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

Catherine Bastien

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

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






Contents

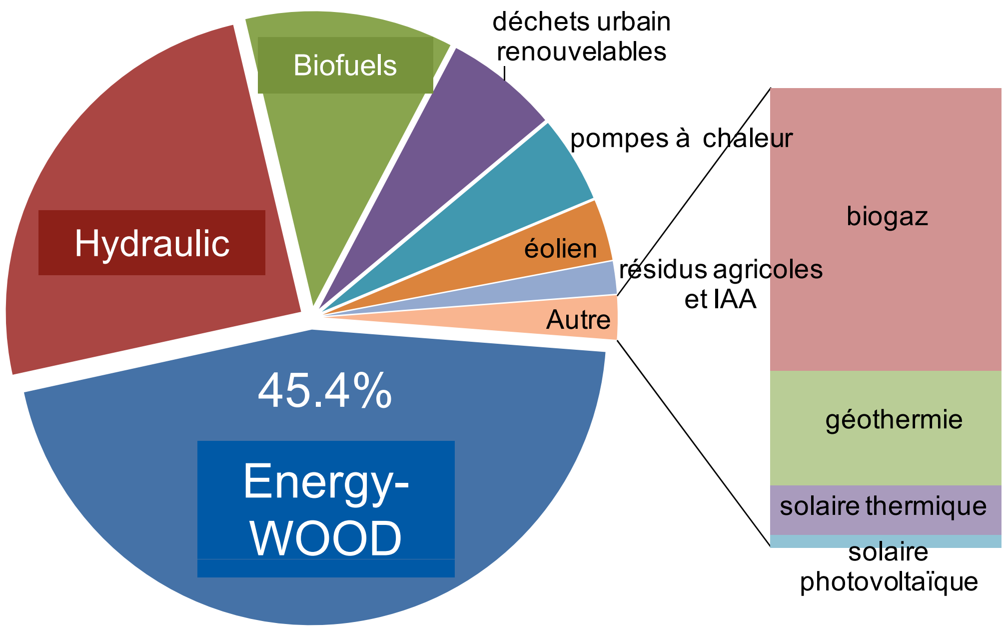
- Potential Wood Biomass resources
- short rotation coppice process
- Definition of performing ideotypes
- Genetic and Breeding Tools
- R&D challenges
- Summary

Wood Biomass Resources

WOOD as Renewable Energy in France

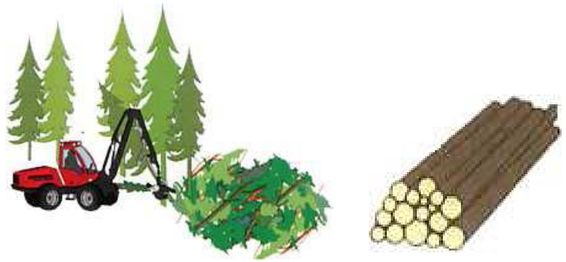
Directive 2009/28/CE related to the promotion of **renewable energies** :

- 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

Annual WOOD Global Availability in France



Today :

Wood sources : fuel logs + pulp logs + branches

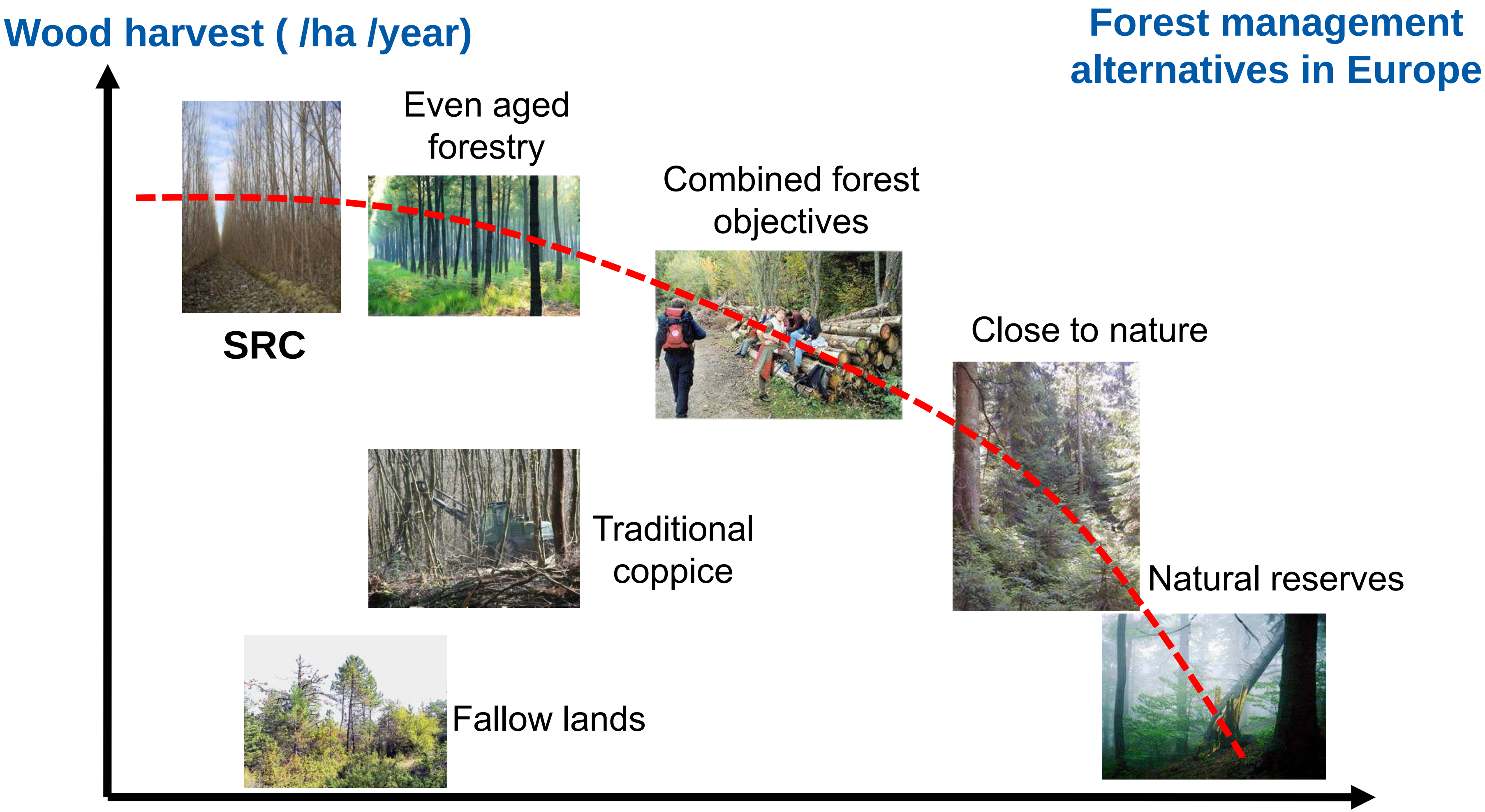
HARDWOODS : 40 millions m^3 / year
SOFTWOODS : 10 millions m^3 / year
Total : 50 million m^3 / year (10 million toe /year)

Additional availability to meet bioenergy demand in 2020:

Total : +19 million m^3 / year (+ 4 million toe /year)

Source IFN, FCBA – 2009

Which additional sources of wood biomass ?



