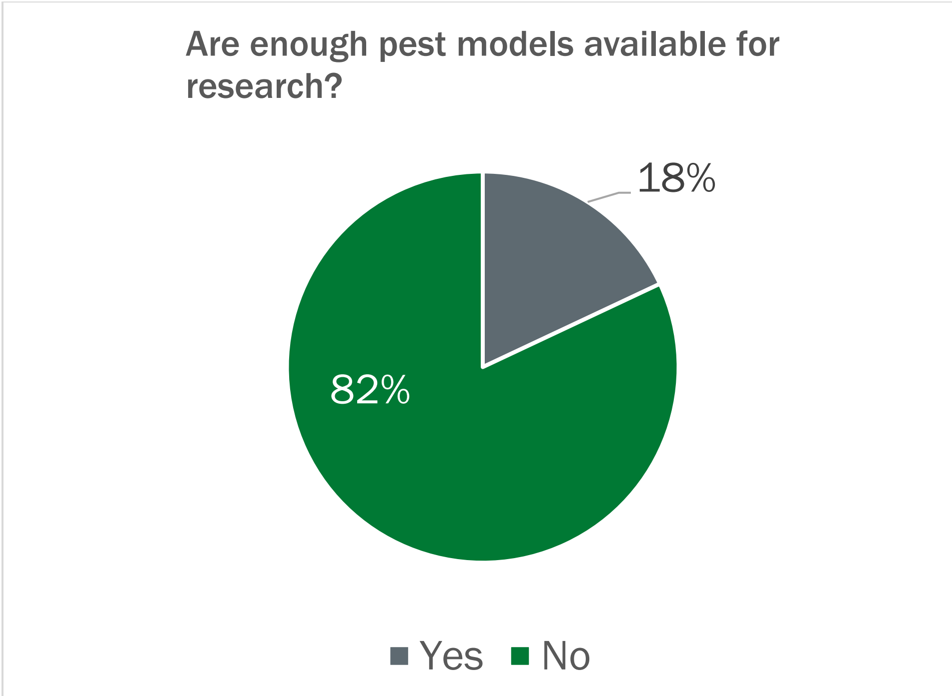


Figure 8. Participants were asked, "Are enough pest models available for research?" Possible responses were yes or no; 23 participants provided ratings.

Priority Research Gaps

Participants were then asked to list and prioritize the current research gaps that need to be addressed to create and refine serviceable pest models. Prioritization accounted for both the feasibility and the impact of addressing each identified research gap. The highest-priority gaps were early-detection methods as well as field-deployable tools for identification, monitoring, and modeling that are low cost, fast, and easy for field operators to use. The next highest priority gaps for research to address were the ecology and biology of fungal pests, particularly pest-host-environment interactions. Omics data would also be necessary for many models. There are few genomes, proteomes, and transcriptomes available to understand mechanisms beyond basic life cycles, and there is no public genomic repository for pest and pond community members. There is also a lack of developed assays with pests to gauge infection, death, and/or productivity declines as well as biotic and abiotic drivers of susceptibility and resistance. Models require sufficient data upon which to base assumptions; and more baseline data are needed for the diversity of possible pests, particularly on fungal and viral pests, as well as on the effect of environmental conditions on pest infectivity.

Priority Research Barriers

After identifying pest model gaps, participants were asked to identify and prioritize the barriers to addressing these research gaps. Prioritization accounted for both the feasibility and the impact of overcoming each identified barrier. According to participants, the main barrier to closing modeling research gaps, particularly garnering sufficient data, is that it is very difficult to isolate pests in culture samples with existing methods, and new method development might be needed. High-throughput, less time-intensive methods of detecting and quantifying pest infections are needed. The unpredictable nature of pest presence over long-term cultivation makes the timing of sampling difficult, and it is difficult to obtain crashed culture samples for isolation and characterization. There is a lack of mechanisms to distribute samples and data from test bed facilities. Additional barriers include insufficient funding to focus on pest model development, lack of time and expertise needed for detailed pest studies, and insufficient communication between algae industry members and algae researchers.

