Gene Flow Matters in Switchgrass (*Panicum virgatum*), a potential widespread biofuel feedstock

Photo credit: D. Mann

ORNL CBES Forum, 17 May 2012

Charles Kwit

University of Tennessee, Department of Plant Sciences

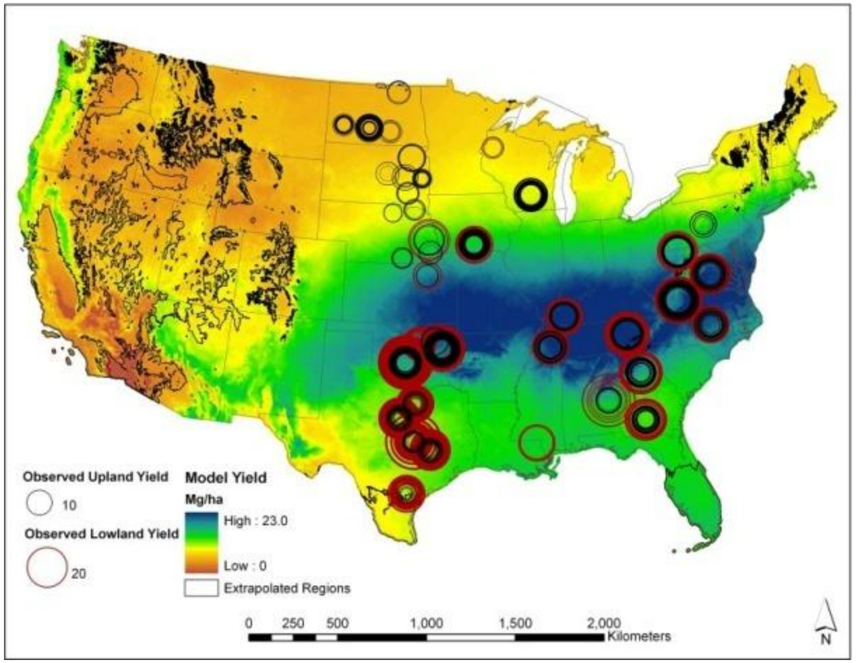
Outline

- Who cares about switchgrass gene flow (and why)?
- What do we know about switchgrass gene flow?
- What steps are underway?

Agronomic switchgrass…

- Attractive *native* bioenergy feedstock (Schmer et al. 2008; Groom et al. 2008)
- Bioenergy interest dates to late 1970s (DOE)
- Extent of its potential also dictated by current energy-related Acts
- Breeding (conventional and molecular) and transgenic efforts for better switchgrass
  - e.g., Rapid growth in spring; high water-use efficiency; partitioning of nutrients to roots

Photo credit: J. Miles Cary



Wullschleger et al. 2010

Who cares about switchgrass gene flow?

- Conservation biologists
- Farmers
- Federal regulators

Photo credit: J. Miles Cary

Who cares about switchgrass gene flow?

- Conservation biologists
  - Wild population "purity" concerns
  - Invasibility concerns
    - Push for wide-scale planting of agronomic switchgrass as a biofuel feedstock + native switchgrass populations
  - Population extinction concerns
    - Demographic swamping (esp. pollen) and "migrational meltdown"