Source : EFORWOOD project Dr. Philipp Duncker

Environmental Value

Which additional sources of wood biomass ?

Wood harvest (/ha /year)

Harvested Fuel wood:
24 M m^3 self consumed

+ ?

Traditional coppice

Large areas of extensive forests … with difficult logging conditions

Source : EFORWOOD project Dr. Philipp Duncker

Environmental Value

Which additional sources of wood biomass ?

Wood harvest (/ha /year)

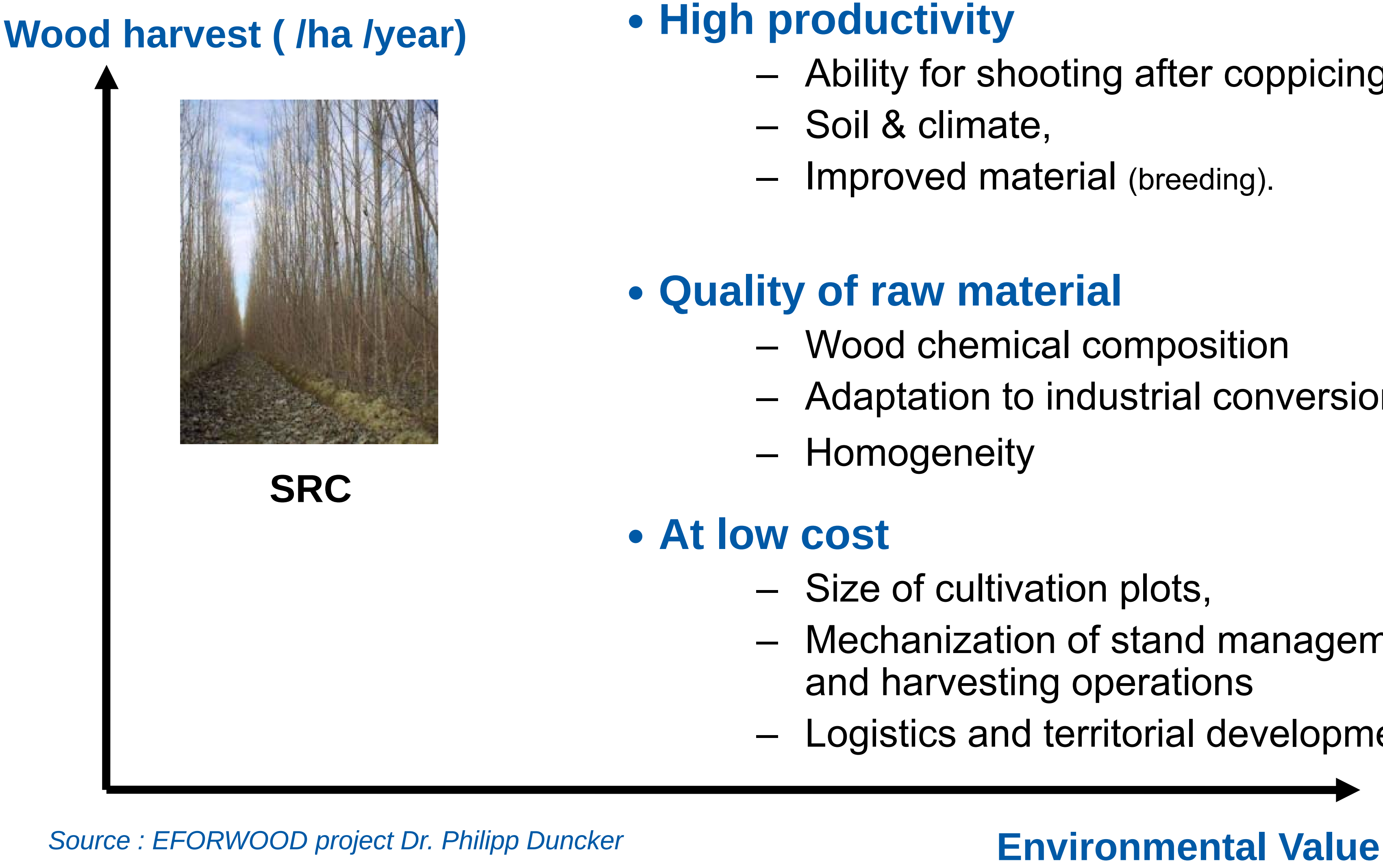
Even aged forestry

- **Even aged conifer 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**

Environmental Value

Source : EFORWOOD project Dr. Philipp Duncker

Which additional sources of wood biomass ?



- **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

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)


Which forest tree species for SRC/VSRC in Europe ?

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

Which forest tree species for SRC/VSRC in Europe ?

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

Which forest tree species for SRC/VSRC in Europe ?

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

Which forest tree species for SRC/VSRC in Europe ?

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

Which forest tree species for SRC/VSRC in Europe ?

