

Developing a National Virtual Biosecurity for Bioenergy Crops Center (NVBBCC)

M. A. Schoonen

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FWP Title: Developing a National Virtual Biosecurity for Bioenergy Crops Center (NVBBCC)

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Lead PI: Martin Schoonen

BER HQ Program Manager: Dr. Resham Kulkarni

FY24 Progress Report



Project Summary:

The goal of this pilot project is to develop a long-range plan to establish a new DOE-BER, complex-wide capability to address biosecurity of bioenergy crops, referred to as the National Virtual Biosecurity for Bioenergy Crops Center (NVBBCC). A mature NVBBCC is envisioned to be a distributed, virtual center with multiple DOE-labs at its core to maximize the use of unique facilities and expertise across the complex. NVBBCC is envisioned to support community-driven plant pathology research as well as broader BER-relevant plant biology research. The research portfolio will be carefully chosen so it is aligned with DOE mission objectives and complements existing efforts by other major stakeholders responsible for general agricultural crop health.

The main deliverables of the project are:

- A roadmap to develop NVBBCC and its research portfolio through community input
- A pilot study on a disease impacting a bioenergy crop
- A prototype computational platform to support a distributed and collaborative research effort
- An investment in a Cryo-Fib capability to advance the ability to study plant tissues and cells and their interactions with microbes and pathogens.
- An investment in state-of-the-art drone sensors to advance disease detection capabilities

Progress on each of these components during FY24 will be summarized below.

Roadmap development

The project team used community input gathered through six workshops in FY23 to write a report that summarized the input and outlined a possible roadmap to establishing NVBBCC. Prior to finalizing the report, the team presented a summary in a hybrid meeting held at BER offices in Germantown on November 13th. The report was submitted to thought-leaders within the complex as well as a leading expert within USDA for comments. The findings were also presented at the PI meeting in April. The final report was submitted to BER and OSTI in June 2024, see references below.

Anthracnose-Sorghum Pilot Study

Through a collaboration with Texas A&M University (TAMU), the team embarked on a study of anthracnose, caused by a fungal pathogen *Colletotrichum sublineola* (Cs), in Sorghum. The team obtained Cs from TAMU and developed a method for infecting single cells using sorghum protoplasts, which are cells without a cell wall. In FY24, the team optimized the protoplast isolation and infection protocols so that the method is suitable for studying sorghum-Cs interactions using imaging techniques at National Synchrotron Light Source II (NSLS-II) and LBMS. In addition, the team performed detached leaf infection experiments and developed a microdissection method for the preparation of sorghum tissues under different infection stages. In collaboration with TAMU, the team performed Cs pathogenicity experiments in the field as well as greenhouse and collected samples for transcriptomics analysis. Omics-sequencing is underway and data analysis will follow after we receive the sequencing results. The pilot study has produced substantial data for the further development of NVBBCC in the protection of bioenergy crops.

The single-cell infection work has led to the development of a cryoFIB-SEM and cryoET workflow (see cryoFIB-SEM capability). The developed workflow is being used to study plant-pathogen interactions supported by two synergistic FY23 BRaVE science projects.

Computational Platform (SciServer) Development

BNL adapted the SciServer concept, originally developed by Johns Hopkins University (see [link](#) for details). SciServer is described as “a fully integrated cyberinfrastructure system with data hosting services and integrated tools developed to support researchers to cope with scientific big data”. The system was set up and expanded at BNL as part of the NVBBCC pilot project to explore the need, scope, and development of a system that could support a mature NVBBCC. Key additions to the environment include the support for AI and HPC applications and resources (hardware & software) and the sharing of community tools. As part of this pilot study, BNL established a dedicated SciServer system, developed several use cases, and identified possible use cases in support of NVBBCC. Supporting these efforts BNL developed software infrastructure components that enable the integration with other DOE facilities and data services such as data transfer capabilities, single sign-on with other organizations and portable workflow infrastructures. Several BRaVE science projects funded in FY23, including a BNL-led effort focused on unlocking the molecular basis of plant-pathogen interaction to create resilient bioenergy crops, are now also supported by the NVBBCC SciServer system. Furthermore, other BER facilities and projects have reached out to explore a closer integration with SciServer to support their user communities and enable users to work across facilities. Three integration efforts are described briefly below.

Cryo-EM workflow integration

BNL has integrated an existing cryo-EM 3D reconstruction workflow package and extended it with a cryo-EM feature enhancement model, integrating experimental maps with deep learning enhancement and AlphaFold predictions to facilitate better cryo-EM image quality and thus enabling a better 3D reconstruction. Sciserver delivers results faster, enhancing speed and precision to support cutting-edge research and high-throughput studies, making it an invaluable tool for structural biologists to accelerate discovery. The powerful GPUs built in SciServer are able to significantly enhance the cryo-EM 3D reconstruction workflow. More importantly, by making this tool available on SciServer, BER domain scientists can benefit from this tool, making it easy for them to bring their own data from non-BNL facilities to the tool and apply it to their experimental data.

KBase integration

The integration of KBase with SciServer will enable two distinct use cases. First, it will enable researchers to combine their data with KBase data for quick prototype analysis, where KBase Apps or a data loader is not yet available, this would, in particular, support the extended use of KBase data with data from other scientific archives or projects for multi-disciplinary research topics such as those tackled in The BRaVE projects. A second use case supports the KBase developers. As in other areas, the use of large language or foundation models is also desirable for KBase; however, to train these systems you need not only significant compute capabilities, but also large fast storage capabilities. With the connection to SciServer, KBase can not only train their initial AI models, but also regularly retrain them when new data has been deposited or otherwise become available. The team has been in discussions with KBase since the GSP meeting earlier this year, early tests have confirmed the usefulness and more detailed plans are currently being developed to start a production service.

Processing and Analysis of Hyperspectral Data

As part of the NVBBCC effort, a distributed team explored the use of hyperspectral remote sensing to detect the emergence of disease within bioenergy crops. The latest generation of drone-mounted hyperspectral imaging detectors opens the way for rapid and repeated data collection to monitor crop status and see the emergence of diseases based on subtle changes in the optical properties of vegetation. The bottleneck, however, is the pre-processing and analysis of the large volume of data that is collected. The team developed the framework for a workflow that can be supported by the SciServer platform. The workflow has not yet been implemented.

Establishing Cryo-FIB-SEM capability

The team identified the need to be able to study plant tissues and cells using Cryo-Electron Tomography (Cryo-ET) in the proposal as a priority. In FY 23, a Thermo Scientific Aquilos 2 cryo-focused ion beam (Cryo-FIB) was procured and installed in the new cryo-EM center at BNL (Laboratory for Biomolecular Structure, LBMS). In FY24, the instrument's science commissioning was completed and protocols were developed to allow for the study of infected plant tissue and single cells. With the samples prepared from the pilot study, the team was able to vitrify the samples, transfer the samples across different instruments, identify and mill the target cells of desired thickness, collect cryo-electron tomography data, and perform the high-resolution 3D reconstruction for identification of cellular and structure features. To support the preparation of tissue samples, the team assisted the LBMS staff in obtaining a high-pressure freezer with support from BER. With the new addition of the Cryo-FIB to the existing LBMS facility, it is now possible to study specific a broad range of plant-pathogen interactions involving plant leaves, stems, or roots using milling, thus retaining context.

Developing remote disease detection capability

The team received two state-of-the-art HYSPEX hyperspectral imaging detectors. The new detectors were integrated into the payload of drones at subcontractor UIUC and BNL. The BNL team also procured additional accessories so that the detector can be used in the laboratory to image plants and plant tissues. Delivery of the accessories is expected to be completed by August so that there is time to make measurements on Sorghum that has been grown in the BNL greenhouse. The detector at UIUC was transferred to PI Andrew Leakey and will support BER research conducted as part of the bioenergy research center he leads at UIUC.

Publications

Schoonen, Martin A., Freimuth, P., Rogers, A., Leakey, A., Mukundan, H., McGraw, R., Aylor, D., Subba, T., Stump, B., da Silva, W., Liu, Q., Paša-Tolić, L., Yoshikuni, Y., Shanklin, J., Coaker, G., Jha, S., and Emrick, A. *Community Input on the Need, Scope, and Development Roadmap of a Proposed National Virtual Biosecurity for Bioenergy Crops Center (NVBBCC)*. United States: N. p., 2024. Web. doi:10.2172/2368825.

Attached as Appendix A.

Presentations and Outreach

Oral Presentations:

Schoonen M., K. Kleese van Dam, J. Shanklin, A. Rogers, R. McGraw, Q Liu, S. Jha, P. Freimuth. (2024) *Developing a National Virtual Biosecurity for Bioenergy Crops Center*. BER PI meeting (4/4/2024), Washington, DC.

Schoonen, M., K. Kleese van Dam, J. Shanklin, A. Rogers, R. McGraw, Q Liu, S. Jha, P. Freimuth. *Protecting Bioenergy Crops: The Need for a National Virtual Biosecurity for Bioenergy Crops Center* (4/23/2024). Innovations in Climate Resilience, Battelle, Washington, DC.

Jha, S. *The Landscape of Data Infrastructure: A National Virtual Biosecurity for Bioenergy Crops Center Perspective*. BER PI meeting (4/4/2024), Washington, DC

Poster Presentation:

Liu Q et al (2024) USDA National Predictive Modeling Tool Initiative Annual Meeting (2/27-28, 2024), Raleigh, NC

Outreach:

Schoonen project overview presentation at Brookhaven Science Associate Board Meeting, 1/16/24.

Schoonen project overview presentation at BNL Management Council meeting, 3/19/24

Management of Project

A team SharePoint site continues to be maintained to facilitate sharing of information. The leadership team of the project meets every Wednesday at 4 PM for an hour to discuss progress and challenges. The PI meets with the Program Manager monthly.

Budget and Personnel Effort

The project received its entire 5M\$ budget in September of 2022, with the intent to perform the project over a period of 18 months, effectively starting on October 1, 2023. A No Cost Extension was granted in FY24Q2 through FY24.

Internally the budget was broken up into 5 activities and a small reserve (\$35,800). The table below provides the financial status of the project at time. We are on track to close out the project this fiscal year. The project supports a large number of staff members fractionally. In total 2.6 FTEs have been supported.

Project	Activity	Activity Description	Initial Funds Available FY24	Sum of Exp YTD (7/15)	UnCosted Balance - Total	Sum of Commit/Re qns	Balance after Commitments	FTEs
26121	26121	Reserve	\$ -					
26121	26592	Leadership and Modeling	\$ 174,562	\$ 174,315	\$ 247			0.23
	26593	Remote Sensing	\$ 659,146	\$ 597,998	\$ 61,148	60,967	\$ 181	0.28
	26594	Pilot Study Bio	\$ 402,442	\$ 288,845	\$ 113,597	\$ 112,500	\$ 1,097	0.77
40095	40095	NVBCC Capital	\$ 247,034	\$ 156,977	\$ 90,057	\$ 90,000	\$ 57	0
26119	26119	Computing Platform	\$ 674,572	\$ 450,442	\$ 224,130	\$ 217,000	\$ 7,130	1.32
			\$ 2,157,756	\$ 1,668,576	\$ 489,180	\$ 480,467	\$ 8,466	2.6

FY24 Collaborations

Current year collaborations			
MSI?	Institution	Last Name	First Name
X	Texas A&M	Rooney	William
	USDA	Widmer	Timothy
	University of Illinois	Leakey	Andrew

FY 2024 Recognition, Honors, and Awards:

None

Unexpected Challenges/Problems encountered (and how these were resolved) and Changes compared to proposed research

Alistair Rogers left in February to take on the role of Chair of Environmental and Ecosystem Sciences at LBL. He was the Leader of the Detection module of the project and led the drone program at BNL. At the time of his departure, he had already concluded the report section he was responsible for. The drone equipment, including a detector bought under this project, was transferred to a support staff member. The subcontract with UIUC was transferred to PI Schoonen and is currently being closed out.

Shantenu Jha left BNL in May for a position at PPPL. He had completed his section of the report and no replacement was appointed to lead the computing module that Shantenu oversaw since the work had been completed. The support for SciServer continued.

FY 2025 Research Plans: None.

We are working to completely close out the project.

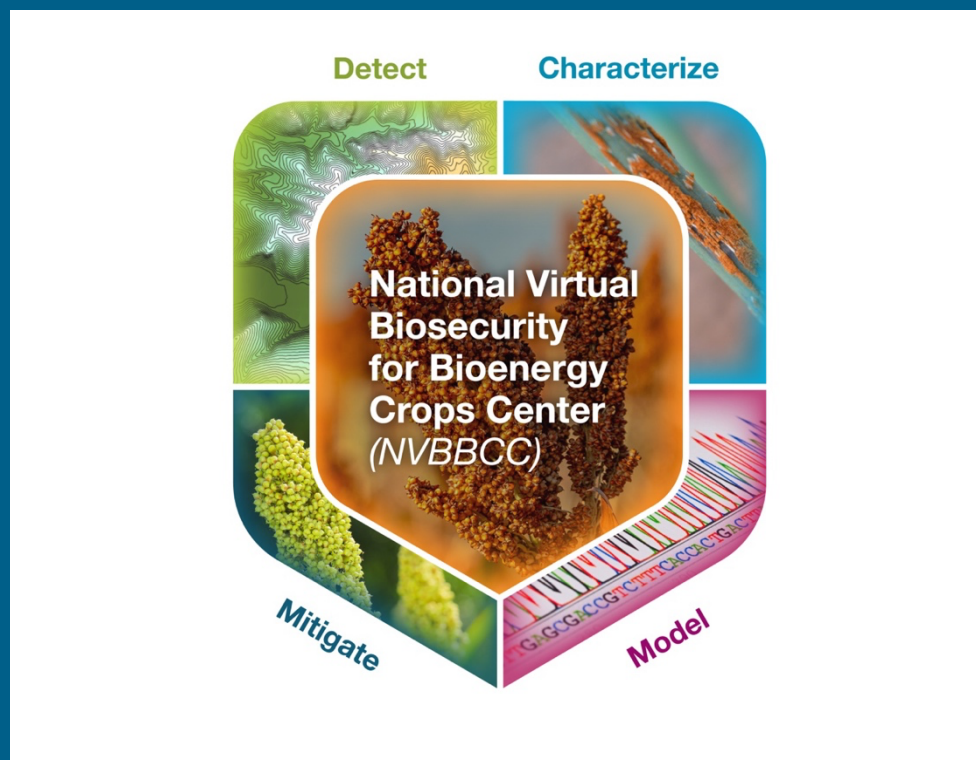
Appendix A. Report

Schoonen, Martin A., Freimuth, P., Rogers, A., Leakey, A., Mukundan, H., McGraw, R., Aylor, D., Subba, T., Stump, B., da Silva, W., Liu, Q., Paša-Tolić, L., Yoshikuni, Y., Shanklin, J., Coaker, G., Jha, S., and Emrick, A. *Community Input on the Need, Scope, and Development Roadmap of a Proposed National Virtual Biosecurity for Bioenergy Crops Center (NVBBCC)*. United States: N. p., 2024. Web. doi:10.2172/2368825.

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COMMUNITY INPUT ON THE NEED, SCOPE, AND DEVELOPMENT ROADMAP OF A PROPOSED NATIONAL VIRTUAL BIOSECURITY FOR BIOENERGY CROPS CENTER (NVBBCC)

May 10, 2024



U.S. Department of Energy
USDOE Office of Science (SC), Biological and Environmental Research (BER)

FY22 DOE- SC-BRaVE Pilot Project

BNL-225522-2024-FORE

Pilot Project Lead: Dr. Martin A. Schoonen, Brookhaven National Laboratory

Federal Program Manager: Dr. Resham Kulkarni

Authors:

Executive Summary. Martin Schoonen

Chapter 1: Introduction and Rationale. Martin Schoonen and Paul Freimuth

Chapter 2: Detecting the Presence and Severity of Disease. Alistair Rogers, Andrew Leakey, and Harshini Mukundan

Chapter 3: Aerial Dispersal of Plant Pathogens. Robert McGraw, Don Aylor, Tamanna Subba, Brandon Stump, and Washington da Silva

Chapter 4: Biomolecular Characterization of Bioenergy Crop-Pathogen Interactions. Qun Liu, Ljiljana Paša-Tolić, and Yasuo Yoshikuni

Chapter 5: Disease Mitigation Strategies for Bioenergy Crops. John Shanklin and Gitta Coaker.

Chapter 6: Enabling Computing Infrastructure. Shantenu Jha

Chapter 7: Establishing and Maintaining NVBBCC Preparedness. Ann Emrick and Paul Freimuth

Chapter 8: Workforce Development and Diversity, Equity, Inclusion, and Accessibility. Shantenu Jha and Martin Schoonen

Chapter 9: Path Forward. Martin Schoonen.

Reference: Community input on the need, scope, and development roadmap of a proposed National Virtual Biosecurity for Bioenergy Crops Center (NVBBCC). BNL Report # 225522-2024-FORE (2024). Schoonen, M.A., Freimuth P., Rogers A., Leakey A, Mukundan H., McGraw R., Aylor D., Subba T., Stump B., da Silva W., Liu Q., Paša-Tolić L., Yoshikuni Y., Shanklin J., Coaker G., Jha S., Emrick A.
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EXECUTIVE SUMMARY

Brookhaven National Laboratory (BNL) was awarded a pilot project in FY22 under the U.S. Department of Energy (DOE) Office of Science Biopreparedness Research Virtual Environment (BRaVE) initiative, to define research priorities, needs, and requirements for a national virtual center devoted to the biosecurity of bioenergy crops. The mission of the proposed center, referred to as the National Virtual Biosecurity for Bioenergy Crop Center (NVBBCC), would be to provide the scientific basis and tools to detect, characterize, model, and mitigate biothreats to bioenergy crops. This function will be essential to ensure the projected increased US reliance over the next few decades on key plant-based energy products, such as biojet fuel. The NVBBCC is envisioned as a distributed, virtual center with multiple national laboratories at its core to maximize the use of existing unique facilities and expertise across the DOE complex.

