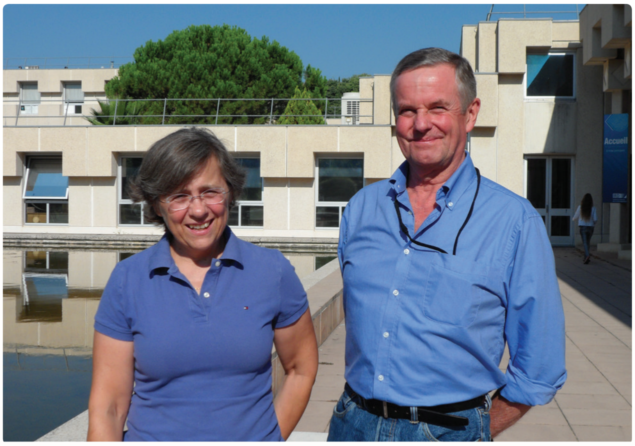
Seed power

In an international collaboration, **Drs Thomas Roscoe** and **Ljerka Kunst** are developing powerful genetic approaches to identify the mechanisms involved in regulating oil production in seeds. Their exciting work will lead to the development of new crops for biofuel production



As joint project coordinators, what are your respective duties in 'Increasing Oil Content by Redirecting Carbon Flux During Seed Development' (SYNERGY)?

We each take part in the supervision of our respective research teams at the Centre National de la Recherche Scientifique within the Institut de Recherche pour le Développement (CNRS-IRD), France and the University of British Columbia, Canada. Several developments have led to us working together: first, the realisation of our overlapping interests in increasing seed oil yield; second, the design of powerful new genetic approaches for the identification of novel transcription factors involved in the regulation of seed oil production in the embryo by the French team; third, the discovery of new information on the regulation of carbon partitioning in the seed coat, and identification of seed coat-specific promoters by the Canadian team; and fourth, an opportunity to apply for joint funding and integrate our individual approaches and expertise in order to pinpoint key regulators of oil accumulation in all seed tissues simultaneously.

Given the pressing environmental and economic issues at stake, do you think there is enough research into biofuels?

There are numerous large national and private sector-funded research programmes in many countries covering many fuel sources, including hydrogen, methane, sugar, starch, ligno-cellulose and seed oils. But if we consider the urgency of the problem, then no, sufficient research is not being carried out at present.

Have you had any success to date in discovering novel genes that help to control carbon partitioning in seeds?

Yes, our work at the University of British Columbia in characterising a high oil mutant of Arabidopsis, has led us to believe that the GLABRA2 (GL2) gene promotes carbon partitioning. Other studies have revealed that the WRI1 transcription factor and an enzyme of glycolysis, a pyrophosphatase, influence carbon partitioning. Comparative genomics and gene mapping in oil palm fruit has demonstrated the importance of transcriptional regulators in influencing partitioning between soluble carbohydrate and oil or structural fibre and oil.

How long will it be before these genes can be introduced into commercial oilseed crop species?

The discovery that inactivation of the GL2 transcriptional regulator in the Arabidopsis seed coat results in higher seed oil content suggests a direct approach to engineering increases in seed oil content in a wide variety of crop species. We are currently employing several reverse genetic techniques to silence GL2 and manipulate seed oil yield in *Camelina sativa*, a close relative of Arabidopsis. In addition, the genetic screens we have performed to date have resulted in a number of candidate regulators, with a potential role in seed oil accumulation. As soon as their role in this process is validated in Arabidopsis, they will also be introduced into *Camelina* in an attempt to increase oil yields.

What are the major scientific barriers to increasing oil yields in seed crops?

Yield is a complex polygenic trait that is subject to developmental and physiological control. Classical breeding and selection have increased seed oil content in an incremental manner consistent with quantitative genetic analysis that has implicated a large number of loci contributing to this trait. Remarkably, there are many reports of the manipulation of single genes that have resulted in increased seed oil content. This apparent contradiction serves to illustrate that lack of knowledge about the regulation that determines the amount of reserves synthesised in the different compartments of the seed remains a major barrier to increasing oil yields in oilseeds. Our approach of employing new efficient genetic screens to discover new components of gene regulatory networks will lead to greater insight into the control of seed reserve synthesis.

How does SYNERGY help to alleviate the food-fuel conflict?

Our focus in the SYNERGY project is on oil yield. Understanding the limitations of carbon channelling towards oil biosynthesis during seed development and identifying key regulators involved in carbon partitioning will allow us to increase overall oil yield in oilseeds for both food and fuel applications. To specifically address the food-fuel conflict, it is our intention to use these novel regulators to enhance oil content in non-food crops that are highly productive on marginal land.

What are the far-reaching implications of your research?

An improved understanding of the regulation of triacylglycerol accumulation and distribution among different seed compartments will lay the foundation for the creation of high yield oilseed non-food crops that are agronomically successful on marginal land, thereby increasing the supply of vegetable oils that are independent of the food supply chain.