Chestnut: *Castanea sativa*

• **a traditional coppicing species with high ability for shooting. Rustic species.**

• **Potential productivity : 6-9** odt /ha/ year

➢**Limits :**

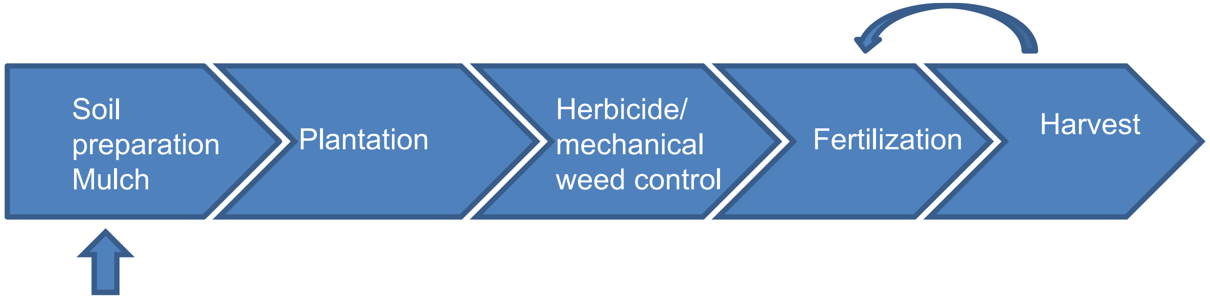
- No breeding program
- pest damages favoured by climate change

➢ **Present situation :**

•France : 780 000 ha

Short rotation coppice Process

Short Rotation Coppice Process



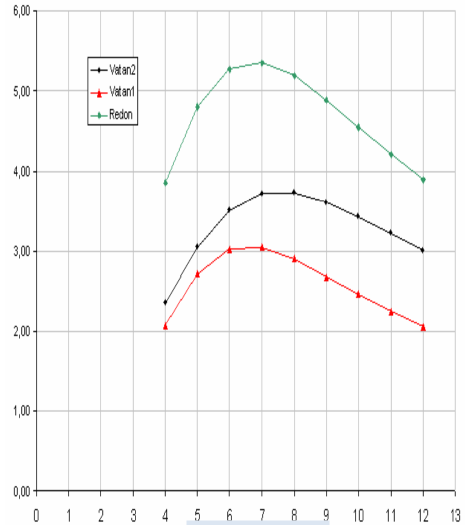
- **Increase rooting performances and reduce competition effects**

Short Rotation Coppice Process

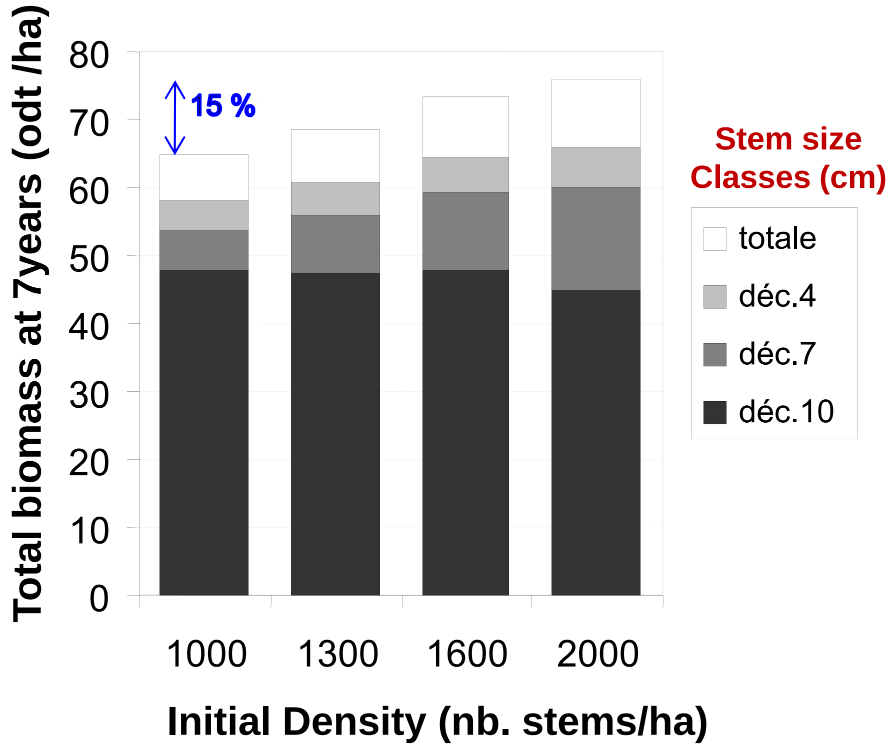
- Soil preparation
- Plantation
- Herbicide/ mechanical weed control
- Fertilization
- Harvest

- **Adapt density plantation to rotation length and size of products**

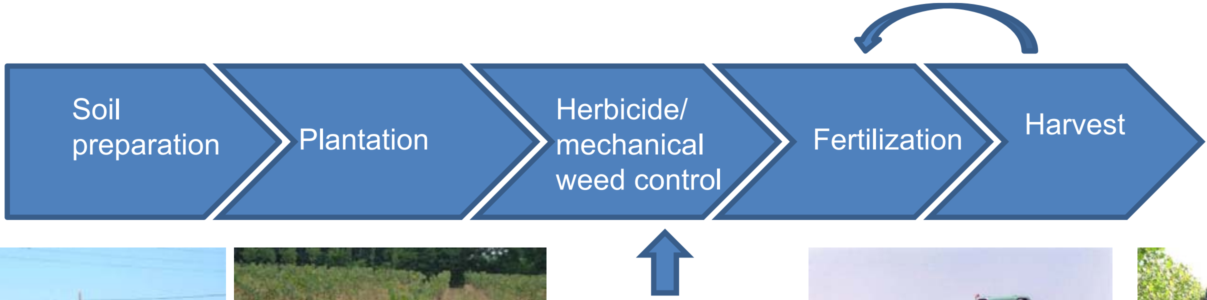
Annual Increment in dry biomass per tree