A major goal of the pilot project was to develop a roadmap for establishing NVBBCC through a series of meetings to gather community input. A total of about 150 individuals, drawn from DOE laboratories, the USDA, academia, NIH, DHS and the private sector participated in six planning meetings held in FY23. Four of the meetings were focused on specific research topics (disease detection, dispersion and disease propagation, biomolecular characterization of plant-pathogen interaction, and mitigation strategies). These four meetings were followed by a meeting that focused on computational needs to support collaborative, data-intensive research within a distributed center as well as workforce development. A final meeting focused on establishing and maintaining preparedness within NVBBCC to respond to an emerging disease within bioenergy crops and how it would collaborate and coordinate with USDA and DHS.

This report captures and summarizes the community input. Participants were charged to: 1) identify key knowledge gaps; 2) prioritize gaps DOE-BER is uniquely positioned to address; 3) outline investments needed to develop the center; and 4) define requirements and processes for the center to respond to an emerging disease.

Four meetings focused on research needs and identified the following high-level key gaps and challenges:

- Improve our ability to detect the emergence of diseases in bioenergy crops and assess their severity using advanced remote sensing techniques to support prediction of disease spread and guide strategies for intervention. (Chapter 2)
- Improve our understanding of aerial dispersion and survival of pathogens under a changing climate accompanied by more frequent extreme events, along with the challenges how to best incorporate key pathogen characteristics and dispersion processes into multiscale computational models and develop techniques to measure specific pathogen loading in air which contains many other bioaerosols. (Chapter 3)
- Develop foundational insights at the biomolecular level into the dynamics of bioenergy crop/pathogen/vector interactions (Chapter 4).
- Develop a foundational understanding of the combined effects of biotic and abiotic stress/threat on bioenergy crops. (Chapter 4)

- Develop a foundational understanding how the engineering of lipid accumulation, manipulation of lignocellulosic components, or other bioproduct accumulation—cornerstones of developing next generation bioenergy crops—will impact plant susceptibility to pathogens and disease progression. (Chapter 5)
- Develop more efficient as well as new approaches to study bioenergy crop-pathogen systems and expand the availability of relevant bioenergy crop and pathogen genomic data. (Chapter 5)

Studies to tackle the challenges outlined above would be performed at multiple sites distributed across the DOE complex in order to leverage expertise and facilities from across the complex. To underpin this collaborative and distributed effort a flexible computational platform that supports high-performance computing (HPC) workflows, data management, and allows for efficiently conducting modeling simulations is needed (Chapter 6). The meeting focused on computing defined these high-level needs:

- Develop an integrated research infrastructure that enables meaningful integration of data, computing, instrumentation, and related resources to allow researchers access to needed computational/data resources from anywhere.
- Employ robust data management systems that can manage diverse data types, to ensure quality while adhering to FAIR principles and supporting better metadata.
- Explore and adopt advanced technologies, such as 5G/6G wireless communication for high-throughput transmission with low latency in poorly connected areas.
- Develop scalable and generalizable models and that link model design to downstream decision-making while using tools and techniques to create a unified, integrated modeling approach.

The network of unique capabilities and expertise necessary for the NVBBCC to conduct the basic research outlined above would be an asset in characterizing new diseases that will emerge in bioenergy crops. To respond effectively to an emerging threat requires the center to establish protocols for material, information, and data transfer among center researchers as well as with the USDA, DHS and other stakeholders. It was recommended that NVBBCC develop a preparedness program based on the general framework of the Federal Select Agent Program. This ensures that there is a reliable, compliant, and efficient capability that can be brought to bear on the biothreat at hand (Chapter 7).

The envisioned center will need a multidisciplinary workforce prepared to work on both long-term basic research and to support a rapid response to emerging diseases in bioenergy crops. Teamwork across multiple laboratories along with researchers in the academic sector and at other agencies will be the norm. Hence, apart from establishing the necessary infrastructure, programs, and procedures to execute the center's mission, it is crucial to attract, train, and retain a workforce that is well prepared and provides a diverse perspective. The center should leverage several existing DOE-Office of Science Programs, such as Reaching a New Energy Sciences Workforce (RENEW), Funding for Accelerated Inclusive Research (FAIR), Visiting Faculty Program (VFP), and Science Undergraduate Laboratory Internships (SULI) to build connections across the academic community (Chapter 8).

In each of the six meetings, participants expressed enthusiasm for the fact that DOE-BER is considering developing a sustained program in biosecurity focused on bioenergy crops with NVBBCC in a central, coordinating and supporting role. A multi-stage development path for NVBBCC is presented in Chapter 9. A first, one-year phase will establish a multi-lab working group and focus on prioritizing the long-term

research challenges identified in the workshops, defining what investments are needed to address the top priorities, as well as establishing the structure and governance of the center. A second, three-year phase will formally establish NVBBCC, initiate work on top research priorities, build up the NVBBCC research infrastructure and response capability, as well as organize an annual meeting for the research community—a recurring suggestion made by workshop attendees. In a third phase, the center would operate as a mature center, with annual reviews of its impact and progress against goals, redirections as needed, and a comprehensive review of the center on a five-year cycle.

A final recommendation that emerged from the workshops is for BER to consider adding a leading Plant Pathologist to the BER Advisory Committee (BERAC) to elevate this topical area.

CHAPTER 1: INTRODUCTION AND RATIONALE

The United States as well as other nations have set net-zero carbon goals which require the development of comprehensive and scalable technologies to replace fossil-carbon energy sources and remove atmospheric carbon through net-negative emission technologies. Expanding the U.S. Bioeconomy is part of the US Government’s “all of the above” approach to meet its net-zero goals ([US Long-term climate strategy, 2021](#))¹. [Presidential Executive Order 14081](#)², published on September 12, 2022, lays out a directive to build the U.S. bioeconomy, which includes enhancing biosafety and biosecurity to reduce risks to the US bioeconomy. In an expanded bioeconomy, a significant fraction of the agricultural sector will be devoted to growing bioenergy crops to support the production of biofuels and bioproducts (see side bar 1.1). The emergence and spread of diseases among bioenergy crops could significantly impact yields and destabilize the U.S. bioeconomy. In line with Executive Order 14081 as well as the [National Biodefense Strategy and Implementation Plan \(2022\)](#)³, which calls for a holistic approach to assessing biothreats, this project explored how DOE-Biological and Environmental Research (DOE-BER) might harness and organize its expertise and facilities to establish a new *National Virtual Biosecurity for Bioenergy Crops Center (NVBBCC)*. While focused on biosecurity for bioenergy crops, the center would also support the National Biodefense Strategy which recognizes that the interrelationships between people, animals, plants and their shared environment shape human health outcomes. The interrelationships between people, animals, and plants and its effect on health is referred to as the [One Health](#) framework⁴.

The US Department of Energy through its investments in [Bioenergy Research Centers](#) and other related programs is paving the way for a bioeconomy capable of producing biofuels and bioproducts at scale, replacing fossil carbon-based fuels and products. The R&D investments have largely focused on development of sustainable and economic, high-yield cropping systems, novel separation and deconstruction processes, and advanced conversion methods to yield the desired biofuels and bio-based products. However, with the projected expansion of bioenergy crops⁵⁶, the risks to crop health associated with the emergence of new diseases, climate-driven spread of known diseases into new regions, and the accidental or deliberate release of plant diseases need to be considered.

The projected large-scale deployment of bioenergy crops changes the interplay between the three factors

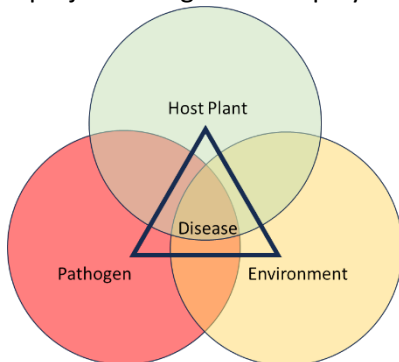


Figure 1. Plant disease triangle.

that control plant disease—host plant/pathogen/environment—as illustrated in the often-used plant disease triangle (Figure 1). The introduction of energy crops at a large scale expands the possibility for a disease to take hold and spread. By selective breeding and/or genetic modification a bioenergy crop might be designed to be less susceptible than current varieties, but the interplay between the three factors evolves over time. Pathogens may become more virulent, pathogens may be introduced from other regions, and changes in weather patterns or climate over a longer time scale can lead to more favorable conditions for a disease to take hold and spread.

The emergence of a new disease among bioenergy crops or the spread of a known disease to a new region would require a response that involves a broad group of stakeholders and decision makers. It is within this context, a new DOE capability to detect, characterize, model, and mitigate biothreats to bioenergy

crops based on experimental and computational facilities as well as expertise within the National Laboratory system would function in coordination with private agriculture sector, local and state agriculture departments, and USDA and DHS. The U.S. DOE’s response to the COVID-19 pandemic in the form of its National Virtual Biotechnology Laboratory illustrates that DOE has a wide range of unique capabilities that can be harnessed⁷, but it also underscored that coordinating material, data, and information sharing arrangements are crucial preparedness components⁸. Furthermore, it is important for a distributed DOE capability focused on biosecurity for bioenergy crops to maintain its preparedness.

The development of a DOE capability to minimize the effects of diseases on bioenergy crops and help respond to an emerging disease can leverage a wealth of expertise and experience in the agricultural crop health community. In fact, many of the research priorities identified by the USDA’s Agricultural Research Service are relevant to the health of bioenergy crops⁹. However, there are specific challenges associated with bioenergy crops that set it apart from food crops. For example, bioenergy crops are projected to mostly be grown on land not used for food production, such as marginal lands. The soil health status of these lands is expected to be lower, which translates into conditions that may reduce the resilience of bioenergy crops to pathogens. Furthermore, bioenergy crops will be selected or genetically modified to yield biomass for biofuel or bioproduct production. It is not known how resistant engineered or selected bioenergy crop plants will be to various diseases. Current research efforts within the USDA are primarily focused on food crops and it is logical for DOE to take the lead in a research program focused on

developing resilient bioenergy crops that can be grown on land not used for food crops. Furthermore, climate-driven spread of diseases will impact bioenergy crops just like food crops. Studies indicate that with warming and changing precipitation and humidity patterns the negative impact of insects, pathogens, and weeds are likely to increase in agricultural ecosystems¹⁰. A higher frequency of hurricanes is likely to promote the spread of pathogens northward¹¹.

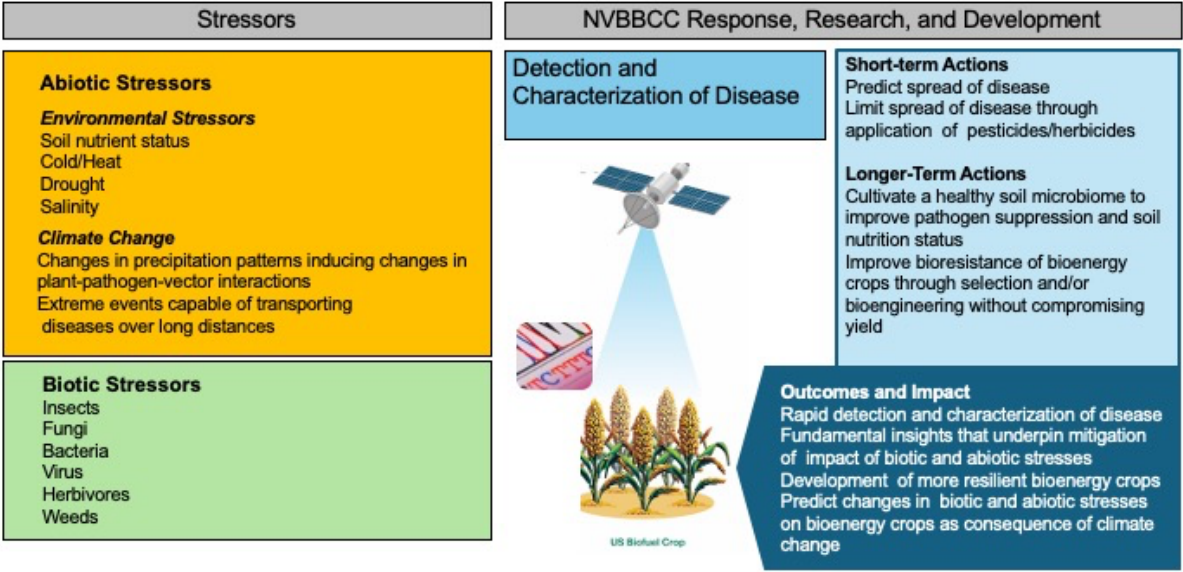


Figure 2. Schematic diagram of stressors on bioenergy crops, disease detection, and possible actions. Figure placeholder.

Similar to DOE’s response to the COVID-19 pandemic in which it rapidly stood up the National Virtual Biotechnology Laboratory and harnessed expertise and facilities from across the complex⁷, it has existing facilities⁸ and expertise within the complex that can be organized in a new virtual center focused on biosecurity of bioenergy crops. For example, DOE’s investments in climate science, genomics, biosystem science, biomolecular characterization and imaging, and high-performance computing can be brought to bear on this complex challenge illustrated in Figure 2.

This report is organized in 8 chapters that summarize the input gathered through a series of virtual planning meetings conducted in FY23, followed by a chapter that outlines possible next steps for the development of NVBBCC. Table 1 provides short description of each of the workshops; a list of participants to each of the workshops is provided in Appendix 1.

Table 1. List of workshops		
Workshop Topic	Lead Organizers	Summarized in Chapter
Detecting the Presence and Severity of Disease	Alistair Rogers (BNL) and Andrew Leakey (UIUC)	2
Aerial Dispersal of Plant Pathogens	Robert McGraw, Chongai Kuang, and Maria Zawadowicz (BNL)	3
Biomolecular Characterization of Bioenergy Crop-Pathogen Interactions	Qun Liu & Sean McSweeney (BNL), Lili Pasa-Tolic (PNNL), Yasuo Yoshikuni (LBNL), Chris Henry (ANL), and Huimin Zhao (UIUC)	4
Disease Mitigation Strategies for Bioenergy Crops	John Shanklin (BNL) Gitta Coaker (UC Davis)	5
Cross-Cutting Workshop: Enabling Computing Infrastructure and Workforce	Shantenu Jha (BNL/Rutgers U), Noel Blackburn (BNL)	6, 8
Establishing and Maintaining NVBBCC Preparedness	Paul Freimuth, Ann Emrick, Martin Schoonen (BNL)	7

Sidebar 1.1. The projected scale of bioenergy crops in 2040

A DOE comprehensive analysis of the potential resources to underpin the US Bioeconomy published in 2023 projects a significant increase of land devoted to bioenergy crops⁶. This is illustrated below in Figures 3 and 4 taken from the data portal associated with the report. Figure 3 shows the extent of potentially available agriculture biomass in 2017; whereas Figure 4 shows a projection for 2040. In 2017, the available agricultural biomass is entirely represented by crop residues, whereas the projections for 2040 show that most of the agricultural biomass could be sourced by dedicated bioenergy crops. An earlier study came to the same general projection⁵.

The impact of a vastly expanded bioeconomy on agricultural land use is illustrated by a case study on the scale of acreage that would need to be devoted to support the growth of crops to produce sustainable aviation fuel, or biojet fuel¹². As a result of DOE’s sustained R&D investments over the past decade, the production of biojet fuel from cellulosic feedstocks is now possible. Using this advanced technology, a crop of miscanthus roughly the size of the state of Wyoming would be required to meet the US demand for biojet fuel in the year 2040. The success of this endeavor will critically hinge on development of a strategy to interpret and integrate data from various crop measurements in real time, to assess crop health and to rapidly deploy measures that effectively block the spread of disease or limit the impacts of adverse environmental events. The NVBBCC is advancing capabilities both in measurements of crop status at multiple scales and in data integration, which will serve as valuable tools to manage large scale bioenergy crops in the future.

