Top of the Poplars

Project Coordinator **Dr Francis Martin** tells us how the special properties of poplars makes them perfect candidates as a 'model species' which could help shape sustainable fuels of the future

Contributor's from left to right: **Francis Martin, Magnus Hertzberg, Andrea Polle, Annabelle Déjardin, Richard Murphy**

How extensively are biofuels currently used as an energy source and what is Europe's position on their development?

Biofuels (liquid transport fuels used mainly for road transport) currently represent only a few per cent of EU road transport fuel (1 to 5 per cent energy basis depending upon country). But under the Renewable Energy Directive (RED), the EC has mandated renewable energy will comprise 15 per cent of the energy mix by 2020 – and that must include renewable energy being 10 per cent of the transport sector by 2020. This is a big increase from renewable energy levels of about 3 per cent at present. In transport, meeting this target suggests deployment of biofuels (included blending with fossil gasoline and/or diesel) at levels of something between 8 and 12 per cent into road transport fuel by 2020 (subject to deployment of renewable in other transport modes (rail, water, air).

Why are poplars particularly attractive 'second generation' bioethanol crops?

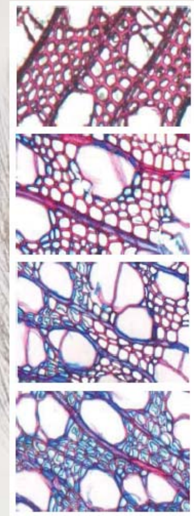
Many problems associated with first generation (1G) biofuels can be addressed through production of biofuels manufactured from agricultural and forest residues, and non-food crop feedstocks. Among these, poplar trees are well adapted to EU and other climates, we have several indigenous species with good biomass yields, and there is lengthy experience of their cultivation. Poplar biomass composition should be reasonable for conversion to biofuels, though this will benefit from optimisation - the major target of ENERGYPOPLAR. The poplar is also a 'model' tree, whose genome is sequenced and amenable to scientific advances and R&D: there is a strong network of European laboratories collaborating on poplar genetics.

Could you explain the importance of lignin in biofuel crops and in your research specifically?

Lignocellulosic biomass has long been recognised as a potential low-cost source of mixed sugars for fermentation to fuel ethanol. However, plant biomass has evolved effective mechanisms for resisting assault on its structural sugars, such as cellulose, from microbial and animal predators. This underlies a natural recalcitrance, creating technical barriers to cost effective transformation of lignocellulosic biomass to fermentable sugars. So there is a clear need to facilitate separation of sugar polymers in cell walls of wood from other lignin-type components. A promising alternative is developing trees with reduced lignin, or with modified lignin easier to extract: previous experience with transgenic poplars shows this to be a realistic goal. However, reducing lignin content excessively would dramatically affect plant growth, so ENERGYPOPLAR is devoted to producing new transgenic poplars, combining genes improving biomass yield, with those reducing lignin content. Within the timeframe of the project, these new transgenic lines will only be tested in greenhouses. If they lead to improved biofuel production, they will be tested in the field beyond this project, pending authorisation.

Will the project promote environmental sustainability of novel energy plantations in Europe?

Unlike agricultural 1G biofuel crops, poplars show a low nutrient demand for their growth, so they can be planted on marginal soils. Their survival relies on mutualistic symbiosis with soil microbes, so-called ectomycorrhizal fungi. These fungi are the link between plant and soil, delivering otherwise inaccessible nutrients to their host tree and acting as 'green' fertiliser. Thanks to this symbiosis, poplars can produce high biomass without industrial fertilisers, lowering greenhouse emissions. ENERGYPOPLAR is committed to evaluating bioenergy poplars' impact on root-associated fungi and soil microbiome.



WOOD STRUCTURES © INRA-ORLÉANS

What place do genetically-enhanced poplars hold in biofuels, as an energy source in the longer-term?

Genetically-enhanced poplar trees with increased yield and cellulose accessibility are within reach. In greenhouse trials, very significant improvements have been made, but additional measurements and evaluation should be carried out. A 30 per cent increase in biomass yield or poplar wood that would require only half of the degrading enzymes currently needed to generate ethanol – both being feasible – would greatly increase economic benefit for the whole industry, from technology providers to farmers, and the liquid biofuel industry. More efficient land use could also increase acceptance of second generation (2G) biofuel crop by the public.