Age



Short Rotation Coppice Process




VSRC

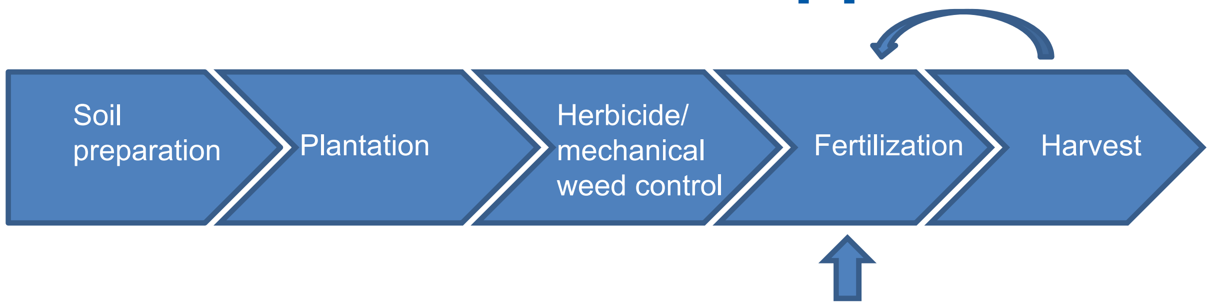

SRC


VSRC

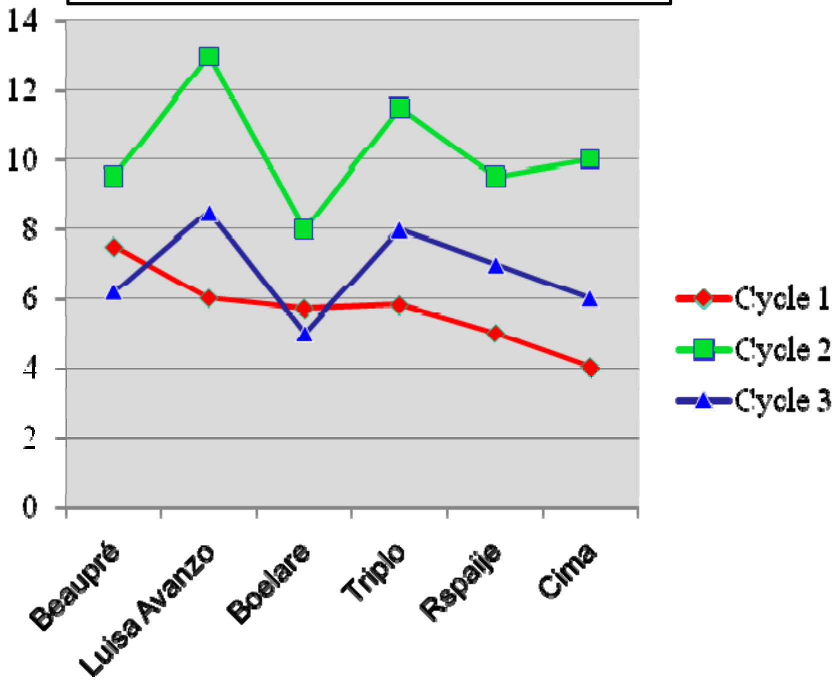

SRC

- **Weed control during plantation establishment is crucial !**

Short Rotation Coppice Process



Annual average production odt /ha/year

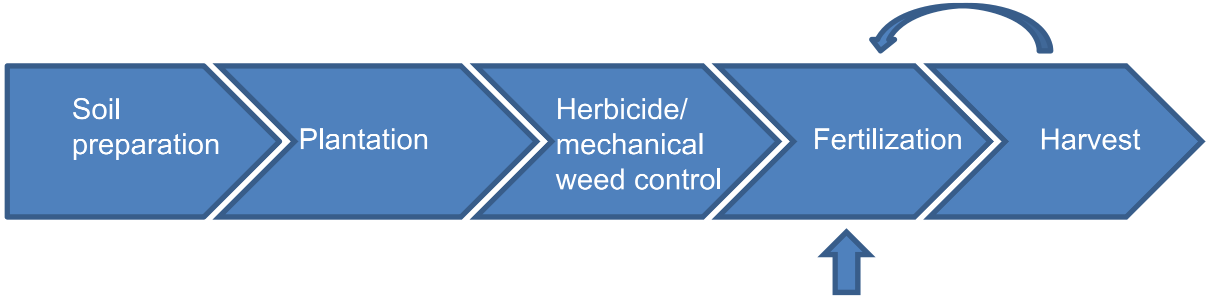


VSRC: Harvest every 3 years

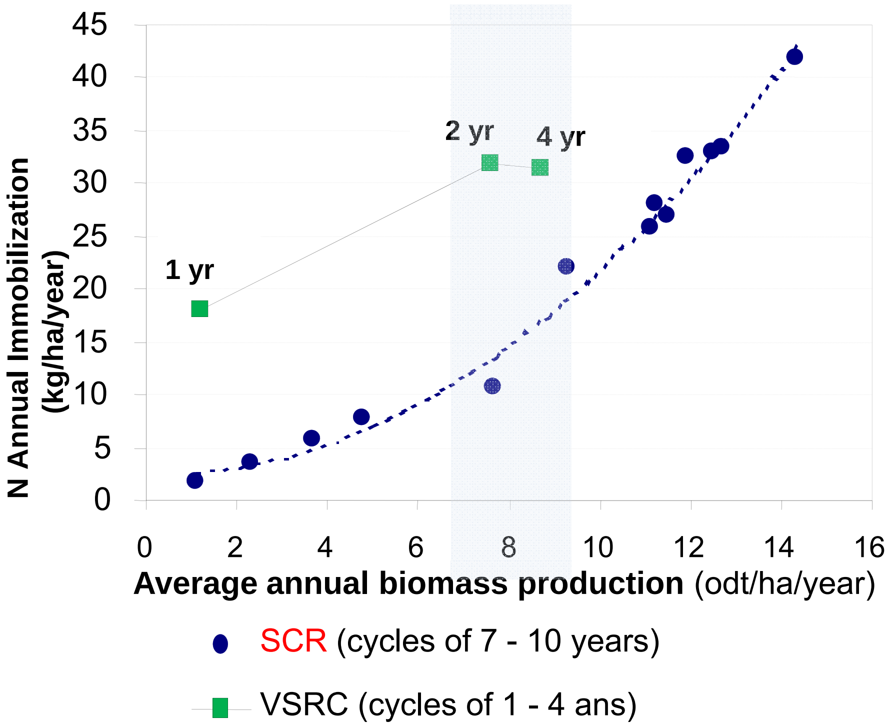
- **Maximum Biomass production obtained after coppicing** (2nd cycle and later)

