Agrofuels and the use of Genetic Modification

A Report by GeneWatch UK

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Executive Summary

In the last few years, rising concern about climate change and the security of oil supplies has led to interest in the production of energy and fuels from biomass. At present, the major feedstocks for liquid biomass fuels are food crops such as soybean, maize and sugar cane. Production is often on a large scale, using chemical-intensive agricultural practices.

As countries have started to set targets for the use of liquid biomass fuel, concerns over their sustainability have increasingly been raised. One response from industry and government has been to invest in the development of so-called 'second generation' biomass fuels claiming they will not only increase output, but allow a broader range of plant based materials to be used as feedstocks.

This report describes the use of genetically modified (GM) crops and micro-organisms in the production of agrofuels. It describes the use of GM crops in current 'first-generation' agrofuels and the research that is being undertaken to produce new 'second-generation' fuels.

GM and 'first-generation' agrofuels

There is no legal requirement to identify agrofuels produced from GM organisms at the point of sale, or to publish information about their use during production. As a result, there is very little information in the public domain about the use of GM organisms in agrofuel production, and the industry provides little or no public information on the subject. However, the evidence suggests that a significant proportion of biodiesel and bioethanol currently on sale is likely to be derived from GM feedstocks. Given that GM foods are viewed unfavourably by consumers in many parts of the world, it could be argued that agrofuels provide a useful outlet for an unpopular product.

The boom in ethanol production in the United States has aided the fortunes of GM seed companies. For example, in response to the ethanol boom, Monsanto is increasing production and raising the price of its GM maize seeds. However, GM seeds being sold to farmers supplying the ethanol market are modified with existing GM traits, such as insect resistance or herbicide tolerance. Agrofuels are providing a useful opportunity for biotechnology companies to increase market share of their existing GM crops. Only one company, Syngenta, has so far produced a GM maize specifically intended for ethanol production, but this is not yet commercially available.

At present, there is no commercial production of GM sugar cane anywhere in the world, because of concerns from the sugar industry about public resistance to GM sugar. However, the agrofuel rush could change this situation and appears to be spurring on the development of GM varieties. In the last few years, companies from Brazil, the United States, Europe and Australia have all started developing GM sugar cane, and it appears these crops are being aimed at the ethanol market. While it remains to be seen whether they will gain commercial approval, some of the companies are claiming their GM sugar cane will be launched by the end of the decade.

GM companies are also claiming that they will soon be launching crops with improved yield or suited to drought prone conditions. However, once again there is little evidence available, so these claims cannot be verified. Historically, GM industry claims for such crops have not come to a great deal, and modifications that seemed successful in the laboratory have not transferred well to field conditions.

GM organisms for 'second-generation' agrofuels

A number of companies are working to develop cellulose enzymes for ethanol production. The ultimate aim is to develop micro-organisms that can digest cellulose and produce ethanol, and while many companies and research groups are making claims to have done so, their work is often so tightly bound by commercial secrecy that little detailed information escapes into the public domain. Approaches include the genetic modification of fungi, as well as bio-prospecting for genes and/or micro-organisms from a range of environments. Projected yields from cellulosic ethanol are dependent, at least in part, upon the abilities of the GM micro-organisms (GMMs) to produce ethanol. So far, the GMMs appear to be struggling to produce the high yields obtained from ethanol production using sugar or grain crops.

Little funding appears to have been allocated for examination of the environmental or plant health issues connected to the development of GM micro-organisms that contain potentially harmful traits. Nor is there any research into whether these traits could be passed on to naturally occurring micro-organisms, or whether they could be released into the environment. Horizontal gene transfer of GM traits is a possibility because the use of feedstocks such as straws or timber will import naturally occurring micro-organisms into the fermentation process. Measures to prevent such gene transfer, or to prevent the escape of GMMs used in cellulosic ethanol production, have not been made public by the industry.

Over millions of years, plants have evolved numerous mechanisms to defend themselves against attack from micro-organisms. These mechanisms act to hinder the breakdown of biomass to sugars. Genetic modification of food crops, trees and energy crops is being proposed as a solution to this problem. However, apart from GM trees, which were already in development for other reasons, the research is still at an early stage. Of the work that has been done, published studies have shown that unexpected impacts are commonplace, including variations in growth rate, survival and decomposition.

The use of GM trees as feedstocks for cellulosic ethanol would pose particular risks of gene escape, because tree pollen and seeds can move long distances. Many species of poplar are also capable of prolific and widespread vegetative (asexual) reproduction. As trees are essentially undomesticated, the spread of GM traits into wild populations is much more of a risk than for crop plants. Lignin modifications have the potential to change the ecological balance of receiving tree populations.

Lignin modifications also have the potential to impact on decomposition rates and carbon cycling in the soil. Results of published studies into this issue are contradictory, but as it is the stated aim of agrofuel production to reduce carbon emissions, further research is required to establish whether such GM crops would reduce carbon sequestration in soil, as has been suggested by some studies.

Another approach to GM crop development is the idea of creating crops that produce cellulase enzymes. There appears to have been little research into the impact on plant metabolism and disease resistance of such modifications. Production of cellulase within plant cells could potentially affect decomposition rates and nutrient cycling in the soil, or important agronomic characteristics such as disease resistance.

At least two US biotechnology companies have now started breeding programmes and genetic modification of energy crops such as miscanthus and switchgrass. These plants naturally display traits that make them good candidates for developing into invasive species, and so genetic modification of such crops needs to be treated with increased caution. Almost no research has been conducted into the potential for these crops to become invasive in the different parts of the world where they could be grown. Until this basic research has been conducted, even preliminary assessments of the environmental impact of GM varieties will not be possible.

1. Introduction

Genetically modified (GM) foods first came to public attention in the late 1990s. In many parts of the world, there has been widespread concern about the impacts of GM crop production on health and the environment. And because the technology is largely within the private sector, there continue to be questions about how, and in whose interests, it will be used. In many countries, such concerns have led to continuing resistance to the growing of GM crops and their use in food, and GM crop use has remained largely confined to the United States, Canada, Brazil, Argentina, Paraguay, South Africa, India and China. [1]

In contrast, there has been relatively little debate around the use of GM micro-organisms for the production of pharmaceuticals or industrial products. Even in the European Union, public attitudes to their use are generally positive. [2] A large number of companies and research institutions are involved in the genetic modification of micro-organisms, and the uses range from medical research to the industrial-scale production of enzymes used in detergents. There are minimal legal requirements to place details about such work in the public domain, and wide provisions to argue that information is confidential. In many cases, there is not even any requirement to inform the regulatory authorities. This means there is limited information about the use of GM micro-organisms, and often the claims made by organisations about their work cannot be verified.

In the last few years, rising concern about climate change and the security of oil supplies has led to interest in the production of energy and fuels from biomass. The use of fossil fuels for heating and electricity accounts for 40% of global CO_2 emissions, and transport accounts for almost 25%. [3] Interest is increasing in the use of biomass to replace fossil fuels for both these purposes. Wind, solar, tidal and geothermal energy can all be used to produce heat and electricity, but government targets on transport generally assume that fuels from biomass are the only near-term substitute for transport fuels such as petrol, diesel and kerosene. [4] And with demand for transportation fuels projected to continue rising, a great deal of attention, not only from governments but from the petroleum industry and venture capital as well, has focused on liquid fuels from biomass.

At present, the major feedstocks for liquid biomass fuels are food crops such as soybean, maize, oilseed rape and sugar cane. Production is often on a large scale, using chemical-intensive agricultural practices. As countries have started to set targets for the use of liquid biomass fuel, concerns over their sustainability have increasingly been raised. Such concerns include greenhouse gas (GHG) savings, competition for crops with food and land use change leading to increased CO_2 emissions. One response from industry and government to limit these negative impacts has been to set up sustainability standards another has been to invest in the development of so-called 'second generation' biomass fuels that will not only increase output, but allow the use of straw, food waste, timber and inedible plants as feedstocks.

Genetically modified plants are already used for the production of agrofuels. For example, GM maize is widely used in the United States as a feedstock for ethanol production, while biodiesel plants around the world may already be accepting GM soybean and oilseed rape. GM crops and micro-organisms are also being promoted for the production of second-generation agrofuels. This report examines the current and future use of genetically modified organisms (GMOs) specifically for agrofuel production, including which companies are involved and whether claims about the value of GMOs for agrofuels are supported by the evidence. It also sets out questions regarding the assessment of potential environmental impacts from using GMOs for agrofuel production.

2. The argofuels context

In recent years there have been growing concerns about the use of fossil fuels. These concerns stem from the overwhelming scientific consensus on the link between global climate change and CO_2 emissions from fossil fuel combustion,[5] the increasing dependence of many industrial countries on fossil fuel imports, the growing oil demand from emerging economies such as China and India,[6] overall rising oil prices[7] and heightening political tensions between western nations and many oil-exporting countries.[8] As public concern has grown, both governments and industry have been increasingly keen to be seen promoting potential solutions.[4]

At the same time, the energy demand for transport is predicted to rise sharply in coming decades. For example, the European Commission has predicted that by 2030 energy demand for freight transport in the European Union will increase by 74%, with the demand for diesel increasing by 51%.[9] An increase in demand approaching 60% is forecast for kerosene, the main aviation fuel. In 2004, the Director of the International Energy Agency stated, 'In the absence of strong government policies, we project that the worldwide use of oil in transport will nearly double between 2000 and 2030, leading to a similar increase in GHG emissions.'[10]

In 2003, the US Department of Transportation published an analysis on reducing GHG emissions from motor vehicles. It concluded that 'the reduction in GHG emissions from most gasoline substitutes would be modest' and 'promoting alternative fuels would be a costly strategy for reducing emissions'.[11] And yet, by 2007, the US administration was promoting just such alternative fuels, and in particular agrofuels.[12] Many commentators argue that the choice of a relatively ineffective and costly means of reducing vehicle emissions is the result of strong corporate lobbying, and not just in the United States.[13]

The success of such lobby groups has been to present agrofuels as a relatively cheap alternative to fossil fuels, and one that can be implemented quickly.[4] Given that the world economy currently relies on the cheap, fast movement of goods, a low-cost and easily implemented alternative to fossil fuels appears an attractive option to governments and consumers. In addition, agricultural interest groups, such as farmers' unions, have promoted agrofuels as a means of protecting rural employment and providing the agricultural sector with secure markets.[14] In the United States and the European Union, both of which already provide significant subsidies to agriculture, support for farmers and rural economies has become a significant driver for the promotion of agrofuels.[15]

The last few years have seen national targets set for percentage mixes of renewable fuels with petroleum-based fuels. There has also been significant public and private investment in research and development. However, these targets and investments have been against a backdrop of growing concern about the environmental and social consequences of agrofuel use.

Biomass, biofuels and agrofuels

Biomass is a term that simply means biological material. So energy from biomass means obtaining heat, light or power from biological sources such as food crops, timber, straw, vegetable oil, animal manures or energy crops. The use of biomass to produce energy is the oldest renewable energy technology available; firewood has been used for cooking and heating for millennia, and its use still supplies 1015% of energy used around the world. [16]

Biomass can be processed to liquids for use in combustion engines, and these liquids are often referred to as biofuels. At the moment, the main sources of biomass for liquid-fuel production are food crops. Oil crops such as soybean, oilseed rape, oil palm and sunflower are used to produce biodiesel, which can be used as a replacement for diesel. Crops such as sugar cane, sugar beet, maize, wheat and barley are used to make ethanol, which can be used as a replacement for gasoline. Ethanol production in 2007 represented about 4 percent of the 1,300 billion litres of gasoline consumed globally. [17]

Recently the term agrofuel has come into use to describe biofuels produced from large-scale, intensive or industrial production. As this report is primarily focused on liquid-fuel production from intensive production of biomass, we will also use the term throughout.

Both the United States and the European Union have now set ambitious targets for the replacement of fossil fuels with agrofuels. In December 2007 the US Congress passed the Renewable Fuel Standard which sets a production target of 36 billion gallons per year (BYG) of 'renewable and alternative' fuels by 2022. [18] Of this, only 15BYG can come from 'conventional' first-generation fuels; the rest must come from cellulosic ethanol and other advanced fuels. [19] Similarly, the European Union has set targets for the use of agrofuels. The EU Directive on agrofuels, 2003/30/EC, already requires each member state to set an indicative target for agrofuel use of 5.75% by 2010. [20] However, as part of the EU's package of measures on climate change, a mandatory target of 10% has now been set for renewable energy in transport. Although the use of electric cars and trains powered by renewably-sourced electricity can count towards this target, the majority of it is likely to be made by agrofuels. The fuels must demonstrate GHG savings of 35% rising to 60% by 2017 although evaluating and incorporating the GHG emissions released by indirect land-use changes still needs to be agreed in future years. A major review of the overall sustainability of this policy is to take place in 2014.

In December 2007 the South African government published its Biofuels Industry Strategy [21] which sets a minimum target of 2% agrofuel use in the next five years. However, this is a reduction of the 4.5% target laid down in the 2005 draft strategy. Other countries around the world have also been setting targets and mandates for future agrofuel use. These include and Indonesian target of 10% by 2010, Malaysian mandate of 5% biodiesel in public vehicles and China the target of 15% of all transport needs by 2020. [22]

Brazil is the world second largest producer of fuel grade ethanol second only to the US [23] and the Brazilian government has taken a lead role in developing an international biofuels market. In November 2008, they hosted the first International Conference on Biofuels and they are working with the US and EU to develop international standards for bioethanol and biodiesel. For Brazil the export market is becoming increasing important, from January to September 2008 ethanol exports were valued at $5.5 billion an increase of 50% on the same period last year.[24]

Large amounts of research funding have been put into agrofuel research and development by various governments. In 2007 the US Department of Energy announced funding totalling more than $700 million into agrofuel research,[25 26] The European Union is providing funding for agrofuel projects through its Framework Programme 7 (FP7) research programme whilst also in 2007 the UK government announced funding of £36 million for research into agrofuels.[27]

As part of the EU's FP7 research programme, its Biofuels European Technology Platform (ETP) was launched at a conference held in Brussels in June 2006.[28] The Vision document, which sets research priorities for the European Union, was developed by the Biofuels Research Advisory Council (BIOFRAC), which includes the biotech industry's lobby group, EuropaBio, and representatives of other industries, including oil, sugar and car manufacturers. The Vision document estimates that between 4 and 18% of the total agricultural land in the European Union would be needed to produce the amount of biofuels to reach the level of liquid fossil fuel replacement required for the transport sector in the European Directive 2003/30/EC.[29] It notes that different sectors food, feed, fibre, chemicals and energy compete for biomass from agriculture and forestry. Therefore, biomass production for energy has to be as efficient as possible per unit area in order to minimise the competition for land. The Vision includes the development of second-generation biofuels and claims that genetics can be used to improve the quality characteristics of the crop, e.g. decrease lignin content, so that whole-crop use becomes efficient.