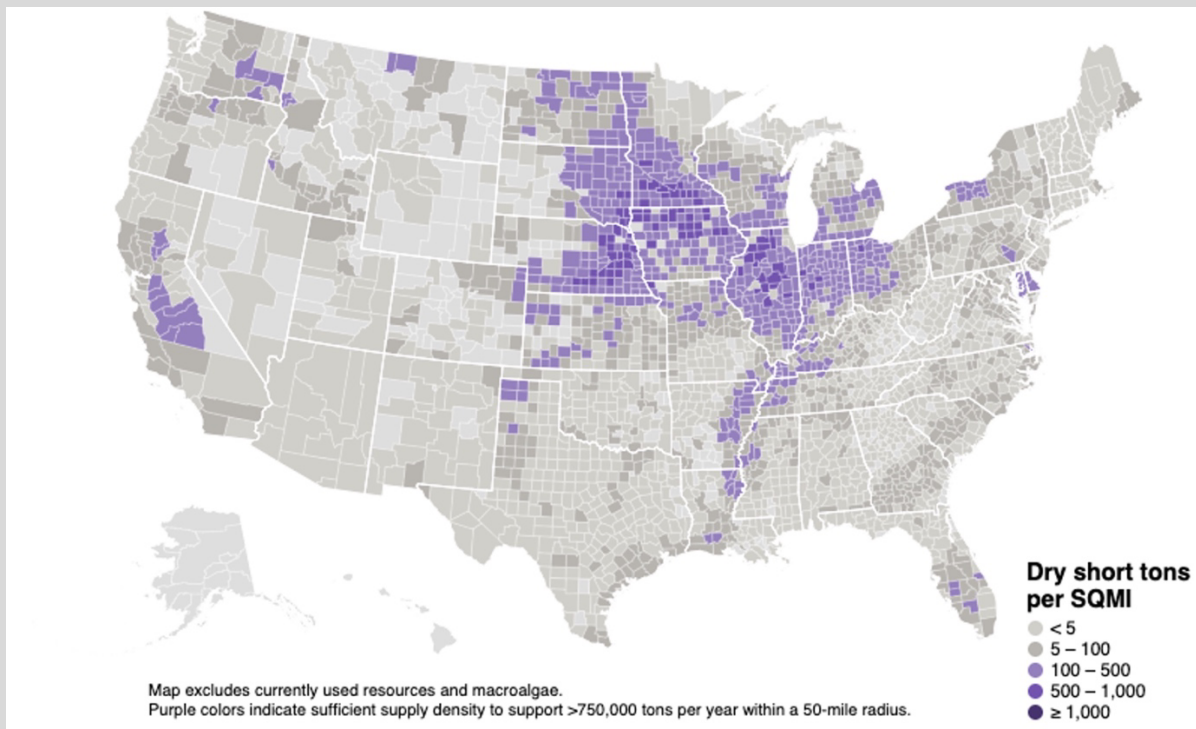
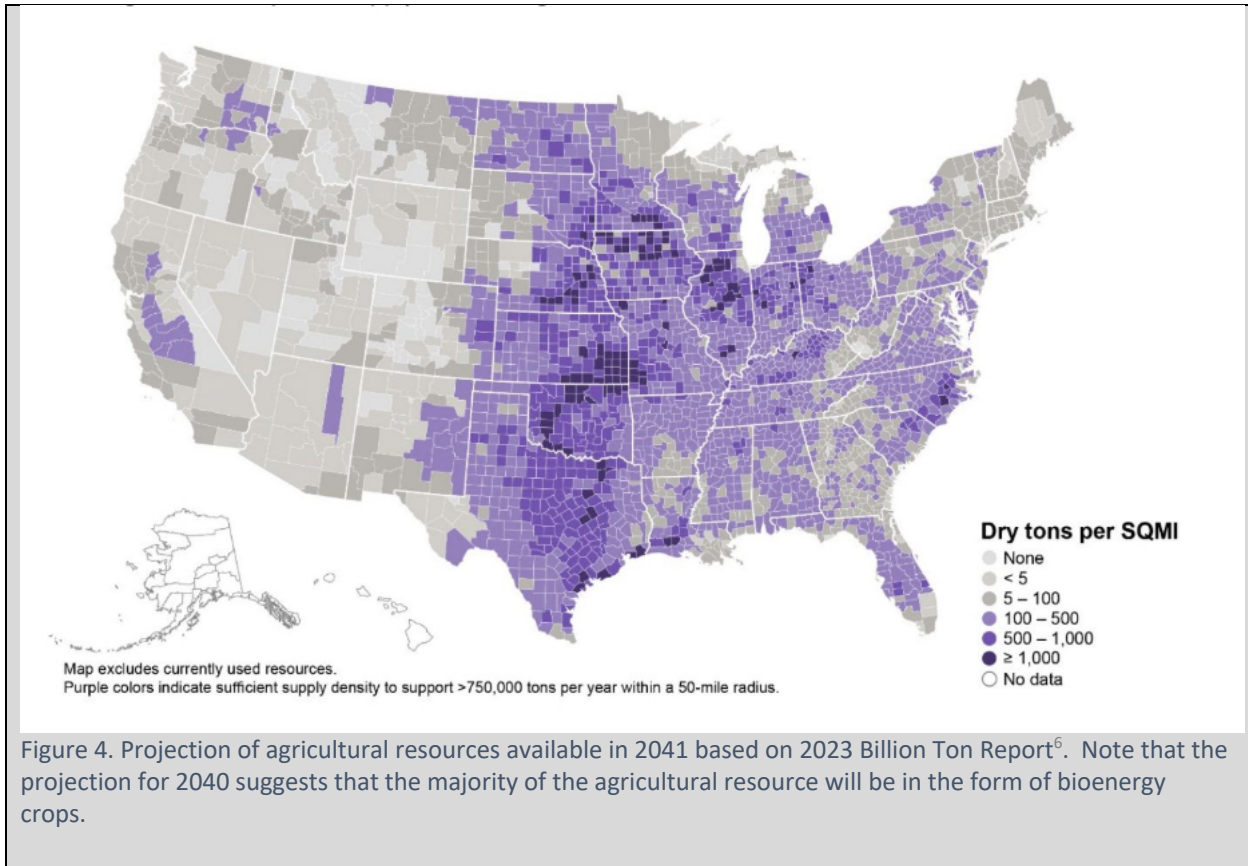


Figure 3. Projection of agricultural resources available in near term based on 2023 Billion Ton Report⁶. Note that crop residue is the only form of agricultural resource available according to this study.



CHAPTER 2: DETECTING THE PRESENCE AND SEVERITY OF DISEASE

Introduction

Infectious diseases are a major threat to biosecurity in the United States and can have significant economic impacts, with current annual losses estimated to be \$21B in the U.S.¹³ and ~\$220B worldwide¹⁴. The emergence of a known disease, new disease races with increased virulence, or a novel biothreat will threaten the biosecurity of a future large-scale U.S. bioeconomy¹⁵, particularly if that crop is grown as a genetically identical monoculture. Currently disease monitoring is siloed in different private, local, state and federal entities with uncertain support¹⁶, and relies mostly on simple, visual assessment by a person on the ground. Disease presence and severity are assessed by applying visual assessment tools such as standard area diagrams and disease progress curves, complemented in rare cases by molecular assays and smartphone apps^{14,15}. Importantly, the approach of requiring a human to seek out and observe disease conditions cannot be scaled to provide assessments at high frequency over large spatial scales. As a result, most diseases can spread for days to months before they are detected, delaying timely mitigation interventions^{14,15}. Fungicides and other control measures must typically be applied early in disease progression to be effective, and applying them after a disease has established decreases the chance of successful management and increases the likelihood that resistance to the pesticide will evolve in the pathogen¹⁷. Therefore, growers often apply pesticides preemptively on a calendar-based schedule incurring significant financial cost, risk of pesticide resistance evolving more quickly, off-target damage to beneficial microbes, — and of note for biofuels - significant associated CO₂ emissions^{14,15,18}. The threat that disease poses to a future bioeconomy demands better tools for detection and monitoring to protect the dependable production of biofuels. Furthermore, improved early detection avoids input waste that will threaten the economic viability of a bioeconomy, and its ability to provide net negative CO₂ emissions in comparison with fossil fuels.

Remote sensing is the only viable approach to identify and track disease *at scale* in a future biofuel production environment in a cost-effective manner. The emerging discipline of plant disease sensing, coupled to proposed investments in earth observing remote sensing satellites^{14,19,20} offers great promise to extend monitoring to previously unachievable resolutions, a basis to construct surveillance networks for early warning, and response at low latency, and an opportunity to mitigate loss while optimizing protection¹⁴.

Plant responses to the biotic and abiotic environment they grow in alter the chemical, physiological, thermal and structural properties of crops, all of which can be detected and quantified through changes in the optical properties of plants using a range of remotely sensed measurements as illustrated in the referenced studies^{21–23}. Importantly, this approach is capable of early, non-destructive detection of plant stress and disease, often before symptoms can be detected by human vision^{24,25}. Therefore, remote sensing of plant health is tractable, and with emerging transformative remote sensing capabilities, will be applicable at a scale and resolution that can enable near real-time monitoring, management and protection of a future U.S. bioeconomy.

Vision of a future detection capability

Satellite-borne sensor packages will monitor biofuel crops and stream data to AI tools that, in near real time, can identify the outbreak and spread of economically important disease in bioenergy crops, enabling

targeted management, further characterization and identification of novel threats to biofuel security. As technology and AI tools advance, it is envisioned that this capability can be realized within about 10 years.

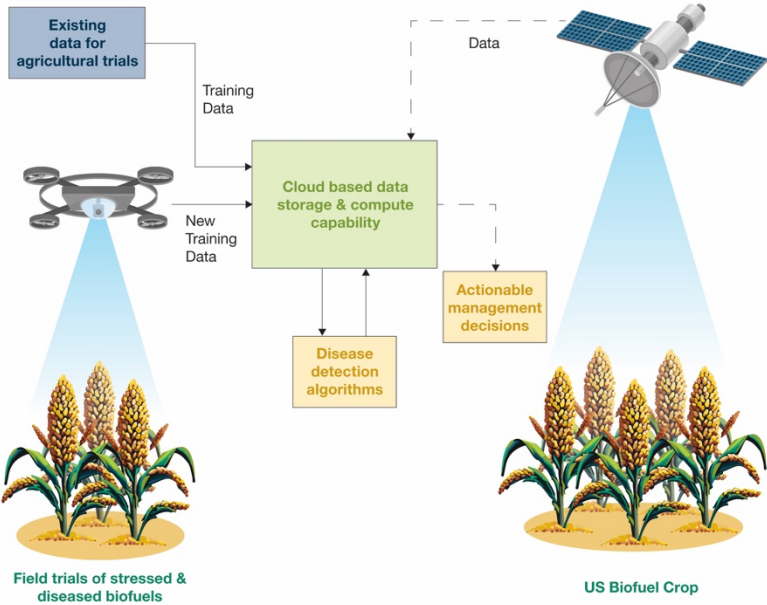


Figure 5. Enabling remote detection of disease in biofuel crops will require use of existing data, and the collection of new data to characterize diseased, stressed, and healthy vegetation. These data will be used to train AI algorithms to recognize disease in next generation satellite imagery.

Barriers to rapid disease detection and monitoring at-scale by remote sensing

We identified three key challenges that need to be addressed to realize a future detection capability.

1. Stress Library

In order to unambiguously detect and monitor the progression of known diseases—and to identify novel biothreats—we need training data to build a robust AI/ML-based detection capability (see AI/ML sidebar). This will require remotely sensed libraries of optical, thermal and structural properties of crops experiencing a wide range of biotic and abiotic stress, including known diseases of biofuel crops. This library will need to be developed under a wide range of environmental conditions and genetic backgrounds of the crops of interest, under a range of stress severity. Previous work in remote detection of plant traits²⁶ has identified the need for a wide range of training data that fills the trait and optical space associated with potential signals. Currently, the limited availability of training data is hampering the development of disease detection and monitoring algorithms.

2. Data sharing

In order to use the data necessary to train AI-based detection algorithms, we need to annotate, store, curate, compress, integrate and share huge and disparate data sets that are highly diverse and inconsistently formatted. Whilst a detection approach could be developed using current technologies, it is inherently inefficient. To operationalize disease detection at scale this challenge must be addressed. In addition to hosting remotely sensed data, a decision algorithm would also need to access meteorological and epidemiological data.

3. Latency

There is currently an unacceptably large time lag between data acquisition and potential management decisions. The current capability is experimental and limited to discovery science and proof of principle. If this approach is to be fully realized, we need to develop automated workflows that can efficiently ingest, process, and analyze data in near real time.

How might DOE play a role in addressing these challenges?

We believe DOE can play a unique role addressing these three challenges. There are two major areas where DOE can contribute:

- Enabling the training of AI-based disease detection tools
- Operationalizing near real-time disease detection.

DOE has an unparalleled record among U.S. federal funding agencies in developing and fostering success among teams of researchers working across traditional disciplinary boundaries to tackle major research challenges. In recent years, this approach has been embodied by the DOE National Laboratories and Bioenergy Research Centers as they have developed and applied AI/ML tools to advance the development of sustainable bioenergy and bioproducts, including the application of AI/ML at the molecular, metabolic, phenotypic, ecosystem and earth-system scales²⁷⁻³¹. Current high-resolution spatial, temporal, and spectral measurements are not yet available for satellite platforms but are readily available for ground based, vehicles, drones and aircraft^{32,33}. These platforms can be used to host a range of instrumentation that can be used in combination with field trials to characterize the fingerprint of diseases known to affect biofuels. DOE support for field trials designed to generate training data will accelerate the development of a detection capability. This would include the development of unambiguous structural, thermal, and spectral fingerprints that can be used to train robust AI tools for disease detection but also inform data compression to aid data storage and sharing and improve computational efficiency. DOE support would enable the development of robust detection algorithms on a time scale that is aligned with the anticipated emergence of transformational new satellite-based detection capabilities. In addition, supporting work to get the maximum value out of data that already exists through synthesis projects will provide a shortcut to developing training datasets and help inform the development of computational workflows.

DOE is a leader in computation and data storage, and it could provide the resources and infrastructure necessary to support the training and operation of an AI-based detection capability that can be used to support biosecurity detection applications. The key will be support for the development of automated workflows capable of ingesting, processing, and analyzing disparate datasets in the cloud where the data is stored.

DOE has leadership in the development and establishment of community-based standards for metadata and data reporting conventions³⁴⁻³⁶. Supporting these activities and investing in new tools to harmonize and combine disparate data is essential to realizing the goal of efficiently using existing and future remotely sensed data products.

Benefits to an emerging US bioeconomy

If fully realized, a new disease detection capability would enable near real-time data sharing that can inform management of the US biofuel crop. This could enable early intervention on emerging diseases, and identification of novel biothreats. In addition, such data can be used to increase the economic viability

of biofuels and reduce associated CO₂ emissions through early and precise application of pesticides and other agricultural amendments, and through avoidance of unnecessary agricultural inputs.

Sidebar 2.1: Development of At-Scale Test-Bed Facilities

Spectral remote sensing technologies can be passive (optical) or active (LiDAR and Radar). More recently, integration of spectral information with other signatures (physiochemistry and taxonomy) has resulted in a new field, spectranomics, offering greater specificity for target identification. Hyperspectral sensing can detect subtle changes in spectral reflectance due to absorption or reflection, facilitating identification of small changes across the electromagnetic spectrum. This is used to identify symptoms of biotic (insects, pesticides, etc.) and abiotic (drought, flood, etc.) plant stresses^{37,14}. Two disruptive technologies have helped realize global use of remote sensing: 1) miniaturization of hyperspectral sensors (e.g., HySpex VNIR (HySpex, Skedsmo, Skjetten, Norway); ProSpectral (Pattern Computer, USA)) have enabled them to be more effectively used in aircraft carriers or Unmanned Aerial System (UAS), making them ideal candidates for scalable remote crop surveillance³⁸. 2) machine learning and artificial intelligence technologies have enabled real-time analysis and output of information for decision making. However, robust use of hyperspectral sensing requires extensive benchmarking and validation— both experimental and analytical, to ensure physiological relevance, quantify uncertainty and ensure reproducibility. This requires at-scale test-bed systems and controlled environmental studies that allow us to interrogate disease and climate-associated changes systematically. The Department of Energy has expertise in bioenergy crops, hyperspectral sensing, machine learning and advanced computing, and at-scale ecological test-beds (e.g., EcoPODs and EcoFABs³⁹); which together can be used to design, deploy and validate the use hyperspectral sensing for reagent-free, scalable, pathogen agnostic interrogation of crops (see Figure 6).

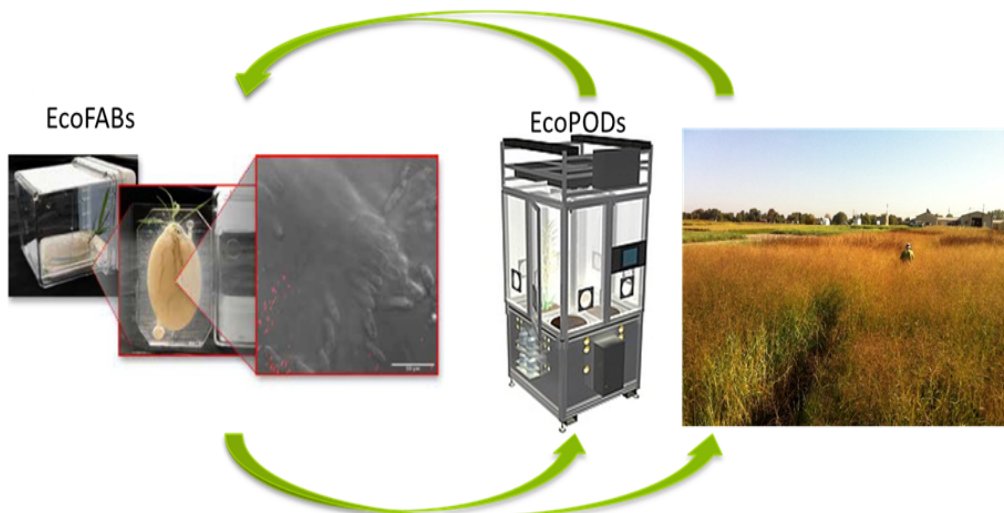


Figure. 6. EcoFABs and EcoPODs, developed at Lawrence Berkeley National Laboratory, allow for the controlled growth of plants in systems that are designed to study the plants using an array of imaging modalities, including hyperspectral techniques in the EcoPODs, which form a bridge to field test plots.

