Michigan State University
AgBioResearch

2013 STATION REPORT

MICHIGAN STATE UNIVERSITY

Northeastern Regional Association of State Agricultural Experiment Station Directors
Sustainable Wood Energy Multistate Project
Malvern, PA
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INTRODUCTION

A thorough summary of woody biomass projects underway at Michigan State University was provided in our 2012 Station Report, submitted at the annual meeting on December 19, 2012. All the projects described therein continue. This year's report will highlight one project that began in 2013 and two that reached significant milestones this year.

WOOD ENERGY ON MSU'S MAIN CAMPUS

The Board of Trustees of Michigan State University adopted an "Energy Transition Plan" in January of 2012 which has set ambitious goals. We intend to achieve a 65% reduction in CO2 emissions and increase to 40% renewable energy by 2030 and move rapidly toward eventual carbon neutrality and 100% renewable energy. The university presently depends on its 95 MW coal-fired CHP plant for heat and electricity. Reducing the use of coal at this plant by substituting torrefied woody biomass has become a focal project. Our research, engineering, and extension resources have been directed to developing and commercializing the technologies required to 1) produce woody biomass in energy plantations and 2) convert woody biomass into a drop-in replacement for coal in existing facilities like our power plant.

Energy Plantations: MSU has begun to establish a functioning 60-acre energy farm on our main campus. This farm will consist of six, 10-acre blocks of hybrid poplars. The first block was established in the spring of 2012 and one additional block will be established in each of the next five years. These management blocks will be harvested and reestablished once each six years. This energy farm will provide research opportunities, yield reliable economic and yield data, and serve as a field laboratory for teaching and extension activities related to woody biomass production.

Torrefied Woody Biomass: Our power plant has several types of coal boilers in which we intend to test densified, torrefied woody biomass. We intend to carry-out an extended test burn of torrefied wood as soon as a supplier can be found. A Request for Proposal was released at the beginning of November with the hope of obtaining 500 tons of densified, torrefied wood for trials at our plant this winter. Obtaining large quantities of torrefied wood like this has proven to be a challenge in the past. We are cautiously optimistic that market and industry factors have matured enough to allow us to move forward. Ultimately we hope to foster the development of a distributed array of torrefaction depots in our state, capable of supplanting an ever expanding portion of the existing coal market.