Agrofuels are similarly attractive to the private sector, with around $2 billion invested in agrofuels from private and venture capital sources in 2006.[30] Companies as diverse as the Japanese car manufacturer Mitsubishi,[31] the investment bank Goldman Sachs[32] and the Chinese oil company PetroChina[33] have all invested in agrofuels. Private finance is also investing in companies developing second-generation agrofuels. For example, in 2002 Shell invested Ca$46 million in Iogen, a company working to develop cellulosic ethanol,[34] while BP has a joint project with the biotech company Mendel Biotechnology[35] to develop new crop varieties for use as agrofuel feedstocks.

In contrast to the enthusiasm for agrofuels from government and industry, there have been growing concerns from civil society groups about the environmental and social impacts of agrofuels.[36]

For example, the RSPB has raised concerns and doubts about the GHG savings and sustainability of a number of agrofuels. It called for minimum GHG standards required by law for all agrofuels that are sold in the UK and in Europe, declaring that it would support only agrofuels which deliver emissions savings of at least 60% above their fossil fuel equivalents based on full life-cycle analysis, including the effects of both direct and indirect land-use change.[37] The new European Directive now means this won't be reached until at least 2017.

A meeting of the UN Permanent Forum on Indigenous Issues in May 2007 was warned that 60 million indigenous people worldwide could be driven off their land to make way for agrofuel plantations.[38] Having first raised concerns in 2007, Oxfam published a briefing paper in June 2008, 'Another Inconvenient Truth'.[39] This report is deeply critical of the policies of richer countries and raises concerns about habitat loss and increasing food prices, arguing that the full scale of GHG emissions from agrofuels have not been properly considered.

Groups from North and South America have highlighted the fact that intensive cultivation of maize for ethanol in the United States has led to soil erosion, nitrate pollution and depletion of groundwater supplies for irrigation. Similarly, the cultivation of soybeans in South American countries has led to serious problems with soil erosion and soil nutrient depletion, and the deforestation of more than 35 million hectares of forest in Brazil, Argentina, Paraguay and Bolivia.[40]

The ambitious EU and US targets for agrofuel consumption will be hard to meet from domestic crop production. For example, a study in the Netherlands found that, even with strong efforts towards energy efficiency, the country has the potential to meet only 10-15% of its 2030 energy needs from biomass.[41] As a result, concerns have been raised that the rush to supply western demand for agrofuel will trigger land grabs in Asia, Africa and South America, the displacement of small farmers by large corporate agrofuel plantations and a reduction of the food available to the world's poor.[42]

During 2008 food prices soared leaving many people unable to afford food,[43] and the UN started to acknowledge the concerns about the indirect effects of biofuel policies by holding a 'High-level Conference on World Food Security: The Challenges of Climate Change and Bioenergy'. Whilst the final declaration did not blame specific policies for increasing food prices it did call for in-depth studies 'necessary to ensure that production and use of biofuels is sustainable in accordance with the three pillars of sustainable development'.[44] But Oxfam is concerned that in the meantime hunger would still be caused by existing policies and targets.[45]

By August 2008 the international Roundtable on Sustainable Biofuels[46] released 'Version Zero' of its proposed standards for sustainable biofuels. Three-hundred experts and representatives of the public and private sector developed the global norms for the economic, social and environmental impact of biofuels. However, concerns have been raised that these standards would always be voluntary, would be difficult to enforce and in any case they are not designed to address the wider questions of whether it is appropriate to develop agrofuels and if so to what extent.

In the UK, the government commissioned Ed Gallagher, chair of the Renewable Fuels Agency, to investigate the indirect effects of increasing agrofuel production. Key findings included:

- There is sufficient land available to meet current increases in agrofuel production until 2020 but the review did not look beyond 2020 when current trends are anticipated to continue and climate change will affect land productivity.

- There should be a slowdown in the growth of agrofuels to avoid conflicts with land that would otherwise be used for agriculture.

- Increased demand for agrofuels does indeed contribute to rising food prices for some commodities but the scale of the effect is uncertain.

- Specific incentives must stimulate advanced technology to increase the GHG savings of agrofuels and that decrease the competition with land for food crops.

Also in the UK, the Renewable Transport Fuel Obligation (RTFO), which came into effect in April 2008, places an obligation on fuel suppliers to ensure that a certain percentage of their aggregate sales is made up of biofuels. The percentage was set to increase year on year until it reached 5% by 2010/2011 but following advice in the Gallagher review this has now been reduced with a 5% target by 2013/14.

The RTFO also contains a reporting mechanism to monitor the programme's seven sustainability principles (five environmental and two social) but, the April - October 2008 report stated that no company met all the required standards.[47] Furthermore, there is concern EU member states will not be allowed to enforce stricter sustainability criteria than in the European Renewable Fuels Directive and this is one of the reasons Friends of the Earth has criticised UK government policy.[48]

A common theme, as issues and concerns have arisen around the use of agrofuels, has been to look to the future and second-generation, or advanced, agrofuels. For example, in an assessment of whether the European Union would be able to meet its agrofuel target, the European Commission assumed that there would be rapid development of new agrofuel technologies to a commercial scale.[49] As previously mentioned the Vision document of the European Biotechnology Platform and the Gallagher review identify the need for research in the areas of advanced agrofuels to reduce the land necessary for production of feedstock and to increase the GHG savings.

2.1 First generation agrofuels

At present, there are two main types of liquid agrofuels: ethanol for use in petrol engines; and fatty acid methyl esters (FAME), or biodiesel, for use in diesel engines. Ethanol is derived from sugar or grain crops and produced by using yeast to ferment sugars into ethanol. Biodiesel is derived from vegetable oils, or sometimes animal fat, using a chemical reaction with methanol. A third type of transport fuel is biogas, produced by using micro-organisms to digest manures or food waste. The gas produced can be used in specially converted engines, but this is not common because of the high cost of conversion.

Agrofuels are increasingly being categorised by their means of production as well as the type of fuel. So, while the end product is still ethanol or biodiesel, fuels are being termed 'first generation' if they are produced using existing production technologies (see Table 1), or 'second generation' if they are produced using technologies under development (see Table 2).

Table 1. First-generation agrofuels[50]

Agrofuel	Biomass feedstock	Production process
Ethanol	Sugar beet/sugar cane	Sugar extraction, fermentation
Ethanol	Cereal grains	Starch extraction, enzyme breakdown to sugars, fermentation
Vegetable oil	Oil crops	Cold pressing/extraction
Biodiesel from energy crops	Oil crops, e.g. Oilseed rape, soybean, Sunflower, palm, jatropha	Cold pressing/extraction and transesterification
Biodiesel from waste	Waste cooking vegetable oil or animal fat	transesterification
Biomethane	Food waste and animal manures	Anaerobic digestion of wastes to produce biogas, purification to biomethane

Ethanol

Ethanol is another name for alcohol, and at present fuel ethanol is produced in a very similar way to beers and wine. High-sugar crops, such as sugar cane or sugar beet, and high-starch crops, such as cereals, are fermented by yeast to produce ethanol. The ethanol is then extracted from the resulting mixture of ethanol and water using heat distillation. There are differences in the processing depending on whether sugar crops or cereal grains are used as feedstock, because yeast can ferment glucose, but not starch.

Glucose can be extracted directly from sugar cane and sugar beet, but cereal grains are made up largely of starch and so further processing is needed before the yeast can be added. First the grain is milled, then it is heated to break open the structure of the starch. After being cooked, a mixture of enzymes is added to break down the starch to glucose. Enzyme mixtures can include alpha-amylase, gluco-amylase and pullanase, which reduce the starch to glucose. Proteases may also be added to make protein available as a nutrient for the yeast.

Whether using sugar crops or cereal crops, fermentation produces a final liquid that is a mixture of ethanol and water. Ethanol levels tend to be around 15%, which is about the same as in a strong wine. But to be suitable as a fuel, the liquid needs to be more in the region of 99.7% ethanol. As a result, heat distillation is used to separate the ethanol from the water, and this uses a considerable amount of energy. The production of ethanol can also produce valuable by-products. In particular, grain residues (often termed distillers grains) can be sold on as animal feed.

Ethanol can be used as a pure fuel, in a mixture with petrol or it can be further processed to produce ethyl tertiary butyl ether (ETBE) and used as a fuel additive.[51] If ethanol is used as a pure fuel, or mixed at high rates, such as 85% blends, the vehicle engine has to be altered. In Brazil, the majority of vehicles have been adapted to drive on either ethanol or petrol, but elsewhere few vehicles have been altered and so high ethanol blends tend not to be supplied. Although ethanol can be used blended at lower rates, in the region of 5-10%, technical problems remain, such as the ethanol separating from the petrol. As a result, processing of ethanol to ETBE is favoured in Europe. However, this adds to the fossil fuel input of the fuel as ETBE is produced by reacting ethanol with iso-butane, a fossil fuel derivative.

Around 13 billion gallons of fuel grade ethanol were produced worldwide in 2007, with United States producing 6.5 billion gallons and Brazil 5 billion gallons. The European Union and China produced 570 and 486 million gallons respectively.[23] In Brazil, ethanol is produced from sugar cane while in the rest of the world it is usually produced from cereal grains such as maize, wheat and barley, with maize being the dominant feedstock.[52] At current production levels, ethanol accounts for only 5% of US petrol consumption, but the National Corn Growers Association has suggested that by 2015, assuming increases in yield and reduced demand from other markets, such as animal feed, domestic US maize grain production could provide around 16 billion gallons of ethanol per year.[53] This would be approximately 8.75% of the US requirement for transportation fuels.[54]

Biodiesel

Biodiesel is the agrofuel used to substitute for petrodiesel, and the majority of diesel vehicles can make use of it without modifications to the engine. First-generation biodiesel is produced from oily crops, such as oilseed rape, soybean or sunflower, but it can also be made from waste vegetable oil or animal fat. Vegetable oils and fats can be burned in the same way as petrochemical diesel, but they have a different viscosity (stickiness) and volatility (tendency to evaporate). If used untreated, they would be ineffective and damaging to standard diesel engines.

As a result, a process known as transesterification is used to lower the viscosity of the vegetable oil to one similar to diesel. This is a catalytic chemical reaction, during which the fatty acids in the vegetable oil react with an alcohol (usually methanol) to produce a compound called a fatty acid methyl ester (FAME) as well as glycerine. It is FAME that is used as biodiesel. This processing adds to the fossil fuel input of the agrofuel, because the methanol is usually derived from natural gas. There is some interest in the European Union and Brazil in switching to the production of fatty acid ethyl ester, in which the fatty acid would be reacted with ethanol, because bioethanol could then be used.[51]

The world's largest producer of biodiesel is the European Union, accounting for 68% of the world's biodiesel production in 2007;[55] historically, the major feedstock for EU biodiesel has been oilseed rape. However, oil crops such as soybean, palm oil and castor oil can also be used. Recently there has been growing interest in the tropical bush jatropha as a source of oil. From 2004 to 2008, the European Union provided a subsidy to farmers growing oil crops for the biodiesel market, and as a result production boomed from 0.31 million ha in 2004 to 2.84 million ha in 2007,[56] although these ended in 2008. US biodiesel also received subsidies, which lead to accusations that cheap US biodiesel was flooding the EU market.[57]

In March 2009 the EU placed temporary import tariffs on these imports.[58] It is predicted that by 2010 the United States will become the largest single country market for biodiesel, accounting for around 18% of consumption, and that new and large markets will emerge in China, India and Brazil.[59]

2.2 Second generation agrofuels

At present, there is no consensus on what actually constitutes a second-generation agrofuel, but the UN energy agency has defined them as being 'made from ligno-cellulosic biomass feedstock using advanced technical processes.[60] More recently definitions of the next stages of agrofuel production include GHG-saving qualities: the US Energy Independence and Security Act of 2007 defines advanced biofuels as a 'renewable fuel other than ethanol derived from corn starch, that is derived from renewable biomass, and achieves a 50 percent GHG emissions reduction requirement'.[19] A wide range of second-generation and advanced agrofuels (and derivatives) is being researched but, in very general terms, the production methods under development fall into two categories.

Cellulosic ethanol

The fermentation of ligno-cellulosic materials into ethanol and other fuels, is often known as cellulosic ethanol. This approach is being investigated for the production of ethanol. Cellulose is extracted from woody and fibrous materials and is converted to sugars using enzyme mixtures. The resulting sugars are then fermented to produce ethanol. By using different fermentation organisms, other fuels can be produced, such as methanol or butanol. In addition, the alcohol can be further processed to produce hydrogen. Cellulosic ethanol is heavily dependent upon the micro-organisms used to ferment the biomass and has been the focus of most GM research on second-generation agrofuels.

Biomass-to-liquid (BtL), also known as gasification[51]

This is a chemical/thermal process, primarily concerned with the production of biodiesel. The first stage is heat treatment. Pyrolisis involves heating biomass to 500°C for a few seconds to produce liquid bio-oils. Alternatively, slow pyrolysis involves heating biomass to around 300°C for about an hour to produce charcoal, which can then be ground into a powder. In the next stage, the oil or powder is converted to a synthetic gas, known as 'syngas', which is mostly made up of hydrogen and carbon monoxide. In the final stage, the syngas is converted to a liquid fuel via a chemical reaction in the presence of a catalyst. This last stage is known as Fischer-Tropsch synthesis and, depending on the catalyst used, produces gasoline, diesel or kerosene.

Hydrogen

In addition to alcohols or diesel fuels, there is also research into methods to produce hydrogen from biomass. It is possible that pyrolisis and gasification could be used to produce hydrogen, by altering the balance of CO and H_2 in the syngas. Hydrogen is not explored in this report.