Photo credit: J. Miles Cary

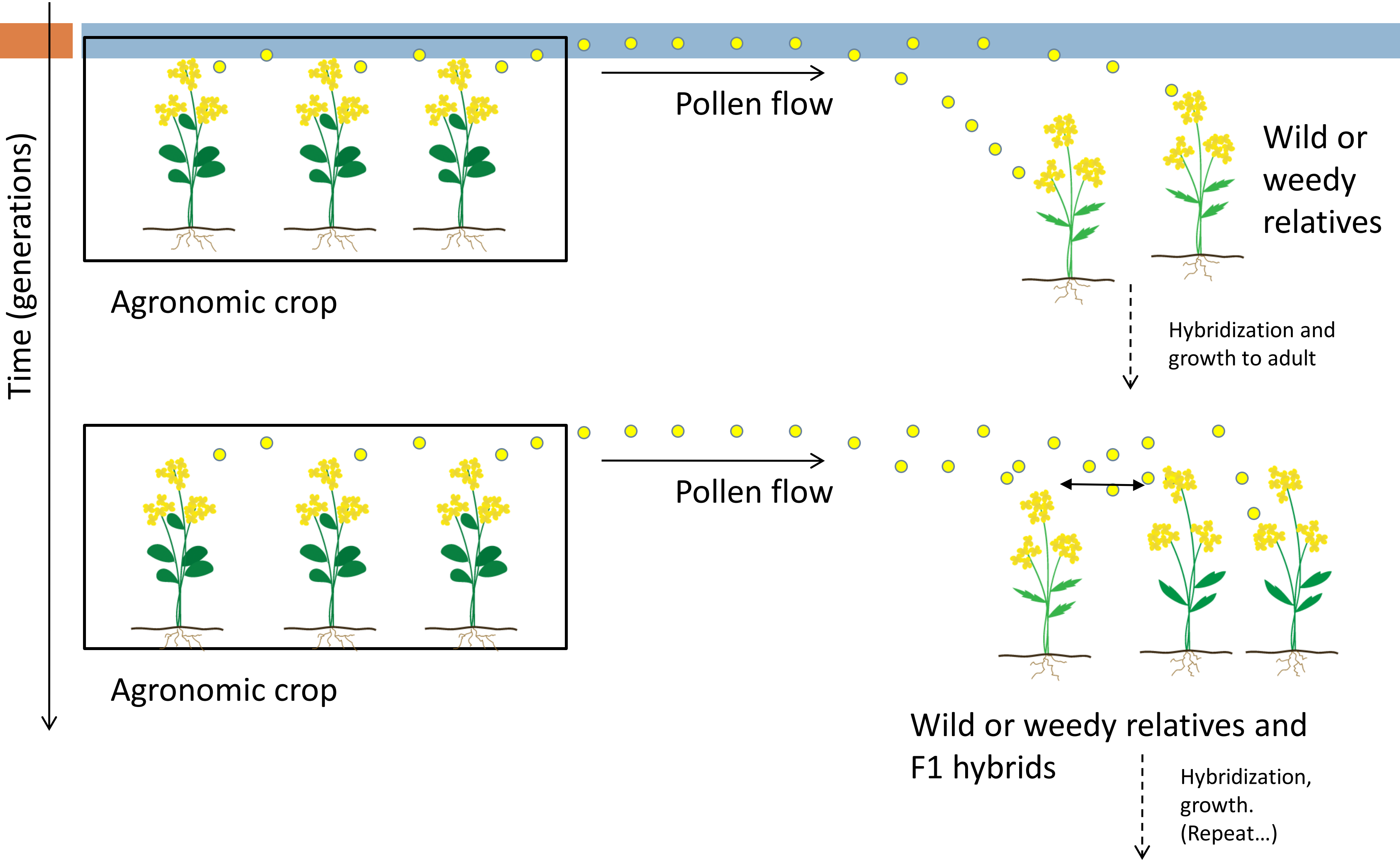
Invasibility (and extinction) concerns

- "Improvement" efforts could lead to switchgrass becoming an **invasive species**
  - Context: species that negatively affects ecosystem processes and functionality, and imparts economic losses
  - Mechanism: planting in novel environment and escape via seed (Raghu et al. 2006) or pollen-mediated introgression (Simberloff 2008)

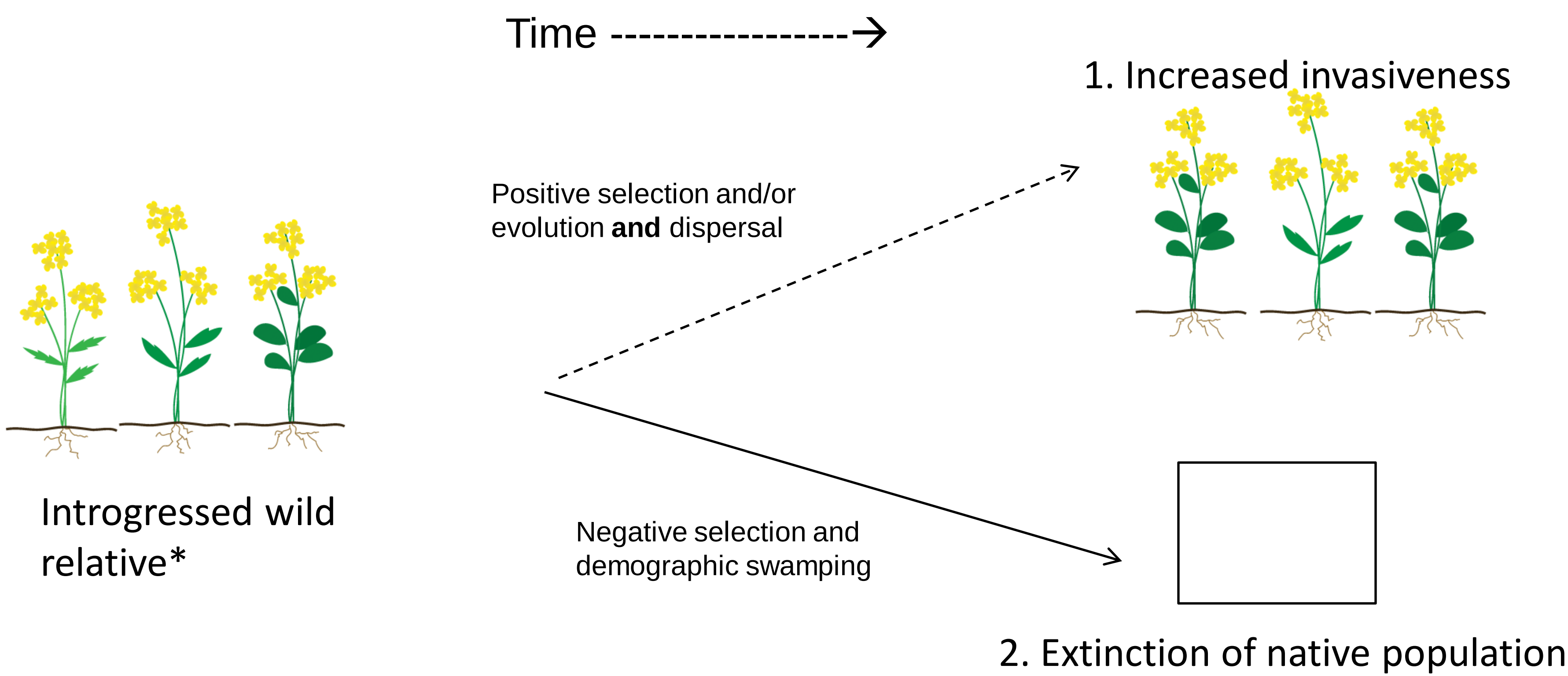
Ideal ecological traits of biomass energy crops (*4*). All traits shown other than perennial growth and sterile seeds are known to contribute to invasiveness. See (*25*).

