NC STATE UNIVERSITY

DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

Next Generation Miscanthus: Hybrid Performance Evaluation and Enhanced, Sustainable Feedstock Production and Supply in the Southeast U.S. for Biofuels and Bioproducts

09 March 2021

Feedstock Technologies

Affordable Sustainable Energy Crops

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Project Team

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Oak Ridge National Lab

Erin Webb, Senior R&D, Environmental Sciences Division

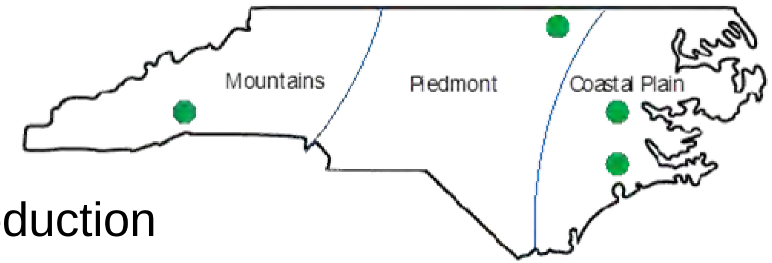
Novozymes North America • Iogen

Key Collaborators: *Carl Crozier, Emeritus Professor Soil Science; Daniela Jones, Biological & Agricultural Engineering*

Project Overview: *Energy Crop Varieties*

- Triploid Hybrids of Giant Miscanthus
  - Perennial grass *Miscanthus x giganteus*
  - 15 advanced high biomass yielding varieties
  - Developed through 10-year breeding program
    - Dry matter yield
    - Propagation capabilities

Project Overview:
Field Locations and Characteristics



- Different geoclimatic regions state
  - Mountains, Piedmont, Coastal Plain
  - Fields less suitable for commodity crop production
    - Soil survey and realistic yield potentials
    - Land capability class ratings—land use limitations (e), (w), (s);
    - NC database of yield potentials—relative productivity of soil map units
    - Land capability class ≤ 3, not labeled as prime farmland
  - Williamsdale Field Lab-Rains fine sandy loam, 3w poorly drained, 0-2% slope
  - Oxford Research Station-Appling Sandy Loam, 2e erosion, 2-6% slope
  - Mountain Research Station-Hayesville loam, 3e erosion 7-15% slope

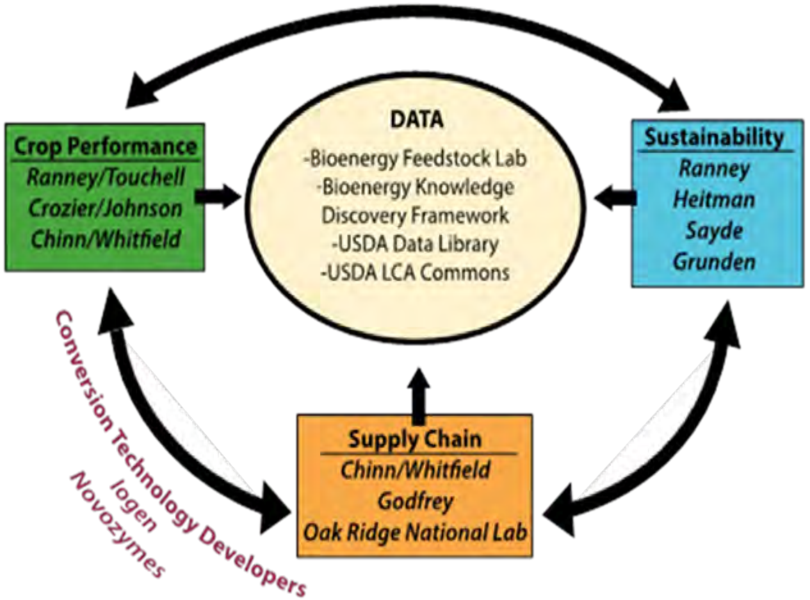
Project Overview: *High Level Project Goal*

The overall goal of the project is to evaluate performance of new miscanthus varieties and improved sustainability within the overall miscanthus biomass supply chain.

- Identify energy **crop production** that is **dependable**, **high-yielding**, **cost-effective,** and environmentally **sustainable**
- Provide **empirical data** on **yields** and **environmental effects** of bioenergy crops associated with different geographic locations, soil types and management practices that is gathered through **field experimentation**
- Enable industry to **enhance energy crop production systems** that will lead to **increased biomass supply** at **decreased cost** to conversion developers of advanced biofuels and coproducts

Project Management:
Groups, Partnerships, and Integration of Activities

- Three project Groups: Crop Performance Group I, Sustainability Group II, and Supply Chain Group III
- Interdisciplinary, complementary expertise
  - *miscanthus breeding, agronomy, soil physics*
  - *environmental and water resource engineering, applied microbiology*
  - *agricultural engineering, process engineering, analytical method development*
  - *logistics modeling, and techno-economic analysis*



Project Management: *Crop Performance Group I*

Objective 1. Evaluate performance of newly developed hybrid miscanthus varieties.

- Establish performance data in different geoclimatic regions state wide
- Characterize reproductive fertility
- Complete genetic testing to confirm breadth of variety genetic diversity and select crops with reduced disease and pest risks and improved regional and climatic adaptability.

Project Management: *Sustainability Group II*

Objective 2. Assess miscanthus production impacts on nutrient and water use efficiency, water movement, carbon fluxes, and soil health and microbial communities.