Table 2. Second-generation or advanced agrofuels [61 62]

Agrofuel	Biomass feedstock	Production process	Status (in 2007)
Cellulosic ethanol	Lignin/cellulose-containing materials, e.g. Wood/straw	Pre-treatment to break cell structure, then breakdown to sugars and fermentation using GM Micro-organisms	Demonstration/pilot plants operating or planned in US, Japan and EU. Industry predicts commercial operations will start in 10-15 years
Bio butanol	Lignin/cellulose-Containing Materials	Use butanol-producing strains of micro-organism to ferment biomass	Pilot plant set up in the UK
Biomass-to-liquid/ Fischer-Tropsch Biodiesel	Lignin/cellulose-Containing materials, e.g. Wood/straw	Heat treatment to convert biomass to oil or charcoal, gasification then conversion to liquid via Fischer-Tropsch process	Pilot BtL plants operating operating in Germany Sweden. Commercial Fischer-Tropsch plants still using coal as the feedstock
Bio-DME (Biodimethyl ether)	As for BtL diesel	Can also be produced from syngas	Production still being researched. DME cannot be blended with petrodiesel and modifications to vehicle engines are required
HTU (hydro Thermal Upgrading) diesel	Wet biomass, e.g. sugar beet pulp, sewage sludge and bagasse (sugar cane waste)	At high temperatures and pressures the biomass is converted to a dense oil called 'biocrude' which is then refined to biodiesel via a catalytic reaction	Only being researched in the Netherlands. Still at very early stage of development
Biohydrogen	Lignin/cellulose-containing materials, e.g. Wood/straw; photosynthetic algae	Various methods including gasification, fermentation, pyrolysis and production from algae	Fuel cell cars not yet available and significant alterations to fuel supply infrastructure are required to distribute hydrogen. Decades more development expected

Biorefineries

For many working in the biotech industry, the aim is not simply to produce fuel from biomass, but to set up biorefineries – facilities that can process a range of feedstocks into a range of different products. So, as well as ethanol or biodiesel, a biorefinery might also produce materials for use in the production of plastics, fibres, solvents, polymers, carriers for pharmaceuticals and so on. In papers exploring the concept of the biorefinery it has been pointed out that the value of the chemical products manufactured from petrochemicals is about the same as the value of transportation fuel, which consumes more than 90% of the crude oil used in the United States,[65] meaning that the economic impact of industrial chemicals is very large. Indeed, some researchers have suggested that if they are to be economically viable, it will be necessary for agrofuel producers to operate like oil refineries, producing a range of products and making use of materials currently viewed as waste.[66] In September 2008 the European Commission published a call for research proposals to develop a biorefinery, with an indicative budget of €57 million, for which industrial partnership was considered essential. Four different European Technology Platforms, including the Biofuels Technology Platform, were involved in a 'partnering event' in Brussels to aid the formation of consortia to submit proposals to the calls. The meeting was organised jointly by EuropaBio and the European Forum on Industrial Biotechnology.[67,68]

In the UK, Northeast Biofuels has developed a business plan to build a 20,000 barrel per day biorefinery, supplying 34% of the UK's 2020 biodiesel targets.[69] But it is not yet clear whether this will utilise a thermochemical or biochemical process. A study carried out for the UK's National Non-Food Crops Centre (NNFCC) assessed the feasibility of a lignocellulosic ethanol plant, and whilst the biorefinery approach is discussed it pointed out that it was too early to tell if a thermochemical or biochemical process would be the most suitable.[70]

2.3 Where does genetic modification come in?

Agrofuel production at present is not dependent upon the use of genetic modification; its use is coincidental rather than essential. But genetic modification is increasingly being portrayed as an essential factor. Genetic modification of yeast and bacteria is being pursued for the development of cellulosic ethanol, while GM crop companies have been promoting their varieties for use as feedstocks for ethanol and biodiesel plants, as well as developing new GM traits specifically for agrofuel production. In August 2007, the chair of the European biotechnology association, Europabio, stated that 'Biotechnology is today one of the most effective and innovative tools we have to attain European targets for agrofuel use in a sustainable way.'[71]

Even in the case of the next generation of agrofuels, genetic modification is not the only technology that is being developed. Methods for producing biodiesel from biomass, hydrogen and agrofuel cells do not rely on genetic modification for their success. Despite this, large sums of private and public funding are being put into genetic modification for agrofuel production. Research areas include:

- GM crops as improved feedstocks for agrofuels plants, for example through increased yield,
- GM crops to act as sources of enzymes for use during ethanol production,
- GM crops that will 'self-destruct' for easier processing for ethanol production,
- GM micro-organisms to produce the enzymes needed to break down cellulose material prior to processing,
- GM yeast and bacteria to make ethanol from woody/fibrous materials.

Industry statements project confidence that GM technologies will help to bring about the rapid commercial production of second-generation agrofuels. However, there is often little information about what is actually being developed, whether the GM technology really will provide all that is hoped for, and whether there will be any environmental consequences from the use of the new GM organisms. The following sections address these issues and look at the use of genetic modification of plants and micro-organisms for the production of agrofuels.

3. GM organisms and first-generation argofuels

First-generation technologies for the production of ethanol and biodiesel pre-date the development of GM organisms by decades. As a result, they are not reliant on the use of GM organisms. However, the use of GM micro-organisms is becoming more common in ethanol production, and companies producing GM plants appear to have noticed the potential of the agrofuel market to provide new opportunities for their products. As a result, existing GM crop lines are already being promoted to farmers growing crops for agrofuels in countries such as the United States and Argentina.

Several GM companies have recently claimed to have GM crops in development specifically for ethanol and biodiesel production. And existing GM traits, such as herbicide tolerance and insect resistance, are being extended into crops viewed as having potential for agrofuel production. Similarly, while the use of GM micro-organisms was relatively uncommon until recently, they are being promoted as a means of cutting out processing steps or increasing the efficiency of ethanol refineries. In this section, we examine the current use of GM micro-organisms and plants in the production of bioethanol and biodiesel, as well as developments of new GM micro-organisms and GM plants for first-generation agrofuels.

It is difficult to find out the extent to which GM micro-organisms are used in agrofuel production, as there is little detailed information in the public domain. This is because legislation around the world allows companies and research institutes a great deal of secrecy about their use of GM micro-organisms. Even in the European Union, which requires publication of information about the use of GMMs, a great deal of information can be classed as confidential business information.[72] It is also rare for GM companies to publish much detail about GM crops while they are in development. And although detailed information is required in support of applications for commercial approval, most GM crops for agrofuel production have yet to reach this stage. As a result, the information in this report has been largely drawn from patents, promotional materials and scientific papers. It is therefore likely to be a significant underestimate of the work actually being conducted and should not be considered exhaustive.

3.1 Current use of GM organisms in agrofuel production

GM micro-organisms

The use of micro-organisms to produce agrofuel is currently restricted to ethanol production. Micro-organisms are not required in the production of biodiesel, because the conversion of vegetable oils to fuel is a chemical reaction. In contrast, fuel ethanol is produced by micro-organisms, which consume glucose and produce ethanol as a by-product of their metabolism – a process known as fermentation. The micro-organism most often, though not exclusively, used is *Saccharomyces cerevisiea*, or brewer's yeast.

There is little public information about the extent to which GM yeast strains are already used in the production of fuel ethanol. However, a little more information is available about the use of GM micro-organisms to produce ethanol for human consumption, because the regulatory requirements are more stringent in this area. In the case of beer production, GM strains of *S. cerevisiea* were first developed in the late 1980s, and approaches were made to the UK authorities in 1990 about the possibility of gaining food approval for GM brewer's yeast. However, no GM brewer's yeast has gained such approval and brewing companies around the world continue to use unmodified varieties.[73] As *Saccharomyces* strains are used in both the brewing and fuel ethanol industries, it is possible that companies producing GM varieties could have found an alternative market in the fuel ethanol sector.

Evidence against this comes from the way commercial yeasts are currently produced. The companies that produce commercial strains commonly hold large numbers of strains and screen them for specific properties in order to find ones best suited to a particular industry.[74] A study in 2004 examined seven yeast strains being sold for fuel ethanol production, and the researchers were able to identify only three distinct genetic profiles.[75] In other words, different companies were selling the same yeast strains. This suggests there has been little emphasis on the use of unique, patented GM strains.

However, recent developments in ethanol production may provide a new impetus to develop GM yeast strains. A process known as very high gravity fermentation uses high temperatures and pressures to increase ethanol production. The process has led to ethanol levels in the fermentation broth increasing from around 10-12% ethanol by volume, up to 16%. It is reported that the yeast company Fermentis is now working on genetically modified strains of yeast that can withstand the high alcohol, temperature and pressure conditions of such fermentation.[74] Whether this approach is taken up by other companies remains to be seen: at present, the primary method for developing new commercial yeast strains still appears to be selection rather than modification.[76]

Another use of micro-organisms is to produce the enzymes needed to break down starch to sugar, prior to fermentation. These enzymes may be derived from GM micro-organisms. Commercial enzyme mixtures can contain alpha-amylase, gluco-amylase and pullunase, which work to reduce the starch to glucose.[77] Other ingredients include proteases, which help to make the protein in the grain available to the yeast. The enzymes in the mixtures are derived from bacteria, which are grown in large fermentation vats and the enzyme extracted from the resulting broth.

The market leaders in the production of enzymes for ethanol production are the US companies Genencor and Dyadic, and the Danish biotech company Novozymes. The use of genetically modified micro-organisms is now a standard practice for these companies. The global market for industrial enzymes was worth $2 billion in 2004, with a predicted growth rate of 4-5% per annum,[78] and some 20-25% of the enzyme market is already made up of the enzymes required for agrofuel production. However, producers of enzymes for industrial purposes are not required to publish detailed information on whether their products are GM or not. Only Novozymes has voluntarily published which of its enzymes are produced from GM micro-organisms, including amylase for use in ethanol production.[79]

GM plants

There are no figures detailing the proportion of ethanol and biodiesel feedstocks that are derived from GM sources, but it is likely that agrofuel production already provides a significant market for GM crops. Maize is one of the primary feedstocks for bioethanol, while soybean and oilseed rape are widely used for biodiesel production. These crops have also been the focus of much GM development; in 2006, GM maize, soybean and oilseed rape made up 87% of all GM crops grown worldwide.[80]

The United States is one of the world's largest producers of ethanol, and the major feedstock is maize. GM maize has been grown widely in the United States since the 1990s, but its use in food has been strongly resisted in the European Union and Japan. In fact, in 2003 the United States complained to the World Trade Organisation that it was losing $300 million annually in maize exports to the European Union because of EU resistance to GM maize.[81] Combined with historical overproduction of US maize, this loss of maize export markets led to the need for other outlets for US maize. Ethanol production may have become important in this respect. Indeed, ethanol production in the United States is largely confined to those states producing surplus maize,[4] areas that would have been hardest hit by the loss of exports. In 2003 it was suggested at a US ethanol industry forum that around 40% of feedstocks were GM maize, and the percentage was expected to increase.[82] By 2006 the levels of GM maize production in Iowa, Nebraska and Illinois were 78%, 79% and 74% respectively.[83] These states were also producing the most ethanol in that year.[84]

As a result of the US administration's announcements of agrofuel targets, there has been a rise in ethanol production capacity in the United States. This has caused a consequent boom in corn production and so seed sales. For biotech companies, the ethanol boom has rejuvenated the GM seed market, and their financial prospects. In June 2007, Monsanto announced it was raising the price and expanding output of its GM maize seeds,[85] and it experienced a 70% rise in its third-quarter profits for 2007,[86] all of which was put down to increased ethanol demand. In fact, the biotech companies are faring better than the ethanol producers, with ethanol prices falling sharply during 2007 because of the expansion in production.[87]

While the dominant ethanol feedstock in the United States is maize, in Brazil ethanol is almost entirely produced from sugar cane. There is currently no commercial production of GM sugar cane anywhere in the world, largely because the sugar industry accepted at the beginning of the decade that public resistance to GM sugar was so strong it could not be marketed successfully.[88] However, in November 2008, Monsanto announced that it had acquired Brazilian-based sugar cane breeding and technology companies, Alellyx and CanaVialis. Their stated interest is particularly in the growing demand for ethanol from sugar cane and as Alellyx in particular has been developing biotech traits for sugar cane, this research is likely to increase with the involvement of Monsanto.[89]

The world's largest producer of biodiesel is the European Union, which accounted for 75% of the world's biodiesel production in 2007.[90] The European Biodiesel Board, which represents the industry, claims that around 90% of the raw materials used for European biodiesel are produced within the European Union.[91] Production of GM oil crops is virtually non-existent in the European Union. However, the rising demand for biodiesel in the European Union, driven by EU targets for agrofuel use, is predicted to lead to a rapid rise in imports. Soybean industry analysts have predicted that oil imports into the European Union from the Americas and Asia will increase by 28 times between 2007 and 2013[92] in order to meet biodiesel demand. EU imports of biodiesel from the United States have already jumped from 90,000 tonnes in 2006 to more than 700,000 tonnes in 2007.[93] More than 75% of US biodiesel is produced from soybeans [94]and, across the United States, more than 90% of soybean plantings are now GM.[95]

Biodiesel production is also increasing in areas where GM crops are grown. For example, In January 2008, the agricultural trading company Louis Dreyfus opened an 80 million gallon biodiesel plant dedicated to using soy as a feedstock.[96] The plant is based in Indiana, where 94% of the soybean production is GM.[97] The same company has made alliances with companies producing biodiesel from soybean in Argentina.[98] In the United States there has already been a rapid increase in the diversion of the soybean crop to biodiesel, from 8% of the crop in 2005/6 (producing around 200 million gallons of biodiesel) to around 20% in 2007/8 (producing in the region of 580 million gallons).[92] According to the US biodiesel association, current capacity to produce biodiesel in the United States is 2.61 billion gallons per year and when the plants currently being constructed come online this will rise to 3.46 billion gallons per year.[99]

Increasing agrofuel yields with GM?

The biotech industry has made claims that GM crops will help improve the economics and energy balance of agrofuel crops by improving yields.[100] However, the evidence from the use of GM herbicide-tolerant and insect-resistant crops shows no clear trends. For example, two reports on the commercial production of GM soybean in the United States, published in 2001, reached conflicting conclusions as to whether there had been yield improvements.[101] Very few studies comparing the yields of GM and non-GM crops have been published in the scientific literature, and those that have relate to cotton, which is not used for agrofuel production. There are, however, ongoing reports of farmers experiencing difficulties with glyphosate-tolerant GM crops: for example, the GM crops are more susceptible to drought stress or disease, may show less efficient pollination and may yield less than conventional crops.[102]

Yield data from government studies are also scarce. In the United States, agricultural extension services experienced difficulties in comparing GM and non-GM cultivars because of limited resources and the complications of the differing pesticide regimes.[103] The herbicides used with GM herbicide-tolerant crops would kill their conventional counterparts, so comparison trials could not be conducted. In fact, many services have since moved to simply testing GM varieties against each other. In the UK, large-scale trials comparing GM herbicide-tolerant and non-GM oilseed rape, sugar beet and maize were undertaken between 2000 and 2003. However, the experiments were designed to assess biodiversity impacts, and yield was not measured.[104]

3.2 Development of new GM organisms for first-generation agrofuels

As well as repackaging existing GM organisms for the agrofuel market, some companies are also making claims to be developing new GMOs for biodiesel and ethanol production. Because companies regard their developments of new GMOs as commercial secrets, there may be little or no detailed information in the public domain. As a result, the examples provided in this section provide a representative list, but it is very likely that more GM organisms are being developed.

Micro-organisms

The focus of research for GM micro-organisms remains the development of strains suited to second-generation agrofuels, and there are only a handful of reports about research directed at current production of ethanol and biodiesel. In the case of ethanol production, the GM developments are aimed at reducing the number of steps required to process starch to ethanol. For biodiesel, the focus is on developing the use of micro-organisms as feedstocks.