Raghu et al. 2006

Introgression…



...leading to invasiveness or extinction



Outline

- Who cares about switchgrass gene flow (and why)? Conservation biologists
  - Wide-scale planting of agronomic switchgrass may carry risk of invasiveness and extinction of wild relative populations
  - From the literature: introgression of crop genomes into wild relatives has been documented; no documentation of invasiveness or extinction

Table 1. Recent (2005–2010) studies that provide molecular evidence of introgression from nontransgenic crops to their wild or weedy relatives

Crop	Relative	Molecular marker	Refs.
Cichorium intybus	*C. intybus*	AFLP	[30]
Glycine max	*Glycine soja*	SSR	[32]
Helianthus annuus var. *macrocarpus*	*Helianthus petiolaris*	RAPD	[87]
Medicago sativa	*M. sativa*	AFLP, SSR	[88]
Oryza sativa	*Oryza rufipogon*	SSR	[89]
Pennisetum glaucum	*P. glaucum*	SSR	[90]
Phaseolus vulgaris	*Ph. vulgaris*	AFLP	[33]
Raphanus sativus	*Raphanus raphanistrum*	Allozyme	[25]
Sorghum bicolor	*Sorghum halepense*	RFLP	[91]
Triticum aestivum	*Aegilops peregrine*	Fragment of noncoding locus	[92]
Vigna unguiculata	*V. unguiculata* ssp. *unguiculata* var. *spontanea*	RFLP	[93]
Vitis vinifera	*Vit. vinifera* ssp. *silvestris*	SSR	[24]
Zea mays	*Z. mays*	SSR	[94]

Abbreviations: RAPD, randomly amplified polymorphism; RFLP, restriction fragment length polymorphism; SSR, simple sequence repeat.

Kwit et al. 2011

- What do we know about switchgrass gene flow?
- What steps are underway?

Who cares about switchgrass gene flow?

- Farmers
  - Seed purity
    - Cultivar purity from seed farms could be compromised by cultivar x cultivar or wild x cultivar cross-pollination
- Federal regulators
  - Containment of transgenes

Photo credit: J. Miles Cary

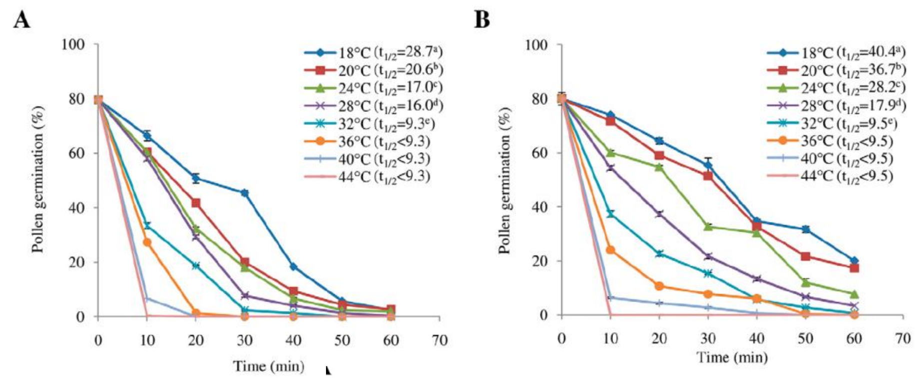
What do we know about switchgrass gene flow?

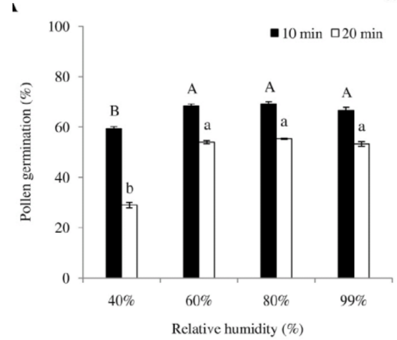
- Very little known, and much assumed
- Information on the following would be helpful:
  - Seed dispersal
  - Pollen dispersal distances and viability
  - Crossing and hybridization potential

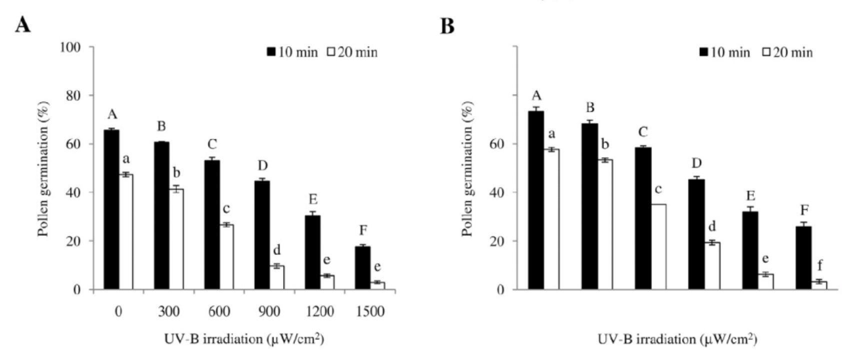
Photo credit: W. Gretz

So little known (and so much assumed)… Pollen dispersal and viability

- Recent information on pollen viability (Ge et al. 2011)
  - Negative effects of increased temperature, decreasing relative humidity, and increased UV-B radiation on pollen germination
  - 150 min under ideal conditions to lose complete viability - similar to other grasses