- **Significant decrease of biomass production if no fertilization**

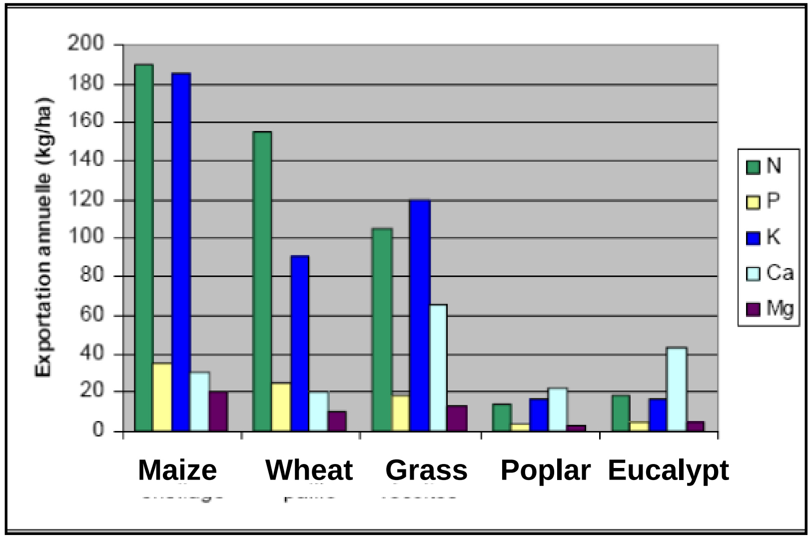
Short Rotation Coppice Process



- **Higher N immobilization in VSRC / SRC**



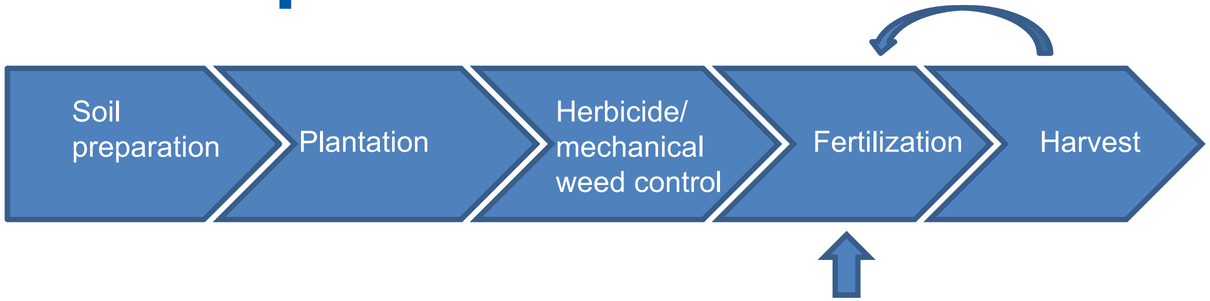
- **Nutrients exports still limited compared to other crops**



Definition of performing ideotypes

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

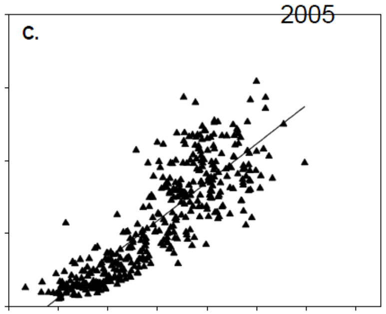
Breeding objectives for Poplars and Willows



- **Adaptation** to soil and climate
- Long-term viability =pest/disease **resistance**s (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 total leaf area

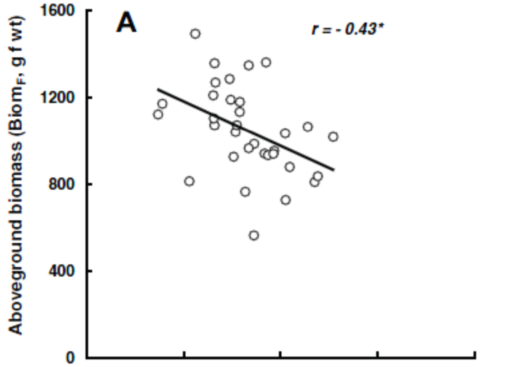


- Maximum Leaf Area (interspecific hybrids)
- Sylleptic branches
- Number of stems (shooting ability)
- Number of leaves

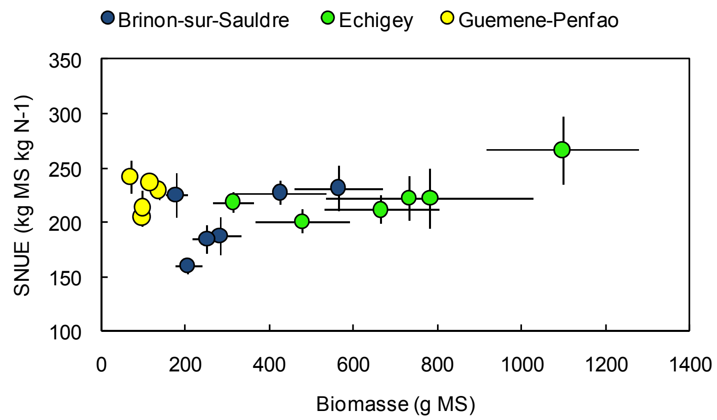
- Canopy Duration (bud flush, leaf fall)

Selection criteria to increase Biomass Yield

Sustainable biomass production if Water and Nutrient Use efficiencies are optimized



Leaf carbon isotope discrimination (Δ, ‰)
Indirect measure of WUE



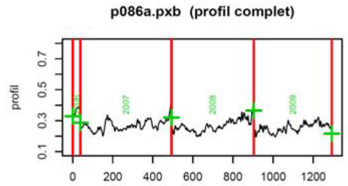
Varying relationships between biomass productivity and Water Use efficiency in *Populus sp. :*

-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)

Selection criteria to increase Biomass Quality

• **Increase specific gravity** (low specific gravity preferred for peeling objectives!)



<u>Clonal Range</u>	<u>Clonal Repeatability</u>
0.266 – 0.370	0.77

Source 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

Selection criteria to increase Biomass Quality

- **Improve chemical composition (lignin, cellulose, hemicellulose)**

Review — Biofpr

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

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; a
Published online in Wiley InterScience (www.interscience.wiley.com); DO
Biofuels, Bioprod, Bioref. 4:209–226 (2010)

➢ Relatively high cellulose content but high lignin content

Table 6. Proportion of cellulose, hemicellulose and lignin (as% dry weight) of poplar and other biomass.

Biomass clone or species	Cellulose (% dry wt.)	Hemicellulose (% dry wt.)	Lignin (% dry wt.)		
			Total	Acid soluble	Acid insoluble
P. deltoides, Stoneville 66[23]	42.2	16.60	25.6		
NM 6[18]	48.95	21.70	23.25	20.95	2.30
CAFI high lignin[74]		20.40	29.10		
CAFI low lignin[74]		21.50	21.40		
Caudina DN 34[71]		19.55	27.23		
DN 182[23]	45.52		23.58		
DN 17[23]	43.65		23.07		
NC 5260[23]	45.08		21.54		
Switchgrass[23]	33.75	27.04	16.80		
Eucalyptus saligna[23]	48.07	12.69	26.91		
Monterey pine[23]	41.70	20.50	25.90		
Corn stover[23]	37.12	24.18	18.20		