Research Techniques

Participants were asked what techniques can be applied to overcome the research barriers identified in the prior exercise and to consider what methods can be applied to better understand pests, what current tools can be adapted for pest research, and whether new methods and tools are required.

To develop cheap and field-deployable tools for pest identification, monitoring, and modeling, qPCR, RT-qPCR, optical spectroscopy, or lateral flow assay techniques can be applied once the specific pest is known. To better understand the ecology and biology of pests, ecologists are needed to support life-cycle lab-to-field studies leveraging functional genomics and visualization tools (microscopy). To effectively research and more deeply understand pest-host-environment interactions, there needs to be an expansion of existing model systems along with a combination of culture-independent and culture-dependent techniques: microbiology and lab inoculations, metabarcoding (or metagenomics), Fluorescence in situ hybridization (FISH), colorimetric metabolic assays/indicators, metabolite profiling of the culture medium, and single-cell genome sequencing. Centralized repositories of these data would be beneficial to the community. It is also likely that the field requires more data mining on existing data sets, in addition to increasing the depth of data being collected at test bed sites, to elucidate trends in the consistencies of pests as well as algae responses to pest presence. These trends can help to identify situations where pests are present but the algae appear to be resistant and to identify when that same relationship becomes virulent.

Early detection methods can include the loop-mediated isothermal amplification assay (from Todd Lane’s presentation), optical methods including remote sensing, nanobioprobes specific to certain metabolites, continuous monitoring probes, and flow cytometers calibrated for certain pests. In general, larger samples help with early detection in ponds, but there needs to be a better way to process large samples.

To address the barrier that it is difficult to get samples and isolates, there needs to be improved long-term storage for sharing samples and optimizing and standardizing lab conditions and methods for collection. Publicly funded research could have a requirement for the deposition of isolated pests into a culture collection (in the same way that some funding requires making omics data or novel strains publicly available). To develop assays with pests to gauge infection, death, and/or productivity declines, markers for programmed cell death and immune response (in both pest and algae to gauge mitigation success) can be leveraged. Higher throughput plate-based assays can be used to monitor algae productivity with additions of different concentrations of the pest, but this requires an "isolated" pest or killing consortia that can be reproduced.

To improve communication between industry and researchers, there must be incentives for collaboration and a way to keep business-sensitive information private. Industry can benefit from researchers working on their mitigation strategies, and researchers can work to anonymize data in publications or to design the research to protect commercial interests while not hindering academic progress. Industry will likely have limited interest in partnership until funding opportunities include emphasis on products other than biofuels.

Session Summary

In summary, participants emphasized that developing pest models takes time and significant data collection effort, but more models are needed to advance the industry. Historically, prior work on pests has helped to inform and benefit current pest-mitigation strategies, and it is promising that the same pests are often seen across sites. Generally, sharing data and information through central, public pest culture collections could help to advance the field by providing baseline model data. This requires improving efforts in isolation and culturing, cryopreservation, and the establishment of data repositories as well as ways to protect commercially sensitive information.

Current and Future Pest-Monitoring Practices

Topic Introduction

The final session of the workshop covered current and future pest-monitoring practices. Pond-monitoring approaches vary by scale, from physically observing pond appearance and characteristics such as smell and color, to small-sample observations of the presence of larger pests (e.g., rotifers by eye or at low magnification), to quantitatively monitoring the presence of small rapidly infectious pests at the molecular level (e.g., qPCR for zoosporic fungi). Some of these approaches have been automated (e.g., imaging cytometers), but the commercial production of algae will likely require rapid, automated, and multifunctional monitoring of pond conditions (if this can be economically realized). This session sought to discuss whether more pest phenotypes could be quantified in a cost-effective manner for a predictive crop protection tool and whether this would be a replacement or an augmentation of current monitoring methods. Some existing monitoring methods might be more suited to research than commercial algae production.