Sidebar 2.2: Advancing Surveillance of Crops and Detection of Diseases by Application of Artificial intelligence (AI) and machine learning (ML) Approaches

Artificial intelligence (AI) and machine learning (ML) are powerful approaches that can accelerate scientific discovery and provide useful tools to society, especially in biological contexts where large-scale, complex problems are commonplace⁴⁰. However, substantial amounts of annotated data from across the spectrum of diseases, disease severity and environmental contexts (i.e. not just baseline monitoring) are required to train AI/ML tools⁴¹. In the case of disease detection and monitoring, gold-standard ratings of disease identity and severity by human scouts need to be available in coordination with imaging of the crop by aerial, remote sensing platforms across hundreds to thousands of field plots. This is especially true if AI/ML tools are to distinguish among a large number of potential diseases in bioenergy crops that are grown across large areas of the landscape where genotype by environment by management interactions will add variation to underlying crop phenotypes^{14,42}. These challenges have proven tractable for emerging bioenergy crops^{19,23,43-45}. Progress will be accelerated by application of data augmentation, pseudo-labeling, and label propagation methods, which each take different approaches to amplifying any human annotated data that is available^{46,47,48}. Emerging techniques in transfer learning, active learning, and semi-supervised learning that can reduce the amount of annotated training data required will increase efficiency of tool development even further⁴⁰.

CHAPTER 3: AERIAL DISPERSAL OF PLANT PATHOGENS

Aerial dispersion of plant pathogens is an important mechanism by which plant diseases spread. The propagation can take place over multiple distances ranging from pathogen release scale, subsequent disease spread within and between adjacent fields, to long-distance transport between continents. The ability to track the movement of these pathogens is essential for forecasting disease spread, making plant-disease management decisions, and establishing effective approaches to mitigation⁴⁹. Descriptions of pathogen dispersal and disease spread range from the phenomenological to highly sophisticated computational models that strive to include meteorological and micrometeorological processes governing particle motion; wind profiles in plant canopies; length and time scales of atmospheric turbulence, storms and precipitation; surface topology and boundary layer processes; weather prediction and assessment of the longer-term impacts from a changing climate⁵⁰. In addition to understanding the aerial dispersion of pathogens, research may also be directed at understanding how disease vectors, such as insects, may spread, possibly driven by climate change.

The contents of this section draw heavily from a virtual gathering of subject matter experts on Aerial dispersal hosted by Brookhaven National Laboratory on March 30-31, 2023. The meeting included 23 invited presentations followed by breakout sessions for group discussion. Emphasis was placed on micrometeorological mechanisms for initiating aerial dispersal, advances in computational models, measurements and instrumentation, and mitigation. Discussion was focused on present understanding, key knowledge gaps, and identifying areas in most urgent need of research and development. Participants included experts from the USDA, the CT Agricultural Station, Universities, NASA, and DOE Laboratories (BNL, LANL, ORNL and PNNL).

TOPIC 1: Mechanisms of Aerial Dispersion

Fungi are highly effective pathogens. In species that produce airborne spores, wheat stem rust for example, these spores can disperse over distances ranging from a few meters to hundreds or even thousands of kilometers⁵¹. A necessary prelude to many plant disease epidemics is the short-range dispersal of spores, which may generate several disease foci within a field. New information is needed on the mechanisms of pathogen spread within and among susceptible plants. A recent article in PNAS showed that jumping dew droplets, working synergistically with low wind flow, can propel the spores of a fungal plant pathogen (wheat leaf rust) beyond the surface layer and disperse them onto neighboring leaves downwind⁵². At the much larger spatiotemporal scales of climate the magnitude and patterns of change remain uncertain despite high scientific confidence that warming and intensification of the hydrological cycle are trending upward with corresponding geographic shifts in both susceptible bioenergy crops and their pathogens expected⁵³. Soybean rust is an example of a disease that have found its way to the US through hurricane Ivan in 2004¹¹, whereas in that same year citrus canker spread throughout Florida as a result of hurricanes Ivan, Frances, and Jeanne⁵⁴. Moreover, ecologists are now addressing the role of plant pathogens and disease vectors in ecosystem processes, with the potential for greater understanding of the large-scale impacts of crop disease⁵⁵.

The following key knowledge gaps were identified:

- Source strength quantification (spores m⁻² s⁻¹) validated with ground truth observations.
- Real-time detection of pathogens in a veritable atmospheric sea of bioaerosols.

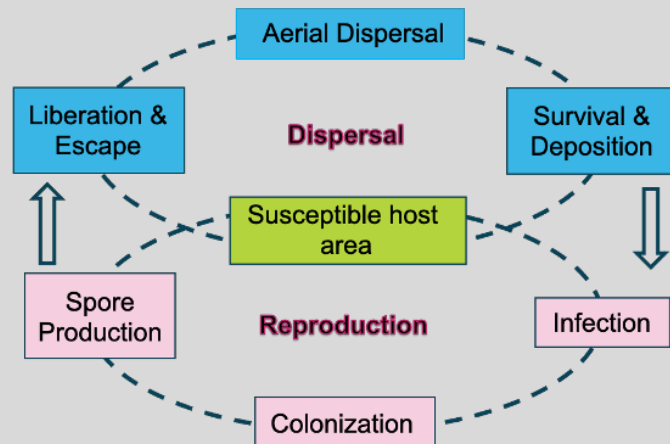
- Dispersal in extreme weather events: Aerosol sampling in tornados, hurricanes and lesser storms to quantify the transport and survival of pathogen inoculum during such events.
- Role of cloud venting above the mixed layer. What is the fate of pathogen propagules not rained or washed out quickly after release? Do those that do return to the surface maintain viability and infectivity?

How might DOE play a role in addressing these gaps?

- Aerial sampling of bioaerosols at large downwind distance scales guided by Lagrangian methods of tracing spore cloud movements.
- ARM-type long-term continuous measurements of pathogen escape, deposition, and transport that would feed into and validate multiscale models such as WRF and E3SM adapted to include local surface topology, parameterizations of leaf canopy, and regional-to-continental scale pathogen spread.
- Strengthen DOE connections to NWS, NOAA, and NCAR for their relevance to disease development, buildup, and inoculum transport. Link weather to transport models in real time to pinpoint coincidence of rainfall events and the passage of a spore cloud (washout) over a crop. (Spores delivered to crops by rainfall are expected to have a markedly higher success of infection.)

Sidebar 3.1. The role of aerial dispersal in the spread of crop disease

Key components needed to create spatially explicit disease management programs are the need for improved understanding of pathogen dispersal across multiple scales as well as accurate and sensitive methods to assess pathogen presence while predicting where the inoculum originated and the probability of deposition and infection.



Achieving these advancements requires significant improvement and integration of our ability to rapidly detect and quantify inoculum with advances in understanding air turbulence at multiple scales⁵⁶. Figure adapted from Aylor⁵⁰.

TOPIC 2: Models and Computation

The transmission of plant pathogens over long distances through the atmosphere is strongly influenced by fluctuations in weather, especially temperature, rainfall, relative humidity, wind velocity and atmospheric turbulence. An example of a comparatively simple phenomenological model is the “infection-threshold finite-leap” dispersal model, which is capable of yielding insights into long-distance

dispersal in absence of available meteorological input. The Aylor monograph gives an especially lucid summary of aerial dispersal with insights into the interplay of biological, meteorological, and changing climate effects on the spread of plant disease⁵⁰. With growing awareness of the role of weather extremes and shifting patterns in climate in the spread of plant disease, more advanced computational models are being brought to the simulation of aerial dispersal of pollen and spores⁵⁷. This growing awareness is also motivating the adaptation of DOE's E3SM towards high-resolution simulations of land-atmosphere interactions in support of its field studies at the Bankhead National Forest in the Southeastern US.

Key knowledge gaps:

- How to best integrate pathogen characteristics, such as: latent period, spore size, and dispersal mechanisms, into models for use as part of an early warning system in time for remediation efforts to save the crop.

How might DOE play a role in addressing these gaps?

- Model development, building on LASSO (LES ARM Symbiotic Simulation and Observation) and high-resolution components of E3SM; model validation studies using bioaerosol and genomic measurements; expanding research focused on land-atmosphere interactions and water cycle; assessment of impacts from extreme weather events and climate variability.

Sidebar 3.2.: Representing the transport and rupture of pollen in the atmosphere

A pollen emission and transport scheme has recently been coupled to the Weather Research and Forecasting Model with Chemistry (WRF-Chem) and used to simulate the emission of pollen and impacts on clouds and precipitation in the Southern Great Plains during a period with both pollen emissions and convective activity. The figure below shows a schematic of the modeling framework along with the coupled chemical, microphysical, land-surface, and meteorological processes included - and will need to be included as part of advanced computational models in support of NVBCC.

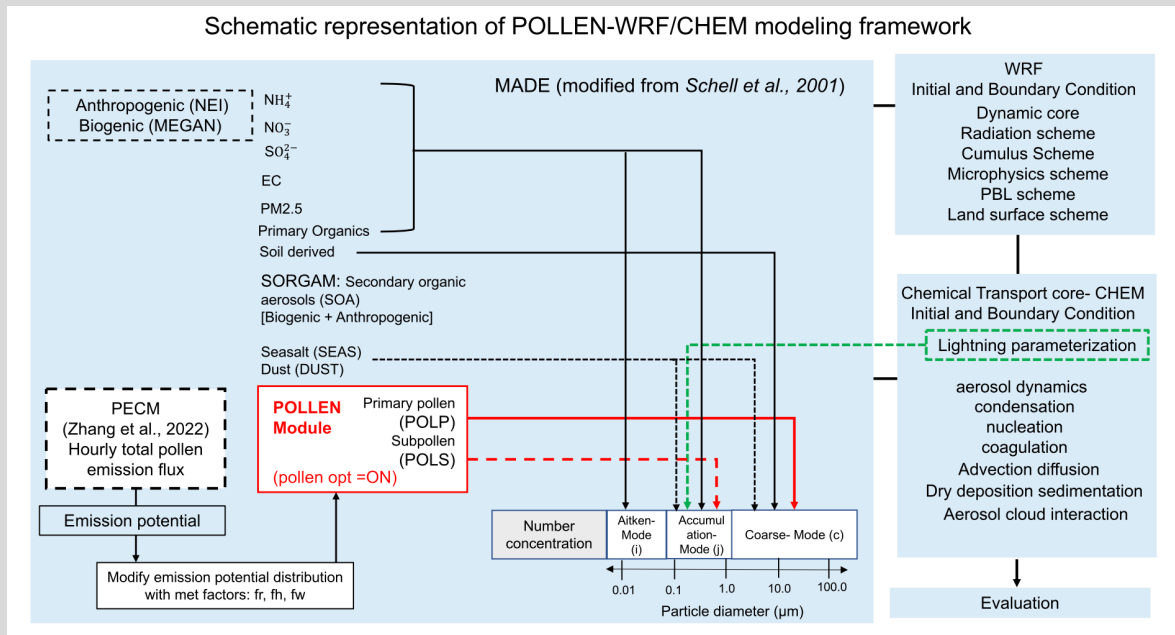


Figure from Subba et al.⁵⁷

TOPIC 3: Measurement and Instrumentation

There are many aerosol types in the atmosphere, while only few particles are relevant for a specific problem. As a result, measurements that are especially selective to detection of the few relevant particles are required. Sensor could possibly be mounted on UAVs, weather towers, and the 530 US Ameriflux towers⁵⁸. Particles captured in a liquid water condensate are especially amenable to a variety of analysis tools including chemical and biological assays, fluorescence spectroscopy for identification of bioaerosols, and genomic analysis for early pathogen detection (similar to the monitoring of community level covid in samples taken from water treatment plants).

Key knowledge gaps:

- Need to integrate DOE atmospheric and bioaerosol measurements into larger-scale measurement/data networks such as maintained by Ameriflux and JGI. Integration of data on bioaerosols with genetics would be a huge asset.

Sidebar 3.3. Water condensation growth capture: Efficient particle collection over 5nm – 10 μ m particle sizes

Applications potentially relevant to BRaVE include high efficiency capture of airborne *P. syringae* and other potential cloud condensation nuclei; viable viral and bacterial particles for biothreat surveillance; and pollen and sub-pollen particles. The University of Florida – Gainesville was first to report viable SARS-CoV-2 found in hospital air using the BioSpot-VIVAS bioaerosol sampler.

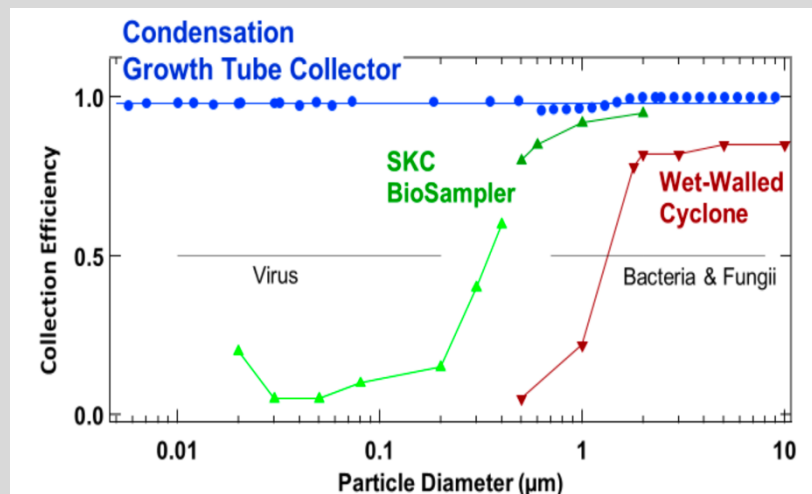


Figure courtesy of Brandon Stump Handix Scientific/Aerosol Devices

Sidebar 3.4. Mitigation involving the aerial deposition of engineered nanoparticles

Modern approaches to pathogen mitigation are rapidly moving towards next-generation sequencing and nucleic acid amplification. The application of these tools is allowing for the detection of pathogen movement and the targeting of management strategies to early stages of epidemic development where there is the greatest opportunity for reducing disease spread and mitigation effort⁵⁶. Sustainable precision agriculture is another way for agricultural communities to target the early stages of disease development through advanced technology and a systems approach to integrating genetics, climate and ecology through trusted communication networks, data acquisition, data management and use⁵⁹.

Recent advances in engineered nanoparticles (NPs) have demonstrated efficacy for treatment of a range of plant pathogens. While this is a comparatively new field, progress in uncovering the mechanisms underlying NP-plant-virus interactions and the antiviral role of NPs is advancing to the level that nanophytovirology is now considered a promising new field with tools development toward sustainable crop protection⁶⁰. Instability of topically applied RNA molecules provides only short-term (order 5 days) protection against virus infection, whereas the use of NPs as RNA carriers can help overcome this limitation by allowing more sustained and targeted release of the RNA⁶¹ [Shidore, et. al. 2021].

Workshop topics and speakers:

- (1) Aerial dispersal (Aylor, Mahaffee, Rouse, Sevanto, Gorris, Schmale)
- (2) Models and computation (Brown, Clifton, Manore, Subba, Kaufeld)
- (3) Measurements and instrumentation (Dana, Kuang, Zawadowicz, Stump, Schmidt, China)
- (4) Pathogen mitigation (da Silva, Podar, Davern, Stoll, Mirsky)

CHAPTER 4: BIOMOLECULAR CHARACTERIZATION OF BIOENERGY CROP-PATHOGEN INTERACTIONS

The development of resilient and sustainable bioenergy crops such as sorghum, poplar, and switchgrass is a focal point within the Department of Energy (DOE) Office of Biological and Environmental Research (BER). Bioenergy crops, like all crops, are susceptible to diseases that can vastly impact yield and quality. With the large-scale deployment of bioenergy crops, pathogen outbreaks will inevitably occur. Grown on marginal lands to avoid competition with food crops and subject to climate change, bioenergy crops are facing an evolving spectrum of biothreats and diseases. Plant pathogens (fungi, bacteria, and viruses) produce a stunning array of virulence-effector proteins and other molecules that interact with and hijack plant defense systems resulting in infection and disease⁶². Conversely, all plants encode intracellular innate immune receptors called nucleotide-binding leucine-rich repeat proteins (NLRs) that recognize effectors to elicit successful immune responses. Insects and other arthropods transmit pathogens in a manner that involves species-specific molecular interactions⁶³. The co-evolution of plants and pathogens drives cycles of infection and immunity⁶⁴.

Built on the lessons learned from the COVID-19 pandemic, the establishment of a DOE-led research environment integrating systems biology, synthetic biology, structural biology and bioimaging, and computational artificial intelligence/machine learning (AI/ML) to provide foundational insights into the dynamic plant-pathogen interactions for bioenergy crop security is urgently needed. This effort will contribute to the development of a resilient bioeconomy through bioengineering, breeding, and biocontrol strategies. The knowledge, technologies, and resources should be rapidly deployable for combating emerging biothreats to food crops, humans, animals, and environments.

The input summarized in this chapter was generated during two half-day virtual meetings held on February 22 and 23, 2023. A total of 43 participants, drawn from the DOE laboratory complex, USDA, NIH, DHS, and academia contributed to the meeting.

What are the key knowledge gaps in understanding plant-pathogen interactions?