Range: 42%-49%

Range: 16%- 23%

Range: 21%- 29%

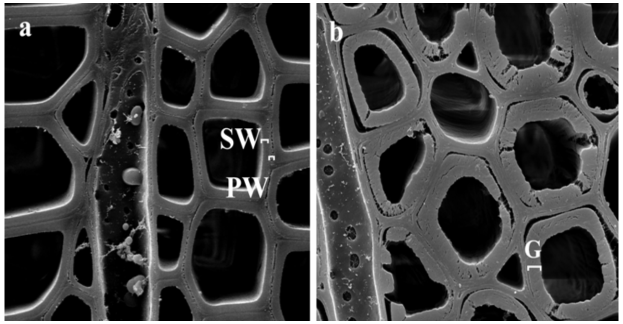
Repeatability: 0.82

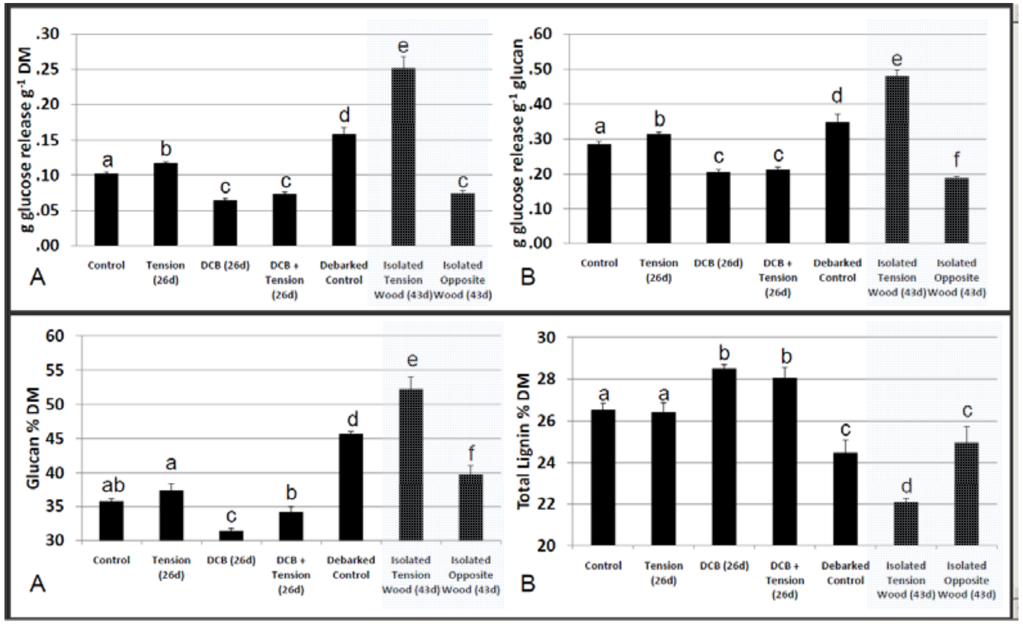
➢ decrease lignin content and increase cellulose

Selection criteria to increase Biomass Quality

- **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



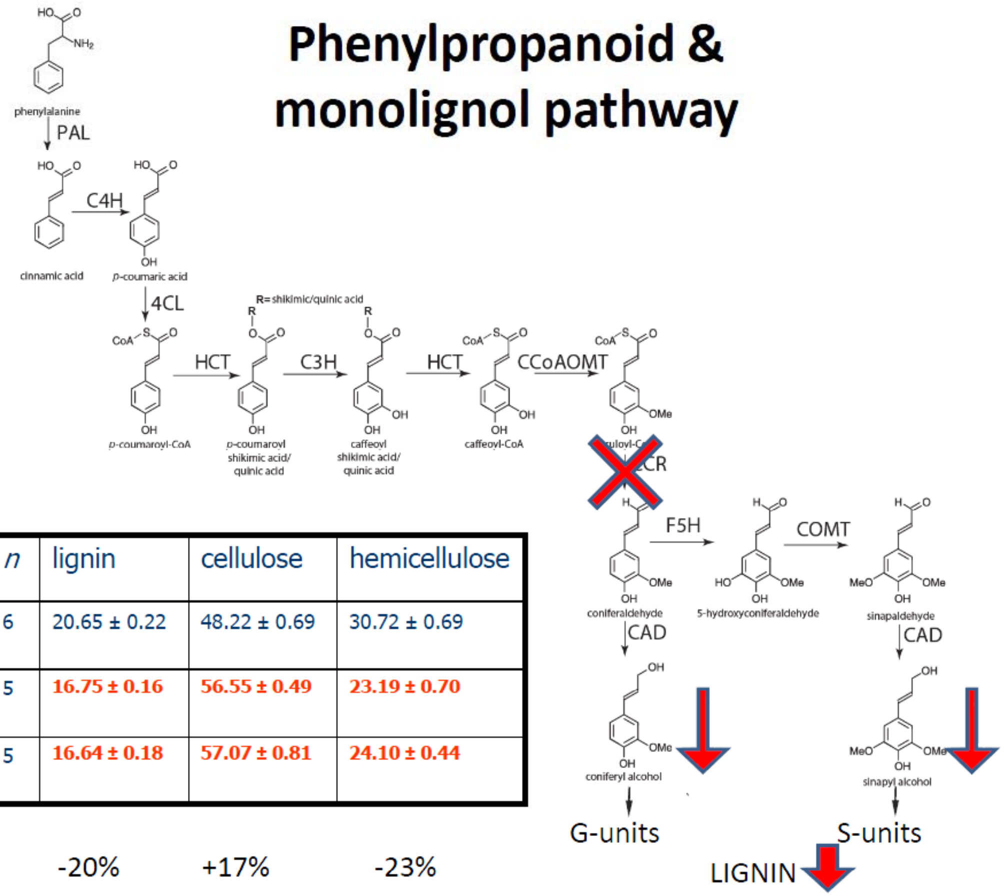


From Brereton et al. 2011 on Salix

Selection criteria to increase Biomass Quality

- **How to decrease lignin content and increase cellulose ?**

2- Alter monolignol pathway in transgenic poplars



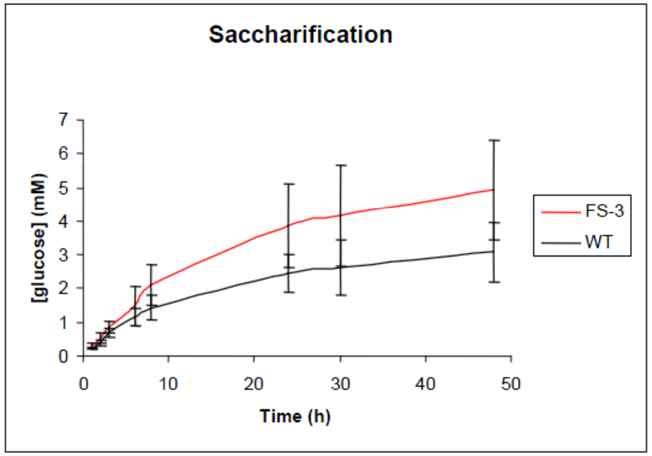
Line	*n*	lignin	cellulose	hemicellulose
WT	6	20.65 ± 0.22	48.22 ± 0.69	30.72 ± 0.69
FS3	5	16.75 ± 0.16	56.55 ± 0.49	23.19 ± 0.70
FS40	5	16.64 ± 0.18	57.07 ± 0.81	24.10 ± 0.44
		-20%	+17%	-23%