Panelist Presentations

Jerilyn Timlin, from Sandia National Laboratories, discussed spectroradiometric monitoring methods; Natalie Cookson, from Quantitative BioSciences, Inc. (QBI), reviewed the capabilities of QBI's water-quality monitory system; and Ryan Simkovsky, from the University of California San Diego, described a mass spectrometry-based detection tool and plans for field deployment. Each presenter emphasized the need for real-time *in situ* monitoring for the early detection of chemical and physiological changes to a culture that would indicate a crash is imminent.

Outlook on Crop Protection, Jerilyn Timlin (Sandia National Laboratories)

Jerilyn Timlin, from Sandia National Laboratories, described her personal outlook on the future of crop protection. She advocated for the importance of both pest-based and host-based monitoring—but not necessarily pest specific and host specific. In many cases, "specific" monitoring strategies can be limiting because of the large number of unknown pests and, importantly, molecular variants. Species-agnostic strategies could be very successful for surveillance and screening by first conducting a broad survey of pond health, then by diving deeper with screening. Early detection will improve any mitigation strategy. In response to species-agnostic pond-monitoring needs, Sandia is developing a spectroradiometric monitoring method that essentially measures the sunlight absorbed and reflected by the contents of the pond. Timlin's team can generate characteristic spectra of the community members of the pond and detect when the spectrum is altered as a result of algae stress. This work began in the lab in 2010 before being moved to a small greenhouse. From 2011–2013, the technology was deployed at Sapphire Energy and AzCATI to simultaneously monitor six ponds and to measure biomass productivity. The system could also observe things affecting the culture prior to crashes, so Sandia started developing methods to exploit the tool to monitor for pest presence. This work was published in 2020, and now the team is seeking to expand this work to additional pathogens while working on controlled crashes in the Sandia crash lab. The advantages of spectroradiometric monitoring are that it is a real-time, *in situ* rapid test with no sampling and no lab access required, and the device does not require extensive calibration. It can also be used at night to monitor respiration. The system's current limitations are that its detection is nonspecific of functional effects on host algae, and it requires characterization of algal optical properties. Cyanobacteria and green algae are easily distinguishable, but similar green algae might be more difficult to differentiate; however, the device detected a diatom at a single-digit percentage contamination in a culture. There is currently a small database of monitoring results, but Sandia is working to increase the data and information available. In the future, the method could be incorporated into a cell phone, drone, or other field-deployable device to monitor commercial-scale algae cultivation.

A Customizable Biosensor for Real-Time Water Monitoring: Applications in Algae Systems, Natalie Cookson (Quantitative BioSciences, Inc.)

Natalie Cookson provided an overview of QBI, a small biotechnology business focused on the development of water-quality technologies for treatment and sensing. QBI has been testing a biosensing platform to monitor water for a suite of contaminants and other targets of interest. The "Qube" biosensor platform uses microfluidic technology to house many sensor strains that fluoresce in response to specific targets, translating the cell responses into a quantification of the targets of interest in the water. QBI uses synthetic biology to develop the sensor strains and has demonstrated the ability to design strains for specific needs. The biosensing strains take 2–3 months to develop and then another few months to test in the laboratory. The Qube can currently detect heavy metals and nutrients in the range of parts per billion. It is customizable and expandable with the ability to pattern up to 100 strains on a single chip. Cells start fluorescing within 5–10 minutes of exposure. Qube runs continuously and immediately provides results without taking samples back to the lab. It was originally funded by the Defense Advanced Research Projects Agency to detect drinking water contaminants, and recently QBI has been looking into algae monitoring as a target for initial market entry. QBI has been validating the sensor at AzCATI for real-time ammonia monitoring. Nutrient sensing can be used to optimize growth. Currently, the team is working to ensure that the Qube can handle harsher weather conditions, and the team is interested in broadening the application to pest detection and early warning for pond crash conditions. Future work could expand the sensor to detect the secretion of fatty acids/carbohydrates and to test the application of nanobodies to detect viruses and bacteria.