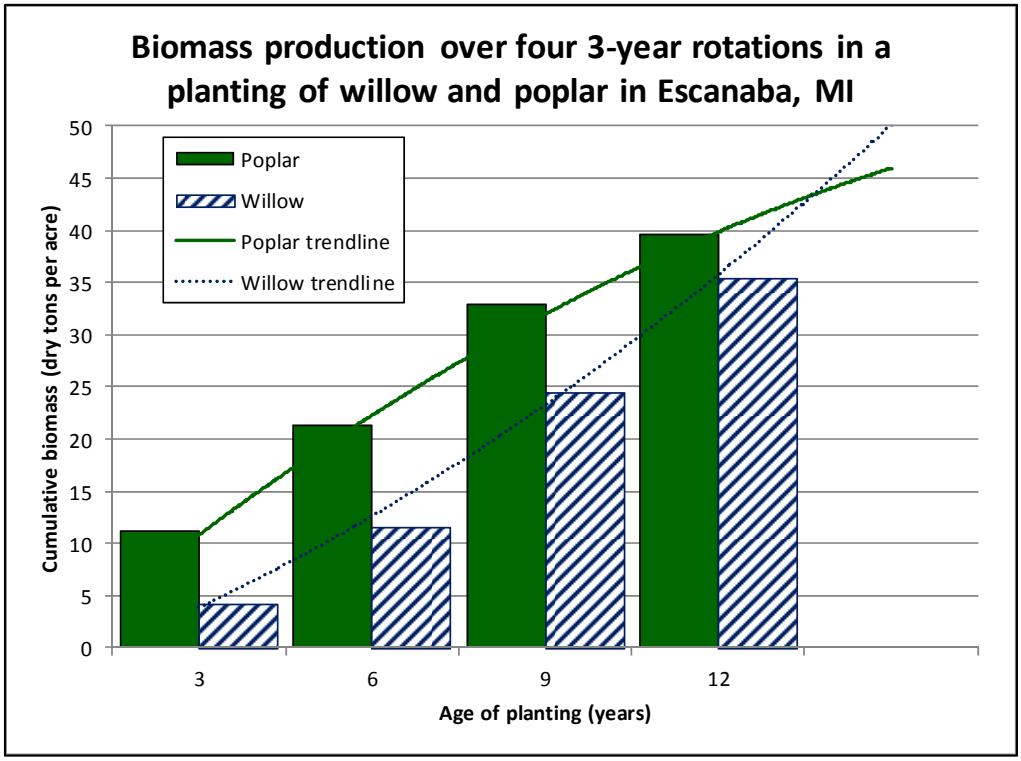
HARVESTING A THREE-ACRE BLOCK OF WILLOW

Willow hybrids are being promoted throughout the northeastern United States as a desirable candidate for energy plantations. Harvesting 3-year-old willow whips requires specialized equipment and constitutes the largest single cost of the production system. A highly productive machine has been developed by Case/New Holland to harvest willow whips but it is large and expensive. Smaller, less productive machines have been developed in northern

Europe which are much less expensive. Michigan State University and Cornell University each own such a machine and have successfully used them to harvest small research plots. It has been difficult to establish productivity data from these small plots, so we at MSU used our machine to harvest a 3-acre block of Tully Champion willow in Escanaba this fall. The operation is just finishing now and data will be made available soon. Some photographs of the operation are included here.

YIELDS OF POPLAR AND WILLOW AFTER FOUR 3-YEAR ROTATIONS

A high-density plantation of 12 willow clones and 2 poplar hybrids was established in Escanaba, MI in 2002. This plantation has been harvested every three years during the intervening 12 years. Annual biomass incremental yields have been tracked and show marked differences in growth strategies between the two genera. Willow has come into its own during the latter half of the trial while poplar seems to be starting a decline in annual productivity. Long running trials like this are rare, and demonstrate the value of sustained support for field plot research. In Michigan we have been fortunate to receive this support from AgBioResearch (formerly the Michigan Agricultural Experiment Station).



NEERA 1005 Sustainable Wood Energy Regional Project
Progress Report for FY2013 – Prepared November 28, 2013
West Virginia University (WVU)

Submitted by Dr. Kaushlendra Singh (NEERA 1005 WVU rep)

Accomplishment Report

Objective-1: Identify and connect relevant persons working in wood energy, both within and outside the Land Grant University System.

Task 1.1- Creation of consortia of a multidisciplinary researchers and industry partners for pyrolysis and bio-oil refining

WVU has put together a research and development team focused on Catalytic Pyrolysis and Bio-oil Refining. The team members are following:

Name of the Expert	Affiliation	Expertise
Kaushlendra Singh	Faculty, Forestry and Natural Resources (WVU)	Pyrolysis
Edwin Kugler	Faculty, Chemical Engineering (WVU)	Petroleum Refining
Dady Dadyburjor	Faculty, Chemical Engineering (WVU)	Catalysis
John Zondlo	Faculty, Chemical Engineering (WVU)	Biomass Torrefaction/ fuel cell
Daniel Carder	Faculty, Mechanical Engineering (WVU)	Engine Testing
Bhaskaran Gopalakrishnan	Faculty, Department of Industrial and Management Systems Engineering (WVU)	Sustainability analysis
J. Richard Hess	Idaho National Lab	Sustainability analysis
Jim Dooley	CTO, Forest Concepts, LLC	Pre-processing
Mr. Ed Aitken	The Process Group LLC	Refining
Mr. Phillip Badger	Renewable Oil International LLC	Pyrolysis

Some selected members of the team had put together a proposal National Science Foundation, Energy for Sustainability Program in the year 2013.

Objective-2: Improve communication within this community as evidenced by high participation in the group by the region's institutions.

Task 2.1- Communication with the land grant universities, federal agencies, and industries was established for partnering American Society of Agricultural and Biological Engineers' (ASABE's) Bioenergy Day held on July 24, 2013 in Kansas City, MO. As result, following partners were recruited:

- United States Department of Agriculture- National Institute of Food and Agriculture

- United States Department of Energy- Bioenergy Technology Office
- AGCO Corporation
- Forest Concepts, LLC,
- University of Georgia, Bioenergy Systems Research Institute,
- Iowa State University, Department of Agricultural and Biosystems Engineering (CBiRC and CenUSA),
- Cornell University, Biofuels Research Laboratory,
- Oklahoma State University, Biobased Products and Energy Center,
- Pennsylvania State University, Biomass Energy Center and Northeast Woody/warmseason Biomass Consortium (NEWBIO),
- South Dakota State University, Center for Bioprocessing Research and Development,
- University of British Columbia, BBRG - Biomass & Bioenergy Research Group,
- West Virginia University, Appalachian Hardwood Center and Bio-based Research Center,
- Center for Alternative Fuels, Engines, and Emissions, WVU
- University of Nebraska-Lincoln, Nebraska Center for Energy Sciences Research
- Michigan State University, Anaerobic Digestion Research and Education
- Great Lakes Bioenergy Research Center, Michigan State University and University of Wisconsin-Madison, and
- Mississippi State University, Energy Institute,