Boosting **biomass**

Uniting research teams in France and Canada, the 'Increasing Oil Content by Redirecting Carbon Flux During Seed Development' project aims to boost oil yields from Brassica seeds, helping alleviate global dependence on fossil fuels

THERE IS INCREASING evidence of the significant damage being caused to the Earth's natural environment by rising anthropogenic CO_2 levels and climate change. As a large part of this problem relates to the burning of fossil fuels including gas and oil; the global community is increasingly turning to alternative fuel sources such as solar and wind power, as well as forms of energy created from living organisms – bioenergy.

Renewable energy sources already contribute to more than 20 per cent of global electricity generation, while biomass constitutes roughly 11 per cent of these renewables. However, questions have been raised about the sustainability of ethanol biofuels derived from starch and sugar. There is constant dispute as to whether any given biofuel produces more energy than was actually necessary to produce it – in other words, whether the biofuel net energy balance is positive. In addition, the widespread adoption of biodiesel is currently being curtailed by cost and limited supply of seed oils.

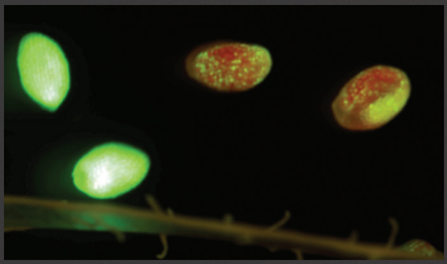
To tackle these issues, researchers have started to search for alternative biomass feedstocks and are looking for ways to increase the biomass yields of seed crops to boost their value for energy generation. However, this research area poses various technical challenges. In addition to ensuring the biofuel has a positive net energy balance, scientists must also take into account the food versus fuel conflict: crops grown for biofuels can take up land that would otherwise be used to feed the world's population. The obvious solution is to develop new plants that can grow in marginal land – areas that are too arid or inhospitable to be used for other forms of agriculture.

The sectors at the left side contain Arabidopsis seedlings expressing a gene that confers metabolic résistance (upper sectors) or susceptibility (lower sectors) under the control of a lipid related promoter. The sectors at the right side of the plate contain wild type seedlings.

HEAVY TRAFFIC

Funded by grants from the French National Research Agency (ANR) and the Natural Sciences and Engineering Research Council of Canada, the 'Increasing Oil Content by Redirecting Carbon Flux During Seed Development' project (Seeds Yield eNERGY, SYNERGY) is jointly led by principal investigators Drs Thomas Roscoe and Ljerka Kunst. Roscoe's group is based at the Centre National de la Recherche Scientifique (CNRS-IRD) accredited laboratory at the Institut de Recherche pour le Développement, Montpellier, France, while Kunst's team is based in the Department of Botany at the University of British Columbia, Canada. Although the research areas of two collaborating teams intersect, they will pursue different avenues of study during the project: "The French team will focus on the interaction of master regulators of seed development with secondary regulators of oil synthesis, whereas the Canadian team will work towards elucidating gene regulatory networks controlling the allocation of carbon between carbohydrate and oil in the seed coat and the embryo," Kunst explains.

The development of any new biofuel, whether made from seeds or algae, can only hope to replace part of our reliance on fossil fuels. Hence the SYNERGY researchers have decided to concentrate their efforts where they believe they will have the maximum impact on sustainability. Specifically, the team wants to develop seeds that produce higher yields of oil that can be used to produce biodiesel for heavy vehicles such as buses and trucks, where the use of bioethanol or electricity is not feasible.



These plants also express a fluorescent protein under the control of a storage protein promoter. The seeds on the left accumulate normal amounts of protein throughout the seed. The seeds on the right have a mutation in one of the regulatory genes that controls the expression of the genes that code for storage proteins. The level of fluorescence is correlated with the protein content.

GOLD-OF-PLEASURE

Transferring the knowledge gained in their earlier work on Arabidopsis, Kunst and Roscoe decided to focus on *Camelina sativa*, commonly known as false flax or gold-of-pleasure. This annual Brassica crop has excellent oil production potential and is rapidly emerging as a dedicated industrial non-food oilseed crop. Another advantage of *C. sativa* from the researchers' perspective is that it can be grown on marginal land, thereby avoiding issues surrounding the food versus fuel conflict. *C. sativa* produces small seeds, but they have high oil content: almost 40 per cent compared to only 20 per cent for soybeans.

As with any other seed, the oil produced from *C. sativa* is predominantly made up of triacylglycerols, or glycerol and three fatty acids. The genes that control triacylglycerol accumulation are not yet fully understood, and developing this understanding is an important objective for the SYNERGY researchers. "We have already identified mutant lines from our initial screens that are affected in seed oil content

and have isolated several transcription factors as candidate regulators of genes that control triacylglycerol assembly," Roscoe reveals.

