Mendel BIOTECHNOLOGY

seeding a sustainable future

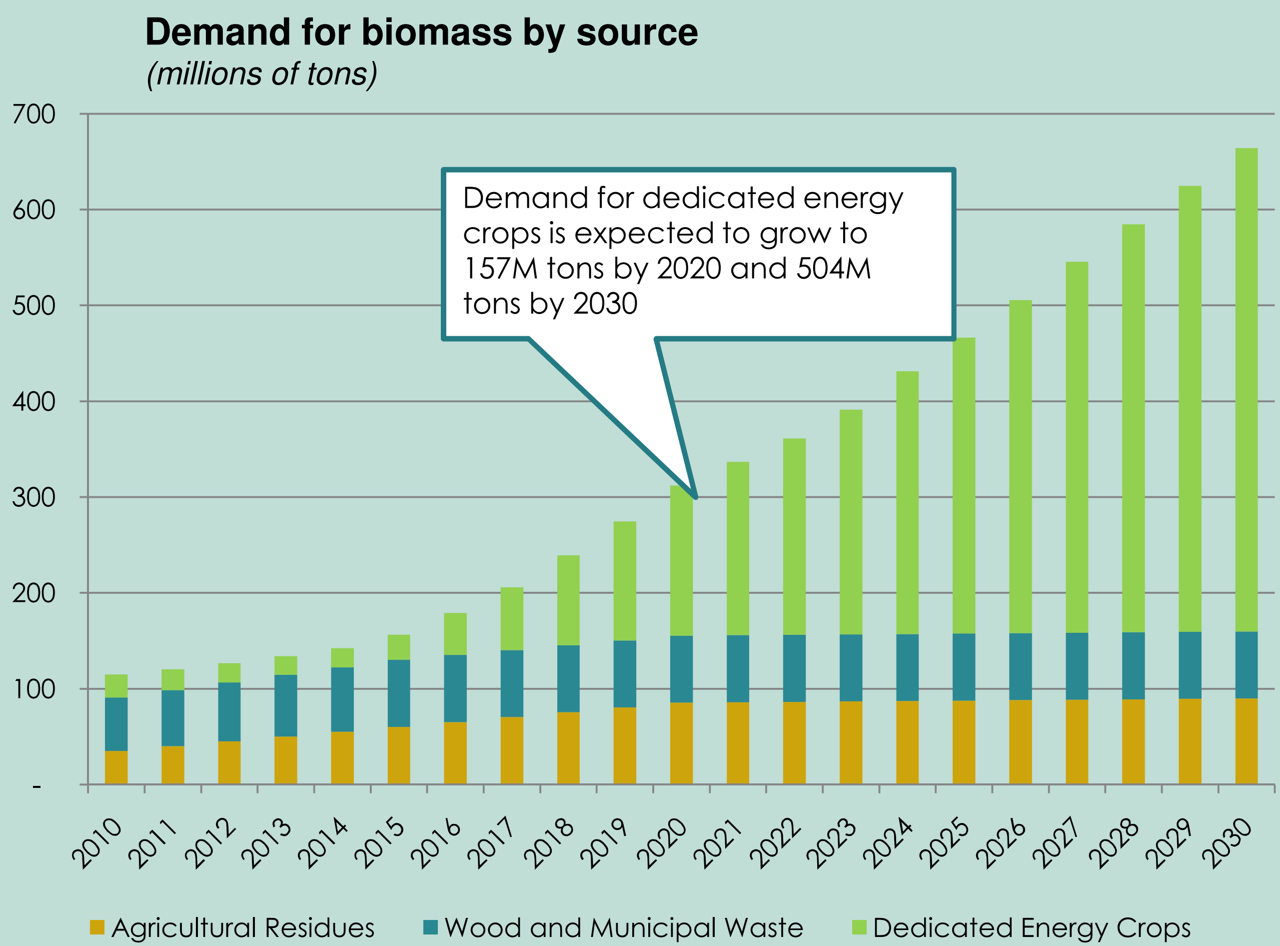
Mendel's seeded miscanthus system: a sustainable and scalable bioenergy feedstock solution

Neal Gutterson
Aug 18th, 2011

CBES Forum

- The Challenge:
  - ~ High volume, energy crop-based feedstock supply systems to meet large-scale renewable energy and materials demands
  - ~ Ensuring that land and other resources are handled in a sustainable manner

Energy Crops Are a Key Part of a Growing Biomass Basket



- **Agricultural residues** are expected to provide up to 100 million tons per year in an economical way, primarily as corn stover and wheat straw
- **Wood and municipal waste** is estimated to contribute 50-100 million dry tons of biomass in a sustainable and economical way
- **Dedicated energy crops** are expected to provide the balance of demand for biomass
  - Perennial crops expected to constitute vast majority of supply

Sources: United Stats Forrest Service study (USDA, Forest Service's Timber Product Output database, 2007) for estimate of biomass from wood and municipal waste
USDA (http://www.ers.usda.gov/) and team analysis for estimate of agricultural residues supply potential

Framework

- Brief intro to Mendel
- Why miscanthus
- Mendel's strategy for broad miscanthus adoption
- Sustainable system considerations
- Stewardship program
- Summary

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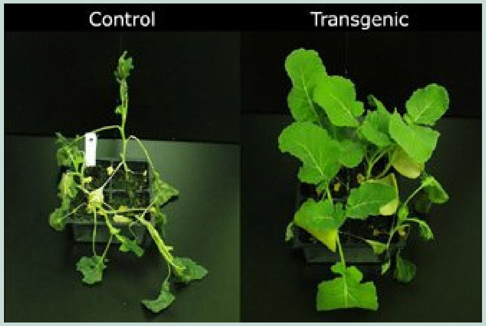
Company Snapshot

Founded in 1997; Headquarters in Hayward, CA

- ~ Additional US operations in Georgia, Indiana, Tennessee & Kentucky
- ~ Research collaborations in China, Germany
- ~ Well-capitalized (key shareholders: BP, Monsanto, ZAM Ventures)

Validated technology platform & products

- ~ Leading trait technology provider to row crop industry for yield and stress tolerance
- ~ Strong intellectual property position in plant gene regulation
- ~ Blue-chip long-term partners (Monsanto, Bayer)



Entered BioEnergy industry 2006

- ~ A new generation of Miscanthus products
- ~ Developing high yield, low input purpose-grown energy crops
  - ~ Sustainable feedstock systems with favorable GHG emission reductions
- ~ Differentiated perennial grass varieties
- ~ Collaborating with BP

Our Business is Founded on Knowledge of Plant Gene Regulatory Networks

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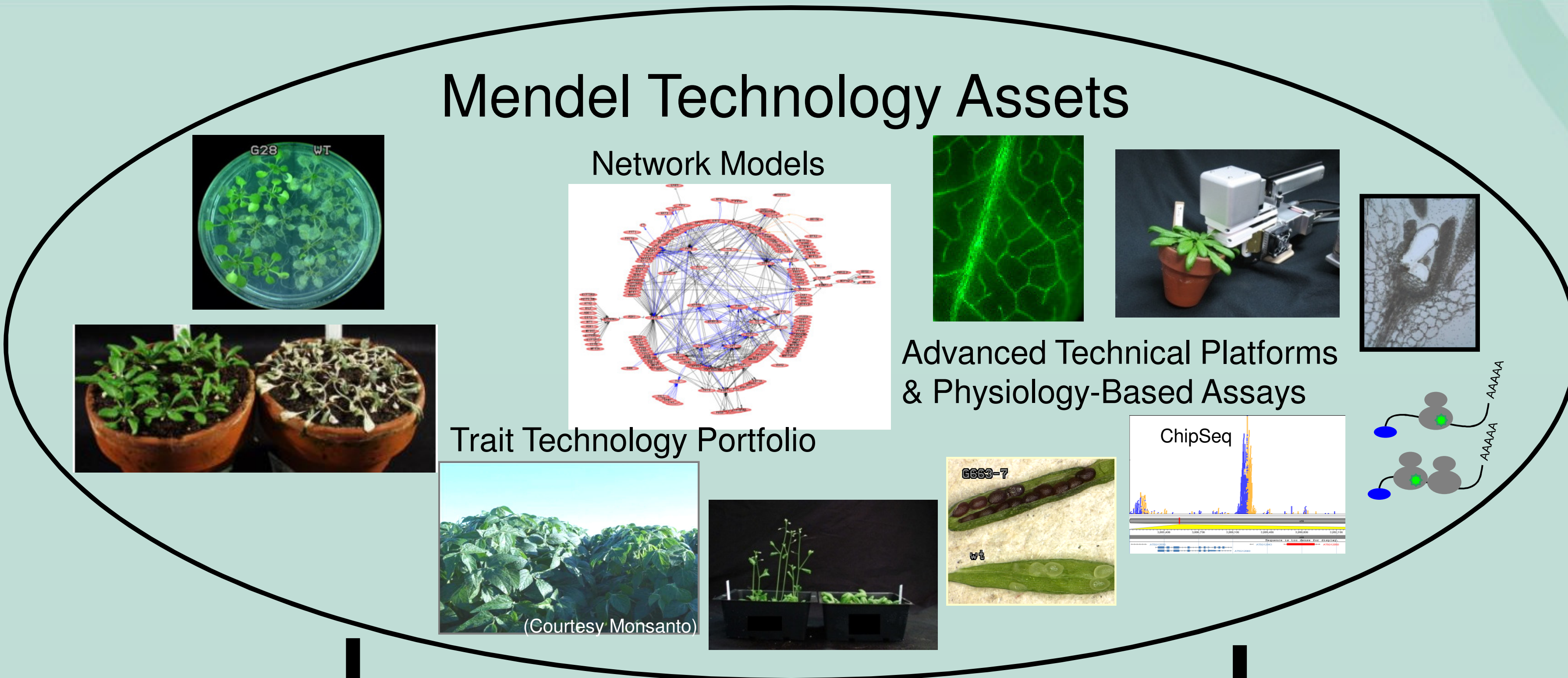

Genetics (Biotech Traits)

MONSANTO

Chemicals for Crop Improvement

Bayer CropScience

Miscanthus Seeds & Services

bp

Our business segments

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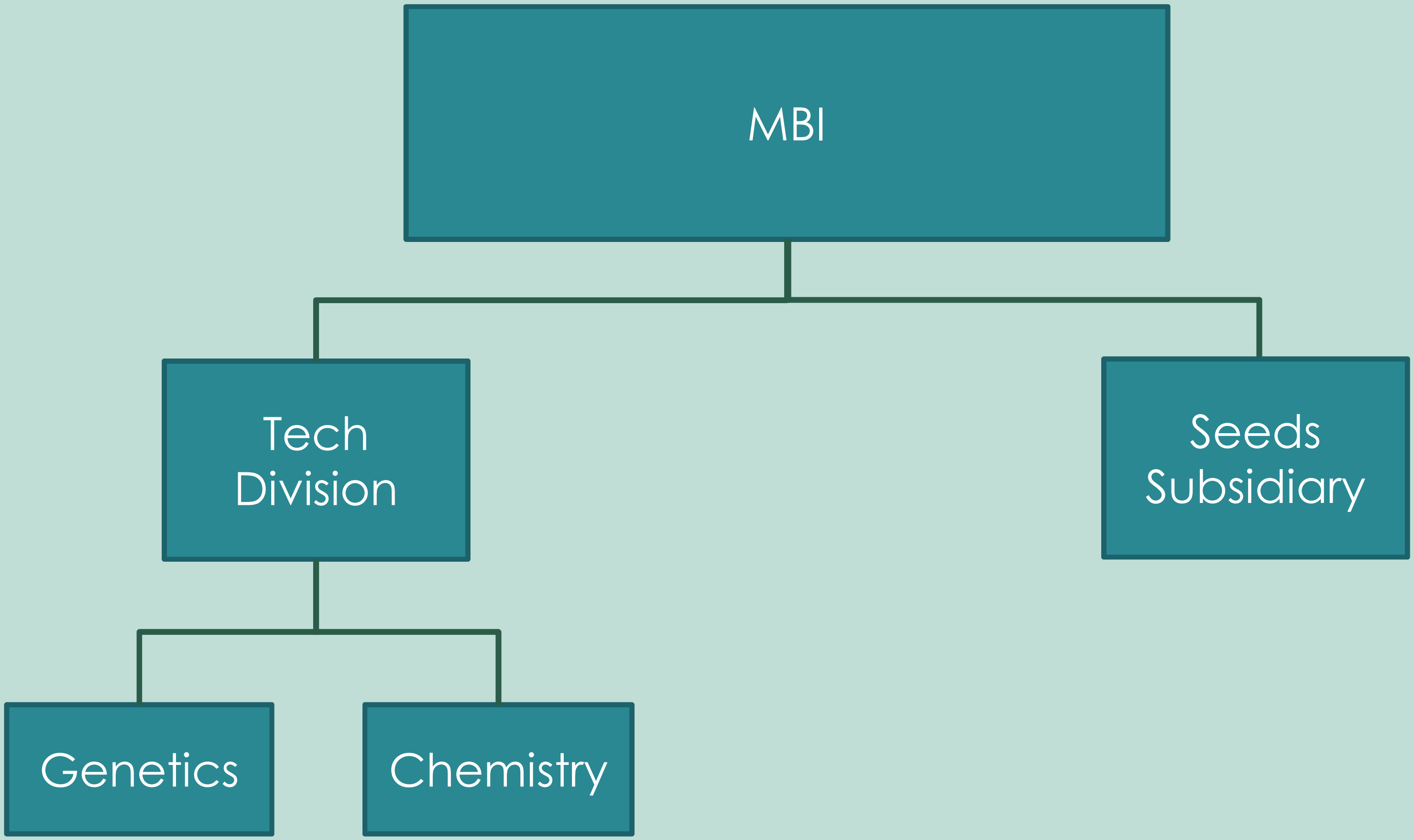
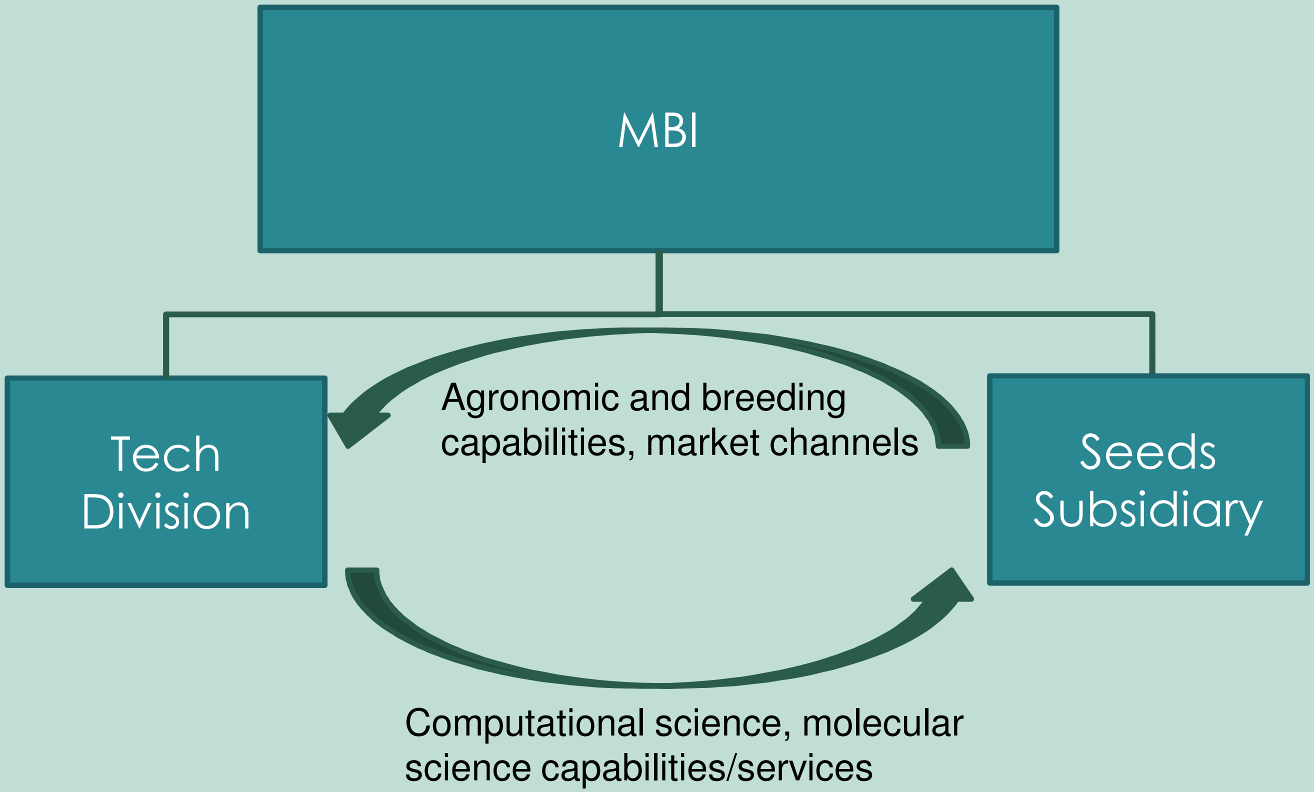


