

Short rotation coppice (SRC): advanced poplar feedstocks for biorefining

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Short rotation coppice (SRC): advanced poplar feedstocks for biorefining

Catherine Bastien, INRA Genetics and Physiology of Forest Trees, Orléans



Biorefining Summer School 2011 : 29/8-1/9/2011, Paris

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- Potential Wood Biomass resources
- short rotation coppice process
- Definition of performing ideotypes
- Genetic and Breeding Tools
- R&D challenges
- Summary





Wood Biomass Resources







WOOD as Renewable Energy in France

Directive 2009/28/CE related to the promotion of renewable energies :

- Constraining objective **for France** of **23%** of renewable energies in the final energy consumption in 2020 (10,3% in 2005)
- Transportation: 2nd generation of biofuels given double credit (Art. 21.2)



Source : French Ministry of Ecology and Sustainable Development, 2009



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Annual WOOD Global Availability in France

Today : Wood sources : fuel logs + pulp logs + branches

HARDWOODS : 40 millions m³ / year SOFTWOODS : 10 millions m³ / year Total : 50 million m³ / year (10 million toe /year)



Additional availability to meet bioenergy demand in 2020:



Total : +19 million m³ / year (+ 4 million toe /year)



Source IFN, FCBA – 2009

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Which additional sources of wood biomass?



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Which additional sources of wood biomass ?

Wood harvest (/ha /year)

Harvested Fuel wood: 24 M m³ self consumed



Traditional coppice

Large areas of extensive forests ... with difficult logging conditions

Source : EFORWOOD project Dr. Philipp Duncker



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Which additional sources of wood biomass ?

Wood harvest (/ha /year)



Even aged forestry

• Even aged <u>conifer</u> plantations are leading the French national production and harvest... with only 20% of the national forest area

 Shortening rotation lengths will increase wood production

But area extension limited

Source : EFORWOOD project Dr. Philipp Duncker



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Which additional sources of wood biomass ?

Wood harvest (/ha /year)



SRC

• High productivity

- Ability for shooting after coppicing
- Soil & climate,
- Improved material (breeding).

• Quality of raw material

- Wood chemical composition
- Adaptation to industrial conversion
- Homogeneity

• At low cost

- Size of cultivation plots,
- Mechanization of stand management and harvesting operations
- Logistics and territorial development

Source : EFORWOOD project Dr. Philipp Duncker

Environmental Value



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Short Rotation Coppices

2 systems :

• SRC :

- 1 000 to 2 000 stems/ha,
- Rotation time : 7 10 years,

- Objective : small diameter trees for three successive rotations (Diameter = 15 cm, Total Height = 15 to 20 m)





• VSRC :

- 6 000 to 12 000 stems/ha,
- Rotation time : 2 3 years,
- Objective : producing the highest number of very small diameter shoots (Diameter = 3 à 4 cm, Total Height = 4 à 6 m)





Poplars: Populus sp.

 Have been cultivated in UE for a long time for peeling and pulp industry. Easy to plant (cuttings). Active breeding

Potential productivity : 10-20 odt /ha/ year

≻Limits :

- Soil constraints and water availability
- Diseases (pests/insects) resistances
- Present situation :
 - •France : conventional stands +230 000 ha 500 ha SRC + >1000 ha VSRC (2010)
 - Italy : 6000 ha VSRC
- > Possible extension:
 - •France: 200 000 ha





Willows: Salix sp.

 Emblematic species for biomass production in VSRC. High ability for shooting after coppicing. Active breeding

Potential productivity : 8-15 odt /ha/ year

>Limits :

- Soil constraints and water availability
- Diseases (pests/insects) resistances

Present situation :

- •France : Recent development of 400 ha VSRC in Northern and Western France
- Poland : 2000 ha VSRC
- UK: 3000 ha VSRC
- •Sweden : 20 000 ha VSRC for the last 15 years







Eucalypt: Eucalyptus sp.