CARBON PARTITIONING

During photosynthesis, plants assimilate carbon and convert it into carbohydrates, lipids and proteins, which are stored in seeds. This process is known as carbon partitioning, although the mechanisms that dictate the process are poorly understood. Each team is responsible for identifying the novel transcriptional factors involved in the regulation of carbon partitioning, and evaluating their effect on oil production in Arabidopsis and in the *C. sativa* seed embryo.

Kunst and Roscoe hope to boost oil yields by re-directing carbon metabolism away from structural components and other storage products, towards oil production in seeds. "By isolating the factors that control carbon partitioning between oil and protein or oil and carbohydrate we will be able to enhance either reserve, depending on the intended use of the crop," Roscoe elucidates. He hopes a deeper knowledge of the regulation of oil accumulation will allow for energy densification in plant biomass.

Once they have defined the factors that control carbon partitioning between oil and protein, the scientists will be able to develop seeds with either high oil/low protein (eg. for use in the production of oil for biofuels) or seeds with low oil/high protein (eg. for use in animal nutrition).

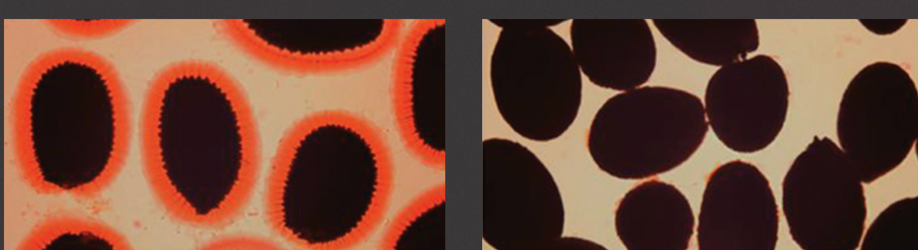
SCREEN THE GENES

To shed light on the underlying mechanisms of carbon partitioning, the SYNERGY researchers have developed powerful novel genetic screens: they use a florescence-based reporter in combination with positive and negative metabolic selections to identify the genetic regulators of the carbon use in developing seeds.

FOOD AND FUEL

As global demand for vegetable oils for food, industrial and fuel purposes continues to rise, finding new ways to boost oil yields is becoming an economic and environmental imperative. In terms of the environment, Kunst and Roscoe's development of seed oils that can be used for the production of biodiesel for heavy transport will help to lower greenhouse gas emissions. In addition, their work will help to offset the conflict between food and fuel by leading to the development of oilseeds that can be grown on marginal land.

The genetic information generated by SYNERGY's study of *C. sativa* also has the potential to be transferred to other oilseeds. Thus the French and Canadian teams' research into the mechanisms that regulate carbon partitioning can be applied to other fruit and seeds as well as tubers and roots. "The knowledge gained in SYNERGY can be translated to enhance the oil yield in tissues of other non-traditional temperate crops – such as maize and oats – and tropical crop species with a high biomass," asserts Kunst.



Carbon partitioning between seed oil biosynthesis and seed coat mucilage production.

INTELLIGENCE

INCREASING OIL CONTENT BY REDIRECTING CARBON FLUX DURING SEED DEVELOPMENT (SYNERGY)

OBJECTIVES

To investigate the extent to which carbon can be redirected from structural carbohydrate and other reserve components of the seed towards triacylglycerol to enhance oil yield in a model oilseed plant and to translate this knowledge to emerging non-food crop platforms which are performant on marginal land.

KEY COLLABORATORS

Dr Martine Devic; **Dr HuiChen, WU** CNRS-IRD, Montpellier, France • **Professor George Haughn**; **Dr Gillian Dean** UBC, Vancouver, Canada • **Dr Mark Smith**, NRC, Saskatoon, Canada • **Matthieu Ingouff**, UM2 Montpelier

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CONTACT

Dr Thomas Roscoe

Epigenetic Regulation and Seed Development Group
UMR232 Plant Diversity, Adaptation and Development Laboratory
Institut de Recherche pour le Developpement
911 Avenue Agropolis
34394 Montpellier Cedex 5
France

T +33 4 67 41 62 63
E thomas.roscoe@ird.fr

DR THOMAS ROSCOE is a research scientist at a Centre National de la Recherche Scientifique (CNRS) accredited laboratory at the Institut de Recherche pour le Développement (IRD), Montpellier. His research has addressed the modification of fatty acid composition of seed oils and the production of unusual fatty acids in the oil of cultivated species for use as industrial precursors. As well as coordinating the SYNERGY project with University of British Columbia, Canada, Roscoe's ongoing research is aimed at understanding the regulation of seed development and how this influences the synthesis of seed reserves.

DR LJERKA KUNST has over 20 years of experience in the molecular genetic analysis of plant lipid metabolism and is regarded as one of the foremost researchers in the study of fatty acid elongation in plants. Her research has led to major advances in the fields of seed oil biosynthesis and biotechnology, and cuticle biology and biochemistry. She has published a number of key papers on these topics in top-tier journals such as *Science*, *Plant Cell*, *PNAS*, *Plant Journal* and *Plant Physiology*, and invited review articles and book chapters on fatty acid, oil and cuticular wax biosynthesis.