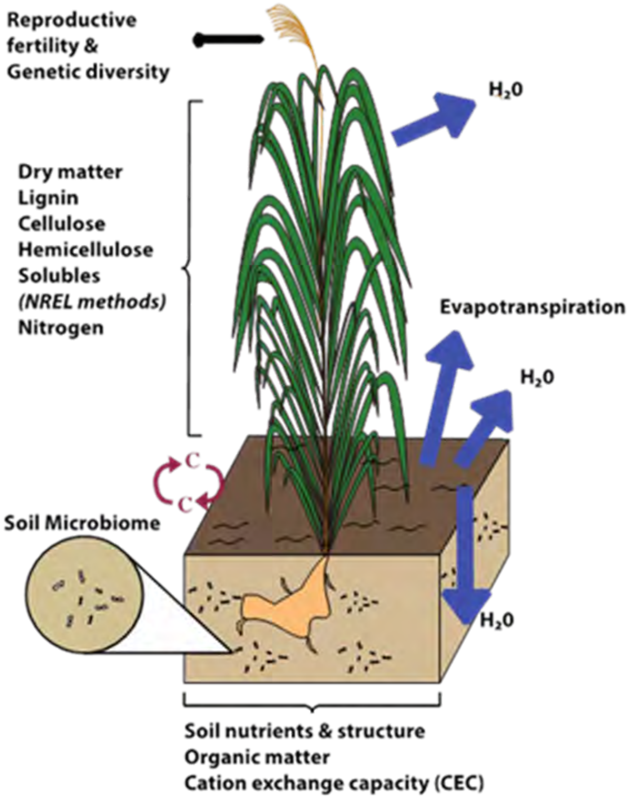
- Support empirical databases used for sustainability assessment
- Quantify impact related to plant, soil and surface water relationships for miscanthus cropping systems
- Investigate microbial communities associated with rhizomes and rhizosphere, recruited by miscanthus and that enhance nutrient provisions

Project Management: *Supply Chain Group III*

Objective 3. Develop cost efficient supply chains to deliver on-spec miscanthus to emerging domestic biofuels and bioproducts producers.

- Fill in data gaps in defining perennial energy crop harvest systems
- Develop NIR algorithms for real-time feedback on biomass composition properties and product value
- Capture economic costs of reasonable supply chain scenarios for delivery at reactor gate for less than $84/dry ton ($2014).

Approach: *Data Collected from Miscanthus Plots*



Project Risks

- Crop establishment
  - Marginal spaces
  - Consistent emergence
  - Mitigation: planting methods
- Weather perturbations
  - Irrigation
  - Weed management
- Limited measureable difference between varieties
  - Value-added research
- COVID-19 Response

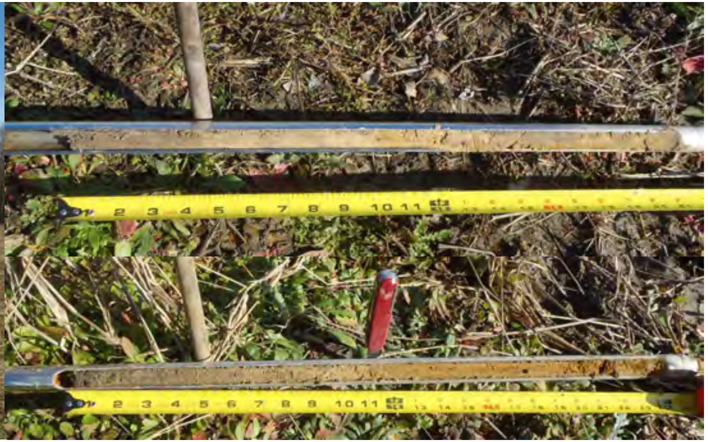
Approach: *Site Evaluation and Soil Quality*

- Soil profile verification
- Probe soil for samples at fixed depths from the surface
  - post-harvest
  - standardized across all sites
    - Initial soil profile sampling indicated similar horizon depths between sites
    - Annual samples from within 0-10 cm
- Nutrients, CEC, pH, organic matter, labile C

Approach: *Site Selection Go/No-Go*

Williamsdale (Coastal Plain)—Noboco loamy fine sand, ~ 4% slope

- Ap: 0 to 4-5 inches thick light brown loamy fine sand, weak structure, weak structure, very friable
- E: 4-5 to 12-17 inch depth light yellowish brown loamy fine sand, weak structure, very friable
- Bt: beginning at 12-18 inch depth, sandy clay loam, yellowish orange to orange (range of variation indicated in two profiles below), medium subangular blocky structure, friable, slightly sticky & plastic



Approach: *Site Selection Go/No-Go*
Oxford (Piedmont)—Helena loamy sand, ~ 3% slope

- Ap: 0 to 6 inches thick light gray brown loamy sand, very friable
- A2: 6 to 10 inch depth light yellowish brown loamy sand, very friable
- B1: 10 to 17 inch depth, yellowish orange clay loam, firm subangular blocky structure
- B21: 17 to 32 inch depth, yellow orange clay with gray mottles, firm structure, plastic & sticky
- B22: beginning at 32 inch depth, variegated clay mostly yellow but gray and red mottles, firm structure, plastic & sticky

Approach: *Site Selection Go/No-Go*

MHREC (Mountains)—Hayesville loam, ~ 8-15% slope

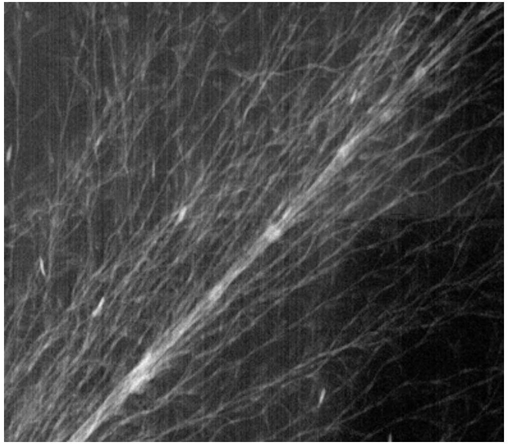
- Henderson Co., Ap: 0 to 4 inches thick, reddish brown loam, weak granular structure, very friable
- AB: 4 to 10 inch depth, reddish brown clay loam, weak granular structure, friable
- B1t: 10 to 24 inch depth, orange clay loam, common mica flakes
- B2t: 24 to 26 inch depth, reddish brown loam, common mica flakes
- C: beginning at 26 inch depth, reddish brown sandy loam, micaceous saprolite