Sources of competitive advantage
- leveraging capabilities



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Miscanthus has excellent biomass properties for biofuels and biopwer

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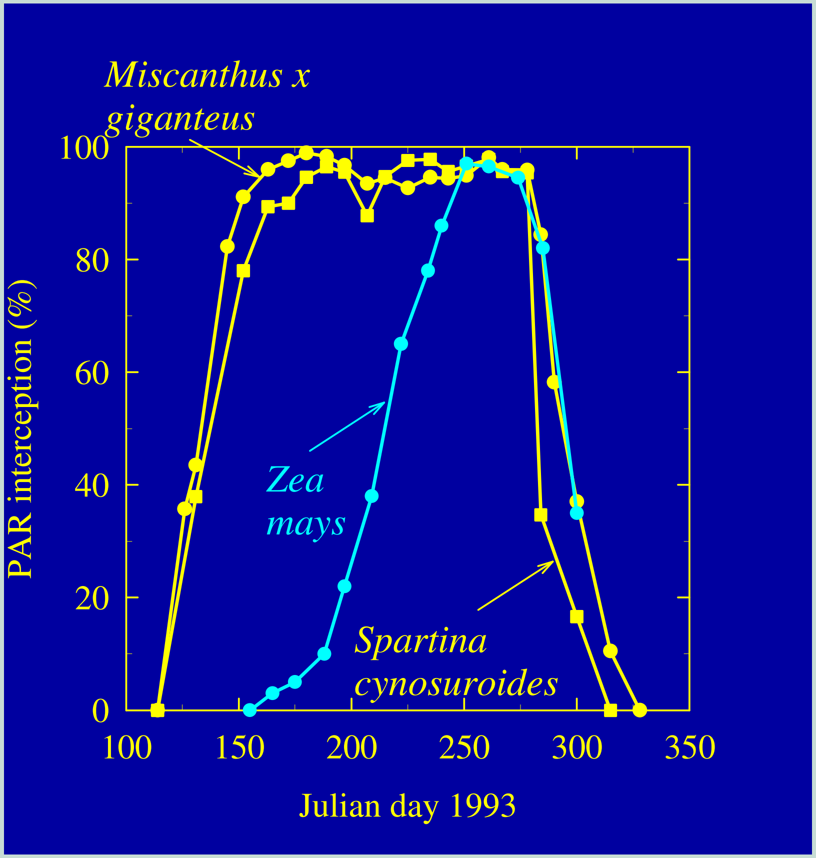
Biomass feedstock	Glucan * %	Xylan* %	Achievable ETOH yield Gal/ton**	Achievable ETOH yield Gal/acre**	Estimated BTU/lb***	MMBTU / acre**
Corn stover	36.1	21.4	105	210	7,800	31
Switchgrass	35.0	21.8	104	728	7,500	105
Sugarcane bagasse	38.6	20.4	108	735	6,200	87
Poplar	43.8	14.9	107	375	8,500	60
Aspen wood	44.8	14.9	109	327	8,500	51
Miscanthus	46.0	19.8	120	1200	8,000	160

* Average value of multiple samples tested
** Assuming biomass yields: 2 dry tons per acre (DT/ac) for corn stover; 7 DT/ac for switchgrass; 7 DT/acre for sugarcane bagasse, 3.5 DT/acre for poplar; 3 DT/acre for Aspen and 10 DT/acre for miscanthus
*** Best available data from literature

Perenniality is an important feature of optimal feedstocks

- High Yield (>15 tons/acre/year)
  - ~ 20% of 25 mi radius = 300M gal/year
- Low Input (fertilizer, water, tillage, pesticides)
- Sustainable
- Stable quality from year to year
- High conversion efficiency



Miscanthus uses resources efficiently

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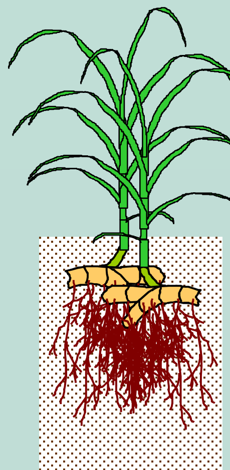
SPRING/ SUMMER

370 kg(N) ha^{-1}

Mineral nutrients



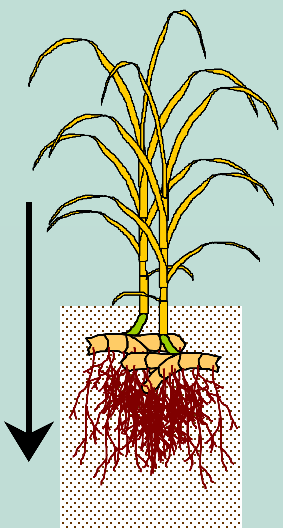
? kg(N) ha^{-1}

Translocation from rhizomes to growing shoot

FALL

270 kg(N) ha^{-1}

Mineral nutrients

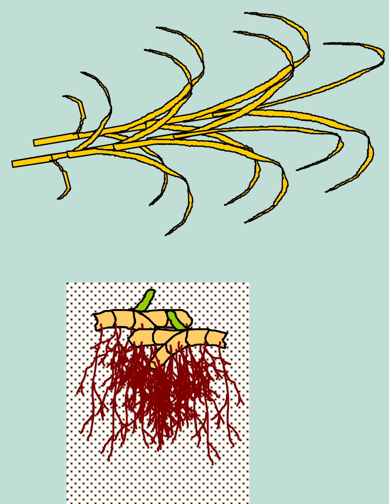


100+ kg(N) ha^{-1}

Translocation to rhizome as shoot senesces

WINTER

42 kg(N) ha^{-1}



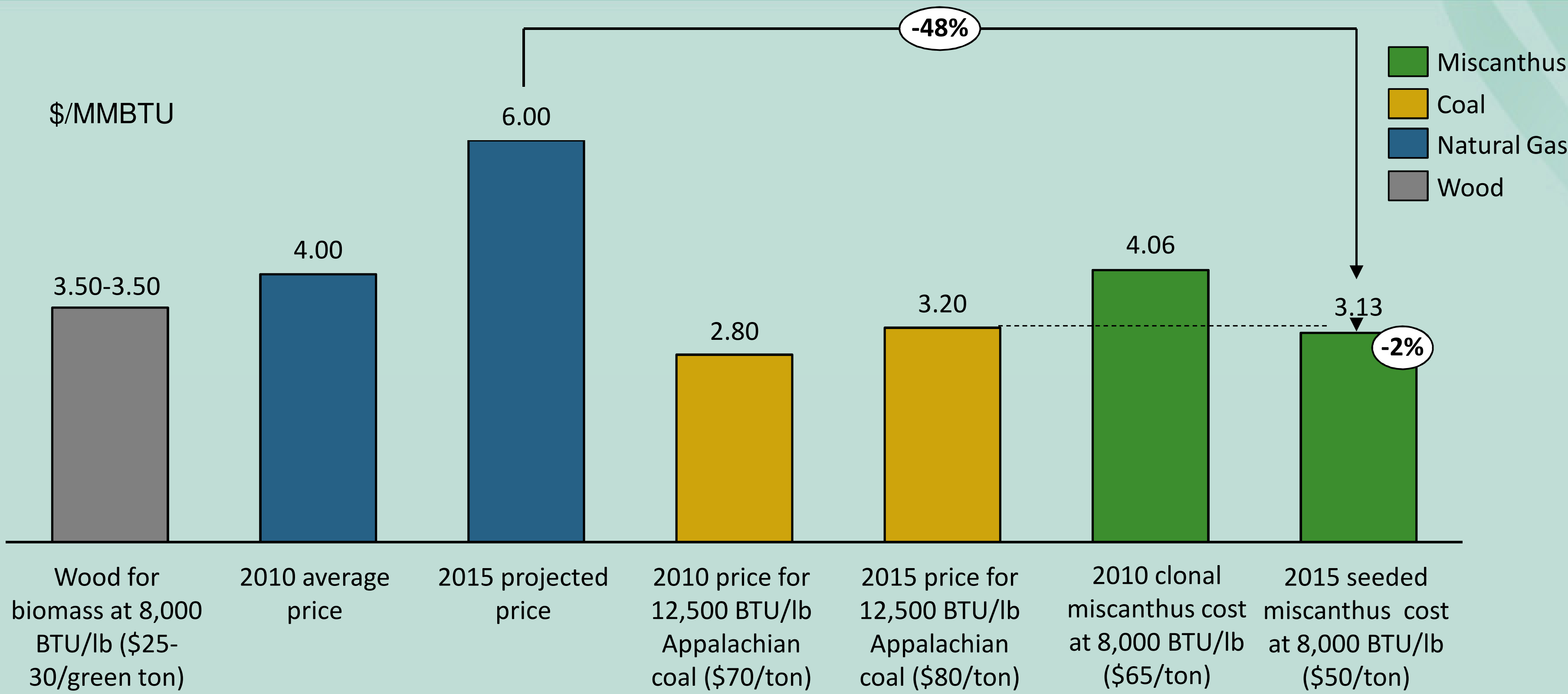
328+ kg(N) ha^{-1}

C harvested in Shoots, N retained in roots, Rhizomes & litter

Courtesy of Steve Long

Miscanthus biomass is cost-competitive with other power generation choices

Projected 2015 costs for miscanthus biomass, Appalachian coal, and natural gas



Sources:

Coal:	NYMEX (November 2010) for future forecast and EIA for current and historic costs
Biomass:	MBS model projections
Natural gas:	Platts (future projections presented at webinar on December 7, 2010), http://futures.tradingcharts.com/marketquotes/NG.html; http://www.cmegroup.com/trading/energy/natural-gas/natural-gas.html; Note: little to no volume supporting the future prices
Wood:	Industry interviews, Timbermart-South.com; costs based on current pulpwood prices; no future projections available