• One of the most cultivated genus worldwide. Adapted to low fertility sites. Active breeding

Potential productivity : 10-13 odt /ha/ year

≻Limits :

- Low tolerance to active Ca
- Frost resistance

> Present situation :

•France : Recent development of 2000 ha SRC in Southern France

• Spain and Portugal : 1 M ha VSRC







Black Locust: Robinia pseudoacacia

- Rustic species. High ability for shooting after coppicing. Nitrogen fixing species
- Potential productivity : 8 10 odt /ha/ year

≻Limits :

- No breeding program
- Favourable environmental conditions badly known
- management practices to be improved

Present situation :

- •France : Recent development of <100 ha SRC
- Europe: 100 000 ha in Hungaria with combined production objectives







Chestnut: Castanea sativa

- a traditional coppicing species with high ability for shooting. Rustic species.
- Potential productivity : 6-9 odt /ha/ year

>Limits :

- No breeding program
- pest damages favoured by climate change
- > Present situation :

•France : 780 000 ha







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• Increase rooting performances and reduce competition effects









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• Weed control during plantation establishment is crucial !

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• Maximum Biomass production obtained after coppicing (2nd cycle and later)

• Significant decrease of biomass production if no fertilization





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N

D P

ΒK

∎Ca ∎Mg

Definition of performing ideotypes



Ideotype – a plant model that will produce a high yield in a given environment Dickman 1985





Breeding objectives for Poplars and Willows Soil preparation Plantation

- Adaptation to soil and climate
- Long-term viability =pest/disease resistances (clonal forestry risks)
- Increased yield per hectare under sustainable management
- Coppice growth, form =ease of harvest
- Ease of establishment =rooting, early growth
- Improved conversion yield



Selection criteria to increase Biomass Yield



Biomass production is tightly linked to







- Maximum Leaf Area (interspecific hybrids)
- Sylleptic branches
- Number of stems (shooting ability)
- Number of leaves



Canopy Duration (bud flush, leaf fall)







Selection criteria to increase Biomass Yield

Sustainable biomass production if Water and Nutrient Use efficiencies are optimized



Varying relationships between biomass productivity and Water Use efficiency in *Populus sp. :*

-unfavourable in DxT hybrids (Monclus et al 2009) -no relationship in DxN hybrids (Monclus et al. 2005)



No antagonism between biomass productivity and N Use efficiency in Salix (J. Toillon & N. Marron INRA)



• Increase specific gravity (low specific gravity preferred for peeling

objectives!)





Clonal RangeClonal Repeatability0.266 - 0.3700.77Source INRA-FCBA, unpublished results on 123 clones at 6 years

Significant genetic gains could be reached thanks to moderate genetic variation and low influence of environmental growing conditions



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• Improve chemical composition (lignin, cellulose, hemicellulose)

Review

Biofper

Poplar as a feedstock for biofuels: A review of compositional characteristics

Relatively high cellulose content but high lignin content

Poulomi Sannigrahi, Arthur J. Ragauskas,* Georgia Institute of Technology, Atlanta, GA, USA Gerald A. Tuskan, BioEnergy Science Center, Oak Ridge Laboratory, Oak Ric

Received October 9, 2009; revised version received December 1, 2009; at Published online in Wiley InterScience (www.interscience.wiley.com); Do Binders, Biomed, Science Science and Lignin (as% dry weight) of poplar and other biomass.

						Lignin (% dry wt	.)	
	Biomass clone or species	Cellulose (% dry wt.)	He (emicellulose (% dry wt.)	Total	Acid soluble	Acid insoluble	
	P. deltoides, Stoneville 6623	42.2		16.60	25.6			
	NM 6 ¹⁸	48.95		21.70	23.25	20.95	2.30	
	CAFI high lignin ⁷⁴	Range:		20.40	29.10			
	CAFI low lignin ⁷⁴	120/ 100/	/	21.50	21.40	Pange.		
	Caudina DN 3471	42 /0-49 /	0	19.55	27.23			
	DN 182 ²³	45.52		Danga:	23.58	21%-2	29%	
nt	DN 17 ²³	43.65	Г А О	Range.	23.07			
IL	NC 5260 ²³	45.08	16	i%- 23%	21.54	Repeata	ability:	
se	Switchgrass ²³	33.75		27.04	16.80		າວງ. ົງ	
	Eucalyptus saligna ²³	48.07		12.69	26.91	0.0	2	
	Monterey pine ²³	41.70		20.50	25.90			
	Corn stover ²³	37.12		24.18	18.20			

decrease
 lignin content
 and increase
 cellulose



How to decrease lignin content and increase cellulose ?