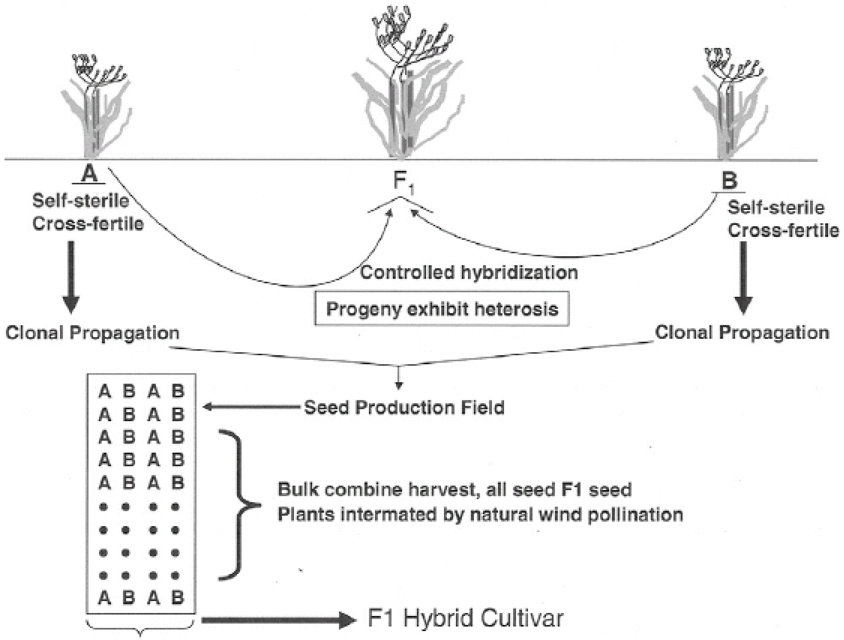






So little known (and so much assumed)… Crossing and hybridization potential

- *P. virgatum* Cultivar x Cultivar crosses
  - requires same ploidy levels [e.g., Lowland tetraploid Kanlow x upland tetraploid Summer (Vogel and Mitchell 2008; Martinez Reyna and Vogel 2008)]
- No published accounts of 'agronomic x "wild"' *or* interspecific hybridization
- Few examples of *Panicum* hybridization



Martinez Reyna and Vogel 2008

Outline

- Who cares about switchgrass gene flow (and why)?
- What do we know about switchgrass gene flow?
  - Remarkably little
  - Pollen viability similar to other grasses
  - Crossing and hybridization potential may be limited to intraspecific crosses involving similar ploidy levels
  - Still need information on pollen dispersal, seed dispersal, intra- and inter-specific crosses
- What steps are underway?

Steps underway

- Genetic diversity of native switchgrass in the Midsouth
- Current USDA BRAG project addressing:
  - Pollen dispersal distances (field-to-field and "general")
  - Intra- and interspecific hybridization

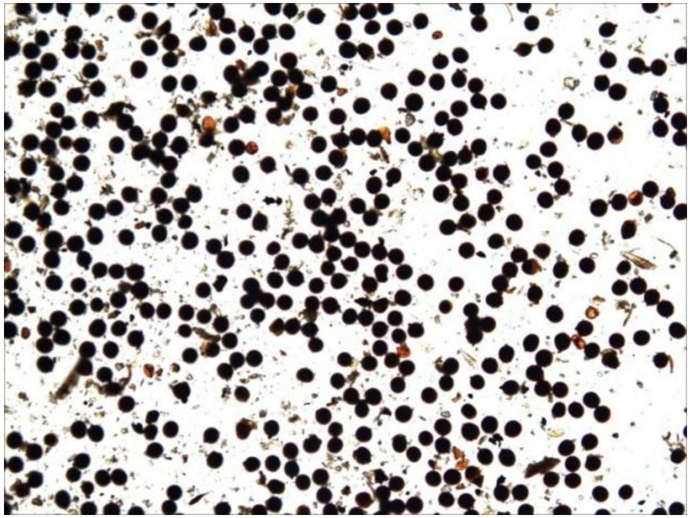
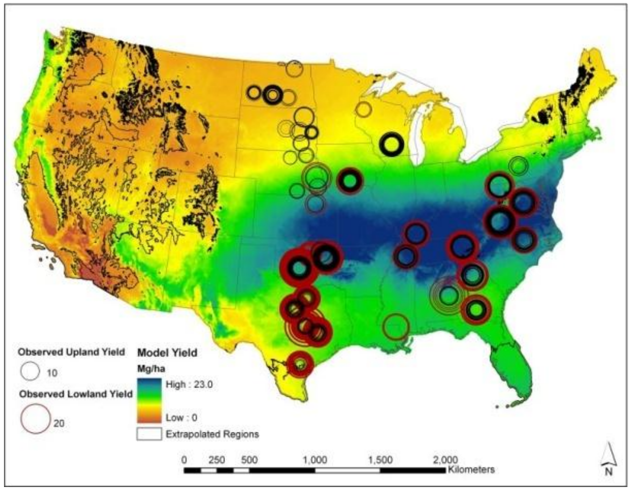
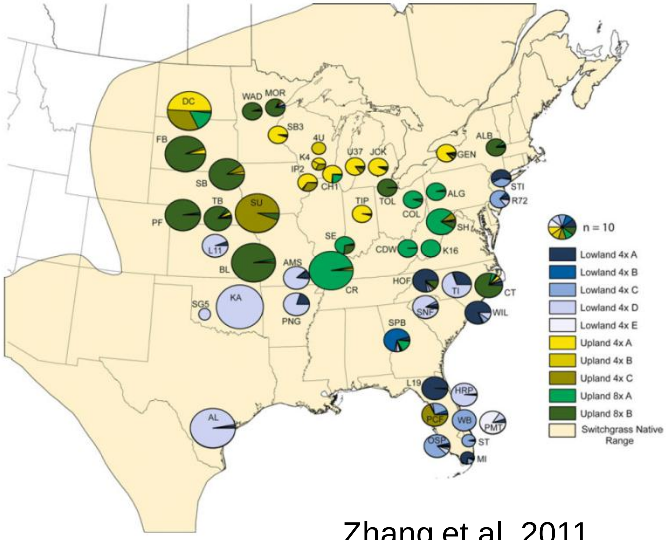