In 2005, the biotech company Genencor launched an enzyme complex that could break down raw, uncooked starch,[105] eliminating the need to cook starch prior to fermentation. Novozymes has taken out patents on similar enzyme mixtures.[106] Neither company has specifically stated these are from GM sources, but both use GM bacteria for the production of their enzymes. In an alternative approach to the same problem, researchers from the US Department of Agriculture are attempting to genetically modify yeast to produce alpha-amylase, in theory enabling the yeast to directly ferment starch.[107] But it is difficult to establish whether ethanol-refining companies are actually taking up these developments, because such information is usually regarded as confidential.

Algae, in particular cyanobacteria, are being considered as sources of oil for biodiesel production. Many microalgae produce oil, with oil contents in the region of 20-50% being quite common, and some even containing oil levels of more than 80%.[108] Microalgae can be grown in tanks or pools on a large scale and using a very small land area in comparison to that required for oil crops. In addition, biomass production is greater and generation time is shorter than for traditional oil crops. Pilot projects to produce biodiesel from algae are underway in China,[109] Europe,[110] Africa and the United States.[111]

While algae do have the potential to provide a sustainable feedstock for biodiesel, this is still a long way from being a commercial prospect,[112] and a number of technical, process and price issues need resolving. As with other areas of agrofuel development, researchers are suggesting that genetic modification be used to increase the oil production and industrial performance of microalgae.[108] However, very little research on the genetic modification of microalgae has been undertaken,[113] and researchers appear still to be conducting basic research, such as developing stable GM strains. The genomes of a number of cyanobacteria and other microalgae have now been mapped,[114] and in 2007 it was announced that the oil company BP is sponsoring research using genetic modification to increase the oil content of one of the most widely studied species of cyanobacterium, *Synechocystis* sp. PCC 6803.[115] Furthermore, the future development of GM algae for oil production in the UK seems doubtful as the recently announced £30 million 'Algae Biofuels Challenge' being run by the Carbon Trust does not wish applicants to use GMOs.[116]

A different approach to using micro-organisms to produce biodiesel is to use GM bacteria instead of algae. A German team has recently produced a GM strain of the widely used gut bacterium *Escherichia coli*, which has been modified to synthesise fatty acid ethyl esters.[117] The researchers' aim is to replace the chemical synthesis of biodiesel with a biological process, which they claim would remove the need to use fossil-fuel-derived methanol in the production of biodiesel. In reality this technology is probably even further from commercial reality than biodiesel from microalgae, and the researchers admit that yields of the fatty acid ethyl ester were low. In addition, unlike photosynthetic microalgae, *E. coli* bacteria would require a source of fatty acids to convert to biodiesel as well as glucose as a nutrient. So this approach would still require the production of agrofuel crops as feedstocks for the bacteria. It could even increase the land area required, because the glucose would have to be produced from grain or sugar crops.

Plants

In the case of GM plants, interest from biotechnology companies in agrofuels appears, in most cases, to be a relatively recent phenomenon. In fact, there are only a small number of examples of new GM crop traits being developed specifically for the production of first-generation agrofuels; almost all the GM crops coming to market continue to be either herbicide tolerant or insect resistant,[1] modifications focused on the production of the GM crop, rather than its final use.

Recently Monsanto started marketing maize varieties in the United States advertised as being better for ethanol production, but they appear to have been selected from existing GM and non-GM maize varieties,[118] rather than having been developed through specific genetic modification. Similarly, the DuPont subsidiary Pioneer HiBred is marketing soy and rapeseed lines selected for biodiesel markets, including GM varieties, but these are still modified for herbicide tolerance or insect resistance, rather than with biodiesel production in mind. So, while the biotech companies are seeking to take advantage of the new agrofuel markets,[119] it appears their GM crop output is still dominated by herbicide tolerance and insect resistance.

However, this is not to say there is no research into GM plants for use in bioethanol and biodiesel production. The key area appears to be the extension of herbicide tolerance and insect resistance traits into crops, such as sugar cane, sorghum or cassava, which are (or could become) important for agrofuel production. The development of new GM traits is a minor activity in comparison. Table 3 gives a summary of some of the claims being made by various companies.

In many cases there is no information in the public domain that indicates when or even whether commercial cultivation is being considered for these GM crops. The fact that a plant can be modified for a specific trait and is being tested in the laboratory does not guarantee that the resulting crop will grow well and safely in field conditions. In fact, out of the large number of GM crops developed in laboratories and tested in field trials, very few are even considered for commercial approval. Apart from Syngenta's Event 3272 maize (see below), it appears that not one of the GM crops listed below is being considered for commercial approval anywhere in the world. Prior to commercial approval, very little detail about the performance and safety of these GM crops is in the public domain. As a result, company statements about their potential cannot be verified.

Maize

GM maize is already widely grown in the United States and is almost certainly being used for ethanol production. However, none of the GM maize varieties currently on the market has been designed for use in ethanol production. As mentioned above, Monsanto has recently started marketing GM maize varieties it claims are better suited to ethanol production, but these appear to have been selected from existing GM herbicide-tolerant and insect-resistant varieties.

There appears to be only one example of a GM maize in development that has been modified specifically for the production of ethanol from maize grain. Developed by the Swiss biotechnology company Syngenta, it is referred to as Event 3272; it has been modified to produce amylase and contains genes from archael micro-organisms, which are single celled organisms often found living in extreme environments. [120] At present, amylase is added to milled maize prior to fermentation in order to break the starch down to glucose. The current method of producing amylase is to use bacteria (possibly genetically modified), which are grown in enclosed vats and then processed to extract the enzyme. Syngenta's GM maize would be grown in fields and the company suggests its GM maize could be added instead of the bacterial enzyme. When milled, the GM maize would release the amylase from within its grains. Results from laboratory tests suggest that replacing 3% of the maize grain with the GM maize would provide sufficient amylase to convert the remaining non-GM maize to glucose, [121] although this does not appear to have been confirmed in industrial conditions.

It is not clear whether this GM maize will really provide a viable alternative to the bacterial enzymes currently used. In the first place, ethanol producers frequently use enzyme mixtures, rather than a single enzyme, in order to maximise the conversion of starch to glucose and provide additional nutrition for the yeast.[77] The GM maize would replace only amylase and so it might not be a particularly attractive alternative. In addition, it would have to be produced at very low cost to make it financially viable; currently enzymes add only 3-4 cents per gallon to the cost of producing maize ethanol. Syngenta has not stated the price at which it expects to sell the seeds of this GM maize.

Table 3. Companies producing GM crops for agrofuel production

Crop	Research claims	Companies undertaking research	Status
Maize	Selection of varieties with high fermentable starch from existing GM lines	Monsanto	Already approved and on sale in US
Maize	GM maize for production of alpha-amylase enzyme	Syngenta	Commercial approval being sought in US and EU. Approved in Canada but rejected by South Africa
Sugar cane	GM sugar cane with higher sugar content	CTC Brazil	Field trials approved by Brazilian government
Sugar cane	GM sugar cane with higher sugar content	CSR, University of Queensland	Field trials being conducted in Australia
Sugar cane	GM sugar cane with altered structure/metabolism/yield	BSES Ltd, Australia	Field trials being conducted in Australia
Sugar cane	GM herbicide tolerance	Monsanto/ Votorantin	Claims that commercial production will start in 2010. This cannot be verified
Sorghum	GM herbicide tolerance	Syngenta	Patented. No information on field trials
Sorghum	GM insect resistance	ICRISAT	Unknown
Cassava	High starch yield	University of Ohio	Field trials conducted
Soybean	Altered fatty acid composition	DuPont	Initial laboratory studies
Soybean/ oilseed Rape	GM varieties modified for	Monsanto/ higher yield	Field trials in Canada Targeted Growth
Soybean/ oilseed rape	GM varieties modified for higher yield	Evogene	Initial laboratory studies
Oil palm	Gene sequenceing as a first step to genetic modification	Synthetic Genomics	Initial laboratory studies

In its application for EU approval, the company accepts that the GM maize may cross-pollinate with maize for food production in the areas where it is grown,[120] and its application to import (but not cultivate) this maize is being made under the European Union's GM food and feed regulation. But, it is not clear what impact contamination with this GM maize would have; aside from the safety issues of introducing a novel protein into the food chain, the amylase enzyme could potentially lead to maize breaking down to sugar during milling to produce food products. The use of GM maize to produce an industrial enzyme creates a high likelihood of contamination because it is wind pollinated.

In addition, a recent scientific paper examining the environmental impact of Syngenta's GM maize concluded that, because the GM amylase is stable at high temperature, it is likely to cycle differently in the soil to naturally occurring amylase. The researchers note that 'if this enzyme were to persist, accumulate and retain activity in the soil environment there is the possibility for indirect effects to be manifested with respect to carbon cycling within agroecosystems where Event 3272 is cultivated'. [122]

It is taking some time for Syngenta to market this maize. It was reported in the US press that the maize line would be available to US farmers by 2007; [123] however by the end of 2008 this had still not happened. [124] In March 2008, the Canadian authorities gave clearance for cultivation, food and animal feed use whilst back in March 2007, the South African government rejected an application for approval to grow the GM maize in South Africa, citing concerns about the safety of the GM maize for food and feed and the commercial impact of contamination. [125] An application to the European Union was made in March 2006, but in July 2007 a request was made for further information.[120] At the time of writing it was not clear if this maize would be commercially available for planting in 2009.

Sugar cane

In 2007 the Brazilian government announced it would be investing 1 billion reais (around €370 million) per year into developing a 'bioeconomy', including investment in agrofuel production; [126] and Brazilian ethanol attracted more than US$9 billion in foreign investment during 2006. [127] As part of its investment programme, the Brazilian government stated a desire to develop GM varieties of sugar cane that would be more drought tolerant, allowing sugar cane production to expand into drier regions of the country. This announcement follows a number of previous moves in Brazil to develop GM sugar cane. In 2003 a project to map the genome of sugar cane was completed. The research, funded by the Sao Paolo state research agency, was conducted by a number of Brazilian universities, and a subsequent collaborative project led to around 200 genes being patented, all related to sugar production in sugar cane. [128]

In February 2007 the Brazilian Centro de Tecnologia Canavieira (Sugar Cane Technology Centre) obtained permission to start field trials of GM sugar cane modified to contain a higher concentration of sugar than non-GM varieties. [129] The company stated its intention to start commercial production by the end of the decade. Other Brazilian biotech companies, including Allelyx and the state-linked EMBRAPA, are also working to develop GM sugar cane varieties for ethanol production.

Outside Brazil, there is also renewed interest in GM sugar cane. The European biotech company Bayer has posted a number of patent claims in the last two years for GM sugar cane with increased sugar content. [130] In 2006, Monsanto announced it would be working with the Brazilian company Votorantim to produce GM herbicide-tolerant sugar cane by 2009. [131] In the same year the Australian government gave a Aus$5 million grant to CSR (Australia's largest sugar refining company) and the University of Queensland to develop a GM sugar cane modified with genes from the bacterium *Pantoea dispersa,* which it is claimed has greatly increased sugar content.[132] In addition the Australian Bureau of Sugar Experimentation Stations (BSES Ltd) was granted permission in 2007 to conduct field trials of up to 2,500 different lines of GM sugar cane modified for altered plant structure, metabolism or yield.[133]

The development of GM sugar cane with increased sugar production is aimed firmly at ethanol production. In fact, given the aversion of the sugar refining industry to any GM sugar cane for human consumption,[88] it seems probable that all GM sugar cane traits are being developed for the ethanol market. As there is no requirement to label GM-derived ethanol at the pump, consumer resistance to GM crops will not be an issue. The World Wide Fund for Nature has highlighted the fact that sugar production already causes serious environmental impacts through habitat destruction, intensive use of agrochemicals, water extraction and soil erosion.[134] None of the GM traits being developed will address these problems. Indeed, the development of drought-resistant GM sugar cane could allow production to expand into the Brazilian Cerrado, a savannah region that is one of the world's oldest tropical ecosystems.

Sorghum

Sweet sorghum (*Sorghum bicolor*) has sugar-rich stems and produces a sugar yield equivalent to that from sugar cane. It has a shorter growing season than sugar cane, as well as much lower water requirements than either sugar cane or maize, and so is being promoted for ethanol production in dry tropical areas.[135] Sorghum is a minor ethanol crop at present; in the United States there are only eight ethanol plants which take sorghum.[136] However, trials of sweet sorghum are taking place in the Phillipines,[137] and the international agency for tropical crop research, ICRISAT, has been working with groups from Japan, Nigeria and China on sweet sorghum for ethanol production.[138]

Sorghum is considered to be one of the more difficult crop plants to genetically modify[139] and common methods for transforming plants do not work very well with this species. Combined with the fact that it is only a minor crop in western agriculture, sorghum has not attracted the same interest from companies and scientists involved in genetic modification as crops such as maize or soybean. However, a small number of GM traits are being developed for this crop: the international tropical crop research agency, ICRISAT, has developed GM insect-resistant sorghum;[140] Syngenta has filed patents for GM herbicide-tolerant sorghum;[141] and Pioneer Hibred has filed a patent for GM dwarf sorghum.[142] None of these traits appears to be aimed specifically at the ethanol market, although they could be marketed as such if sorghum becomes more widely used as an agrofuel crop. That said, the GM lines that have been developed appear to be a long way from commercial development, and there are no commercial plantings of GM sorghum anywhere in the world.

A serious concern regarding the genetic modification of sorghum is that it is wind pollinated and has close wild relatives in many areas where it is grown. In particular there is concern about the plant Johnsongrass (*S. halapense)* which is already a difficult weed in parts of

Africa. A study examining gene flow from sorghum concluded there was strong evidence that traits introduced into GM sorghum would cross into related plant species if GM sorghum were grown commercially.[143]

Cassava

Cassava is a staple food crop in many parts of Africa and Asia, and produces a very high yield of starch. It is often considered a food for the very poorest people as it can grow on very low-quality agricultural land and is cheap in comparison to other staple foods. There has been interest in its use as a feedstock for ethanol production. In 2006, China announced the development of a fuel ethanol refinery based on cassava,[144] and the Thai government announced that 12 new cassava-ethanol refineries would be opened over the next two years.[145] There is also growing interest in producing ethanol from cassava in the tropical countries of Africa.[146] At the same time, there has been a growth in demand for cassava starch for industrial uses, and concern has been raised that demand for cassava for industrial starch and ethanol could push up prices of a crop mainly eaten by the world's poorest people.[147]

The development of GM cassava has a controversial history, particularly in Africa. While previous research had the stated intention of supporting cassava production for food, the emphasis now appears to have shifted to ethanol and industrial starch. For example, the US Department of Energy is funding research to sequence the genome of cassava with the expressed intention of eventually using genetic modification to improve its use as a feedstock for agrofuel.[148] Researchers at the University of Ohio recently reported they had produced GM cassava plants with a significantly increased starch yield.[149] However, there is already wide genetic variation in starch yields among existing cultivars of cassava, and several programmes have successfully identified high starch yielding varieties of cassava without the need for genetic modification.[150]

Oil plants

Biodiesel is expensive. A study in 1999 estimated that the cost of production for integrated crushing and biodiesel plants was in the range of US$0.30/l using soybean as a feedstock, and up to US$0.69/l using oilseed rape.[151] In a more recent study it was estimated that if soybean oil were bought on the open market, this alone contributed more than 80% of total production costs and placed the price of the finished biodiesel well above the price of petrodiesel.[152] Reduction in the cost of oil production has therefore been targeted as an area for genetic modification of biodiesel crops, primarily via the increase in oil yield. Indeed industry claims are that GM varieties could increase oil yields by 10%.[153] Nevertheless, genetic modification of oil crops for biodiesel production remains a minor area of research when compared to the development of agricultural traits such as herbicide tolerance and insect resistance.