Miscanthus production is excellent in key U.S. markets

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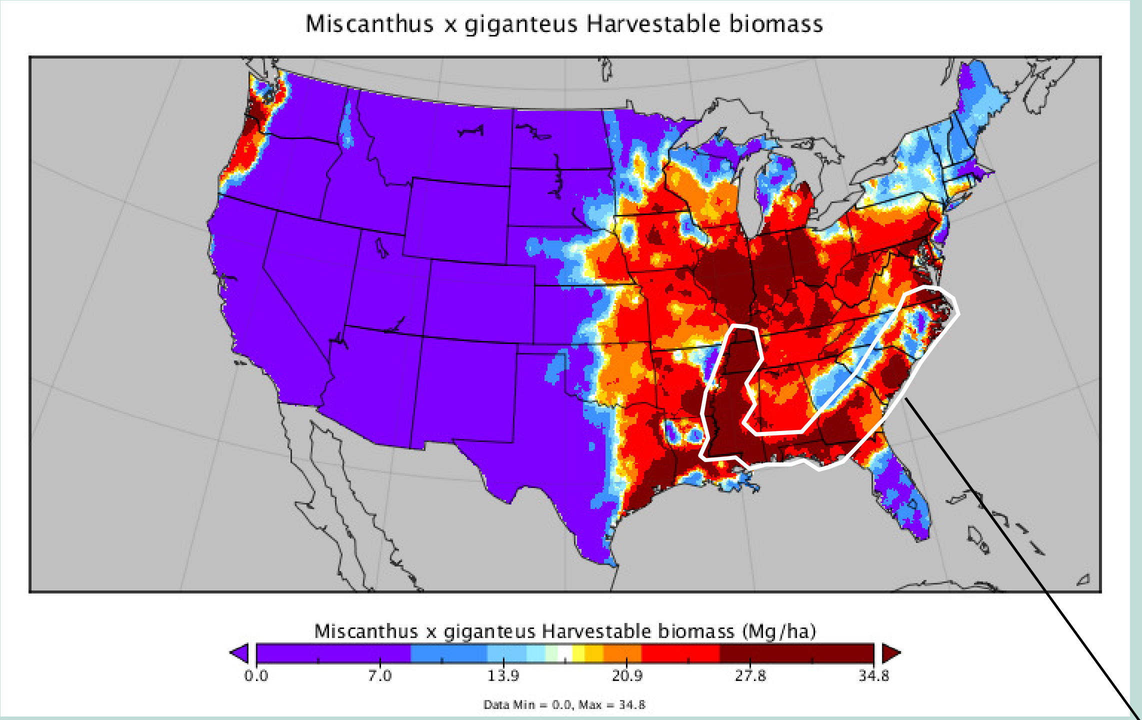

Work of F. Miguez, Iowa State. Published in review: C Somerville, H Youngs, C Taylor, SC Davis, SP Long - Science, 2010

Miscanthus revenues compete well with current crops

Cost-effective systems for harvesting miscanthus are in development

- Successful test of large harvest equipment (Mendel's, Kentucky location)
- One-pass harvester tests are being conducted

Framework

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What limits miscanthus as a feedstock?

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- Establishment costs
  - Need a cheaper source of planting material
  - Need supply chain for production of planting material
- Ease of scalability
- Harvest system – post-harvest treatments
- Supply chain integration
- Competition with first generation feedstocks

- ***Conclusion: The paradigm for miscanthus field establishment must be changed***

Seeded Miscanthus System Advantages

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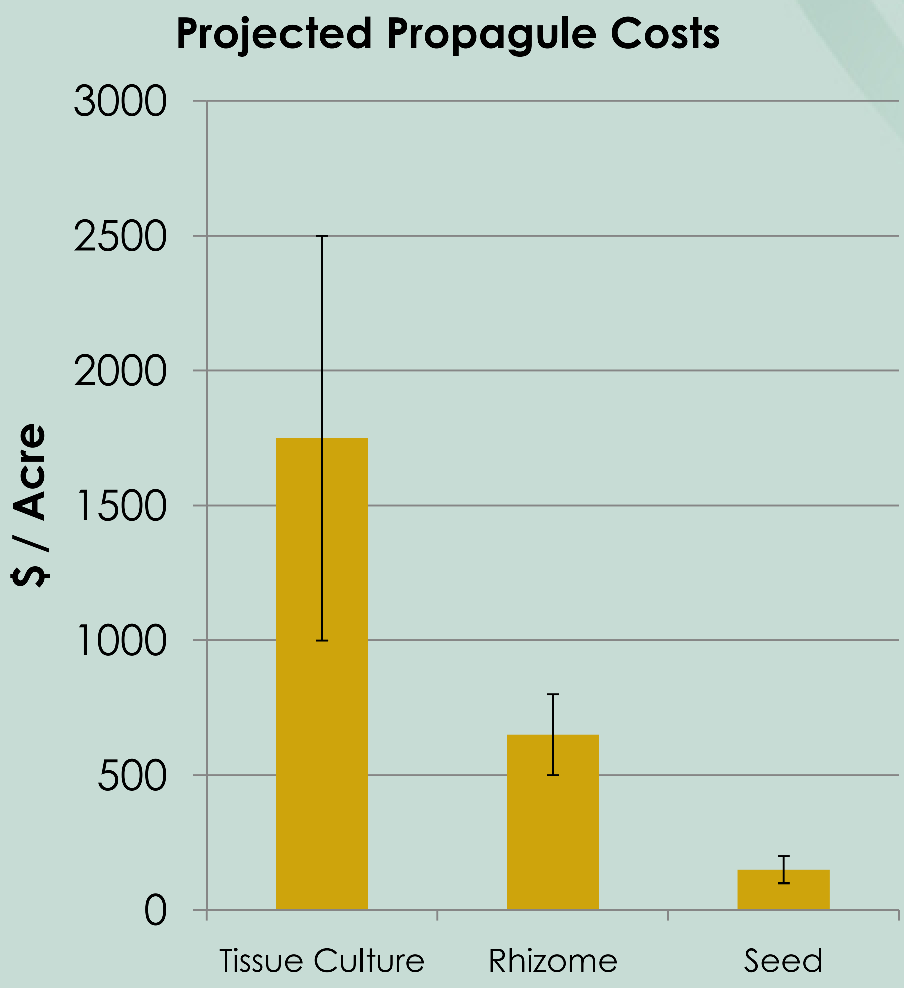
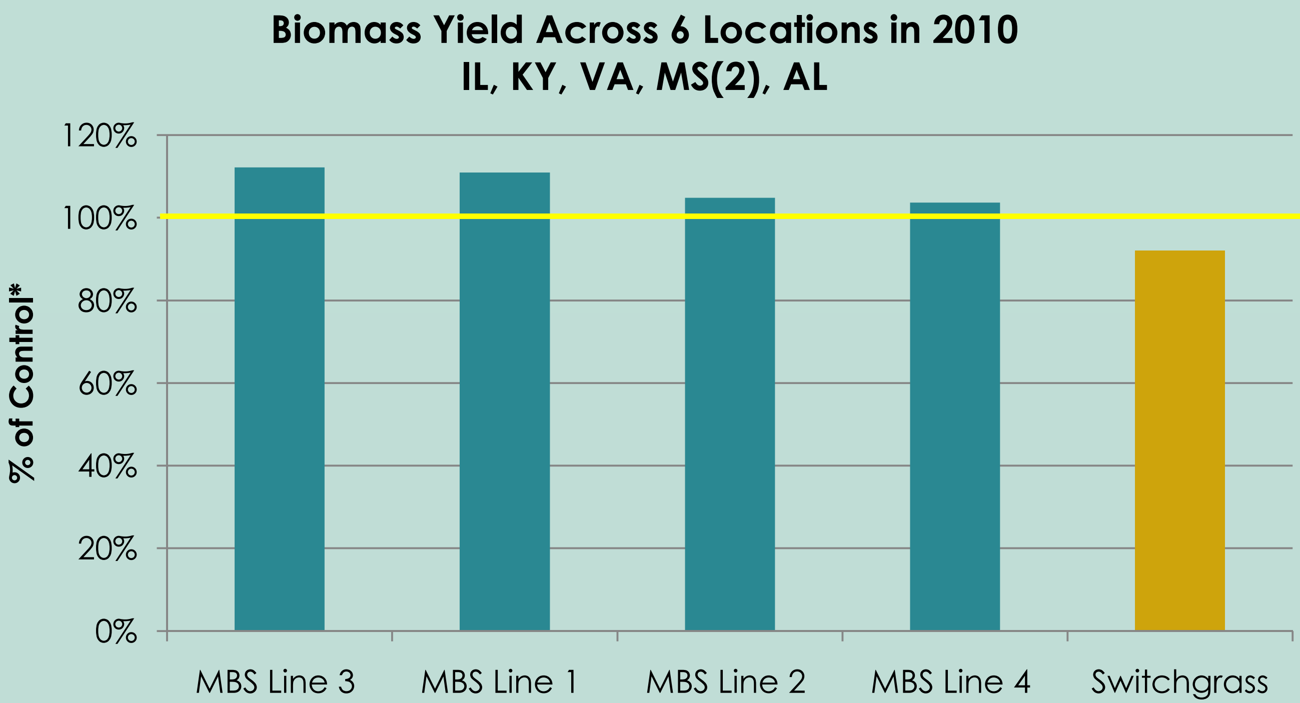
- Lower plant and establishment costs than clonal miscanthus
- Easily scalable for market introduction and rapid expansion
- Yields comparable to or better than current clonal miscanthus (*M. x giganteus* cv. *Illinois*)



First Seeded Miscanthus Products Identified



* Control Variety = 'Illinois'

MBS Line Avg. (Dry Ton/A)

Location	Yield
Leland, MS	13.6
Champaign, IL	12.5
New Castle, KY	9.2

Early commercial miscanthus products are sterile *M. x giganteus* triploids

Miscanthus sinensis

"Diploid"

2n=2x=38

+

Miscanthus sacchariflorus

"Tetraploid"

2n=4x=76

=

Miscanthus x giganteus

"Triploid"

2n=3x=57

STERILE

Building a *M x giganteus* variety that can be produced from seed

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Diploid
M. sinensis
2n=2x=38

X

Tetraploid
M. sacchariflorus
2n=4x=76

NON-REDUCTION OF GAMETES?