Application of Chemical Ionization Mass Spectrometry for Rapid Multivessel Monitoring of Algal Health and Grazer Infection State, Ryan Simkovsky (University of California San Diego)

Ryan Simkovsky presented on his work applying chemical ionization mass spectrometry for the rapid monitoring of algal health and grazer infections. This project started 4–5 years ago based on anecdotal discussion of operators being able to smell changes in ponds before they crashed, so Simkovsky's team developed chemical ionization mass spectrometry techniques for monitoring this phenomenon. The team built hardware and software to simultaneously sample the headspace of multiple cultures. When the airstream (even from open ponds) comes into the machine, it is bombarded by reagent gas, and the machine can select different compounds to test for. This selection filter can be modified and designed to monitor chemicals of interest before deployment. With minimal to no wait time, the chemical ionization mass spectrometry can collect 1 spectra per second, and it can continuously run for 2 months with little intervention. The system is automated with no manual sampling or liquid sampling required, and it is scalable (it can monitor multiple sources at a time—10 sources/ponds every hour). Chemical ionization mass spectrometry has detected predation days before a crash and can use predator-specific signatures to detect them photometrically. It can also be used to monitor the general state of pond health. The team has observed circadian rhythms and signatures related to exponential growth in the data sets. In collaboration with Thermo Fischer Scientific, the team is working on a smaller, field-deployable prototype.

Need for a Pest Database

As an introduction to frame the discussion session, participants were asked whether a pest database, identification tool, or similar service would be useful. Participants were unanimous in agreeing that it would be useful, but it would be difficult to develop and might need to be quite complex. Pests can be geographically specific or specific to certain crops. Additionally, growers might be using different technologies for identification, and thus training or standardization might need to be required both at the service and at the grower sites to ensure comparable data for pest identification. It is likely that diseases and pests have gone unreported in micro- and macroalgae cultivation because many growers might not know what to look for in their cultures. Participants suggested beginning by using functions that already exist at established culture collections.

Monitoring Methods

Participants were then asked whether an algae farmer can rely on a single monitoring method (Figure 9). Participants largely agreed that although that would be nice, it would not be practical. Participants noted that any system that relies on only one monitoring method is likely weak because there is a single point of failure. Because of the diversity of pests in an algae culture, relying on a single detection method would risk missing some pests. Additionally, although some methods might be very good at predicting imminent catastrophic crashes, monitoring multiple signals will provide more accurate predictions of contaminations that do not ultimately lead to a full crash but that could impact algae productivity and product yield. Monitoring multiple parameters can also help to minimize false positive results and user bias.

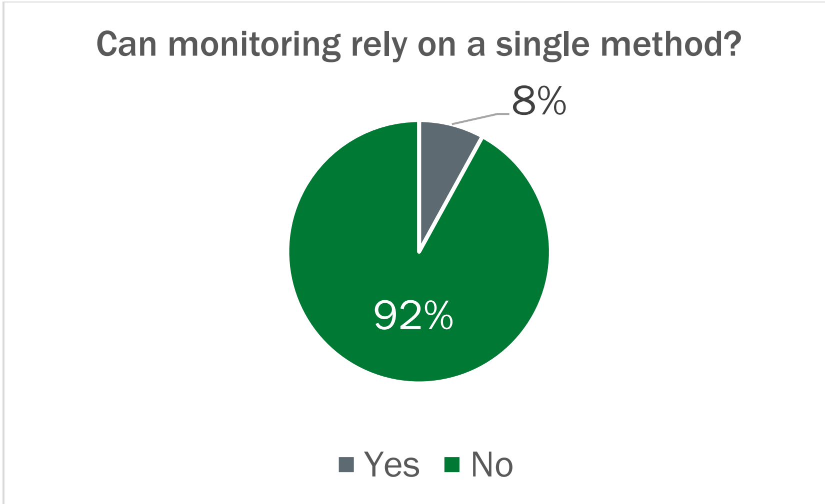


Figure 9. Participants were asked, "Can monitoring rely on a single method?" Possible responses were yes or no; 53 participants provided ratings.