Photo credit: H.S. Moon

Genetic diversity of native switchgrass in the Midsouth

- Little is known about switchgrass population genetic structure where it has its highest potential yields
- Baseline data needed for future monitoring

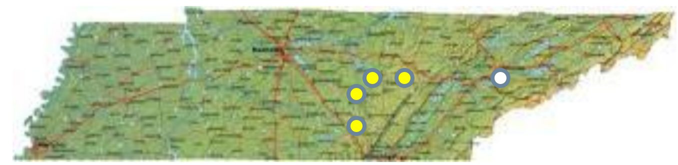


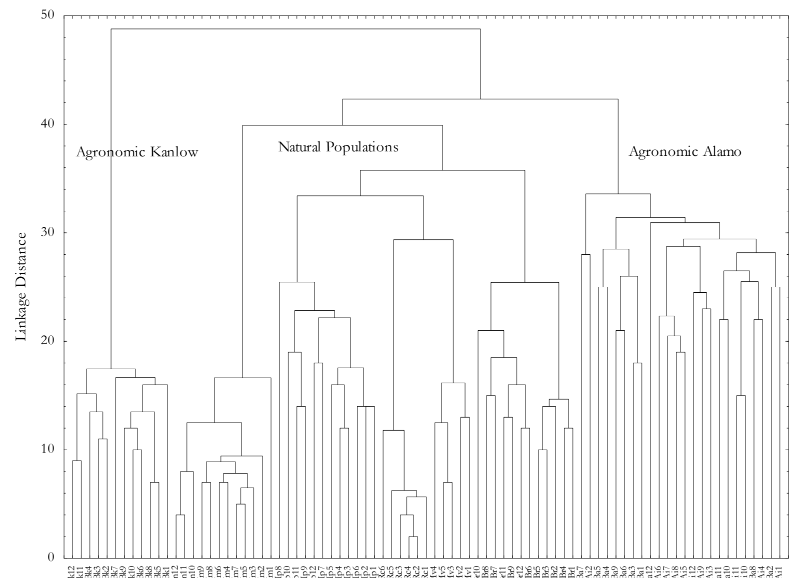


Zhang et al. 2011

Genetic diversity of native switchgrass in the Midsouth

- 8 RAPD markers across 5 native TN populations and 3 agronomic “populations” in east TN
- Similar mean number of loci/primer (15.1) and diversity within and among populations as other RAPD studies
- Most diverse native population not as diverse as some agronomic “populations”
- NSF and NPCI proposals in review for expansion of this using SSR markers

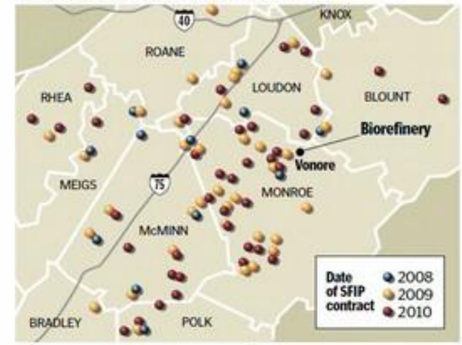




Nageswara Rao et al., *in review*

Steps underway: USDA BRAG

- Field-to-field pollen dispersal distances
  - Take advantage of new plantings of improved Kanlow (EG1102) in east TN
  - Assignment analysis (via diagnostic SNPs) of offspring from maternal Alamo plants at numerous distances from the Kanlow source: realized probability of outcrossing
  - Relevant to seed purity



County	Total acres	Farmers
Monroe	2,205.3	26
McMinn	818.1	10
Loudon	553.9	6
Blount	469.5	4
Bradley	354.7	2
Polk	291.9	3
Rhea	258.1	5
Roane	118.8	4
Hamilton	58.6	1
Meigs	33.3	2
Totals	5,162.2	63

Year	New farmers	Total production in tons
2008	16	1,000
2009	24	6,000
2010	21	*15,000

*Estimated

Farmer type	Farmers
Full time	31
Part time	30

Source: Genera Energy LLC NEWS SENTINEL

S. Jackson

Steps underway: USDA BRAG

- Pollen dispersal distances in switchgrass
  - Nelder wheel design
  - Source clones of RFP Alamo ST1
  - Receptor clones of Alamo 2 along rays
  - Pollen traps to measure RFP pollen grains as a function of distance
  - Seeds (verified by RFP seedlings) as a function of distance