1- Favour tension wood formation: tension wood (consider as defect for mechanical properties) is rich in cellulose





From Brereton et al. 2011 on Salix



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How to decrease lignin content and increase cellulose ?

2-Alter monolignol pathway in transgenic poplars



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2-Alter monolignol pathway in transgenic poplars

Saccharification and SSF of FS-3 and WT (with acidic pre-treatment)



- Improvement of chemical composition (lignin, cellulose, hemicellulose) requires high through-put phenotyping tools to screen large populations under selection
 - Standard laboratory methods are labor intensive, difficult, time consuming, and limited to restricted population sizes,
 - Focus on FTIR and NIR Spectroscopy (NIR) indirect Evaluation.



Choice of the calibration panel is important



Some calibration equations already available on poplar and willows

Maranan, M. C. and M.-P. G. Laborie. **2008.** Rapid p traits of hybrid poplar with near infrared spectroscop *and Bioenergy*, **2: 57-63.**



2010 (18) special issue on wood and wood products

Zhou et al. Plant Methods 2011, 7:9 http://www.plantmethods.com/content/7/1/9

RESEARCH

FTIR-ATR-based prediction and m lignin and energy contents revea intra-specific variation of these ti poplars

Guanwu Zhou^{1,2}, Gail Taylor³ and Andrea Polle^{1*}



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Genetic and Breeding Tools







Breeding is likely to improve Productivity of Poplars and Willows

- Vegetative propagation is easy and clonal forestry has been adopted for decades
- Little domestication and high genetic diversity between and within species
- Short generation time (3-6 years)
- Interspecific hybridization benefits : complementation (growth/rooting/disease resistances) hybrid vigour (+35% - +73% for biomass production)

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P.nigra





P. deltoides

P. trichocarpa



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Genomics and biotechnology will be of significant help for Salicaceae (Poplars + Willows) Improvement

• An annotated Reference Genome available in P. trichocarpa

• Functional candidate genes

• Large lists of expressional candidate genes

Populus: A Model System for Plant Biology Annu. Rev. Plant Biol. 2007. 58:435–58

Science



• Possibility of Genetic transformation for some clones







Up to now, prediction of genetic value is based on :



phenotypes and information about relatives

Identification of functional gene polymorphisms will :

- Increase precision of estimated genetic values
- Increase genetic gain on targeted traits
- Allow monitoring of genetic diversity along selection process







Two strategies to identify functional gene polymorphisms involved in biomass traits

 $\frac{Top - Bottom}{Phenotype \longrightarrow QTL \longrightarrow Genes}$

QTLs and Gene Mapping



From Rae et al. Tree Genetics and Genomes, 2008



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QTL Mapping of Enzymatic Saccharification in Short Rotation Coppice Willow and Its Independence from Biomass Yield

Nicholas J. B. Brereton • Frederic E. Pitre • Steven J. Hanley • Michael J. Ray • Angela Karp • Richard J. Murphy Include QTL information in selection



- Markers are often family-dependent
- Confidence intervals for QTLs are large
- A lot of QTLs to select to explain less than 60% of the observed variation
- Marker based selection still not possible!





Improve accuracy of QTL position Marker Assisted Selection



 Such markers are under discovery for most traits of interest thanks to large candidate gene re-sequencing programs

The Plant Journal (2011) 67, 736–745	doi: 10.1111/j.1365	of CAD4,
TECHNICAL ADVANCE		Cono
Large-scale detection of rare variants via multiplexed next-generation sequencing	pooled : towards	CAD4 HCT1
next-generation Ecotilling		C3H3 CCR7
Fabio Marroni ^{1,1,*} , Sara Pinosio ^{1,2,†} , Eleonora Di Centa ¹ , Irena Jurman ¹ , Wout Boeri	an ^{3,4} . Nicoletta Felice ² .	4CL3