The lack of understanding of molecular mechanisms that control plant infection and colonization by pathogens (fungi, bacteria, viruses) within any specific environment is the biggest knowledge gap in developing novel resilience and therapeutic strategies. Examples of research questions include:

- What virulence effectors are produced by pathogens that are utilized to manipulate hosts for promoting infection and colonization?
- What host factors, including proteins, nucleic acids, and lipids are involved in response to pathogen infection and colonization?
- What are the host and environmental factors contributing to disease resistance?
- What are the structural bases of molecular interactions between hosts, pathogens and arthropod vectors contributing to pathogenicity, disease symptoms, transmission, disease progression and epidemiology?

Research Strategy

To improve our understanding of the molecular mechanisms that control plant-pathogen-vector interactions and plant immunity will require a research strategy that makes use of the following components:

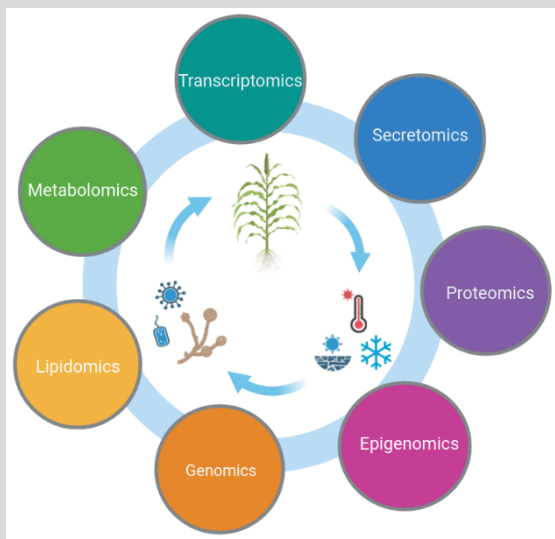
- a systems biology approach and spatiotemporally resolved measurements at (sub)cellular resolution preferably from the same samples and *in situ*,

- bioimaging approaches to visualize the dynamics of vector-plant-pathogen interactions at multiple scales with high resolution, and *in-situ*,
- structural biology to determine the structures and dynamics of specific molecular interactions,
- synthetic biology to validate the interactions and to test the mitigation strategies,
- automation and robotics for HTP interactomes measurement of inter and intra-species,
- integration of data across different modalities and scales for predictive modeling of pathogen infection and remediation.

Specific Research Needs

Multi-scale multi-omics systems approach to link genotype to phenotype. Plant organs and tissues contain a variety of cell populations and subpopulations, which are well-organized in defined patterns to implement critical biological functions such as homeostasis and response to environmental stressors and pathogens. Identifying the molecular basis of plant-pathogen interactions requires in-depth knowledge of spatiotemporal organization and interaction of (populations of) biomolecules within biologically relevant systems. Accomplishing this requires multiscale and multimodal omics measurements with small sample amounts (down to single cells and single molecules), increased throughput of measurement (tens of thousands of samples), and optimized informatics tools, including metadata. Most existing single-cell technologies only capture single modalities of molecular information and therefore provide only a partial picture of a cell's phenotype, which is determined by the interplay between the genome, epigenome, transcriptome, proteome, and metabolome. Novel spatial and single-cell multi-omics technologies are

Sidebar 4.1. Multi-omics systems approach to link genotype to phenotype

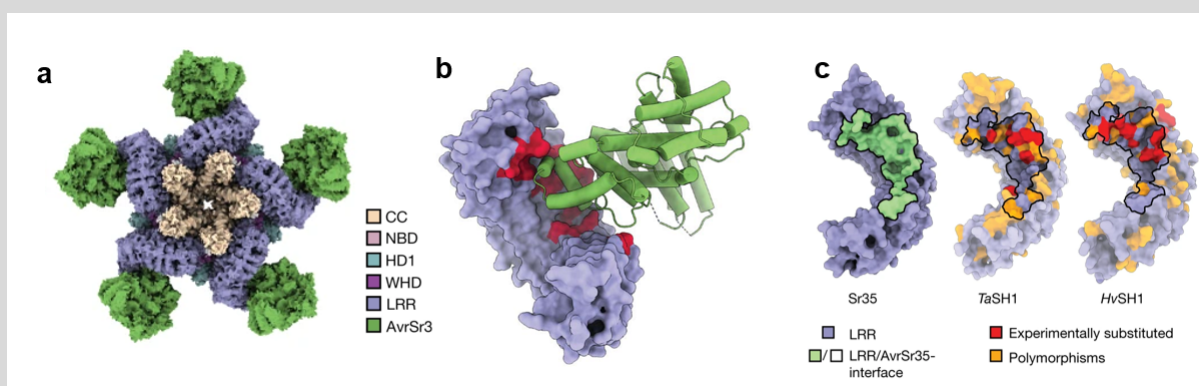


Plant-pathogen-vector interactions are dynamic and specific, requiring a systems approach to interrogate both the spatial and temporal spaces within the specific environment (cold, drought, heat). The dynamic evolution of host and pathogen genomes and epigenomes necessitates multi-omics approaches to fully understand the interactions and changes of the hosts, pathogens, and the environment. Such a systems approach may allow for the understanding of genetic and molecular basis of pathogenicity and identification of virulence factors conferring plant disease. This knowledge helps develop both engineering and biocontrol strategies for disease mitigation.

required to attain comprehensive insights into gene regulatory networks, cellular metabolism, and temporal dynamics that underlie plant-pathogen interactions. Some of the approaches discussed included expanding capabilities for probing posttranslational modifications, ligand/metal binding, protein folding, and complex stoichiometry and dynamics as they relate to biological function. Systems approaches will deliver a more holistic understanding of plant defense mechanisms that can be harnessed for controlling diseases and engineering resilient bioenergy crops.

Structural biology and bioimaging approaches to visualize the dynamics of plant-pathogen-vector interactions with high resolution, and in-situ. Cell architecture dictates function and phenotype, but disentangling this relationship remains one of the great challenges for understanding, predicting, and controlling plant-pathogen interactions. Bridging this gap will require visualizing the spatial organization of all cellular components, from organelles down to single molecules and single atoms. This could be accomplished through multimodal measurements leveraging recent advances in micro-crystal and time-resolved x-ray crystallography, state-of-the-art cryo-electron microscopy with near-atomic resolution cryo-electron tomographic imaging capabilities, optical imaging including using [quantum-enabled bioimaging and sensors \(quantum dots\)](#), and fluorescence tags, and deep-penetration x-ray imaging. Plant pathogens secrete toxins, such as aflatoxin from fungi. Structural biology can help us better understand these toxins and how to regulate their production for disease control. Each cell is different and changes constantly during its lifetime. Assessing cell heterogeneity and dynamics requires multimodal measurements of the same cell types or the same cells. Innovative real-time high-resolution measurement capabilities are needed to enhance our understanding of complex vector-plant-pathogen interactions in situ. For example, cryofixation of biological samples is an effective way to reduce measurement-caused radiation damage, and there is an urgent need to develop cryofixation methods for studying vector-plant-pathogen interactions at both, single-cell and bulk levels. Achieving contextual visualization in conditions of heterogeneity “real world conditions” is especially important for accurate interpretation of results.

Sidebar 4.2. Harness molecular interactions between host NLRs and pathogen effectors to enable engineered disease-resistant plants



Atomic-resolution molecular interactions between plants and pathogens provide structural basis for creating disease-resilient plants. Structural biology was used to elucidate atomic details of how a wheat nucleotide-binding leucine-rich repeat (NLR) receptor Sr35 forms a complex upon recognition of its pathogen effector AvrSr35 to confer disease resistance (a). The specific interaction surface (red) shown in (b) allowed the engineering of functionally unknown NLR receptors in bread wheat (*TaSH1*) and barley (*HvSH1*) to confer disease resistance (c)⁶⁵.

Synthetic biology to understand the dynamics of vector-plant-pathogen interactions. Our ability to manipulate both DOE-relevant plant species and microbes needs to be improved. For example, to study

vector-plant-pathogen interactions, there is a lack of bioimaging tools at the single-cell level. Fluorescence and affinity tags may be used for bioimaging and structural characterization of plant-pathogen interactions. However, transforming plants with a genetic tag is challenging. Therefore, there is an unmet need for high-throughput transformation of arthropod vectors, plants and pathogens with increased transformation efficiency. There is also a need to use synthetic strategies such as CRISPR-Cas9 genome editing, to explore vector-plant-pathogen interactions through up- or down-regulation of gene expression. Such advanced synthetic strategies will lead to the creation of disease-resistance bioenergy crops or biocontrol of pathogens and vectors.

High throughput (HTP) interactome measurements for inter- and intra-species and between all biomolecules. Systems approaches are key to the discovery of the molecular basis of interspecies interactions. Cells utilize a wide range of molecular machines (e.g., multiprotein complexes) in a coordinated fashion to perform complex biological functions such as response to a pathogen and/or environmental stressors. Vector-plant-pathogen interactions are highly heterogeneous varying at cellular and species levels and are modulated by environmental conditions and development stages. Unlocking the molecular mechanisms that control pathogen infection, colonization, and subsequent symbiosis requires the characterization at individual organism and cell levels. To get population meaning, the number of interacting data to be acquired will be immense. Addressing this need will require advances in automation and robotics for measuring the interactome including inter-, intra-species, and between all biomolecules that interact with each other. Three key research needs exist for studying vector-plant-pathogen interactions: 1) automated capabilities for high throughput screening, sample preparation for multiomics, phenotyping, protein expression, and validation of molecular interactions between plants and pathogens; 2) automation for connecting individual measurement steps; 3) creation of a mission-driven automation infrastructure for studying vector-host-pathogen interactions that is flexible enough to address future biothreats.

Computational integration of data across different modalities and scales to fully understand vector-plant-pathogen interactions. Connecting the structure and bioimaging data to global and single-cell multiomics data (genomics, transcriptomics, proteomics, lipidomics, metabolomics, and metallomics) with the needed spatiotemporal resolution is prerequisite for deciphering molecular basis of vector-plant-pathogen interactions and diseases. When considering data integration for plant-pathogen interactions, we are immediately faced with the dilemma of lacking much of the high-quality data that we would need to integrate. We have a significant challenge in integrating data and insights between experiments done in the lab versus experiments done in the field. As we scale up the collection of data itself, we must also scale up and improve our mechanisms for integrating data into data repositories. We need to improve data standards with consistent metadata collection and labeling across experimental and computational labs. Metadata must be collected at all stages of the experimental and computational processes. AI/ML is making great strides in data integration and analysis, but current algorithms are black boxes with little explanation of how predictions are made. We need explainable AI/ML and to develop more AI/ML methods for predicting plant-pathogen interactions and pathogenicity of microbial strains. Graph theory and hypernetworks are essential tools, and image and text data can be integrated using large foundation models such as GPT-4, DALLE-2, and BERT. Mechanistic modeling approaches also need work to build a deeper understanding of these systems. Ultimately, we need to integrate mechanistic and AI/ML methods for the best results.

How DOE may play a role in addressing these gaps?

Seamless integration of experiments and computing is required to support and grow emerging capabilities necessary for elucidating molecular components and their interactions in the context of intra- and intercellular interactions. The most pressing needs identified included:

Coordinating available resources through the organization of match-making workshops, networking opportunities, etc. to bring together expertise and capabilities. DOE can play a lead role in organizing regular workshops, for example, annual or biannual, to bring together experts from DOE national labs, academia, and industry, and support team-based projects. DOE can help organize working groups for data standardization, validation, and integration. Following the workshop report, DOE can release funding calls to address identified science and technology gaps led by DOE labs in partnership with other institutions.

Developing new technologies to address current gaps and integrate across different measurement modalities. DOE could develop new technologies to bridge gaps between measurement and computational modalities, such as multi-omics, bioimaging, automation, systems analyses, structural biology, and computation. This could enable the development of surveillance programs for early pathogen detection and field-deployable technologies. DOE should develop explainable AI/ML, linking laboratory and field work, integrating cell atlas and microscopic data, and improving sequencing and synthetic biology methods. Investment is also needed in combining AI/ML and domain research and integrating AI/ML into data integration platforms, building on existing efforts at DOE light sources and nanocenters, and aligned with the objectives of DOE-BER's Unified Data Infrastructure initiative⁶⁶.

Building and operating large-scale automation instruments for scientific research. With the identified research gaps for automation and robotics, DOE can lead the development of prototyping automated systems to study plant-pathogen interactions. DOE can bring together experts in biology, computational science, and automation engineering from DOE national labs, academia, and industry to support team-based projects. DOE should fund the automation groups to provide a service to the broad community. DOE could also develop capabilities to transform bioenergy crops and non-model pathogens and discover new microbes with desired traits. Such capabilities could be part of the automation facility. Furthermore, DOE could integrate individual lab efforts with their existing user facilities or create new user facilities to allow automation using robotics and AI/ML for autonomous DBTL (design, build, test, and learn) cycles for bioenergy and agriculture biosecurity. Additionally, abilities to grow plants and monitor plant-pathogen interactions under controlled and real-world conditions could be improved for high-throughput phenotyping and standardized data collection.

Developing bioimaging techniques. DOE could facilitate special and temporal research of plant-pathogen interactions by developing cellular imaging tools that can deal with limited samples and insufficient sensitivity. Cryo-FIB-SEM (cryogenic focused ion beam scanning electron microscopy) based electron tomography and x-ray tomography are of interest for high-resolution imaging across scales. To circumvent the limitations on sample thickness, near-infrared, hard x-ray imaging, and biosensors including fluorescence tags might be developed. To reduce radiation damage during imaging data acquisition, cryofixation technologies needed to be developed and implemented at DOE electron and x-ray imaging facilities. To further promote collaboration and scientific productivity, DOE should establish a virtual bioimaging research environment to foster close collaboration between DOE user facilities and individual research labs. This is especially important for biopreparedness, which requires timely collaborative research and coordination in rapid response to emerging biothreats.

Integrating facilities, users, and data. Given that DOE-funded facilities play a key role in data collection (JGI, EMSL, APS), data integration (JGI, EMSL, NMDC, KBase, PDB), predictive modeling (KBase), and the under construction Microbial Molecular Phenotyping Capability (M2PC), DOE has an enormous opportunity to aid in addressing data integration gaps. These user facilities can leverage their roles as hubs in the scientific process to provide some leadership. Facilities can organize and engage with scientific communities through workshops, which can be applied to aid in the development of data standards. Facilities can also encourage and enforce data standards. Facilities can ensure FAIR data and offer mechanisms for data sharing and publication that ensure data generators are properly credited and engaged in analysis at all stages. Finally, facilities can be expanded and improved to support large-scale, high-throughput, low-latency data integration and continuous data modeling to improve data quality and validation. All of this requires greater cooperation between the various DOE-funded facilities.

Enable data collection at a large scale across a wide range of spatial conditions and plant strains with time series to permit dynamic analysis. DOE could create a data collection system that will span a wide range of spatial conditions and plant strains. This data, standardized and benchmarked to support effective analytics, will be used to create time series which will allow for dynamic analysis. This data will be used to develop predictive models that can accurately predict the behavior of a variety of biomolecules. The goal is to be able to anticipate potential bio-crises and respond to them quickly and effectively. DOE can also build the necessary infrastructure and models to stay ahead of any potential crises and prevent them from escalating. Such work may allow the prediction of the transferability of pathogens and immune responses between organisms (and individuals) and also the prediction of virulence from the genome (towards specific crops and under different (a)biotic stresses).

What investments are needed so DOE can play its role in addressing the knowledge gaps?

DOE national laboratories and user facilities offer state-of-the-art capabilities including x-ray and neutron sources, omics resources, high-resolution electron microscopy and imaging resources, advanced supercomputing, etc. While these capabilities have been invaluable for scientific discovery, and enabling rapid response to threats (e.g., COVID-19 pandemic), there are significant needs for additional modern large-scale resources to facilitate lab and field studies.

- ***Developing and implementing novel bioimaging tools.*** We need to develop bioimaging tools with a wide field of view to image larger samples such as the microenvironments surrounding roots and tissues, and to detect and characterize plant-level pathogen infections. Single-molecule bioimaging tools are needed for plant research, which can be adapted for studying biothreats when necessary. Mathematical formalisms are needed to quantify imaging that works at different scales, moving from observations and correlations to the relationships between features in images at the same scale and then images at different scales, to truly become multiscale. Multi-scale structural biology and bioimaging capabilities are needed to connect information from the atomic to meso- and micro-scales for the same samples, activities, genotypes, and phenotypes.
- ***Developing AI-driven and HTP-enabled microbial communities and energy crops for desired traits.*** We need to develop AI-driven and HTP-enabled model systems of microbial communities and bioenergy crops with advanced engineering strategies. These model systems could be used to study genes of these microbes, vectors and plants and their regulatory mechanisms, as well as plant and vector immune systems, pathogen transmission by arthropod vectors and the complex genomic responses to pathogens that are important for resistance to diverse stressors in plants.