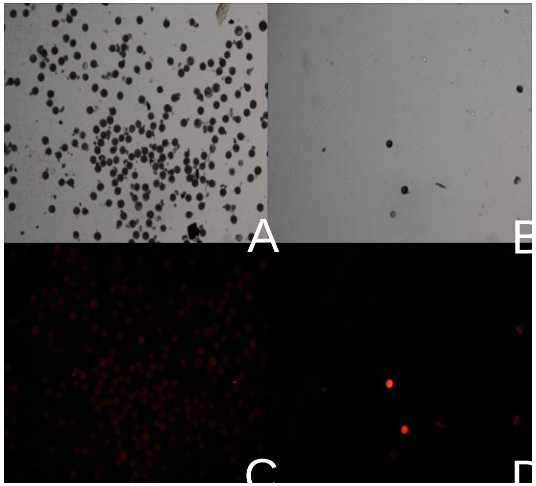


Photo credit: J. Burris

Steps underway: USDA BRAG

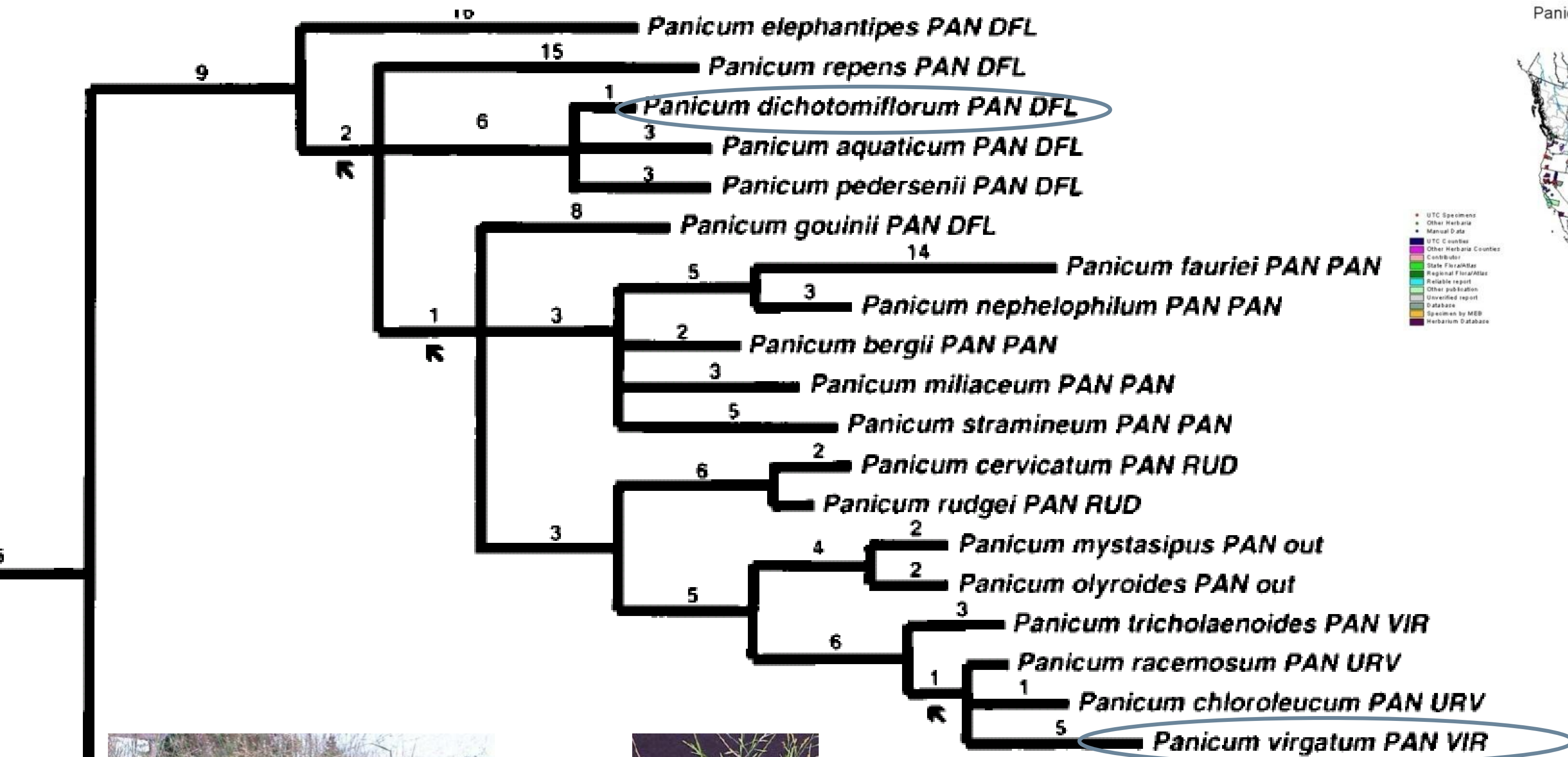
- Intra- and interspecific crosses involving *P. virgatum*
  - cv. Alamo x wild
    - Compared to offspring of cv. Alamo x cv. Alamo and wild x wild
  - Interspecific crosses…

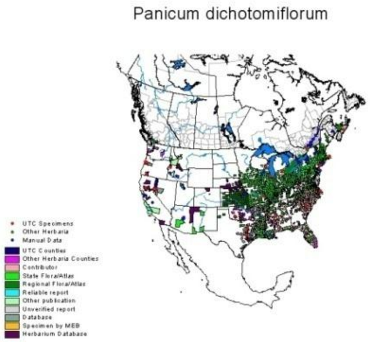
Steps underway: USDA BRAG

- Intra- and interspecific hybridization

Aliscioni et al. 2003

Plans to cross switchgrass with its closest existing congener in TN





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Outline

- Why should anyone care about gene flow and hybridization in switchgrass?
- So little known (and so much assumed)…
- Steps underway
  - Genetic diversity and structure of local Midsouthern populations
  - Pollen dispersal distances & field-to-field ‘realized’ pollen dispersal success
  - Intra- and inter-specific hybridization

Pressing questions for the CBES Forum

- How should elements of gene flow be incorporated into sustainability indicators for bioenergy systems?
- Should crop genes be contained? If so, where, when, and how?
- Can the risk associated with invasiveness (genes and/or organisms) be minimized? If so, how?