The Canadian company Targeted Growth claims it has discovered a method to genetically modify oil plants for increased yield, producing greater numbers of seeds per plant. Although this is not specifically aimed at agrofuel production, the company does mention agrofuels as a potential market in its promotional material.[154] However, Targeted Growth has not put forward any applications for commercial approval of any GM plants, and whilst in 2006 it conducted more than 100 field trials of GM oilseed rape and soybean,[155] by 2008 this was reduced to fewer than 50.[156] The company has licensed its technology to Monsanto[157] although neither company has made any statement about when or if these GM crops will be coming to market.

The Israeli company Evogene[158] also claims to have identified genes that will produce higher yields in oilseed rape and soybean. However, the work appears to be in the early stages and the company is still working on establishing whether the genes will actually work when inserted into GM plants. For both Targeted Growth and Evogene, it remains to be seen whether yield improvements in the laboratory will actually translate into yield improvements in the field.

Another area of GM research is the production of more stable oils that are better for processing to biodiesel, in particular high oleic acid oil. DuPont has sponsored research to develop GM soybeans with an altered fatty acid composition so that the resultant biodiesel performs better in cold conditions.[159] Both sunflowers[160] and soybeans[161] have been genetically modified to produce high oleic acid oil, which is considered preferable for processing to biodiesel. However, there is no information in the public domain to indicate when or even whether these crops might be approved for commercial production. In the case of sunflower, which is an open pollinated species native to North America, the probability of cross-pollination to native varieties and commercial sunflower crops means a GM sunflower might be viewed unfavourably for commercial cultivation.[162]

As with crops for ethanol production, there are also moves to insert patented GM traits, such as herbicide tolerance, into biodiesel crops. Production of GM insect-resistant oil palm has been undertaken in the laboratory,[163] and in July 2007 the US biotech company Synthetic Genomics announced it had formed a partnership with an oil palm plantation company in order to undertake gene sequencing of oil palm with a view to producing genetically modified varieties.[164]

Other modifications of agrofuel crops

Concerns have been expressed that the growth in agrofuels will cause high-quality crop land to be diverted from food production, in order to produce fuel.[165] As a counter to these concerns, it has been suggested that genetic modification could allow crops for agrofuel to be grown on land currently not suited to agriculture.[166] Proponents of GM agrofuels point to research aimed at producing drought-tolerant crops. In 1998 a researcher at the University of

Toronto isolated a gene claimed to control drought tolerance in plants.[167] Since then, research programmes have been put in place by various GM companies including Monsanto, Bayer, Syngenta, Dow, BASF and Dupont, as well as research organisations including the international maize and wheat improvement centre (CIMMYT), the Agricultural Genetic Engineering Research Institute in Cairo and a number of research institutes in South Africa.[168] Monsanto recently announced it would be marketing drought-tolerant cotton and maize varieties in India,[169] and it has already been suggested in South Africa that drought-tolerant soybean could be used as a feedstock for biodiesel production.[168]

However, there is little evidence that genetic modification will actually be able to address the difficult problem of water stress. Dozens of different physiological traits (such as plant leaf area, rooting depth and chemical responses to sunlight and water) affect a plant's response to drought; increasing grain yield under water-stressed conditions is therefore very difficult.[170] Plants displaying natural drought tolerance do so by means of a number of different mechanisms, some of which are not desirable traits for crop plants. For example, slow growth could help the plant survive drought, but would be unacceptable to farmers if the crop did not yield well by the end of the growing season. Historically, attempts to develop GM drought-tolerant crops have often been unsuccessful when transferred to the field. For example, claims were made that GM crops were drought tolerant when they had been tested using 'shock' treatments, such as sudden water deprivation. But in the field, drought tends to be a slow process, gradually drying out the crop.[170] Similarly, GM drought tolerance claims have been made based on simple survival under drought conditions, without assessing the ability of the GM plant to yield well. The understanding of the physiology of plant yield in water-limiting conditions is still rudimentary and there is so far little sign of any successful drought-resistant GM crops.[171]

As with many areas of GM technology, there is little information in the public domain to allow a critical assessment of the claims that GM drought-tolerant crops will soon be available. A small number of studies have been published in the scientific literature but only one, using oilseed rape, is relevant to agrofuel production.[172] Otherwise, claims for successful drought tolerance through genetic modification are difficult to verify because of a lack of field trial data in the public domain.[170] So while companies, such as Monsanto,[169] are making media statements that such crops will soon be in production, the reality is that no GM drought-tolerant crops have been approved anywhere in the world. It therefore appears unlikely that, in the near future, GM crops will allow agrofuel production on marginal or drought-prone land. And this may be no bad thing, because drought-tolerant GM crops could allow agrofuel production to spread into sensitive habitats.

3.3 Summary

There is very little information in the public domain about the use of GM organisms in agrofuel production. It seems probable that GM micro-organisms are used to produce some proportion of the enzyme additives used in ethanol production while the use of GM yeast for ethanol production appears to be uncommon at present. But none of this can be confirmed as the ethanol industry provides little or no public information on the subject. With respect of the use of GM crops, the evidence suggests that a significant proportion of biodiesel and bioethanol currently on sale is likely to be derived from GM feedstocks.

The proportion of agrofuels being derived from GM sources will vary depending on location, and is likely to be highest in those countries producing GM crops, such as the United States and Argentina. However, GM agrofuels are also likely to be sold in countries that do not grow GM crops. For example, recently there has been a large jump in imports of biodiesel into the European Union, and imports coming from the United States or Latin America are probably derived from GM sources. Given that GM foods are viewed unfavourably by consumers in many parts of the world, it could be argued that agrofuels provide a useful outlet for an unpopular product.

GM maize lines are now being sold to farmers supplying the ethanol market. In many cases, this is simply the repackaging of existing GM lines. Syngenta, is the only company so far to have produced a GM maize specifically modified for ethanol production, but its 3272 maize is not yet grown commercially.

There is a small amount of work to develop GM micro-organisms for ethanol production, but it is not known whether these are being taken up by ethanol refiners. Research to develop GM algae and bacteria for biodiesel production is at the preliminary stages and, in any case, the use of micro-organisms as feedstock for biodiesel is restricted largely to pilot projects.

Developments of new GM crops aimed at the agrofuel market fall into two categories. First, proprietary agronomic traits, such as herbicide tolerance, are being inserted into crops seen as having potential for agrofuel production. Second, crops already used as feedstocks are being modified with a view to making them more suitable for agrofuel production. In both cases, it is generally difficult to find out a great deal about what is actually being developed because very little reliable information is in the public domain.

In the case of ethanol from sugar cane, it appears that the rising interest in agrofuels is spurring the development of GM varieties. At present there are no commercially available GM sugar cane lines, largely because of the sugar industry's concerns about consumer rejection of GM sugar. Such concerns are not relevant to GM sugar cane for use in ethanol production, because around the world there are no requirements to provide any information about whether or not an agrofuel is derived from a GM source. And in the last couple of years a number of Brazilian, US and Australian companies have started developing GM sugar cane varieties, several aimed specifically at ethanol production. Some of these companies are claiming that GM sugar cane will be launched by the end of the decade.

Claims that GM technologies will boost oil production from oilseed rape and soybean cannot be verified because the companies involved have not placed any detailed information about their GM crops in the public domain. Historically, modifications to yield that have seemed successful in the laboratory have not transferred well to field conditions. Despite this, one of the companies involved has licensed its technology to Monsanto. Similar claims are being made for the development of drought-tolerant GM crops, but again there is little reliable data in the public domain as to whether such crops can grow successfully under field conditions.

4. Genetic modification of micro-organisms for cellulosic ethanol

At present, agrofuel production provides only a small percentage of global transport fuel requirements. Despite this, it is already controversial: there are concerns about whether current agrofuels really are carbon negative and questions have been raised as to whether it is morally or economically sensible to divert an increasing proportion of the world's food supply to fuel production. So-called second-generation agrofuels are being promoted as the answer to these concerns: fuel will be provided from a range of biological materials currently regarded as waste; carbon savings will be far greater than when using food crops; and food supplies can be reserved for feeding the world's growing population.

The United Nations has defined second-generation agrofuels as being 'made from ligno-cellulosic biomass feedstock using advanced technical processes'.[60] In general, the two main approaches being taken are the thermo-chemical approach, often termed biomass-to-liquid (BtL), and the biochemical approach, termed cellulosic ethanol (CE, see Section 2). The thermo-chemical processes used for BtL are not dependent upon GM organisms, either as feedstocks or for processing. In contrast, the biochemical approach of cellulosic ethanol production has been the focus of a great deal of GM research. GM micro-organisms are being developed to produce enzymes to break down the biomass and to ferment it, while GM plants are being developed to make processing easier. As this report is concerned with the use of genetic modification, only cellulosic ethanol is examined in detail.

4.1 The push for cellulose

There are a range of technologies competing to become the future of transportation fuel, and cellulosic ethanol is often presented as being one of the closest of these to commercial production. For example, the Agrofuels Initiative of the US Department of Energy (DOE) has the stated aim of making cellulosic ethanol production cost competitive by 2012. But, this may be some way off as there are only a small number of operational cellulosic ethanol pilot projects and the first demonstration plant, which opened in May 2008 and is operated by the company Verenium, has an output of only 1.4 million gallons per year.[173] In 2007 the United States consumed over 20 million gallons of crude oil and petroleum products per day.[174]

Spurred on by government enthusiasm for the technology, companies from the biotech, agrofuel and petrochemical industries have been forming a series of corporate partnerships aimed at the production of cellulosic ethanol. Examples are given in Table 4.

Table 4. Examples of corporate partnerships for the development of cellulosic ethanol[131]

Company	Description	Partnerships for producing cellulosic ethanol
Abengoa Bioenergy	Spanish agrofuel company	Has two CE plants planned in the US; one has US government funding. Also partnered with Ethanol Technologies, Australia
Dyadic	US microbial biotech company	Partnership with Abengoa to set up large-scale production of enzymes needed for CE. Partnered with Royal Nedalco to develop CE plants in Netherlands
Genencor	US enzymes company	Has CE partnerships with Mascomer (US agrofuel company) and Cargill Dow
Iogen	US biotech company developing cellulosic ethanol	Royal Dutch Shell, Goldman Sachs
Novozymes	Danish microbial biotech company; enzyme producer	Partnered in the US with Dupont and POET; in Denmark with Biogasol; in Brazil with Centro de Tecnologia Canavieira
PetroChina	Chinese petrochemical company	Has initial agreement with China's state forestry administration to develop ethanol from forest products
POET (formerly Broin)	US agrofuel company	Has CE plants planned with Dupont and Novozymes
Honda	Japanese car manufacturer	Developing pilot CE plant with Japanese Research Institute of Innovative Technology for the Earth (RITE)
SunOpta	North American agrofuel development and food distribution company	Partnered with China Resources Alcohol Corporation; in Spain with Abengoa; and with Celunol and others in US
Verenium	US company, born of merger of Diversa and Celunol Corporations, two biotech firms, focused on developing Cellulosic Ethanol	Key partners include BASF, BP, Bunge, Cargill, Danisco, DuPont, Fermic, Maurubeni, Syngenta, Tsukishima Kikai Corporation and University of Florida

The US administration has shown great enthusiasm for cellulosic ethanol, and these corporate projects have benefitted from significant public funding. In February 2007 the US

DOE announced that up to $385 million would be allocated to six projects aimed at producing pilot cellulosic ethanol plants,[25] and shortly afterwards it announced another $375 million would be provided for the establishment of three agrofuel research centres.[26] By October 2008 the DOE had announced plans to invest nearly $1 billion to develop and deploy advanced biofuel technologies by 2012.[176]

Producing cellulosic ethanol

Plant materials such as straws or timber are not made up of easily processed starch or sugar. Depending on the material, they are composed of cellulose (30-50%), hemicellulose (20-40%) and lignin (15-30%).[52] The idea behind making ethanol from cellulosic materials comes from the fact that cellulose and hemicellulose are essentially complex crystals made of sugar molecules. In theory, if they can be broken down into sugar, this can then be fermented into ethanol. And because cellulose and hemicellulose are major structural materials in plants, much more of the biomass could be converted into ethanol than is possible when only the starch or free sugar content is used.

Cellulosic ethanol is being widely promoted as the future for agrofuel production. Some refer to cellulosic biomass as the 'oil wells of the twenty-first century'.[177] The US Energy Independence and Security Act of 2007 calls for over half of the 36 billion gallon a year target for biofuels to come from cellulosic ethanol.[19] In addition, it is claimed that cellulosic ethanol would make use of plant material not currently being utilised for food, feed, timber, fibres etc, and that the fuel production possible from each hectare under cultivation would be significantly higher than from corn grain ethanol or from soy biodiesel.[178] However, such a view assumes that the plant material currently considered to be waste, for example, straw, grain husks, wood off cuts, sawdust etc, has no direct economic benefit to the human race. But where these by-products are not used by humans they become the very fabric of the soils we wish to continue to utilise; furthermore they are the nutritional and structural habitat for a large number for flora, fauna and fungi. Before using this material very careful consideration should be given to the effects on soil and biodiversity.

The steps necessary for cellulosic ethanol production, with their related difficulties, can be summarised as follows:

Production of feedstocks

In theory, any material containing cellulose can be used to produce ethanol. Feedstocks being tested include rice straw, wheat straw, corn stover, wood pulp, sugar cane pulp, urban refuse and crops grown specifically for the purpose, such as willow, poplar, miscanthus and switchgrass.

Pre-treatment

As well as cellulose and hemicellulose, the other important structural material in plants is lignin, the main constituent of wood. Lignin is not made of sugar and so cannot be converted to ethanol, but cellulose is often linked to lignin by structural and chemical bonds. Treatments using harsh chemicals and high temperatures are required to break apart these bonds, and this can take several hours to several days depending on the process used.[179] Pre-treatment can also produce by-products, such as organic acids and phenolic compounds, which can be toxic to the micro-organisms used at the fermentation stage.