- ***Developing a new program on plant transformation and growth.*** Such a program could develop tools and methodologies for genetic engineering and transformation of energy crops. The programs could also automate the growth of engineered plants and monitor their growth and physiology in real-time. The platforms could be upgraded to autonomous laboratories to support AI-driven experimentation on plants. The program could develop model plant–pathogen systems and perform longer-term experiments to increase understanding of their interactions. Additionally, such a program could create a database for gene functions and their regulatory mechanisms based on the experiments carried out through this program and curating information obtained from other databases.
- ***Enhancing DOE user facilities and investment for studying plant-pathogen interactions including early detection, characterization, and remediation.*** To address many of the gaps identified, DOE facilities need additional investment to support computation, data standards, data curation, data sharing, distributed learning, publication, ontologies, community development, and new methods development. DOE also needs to invest in more automated large-scale infrastructure that can be used for rapid screening for phenotyping and bioengineering. DOE could invest in new beamlines and instrumentation, support early-career scientists, and provide training to new users for multi-disciplinary research. DOE could invest in its existing bioimaging user facilities for studying vector-plant-pathogen interactions and support instrumentation for cryo-imaging and real-world imaging. Additionally, DOE could invest to support lab and field studies of emerging biothreats.
- ***Developing biosafety contained large green-house and field trial facilities for plant disease research.*** USDA APHIS has defined procedures to handle plant pathogens, with the BSL-3 required for seven plant pathogens listed as Select Agents ([USDA Plant Protection and Quarantine Select Agents and Toxins list](#)). Within the “One Health” concept, some plant pathogens may adapt and jump to humans. The participants highlighted the need for more investments in national labs and facilities to create and maintain robust but adaptable biosafety infrastructure and support basic and applied research, including the development of new technologies and establishment of new (or expansion of existing) user facilities and programs to study plant-pathogen interactions. Investment is also needed to develop plant biosafety control standards in handling and disposing of plant pathogens.

What barriers exist in collaborating with other stakeholders and how can they be addressed? Combating the emerging biothreats requires national and international collaborations on the detection, analysis, and mitigation. This is a lack of tools for fostering pathogen-research collaborations, such as universal/ accessible, and shareable platforms and access to advanced facilities and resources. There is a barrier to data standardization, sharing, and credit distribution among DOE labs and facilities and with other stakeholders. The paperwork needed for material transfer and fieldwork, USDA and CDC permits for pathogen research, access to appropriate BSL facilities, and safety protocols in different facilities can slow down progress. There is a lack of technology transfer harmonization and material transfer processes (sample, data, protocols, and results) within DOE labs and with other agencies.

Funding for collaborations among researchers with diverse backgrounds is limited. The partitioning of research into disparate silos was recognized as a barrier to progress and further investments to bring national labs and facilities together and encourage inter-agencies collaborations are needed to bridge the divide and strengthen multidisciplinary science. Collaboration between DOE user facilities and research labs presents opportunities, but efficient access and facilitation among participating collaborators

become critical to maximize their benefits. The USDA and university expertise with specific host cultivars and strains of potential pathogens may create constraints in conducting comprehensive studies at DOE facilities and resources.

What facilities and expertise are available currently across DOE, USDA, DHS, and the academic sectors?

Many labs have automation efforts and lots of domain expertise may be utilized and integrated for studying vector-plant-pathogen interactions. For example, USDA has many greenhouses, field sites and has diverse expertise in multitrophic plant-vector-pathogen interactions from lab to field which should be brought into the campaign of developing automation and robotics to study plant crops biosecurity. DOE has user facilities of JGI, EMSL, x-ray, electron and neutron sources (ALS, SSRL, APS, NSLS-II, SNS, HFIR), structural biology, bioimaging, and microfluidics capabilities⁶⁷. How to integrate them for mission-based research through automation labs such as Agile Biofoundry (LBNL) and iBioFAB (UIUC) will be in high demand.

DOE and NIH have extensive bioimaging resources but not USDA and DHS. DOE, USDA, and DHS may work together to establish cross-agency facilities and capabilities at DOE national laboratories. For example, USDA and DHS could invest to enhance existing facilities or add new capabilities to DOE user facilities, such as x-ray and neutron facilities, and cryo-EM facilities.

DOE user facilities have extensive and leading expertise in biological data aggregation and predictive modeling, but they have been operating largely in isolation. Efforts to harmonize with resources outside of DOE are happening, but mostly through informal working groups. NIH, NSF, USDA, and NASA all have facilities and expertise that DOE could leverage. RCSB-PDB is an example of a facility that operates effectively across multiple agencies, with significant progress made in structural biology data standards development. Significant expertise in data integration, visualization, and large-scale modeling is also present in high-performance computing facilities, and an effort should be made to bridge this expertise with biologically oriented facilities. KBase is a major hub for all of the needed expertise as this particular facility can be used for data integration and predictive modeling.

What are longer-term research questions DOE should focus on?

A central component of the envisioned center is a research portfolio aligned with DOE's mission goal to develop resilient bioenergy crops that can be grown on land not used for food crops. Long-term research priorities focused on bioenergy crops and plant-pathogen interactions that DOE is well placed to lead include:

- ***Eco-evolutionary dynamics of plant-microbe-vector interactions.*** Dynamic interactions between plants, vectors, and microbes have both beneficial and detrimental effects, and research on these interactions can promote sustainable agricultural practices for the cultivation of bioenergy crops.
- ***Breed for a "healthy microbiome."*** Understanding the nature of disease in mixed genotype fields and wild progenitors and breeding growth-promoting stress-alleviating and pathogen suppressing commensals will create a 'healthy microbiome' for deploying large field of bioenergy crops.
- ***Programming plants as sensors and early detection systems.*** Smart bioenergy plants can be programmed to respond quickly or self-destruct when exposed to pathogens, which will involve breeding, CRISPR-Cas genome editing, and new plant biotechnology methods, including the development of efficient plant transformation systems using *Agrobacterium*.

- ***Enabling plants to respond to emerging threats in real time.*** New technologies on the development and delivery of novel disease therapies will enable plants to modify their phenotypes to adaptively respond to threats in real time, providing an alternative to plant transgenesis for the development of disease resistance.
- ***Understand metabolic pathways and regulatory networks that govern biochemical processes within living organisms.*** Research is needed to create whole-cell metabolic models and their application to investigate plant-microbe/pathogen/vector interactions, enabling a deeper understanding of the complexities of these plant-pathogen-vector relationships.

CHAPTER 5: DISEASE MITIGATION STRATEGIES FOR BIOENERGY CROPS

Introduction

Plants can be infected by all pathogen classes, with annual crop losses due to pests and pathogens across five major food crops ranging from 17.2-30% annually⁶⁸. Not only are plants the major source of human food, clothing, and shelter, but they also comprise biomass/biofuel crops for environmentally sustainable energy production. Climatic and resource changes are predicted to impact crop loss due to plant disease. Over the past 30 years, scientists have garnered rich foundational knowledge of how plants restrict pathogens by activating immune receptors and defense responses⁶⁹. Furthermore, scientists have identified promising targets that can be modified using biotechnology for pathogen restriction. Development of tools for mitigating disease in biomass/biofuels crops including screening pathogen populations, plant germplasm collections, and transformation technologies is needed. Below, we outline how foundational knowledge of disease resistance, advancements in sequencing/computing capabilities, and genome editing provide a strong foundation for focused implementation of disease mitigation strategies.

Overview of the meeting. A two-hour virtual meeting discussion with 11 scientists was held on August 17, 2023. It was co-hosted by **John Shanklin**, Chair of Biology, BNL, with expertise in lipid biochemistry and engineering biofuel crops, and **Gitta Coaker** from the University of California Davis with expertise in plant immunity, bacterial pathogens and insect-transmitted pathogens. Panelists included **Peter Balint-Kurti**, USDA-ARS at North Carolina State University, with expertise in monocot disease resistance, quantitative disease resistance, and microbiome research; **Savithamma Dinesh-Kumar**, Professor, University of California Davis, with expertise in immune receptor function and recognition of bacterial/viral pathogens, inter-organellar communications during immunity, VIGS/genome editing; **Xinnian Dong**, Professor, Duke University with expertise in plant immunity, systemic acquired resistance and bacterial pathogens; **Ksenia Krasileva**, Assistant Professor, University of California, Berkeley, with expertise in plant NLR-mediated resistance, fungal pathogens and genomics; **Doreen Ware**, USDA ARS and Adjunct Professor, Cold Spring Harbor Laboratory, with expertise in comparative plant genomics, domestication/breeding and the sustainable production of food and fuel; **Timothy Widmer**, National Program Leader, Plant Health USDA/ARS; **Andrew Leakey**, Professor and Director of the Center for Advanced Bioenergy and Bioproducts Innovation, University of Illinois Champaign Urbana, with expertise in integrative plant physiology, genetics and genomics; **Bing Yang**, Professor, Donald Danforth Plant Science Center and the University of Missouri, Columbia with expertise in genome editing, disease biology, genetic engineering of improved traits, and monocot genetics; **Paul Freimuth**, a BNL virologist, and BRaVE Co-PI was present as an observer.

Focus of the meeting: Shanklin introduced the Bioenergy Research Centers and DOE's interest in developing a bioeconomy for the production of biofuels and bioproducts in sorghum, Miscanthus, switchgrass, poplar, and energy cane, and how these activities have generated interest in biopreparedness for which BNL was awarded a National Virtual Biosecurity for Bioenergy Crops Center (NVBBCC) Pilot grant in part to conduct a roadmap exercise to identify knowledge gaps with respect to biopreparedness. He also introduced key DOE facilities including JGI, EMSL and DOE's Structure and Imaging capabilities. Gitta Coaker then led the discussion in which panelists identified key knowledge gaps

in disease mitigation strategies and what role(s) DOE might play in addressing the gaps. Discussion was roughly divided into: Identification of the most important pathogens and potential emerging threats; Strategies for identification of promising targets considering pathogen diversity; Roles of genome editing and engineering approaches for pathogen control; and How DOE's model flagship organisms could be leveraged to increase progress. They also considered what investments from DOE could help in addressing these knowledge gaps.

Disease mitigation strategies

Disease resistant plants have been developed using multiple approaches, utilizing different strategies including transfer and stacking of immune receptors, quantitative resistance, and mutagenesis. The majority of resistant plants have been developed using traditional breeding techniques, which can now be accelerated through speed breeding (altering light/temperature for fast cycling) as well as genomic selection. More recent technological advancements enable precise genome editing, receptor engineering, and modification of protein translation. Biotechnology-based approaches will benefit from the development of improved transformation, viral expression systems, and screens for target effectiveness.

Key knowledge gaps

The following knowledge gaps were identified:

- How does the engineering of lipid accumulation, manipulation of lignocellulosic components, or other bioproduct accumulation affect susceptibility to pathogens and disease progression?
- What are the best approaches to develop novel disease mitigation strategies? How can JGI's flagship models be deployed to facilitate high-throughput screening systems to accelerate the development and testing of the efficacy of different mitigation strategies?
- What is the genetic diversity of important current and potential pathogens, their wild hosts and cultivated hosts? While some biomass/biofuel crops (e.g., Sorghum) have more detailed information on variation in disease resistance and germplasm collections, others may not. Additional resources for key crops are necessary to drive breeding efforts and receptor engineering.
- What is the potential for engineering novel disease mitigation strategies? Recent advances in targeted, e.g. immune receptor engineering, as well as broad strategies, e.g. the regulation of protein translation, can be leveraged for disease mitigation (sidebars 2 and 3).
- Why are some bioenergy crops amenable to transformation and viral expression, while others are more recalcitrant to transformation?

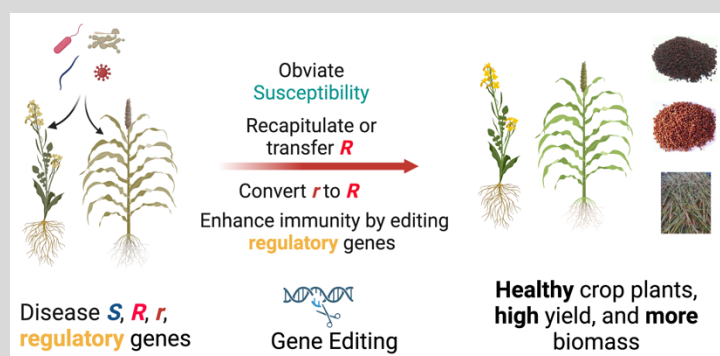
How might DOE play a role in addressing these gaps?

The following strategies are well aligned with DOE-BER's mission and capabilities:

- Develop a toolbox to facilitate disease mitigation strategies: (1) improved transformation and viral expression systems in 1-2 bioenergy crops, (2) contained rapid phenotyping platform to test efficacy against different pathogens, (3) genetic switches that can precisely tune protein translation to boost defense or plant resilience under pathogen attack^{70,71}.

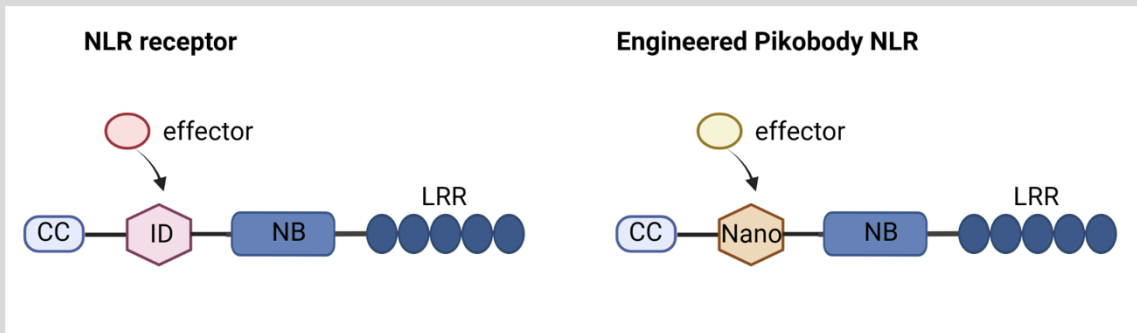
- JGI genomic support: (1) sequence breeding populations of BER target bioenergy crops that have been phenotyped for disease resistance/susceptibility to identify candidates for mitigation efforts, (2) sequence current and emerging pathogen populations to understand virulence and identify targets for disease control.
- Incorporate high performance computing to: (1) understanding ligand-receptor binding for pathogen recognition using plant pathogen specific datasets (receptor engineering), (2) deploy AIML to understand factors that contribute regulation of translational efficiency, and (3) *in silico* experiments to identify docking onto target enzymes.
- Consider the inclusion of a leading Plant Pathologist on BERAC to provide ongoing expertise and guidance to DOE regarding cutting-edge research opportunities in biopreparedness.

Sidebar 5.1. Programmable genome editing



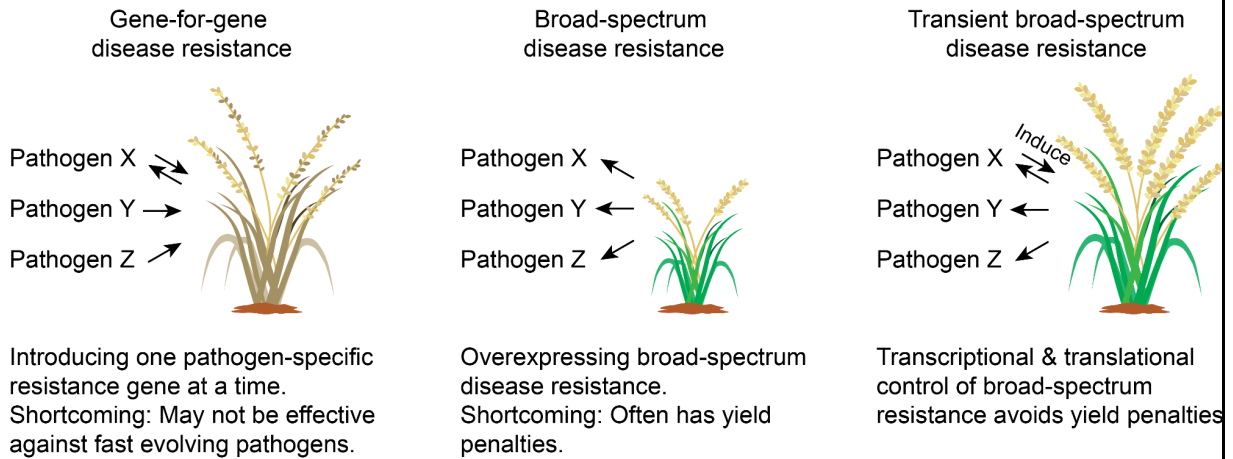
Programmable gene editing, in contrast to conventional mutagenesis and breeding, is capable of making targeted mutagenesis and precise DNA sequence changes (insertions, deletions, substitutions) in the genome of interest in a relatively short time scale. The gene editing reagents can be eliminated, leading to transgene free, non-regulated gene-edited biomass/biofuel crop germplasm⁷². Multiple mitigation strategies can be exploited for engineering disease resistance through genome editing in biomass and biofuel plants. Targets for genome editing include disease susceptibility (*S*) genes, disease resistance (*R*) genes, as well as recessive (*r*) genes or regulators that modulate immunity. The resulting gene edits can lead to high-yielding disease resistant plants.

Sidebar 5.2. Receptor engineering



Plants utilize two general classes of immune receptors: intracellular NLR receptors that recognize pathogen effector proteins delivered inside plant cells and surface localized pattern recognition receptors that can recognize damage, pathogen patterns, or extracellular pathogen effectors as non-self⁶⁹. Recent advancements in immune receptor structure enable engineering of intracellular NLRs by domain swapping to recognize new pathogen effectors, shown above⁷⁰. Receptor engineering approaches are also being deployed for surface localized receptors to enable recognition of novel pathogen features. Receptor engineering now enables detection of conserved pathogen virulence factors and design of novel receptors can be informed by understanding the genomics of pathogen populations.