Approach: *Crop Productivity*

- Variety Trial (*Mar-Dec*)
  - Statewide replicated trials
  - 5 hybrids, 1 commercial (4 reps)
- Plant emergence, heights, spread, survival, culm count
- Fresh and dry matter yields
- Fresh and dry matter yields
- Subsample for tissue analyses
  - Macronutrients
  - Composition analysis (cellulose, hemicellulose, lignin)
  - NIR scans (fresh, dry, stored)

Typical Rhizome Piece—2019

Approach: *Genotyping and Fertility*



- Marker systems for genotyping
  – DNA extraction and testing primer sets
  – Sequencing for profiles
  – Characterization of hybrids

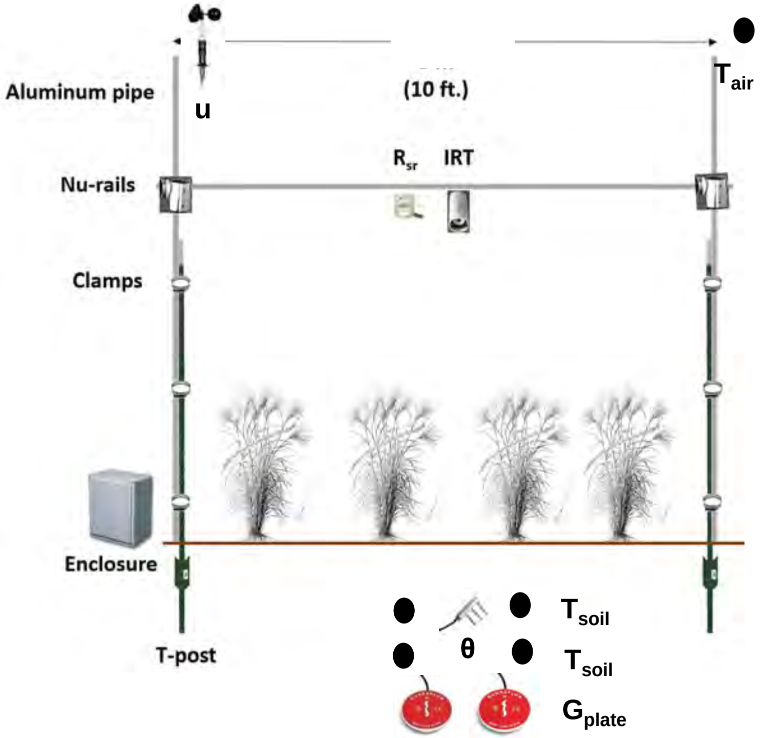
- Reproductive fertility of hybrids
  – Pollen viability
  – X-ray imaging of seed sets
  – % Reduction compared to control

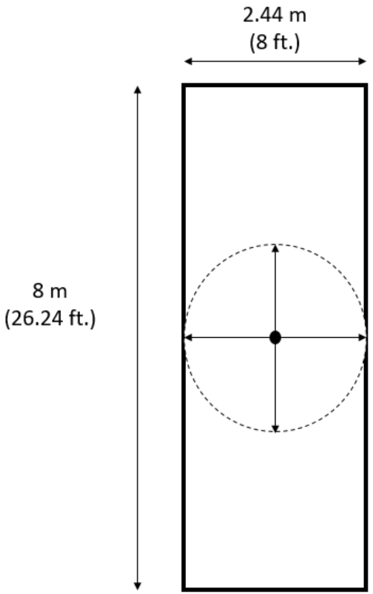
Approach: *Hydrologic Parameters*

- Use field lysimeters to capture key parameters
  - Water use efficiency (WUE)
  - ET
  - Stomatal conductance
  - Canopy interception
  - Deep percolation
  - Soil moisture
- Infiltration; Soil water retention
- Biophysical measurements

Experimental Set Up: Miscanthus (VT) plots

- R_s and LW_i are obtained from weather station pyranometer and pyrgeometer
- R_{sr} and IRT are kept at 0.7 m above the canopy. IRT is at °55 view angle





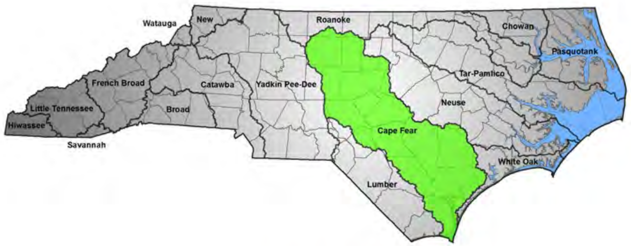
Approach: *Water Quality and Watershed Monitoring*

	Year 1 (2019)					Year 2 (2020)			
	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec
Task 10 Watershed hydrology									
Progress as of 12/2020								75%	

	Year 1 (2019)					Year 2 (2020)			
	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec
Task 11 Carbon exchange									
Progress as of 12/2020								75%	

	Year 1 (2019)					Year 2 (2020)			
	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec
Task 19 Water Quality Assessment									
Progress as of 12/2020								60%	