Priority Pest-Monitoring Tool Attributes

Participants were asked to design their ideal pest-monitoring tool by listing and then prioritizing key attributes. Prioritization was based both on the attribute's technical feasibility as well as on potential impact in advancing algae R&D. The highest-priority tool attribute was robustness—at commercial algae production sites, tools must be able to be deployed in the field and used by pond operators with minimal training (Figure 10). The second highest priority attribute was cost. Tools must be affordable for wide deployment at a site, or at least able to pay for themselves through improvements to overall productivity. Another key attribute was sensitivity. Monitoring tools must be sensitive enough to detect the conditions for a crash within sufficient time that the event can be prevented or mitigated—the sooner, the better. Additionally, tools must be scalable for use both in the lab and in the field. Detection tools need to be able to monitor a diversity of pests—protozoa, bacteria, and viruses. Ideally, tools could also incorporate multiple data sources, including parameters such as weather and temperature, into their readings. Monitoring might need to focus only on pests that are doing harm or have the potential to do harm, leveraging functional presence methods rather than detecting all presence.

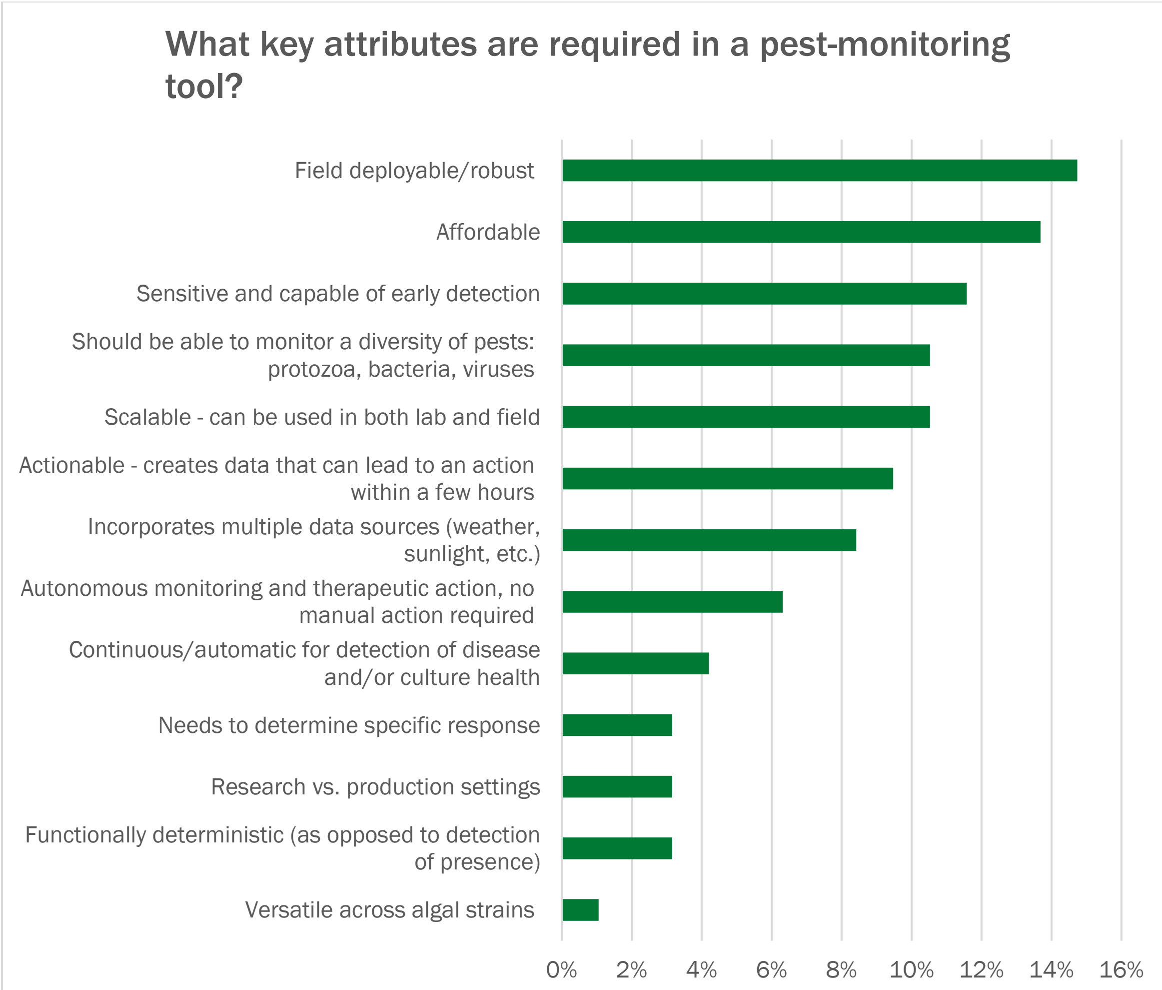


Figure 10. Participants were asked, "What key attributes are required in a pest-monitoring tool?" Then participants were asked to vote for the most impactful responses.

Priority Pest Identification Tools and Service Functions

Participants were then asked what functions would be desirable in a pest database or identification tool/service. Participants responded that the information should include the isolation method and infection assay methodology so that these can be replicated at their own site. Key information would also include the phenotype of the algal host and pest, the pest growth conditions, and information on infectious dose so that the algae grower can determine if their own algae are susceptible. As with the monitoring tools, the ability to deploy the tool in the field by a pond operator who might not be a specialist would be useful. This could take the form of a user-friendly application based on machine learning built on an image/taxonomy database, for example. Strategies for mitigating each pest would also be beneficial.

Session Summary