Sidebar 5. 3. Pathogen-inducible broad-spectrum resistance



A challenge in engineering broad-spectrum disease resistance is the associated fitness cost because this type of resistance often involves induction of hundreds or thousands of defense genes which can have deleterious effects on yield. Translational regulation allows rapid, but transient, expression of immune proteins only upon pathogen challenge^{73,71}. By applying new techniques like Ribosome profiling and SHAPE-MaP, novel mRNA features that govern protein production during plant disease resistance have been identified. Such molecular switches precisely activate translation of defense genes only in response to pathogen infection, thereby minimizing potential yield losses⁷¹. Computational algorithms have been developed in analyzing the large-scale datasets and defining these regulatory switches in mRNAs, allowing the use of rapidly improving genome-editing tools to rationally design defense protein production to enhance crop resistance to multiple pathogens without incurring yield penalties. With DOE's class-leading facilities and resources in HPC, research and applications of such molecular switches can be rapidly expanded to include those activated by biotic stresses i.e., disease, and abiotic stresses.

CHAPTER 6: ENABLING COMPUTING INFRASTRUCTURE

Introduction

To bring together experts from various perspectives and institutions and identify partnerships within and outside DOE, as well as any necessary future investments, the NVBBCC organized four science-focused workshops. These meetings, summarized in the preceding chapters, have aimed to establish the full capabilities of an end-to-end biosecurity platform. The fourth meeting in the series focused on computing infrastructure. Specifically, a two-day virtual computing and cross-cutting meeting was held May 17-18, 2023, to elicit computational requirements for a computational platform and form a deeper appreciation of the computing landscape in which NVBBCC will operate.

The workshop afforded a comprehensive effort to address various computing aspects associated with biopreparedness, from high-performance computing (HPC) workflows to data management and modeling simulations. It also emphasized the importance of diversity, equity, and inclusion in workforce development and accessibility. The workshop began with an opening session that included introductions, keynotes, and an overview of the agenda/mission. The opening session included a talk by Jim Brase (Lawrence Livermore National Laboratory), who highlighted several related and relevant programs and suggested possible collaboration areas. The six workshop sessions were:

- High-performance and End-to-End Workflows
- Distributed infrastructure and Integration with Facilities
- Software, Services, and Analytics
- Data Life Cycle Management, Sharing, and FAIR (Findable, Accessible, Interoperable, Re-usable) Principles
- Modeling and Simulations
- Diversity, Equity, Inclusion, and Accessibility (DEIA), Workforce Development

Each session's co-chairs were responsible for developing the topics, inviting speakers and participants, leading the discussion components, and summarizing and contributing to the planning report related to their respective sessions. Each session had up to four speakers with time dedicated to discussing key challenges and opportunities. For example, the High-performance and End-to-End Workflows session discussed real-time, streaming-based coupling to simulations, integration with computing facilities, and preconfigured workflows/skeleton campaigns. Meanwhile, the Modeling and Simulations session discussed artificial intelligence/machine learning (AI/ML) capabilities and workflow integration, sharing AI models and workflows, and predictive model-data fusion.

Infrastructure Requirements

The first session primarily covered the challenges and opportunities of creating a distributed infrastructure for the NVBBCC. Some recurring concerns included the challenges in integrating data collected from different sites for distinct purposes, which often vary in quality and have unclear provenance. There is a need for better metadata and a digital archive of historical data, including the time evolution of genomes. While significant advances have been made in accessing data and computing resources, progress on integrated policies could be more advanced. Methods for secure and reliable remote access to scientific instruments are in their infancy. NVBBCC should commit to developing an

integrated research infrastructure that enables meaningful integration of data, computing, instrumentation, and related resources to allow researchers access to needed computational/data resources from anywhere.

Development of Workflows and Workflow Systems

The second session discussed workflows and workflow systems, including critical infrastructures for scientific domains that involve high-performance, data-intensive computing. For example, the Joint Genome Institute (JGI) Analysis Workflow Service, or JAWS, is used extensively for complex computational workflows with support for distributed computation by numerous BER facilities across multiple HPC-enabled sites. However, several open requirements for the NVBCC community remain. For example, there are unique challenges in handling extensive remote agricultural field data in real-time streaming analysis with a performance guarantee. Notably, it requires: 1) good network connectivity to transmit large volumes of field phenotype data (i.e., drone flying with hyperspectral imaging), 2) intelligence surveillance planning (i.e., optimal scanning strategies), and 3) efficient real-time streaming processing at the edge. The current bioinformatics, HPC, or cloud-based workflow management systems must be able to manage these capabilities. These workflow challenges also pose an opportunity to incorporate and/or improve 5G or upcoming 6G wireless communication to enable (1) high-throughput transmission with low latency and large coverage area, (2) autonomous data curation/collection using AI or OED (Optimal Experimental Design), and (3) real-time edge processing capabilities.

Data Analytics Challenges

The Software, Services, and Analytics session emphasized the need for data analysis from various sources, e.g., satellite imagery, drone or robot data, historical weather and climate data, biological pathogen surveillance data, and experimental results (see also Chapters 2-5). These datasets will be significant and require analysis techniques such as ML, HPC simulation, database query, and traditional statistics. SciServer, a platform from Johns Hopkins University, was introduced as a solution for handling some of these pertinent requirements.

For advanced software services and analytics to play an essential role in the biosecurity of bioenergy crops, additional challenges must be addressed, such as model validation, sparse datasets that cause ML training difficulties, and integrating data from different modalities to create interoperable datasets. Some notable considerations for such services include: *Given the historical inputs to a specific growing block, what will the crop yield be when the block is harvested?* and *What inputs maximize crop yield?*

Data Management, Sharing and Governance

The fourth session covered the importance of data management, sharing, and FAIR principles in biosecurity and bioenergy crops. The session reiterated the importance of data quality, including metadata and provenance, in biosecurity and biopreparedness. This includes curated data, metadata, and provenance that associate datasets used in modeling and simulation with their origin and lineage of transformation. Tools facilitating data quality control are needed to provide users contextual information about datasets; standard use formats; and metadata that makes blending data more tractable, for example, the ability to answer questions about the data life cycle, such as the technical specifications of the sensors that produced the data, calibration measurements of the sensor, how a particular quantity was derived, and in what analyses have the data been used. The session also identified that the social science component of effective data management and operationalizing the FAIR principles are vital. An environment where developing meaningful data management resources, practices, and tools is

prioritized will become essential to enabling effective data management at scale. Achieving FAIRness is a costly undertaking. Still, without proper support and incentives, this may not happen organically.

Modeling and Simulation Requirements

The Modeling and Simulations session focused on agricultural and bioenergy crop security, especially in the epidemiological setting, highlighting diverse models considering hosts, pathogens, and environmental factors. These models, including monitoring, predictive, and process models, have a range of applications. Yet, they usually focus on single elements targeting specific aspects such as statistical regression, spatial dynamics, or systems biology. Some known gaps include model specificity, lack of integrated frameworks, limited data, and inadequate uncertainty quantification (UQ).

The priority for future research includes leveraging existing crop models, developing scalable and generalizable models, linking model design to downstream decision-making, and integrating models for biosecurity needs. Future work also involves systematic model comparison, validation protocols, and UQ. Areas requiring additional clarity include the role of data-driven and mechanistic models, the limits of predictability, and OED. The goal should be to create a unified, integrated modeling approach for bioenergy crop security. For example, model design should be closely tied to the needs of downstream decision-makers, such as farmers or federal planners. Providing valuable feedback for model improvement requires considering the information flow and interactions—social and technical—between modelers, field researchers, and growers. Some challenges in integrating different models involve model coupling, multi-model uncertainty, and data-model fusion. Models must connect information across various scales from regional to intra-plot and even to plant or cellular biology levels.

Overarching Recommendations

Integrating capabilities across the workshop's five technical tracks is necessary for a comprehensive infrastructure for biosecurity for bioenergy crops. These include but are not limited to:

- Integrated research infrastructure and advanced workflow capabilities.
- Robust data management systems that can manage diverse data types to ensure their quality while adhering to FAIR principles and supporting better metadata.
- Exploration and adoption of advanced technologies, such as 5G/6G wireless communication for high-throughput transmission with low latency in poorly connected areas.
- Developing scalable and generalizable models, validation and standardization of models, and linking model design to downstream decision-making while using tools and techniques to create a unified, integrated modeling approach.

Such integrated capabilities will enable the NVBBCC to leverage diverse resources and make informed, data-driven decisions, ultimately enhancing its ability to address biopreparedness and biosecurity concerns.

Ultimately, the NVBBCC must adopt and integrate advanced computing technologies to ensure it can advance research and innovation in biosecurity for bioenergy crops and reduce response times through integrated knowledge sharing. Integrating the advanced technologies required to deliver a comprehensive infrastructure for biosecurity for bioenergy crops is equally essential. Integrated capabilities will enable the NVBBCC to employ an array of national-laboratory-based resources and make

informed, data-driven decisions that enhance its ability to address biopreparedness and biosecurity concerns impacting the nation and world.

Sidebar 6.1. Citrus Greening and Advanced Biosurveillance

At UCSB, a team has been working with citrus growers in the California Central Valley to understand how to detect and remediate the Asian Citrus Psyllid which is a vector for the Huanglongbing (HLB) disease also known as Citrus Greening Disease, see image from USDA to the right⁷⁴. HLB has largely destroyed the citrus capacity of the state of Florida which was the largest producer of citrus juice in the US before the spread of the disease.



California grows almost all of the citrus not used in juice production in the US and the California Department of Agriculture is monitoring transportation pathways for the presence of the Asian Citrus Psyllid. It has been located along the I-5 corridor leading from the Los Angeles Area to the southern end of the San Joaquin Valley, which is the region in which most of the citrus is grown. Since monitoring began, Psyllid detection has moved steadily northward from Los Angeles and it is currently detected in Ventura County, at the north end of the Los Angeles Basin.

The UCSB SmartFarm effort is working with citrus researchers to study Citrus Under Protect Screening (CUPS) -- an effort to understand the science, engineering, and economics of growing citrus in large, screened enclosures. Unlike greenhouses, CUPS are uncontrolled growing environments. The screens are dense enough to prevent the Psyllid from infecting the enclosed trees, but otherwise porous, allowing smaller (less harmful) insects to pass. The growing conditions, at scale, are not well understood. The screens attenuate wind, UV light, and solar radiation while trapping humidity and warmth.

The UCSB team has developed monitoring and alerting infrastructure for the first CUPS that has been deployed at production scale in California. The goal of the effort is to provide both environmental and bio surveillance over the multiyear lifetime of the trees that are housed in the test CUPS. This robust and long-lived sensing and analytics infrastructure is informing growers, entomologists studying the proliferation of fauna in the CUPS growing environment, and maintenance personnel who must continually maintain the integrity of the structure.

The California Department of Agriculture does not yet provide a timeline for the arrival of the Asian Citrus Psyllid in the San Joaquin Valley, but many growers believe that whatever HLB prevention technology will prove effective and commercial scale will need to be in place within the next 3 to 5 years. The Cyberinfrastructure of Things (CYoT) pioneered at UCSB is accelerating the ability to deploy CUPS as the leading methodology for the prevention of HLB in California.

CHAPTER 7: ESTABLISHING AND MAINTAINING NVBBCC PREPAREDNESS

A meeting to discuss how NVBBCC should establish and maintain preparedness was held on June 12, 2023. The meeting brought together the following individuals:

Martin Schoonen, BNL, Principal Investigator NVBBCC

Paul Freimuth, BNL, Virologist

Matthew Cowan, BNL, High Performance Computing

Ann Emrick, BNL, Biology Chief Operating Officer

Brian Gallagher, BNL, Office of Emergency Management

Clayton Hamilton, BNL, Quality Management Office

William Hurst, Jr., BNL, Manager, Office of Emergency Management

Lee Michel, BNL, DOE NNSA Radiological Assistance Program Training and Preparedness Manager

John Searing, US Dept of Homeland Security, Deputy Center Director, Plum Island Animal Disease Center

Introduction

As the nation's investment and reliance on the bioeconomy increases, it will become an ever-higher priority to ensure the biosecurity of bioenergy crops. This task will be particularly complex considering the massive scale of crop growth that eventually will be required. For example, it was estimated that a miscanthus crop the size of Wyoming would be required to meet the nation's demand for biojet fuel by the year 2040¹². Furthermore, global warming will likely increase the dynamic range of crop plant exposures to both adverse environmental conditions and to pathogens.

Key functions of the NVBBCC will be to identify threats likely to emerge as bioenergy plants are grown at commercial-scale, and to develop effective countermeasures. While valuable lessons can be learned from the USDA's long experience in securing agricultural crops, the growth of bioenergy crops under marginal conditions will expose these plants to a unique set of threats that in turn will require the development of novel approaches to ensure crop security. This would include advancing current capabilities to measure crop and plant health at multiple scales, ranging from satellite and aerial (drone) imaging of crops to subcellular analysis of pathogen-host interactions. High performance computing will play a central role in the NVBBCC by integrating data from these measurements, thus helping guide the efforts of scientists and commercial growers to fully assess threats to bioenergy crops and to develop effective countermeasures.

While the NVBBCC capabilities outlined above clearly will support fundamental studies of plant and crop health, they also could be powerful resources to deploy in response to unanticipated outbreaks of disease in any ecosystem, including human. The effective use of NVBBCC resources during emergency situations, however, could be hampered by several technical or administrative obstacles. Such obstacles could include, for example, the lack of permits required for shipping and receiving biological material, the lack of laboratory space approved for safe handling of biological samples (e.g., BSL-3 lab space for working with exotic pathogens), the lack of pre-approved standard operating procedures to study biological sample, and issues concerning intellectual property and data sharing. A planning meeting therefore was convened by the NVBBCC PI, Martin Schoonen, to identify obstacles and discuss possible ways these obstacles could be eliminated or reduced.

Structure of Meeting

The preparedness meeting was attended by preparedness experts both from within BNL and from outside institutions. The discussion started with the workflow of the NVBBCC to include the samples, equipment, and data. Each of these drive requirements. The following requirements were identified.

- Samples – samples may include plants, plant parts, spores, insects or microorganism. This includes pre-approval from USDA to receive shipments of plant pathogens (mainly fungal spores) from outside collaborators and a receiving area to open and process samples that has the appropriate containment procedures. Data collection facilities that meet all safety requirements. Reliable environmental conditions for storage of samples and an approved disposal process.
- Equipment –this includes standard laboratory equipment, sample preparation equipment and data collection and storage. There needs to be reliable, redundant power, maintenance contracts. Airborne detection may require permits (e.g., FAA). In addition, there needs to be prioritized access to NVBBCC resources during crisis periods.
- Data –compute for data analysis and storage must be in a reliable data center with data management and cybersecurity plans, redundant power and cooling systems and a duplicate off-site (e.g., cloud) storage location.

Preparedness Frameworks

There are several preparedness frameworks that can be used to test the program’s resilience and continuity. These include:

- DOE Order O 151.1D, Comprehensive Emergency Management System
- Federal Select Agent Program (USDA, CDC) – 7 CFR Part 331, 9 CFR Part 121, 42 CFR Part 73.
- FEMA Business Process Analysis and Business Impact Analysis
- Continuity of Ops DOE 150.1B
- DOE- NE Nuclear Forensics
- DOE-NNSA Radiological Assistance Program

Each of these frameworks has elements that are suitable for use in developing the preparedness program for the NVBBCC. However, the Federal Select Agent framework is considered the best fit. While it is intended and required for high-risk biological agents it provides a comprehensive program that can be scaled to match the needs of the NVBBCC. The program includes guidance for cradle to grave management of samples including shipping and permit requirements, information, cyber and physical security planning and incident response planning.

There are different types of exercises that can be used to evaluate program plans, procedures and capabilities including walkthroughs, tabletop exercises, functional exercises and full-scale exercises.

Recommendation

A preparedness program that uses the general framework of the Federal Select Agent Program as a guide will ensure that there is a robust program to ensure reliability, compliance, and efficiency of the NVBBCC. The new center can draw upon existing expertise and experience within the USDA on how to handle plant pathogens, including the ones on the [USDA Plant Protection and Quarantine Select Agents and Toxins list](#).

The center will also benefit from close coordination with the [USDA Plant Pathogen Confirmatory Diagnostic Laboratory](#) as it builds up its infrastructure and capabilities.

In addition to developing a preparedness and response component, NVBBCC could play an important role in bringing the community together annually or semi-annually to discuss potential threats, knowledge gaps, and how the community would respond to a rapidly spreading pest or a bioenergy crop collapse. These meetings would also serve as a community networking activity among experts distributed among the DOE labs, USDA, DHS, academia and the private sector. Developing this network is an important aspect of preparedness and these NVBBC-led meetings can contribute to national risks assessments.

CHAPTER 8: WORKFORCE DEVELOPMENT AND DIVERSITY, EQUALITY, INCLUSION AND ACCESSIBILITY (DEIA)

The last session of the Enabling Computing Infrastructure and Cross-cutting themes workshop examined DEIA to raise awareness about promoting diversity, equity, inclusion, and accessibility in STEM (science, technology, engineering, and mathematics), particularly in scientific computing. Diverse teams correlate with increased productivity and reduced turnover in computing-heavy teams, indicating that pushing for DEIA-forward, inclusive practices is the right thing to do and good business.

Key general findings and recommendations from the session included:

- mentorship, particularly peer mentorship, is an effective and low-cost mechanism at different stages of career development toward a more diverse and inclusive workforce;
- to build a more diverse and inclusive workforce, it is essential to intervene and engage early and proactively, ideally before college; and
- the most significant barriers to DEIA are the cultural and mindset changes needed to break people from the expected (or accepted) status quo.