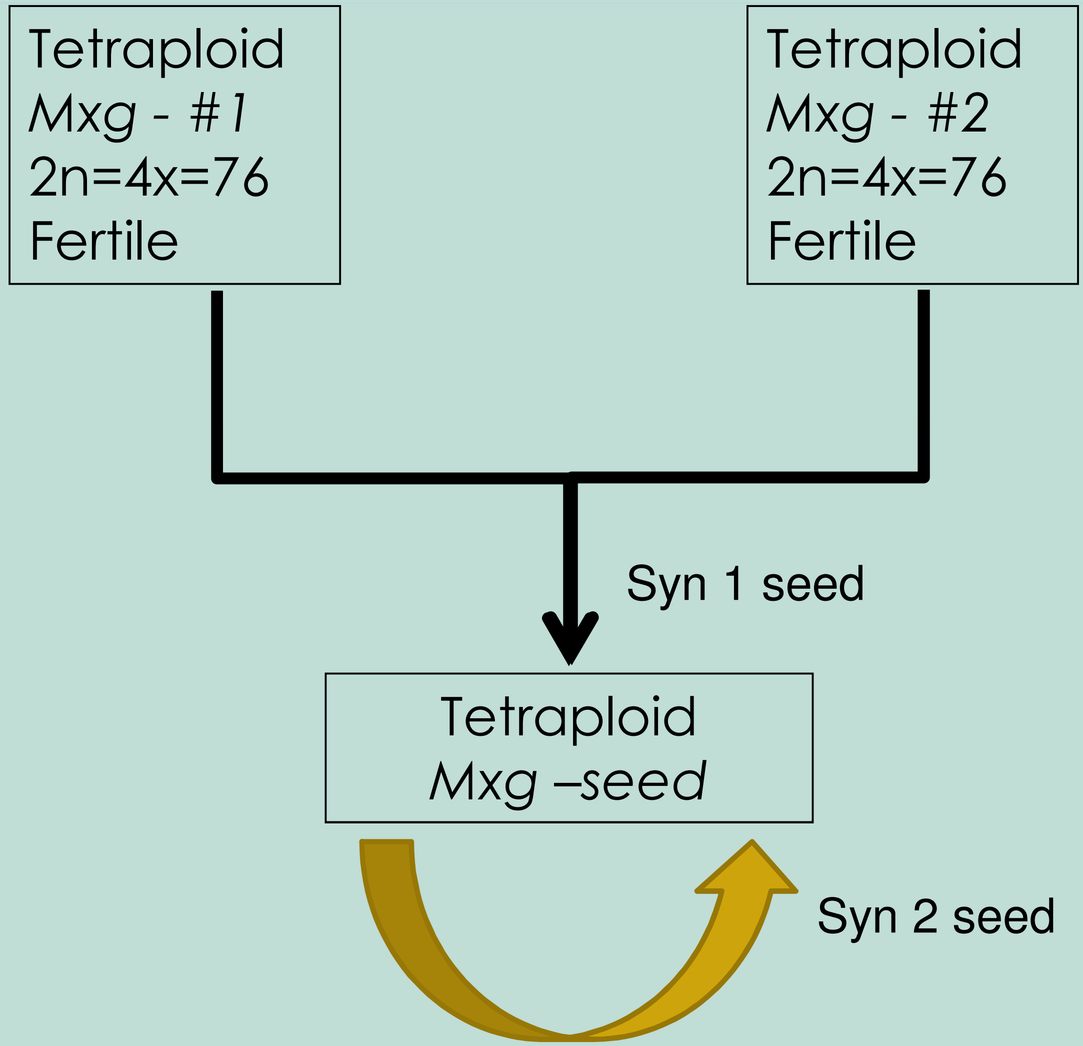
Likely
1x = sin
2x = sacch

Triploid
Mxg
2n=3x=57
Sterile

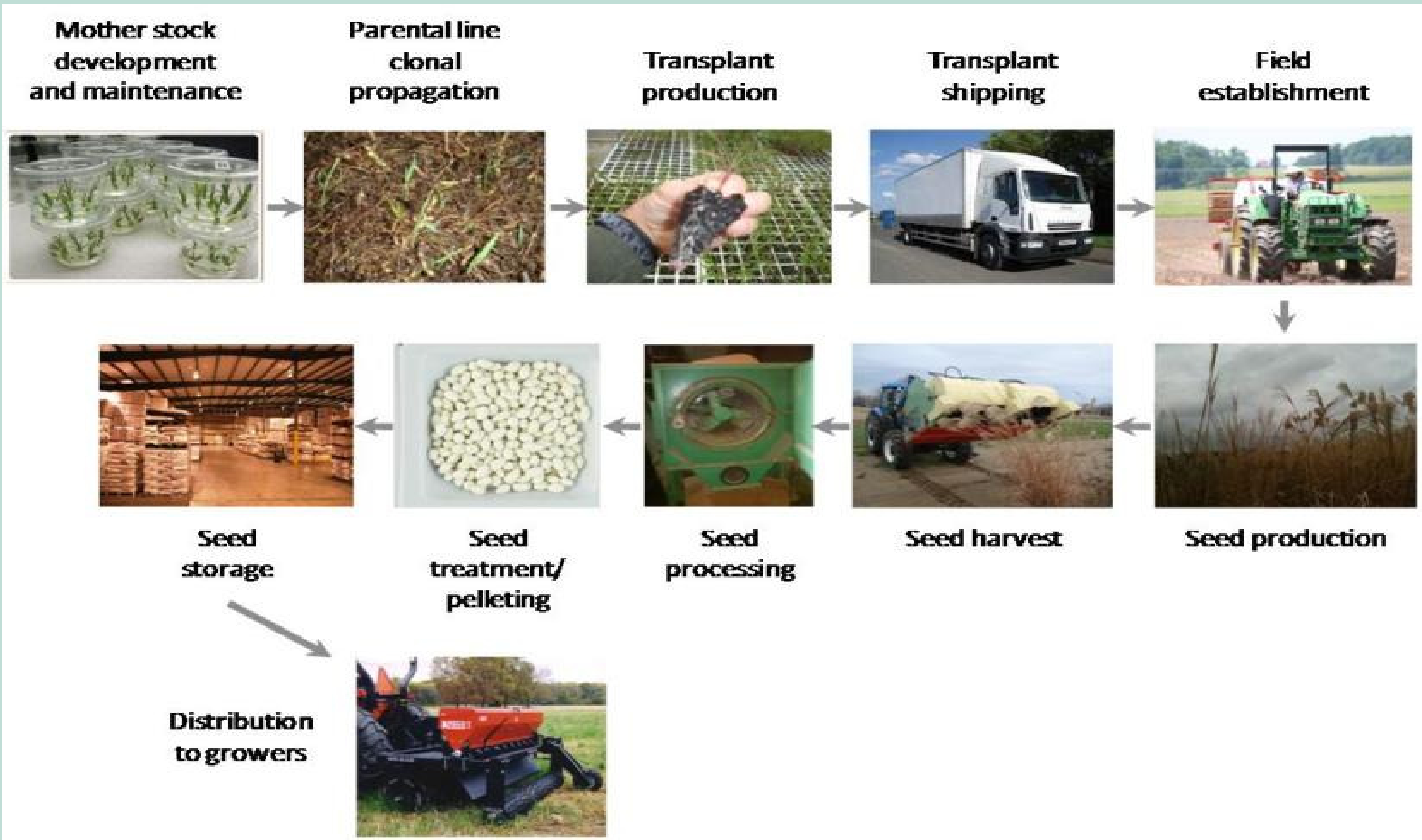
Tetraploid
Mxg
2n=4x=76
Fertile

Likely
2x = sin
2x = sacch

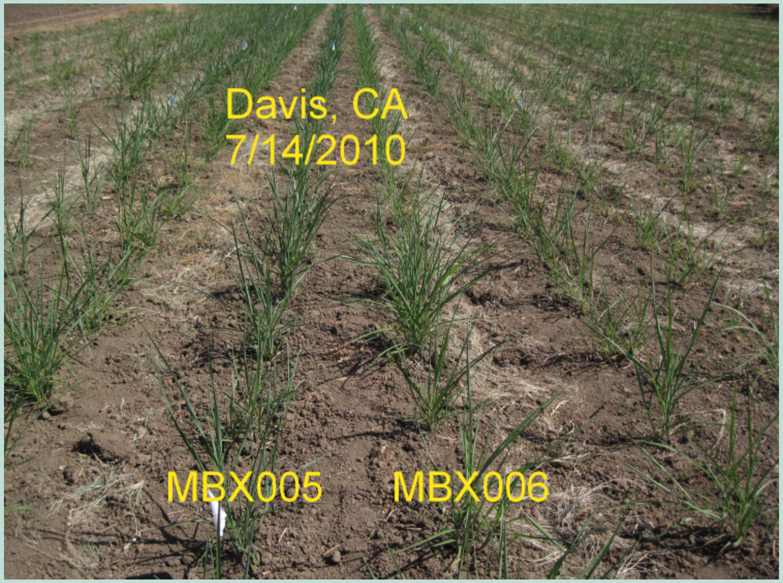
Seed production approach



Building the Supply Chain for High-Quality Seed Production



Typical production field
(Davis, CA)



1st Year, two months after transplanting

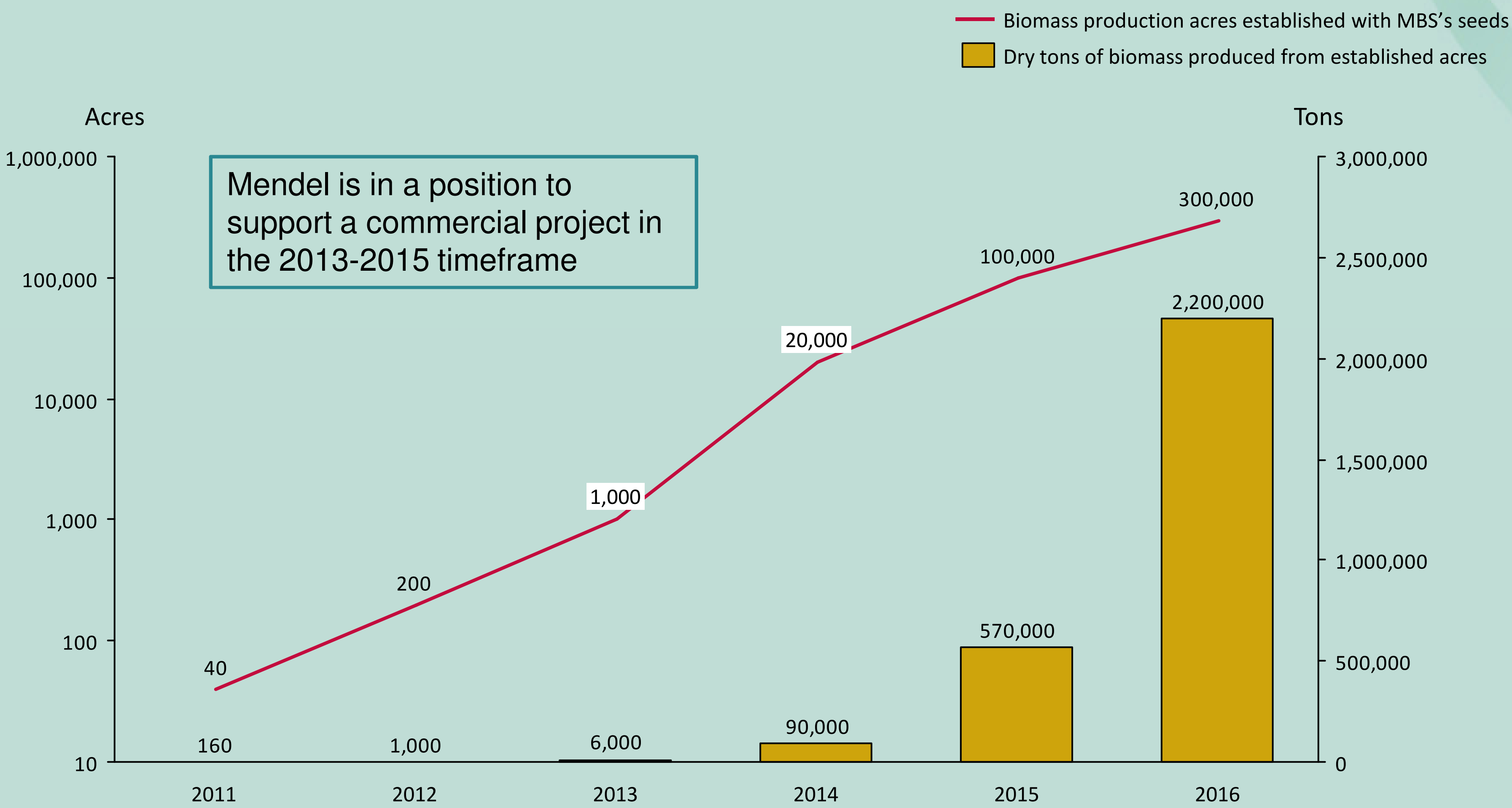
2nd Year

Creating Seed Production and Conditioning Systems

- Successfully produced seed in key seed production regions
- Drafted first <u>*Miscanthus Seed Production Management Guide*</u>
- Identifying equipment and manufacturing partners for top quality seed conditioning

Panicle with excellent seed set in Lost Hills, CA.

Ramping up to commercial launch and material scale



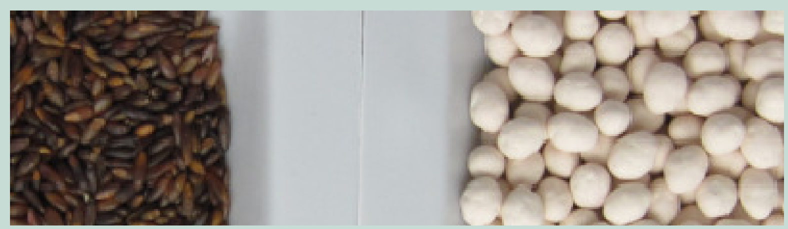
Note: Mendel can supply virtually any acreage post 2016

Conducting Studies on Seed Quality and Germination Enhancement

- Improving miscanthus seed germination and vigor
- Developing seed treatments
  - ~ Fungicide and nutrient packages
  - ~ Seed pelleting