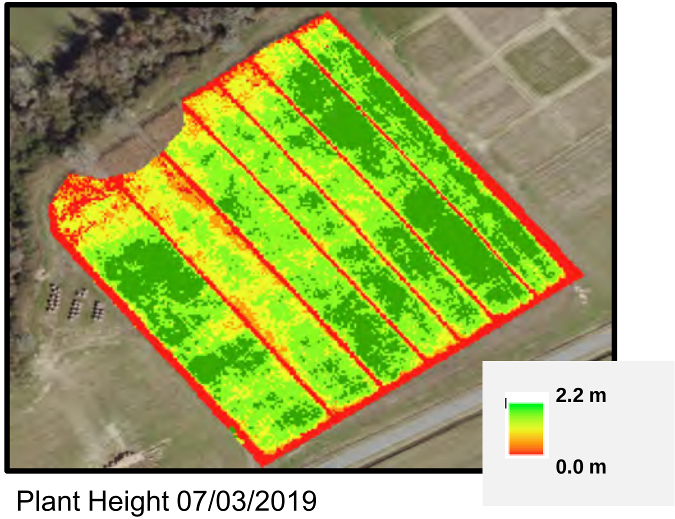
- Nutrient loading- Nitrogen and Phosphorus
  - Surface water
  - Groundwater
- Edge of field monitoring
- Wick lysimeters
- Energy and Mass Flux
- SWAT Modeling (BP 2 & 3)
  - Simulate miscanthus growth
  - Environmental impact



Cape Fear River Basin

Watershed Site

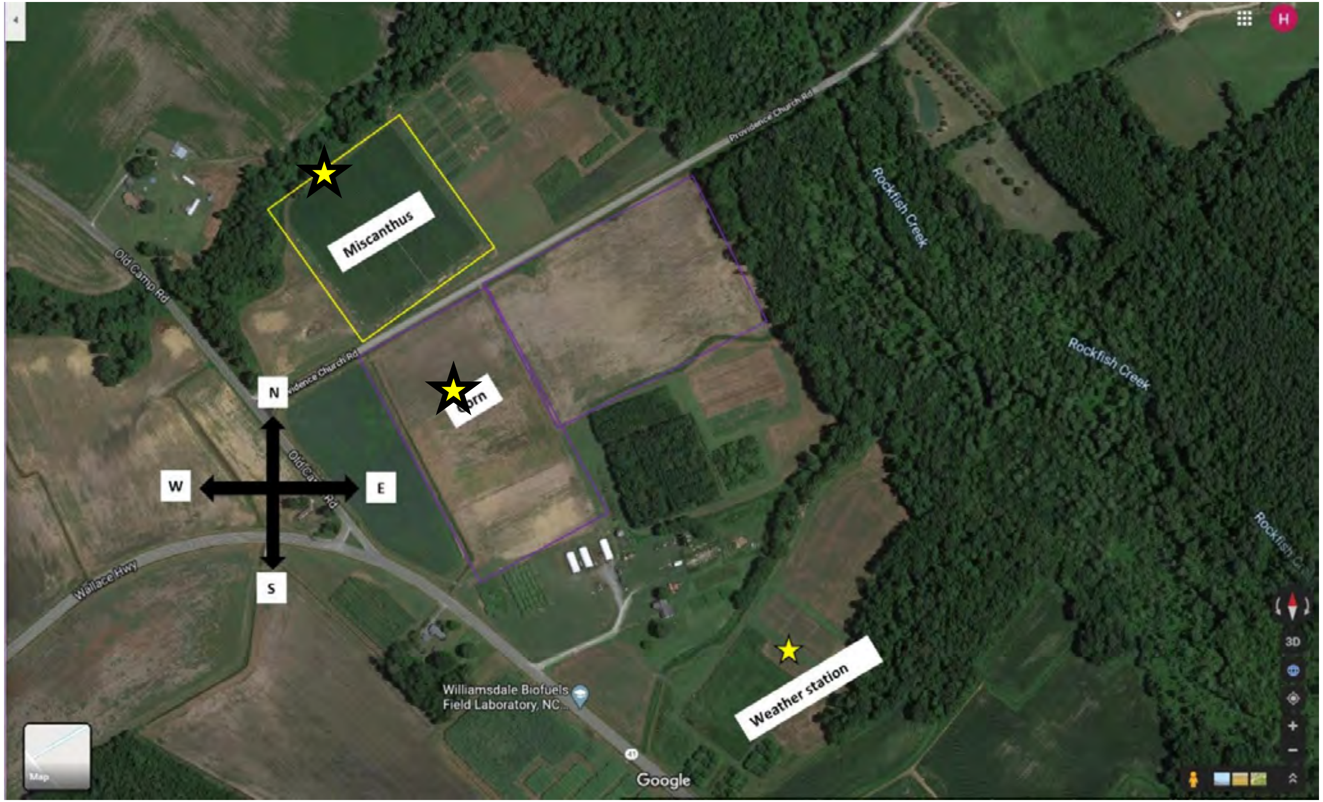
- Remote sensing to identify ecosystem scale interaction plant-soil-climate.
- Hot spots identified for targeted instrumentation and for monitoring changes in soil hydrological properties (fiber-optic cables)



Plant Height 07/03/2019

NDVI 07/03/2019

Energy & Mass Fluxes: Miscanthus vs. Corn



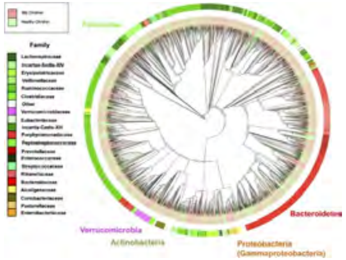
Energy & Mass Fluxes: Field set up

July 2020

Approach: *Biodiversity—Miscanthus Microbiome*

	Year 1 (2019)					Year 2 (2020)			
	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec
Task 3 Associated Microbial Communities									
Progress as of 12/2020						67%			