It is a reasonable prediction that 2G and 3G biofuels, mainly from lignocellulosic feedstocks like SRC, SRF, agricultural residues and post-consumer waste, will provide between 20 and 30 per cent of world liquid transport fuel and after 2030. They will be significant for trucks, rail, sea transport and likely aviation. Light vehicles could progressively shift to plug-in technologies after 2030 based on a decarbonised electricity supply and potential H_2 and fuel cell development.

Taking on **Fuel**

Through pioneering work on the poplar tree, the **ENERGYPOPLAR** consortium could break down barriers to viable and price-competitive second generation biofuel production in Europe

WITH WORLD ENERGY demand in 2050 set to increase by 84 per cent on the 2007 level, and liquid fuel demand and CO_2 emissions set to double from 2010 levels, the search for greener alternatives to fossil fuels has never been more urgent. To limit climate change's impact to a global temperature rise of 2.0 to 2.4°C, the Intergovernmental Panel on Climate Change (IPCC) predicts a 50 per cent reduction of CO_2 emissions is needed. While biofuel production has increased, the availability of hitherto cheaper fossil fuels has led to them always being second choice, but in a sustainable future, by 2050 they could represent 30 per cent of transport fuel. Biofuel's advantages are manifold, ENERGYPOPLAR coordinator Dr Francis Martin explains: "Biofuels can be derived from many biomass sources: corn, Miscanthus, trees, agricultural and forestry residues. They can provide gasoline and diesel-type fuels derived from non-food crops like poplars, but also agricultural residues. These fit into existing infrastructure and are low 'well-to-wheels' emissions". With extensive experience in plant biology, biochemistry, biophysics and presently heading up the Ecogenomics of Interactions lab of the French National Institute for Agricultural Research, Martin is perfectly placed to coordinate a project whose expertise may lead to a range of second generation (2G) poplar biofuels representing ecological, economic and social benefits.

DEVELOPING POPLAR AS BIOENERGY

One barrier has been the perceived conflict between food crops – another ever-increasing demand – with the land needed to produce biofuels. However, species like poplars thrive in wide-ranging habitats, including marginal land not suitable for agricultural crops, and could prove appealing to farmers for reduced input costs and optimised land management. For this reason one of ENERGYPOPLAR's major aims is poplar's development as a bioenergy short rotation coppice crop (SRC) for large-scale European deployment on marginal land.

As Martin puts it, the initiative's ambitions rival the challenges it addresses: "The consumerist mantra of 'bigger, better and more' could summarise our research goals: the modifying of tree species like poplars for sustainable biomass production. We aim to unravel mechanisms controlling cell wall structure and composition, to design new poplar trees with enhanced agronomical traits for industrial production of bioethanol". Crucial in bioethanol production is lignocellulosic mass - the plant biomass composed of cellulose, hemicellulose, and lignin – for fermentation: the higher the yield, the more efficient the fuel. So lower-lignin varieties provide more efficient biofuel crops. ENERGYPOPLAR therefore aims at better understanding mechanisms regulating synthesis of cell wall lignocellulosic polymers, as well as establishing assays to demonstrate genetic and genomic traits of high or low-lignin trees.

With biodiversity a major concern in biofuel monocultures, alongside its genetic work ENERGYPOPLAR is also establishing tools for assessing environmental sustainability, conducting full Life Cycle Assessment of biofuels from poplar biomass in different scenarios. These also consider climate change impact on such SRCs and crop effect on soil microbial diversity and water quality. Given the project's engagement in promoting environmental sustainability of novel energy plantations, Martin is confident of these poplar crops' potential for European agriculture: "SRC poplar forests would not only be environmentally beneficial, but offer diversity in rural landscapes – which has been found to support increased biodiversity with other SRC crops compared with arable crops – and present an opportunity for rural income, social cohesion and other social benefits".