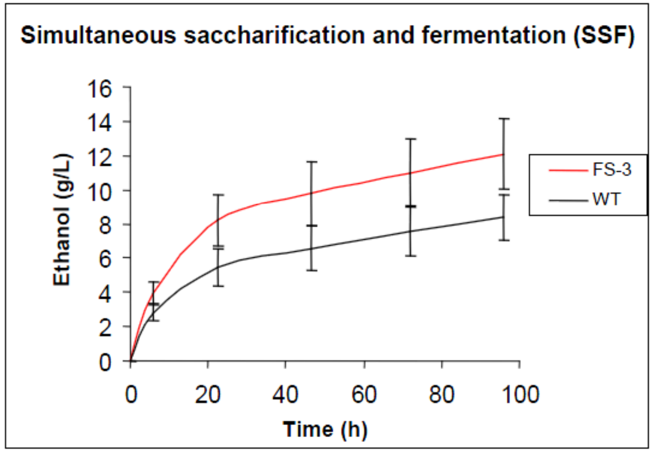
Selection criteria to increase Biomass Quality

2- Alter monolignol pathway in transgenic poplars

Saccharification and SSF of FS-3 and WT

(with acidic pre-treatment)





-> 60 % more glucose released by CCR-downregulated poplar

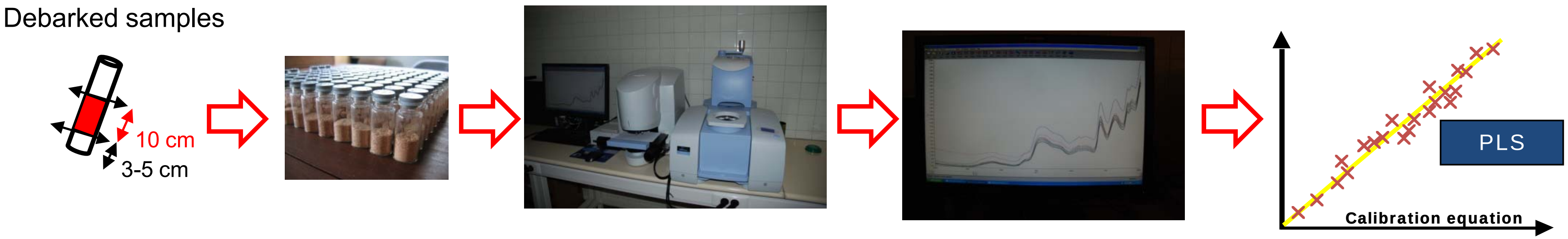
-> 50 % more ethanol released by CCR-downregulated poplar

From Van Holme, Van Acker, Piens & Boerjan, Energy-Poplar project www.energy-poplar.eu

Selection criteria to increase Biomass Quality

- **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**

Selection criteria to increase Biomass Quality

- **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 t
poplars

Guanwu Zhou[1,2], Gail Taylor[3] and Andrea Polle[1*]

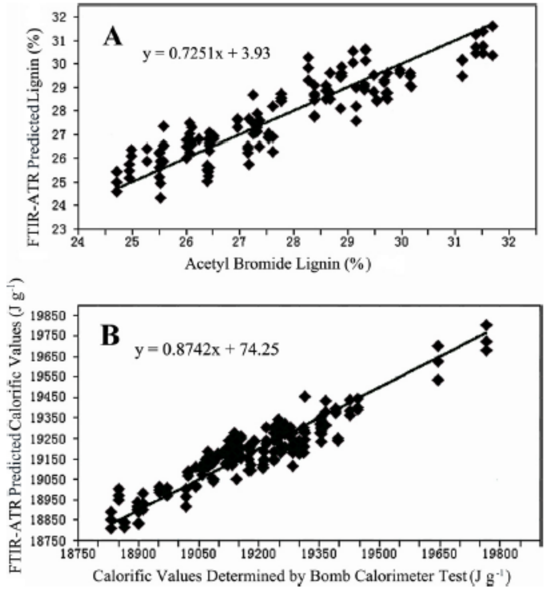
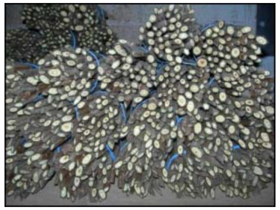


Figure 3 Partial least square regression (PLSR) models for the prediction of lignin (A) and energy (B) content in extractive-free poplar wood exhibiting a natural range of variability. Lignin was measured with the acetyl bromide method. The energy content was determined with a combustion calorimeter. Plots of measured versus predicted values for lignin (**A**) and energy (**B**) content were calculated with the best models with cross-validation results after data preprocessing and automatic wavenumber selection (Table 1 and Table 2). Solid lines represent regression line of best fit between measured and predicted values.