Acknowledgements

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- All references available upon request

THE UNIVERSITY of TENNESSEE UT INSTITUTE of AGRICULTURE

USDA NIFA The National Institute of Food and Agriculture

Photo credit: J. Miles Cary

Questions?

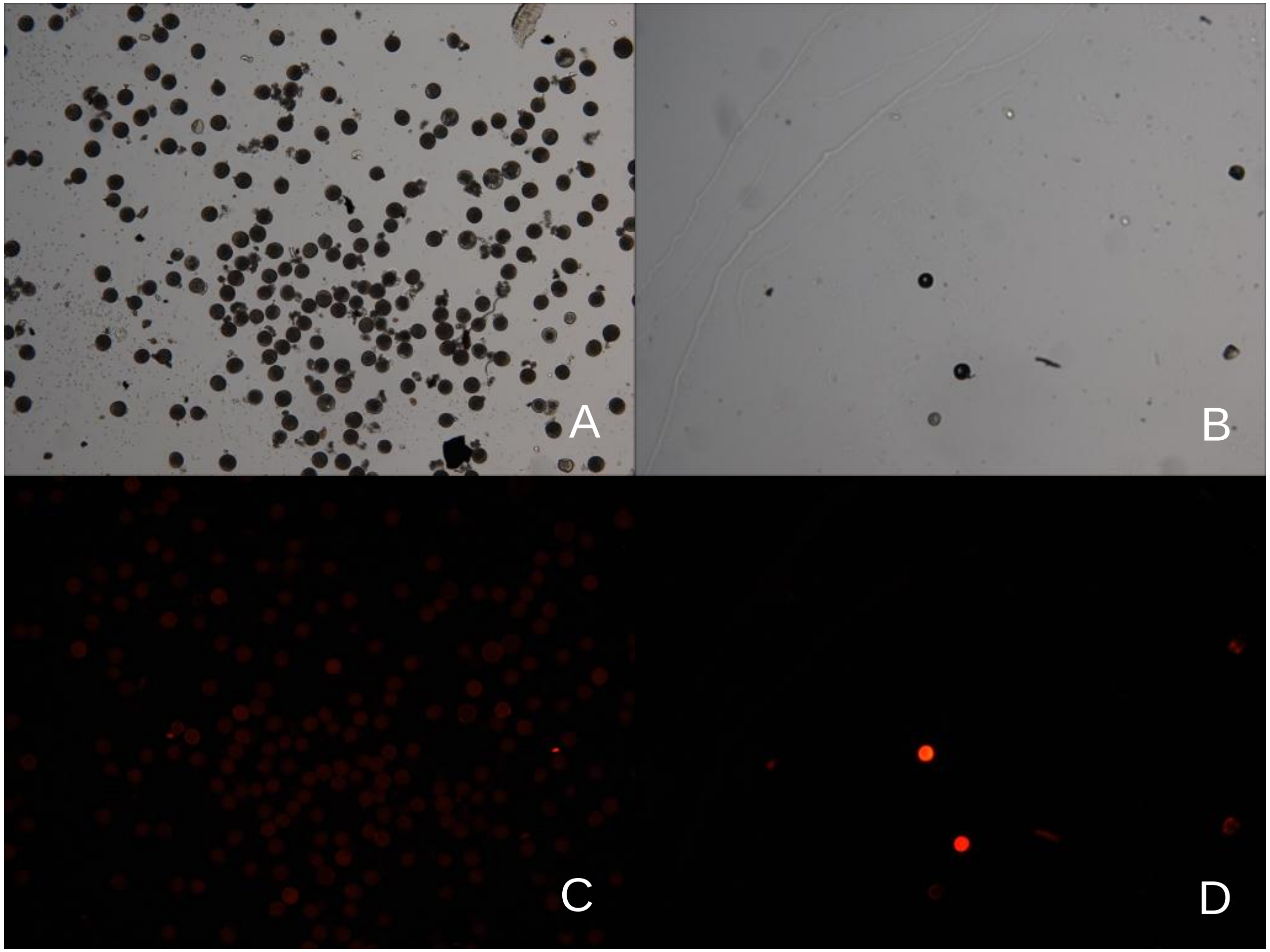
D. Mann

Groom et al. (2008)

Table 1. Comparison of estimated feedstock efficiencies, environmental impacts, and land-use requirements to produce 50% of U.S. demand for transportation fuels from various biofuel crops.