MBS Seed Treatment

Control



Raw Seed

Pelleted Seed – 12x increase in size

Defining Protocols for Field Establishment from Seed

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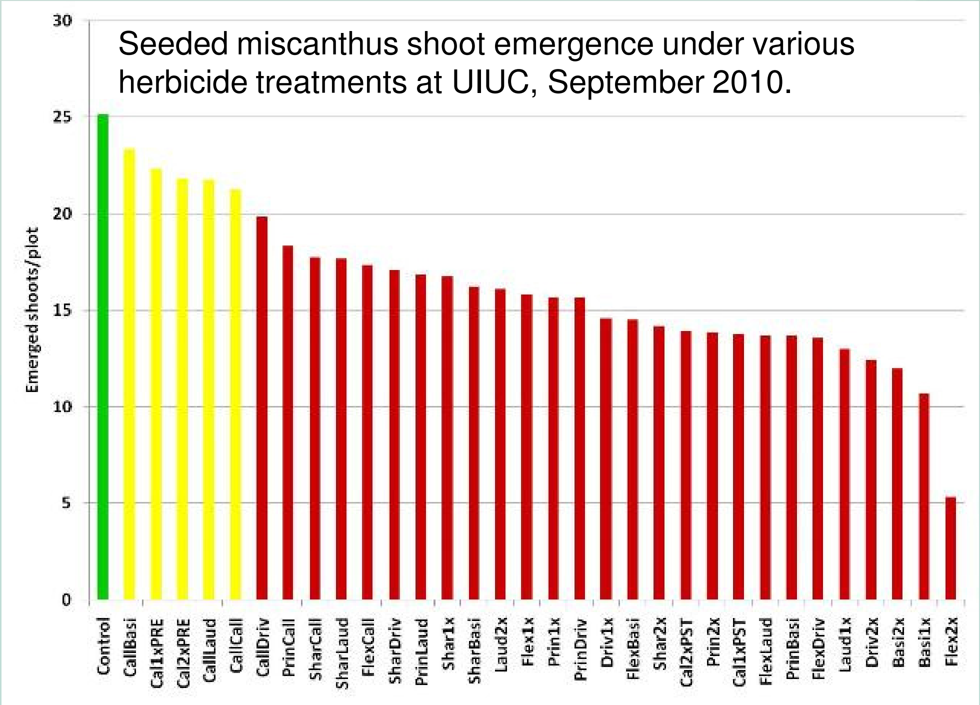
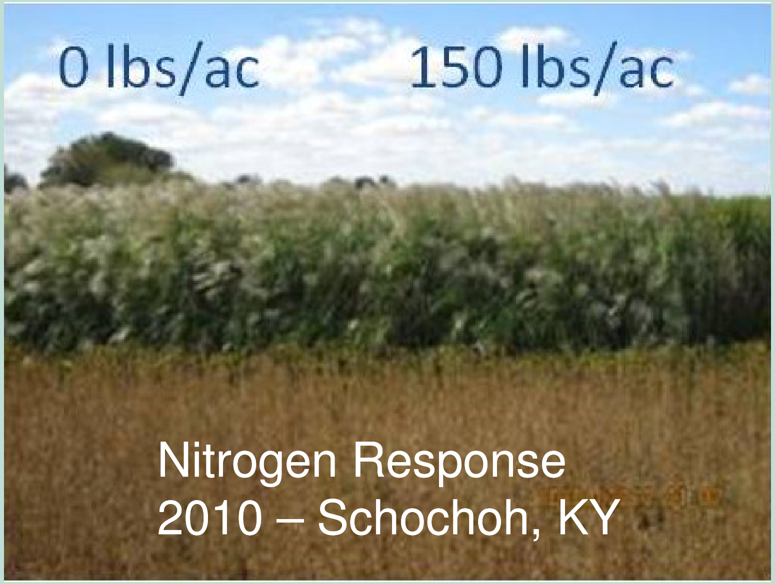
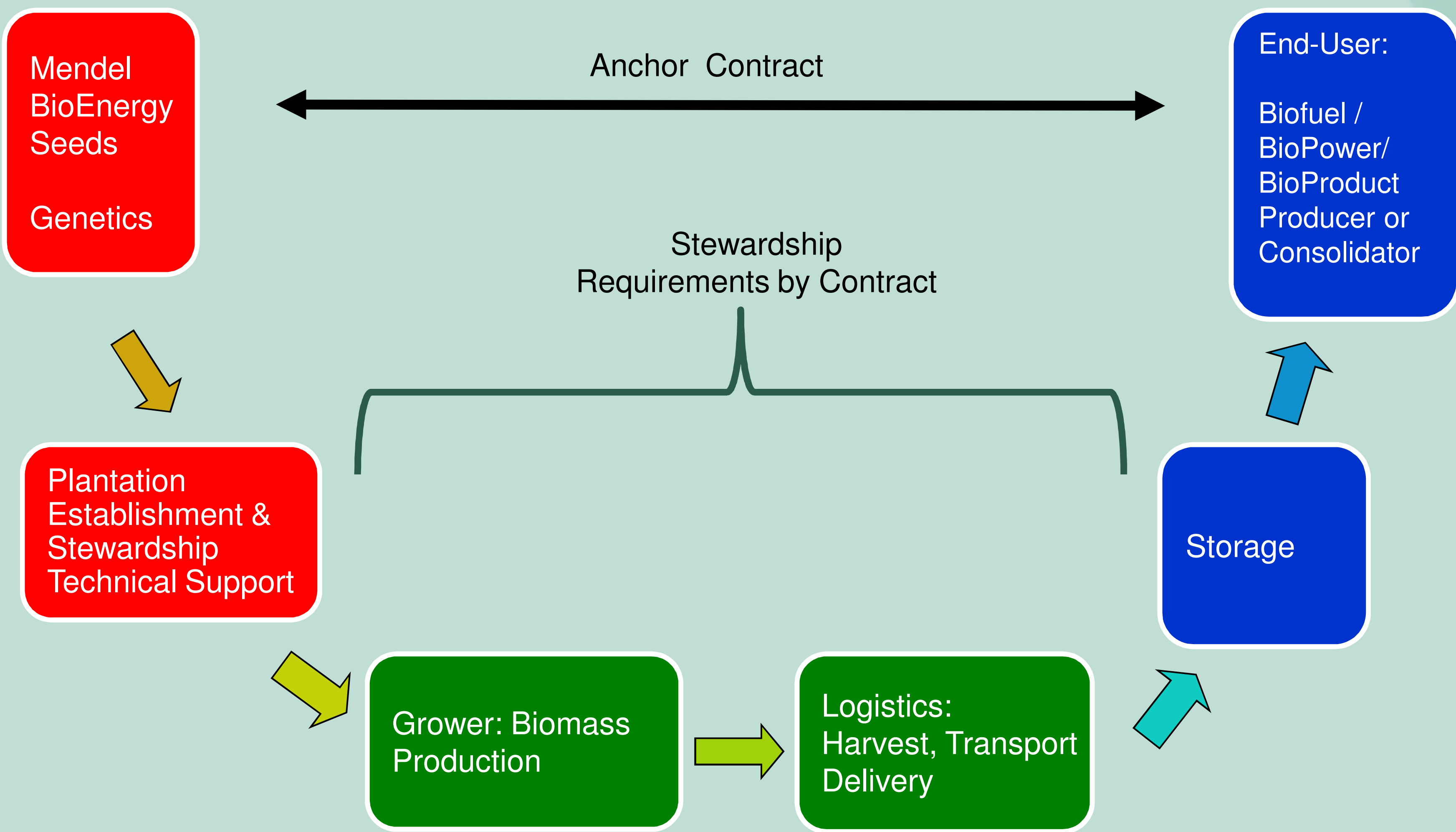
- Weed control programs
- Fertility programs

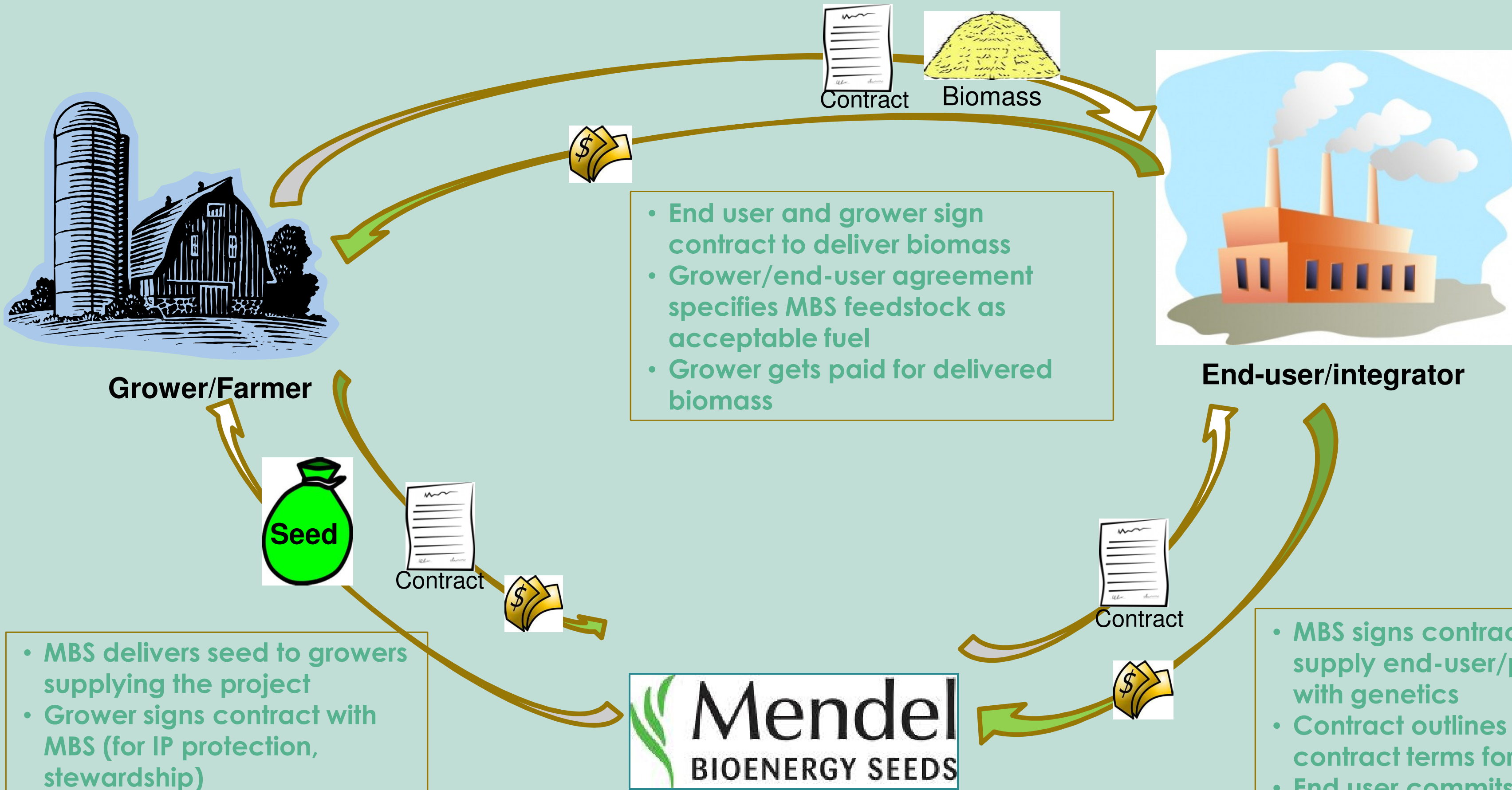




Mendel's role in the supply chain



MBS commercial model



- End user and grower sign contract to deliver biomass
- Grower/end-user agreement specifies MBS feedstock as acceptable fuel
- Grower gets paid for delivered biomass

- MBS delivers seed to growers supplying the project
- Grower signs contract with MBS (for IP protection, stewardship)
- Grower pays MBS for delivered seed*
- MBS provides ongoing support

- MBS signs contract to supply end-user/project with genetics
- Contract outlines seed contract terms for growers
- End user commits to annual payments to MBS (framed as license fee, financing fee, etc.)

* Initial payment for delivered seed could be end-user responsibility depending on individual project arrangements

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Sustainable system considerations

- Impacts on:
  - ~ Water
  - ~ Soil
  - ~ Nitrogen
  - ~ Biodiversity
- Biodiversity translates into:
  - ~ Land use patterns
  - ~ Potential for invasiveness

Water impact

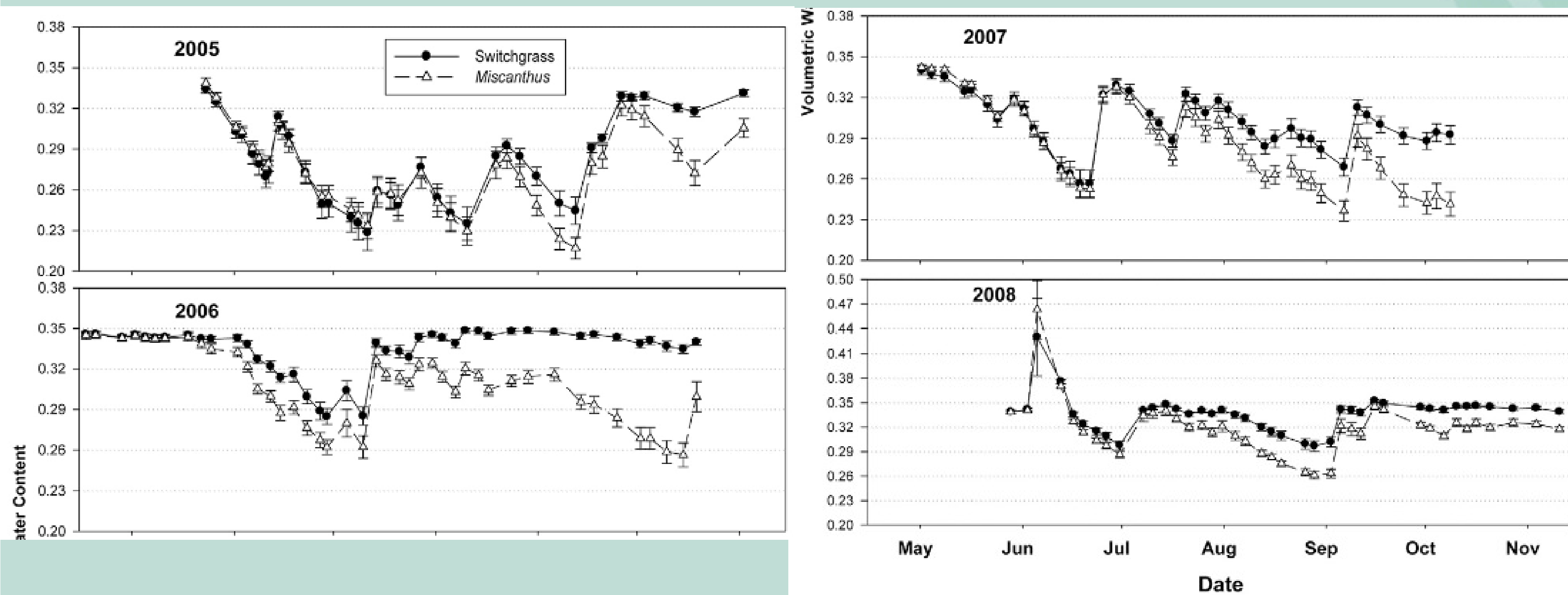

Fig. 1. Mean and 95% confidence interval of volumetric soil moisture content (0–90 cm) in mature switchgrass and *Miscanthus* measured in small plots (0.01 ha) during the 2005 to 2008 growing seasons. Error bars indicate 95% confidence intervals for the mean value.

McIsaac et al. J. Environ. Qual. 39:1790–1799 (2010)

Nitrogen impact

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Table 3. Mean ± 95% confidence limits of the annual leaching fluxes of nitrate, ammonium, and total inorganic nitrogen recovered in the ion exchange resin lysimeters at 50 cm depth under maize–soybean, switchgrass, and *Miscanthus*.

Collection period†	Maize–soybean	Switchgrass	*Miscanthus*
		kg N ha^{-1} yr^{-1}	
		NO_3	
2005–2006	41.2 ± 12.6	0.3 ± 0.3	ND‡
2006–2007	34.2 ± 6.5	0.4 ± 0.3	6.6 ± 2.0
2007–2008	45.9 ± 12.9	3.9 ± 3.2	1.6 ± 0.7
2008–2009	43.1 ± 8.9	1.1 ± 0.5	1.5 ± 0.7
2006–2009 avg.	40.4 ± 5.2	1.4 ± 0.7	3.0 ± 1.0
		NH_4–N	
2005–2006	2.8 ± 2.8	0.1 ± 0.05	ND
2006–2007	2.4 ± 0.6	4.2 ± 1.3	1.3 ± 0.2
2007–2008	2.3 ± 0.4	3.9 ± 0.7	2.3 ± 0.7
2008–2009	2.7 ± 0.5	4.0 ± 1.2	1.8 ± 0.4
2006–2009 avg.	2.4 ± 0.3	4.0 ± 0.6	1.8 ± 0.3
		TIN§	
2005–2006	45.4 ± 14.4	0.5 ± 0.3	ND
2006–2007	36.5 ± 6.8	4.6 ± 1.3	7.9 ± 2.0
2007–2008	48.3 ± 13.0	7.8 ± 3.6	3.9 ± 1.2
2008–2009	46.0 ± 9.0	5.1 ± 1.6	3.3 ± 0.9
2006–2009 avg.	43.0 ± 5.4	5.7 ± 1.6	4.8 ± 1.0

† The collection periods began in mid-April to early May and continued to approximately the same date in the following year.