The organizing team included Drs. Dr. Keri Cantrell, USDA; K. C. Ting, UIUC; Sam Tagore, USDOE; Kaushlendra Singh, WVU; Shahab Sokhansanj, ORNL; Raymond Huhnke, OSU; Ajay Kumar, OSU; Ganti Murthy, Oregon State University; Darrin Drollinger, ASABE Executive Director, and Sharon McKnight, ASABE Liaison. The organizing team met on conference call several times during the year.

Objective-3: Coordinate existing and planned Research, Extension, and Education projects in the region, as evidenced by new collaborations formed.

Task 3.1- Education/Extension- In year 2013, Dr. Singh led the organization of the Bioenergy day, sponsored by the American Society of Agricultural and Biological Engineers (ASABE) on July 23, 2013. The Bioenergy Day featured following events:

1. Keynote session- Dr. Sonny Ramaswamy, Director, USDA-NIFA

2. **Panel-1 "Supply of High Tonnage Feedstock"**

   Objective: *To review recently developed innovative equipment and systems to overcome barriers in delivering high tonnage biomass for cellulosic biofuel production.*

   Panel Abstract- The U.S. Department of Energy (USDOE) announced the selection of five projects back in 2009 to develop supply systems to handle and deliver high tonnage biomass feedstocks for cellulosic biofuels production. The awards were part of the DOE's ongoing efforts to reduce U.S. dependence on foreign oil, spur the creation of the domestic bio-industry, and provide new jobs in

many rural areas of the country. The chosen awards were selected as the best projects to stimulate the design and demonstration of a comprehensive system to handle the harvesting, collection, preprocessing, transport, and storage of sufficient volumes of sustainably produced feedstocks. Feedstocks or combinations of feedstocks that were considered included: agricultural residues, energy crops (e.g., switchgrass, miscanthus, energycane, sorghum, poplar, willow), forest resources (e.g., pine plantations), and short rotation woody crops (willow and poplar). These five projects are nearing their completion. The ASABE has invited the lead project investigators of these logistics projects to participate in a panel to discuss innovative equipment and systems that these projects have developed.

Panelist- Sam Tagore (USDOE), Kevin Corner (FDCE), Maynard Herron (AGCO), Steve Taylor (Auburn University), and K. C. Ting (UIUC).

3. **Panel-2- "Critical Needs in Biorefining Research and Development"**

Objective: To highlight the collective roles and responsibilities of research institutions, federal agencies, and industries in addressing R&D hurdles biorefineries must overcome.

Panel Abstract- Advanced biofuels are near commercialization and bioenergy systems are being employed on a very selective basis. Still, many issues and hurdles remain which must still be addressed before biobased refineries are considered economically viable and environmentally sustainable on a large-scale commercial basis. Many of the solutions are and will continue to be identified and addressed through the collective R&D of research institutions, industry and federal agencies. Members of this panel will explore opportunities and activities from basic to applied research in the feedstock development, production, supply and conversion.

Panelist- Raymond Huhnke (Moderator), Ram Gupta (NSF), Jim Rekoske (UOP), Quang Nguyen (Abengoa), Steve Thomas (DOE, BETO), Tim Rials (University of Tennessee), and Ralph Cavalieri (Washington State University).

4. **Poster session**- New Frontiers of Bioenergy featured more than 73 posters.

Task 3.2- Standard Development: A project proposal (Project X516) was approved by ASABE to develop a new standard on "Terminology for Forest Operation and Equipment". The working group for this standard development consists of following members:

Name	Company	Interest Area
Kaushlendra Singh	W Va Univ	Processing Equipment
William Elliot	USDA Forest Service	Harvesting Equipment
Jim Dooley	Forest Concepts LLC	Harvesting and Processing Systems
Steve Taylor	Auburn Univ	Harvesting Systems

Bryce Stokes	Dept of Energy	Harvesting and Processing Biomass
Shawn Grushecky	W Va Uni	Harvesting
Joseph McNeel	W Va Uni	Harvesting Systems
Aaron Yoder	Penn State	Safety

2013 North Dakota Report
NEERA-1005

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Project: NEERA 1005: "Sustainable Wood Energy" regional project group.
North Dakota Report for the Period: 10/1/12 – 9/30/2013.