FIELD TRIALS AND GENETIC IMPROVEMENT

The ENERGYPOPLAR team is establishing field trials, planting trees at high density for maximum biomass production. Since lignin is the main factor limiting saccharification (sugar production) for fermentation, the team are developing new transgenic poplars combining genes improving yield with those that reduce lignin. ENERGYPOPLAR has new field sites in France, Belgium, the UK and Germany, and has identified high yield genotypes being studied further to elucidate key genetic control points underlying wood production. A key focus has been the below-ground aspects of yield, including the role of ectomycorrhizal fungi and C sequestration, which may have beneficial effects on tree yield. Progress has been made

on novel transgenic trees for improved yield and quality, through targeting developmental processes including cell division, dormancy and canopy architecture. Further phenotyping analysis will be undertaken to develop material for future field trials beyond this project.

Through conventional breeding as well as genetic engineering, the project aims for a deeper understanding of cell wall parameters determining saccharification potential. High-throughput tools to determine cellulose and lignin content and saccharification potential are being optimised, using genotypes with varying cell wall compositions. Significant progress has been made: five transcription factors with a putative role in lignification have been identified and altered, calibration models have been constructed to measure poplar lignin, a saccharification protocol to process woody stems has been established, and lignin and cell wall carbohydrates have been measured through wet chemical analyses. This should provide the basis for optimising saccharification potential by altering cell wall composition.

NOVEL GENOTYPES

The project aims to identify novel genotypes and, in particular, genotypes carrying desirable characteristics for bioethanol production in three ways: screening for natural variation; identification of genomic regions associated with particular traits; and use of transgenic poplars. Martin points out some exciting fresh developments in this area: "A recent key achievement is the discovery of natural mutant alleles in genes related to lignin synthesis through state-of-the-art next generation sequencing. This major finding opens new possibilities of lower lignin-content plants without using genetically-modified poplars".

The research has created new information on saccharification variation from a range of poplar genotypes – a good indicator of bioethanol potential – and will generate new indicators for clone breeding and improvement for global biofuel production. The project also aims to identify genomic regions involved in heterosis using experimental crosses between individuals, as well as producing transgenic trees through gene-stacking, a technique which will create trees with altered lignification but no substantial biomass loss. The most promising trees will then be used in experimental crosses producing elite offspring, further enhancing their potential to become biofuel crops of tomorrow.

ENHANCED COMPETITIVENESS

ENERGYPOPLAR's consortium comprises an interdisciplinary group of 10 public and private partners from six European countries, with expertise across tree biotechnology

POPLAR LEAVES © FRANCIS MARTIN

and breeding, hydrolysis and fermentation, environmental science, as well as scientists engaged in commercial-scale development of lignocellulose for ethanol. The consortium's findings are disseminated to academics, as well as stakeholders, through patents, publications, reports, newsletters and conferences: it is even being featured as a EURONEWS TV documentary to a worldwide audience. Next year, ENERGYPOPLAR will organise an international symposium on bioenergy trees from Europe, North America and China.

Martin believes their results will be of strong interest to 2G biofuel developers. They are currently investigating GM-poplar trees that, based on greenhouse data, give 100 per cent increased biomass production and 100 per cent increase in saccharification. Environmental impact assessment of new poplar lines will help industry, governmental agencies and farmers make good choices between technologies. For companies aiming to develop high value poplar trees for wood and bioenergy production, the research will be invaluable.

While reaching scientists, policy-makers and the commercial sector is important, ENERGYPOPLAR could enthuse an economically-challenged public about biofuels in one crucial way: cost. Martin explains: "Advances through ENERGYPOPLAR will enhance price competitiveness of 2G biofuels with gasoline through enhanced biomass yields (lower biomass costs) and reduced processing costs (better sugar release and yield for fermentation to biofuels). 2G lignocellulosic biomass should price compete with gasoline".

INTELLIGENCE

ENERGYPOPLAR

ENHANCING POPLAR TRAITS FOR ENERGY APPLICATIONS

OBJECTIVES

ENERGYPOPLAR is designed to develop new poplar trees having both desirable cell-wall traits and high biomass to be used as an efficient, renewable and sustainable source of lignocellulosic feedstock for industrial production of bioethanol.

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FRANCIS MARTIN received a bachelors degree from the Plant Biology Department of Nancy University in 1979, a PhD from the same University in 1982, and a Science Doctorat from Paris XI-Orsay University in 1986. He joined the INRA Forestry Center in 1981 where he built up a research group on the physiology and molecular biology of the ectomycorrhizal symbiosis. He headed the Tree-Microbes Interactions Department from 2001 to 2008 and is now leading the Ecogenomics of Interactions lab.

energy poplar