‡ ND, no data collected due to establishment failure.

§ TIN, total inorganic nitrogen.

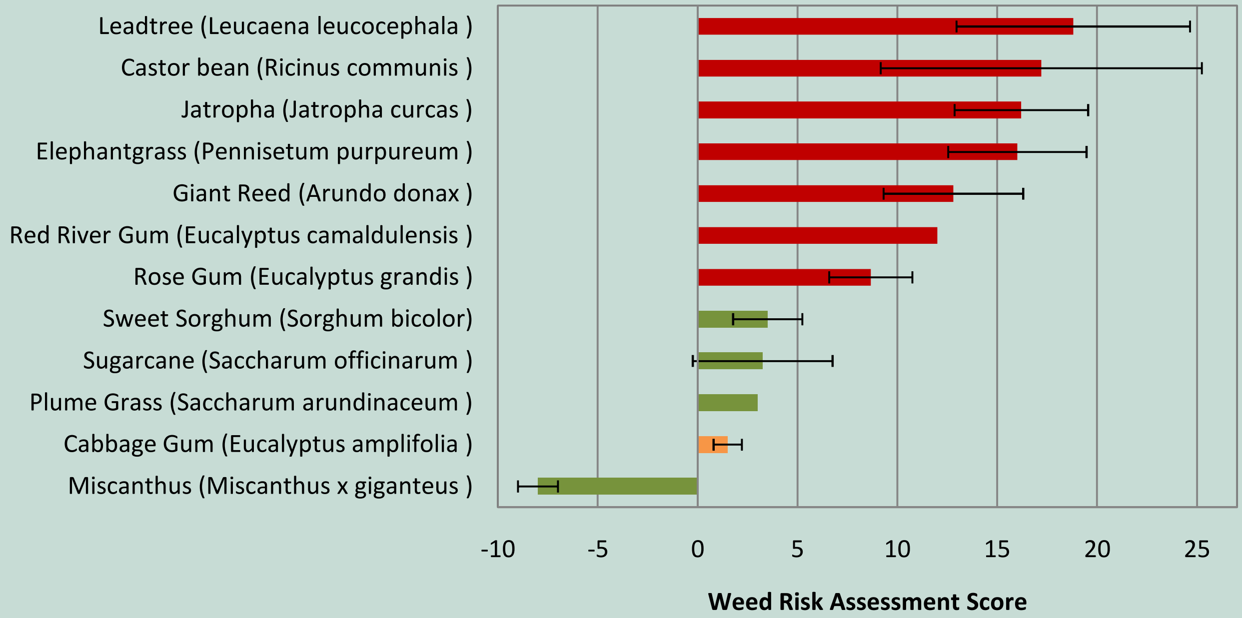
McIsaac et al.: *Miscanthus* and Switchgrass in Illinois 1797

McIsaac et al. J. Environ. Qual. 39:1790–1799 (2010)

Can Miscanthus be invasive?

A 2011 study by The Nature Conservancy & University of Florida lists sterile *Miscanthus* x *giganteus* as the lowest probability of becoming invasive among current & potential biomass crops

- Mean (± 1 standard deviation) for 2-5 published Weed Risk Assessment (WRA) scores
- Outcome:
  - **Reject**
  - **Evaluate**
  - **Accept**
- Data from Gordon et al. (2011 Biomass & Bioenergy 35: 74) and citations within



Breeding & management approaches to invasive risk

Table 2 Hierarchy of invasive-related traits which could be targeted in a *Miscanthus* breeding and management program to minimize invasive potential, along with the commercial implications

Breeding & management approaches	Non-invasiveness goal	Commercial implications	Invasion risk
Triploid sterility Non-flowering Self-incompatibility Functional non-flowering due to daylength No functional seed production due to daylength	Preventing seed production	Higher clonal establishment costs or inter-species F1 seed production costs Regulatory requirements defining distance from compatible pollen source Regulatory requirements to define planting regions	Lower
Non-shattering seeds "Flightless" seeds	Preventing seed dispersal	Lower seed establishment costs	
Non-germination No survival to maturity	Preventing seed establishment	Higher regulatory risk	
Non-dormancy Inviable vegetative propagules	Ease of eradication	Higher regulatory risk	Higher

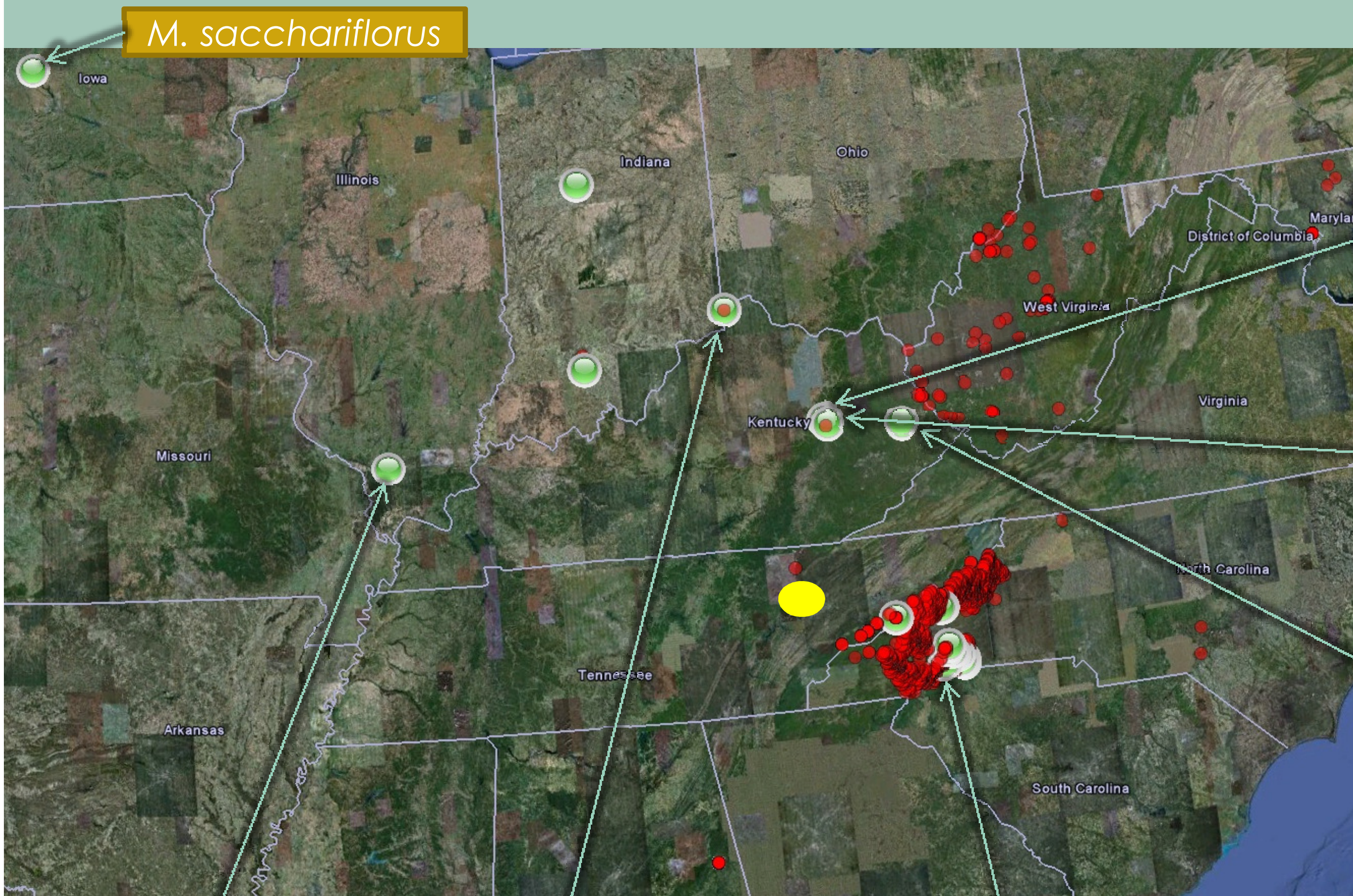
LAUREN D. QUINN*, DAMIAN J. ALLEN† and J. RYAN STEWART GCB Bioenergy (2010)

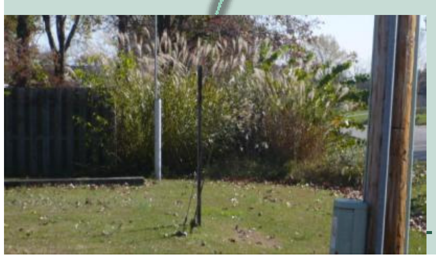
History of miscanthus in United States

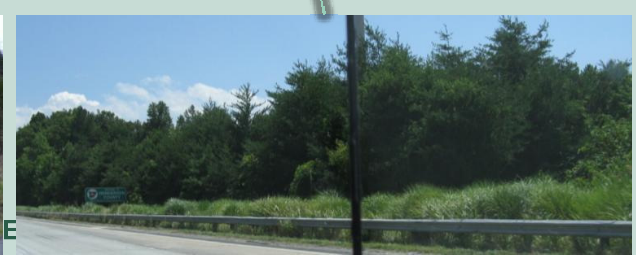
- Introduction of the genus as an ornamental over 100 years ago
  - Primarily *M. sinensis*
  - Secondarily *M. sacchariflorus*

- Biomass types only introduced in past decade
  - *M. x giganteus* introduced about a decade ago
  - Several *Miscanthus* species for biomass production introduced in the past decade

Understanding existing naturalized ornamental *Miscanthus*







SE-EPPC *M. sinensis* volunteers
http://www.eddmaps.org/southeast/distribution/viewmap.cfm?sub=3052

Sites sampled by MBS for molecular marker analysis

Miscanthus cross-pollination is primarily wind driven

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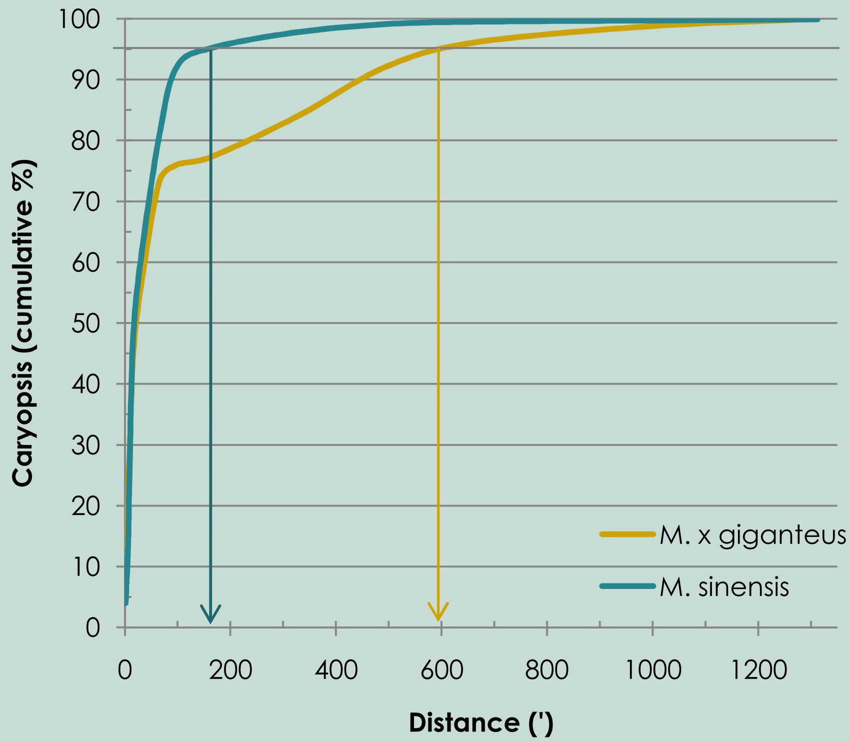
Wind Pollinated
(predominant)

Bee Assisted

Detecting miscanthus "seed" dispersal range

Cumulative distribution of distance traveled

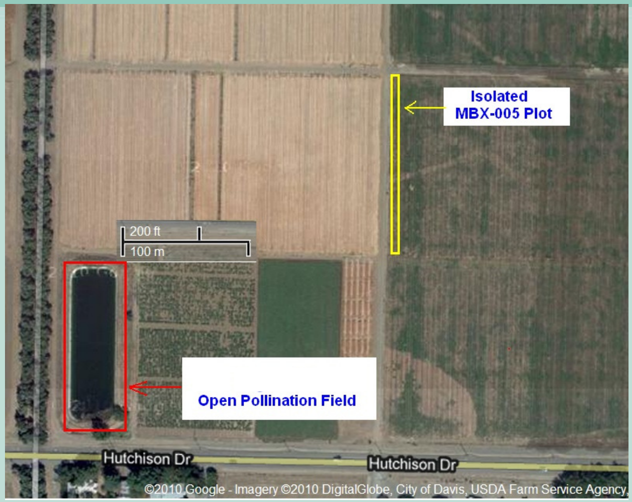


http://www.weedcenter.org/wab/2010/docs/presentations/Session-04/Matlaga/4-1-Matlaga.pdf