Gene	Coding sequence (bp) ^a	SNPs (<i>n</i>)	Mis-sense (<i>n</i>)	Stop (<i>n</i>)
CAD4	1074	19	8	0
HCT1	948	13	5	1
СЗНЗ	1527	12	6	0
CCR7	1017	15	9	0
4CL3	1623	25	8	0

 As Linkage Disequilibrium is decreasing rapidly, very high density of SNPs is required

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Federica Cattonaro^{1,5} and Michele Morgante^{1,2}

Identify causal mutations responsible of genetic variation



Gene and exact polymorphism (QTN) identifi

 Under discovery for most traits of interest through association genetics based on candidate gene approach in large natural populations

"A mutation that causes a premature stop codon (C243*) in HCT1 has been detected in a set of 768 *Populus nigr*a. This mutation was present in homozygous state in one accession. *From Marroni, Boerjan, Morgante, The plant Journal 2011*



Significant differences between phenotypes are observed

 Most of causal mutations detected have low effects and need validation in different genetic backgrounds

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R&D Challenges







SRC perspectives in France

R & D needs :

- Genetic improvement and biotechnology (biomass yield, architecture, pest resistance, water/nitrogen use efficiency, wood quality)
- Industrialization of plant production (seedlings and cuttings)
- Mechanization of stand management and adapted logging/harvesting operations
- Bioconversion process (lignocellulose pretreatment, enzymatic hydrolysis, C5 sugars fermentation, lignin valorization)
- Logistics and territorial development (acceptability, ecological impacts)
- Impacts on soil and water resources

Economical and political aspects :

- Synergy vs competition with the existing Wood Chain
- Moving toward an industrial dimension of biomass supply / use : flows, quality, prices
- Dissemination: public, stakeholders,...



Forest Tree Breeding and Physiology research unit

(in collaboration with EEF & IAM research units of INRA Nancy)

Some running research projects on woody biomass

ENERGY POPLAR (UE FP7 – 2008-2011):

- Identify important transcription factors and other candidate genes regulating cellulose and lignin content & nutrient uptake
- Establish a platform for rapid gene discovery and testing using systems biology approaches to identify novel transcripts for traits of interest
 Establish tools for environmental sustainability assessments of SRC Poplar growing systems with respect to soil microbial diversity, GHG

mitigation, water and other inputs relevant to a changing climate

SYLVABIOM (ANR – 2010-2013:

- Identify site and sylvicultural treatment effects for biomass production and water/nutrient use efficiency on poplar, willow and black locust
- Evaluate the perspective of genetic selection to improve these 2 traits
- Creation of site and sylviculture-dependent biomass yield models



Forest Tree Breeding and Physiology research unit (in collaboration with EEF & IAM research units of INRA Nancy) Some running research projects on woody biomass

CREFF (ERA-Net Bioenergy – 2008-2011) : Cost REduction and EFFiciency improvement of short rotation coppice. Implement cost-efficient and consumer-oriented SRC-value-chains in regions with unfavorable conditions.

FUTUROL (2008-2016) : Develop and validate a "second-generation" bioprocess for ethanol production by using lignocellulose as a feedstock.

TREE-FOR-JOULES (KBBE – 2011-2014) : Improving Eucalypt and Poplar wood properties for bioenergy (genomics, genetics, HT phenotyping).





Summary

1. Substantial developments in Biorefinery industry featuring biochemical conversion of wood biomass are likely over the next few years especially with poplar and willow resources.

2. Ongoing improvement of biomass yield and composition is necessary to lower the cost of delivered feedstock on a per unit carbohydrate basis. Revision of selection criteria from conventional breeding is necessary.

3. Chemical constituents important to the economics of fuel production via biochemical processes should show significant selection responses in breeding populations as large individual variation has been observed.

4. Indirect assessment method for conversion ability likely will be an important tool to allow evaluation of large segregating populations.

5. Genomic-assisted breeding program has an appeal to accelerate and precise selection for wood chemical composition. Genetic transformation on key genes could significantly help breeding



Thank you for your attention

Willow - Reigate Priory

Poplar - Monet