Biofuel crop	*Energy conversion efficiency*[a]	*GHG emissions*[b] *(kg CO2/MJ)*	*Water use*[c]	*Fertilizer use*[d]	*Pesticide use*[e]	*Energy inputs*[f]	*Fuel yield*[g] *(l/ha)*	*Land area needed to meet 50% of U.S. transportation fuel demands*[h] *(million ha)*	*(% U.S. cropland)*	*Additional considerations*[i]	*Sources*[j]
Grasses → ethanol											
Corn	1.1–1.25	81–85	high	high	high	high	1135–1900	290–485	157–262	A, F	3, 5
Sugar cane	8–10.2	4–12	high	high	med	med	5300–6500	85–105	46–57	A	4,7,12
Switch grass	1.8–4.4	−24	med-low	low	low	low	2750–5000	110–200	60–108	P, N, R	1,5
Native prairie grasses	est. 5.44	−88	low	low	low	low	est. 940	585	316	P, N, W, Expt	5,11
Woody biomass → ethanol/synfuel											
Poplar & willow spp.	10	−24 to 11	low–med	low–med	low	low	5500–9000	60–100	32–54	P, W	1,10
Fischer-Tropsch (2nd generation fuel)	18–64	−24 to 11	low–med	low–med	low	low	30,000–50,000	11–18	6–10	P, W	1
Residues → biodiesel/ ethanol											
Wood residues	20–40	—	med	low	low	low	1150–2000	275–475	150–250	P, W	8, 10
Corn stover	5–11	81	med	high	high	low	0.25–0.3 l/kg	—	—	S	1, 8
Wheat straw	2–5	—	low	med	med	low	0.3–0.5 l/kg	—	—	S	8, 10
Oil crops → biodiesel											
Soybeans	1.9–6	49	high	low–med	med	med-low	225–350	330–450	180–240	A, D, F	2,5
Rapeseed or canola	1.8–4.4	37	high	med	med	med-low	2700	55	30	A	2,6
Oil palm	9	51	high	med	low	low	4760	34	18	P, D	2
Microalgae → biodiesel	—	−183	med	low	low	high	49,700–108,800	1.5–3.2	1.1–1.7	Expt	2,9

Transgenic Pollen vs. Non transgenic pollen



Photos A and B (Non-transgenic vs. Transgenic ST1) were taken under white light with 10 ms exposure. Photos C and D were excited with 535/30 nm light and emissions filter 600/50 nm for 2 s.

Switchgrass

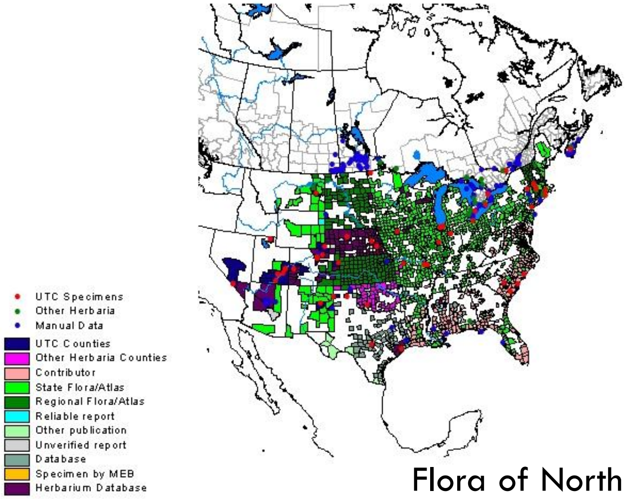
Name	Ecotype	Range	Target Uses	Comments
EG 1101	Lowland	Southern Range and lower limits of Midrange; about 20-25" min. rainfall during the growing season, or irrigation.	Biochemical Thermochemical Cofiring/ Cogeneration Digestion	Improved Alamo type; bred for greater biomass yields and better vigor/establishment. High biofuel conversion potential; has shown superior conversion characteristics for biochemical and thermochemical processes. Moderate resistance to rust (*Puccinia spp.*) observed. May work well in a 2-cut system in high rainfall areas.
EG 1102	Lowland	Southern to Midrange; does well in northern limits of Southern Range; about 20-25" min. rainfall during the growing season, or irrigation.	Biochemical Thermochemical Cofiring/ Cogeneration Digestion	Improved Kanlow type; adapted farther north than EG 1101. Bred for high-biomass yields and better vigor/establishment; high biofuel conversion potential; has shown superior conversion characteristics for biochemical and thermochemical processes. Moderate resistance to rust (*Puccinia spp.*) observed.
Blackwell	Upland	Midrange; about 20-25" min. rainfall during the growing season, or irrigation.	Biochemical Thermochemical Cofiring/ Cogeneration Digestion	Late-maturing and productive; rust resistance observed; suited to drier areas.
Trailblazer	Upland	Midrange and lower limits of Northern Range; about 20-25" min. rainfall during the growing season, or irrigation.	Biochemical Thermochemical Cofiring/ Cogeneration Digestion	Pathfinder type with improved digestibility; late-maturing, vigorous and winter hardy.
Sunburst	Upland	Northern Range; about 20-25" min. rainfall during the growing season, or irrigation.	Biochemical Thermochemical Cofiring/ Cogeneration Digestion	Selected for good seedling vigor; medium-to-high yield potential and winter hardy.

http://newenergyandfuel.com/wp-content/uploads/2009/05/ceres-switchgrass-seed-chart.jpg

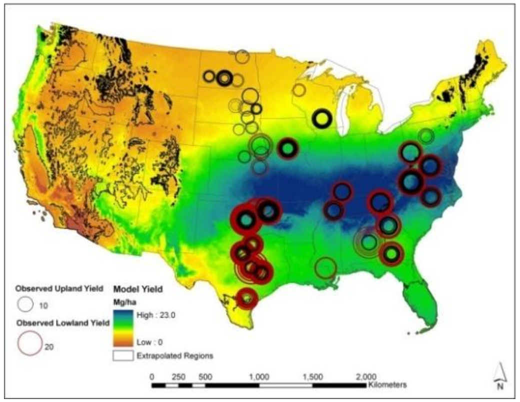
Native switchgrass

- Native in numerous habitats, mainly eastern U.S.
- History of use: ornamental, erosion control, forage, wildlife habitat
- As a *Panicum*, it has a history of hybridization and **introgression** (Flora of North America)
- Overlap of populations with target areas for agronomic potential

Panicum virgatum



Flora of North America



Wullschleger et al. 2010