Seed capture

- 1+ million caryopses wind dispersed for ~6 weeks
- 95% of seeded (heavier) *M. sinensis* captured within 160' and only 0.2% at ¼ mile
- 95% for seedless (lighter) *M. x giganteus* captured within 600' and only 1.3% at ¼ mile
- Adapted from Quinn et al. 2011. Invasive Plant Science and Management 4:142

Understanding the extent of self-fertility



Seed Set Rate: 0.047%
(395 seeds out of 846,456 spikelets)

11/17/2010

Seeds of Mendel's products have a very short dormancy window

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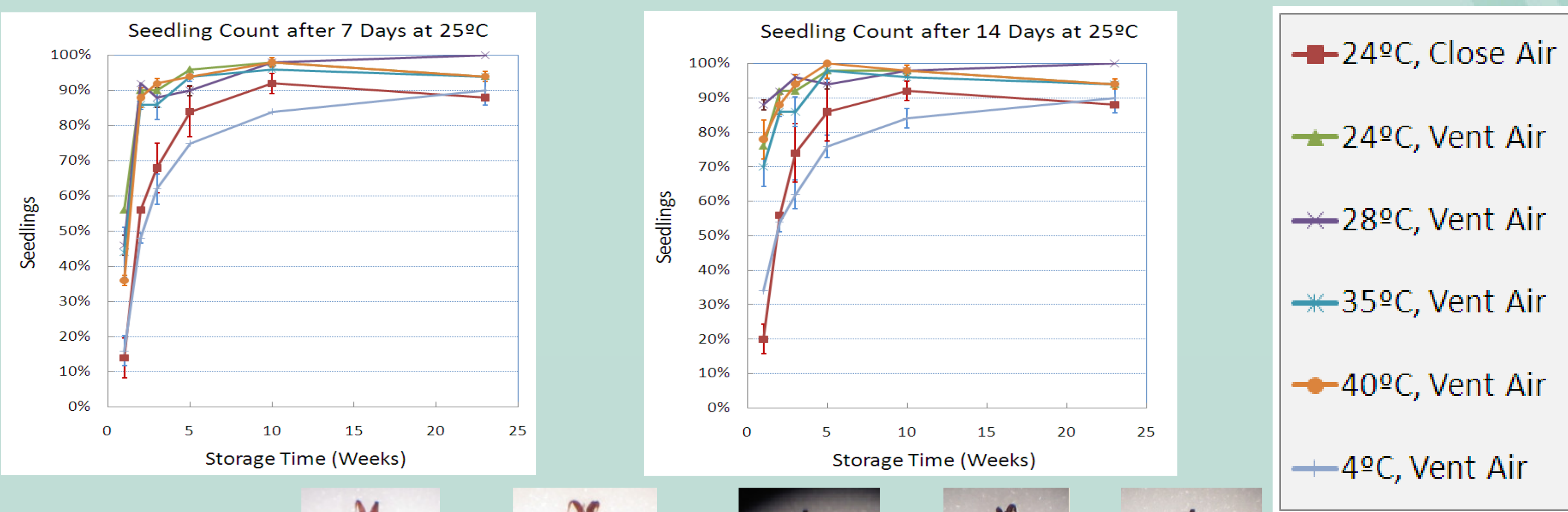
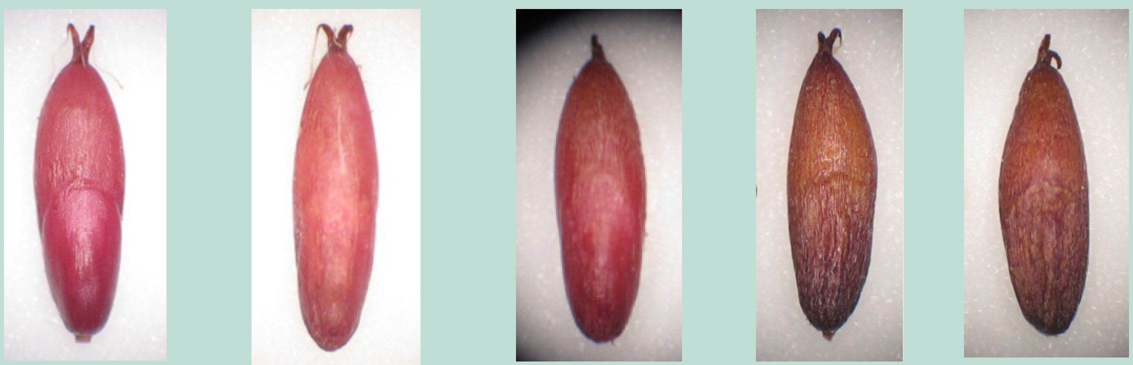




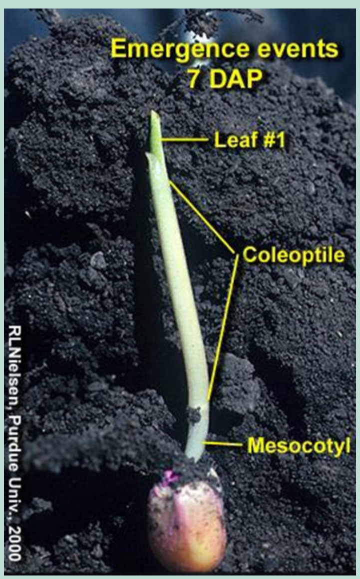
26 30 38 47 52

Days After Pollination

Assessing miscanthus seedling vigor: Shoot structures of various grass seedlings

Timothy

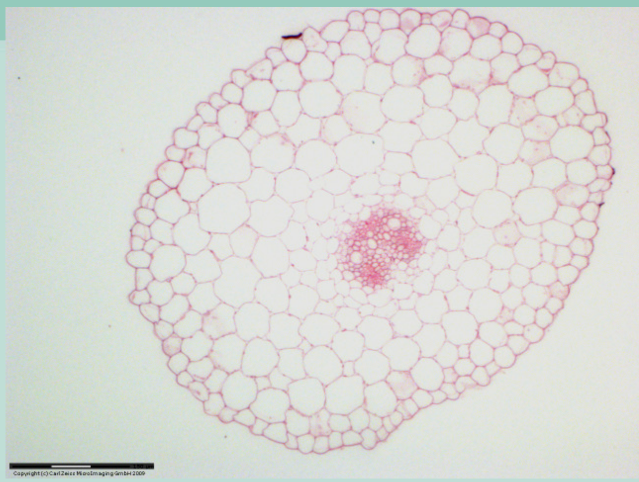
Sorghum



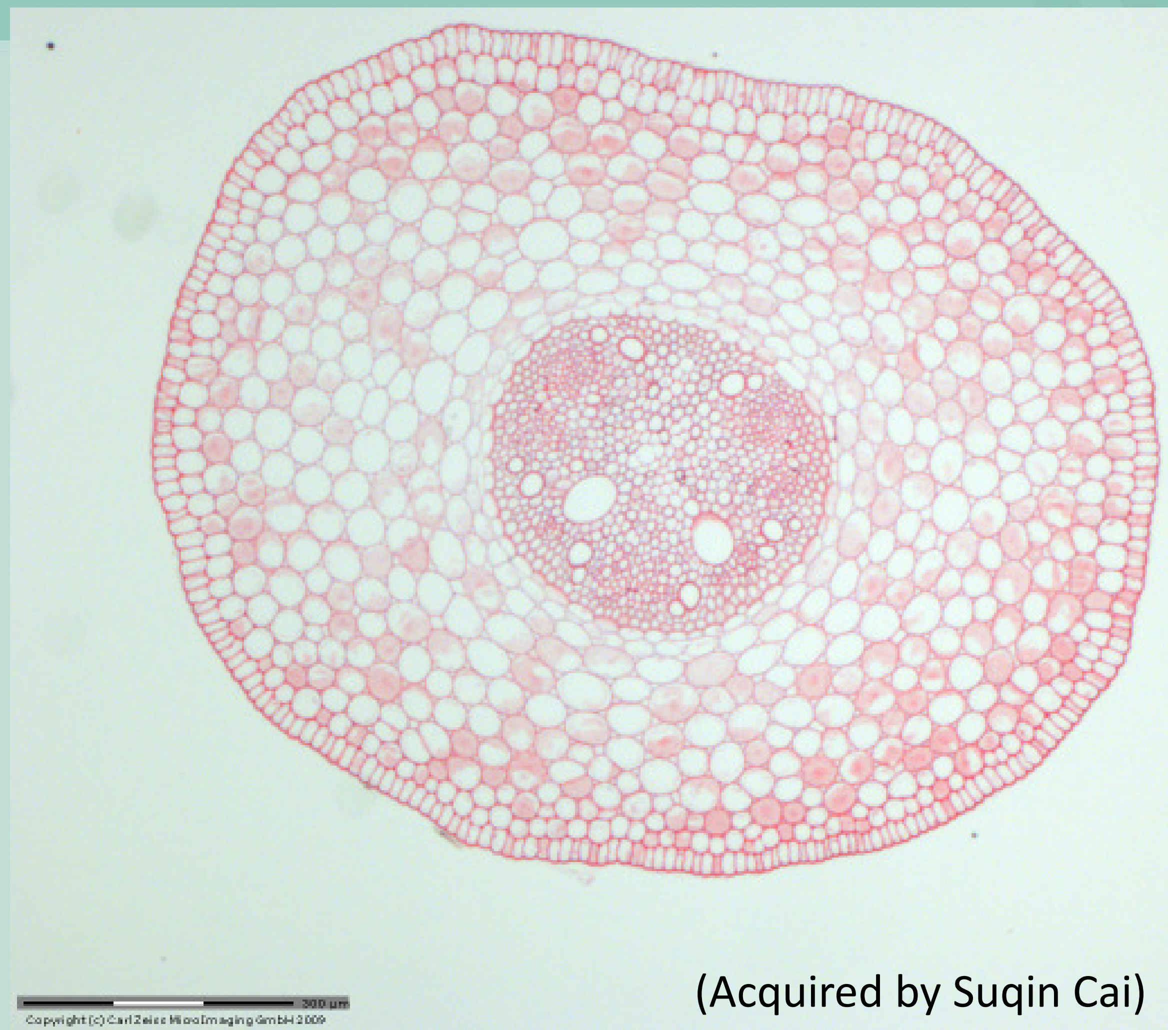
Corn

Barley

Mesocotyl of Miscanthus and Sorghum

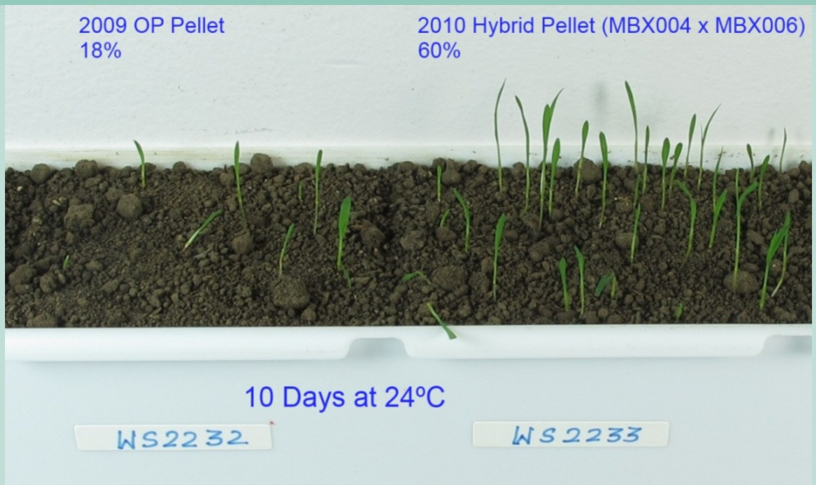


Miscanthus mesocotyl

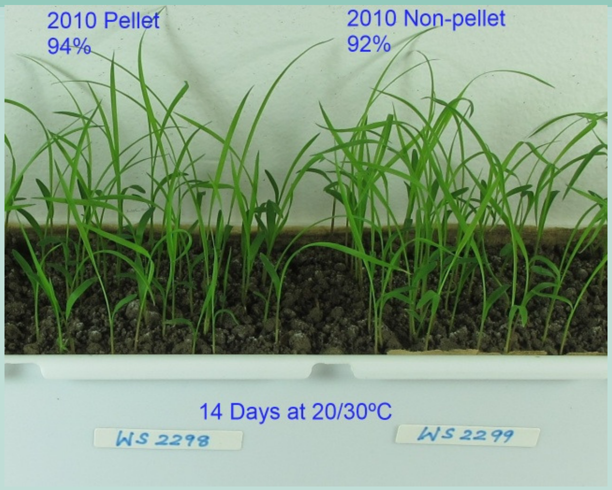


Sorghum mesocotyl

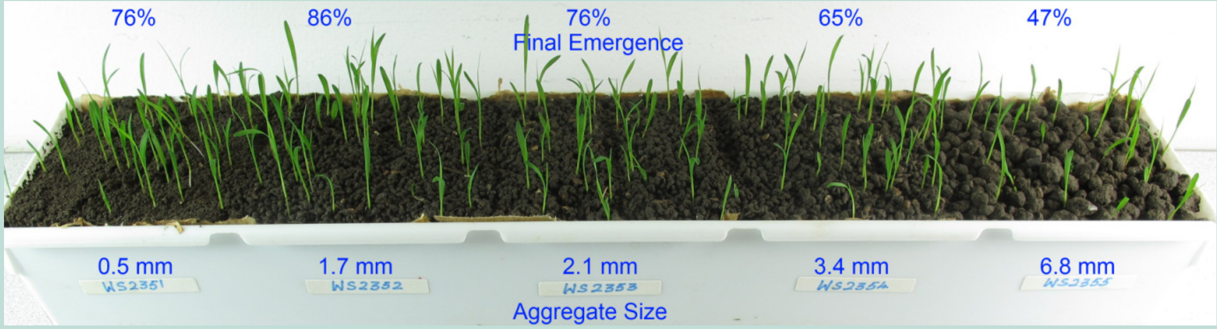
Seedling emergence impacted by quality, temperature and soil "size"