	Year 1 (2019)					Year 2 (2020)			
	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec
Task 9 Metatranscriptome profiles									
Progress as of 12/2020								75%	



- DNA sequences (16S rDNA/ITS libraries)
  - microbial community structure → *Who is there?*
- RNA sequences (isolated RNA to cDNA libraries)
  - metatranscriptome of microbiomes → *What they're doing?*
- Sampling rhizomes and rhizosphere soils
  - Before planting, Mid-season, Post-Harvest

Challenges:

- Sequencing read quality metrics
- Blocking amplification of host plant
- Co-extraction of RNA and DNA for copy number comparison
- Access to lab kits

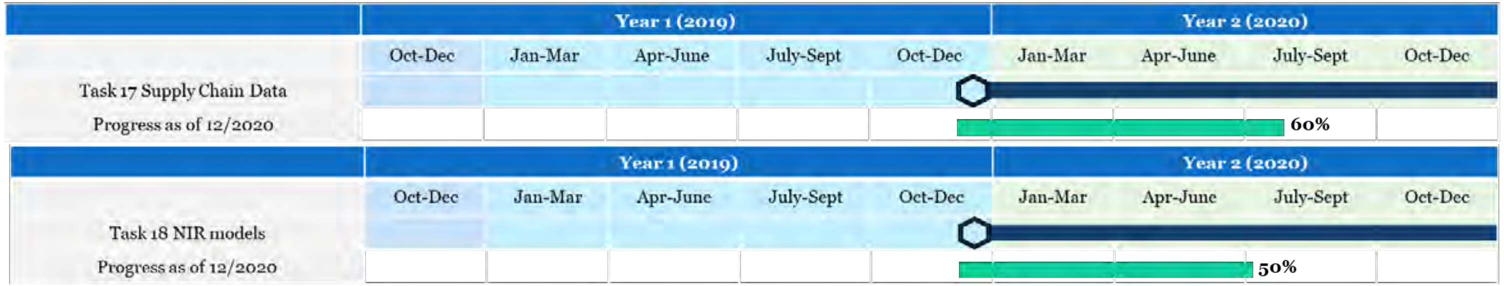
Approach: *Miscanthus-Harvest System Evaluation*

	Year 1 (2019)					Year 2 (2020)			
	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec
Task 8 Supply chain scenarios									
Progress as of 12/2020									85%

	Year 1 (2019)					Year 2 (2020)			
	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec
Task 15 Yield efficiencies of harvest ops									
Progress as of 12/2020									80%

- Data Collection
  - Hand harvest yields
  - Mechanical harvest losses
  - PTO Torque/Hp requirements
  - Fuel Usage
  - Time of Operations
- Established Plots-2 varieties including commercial
- Two harvest times
- Three operations
  - Mow and Bale
  - Forage Chopping
- Densified Storage (BP2 and BP3)

Approach: *Biomass Analysis*



- Lignin, Hemicellulose, Cellulose
- Other carbohydrates
- Combustion
  - Proximate and Ultimate Analysis
- Near Infrared Spectroscopy
- Conversion Developers—Iogen and Novozymes
  - On-spec feedstocks
    - quality and suitability

- Variety effects
  - Agronomic samples
  - Stock plot samples
  - Deposits to INL Feedstock Library
- Storage effects
  - Harvest samples
  - Storage method samples (with time)

Approach: *Logistics and Techno-Economic Analysis*

	Year 1 (2019)					Year 2 (2020)			
	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec
Task 8 Supply chain scenarios									
Progress as of 12/2020									85%
	Year 1 (2019)					**Year 2 (2020)**			
	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec
Task 13 Simulation modules									
Progress as of 12/2020									80%

- Oak Ridge National Labs
- IBSAL Model Enhancement for Miscanthus
  – Multiple supply chain scenarios
  – Field operation data
  – Feedstock parameters
- Delivery to reactor gate costs
  – $84/dry ton *($2014)*

Project Impact
End Goals

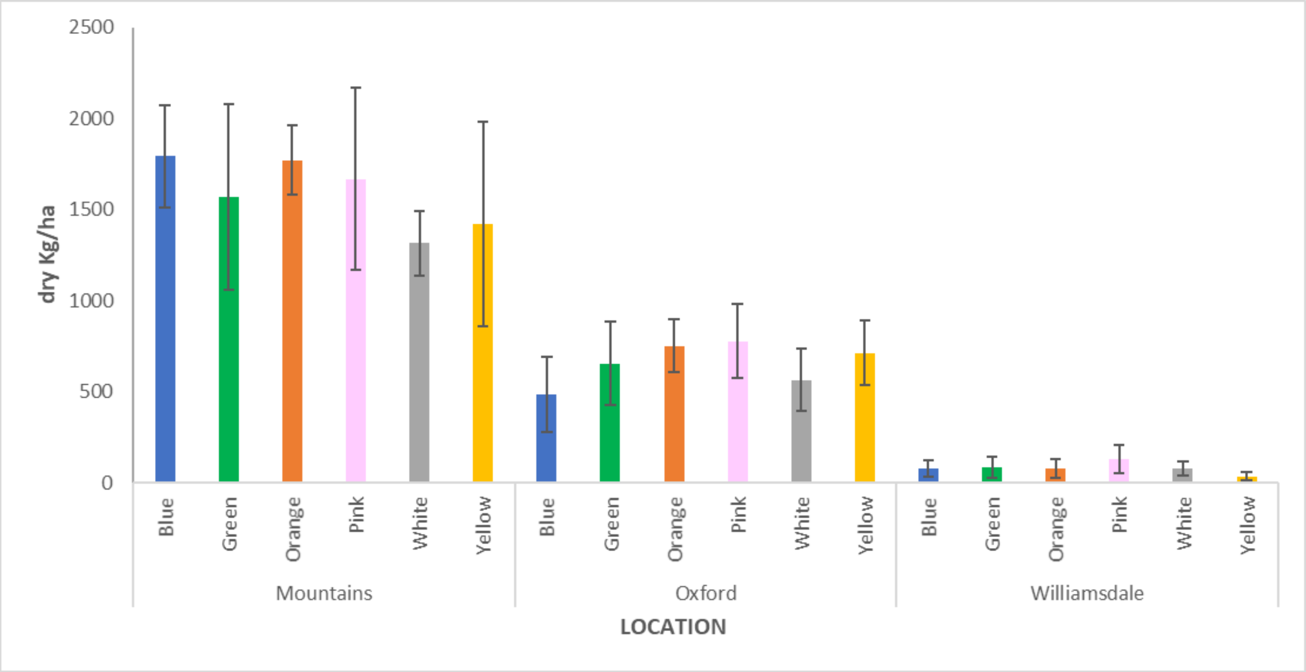
- *Variety Assessment* – Identify new varieties that outperform commercial varieties by 50% dry matter yield, higher propagation capabilities, and are sterile
- *Sustainability* – Capture localized environmental data on microbial-soil-water-miscanthus interactions from multiple regions to promote environmental stewardship decisions
- *Supply Chain* – Define regionally specific supply chain scenarios to meet delivery cost and quality performance metrics
- *Overall* – Dissemination of miscanthus cropping systems and supply chain parameters to databases serving advancement of a US bioeconomy