Genetic and Breeding Tools

Breeding is likely to improve Productivity of Poplars and Willows

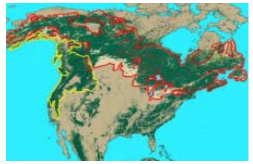
• Vegetative propagation is easy and clonal forestry has been adopted for decades




• Little domestication and high genetic diversity between and within species


P.nigra


P. deltoides


P. trichocarpa

• Short generation time (3-6 years)


• Interspecific hybridization benefits :

complementation (growth/rooting/disease resistances)

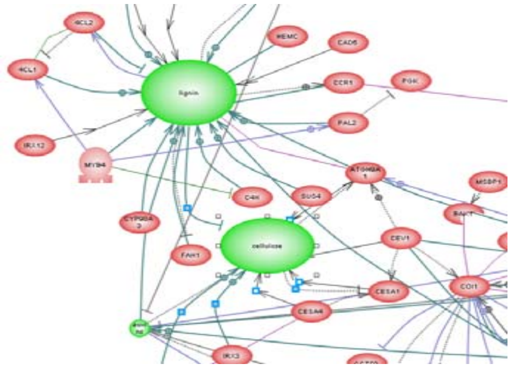
hybrid vigour (+35% - +73% for biomass production)

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

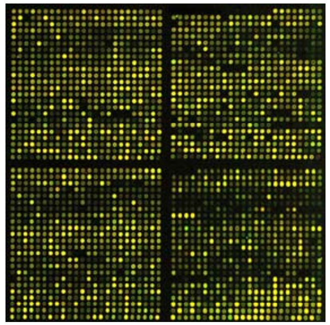
- An annotated Reference Genome available in *P. trichocarpa*

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

- Functional candidate genes



- Large lists of expressional candidate genes



- Possibility of Genetic transformation for some clones

Accelerating breeding thanks to genomics and biotechnologies

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

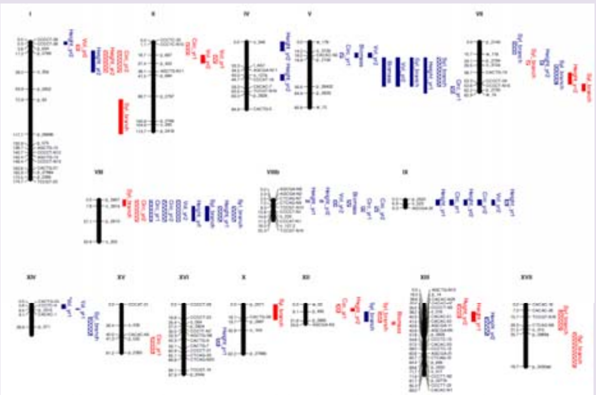
Accelerating breeding thanks to genomics and biotechnologies

Two strategies to identify functional gene polymorphisms involved in biomass traits

Top – Bottom

Phenotype → QTL → Genes

QTLs and Gene Mapping



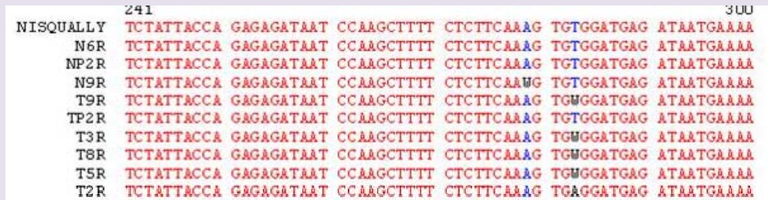
From Rae et al. Tree Genetics and Genomes, 2008

Bottom – Up

Genes → Nucleotide polymorphism → Phenotype

Association Genetics
Ecotilling

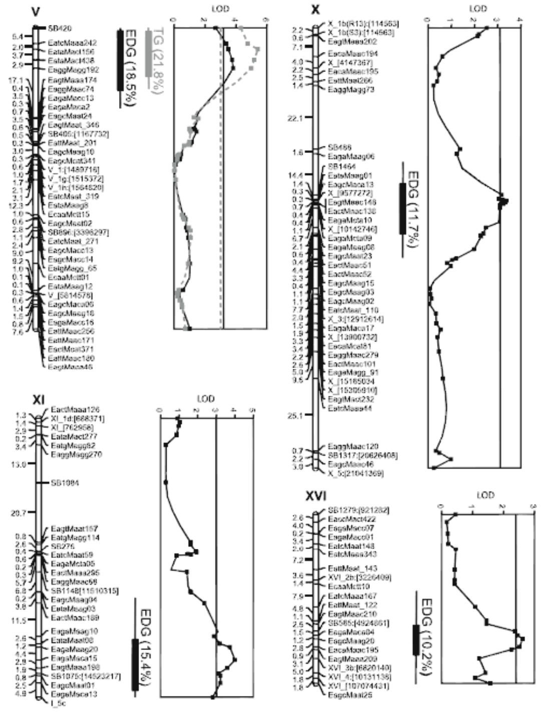
SNP = single nucleotide polymorphisms



More than 3 Millions SNP identified in P.nigra

Accelerating breeding thanks to genomics and biotechnologies

QTLs and Gene Mapping



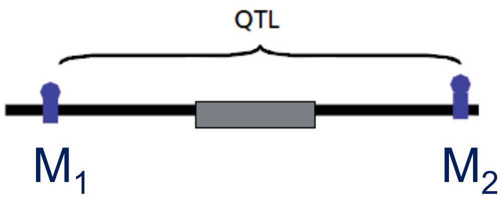
Bioenerg. Res. (2010) 3:251–261
DOI 10.1007/s12155-010-9077-3

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

→ **M**arker **A**ssisted **S**election



- 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!

Accelerating breeding thanks to genomics and biotechnologies

Improve accuracy of QTL position **M**arker **A**ssisted **S**election

- 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-

TECHNICAL ADVANCE

Large-scale detection of rare variants via pooled multiplexed next-generation sequencing: towards next-generation Ecotilling

Fabio Marroni[1,†,*], Sara Pinosio[1,2,†], Eleonora Di Centa[1], Irena Jurman[1], Wout Boerjan[3,4], Nicoletta Felice[2], Federica Cattonaro[1,5] and Michele Morgante[1,2]

Table 1 Summary statistics for SNPs identified in coding sequences of *CAD4, HCT1, C3H3, CCR7* and *4CL3*

Gene	Coding sequence (bp)[a]	SNPs (*n*)	Mis-sense (*n*)	Stop (*n*)
CAD4	1074	19	8	0
HCT1	948	13	5	1
C3H3	1527	12	6	0
CCR7	1017	15	9	0
4CL3	1623	25	8	0

[a]Length of coding sequence (in base pairs).

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

Accelerating breeding thanks to genomics and biotechnologies

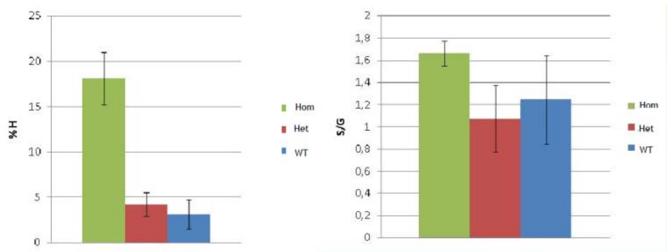
Identify causal mutations responsible of genetic variation

Gene and exact polymorphism (QTN) identifi

M_1

- 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 nigra*. 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

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

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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