Enzymatic hydrolysis/saccharification

This process uses enzyme mixtures to break down the cellulose and hemicellulose to their constituent sugars. Cellulose breaks down to sugar molecules containing units of six carbon atoms, such as glucose; hemicellulose breaks down to sugar molecules containing units of five carbon atoms, such as xylose. Because of the complex structures of cellulose and hemicellulose, large quantities and numbers of enzymes are required to reduce them to sugars.[180]

Fermentation of the sugars to ethanol

Yeast can digest glucose to produce ethanol, and this is the basis of brewing. However, the saccharification of cellulosic materials produces a mixture of different sugars as well as other by-products, many of which are toxic. Natural yeast cannot make use of these other sugars, while micro-organisms that can often don't produce ethanol, or do so only in very small quantities.

Separation of the ethanol from the fermentation broth

As with conventional ethanol production, the end product is a dilute mixture of ethanol and water, although the proportion of ethanol in the final liquid may be much lower than for ethanol derived from grain or sugar. Heat distillation is used to extract the small proportion of ethanol; this process can consume large amounts of energy.[181]

The ultimate aim of cellulosic ethanol technology is to ferment woody or fibrous materials and make alcohol. Obviously, if this were a straightforward biological process, there would be a thriving drinks industry based upon it. Unfortunately, it is actually extremely difficult and inefficient using naturally occurring organisms. As a result, it has become a focus for GM research. Different approaches are being taken by different companies to address the problem. One strand of research focuses on developing micro-organisms able to produce enzymes to break down biomass and others to produce ethanol from the resulting sugars. Alternatively, other researchers are attempting to develop micro-organisms that can break down the biomass and produce the ethanol. Finally, modifications are also being made to plants in the hope of producing feedstocks that will be easier to process (see Section 5).

Biomass recalcitrance

The difficulty in converting plant materials into usable sugars is increasingly being referred to as 'biomass recalcitrance'.[180] This recalcitrance is another term for the mechanisms plants have evolved to resist pest and disease attack. These mechanisms include the structural complexity and diversity of materials inside plant cells, the production by plants of enzymes to inhibit fermentation, and the high resistance of cellulose to chemical or biological attack. The goal of fermenting fibrous material has been pursued for at least 20 years, yet a lot of fundamental research still needs to be done. For example, the biochemistry underlying particular stages in the enzymatic breakdown of biomass is poorly understood,[180] and there is still a large amount of investigation required to understand how micro-organisms break down plant materials using enzymes. Yet, increasing efficiency and reducing costs in this area is seen as one of the key factors in making cellulosic ethanol economically competative.[178]

4.2 GM micro-organisms for enzyme production

Enzyme mixtudres are already used in the processing of grain to ethanol, primarily to break down the starch to glucose. But these mixtures are cheap and simple when compared to those needed to break down cellulosic materials. This is because starch has an uncomplicated structure and is open to enzyme attack. In contrast, cellulose and hemicellulose have a crystalline structure that is very compact and highly resistant to biological attack. Not only this, but the composition of hemicellulose varies depending on its source. So, for example, the hemicellulose in herbaceous plants, such as maize or switchgrass, consists mainly of arabinoxylan; in hardwoods it is mainly glucuronoxylan; and in softwoods it is mainly galactoglucomannan.[182] Different enzyme mixtures are needed in each case.

The difficulty of breaking down these materials is illustrated by the diversity of enzyme mixtures and complexes produced naturally by bacteria and fungi to digest woody materials. Some species produce hundreds of enzymes and non-enzymatic substances to achieve this.[183] Requirements for commercial cellulases include endo-1,4-ß-glucanases, exo-cellobiohydrolases and ß-glucosidases to break down the cellulose, and xylanases, a-L-arabinofuranosidases, feruloyl and acetylxylan[184] esterates and ß-xylosidases to break down the hemicellulose.

As many naturally occurring enzymes would be inactivated by the high temperatures used during the processing of biomass, one focus of research since the 1990s has been the identification of enzymes that remain active at higher temperatures. These have been found in some thermophilic micro-organisms, particularly those able to withstand temperatures greater than 80°C.[63] The genes for these enzymes may then be transferred into genetically modified micro-organisms, such as the commonly used *E. coli*, for large-scale production. In addition, researchers from the University of Florida, Caltech, the biotech company Verenium (previously Diversa), the Brazilian Instituto Nacional de Biodiversidad and the Joint Genome Institute are all working to identify cellulase enzymes from the intestines of termites.[185 186]

Cellulase enzyme mixtures already have a number of industrial applications, including in detergents. The market leaders in the production of these enzymes include the Danish biotech company Novozymes, which started working on these enzymes in 2001, and the US company Genencor. Both already make cellulases based on *Trichoderma* species of fungi, and both have developed GM micro-organisms for their production.[187 188]

Another major US enzyme company, Dyadic, has patented a strain of the *Chrysosporium lucknowense* fungus[189] for cellulase production and is working with the US DOE to develop genetically modified fungi that can produce greater volumes of enzymes.[190] The company is also collaborating with the Spanish agrofuel company Abengoa Bioenergy to start large-scale production.[191] The oil giant Shell has invested in the US enzyme company Codexis, which claims it can develop new enzymes for biomass breakdown using what it terms 'directed evolution'.[192] Dyadic has recently announced a licence agreement with Codexis for use of Dyadic's 'C1'enzyme production technology platform.[193]

The biofuels company Verenium has been prospecting for micro-organisms producing cellulases in extreme environments, and it claims to use mutagenesis and genetic modification to produce bacterial strains able to produce enzymes with the desired activity under different conditions of temperature and acidity.[194] Similarly, Sandia Corp, a division of Lockheed Martin, is being funded by the US DOE to use mutagenesis to manipulate micro-organisms from extreme environments to produce enzymes suitable for cellulosic ethanol production.[195]

At present, commercial cellulase products tend to be based on those produced by fungi, which produce complex mixtures of the cellulases and hemicellulases containing tens of different enzymes. But because of the resistance to attack and complexity of the biomass materials, very large doses of these enzyme mixtures are required current estimates are 100g of cellulase needed for every gallon of ethanol produced,[52] although the Danish enzyme company Novozymes estimates the figure at 15g of enzymes for every gallon when using maize stover.[196] In comparison, maize grain requires only 1 gram per gallon.

However, the enzyme mixtures are expensive and not as potent under commercial conditions as they are in the laboratory.[197 198] Even after significant research funding from the US government, the enzyme mixtures required for cellulosic ethanol cost in the region of 20-30 US cents per gallon, compared to costs of around 3-4 US cents per gallon for the enzymes used in the production of ethanol from grains.[198] Novozymes claims it has been able to reduce those costs to under 20 cents per gallon in laboratory tests,[196] but, this price is regarded as being too high for viable commercial production, and the companies involved are working to establish which mixtures of enzymes work best for particular feedstocks in industrial conditions.[199] In February 2008, the US DOE announced the four projects that will receive funds of $34 million towards developing enzymes for cellulosic ethanol production. They are the DSM Innovation Center, Genencor, Novozymes, Inc. and Verenium Corporation.[200]

The prospects for cellulosic ethanol

It seems unlikely that cellulosic ethanol will be able to significantly increase agrofuel production within the short time needed to address climate change. While the US government has suggested that, in theory, 30% of US energy needs could come from biomass in the future,[201] the predictions from the Department of Energy are that cellulosic ethanol will replace a mere 5% of US gasoline consumption by 2030.[202] In the near term, the projections for cellulosic ethanol, even from those companies developing the technology, are underwhelming: for example, six projects being funded by the US government are predicting that, when up and running, they will produce a total production of 133 million gallons of ethanol per year. This equates to enough fuel to supply US consumers for one third of one day.[54]

The US government is providing significant research funding (over $1 billion in the next few years),[203] but this technology has a long way to go before being economically viable. For example, a 2007 study by researchers from Iowa State University concludes that at $756 million the capital cost for a cellulosic ethanol plant would be nearly seven times greater than a comparable grain ethanol plant, while the operating cost would be around $1.76 per gallon of ethanol produced[204] (at 2005 prices) compared to $1.22 per gallon for grain ethanol. The researchers point out that such high start-up costs would be a serious disincentive to private development of the technology. Many pilot projects are currently supported by government funds.

A study by the University of Oregon[205] concludes that the costs for production of cellulosic ethanol appear to be around 25% higher than revenues. And the European Commission estimates that cellulosic ethanol production is currently 30% more expensive than first-generation ethanol production.[49] In addition, the University of Oregon analysis concludes that the use of biofuels to reduce fossil fuel use is 6 to 28 times more costly as a policy option than increasing fuel tax or tightening vehicle efficiency standards. Nevertheless, it is argued by the industry that the high costs of production will come down as the technology advances. Unfortunately, it is impossible to evaluate such claims because much of the detailed operations of pilot schemes for developing cellulosic ethanol remain closely guarded commercial secrets.[206] As a result, estimates of future profitability can only be treated as speculation, because they cannot be verified.

4.3 GM micro-organisms for fermentation

Natural strains of brewer's yeast (*Saccharomyces cerevisiea)* can only convert glucose to ethanol, not the range of sugars produced from the breakdown of cellulosic material. Conversely, micro-organisms that can digest the range of sugars available from biomass often don't produce ethanol or do so only in very small quantities. In addition, any micro-organism suitable for cellulosic ethanol production will need to be able to withstand taxing conditions, such as high levels of ethanol, which is itself toxic to many organisms; high temperatures; acidic or alkali conditions, depending on the pre-treatment; potentially toxic by-products of pre-treatment; and harsh physical conditions, such as movement through pipes. In all, this is a demanding list for any micro-organism.

In contrast to first-generation ethanol production, where the use of GM yeast appears to be uncommon, GM micro-organisms appear to be the norm for companies developing cellulosic ethanol. For example, researchers at Purdue University in the United States have developed a GM yeast strain modified to ferment the C5 sugar xylose. This was licensed to the Canadian company Iogen Corp in 2004.[207] A patent for yeast able to ferment xylose was also filed by the Dutch company Royal Nedalco,[208] one of Europe's largest ethanol producers, and this has since been licensed to US ethanol companies SunOpta[209] and Mascoma.[210] Another yeast species *Pichia stipitis*, which is found in the gut of the stag beetle, is able to convert xylose to ethanol, and the company Xethanol has licensed strains of this micro-organism developed by the University of Wisconsin.[211] In the United States, the government's Agriculture Research Service is conducting high-throughput genetic modification of yeast with the aim of producing GM strains suitable for cellulosic ethanol production.[190]

The University of Florida has filed patents for a number of different GM bacteria modified to produce ethanol,[212] and has licensed some of its GM micro-organisms to the biofuels company Verenium.[213] Another micro-organism, called *Zymomonas mobilis,* has been modified by researchers at the US National Renewable Energy Laboratory,[214] and this has been licensed to DuPont. However, there are problems associated with the use of the bacteria instead of yeast. Non-yeast organisms which work well in the laboratory do not necessarily thrive in industrial conditions. For example, commercial strains of unmodified *Z. mobilis* for use in ethanol production have been around for 50 years, but they are not used in any commercial ethanol facility because *Z. mobilis* is not very resilient and it dies rapidly, making it hard to harvest and recycle.[211] In addition, GM bacteria can be slow to produce ethanol: fermentation can take days, compared to a few hours for yeast working with glucose.[30]

A major difficulty in using non-yeast micro-organisms appears to be their low tolerance of ethanol. While some strains of brewer's yeast can tolerate high alcohol volumes, up to 20%, most other organisms tolerate much lower concentrations.[52] For example, researchers at the University of Florida were among the first to modify *E. coli* strains that could digest biomass, but these could only tolerate 4% ethanol in the fermentation broth. They have since developed strains able to tolerate up to 6.4% ethanol, but this is still much less than yeast. In 2006, Dupont announced it had engineered a strain of the bacterium *Z. mobilis* able to tolerate ethanol levels of 10%,[30] but this is still lower than ethanol concentrations in commercial refineries. And DuPont's claim is not supported by evidence from commercial production.

If the GM micro-organism is killed off at low ethanol concentrations, then the yield of ethanol in the final mixture will inevitably be lower. A recent analysis of ethanol production figures from Iogen's cellulosic facility suggests that the company was probably achieving ethanol yields in the region of 3-4%.[215] Lower ethanol yields mean more energy must be expended to extract the ethanol, and this has significant consequences for the net energy balance of the final fuel.

According to researchers in the field, approaches using single genes or small numbers of genes have made little progress in engineering bacteria able to withstand higher levels of ethanol and recent evidence has been accumulating that ethanol tolerance is controlled by multiple genes,[216] raising the question of whether such tolerance can even be achieved using current genetic modification techniques.

4.4 Consolidated biomass processing

Recently, a process referred to as consolidated biomass processing (CBP) has been proposed as a means of developing cellulosic ethanol. The aim would be to reduce the number of stages involved in the production of ethanol from cellulose by developing GM micro-organisms that are able to ferment cellulose directly, without the need to break it down to sugars first. Two approaches are either to genetically modify yeasts so they can break down the cellulose themselves, or else to modify bacteria and fungi capable of breaking down cellulose, such as *Clostridium thermocellum* or *Trichoderma* species, so they can produce ethanol.

A review in 2005 stated that no such organisms were available at that time.[217] But in 2007 the University of Massachusetts announced it had developed CBP techniques based upon *Clostridium phytofermentans*, which has been licensed to the US agrofuel companies Sunethanol and VeraSun.[218] A team at the University of Dartmouth in the United States is also working to develop a GM yeast capable of CBP.[219] Commercial R&D is being carried out by Mascoma with investment from General Motors[220] to develop a new generation of microbes and processes for economical conversion of cellulosic feedstocks into ethanol.[221]

However, the development of this process has come up against difficulties in engineering micro-organisms capable of producing cellulases in sufficient quantity. The breakdown of cellulosic material required for ethanol production requires very large amounts of the enzymes. It has been suggested that CBP may not be feasible because the cells of the GM micro-organism could not contain the volume of enzyme required, or pass it out sufficiently quickly into the biomass material.[198] A 2007 review of the state of the technology comments: 'the development of a CBP requires more fundamental research in many areas',[222] and a later review concludes success depends upon 'detailed understanding of the extremely complex genetic, enymatic and thermodynamic mechanisms that direct carbon flow'.[223]

An alternative approach is taken by Coskata Corporation whose process involves the gasification of lignocellulosic feedstock to produce a syngas of carbon monoxide (CO) and hydrogen (H_2), this syngas is directly converted to ethanol by 'proprietary microorganisms' (it is not known if they are genetically modified). Coskata claims it will be able to produce ethanol for under $1 per gallon.