Project Impact

The high yielding characteristics of **newly developed varieties** will be exploited to:

- Capture relevant data that **address gaps in knowledge** surrounding impacts of bioenergy crops on local and watershed scale soil and water ecosystems
- Generate field data to **address barriers** in harvest operations, handling and transportation options that affect affordability of ***giant miscanthus*** as a viable feedstock in different US regions**.**
- Engage commercial **biomass producers** and **conversion technology developers** to **stimulate new industrial interests** and enhance market transformation.
- **Support a diversified portfolio** of crop options and contribute to further advancements in research driven models and bioeconomy development decisions

Progress & Outcomes
2019 Establishment Yields



Williamsdale

2019-2020 Survivability

Red text indicates a replant event has been triggered

As of 6/25/2020 only X1B and X3B are below threshold

	401	402	X4A	403	404	405	406	X4B
2019 Emergence	56%	27%	17%	37%	19%	19%	37%	62%
2019 Final Stand	44%	88%	83%	83%	83%	73%	65%	73%
2020 Emergence	33%	63%	79%	62%	75%	60%	56%	31%
2020 Post Replant	96%	98%	90%	96%	90%	92%	96%	108%
2020 Post 2nd Replant	98%	100%	100%	98%	94%	96%	94%	104%

	301	302	X3A	303	304	305	306	X3B
2019 Emergence	63%	65%	67%	44%	15%	56%	54%	81%
2019 Final Stand	54%	42%	40%	56%	87%	50%	62%	58%
2020 Emergence	46%	35%	27%	58%	83%	38%	37%	46%
2020 Post Replant	85%	88%	92%	94%	96%	92%	90%	96%
2020 Post 2nd Replant	94%	94%	98%	94%	98%	96%	98%	92%

	201	202	X2A	203	204	205	206	X2B
2019 Emergence	83%	56%	63%	75%	60%	25%	25%	73%
2019 Final Stand	35%	52%	50%	40%	44%	90%	85%	58%
2020 Emergence	19%	62%	46%	19%	42%	92%	79%	38%
2020 Post Replant	94%	87%	92%	96%	73%	94%	96%	96%
2020 Post 2nd Replant	98%	94%	96%	100%	94%	94%	98%	100%

	101	102	X1A	103	104	105	106	X1B
2019 Emergence	58%	65%	52%	31%	40%	27%	52%	46%
2019 Final Stand	52%	46%	75%	87%	71%	98%	62%	58%
2020 Emergence	38%	35%	58%	83%	73%	87%	60%	73%
2020 Post Replant	96%	87%	96%	94%	92%	100%	92%	85%
2020 Post 2nd Replant	96%	94%	96%	94%	94%	100%	94%	92%

Oxford

2019-2020 Survivability

Red text indicates a replant event has been triggered

	X2B	104	202	206	304	X4B	402	406
2019 Post Replant	-	92%	77%	94%	92%	-	96%	90%
2020 Emergence	77%	100%	100%	100%	100%	98%	100%	100%
2020 Post Replant	77%	100%	100%	100%	100%	-	100%	100%

	X2A	103	201	205	303	X4A	401	405
2019 Post Replant	-	94%	96%	96%	92%	-	92%	85%
2020 Emergence	69%	100%	100%	100%	100%	98%	100%	100%
2020 Post Replant	73%	100%	100%	100%	100%	-	100%	100%

	X1B	102	106	204	302	X3B	306	404
2019 Post Replant	-	88%	92%	96%	90%	-	94%	92%
2020 Emergence	77%	100%	100%	100%	100%	100%	96%	100%
2020 Post Replant	77%	100%	100%	100%	100%	-	100%	100%

	X1A	101	105	203	301	X3A	305	403
2019 Post Replant	-	94%	96%	92%	98%	-	94%	94%
2020 Emergence	77%	100%	100%	100%	98%	96%	100%	100%
2020 Post Replant	77%	100%	100%	100%	100%	-	100%	100%