In summary, participants agreed that there is a need for both monitoring methods as well as a pest database/identification tool. Monitoring strategies need to be field deployable and able to detect conditions impacting pond productivity within sufficient time that mitigation strategies can be employed. Pest identification tools must be comprehensive, accessible, and complete with metadata on how the pest information was obtained.

Conclusions

Through presentations from invited speakers and facilitated discussions with participants, a number of common themes emerged as key findings of the workshop. Pests are a threat to all algae growers, and we do not currently have sufficient strategies to combat them. The cultivation of algae outdoors inherently requires active pest management approaches, and there are significant knowledge gaps in crop protection research, such as:

- The mechanism of host-pest interaction and recognition is unknown for most pests.
- A better understanding of how microbiome population dynamics are affected by the introduction of pests or how to beneficially manipulate these communities is needed.
- Few pests genomes, proteomes, or transcriptomes are available, and there is no public genomic repository for pest and pond community members.

One reason for these knowledge gaps is that there are numerous algal strains of interest, and researchers are currently identifying and developing new ones. This means that pest issues vary among growers and continually change, making pest research difficult to establish. Additionally, companies and researchers are disincentivized to share pest information because of desires to maintain a competitive advantage and to mitigate negative press about infections.

To close these gaps, the field would benefit from:

- Dedicated crop protection research conducted at commercially relevant scales and systems. This includes relevant media compositions and water chemistries under varying conditions (including water/media recycling) over a meaningful length of time, coupled with laboratory-scale omics research.
- A better understanding and modeling of the economic impact of both pests and mitigation strategies
- Improved communication and data sharing through centralized public culture collections to support modeling with robust baseline data.

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Appendix A: Attendees

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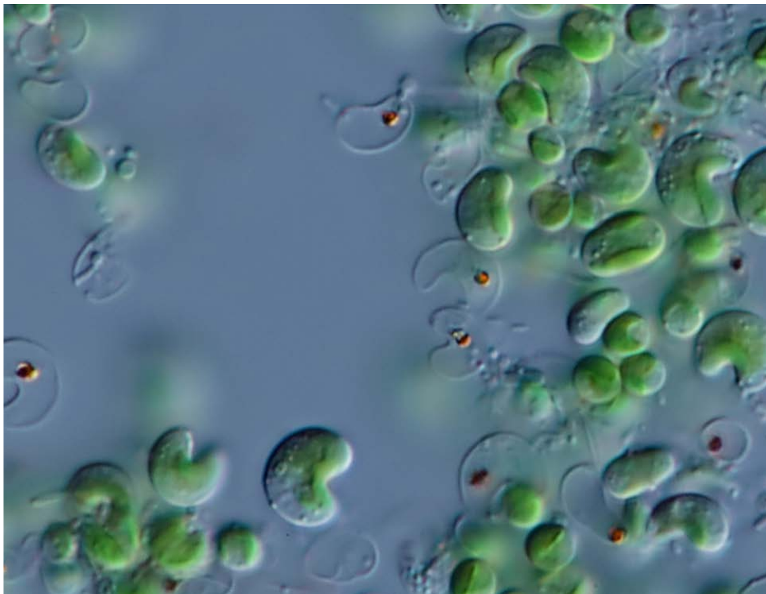