Biobutanol

Instead of trying to create organisms to produce ethanol from cellulosic material another approach has been to produce butanol instead. Butanol has advantages over ethanol as a fuel. Its has an energy content about 40% higher than ethanol energy density which means vehicles running on butanol will travel further per litre of fuel. Unlike ethanol it does not absorb water (ethanol cannot be distributed using existing pipelines and high-percentage blends with petrol require alteration of vehicle engines). It can be used by itself as a fuel in conventional engines. However, it is difficult and costly to produce. Bacteria, in particular certain *Clostridium* species, can produce butanol from a range of sugars.[225] There are a number of problems associated with using the *Clostridium* species. The butanol becomes toxic to it at low concentrations, resulting in low yields, and it is relatively slow growing and has a spore-forming life cycle both of which create problems for producing economic quantities of butanol on an industrial scale. Furthermore, its relatively unknown genetic system and complex physiology present difficulties in engineering its metabolism for optimal production of butanol.[225 226]

In 2007, BP and Dupont announced plans to set up a pilot plant for the production of biobutanol in the UK, probably for use as a fuel additive.[227] Although the companies mention using agricultural waste as a feedstock, this first plant will in fact use glucose from sugar beet.[226]

Synthetic biology

Increasing numbers of researchers are turning to synthetic biology approaches as limitations are met in attempting to manipulate existing single pathways within species.[228] According to the UK's Royal Society, 'Synthetic biology is an emerging area of research that can broadly be described as the design and construction of novel artificial biological pathways, organisms or devices, or the redesign of existing natural biological systems. Biologists have traditionally sought to understand how life works. In contrast, synthetic biologists seek to design and build new biological systems.'[229]

A common approach is to start with industry's two most commonly used micro-organisms, *Eshcerichia coli* and *Saccharomyces cerevisiae*, due to their comparatively well understood genomes, fast growth rate and successful survival in other large-scale industrial processes.

These organisms are being explored for production of a whole range of fuels and petrochemical replacements, such as fatty acid synthesis for biodiesel production[230] and isobutanol.[224] However, these approaches face many of the same issues that have been described above. Even where useful metabolic pathways are identified, they must remain functional as by-products are produced and be capable of synthesising the target product at sufficiently high levels in a reasonable time frame for the process to be economically viable.

4.5 Environmental impact

Within the European Union, the use of genetically modified micro-organisms (GMMs) is governed by the Contained Use Directive (98/81/EC). This Directive requires that users of GMMs undertake a risk assessment to identify potential hazards to human health and the environment. Following the risk assessment, activities involving GMMs are then assigned to Classes 1 to 4 with Class 1 representing the lowest risk and Class 4 the highest. Because the GMMs being developed to degrade cellulose do not possess known pathogenic properties it is likely they will be placed in Class 1.

Survival in the environment

Containment measures applied to Class 1 organisms can be physical, chemical or biological and are not necessarily designed to ensure absolute containment but aim instead to 'limit their contact with humans and environment'.[231] In a UK government study of waste streams from industrial facilities in 2000 it was found that some facilities were releasing around 10,000 viable GM micro-organisms per litre of waste.[232]

Whilst it is common for GMMs to be based upon strains that have been 'disabled' to limit their reproductive abilities, micro-organisms for cellulosic ethanol production will have been modified to withstand extreme environments and will be designed to break down cellulose more efficiently than wild-type micro-organisms.

Horizontal gene transfer

Micro-organisms are able to pass genetic material between themselves, including to other species, via three main mechanisms:[233] transformation, in which free DNA is taken up by the cell; conjugation, which is the transfer of genetic material following cell-to-cell contact; and transduction, during which DNA is transferred between bacteria by viruses. In addition, there is evidence that yeast and other fungal organisms are able to acquire genetic material through horizontal gene transfer,[234] although this is still an area of debate.

It is possible that horizontal gene transfer may take place once the GMM is released into the environment; however, in the case of cellulosic ethanol production it may also happen at an earlier stage. The fermentation of cellulosic materials brings into proximity large quantities of naturally occurring micro-organisms and GM micro-organisms. The plant materials used as feedstock will bring with them vast numbers of the micro-organisms that live on plant surfaces, including plant disease organisms. During fermentation, the GM micro-organisms will be added to the mix. One report from a US pilot developed in the mid-1990s, describes attempts to use GM yeast to produce ethanol from maize stover.[235] The authors note that one of the major problems was contamination of the main process fermenters with lactic acid bacteria, which rapidly out-competed the GM yeast. Standard sterilisation procedures, such as steam cleaning, did not eliminate the contaminating bacteria, and the conclusion was that the bacteria were being brought in on the maize straw.

GM micro-organisms for cellulosic ethanol production are being provided with characteristics that could also be useful for plant disease organisms; in particular, the ability to produce enzymes that can break down plant material and/or the ability to make use of a wider range of substrates than naturally possible. Whilst there are plenty of research papers in the scientific literature detailing developments in the genetic modification of fermentation micro-organisms, our searches have not revealed any examining the potential risks. Nor have the companies involved in producing GMMs for cellulosic ethanol provided any detail as to their proposed containment practices. Even in the European Union, which has relatively stringent requirements, companies working with species classed as non-pathogenic, such as *E. coli* and *S. cerevisiae*, are required only to provide an initial notification. Further GM work can be carried out afterwards without further approval. The result is much of the detail about work on GM micro-organisms is known only to the companies involved. This is exemplified in a comment from a report by the UK's Department of the Environment Food and Rural Affairs (DEFRA), in which it is noted that even 'the total number of activities involving GM micro-organisms in the UK in containment is unknown'.[236]

4.6 Summary

Cellulosic ethanol is being widely promoted as the solution to many of the problems posed by first-generation agrofuels, such as concerns about diversion of food supplies, availability of agricultural land and the absolute limits imposed by the amount of sugar and grain crops that can be grown. In both the European Union and the United States, government strategies for the development of agrofuel production are based upon industry projections of when second-generation agrofuels, including cellulosic ethanol, will become a commercial option. For example, an assessment by the European Commission states that cellulosic ethanol production will 'take off' from 2014 onwards, and that all second-generation agrofuels will contribute 30% of domestic agrofuel requirements by 2020.[49] The result of these assumptions is that large amounts of funding are being directed into research aimed at developing cellulosic ethanol, including projects to develop GM organisms.

Research in this field has recently increased, particularly in the United States, where the government has provided large research grants. Enzyme preparations for breaking down cellulosic materials are already being produced, but until recently their main applications have been in paper making, detergents and animal feed. The production of ethanol from cellulose material demands different properties from enzyme mixtures and much greater volumes of enzyme. At present, even the market leaders in enzyme production do not have the production capacity to produce the amounts required for commercial-scale production of cellulosic ethanol.

A number of companies are working to develop cellulase enzymes for ethanol production. Approaches include genetic modification of fungi already being used in industry as well as bio-prospecting for genes and/or micro-organisms from a range of environments. However, the success of these efforts is difficult to establish because the details of work are often closely guarded by the companies involved, and requirements to place information about GM micro-organisms into the public domain are weak. What is clear is that, as yet, the high cost of cellulase enzymes make cellulosic ethanol uncompetitive with grain ethanol or petroleum.

There are a number of challenges to cellulosic ethanol production before commercial production can become a reality, such as the high capital costs, including the infrastructure investment required to produce and transport large quantities of biomass. And one of these hurdles is the dependence of cellulosic ethanol upon GM micro-organisms. The ultimate aim is to develop micro-organisms that can digest cellulose and produce ethanol, and while many companies and research groups are making claims to have done so, their work is rarely in the public domain. Projected yields from cellulosic ethanol are dependent, at least in part, upon the abilities of the GM micro-organisms to produce ethanol. So far, the GMMs appear to be struggling to produce the high yields obtained from ethanol production using sugar or grain crops.

The US government recently provided more than $700 million to assist the research and development of cellulosic ethanol. As yet, none of this appears to have been allocated for environmental risk assessment. The situation in the UK appears to be the same, and searches of the scientific literature by GeneWatch UK have failed to produce any published data.

The development of cellulosic ethanol is likely to be dependent on the successful genetic modification of micro-organisms that will alter their ability to survive in extreme environments and the substrates they utilise. Any large-scale cellulosic ethanol refinery will release some of these microbes into the environment. Prior to commercial development of cellulosic ethanol, it will be important to undertake a thorough environmental risk assessment. The financial and energy costs of any resultant containment measures will affect the viability of this technology.

5. Genetic modification of crops for cellulosic ethanol

Because of the difficulty of producing ethanol from cellulosic material, there is interest in developing GM plants whose biomass would be easier to process. The two approaches appear to be the production of GM plants with altered lignin composition and plants that will 'self-destruct' before or during processing. In the case of plants with altered lignin content, such research has been going on for some time. As with other areas of agrofuel research, it was initiated for other reasons, in this case with the aim of reducing costs for the paper industry. Because the driver was originally the paper industry, the focus of research was initially on trees. In the case of self-destructing crops, this approach has developed more recently, and most are still at the laboratory stage.

5.1 GM crops with reduced lignin

Lignin is the material that makes up the woody or fibrous part of most vascular plants. It is a complex polymer that can be found in different forms and is linked by chemical bonds to cellulose and hemicellulose, giving physical strength to the cell walls and the plant as a whole. Lignin is a key compound in the formation of xylem, the internal tubes that transport water within the plant, and it may also provide a defence against bacterial and insect attack. However, as it cannot be broken down into sugars, it cannot be converted to ethanol. From the perspective of ethanol production, lignin is undesirable. There is already a large body of research into the lignin content of trees, due to its importance to the paper industry. But, more recently, researchers have started to cite cellulosic ethanol as a rationale for this work.

Even a couple of years ago, the development of GM trees appeared to be in the doldrums. In 2005, according to researchers from universities in the United States and Chile, investment in GM research into trees was being cut around the world and companies in Chile were backing away from the use of GM trees because of concerns about public opinion in important markets such as the European Union.[237] Since then, there appears to be a renewed enthusiasm for research and development of GM trees, possibly spurred on by the interest in cellulosic ethanol. See Table 5. In 2005, Beijing University filed a patent for a GM white poplar with reduced lignin content.[238] In 2007, a joint Chinese and US research programme announced it had developed fast-growing GM eucalyptus trees with reduced lignin content.[239] Also in 2007, the University of Oregon gained permission to conduct field trials of GM reduced-lignin poplar trees and to allow them to reach flowering stage.[240]

By September 2007, 41 applications to conduct outdoor trials of GM plants with altered lignin composition had been notified to the US government,[241] although details of the area and numbers of trials being conducted is not publicly available. Just over a quarter of the notifications are for forage crops, but the rest are for GM poplar, pine and eucalyptus and other trees. One of the companies with most applications is ArborGen, which is owned by a consortium of paper and packaging companies. Both the University of Oregon and Michigan Tech University have been undertaking trials of GM trees; and in 2007 the US biotech company Mendel Biotechnology announced it would be extending its collaboration with the Swedish biotech company SweTre technologies on development of GM poplar trees, including lines with altered cellulose production.

Table 5. Companies undertaking work on GM trees with altered lignin synthesis

Company/organisation	Species modified	Genes taken from
ArborGen, California	Eucalyptus hybrids, [242] *Eucalyptus grandis*,[243] *Eucalyptus camaldulensis*,[244] Loblolly pine,[245] Pitch pine x loblolly pine,[246] Sweetgum[247]	Not known listed as confidential business information
GenFor Santiago, Chile	*Pinus radiata*[248]	Not known
INRA, France	*Populus alba* x *P. tremula*	Cauliflower mosaic virus, poplar
Mendel Biotechnology/ SweTre Technologies	Poplar[249]	Not known detail not in public domain
Michigan Tech University	Poplar[250]	Poplar, *E. coli*
Taiwan Forestry Research Institute and University of California	Eucalyptus hybrids[251]	Not known
University of Oregon	Poplar,[240] *Populus tremula* x *P. Alba*[252]	*P. tremuloides*, *E. coli* *P. tremuloides*, *E. coli*

Lignin synthesis is a complex metabolic process, and genetic modifications can result in unexpected effects. This is because altering the genes involved in lignin production can affect the expression of other genes in a number of metabolic pathways.[253] For example, when poplar trees were modified to produce less of one of the enzymes involved in lignin formation (cinnamyl alcohol dehydrogenase, CAD) the resulting GM trees contained slightly less lignin than normal and this was easier to extract during chemical processing. However, when poplar trees were modified to produce less of a different enzyme (caffeic acid *0*-methyl transferase, COMT), the resulting GM trees produced the same quantity of lignin as unmodified trees, but the structure and composition were very different.[254] Because lignin is part of the plant's defence against herbivorous insects and fungal diseases, altering lignin content has the potential to make GM plants more prone to pest attack.[255]

Altering lignin composition has also been shown to have wide-ranging impacts on the ability of the GM plants to grow normally. For example, studies by the French research institute INRA found that altering lignin content showed immediate effects on the ability of trees to support themselves. The impacts could be so severe that 'some lignin modified poplars that were shown to grow normally in the greenhouse ... were unable to do so in the nursery ...

and some transgenic lines were even unable to survive'.[256] In contrast, a group conducting genetic modifications on aspen (*Populus tremuloides*) found the GM trees grew significantly faster than unmodified lines.[257]

In comparison with crop plants, such as oilseed rape or wheat, trees are undomesticated. Breeding of plants for crop use often leads to the reduction of 'wild' traits that aid the plants' survival, such as effective seed dispersal, but these traits have not been removed from tree species. For example, poplar species are long lived and are able to regenerate vegetatively as well as by seed production, while aspen can reproduce using root suckers. And the hybrid poplar commonly called cottonwood (*Populus trichocarpa* x *P. deltoids*) produces offshoots that can be dispersed by water.[258]

There is a much greater risk of the transmission of GM traits from GM trees to wild tree populations. Poplars are completely out-crossing; in other words they must have pollen from another plant in order to produce seeds.[258] This means they have developed pollination forms designed to disperse the pollen as far as possible and, as they are wind pollinated, this can lead to long-distance movement of genes. In addition, the seeds of the poplar are themselves encapsulated in a cotton-like material, which enables them to be carried on the wind and by water. In the case of pine trees, it has been estimated there is a 100% probability of dispersal of transgenic pine pollen and seeds at distances greater than 1km. Because of their longevity, gene flow in trees can be far more complex than in annual crops [259] and natural or plantation woodland near to tree crops may contain related wild species.

Because of the major and unpredictable alterations to plant metabolism caused by genetic modification of lignin production, gene escape is a serious issue. For example, if tree seedlings fail to grow due to altered lignin composition this could affect replacement rates in forests. Or if GM trees grow faster than normal this could allow them to become invasive pests. Some researchers have suggested producing GM trees that lack the ability to flower, but tree pollen is an important food source for many organisms in forest ecosystems and so this itself could have adverse impacts on biodiversity.[260] Furthermore, as discussed above, many tree species can reproduce vegetatively so the trees would still have the ability to spread and propagate in the natural environment

Modification of lignin content is not limited to tree crops. In 2003 the company Biogemma applied to conduct field trials of maize and fescue grass with altered lignin content.[261] Research has been published on GM alfalfa with modified lignin levels which shows that more sugar could be extracted for ethanol production without chemical pre-treatment.[262] This development has been hailed as a step forwards for the production of ethanol from cellulose because pre-treatment is an expensive stage in the process. However, the researchers also found that some of the modified alfalfa lines showed yield reductions of up to 40% and increased branching. Further, the plants were not tested for their ability to grow in field conditions or their ability to resist pests.