Impact of Seed Quality

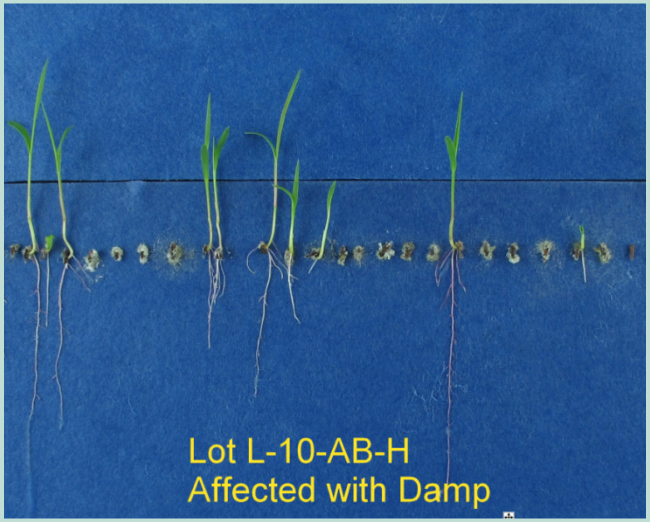


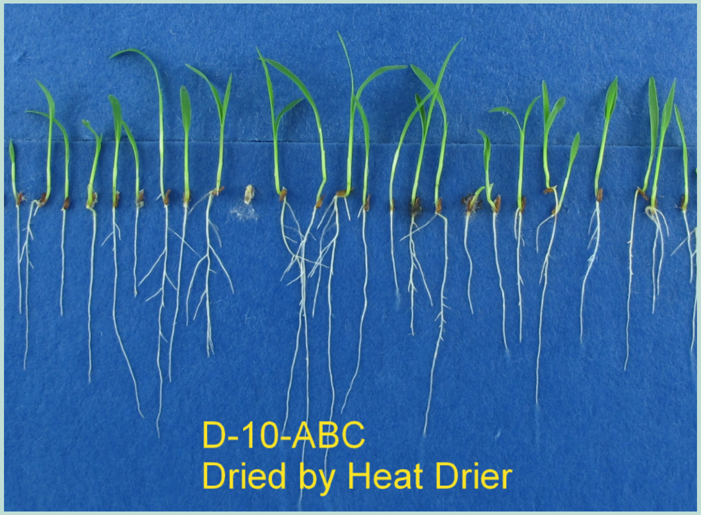
Impact of Temperature



Impact of Soil Aggregate Size

Moisture can negatively impact seed germination and quality





Assessing Mendel's seeded product for potential invasiveness

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- How much viable seed is made?
  - Harvest between seed maturity and shattering
  - Measure seed number and germination rate
  - Measure Gen 1 products 2011-13 (11RYTs)
- Competitive ability outside of Miscanthus fields
  - Depends on what's growing there now & how it is managed
  - Measure seed germination and plant establishment % in major land uses without interfering with management (tillage, mowing, spraying, planting etc)
  - Measure Gen 1 proxy 2010; Gen 1 products 2011-13
- Genotype x Environment interaction
  - All genotypes across all regions expect to launch

Testing for volunteers in managed landscape

- Add seed without disturbing existing management
- 100 seed in replicated1m^2 plots
- KY & IN
- e.g. IN:
  - ~ Mowed waterway/ditch
  - ~ Soybeans
  - ~ Wheat
  - ~ Tree line
  - ~ Corn
- No volunteers observed in 2010

2008 M. sinensis trial Lafayette, IN
2010 Volunteers

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Turf grass Grass weeds Trial plants

Miscanthus volunteers

2009 Breeding Trial Lafayette, IN 2010 Volunteers

- Volunteers in alleys within field
- Volunteers in plots where there are missing plants
- No volunteers observed on edge or outside trial
- In alleys, approximately 1 volunteer every 10 sq ft (4,800 plants/ac)

Miscanthus volunteers

Summary observations: implications for potential invasiveness

- Seeds of Mendel miscanthus varieties have a brief period of dormancy, likely leading to a short longevity in soil;
- Static coleoptile and weak mesocotyl prevent seedling emerging from deep soil;
- Pollen is short-lived (30'); 500-1,000 feet is a good seed production separation distance

Long-term product strategy: reduce seed propagule load to reduce risk

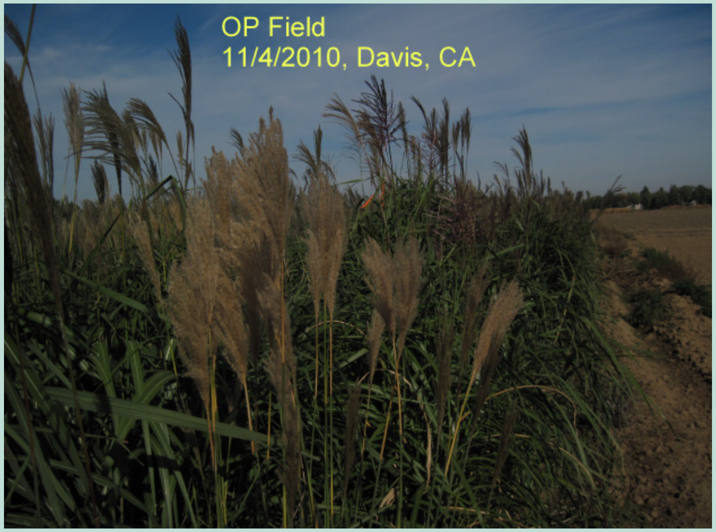
- Create varieties that *create and combine* various approaches to reduced seed production or dispersal

- “Functional sterility” by delayed flowering
- “Incompatibility-based sterility” in hybrid type system
- “Ploidy-based sterility” through odd ploidy product

- 2nd and 3rd generation products launched as market grows
  - ~ Enables reduced cost of stewardship as welll

Diversity of flowering time in Mendel's breeding populations

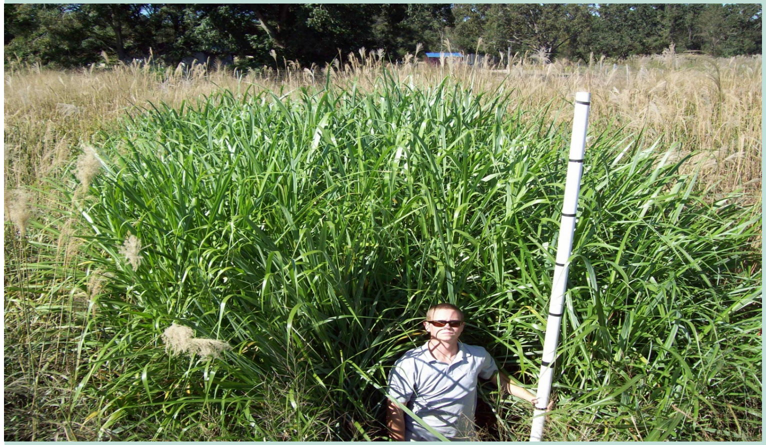
Breeding nursery



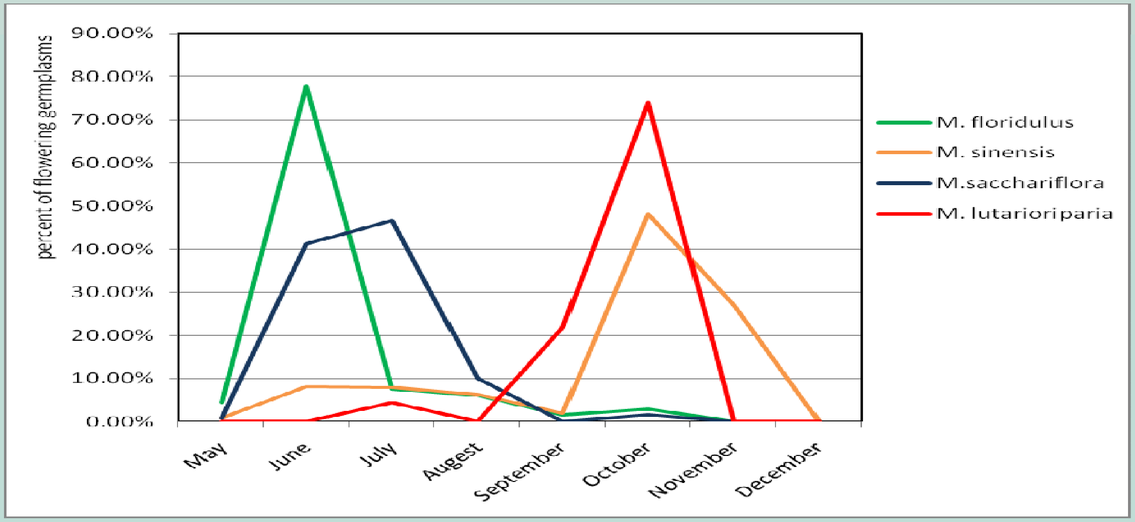
Panicles harvested Nov 18, 2010

"Functional sterility" as one strategy to better manage propagule pressure

- Improved control of flowering
  - ~ Late flowering for increased biomass
  - ~ Maturity groups tuned to each region
  - ~ Flowering late enough to avoid production of viable substantial amounts of viable seed



Functional sterility demonstrated in a genotype derived from *Miscanthus sinensis* at Mendel's Auburn, AL facility (33° N), through identification of a non-flowering and high-biomass breeding line that was grown from seed (foreground plot) in comparison to flowering genotypes (background). Lines from Texas A&M under MTA.



1. ***M. sinensis* northern type flowers in May-Aug**
2. ***M. sinensis* southern type flowers in Sept-Dec**
3. ***M. sacchariflorus* flowers in May-Sept**
4. ***M. lutarioriparium* flowers in Aug-Nov**
5. ***M. floridulus* flowers in May-July**

Data from Xiao Liang, collected in Hunan, 2008 – Mendel collaboration

Odd ploidy-based sterility

- Mendel now has Mxg lines with a wide range of ploidies to test approach
  - ~ 2x -> 8x
  - ~ Can create 3x, 5x, 7x varieties

- Higher ploidy does not necessarily lead to more vigorous, higher biomass products

- Tests of multiple odd ploidy strategies in progress

Incompatibility-based sterility via inbred-type system

- Production of “in-breds” through anther culture
- A range of ploidies has been achieved with these inbreds
- Seed derived by crossing two different “in-bred” lines would have identical incompatibility group
  - A field established with these seed, at more than quarter mile (?) from any other fertile miscanthus, would not be fertile
- Seed similarly derived, but from parents of different ploidy, would have two mechanisms for reduced production field fertility

Framework

- Brief intro to Mendel
- Why miscanthus
- Mendel's strategy for broad miscanthus adoption
- Sustainable system considerations
- **Stewardship program**
- Summary

Importance of Product Stewardship

- New species for U.S. production agriculture
  - Miscanthus species have been planted as ornamentals for 100+ years
  - 10+ years of miscanthus experience in U.S. academia
  - Gathering information to help predict miscanthus reaction in different environments
- Ensure longevity of the system
  - Examples: insecticidal genes in corn / cotton
- Enable crop rotations

Internal Stewardship Program Established

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- Scouting and reporting protocols established and underway
  - ~ 48 North American locations under protocol
- Eradication protocols underway at ≈ 20 locations in 2011
- Internal stewardship studies underway

KY Demonstration Farm – Eradication Demo

- Knee-high 2nd year sterile Miscanthus growth
- 1st Round-up application & mowing eliminated all live above ground tissue
- 2nd application during bean growth
- A few survivors visible when beans senesced

External Stewardship Research & Input

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- Funding several stewardship-related research projects in Southeast, Mid-Atlantic and Midwest
  - Notasulga, AL site designated for use by Auburn University for miscanthus eradication and stewardship studies (Stephen Enloe)
  - Miscanthus propagule biology studies at Virginia Tech (Jacob Barney)
  - Providing seed for other studies
- Seeds subsidiary Scientific Advisory Board includes an expert on weed physiology and management (Joseph DiTomaso)
- Forming an Advisory Group, consisting of government, academic and industry experts
- Participating in CSBP – Council for Sustainable Biomass Production

Stewardship systems are critical for a new crop production system

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- Elements of Mendel's stewardship strategy:
  - ~ Invest in basic understanding of seed germination biology
  - ~ Determine the potential for seed dispersal
  - ~ Develop methods for eradication of plants arising from dispersed seed outside of production zones
  - ~ Monitor all test sites
  - ~ Use commercial agreements requiring grower stewardship
  - ~ Develop low-fertility varieties as foundation for long-term product strategy

Commercial considerations

- Seed label use restrictions represent one important approach to managing potential risk
  - Geographical restrictions (variety adaptation by region)
  - Movement restrictions of harvested biomass with seed
  - Monitoring & mitigation (stewardship) regimes
- Assuring compliance with any label use restrictions
  - The "closed loop" commercial systems should facilitate compliance

Summary/Conclusions

- Seeded miscanthus is a highly desirable production system for purpose-grown biomass
- First generation products (2014+) are fertile, but good stewardship, under contracts (including choice of production sites), can effectively manage invasiveness risk
  - Continued study and collaborations are needed to develop best stewardship practices
- 2nd and 3rd generation products (2018-20) will reduce potential for invasiveness & stewardship costs

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seeding a sustainable future

Questions?