Oxford 2020

April | May | June

Pink
Plot 102



Yellow-Illinois
Plot 106



Blue
Plot 103

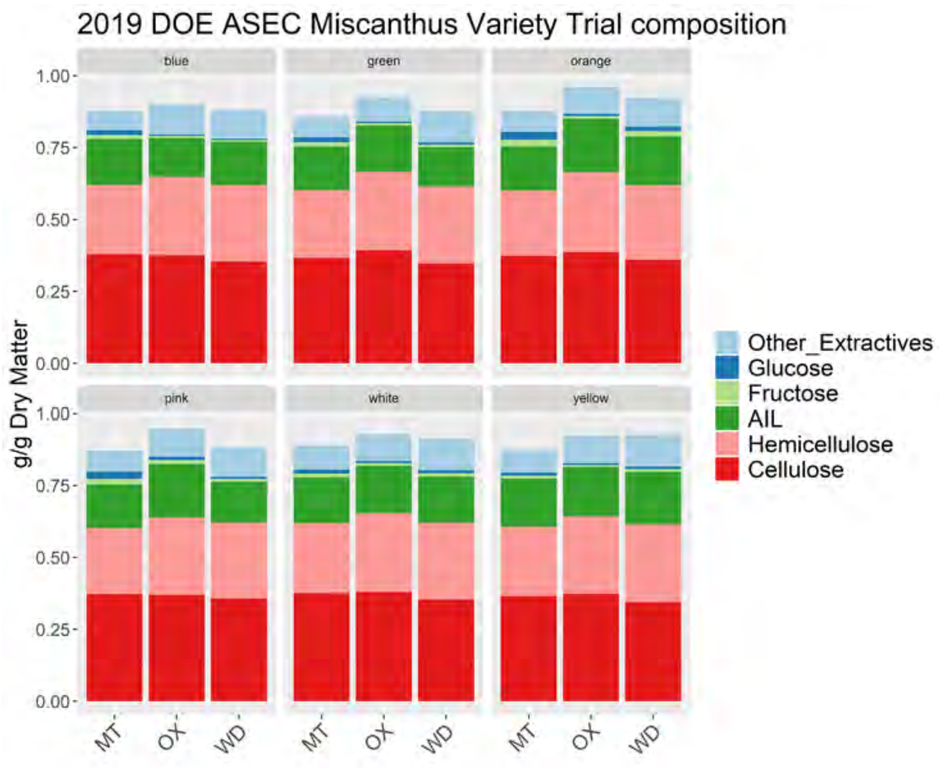


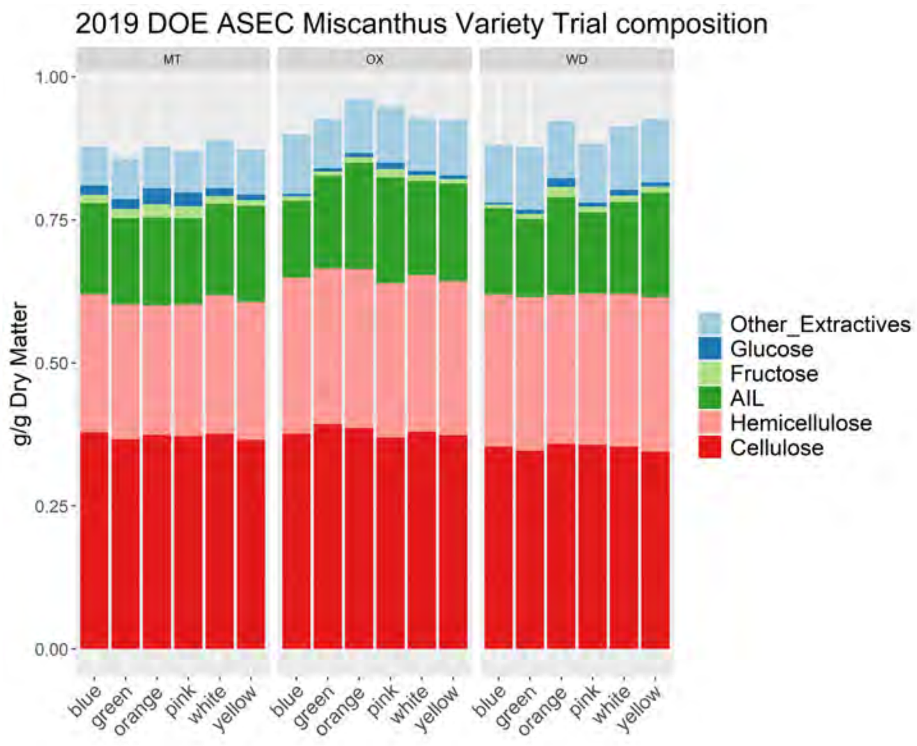
Biophysical Differences—July 2020

Yield Info—All Sites

Site	Accession	Color	Dry Yield (kg/ha)	Culm Height (m)	Dry Weight per Culm (g/plant)	Dry Weight per Height (g/m)	Relative Dry Yield to Check (yellow)
			average				
Williamsdale	2006-134	yellow	4632.12	2.57	23.78	9.29	100.0%
Williamsdale	H2015-004-074	white	6872.93	2.79	25.99	9.22	148.4%
Williamsdale	H2015-004-049	blue	6476.79	2.85	19.75	6.81	139.8%
Williamsdale	H2015-004-019	green	6962.39	2.89	20.18	6.96	150.3%
Williamsdale	H2015-004-087	pink	7242.12	3.03	16.56	5.43	156.3%
Williamsdale	H2015-004-096	orange	6810.65	3.03	21.89	7.24	147.0%
Site	Accession	Color	average				
Oxford	2006-134	yellow	8779.86	3.28	31.08	9.44	100.0%
Oxford	H2015-004-074	white	9578.99	3.43	23.92	6.95	109.1%
Oxford	H2015-004-049	blue	7481.11	2.97	18.60	6.18	85.2%
Oxford	H2015-004-019	green	9562.98	3.34	21.97	6.52	108.9%
Oxford	H2015-004-087	pink	12256.58	3.46	22.56	6.51	139.6%
Oxford	H2015-004-096	orange	11472.04	3.42	27.31	8.00	130.7%
Site	Accession	Color	average				
Mills River	2006-134	yellow	6421.08	3.23	20.00	6.21	100.0%
Mills River	H2015-004-074	white	5837.96	3.23	19.08	5.89	90.9%
Mills River	H2015-004-049	blue	5701.17	3.27	20.45	6.27	88.8%
Mills River	H2015-004-019	green	6591.14	2.98	18.34	6.16	102.6%
Mills River	H2015-004-087	pink	5099.35	2.94	19.78	6.70	79.4%
Mills River	H2015-004-096	orange	7691.91	3.29	23.72	7.20	119.8%