Lignin composition of plants is related to carbon cycling and sequestration in the soil. A laboratory study of GM tobacco with modified lignin content showed increased rates of decomposition in soil,[263] and two studies with GM poplar trees have shown contradictory results, with one showing increased carbon dioxide production from decomposing GM material,[254] and the other not finding any differences.[264] Alterations to rates of decomposition, or the stability of carbon reserves in soil after harvest, could have an impact on how much carbon dioxide is stored in the soil when crops or trees are grown for agrofuels, and how much is released when they are harvested. It is frequently stated that a major reason for developing agrofuels is to reduce carbon emissions; therefore, the impact of genetic modification upon soil carbon cycles should be an important consideration.

Bt maize – too much lignin?

One of the most widely commercialised GM traits is insect resistance using genes derived from *Bacillus thuringiensis* (Bt). GM Bt maize has been found to contain significantly higher levels of lignin in its straw than unmodified maize. A study in 2001[265] found higher lignin content in all the varieties of GM Bt maize tested, whether grown in the laboratory or in the field. Levels of lignin ranged from 33% to 97% greater than unmodified versions of the same maize varieties. Reduction in lignin content is a key aim for the producers of cellulosic ethanol. So, if cellulosic ethanol ever does become a commercial reality, Bt maize could become an unattractive proposition for farmers hoping to supply the agrofuel market.

5.2 Self-destructing crops

One of the stages in the production of ethanol from plant biomass is the use of enzymes to break down the cellulose to its constituent sugars. This is a complex biochemical reaction involving a number of enzymes. Various companies and institutes are focused on GM micro-organisms to produce these enzymes (see Section 4), but there is also a strand of GM research focused on the idea of modifying plants to produce the enzymes within their cells. Clearly, if the plants produce such enzymes throughout their cells, the cellulose they produce would be degraded and the plant would die. So the GM plants have been engineered to store the enzymes within sealed cell structures.[266] Once released, for example by the application of a chemical at a particular stage of the plant's life, or during processing after harvest, the stored enzymes would cause the plant's cellulose to break down. It is hoped this would make the biomass easier to process into ethanol. Alternatively, the enzymes could be extracted and added to other non-GM biomass.

In 2006 a research group from the University of Michigan filed a patent for just such GM plants. The patent stated that the plants would be modified with bacterial genes, allowing them to accumulate cellulose-degrading enzymes.[267] It was suggested that milling the crop would break the plants' cells; releasing the enzymes and so degrading the plants' cellulose to sugars suitable for fermenting to ethanol. A patent for a similar approach was filed in 2007 by the South Korean research organisation Postech;[268] and laboratory tests have been conducted with GM tobacco,[269] potato,[270] barley,[271] maize[272] and rice[273 –] all producing cellulase enzymes within their tissues. The Swiss biotech company Syngenta has filed a patent for GM plants that would produce cellulose-degrading enzymes in response to the application of a chemical.[274] It has also formed a partnership with the biotech enzyme company Diversa, giving Syngenta exclusive access to the company's industrial enzymes for use in its GM crops.[275]

The research group at the University of Michigan has modified its GM plants so that the cellulase enzymes are stored in the space within the cell walls, known as the apoplast.[276] Plants use this space for the transport of water and as a route for absorbing and moving nutrients, hormones and carbon dioxide. The apoplastic space is also used for communication between cells and within the plant body as a whole, particularly in response to stress.[277] The projects modifying plants to produce cellulose enzymes within this space were confined to the laboratory and primarily focused on whether the modification was possible. While the researchers claim the plants were unaffected, it is not clear what level of assessment, other than visual, was undertaken to reach this conclusion.

The researchers argue the enzyme is safely contained within the cell walls; however, other scientists have commented that evidence for the structure and chemistry of plant cell walls is 'largely anecdotal'.[180] This raises questions about the prudence of introducing GM enzymes into a plant structure that is largely unstudied but is known to be important for plant metabolism. It also suggests there will be major gaps in the ability to assess the impact of the genetic modification. Further research is clearly required to establish the impact of producing cellulase enzymes within key plant structures. In spite of this, Michigan State University announced in 2007 that ten companies were interested in licensing their GM maize, and that field trials could begin within two or three years.[278]

Given the impact of this modification upon the growth, metabolism and processing properties of the crop, contamination of this trait into non-GM maize would be of serious concern. Maize is very prone to contamination as the pollen can travel a long way,[279] so the prospect of contamination of food crops with maize GM for ethanol production is a very real concern. The researchers claim the modifications are carried within the chloroplast DNA and the novel enzyme is not found in the seed kernel. Because chloroplast DNA is inherited maternally (i.e. not through pollen) they argue this eliminates concerns about contamination through pollen transfer. However, recent studies[280 281] have found that chloroplast DNA can be naturally transferred into the nucleus of plant cells at high rates. One of the research teams identified plants that could only have inherited genes from the chloroplast DNA of their parent plants,[281] suggesting that GM genes inserted into chloroplast DNA could be transferred through cross-pollination within a single generation. This calls into question the effectiveness of inserting genes into the chloroplast as a method of preventing their transfer to non-GM plants.

5.3 GM energy crops

The rising profile of biomass for heating, electricity generation and agrofuels has led to interest in so-called energy crops, such as switchgrass and miscanthus. Such crops could be used to supply a range of different biomass technologies. In March 2007 the biotech company Mendel Biotechnology acquired the entire miscanthus breeding programme of the German plant-breeding company Tinplant Biotechnik.[282] In June 2007 the petroleum giant BP became a shareholder in Mendel Biotechnology and announced it was collaborating with Mendel to develop perennial grass crops as energy feedstocks,[35] including breeding programmes in the United States, Germany and China. In April 2008, Mendel announced a collaboration with Monsanto to further develop perennial grasses for cellulosic fuel production.[283] In July 2007 another US biotechnology company, Ceres, announced it would be developing GM energy crops, including sorghum, switchgrass, miscanthus and energycane (sugar cane with high biomass).[284]

Energy crops such as switchgrass and miscanthus are not well studied, and are essentially undomesticated wild plants. There are already concerns that many of the proposed energy crops display natural traits similar to known invasive species.[285] One of the researchers who highlighted the issue commented: 'Most of the traits that are touted as great for biofuel crops – no known pests or diseases, rapid growth, high water-use efficiency – are red flags for invasion biologists.'[286] Given that the invasiveness potential of these plants has not been studied, great caution should be taken over the introduction of GM traits, such as insect resistance or increased yield, that could potentially increase the competitive ability of these plants.

Invasive species are one of the greatest threats to biodiversity worldwide. Further research is needed into the impact of large-scale plantings of the unmodified strains of the perennial grasses before even preliminary assessments can be made of the environmental impact of growing GM lines. The EU's Biofuels Research Advisory Council pointed out that 'the use of energy crops requires that bio-diversity and impact studies are carried out with a long lead time. Studies must start now for full implementation in 2020 and beyond.'[50] However, as yet there do not appear to be any funded research projects into the risks of growing GM energy crops.

5.4 Summary

Producing ethanol from biomass is a difficult process. Over millions of years, plants have evolved numerous mechanisms to defend themselves against attack from micro-organisms. These mechanisms act to hinder the breakdown of biomass to sugars. Genetic modification of food crops, trees and energy crops is being proposed as a solution to this problem. Apart from GM trees, which were already in development for other reasons, the research is still at an early stage. However, there is a range of issues that must be addressed well in advance of any releases of these GM plants.

Modification to alter the lignin content of GM plants affects an extremely complex aspect of plant metabolism. Published studies have shown that unexpected impacts are commonplace, including variations in growth rate, survival and decomposition. Lignin-modified GM trees pose particular risks in terms of the possibility of gene escape because tree pollen and seeds can move long distances. As trees are essentially undomesticated, the spread of GM traits into wild populations is much more of a risk than for crop plants. Many species of poplar are also capable of prolific and widespread vegetative (asexual) reproduction. Lignin modifications appear to have wide-ranging effects on the plants, and so the spread of such traits could change the ecological balance of receiving tree populations.

Lignin modifications also have the potential to impact on decomposition rates and carbon cycling in the soil. Results of published studies into this issue are contradictory. As it is often the stated aim of agrofuel production to reduce carbon emissions, further research is required to establish whether GM modification of lignin production could reduce carbon sequestration in soil, as has been suggested by some studies. If this were the case, then GM crops could actually reduce the carbon savings claimed for agrofuels.

Another approach to GM crop development is the idea of producing crops that produce cellulase enzymes. It is hoped such crops will reduce the need to add such enzymes during processing. However, there appears to have been little research into the impact on plant metabolism and disease resistance of such modifications. Production of cellulase within plant cells could potentially affect decomposition rates and nutrient cycling in the soil, or important agronomic characteristics such as disease resistance.

A number of plant species are now being considered as having potential to be 'energy crops'. These include perennial grasses such as miscanthus, switchgrass and sorghum. Although there is relatively little information in the public domain, at least two US biotechnology companies have started breeding programmes and modification of these plants. Plant species seen as useful for biomass production are chosen for their fast growth, resistance to disease and high biomass yield. Unfortunately, these are also traits that make them good candidates for developing into invasive species. Almost no research has been conducted into the potential for these crops to become invasive in different parts of the world where they could be grown. Until this basic research has been conducted, even preliminary assessments of the environmental impact of GM varieties will not be possible.

Development of GM crops and plants as feedstocks for second-generation agrofuels is concentrated in the United States. The information about these GM crops that is in the public domain is often limited to press releases provided by research institutes or private companies. The detail about the real success of the modifications, or the ability of the GM plants to grow well in field conditions, is generally classed as confidential business information. This means that claims made for the potential of these GM crops cannot be verified. At the same time, there is very little research into the environmental impact of introducing these crops, and in many areas fundamental research has yet to be undertaken. Taken together, the available evidence suggests that GM crops being developed for cellulosic ethanol are unknown quantities, and there is little capacity at present to assess their environmental or health risks.

6. Conclusions

Both current practice and future proposals for the production of agrofuels raise a wide range of important issues. Potential impacts are as wide ranging as the agriculture (and, in the future, forestry) upon which this new industry is based.

Powerful vested interests from the oil, car-manufacturing, agricultural and finance sectors are all involved in the current rush to develop agrofuel production. Environmental groups, aid agencies and community groups in affected areas are also trying to influence the course of its development. Policy makers are being required to make decisions on whether agrofuels really do reduce carbon emissions, whether they are fuelling habitat destruction, whether they are a viable route of development for developing countries, and whether they are distracting attention from other, more valuable, technologies. Against this background, the use of genetic modification in agrofuel production is only one technology amongst many. However, a clear understanding of the various technologies, their potential and their limitations should be central to assessing energy options and making policy decisions.

Assessing the pros and cons of agrofuels depends on a number of key issues:

1. Impact on reducing carbon emissions

The first generation of agrofuels has been widely criticised for making over-optimistic assumptions about the claimed benefits for mitigating climate change. Recent assessments suggest that burning some existing agrofuels, in some circumstances, may even be worse than burning oil. Although second-generation agrofuels are intended to address this problem, there is little evidence that any serious attempt has been made to thoroughly assess the likely climate impacts.

2. Impact on biodiversity

Industrial-scale production of agrofuels, whether GM or not, may have serious negative environmental impacts, associated with the use of intensive agriculture and monocultures. The use of a new generation of GM crops and micro-organisms raises new areas of concern, including the likely introduction of invasive traits; impacts on sensitive ecosystems on marginal land; the contamination of non-GM plants and micro-organisms and the potential spread of undesirable traits. The possible survival and spread in the environment of genetically modified micro-organisms designed to break down plant material is of particular concern.

3. Impact on food supply and land use

The production of first-generation agrofuels is having significant effects on land use and food prices, with serious negative consequences for poor people. Although second-generation agrofuels are intended to increase the use of non-food crops (such as grasses and trees) and agricultural waste (such as corn stalks), both these practices could still have major impacts on land use. Some GM plants grown for agrofuels could also cross-contaminate food crops, introducing new traits into the food chain with unknown consequences for human health.

4. Technical feasibility, costs and impact on alternatives

The use of agrofuels in general raises issues about whether this approach will undermine alternatives, such as better transport policies and planning and more efficient use of fuel. There are major technical limitations to producing second-generation agrofuels, and the likelihood that they will be developed in time to make a significant impact on climate change appears slim. The cost-effectiveness of these technologies is another issue, raising questions about whether money invested in research and development is being wisely spent.

A significant amount of research funding, both public and private, is being put into GM methods to develop agrofuel, particularly cellulosic ethanol. At the same time, almost no funding is being put towards an evaluation of the safety of these methods or their environmental impact.

The push for the GM route to agrofuel production is largely coming from the United States, but governments around the world are also succumbing to the appealing prospect being presented for cellulosic ethanol. Virtually every development in cellulosic ethanol is being patented, not least those relating to GM organisms.[287] Combined with the accepted practice of allowing companies to prevent publication of details of their technology on the grounds of commercial confidentiality, this means that the large quantities of research funding going into GM developments for agrofuels has produced only a trickle of publicly available data.

In the absence of evidence, policy makers are largely reliant upon statements and projections made by the industry. So claims are made for the ability of GM micro-organisms to efficiently convert biomass to ethanol; or that GM crops will increase yields of oil crops; or that GM biomass crops can be developed that will be easy to process into ethanol. Very little hard evidence is provided in support of these claims. Yet they feed into projections by the agrofuel industry for future production and the lead time required for commercialisation of second-generation agrofuels. In turn, these projections are used to determine policy and shift economies in the direction of agrofuel use.

6.1 Policy recommendations

The development of GM agrofuels raises serious questions in two important areas: whether research money is being wisely spent, and whether potential environmental impacts are being thoroughly considered. GeneWatch UK recommends:

1. A more realistic and independent appraisal of the potential impact of second-general GM biofuels is needed to inform policy decisions. This should include an assessment of the likely performance against key criteria, including: impact on climate, biodiversity, food supply and land use, and technical feasibility. It should be open about uncertainties, economic interests and how different social values (such as how people value biodiversity and impacts on food supplies in poorer countries) are likely to affect policy decisions.

2. Important gaps in research and regulation should be addressed. These include:

   - research on environmental impacts, including invasiveness, energy balance and the impact of factory-scale waste streams containing genetically modified micro-organisms;

   - consideration of major gaps in regulation, including regulation of waste streams containing genetically modified micro-organisms, and how the possible contamination of food crops with new traits from GM agrofuels will be addressed.

In general, more public involvement and debate is also needed to ensure that policy decisions, including research funding decisions, are not driven by a narrow range of vested interests.

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