The center should leverage existing DOE-Office of Science education and capacity-building programs such as its Visting Faculty Program, SULI, RENEW and FAIR to build meaningful connections with academic institutions with programs that align with its mission. Through targeted outreach in advance of the release of annual call for internship programs long-lasting connections can be established with academic institutions that have strong program in areas such as: crop science, plant pathology, digital agriculture, drone technology, and multiscale modeling. In addition, the center will develop its own PIER program. For starters it can build on the existing involvement of Texas A&M in the pilot program and the connections made through workshops. In addition, USDA has a program that connects agency staff scientists with researchers at 1890 institutions. This extensive network could be leveraged by NVBBCC.

NVBBCC faces some additional challenges in that it is envisioned as a collaborative, but distributed center. Collaborative research across different nodes is common, but responding to a new plant disease will require that the distributive team can respond effectively, leveraging necessary NVBBCC expertise and facilities. In addition, the team will need to be able to coordinate with stakeholders, such as DHS and USDA. To meet this challenge, four specific recommendations were suggested:

1. Build or strengthen existing mentorship programs that pair mentees with peer mentors (for psychosocial support) and senior mentors (for career development opportunities) within each node of the distributed center.
2. Develop intra-laboratory research efforts to develop lasting ties among staff within the distributed center.
3. Explore the possibility of personnel rotations among center nodes or extended visits to build team cohesiveness.
4. As part of the center's activities develop a diverse network of academic partners and build meaningful relationships by leveraging existing DOE programs in a strategic way. This may include

proactive outreach, developing formal ties, and building capacity at partner institutions to conduct research that is aligned with center goals.

5. Host lectures, seminars, and social events to emphasize respect and community building.

CHAPTER 9. PATH FORWARD

The workshops held as part of this BRaVE pilot project identified the need for the development of a long-term research and development program with as goals to develop: 1) high-yielding and resilient bioenergy crops based on new biomolecular insights of plant-pathogen-vector interactions; 2) the scientific basis for rapid identification of disease in the field at a regional scale to support interventions and prediction on the progression of disease; and 3) a capability to predict how diseases may spread as climate is changing. Should a new biothreat emerge in bioenergy crops there is also a need to rapidly identify the disease, predict the spread of the disease, and work with stakeholders on short-term and longer-term counter measures to minimize the impact on the bioenergy crops. Establishing a virtual, distributed center focused on biosecurity for bioenergy crops could support both the need for a long-term research program and a response role by leveraging a wide range of existing expertise and facilities across the laboratory complex.

While the six workshops provided a wealth of perspectives and input, this report also benefitted from executing an exploratory study and establishing some new capabilities as part of the pilot project. The exploratory study focused on the disease anthracnose which affects sorghum, an energy crop. Executing the study provided new insights into what facilities, workflows, and permits need to be in place while working on a plant disease. Investments in new detector systems flown on Unmanned Aerial Systems (UAS) provided insights in data needs, data volume, and data analysis workflow pipelines that need to be established. Similarly, an investment in a new Cryo-Focused Ion Beam-Scanning Electron Microscope provided insight in how to work with infected tissue and the workflows involved. Finally, an initial investment in a computer system (hardware and software) made it possible to integrate some the workflows that were developed as part of the pilot and provided insights in how it can be scaled for a mature center with a distributed workforce.

Establishing a new virtual center that leverages existing facilities and expertise will require extensive planning and a prioritization of effort. A possible path toward a center is laid out below in Table 2. The workshops conducted as part of the pilot focused mostly on research needs that DOE is uniquely positioned to take on, but these needs were not prioritized nor did the workshops focus on how to establish a new center. Since the conclusion of the workshops, DOE-BER has also awarded several new three-year projects and some of these address research questions raised during the workshops. Furthermore, some of the FY23 projects make use of research and computing infrastructure established as part of this pilot project.

Given these developments, we envision that Phase 1 prioritizes research needs for the center through planning meetings, develops out-year budgets, and sets up the center's operational and governance structure, while at the same time continuing the SciServer platform that supports several FY23 BRaVE projects and is critical to underpin a distributed center. A multi-lab working group drawn from the thought leaders who worked on developing the workshops and this report, along with additional Subject Matter Experts and DOE-lab staff with expertise in building multi-institutional and multidisciplinary programs will need to be established to execute Phase 1. BER will need to provide a modest amount of funding to support the planning effort as well as the research and computing infrastructure that was established as part of this pilot project. From this working group a leadership team will be formed that

will lead the project into Phase 2. Phase 2 is essentially a three-year ramp up period for the center. In Phase 3, the center is fully operational, including its response capability, and it would transition into a five-year review cycle.

Phase	Objectives	Timeline
Phase 1: Follow-on planning meetings and maintaining current infrastructure	<ul style="list-style-type: none"> • Develop <u>prioritized</u> research and development (R&D) needs • Identify DOE expertise and facilities that can contribute to the R&D needs • Determine gaps in capabilities to address R&D needs • Establish out-year budgets for initial R&D portfolio, center build out, and operations. • Establish operational and governance structure of new center and appoint leadership team • Establish framework for measuring progress on R&D goals • Support and expand current SciServer platform and other critical infrastructure to the benefit of new center and ongoing BRaVE research efforts started in FY23. 	FY25
Phase 2: Initiate center	<ul style="list-style-type: none"> • Integrate relevant FY23 BRaVE research efforts into new center • Initiate highest priority R&D not addressed by FY23 BRaVE project • Initiate the development of a response capability • Measure progress on R&D goals and conduct external expert reviews • Initiate an annual research community gathering, preferably by leveraging existing DOE and USDA meetings • Establish advisory committee that represent a broader stakeholder group 	FY26-28
Phase 3: operate mature center	<ul style="list-style-type: none"> • Re-evaluate R&D priorities for five-year cycle and adjust science plan • Adjust center operations and infrastructure to meet new R&D goals • Test response capability on annual basis • Continue to hold research community gatherings, preferably by leveraging existing DOE and USDA meetings 	FY29 onward

NVBBCC is envisioned as a distributed center that will make extensive use of already existing research facilities and planned ones as well as ongoing programmatic efforts. For example, BER-funded user facilities, such as the Joint Genome Institute, the Environmental Molecular Science Laboratory, KBase, various investments in structural biology and bioimaging capabilities, and the planned Microbial Molecular Phenotyping Capability at PNNL will play a critical role in the NVBBCC to conduct its research agenda and respond to new threats. Programmatic efforts such as the Bioenergy Research Centers, the Environmental Microbiome Science program, and the E3SM climate model development will also be leveraged. NVBBCC will require some specific investments in dedicated facilities to work with plant

diseases, but much of its research agenda is expected to leverage existing facilities, which makes establishing NVBBCC feasible without developing a completely new research infrastructure. In addition, there are assets, such as BSL-3 greenhouse facilities operated by USDA that may support joint research efforts.

It is also important to point out that the Pilot Project was managed by BER's Biological System Science Division, but the workshops identified research topics and capabilities aligned with and stewarded by BER's Earth and Environmental System Science Division (e.g., climate-driven spread of disease and the capabilities at the Environmental Molecular Science Laboratory). Hence, it is important to involve both divisions as plans for this new center evolve.

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APPENDIX—A: LIST OF PARTICIPANTS IN NVBBCC PLANNING MEETINGS

BIOMOLECULAR CHARACTERIZATION MEETING, February 22-23, 2023

HOST: Qun Liu, Brookhaven National Laboratory

Jeff Dangl, University of North Carolina

Benjamin Cole, Lawrence Berkeley National Laboratory

Amy Marshall Colon, University of Illinois at Urbana-Champaign

Clint Magill, Texas A&M University

Chang Liu, University of California, Irvine

Douglas Densmore, Boston University

Hector Garcia Martin, Lawrence Berkeley National Laboratory

Nathan Hillson, Lawrence Berkeley National Laboratory

Michelle Heck, U.S. Department of Agriculture/Cornell University

Robert Shatters, U.S. Department of Agriculture/ U. S. Horticultural Research Laboratory

Carolyn Larabell, Lawrence Berkeley National Laboratory

Chris Jacobson, Argonne National Laboratory/Northwestern University

Carrie Eckert, Oak Ridge National Laboratory

Jennifer Brophy, Stanford University

Sheng Yang He, Duke University

Elizabeth Rogers, U.S. Department of Agriculture/ Foreign Disease-Weed Science Research

Xong Xiong, Yale University

Andrzej Jaochimiak, Argonne National Laboratory

James Fraser, University of California, San Francisco

Lee Ann McCue, Pacific Northwest National Laboratory

Daniel Jacobson, Oak Ridge National Laboratory

Arvind Ramanathan, Argonne National Laboratory

Hyun Seob Song, University of Nebraska–Lincoln

Martin Schoonen, Brookhaven National Laboratory

Kerstin Kleese Van Dam, Brookhaven National Laboratory

Shantenu Jha, Brookhaven National Laboratory

Francis Alexander, Brookhaven National Laboratory

Paul Freimuth, Brookhaven National Laboratory

Sean McSweeney, Brookhaven National Laboratory

Ljiljana Paša-Tolić, Pacific Northwest National Laboratory

Yasuo Yoshikuni, Lawrence Berkeley National Laboratory

Qun Liu, Brookhaven National Laboratory

Huimin Zhao, University of Illinois at Urbana-Champaign

Chris Henry, Argonne National Laboratory

Tim Widmer, U.S. Department of Agriculture

Louis Prom, U.S. Department of Agriculture
Trevor Glaros, Los Alamos National Laboratory
Douglas Sheeley, The National Institutes of Health
Krista Versteeg, Department of Homeland Security
Shawn Starkenburg, Los Alamos National Laboratory
Thomas Metz, Pacific Northwest National Laboratory
Robert G. Egbert, Pacific Northwest National Laboratory
Kim Yongchang, Argonne National Laboratory
Changsoo Chang, Argonne National Laboratory
Finfrock, Zou, Argonne National Laboratory

AERIAL DISPERSAL MEETING, March 30-31, 2023

HOST: Robert McGraw, Brookhaven National Laboratory

Walt Mahaffee, U.S. Department of Agriculture
Matt Rouse, U.S. Department of Agriculture
Scott Adkins, U.S. Department of Agriculture
David G. Schmale, Virginia Tech
Olivia Clifton, NASA Goddard Institute of Space Studies
Jon Schwenk, Los Alamos National Laboratory
Kim Kaufeld, Los Alamos National Laboratory
Chongai Kuang, Brookhaven National Laboratory
Braden Stump, Aerosol Devices
Swarup China, Pacific Northwest National Laboratory
Rob Stoll, Computational & Environmental Fluid Dynamics Lab, University of Utah
Steven Mirsky, U.S. Department of Agriculture
Donald Aylor, Agricultural Station, New Haven, CT
Sanna Annika Sevanto, Los Alamos National Laboratory
Morgan Gorris, Los Alamos National Laboratory
Michael Brown, Los Alamos National Laboratory
Carrie A. Manore, Los Alamos National Laboratory
Tamanna Subba, Brookhaven National Laboratory
Bob Dana, Nano Engineering Corp., New Haven, CT
Maria Zawadowicz, Brookhaven National Laboratory
Jerry Schmidt, Nano Engineering Corp., New Haven, CT
Washington daSilva, CT Agricultural Station, New Haven, CT
Sandra Davern, Oak Ridge National Laboratory
Paul Freimuth, Brookhaven National Laboratory

DETECTION PLANNING MEETING, May 3, 2023

HOST: Alistair Rogers (Plant Physiology), Brookhaven National Laboratory

David LeBauer, (Big Data, Agriculture), University of Arizona

Nathaniel K Newlands, (Precision Agriculture, Remote Sensing), Canadian Gov

Bill Rooney, (Sorghum Breeding & Genetics), Texas A&M University

Alina Deshpande, (Pathogen detection and surveillance) Los Alamos National Laboratory

Shawn Serbin, (Remote sensing) Brookhaven National Laboratory

Andrew Leakey, (Plant physiology, high throughput phenotyping) University of Illinois Urbana-Champaign

Kaiyu Guan, (Digital Twins of Agroecosystems, Remote Sensing), University of Illinois Urbana-Champaign

Katie Gold, (Plant Pathology, Early Disease Detection), Cornell University

Turin Dickman, (Plant ecophysiology) Los Alamos National Laboratory

Yan Zhu, (crop phenotyping with UASs) University of Illinois Urbana-Champaign

Sebastian Varela, (Data Science, Geospatial Analytics, Agronomy) University of Illinois Urbana-Champaign

Jeremiah Andersen, (UAS Operations) Brookhaven National Laboratory

Sheng Wang, (Crop Monitoring, Spectroscopy), University of Illinois Urbana-Champaign

Shah-Al Emran, (Crop monitoring, remote sensing) University of Illinois Urbana-Champaign

Eric Bishop Von Wettberg, (Crop breeding, Genetics, Agro-terrorism), University of Vermont

Zhao Jiang (Deep Learning, UAS imagery), University of Illinois Urbana-Champaign

COMPUTING AND CROSSCUTTING ISSUES PLANNING MEETING, May 17-18, 2023

HOST: Shantenu Jha, Chair, Computation and Data-Driven Discovery, Computational Science Initiative, BNL

Speakers:

Arik Mitschang, IDIES/John Hopkins University

Belinda Akpa, Oak Ridge National Laboratory

Benjamin Mintz, Oak Ridge National Laboratory

Christine Kirkpatrick, University of California San Diego

Erick De Wolfe, Kansas State

Jaime E. Combariza, John Hopkins University

Jim Brase, LLNL/Livermore Computing

Jon Taylor, Oak Ridge National Laboratory

Mary Ann Leung, Sustainable Horizon Institute

Noel Blackburn, Brookhaven National Laboratory

Rachna Ananthkrishnan, Globus/Chicago

Reed Milewicz, Sandia National Laboratory

Rich Wolski, University of California, Santa Barbara

Ryan Chard, Argonne National Laboratory

Sophie Kuchynka, Rutgers-Newark

Steve Chan, JGI/NMDC

Steven Hofmeyr, ExaBiome/Lawrence Berkeley National Laboratory
Vikram Adve, University of Illinois at Urbana-Champaign

Participants accepted:

Arvind Ramanathan, Argonne National Laboratory
Brian Stucky, U.S. Department of Agriculture
Chonggang Xu, Los Alamos National Laboratory
Dale Kreiter, Brookhaven National Laboratory
Emmanuel Miguel Gonzalez, University of Arizona
Erin Acquesta, Sandia National Laboratory
Heather Savoy, U.S. Department of Agriculture
Hector Garcia Martin, Lawrence Berkeley National Laboratory
Ian Goethert, Oak Ridge National Laboratory
Ioana Danciu, Oak Ridge National Laboratory
Jay Loftstead, Sandia National Laboratory
Jean Jakoncic, Brookhaven National Laboratory
Jo Anne Crouch, U.S. Department of Agriculture -ARS
Lee Ann McCue, Pacific Northwest National Laboratory
Logan Ward, Argonne National Laboratory
Matthew Hudson, CABBI/UIUC
Nik Grunwald, U.S. Department of Agriculture
Patrick Chain, Los Alamos National Laboratory
Peeyush Kumar, Microsoft
Qianyu (“Cherry”) Li, Brookhaven National Laboratory
Robert Cottingham, Oakridge National Laboratory
Tim Germann, Los Alamos National Laboratory
Vaishnavi Ranganathan, Microsoft

NVBBCC and Organizing Team:

Alina Deshpande, Los Alamos National Laboratory
Frank Alexander, Brookhaven National Laboratory
Gerard Lemson, John Hopkins University
Ian Foster, Argonne National Laboratory
John Shanklin, Brookhaven National Laboratory
Katherine Knight, Oakridge National Laboratory
Kerstin Kleese van Dam, Brookhaven National Laboratory
Line Pouchard, Brookhaven National Laboratory
Matt Carbone, Brookhaven National Laboratory
Meifeng Lin, Brookhaven National Laboratory
Nathan Urban, Brookhaven National Laboratory
Nick D’ Imperio, Brookhaven National Laboratory
Paul Freimuth, Brookhaven National Laboratory

Qun Liu, Brookhaven National Laboratory
Robert McGraw, Brookhaven National Laboratory
Shinjae Yoo, Brookhaven National Laboratory

PREPAREDNESS PLANNING MEETING, June 12, 2023

HOSTS: Paul Freimuth and Ann Emrick, Brookhaven National Laboratory

Martin Schoonen, BNL, Principal Investigator NVBBCC
Matthew Cowan, Brookhaven National Laboratory
Brian Gallagher, Brookhaven National Laboratory
Clayton Hamilton, Brookhaven National Laboratory
William Hurst, Jr., Brookhaven National Laboratory
Lee Michel, Brookhaven National Laboratory
John Searing, US Dept of Homeland Security, Plum Island Animal Disease Ctr.

DISEASE MITIGATION STRATEGIES MEETING, August 17, 2023

HOST: John Shanklin, Brookhaven National Laboratory

Gitta Coaker, University of California
Peter Balint-Kurti, North Carolina State University
Savithamma Dinesh-Kumar, University of California Davis
Xinnian Dong, Duke University
Ksenia Krasileva, University of California, Berkeley
Doreen Ware, U.S. Department Of Agriculture/ARS, Cold Spring Harbor Laboratory
Timothy Widmer, U.S. Department Of Agriculture/ARS
Andrew Leakey, University of Illinois at Urbana-Champaign
Bing Yang, University of Missouri
Paul Freimuth, BRaVE project coordinator, observer, Brookhaven National Laboratory