OUTPUTS / ACCOMPLISHMENTS:

1. Understanding disease resistance to Septoria canker in hybrid poplar. Collaborators: Ruqian Qin (M.S. Student), and Glen Stanosz (University of Wisconsin – Madison).

We tested the relationship between field and greenhouse resistance to Septoria canker by conducting non-wounded inoculations of 15 genotypes of hybrid poplar using the protocol developed by LeBoldus *et al.* (2010). These genotypes have known levels of resistance to Septoria canker (Low, intermediate, and High, canker length, and % girdle – provided by Dr. Stanosz). Following inoculation several disease severity parameters were measured (lesions/cm, % necrotic area, lesion number). Logistic regression using % necrotic area and lesion number as a predictor of susceptibility category (low, intermediate, and high) indicated that the non-wound inoculation protocol was a good predictor of the most resistant genotypes under field conditions.

A second study characterizing the mode of infection of *Septoria musiva* into host tissue is being conducted. Two experiments were conducted using a resistant (DN 34) and a susceptible (NC11505) genotype. For both experiments inoculations were conducted as described by LeBoldus *et al.* 2010. In experiment 1 Stem tissue was collected 6 h, 12 h, 24 h, 72 h 1 week, and 3 weeks post inoculation. Scanning electron microscopy was used to examine the infection biology of the pathogen a determined that the majority of new infections occur via lenticels and that the pathogen appears to penetrate host tissue within 12 h of inoculation. In the second experiment trees were harvested at 3 weeks, 5 weeks, and 7 weeks fixed, embedded, sectioned and stained. Responses between the resistant and susceptible genotypes will be compared.

2. IPM strategies for Septoria canker on hybrid poplar farms in the north central region. Collaborators: Kelsey Dunnell (Ph.D. student), Nivi Abraham (Ph.D. Student), Achala Nepal (Research Specialist), Bernie McMahon (NRRI University of Minnesota – Duluth), and William Bergeson (NRRI University of Minnesota – Duluth).

In order to characterize the variability in *Septoria* canker resistance, fifty genotypes of *P. nigra*, a common parent in hybrid poplar breeding programs, were inoculated with six isolates of *S. musiva*. Three weeks following inoculation the number of cankers cm^{-1} and a disease severity rating (1 resistant - 5 susceptible) were recorded. A wide range of variation from completely resistant to highly susceptible was

detected among genotypes and among provenances; with some provenances showing on average more resistance. A second experiment was conducted to determine the relationship between disease severity and mortality. Thirteen genotypes representing the full range of disease severity ratings were inoculated with a bulk spore suspension of six isolates of *S. musiva*. Disease severity and cankers cm^{-1} were evaluated at three weeks as described above. Subsequently, trees were evaluated for mortality on a weekly basis. The mean disease severities of each genotype were similar between the two experiments ($R^2 = 0.8798$) and trees assigned a disease severity of 4 and 5 had a high probability of mortality.

In the spring of 2013 a field trial evaluating the relative tolerances of 75 different genotypes of *Populus* (40 *P. deltoides* x *P. nigra* hybrids and 35 *P. nigra*) to Septoria canker was established. The experimental design is a randomized complete block design with 5 blocks. Trees were planted at 3m spacing and the entire trial was surrounded by two rows of border trees. Genotypes were randomly arranged within each block as two tree plots. In the spring of 2014 one tree in each paired plot will be wound inoculated with a virulent isolate of *S. musiva*. Growth and survival will be monitored at the beginning and end of each growing season. This information will be used to compare the relative tolerances of each genotype to Septoria canker.

A quantitative real time PCR assay is also being developed to quantify *S. musiva* growth in host tissue following inoculation. This is a multiplexed assay using the Beta-tubulin gene in the fungus and Initiation factor gene in the pathogen. To date this assay has been demonstrated to be both sensitive and specific. A time course experiment (0h, 24h, 48h, 72h,1wk, 3wk, 5wk, Control) is currently being conducted, using four genotypes of hybrid poplar (1 resistant, 1 susceptible, and 2 intermediate), to determine the best time point to distinguish between resistant and susceptible genotypes. This assay will be used to phenotype both leaf spot and stem canker symptoms.