Composition Analysis





Upcoming Go/No-Go

	Year 1 (2019)					Year 2 (2020)			
	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec	Jan-Mar	Apr-June	July-Sept	Oct-Dec
Task 23 Variety Down Selection									
Progress as of 12/2020									75%

Task 23: Variety Down Selection—Initiate Agronomic Trial

*Task 14 & 21. Down selection of 3 varieties/site; 20% dry matter increase, rhizomatousness, 15x propagation-**DATA COLLECTION COMPLETE***

*Task 12. Reproductive fertility for down selection of varieties; low or no fertility for field trial-**80% COMPLETE, on schedule to meet targets***

Agronomic Trial-Planning Stages

- Starting Budget Period 2
- Target factors: Plant spacing, N fertility
- 3 Varieties—2 hybrids, 1 commercial
  - Yield
  - Shoot density
  - Rhizomatousness
  - Reproductive fertility (lack of)
- Plant by rhizome (March)
- Post senescence harvest (Dec)

Summary

- Integrated, multidisciplinary approach to characterize and enhance production knowledge of new perennial miscanthus varieties
- Offer guidance in defining “marginal” spaces and addressing alternative land use opportunities
- Well-positioned to define crop production and management practices around a miscanthus cropping system, including decision making opportunities in variety selection, land use, water use and quality and soil health impacts, and harvest methods, storage, and siting
- Promising results in crop performance with new hybrids relative to the commercial varieties
- Generating interesting data sets that can be evaluated and incorporated within and across supply chain functional efforts

Timeline

- October 2018
- December 2023

	FY20 Costed	Total Award
DOE Funding	**$1,937,925**	**$4,627,161**
Project Cost Share	**$435,685**	**$1,184,345**

Project Partners*

- Oak Ridge National Labs
- Novozymes North America
- Iogen

End of Project Goals & Milestones

At the end of this project we hope to meet the following ***End of Project Goals***:

Variety Assessment **–** Identify new varieties that outperform commercial varieties by 50% dry matter yield, higher propagation capabilities, and are sterile

Sustainability **–** Capture localized environmental data collection on microbial-soil-water-miscanthus interactions from multiple regions to promote environmental stewardship decisions

Supply Chain **–** Define regionally specific supply chain scenarios to meet delivery cost and quality performance metrics

Overall **–** Dissemination of miscanthus cropping systems and supply chain parameters to databases serving advancement of a US bioeconomy

Funding Mechanism

2018 Affordable and Sustainable Energy Crops (ASEC) DE-FOA-0001917

*Only fill out if applicable.

Additional Slides

Publications

- Crozier, C., D. R. Carvalho, H., Johnson, A., Chinn, M. and Heitman, J.L. 2021. *Appropriate "Marginal" Farmlands for Second Generation Biofuel Crops in North Carolina*. Agricultural & Environmental Letters. Accepted.

- Shehata, M., Heitman, J., Ishak, J. and Sayde, C., 2020. *High-Resolution Measurement of Soil Thermal Properties and Moisture Content Using a Novel Heated Fiber Optics Approach*. Water Resources Research, 56(7), p.e2019WR025204.

- Hillman, A., Young, S., Chinn, M., Sayde, C., 2021. *Miscanthus: An Environmental Choice for Marginal Lands*. NC State Extension Publications, under review.

Presentations

- D. R. Carvalho, H., Heitman, J.L., Sayde, C., Johnson, A., Crozier, C. and Chinn, M. 2020. *Estimating Energy Balance Components and ET at the Plot Scale.* ASA-CSSA-SSSA International Virtual Meeting. Oral presentation.
- Sayde, C., 2019. *DOE Giant Miscanthus Project: technologies for the environmental assessment of Miscanthus cultivation*. NC ASABE State Section Meeting, Oral presentation.
- Shehata, M., Ishak, J., Heitman, J., Sayde, C., 2019. *A Novel Heated Fiber-Optic Technique to Measure Variability in Soil Thermal Properties and Moisture Content over a Wide Range of Spatial Scales*. 2019 American Geophysical Union Annual Meeting, Oral presentation.
- Hillman, A., Sayde, C., Young, S., 2020. *High Resolution Assessment of Miscanthus Production Environmental Impacts*. 2020 ASABE Annual International Meeting, virtual poster presentation.
- Ackett, R., Sayde, C., 2020. *Novel Low-Cost Weighing Lysimeter to Monitor Crop Water Use and Deep Percolation*. 2020 ASABE Annual International Meeting, virtual poster presentation.
- Shehata M., Sayde, C., 2020. *Measuring Soil Thermal Properties and Moisture Content over a Wide Range of Spatial Scales Using Novel Fiber-Optic DTS Techniques*. 2020 ASABE Annual International Meeting, virtual oral presentation.