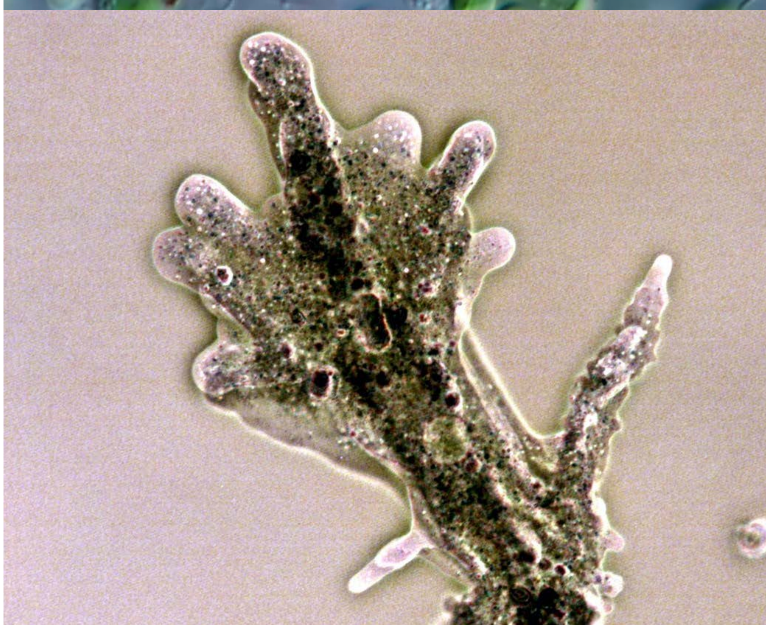

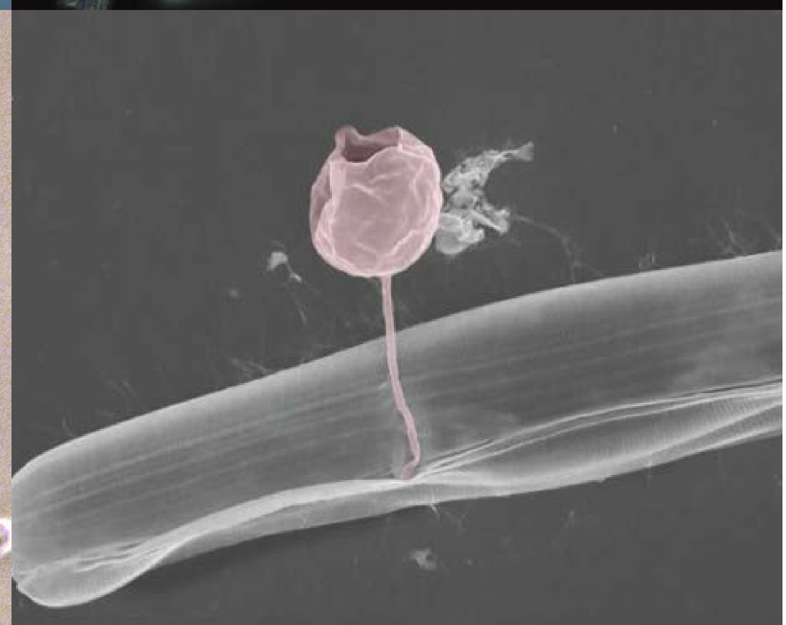

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