Research to characterize the genetics of pathogen populations has been limited by the availability of adequate genome resources and species characterization at a molecular level. To overcome these bottlenecks, a non-resource prohibitive method of deep coverage genotyping of pathogen populations is required. Here we describe the use of a two-enzyme genotype-by-sequencing (GBS) method adapted for Ion Torrent sequencing technology. Utilizing this method, 2,373 and 5,960 unique loci,"sequence tags", containing 9,992 and 17,193 SNPs were identified and characterized from natural populations of *Septoria musiva* and *Pyrenophora teres* f. *maculata*, respectively. *S. musiva* is a necrotrophic fungal pathogen that causes leaf spot and stem canker of poplar hybrids and *P. teres* f. *maculata* is a necrotrophic fungal pathogen that causes spot form net blotch of barley. Based on the estimated sizes of the *S. musiva* and *P. teres* f. *maculata* genomes, ~30 and 34 Mb, respectively, this GBS analysis placed a SNP marker on average approximately every 12.6 to 5.7 kb in the respective fungal genomes. This high-density of markers is adequate for association mapping in natural populations and positional cloning efforts in bi-parental fungal populations. Blast searches of the sequence tags generated from the *S. musiva* genome revealed that ~95% represent predicted genes, providing ~23% coverage of the total predicted genes present in the genome. This genotyping can be done at a relatively low cost per fungal isolate or progeny making it an option for researchers with limited resources and/or working on pathogens with no or limited genome information available.

OUTCOMES/IMPACTS:

The ability of the greenhouse screening protocol to detect the most resistant and susceptible genotypes of hybrid poplar will allow forest companies to focus resources for expensive and time consuming field testing on the most resistant genotypes.

The study characterizing the mode of infection of Septoria canker into hybrid poplar stems and the qPCR assay will allow us to improve our understanding of this pathosystem and improve current disease resistance screening strategies.

Disease tolerance is an often overlooked tool which could be used to manage disease. The field experiment will be used to evaluate the potential of disease tolerance as a management tool for Septoria canker of hybrid poplar.

The low cost genotyping assay for the pathogen will be used to identify virulence factors and further improve our understanding of host pathogen interactions and disease resistance in *Populus*. This information will be used to improve isolate selection for screening assays.

My program is training: One M.S. student (Ruqian Qin), Two Ph.D. students (Kelsey Dunnell and Nivi Abrabham), one post-doctoral fellow (Achala Nepal).

REFEREED PUBLICATIONS (2):

LeBoldus, J.M. Isabel N., Floate K.D., Blenis P.V., and Thomas B.R. Testing the 'hybrid susceptibility' and 'phenological sink' hypotheses using the *P. balsamifera – P. deltoides* hybrid zone and Septoria leaf spot [*Septoria musiva*] PLoS ONE. (*In press*)

LeBoldus, J.M. Zhang, Q. and Kinzer, K. (2012). First report of dollar spot caused by *Sclerotinia homoeocarpa* on *Agrostis stolonifera* in North Dakota. Plant Disease 96: 1071.

ABSTRACTS (5):

LeBoldus JM, Stanosz GR, Qin R, and Faria O. 2012 Screening hybrid poplar for resistance to *Septoria musiva*: Greenhouse to field correlations using a novel inoculation procedure. Oak Ridge, TN.

Brueggeman R, Friesen T, and **LeBoldus JM**. 2012. Genotype by sequencing and Marker Assisted Selection: Breaking the Bottleneck. Proceedings of the National Fusarium Head Blight Forum. Orlando, FL.

Dunnell KL, Nepal A, and **LeBoldus JM**. 2013. A medium thouput greenhouse phenotyping assay of *Populus* spp. for Septoria canker resistance. Southern Forest Tree Improvement Conference. Clemson, SC.

Nepal A, Friesen T, **LeBoldus JM**. 2013. Polyethylene Glycol (PEG) mediated transformation of *Septoria musiva*. North Central - American Phtypathological Society Regional Meeting. Manhattan, KS.

Qin R, Stanosz GR, **LeBoldus JM**. 2013. Non-wound greenhouse screening of hybrid poplar trees with *Septoria musiva.* America Phytopathological Society. Austen TX.

EXTENSION PUBLICATIONS (2):

Walla, J., **LeBoldus J.M.**, and Bergdahl A. 2013. The old and the New: Two needle diseases of spruce in North Dakota. F1680.

LeBoldus, J.M., Bergdahl, A., Knodel, J., and Zeleznik, J. 2012. Dutch Elm Disease in North Dakota: a new look. PP1635.

FUNDING:

USDA Forest Service – North Dakota Forest Service. $45,000. Riparian Forest Health Assessment. 3 yrs.

USDA NIFA. 2012-2015. $98,190. Integrated Pest Management of Septoria Canker on Hybrid Poplar Farms in the North Central Region. J.M. LeBoldus, G.R. Stanosz, and B. Berguson. 3 yrs.

USDA NIFA. 2013-2016. $61,120. North Dakota eIPM – CS Program. Knodel J, Beauzay P, LeBoldus JM.