The days of factories filled with vats fermenting medicines may be numbered. **Becky McCall** talks to the new breed of scientists designing plants and fungi that naturally manufacture cleaner, cheaper and safer drugs.

Pharmland

Could fields of sunflowers grow the next cancer drugs? Scientists are looking to plants to create the pharmaceuticals of the future.

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Pharmaceuticals produced in plants are less likely than animal-produced drugs to pass on any viral contamination to humans.

JOSHUA MYLNE

IT SHOULD HAVE BEEN a turning point in drug manufacturing. In the 1950s and '60s, a polio vaccine was given to millions of U.S. citizens in an effort to halt the spread of this disabling and deadly disease. But the vaccine was contaminated with Simian virus 40, a virus found in both monkeys and humans that enters the DNA, and which has since been controversially associated with aggressive bone, lung and brain cancers.

The contamination was due to the production of the polio vaccine in animal cells derived from rhesus monkeys originally caught in the wild.

Skip forward more than half a century, and in a similar scenario in 2010, the facilities of U.S.-based biotechnology company Genzyme were repeatedly shut down because of the viral contamination of an animal cell production line used to make a drug containing the enzyme glucocerebrosidase, to treat the rare genetic condition Gaucher disease.

Motivated by these incidents, biotechnology companies and drug regulatory agencies are shifting the focus from the use of animal cell lines for drug design and looking at the cheaper, cleaner alternative offered by plants.

"It's been argued for years that animal cell cultures may contain pathogens that could potentially enter the patient," says Pam Weathers, a plant biotechnologist at Worcester Polytechnic Institute in Massachusetts. "But with plant systems, that problem is eliminated.

"Unfortunately, it's taken major contamination incidents for this to come to a head, and for people to come to their bio-manufacturing senses, realising plants offer some real opportunities."

The fields of sunflowers that Mylne's grandfather grew for oil could one day act as bio-factories yielding cancer-fighting drugs.

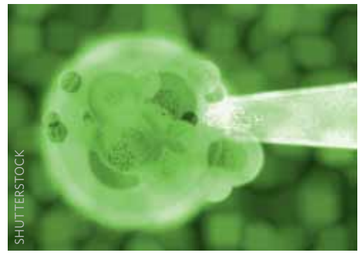
In the past 15 years or so, there has been an unprecedented surge in the development of highly efficacious protein and antibody-based drugs (collectively termed 'biologics'), which tackle diseases left untouched by older drugs. For example, rheumatoid arthritis was treated with the same drugs for decades until 2000, when a monoclonal antibody drug, etanercept, was developed in Chinese hamster ovary cells. Etanercept has revolutionised the treatment of this disease.

Like etanercept, most biologics are manufactured in living mammalian cell cultures. The most common way to manufacture drugs or vaccines in cell cultures involves inserting a protein's DNA into a bacterial or animal (often mammalian) cell. When the living cell replicates, the protein does too.

RECENTLY A PRODUCT similar to Genzyme's but made in plant-based systems, called taliglucerase alfa, has become the first plant-derived human pharmaceutical to enter the commercial market. Close on its heels is a vaccine for non-Hodgkin's lymphoma, which is now in an early phase clinical trial. The vaccine is created by 'infecting' a tobacco plant with bacteria containing the required genetic blueprint or therapy, and the plant then produces the target protein.

While some plant-derived drug proteins are best produced in bioreactors or vessels containing living plant cells or algae, others prefer whole plants grown in the field or in a special greenhouse, says Weathers. Moss, duckweed, clover, lettuce, alfalfa and tobacco have all been used this way.

The products – generally proteins – are usually made in the leaf tissue, but seeds also offer a viable route. Unlike leaves, seeds provide a naturally protective environment, which guards the target protein from enzyme breakdown and temperature change. Seeds are also geared up to naturally produce high levels of proteins, and so by default produce a high yield of the manufactured – or target – protein. >>



Some plant cells can be modified to produce therapeutic proteins in their seeds or leaves. If enough protein can be made, it can lead to new medicines to target disease.

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>> **SUNFLOWER SEEDS**, both natural and genetically modified, could be the perfect vehicle. Earlier in 2011 at the International Botanical Congress in Melbourne, Australian researchers suggested that sunflower seeds could produce a protein that's active against breast and prostate cancers. The beauty of the sunflower seed protein, known as sunflower trypsin inhibitor-1 (SFTI-1), is that it has the potential to also be effective against many other forms of cancer.

Joshua Mylne, from the Institute for Molecular Bioscience at the University of Queensland in Brisbane, is leading the research. Interestingly, Mylne has more than a passing interest in sunflowers. His grandfather was the first farmer to import and breed oil sunflowers on the Australian continent, two generations ago. The fields of sunflowers that Mylne's grandfather grew for oil could one day act as bio-factories yielding cancer-fighting drugs.

SFTI-1 was discovered in 1999 and works by stopping the activity of certain enzymes, called proteases, that break down proteins. SFTI-1 specifically inhibits a protease called trypsin, known to play a central part in the digestion of protein in mammals. In sunflowers, the presence of SFTI-1 in the seed is thought to act as a defence mechanism against insect attack. But in human cancer cells, SFTI-1 inhibits the proteases that digest healthy tissue around a tumour to create more room for it to grow.

Soon after its discovery, SFTI-1 was shown to be effective against a protease found in breast cancer. But despite considerable effort to design a synthetic drug based on its structure, no further significant advances were made for a decade. In 2009, a synthetically re-engineered version of SFTI-1 was shown to be effective against prostate cancer cells, but manufacturing costs and other difficulties have stifled development.

This is where Mylne's research comes in. "As a plant biologist, I wondered how plants actually made their SFTI-1. I work with a drug designer whose lab had to make a gram of peptide drug, and it was expensive and took them several months," Mylne recalls. "I just thought, 'There must be a better way to do this'."

He also wanted to understand if it was possible to modify the SFTI-1 precursor gene and produce tailor-made drugs in the plants themselves. By identifying the SFTI-1 gene, which was cleverly buried within another gene called PawS1, he was able to not only transfer this gene into another plant (*Arabidopsis* – known as thale cress) but also genetically modify it to specifically inhibit prostate cancer proteases.

In Joshua Mylne's laboratory, sunflowers are being grown that contain proteins that can inhibit cancer.

JOSHUA MYLNE

It's an early yet important step towards growing STF-1 within a high-yield plant crop, which could help produce much-needed cancer therapies. The next step is a leap from the laboratory bench to wide-scale production: where plant science meets the economics of mass manufacture.

PETER ROMAINE, a plant pathologist at Pennsylvania State University in the U.S., is working along similar lines, albeit with a less attractive production vehicle: mushrooms. In 2007, Romaine patented technology that enabled the genetic modification of mushrooms (*Agaricus bisporus*) into factories for producing therapeutic proteins, such as vaccines, monoclonal antibodies, hormones such as insulin or commercial enzymes such as cellulose for biofuels.

> "Pharma is resistant to change because the chicken-egg system is a rubber stamp. Regulatory agencies are also familiar with the old system."

Romaine's transgenic mushrooms are created by adding the desired gene to a stretch of circular DNA (termed a plasmid), which is inserted into a bacterium called *Agrobacterium*. When the bacteria mix with mushroom gill tissue, the DNA with the desired gene crosses into the mushroom. To date, this has been successful with 40 genes for different proteins.

The advantage of mushrooms over other genetically modified plants is the containment factor – mushrooms are more easily grown indoors. "If you move the plant indoors, then there's extra cost to contain the plants and supply lighting and maintenance," says Romaine. "Mushrooms are unique because they do not need sunlight, they can be grown in closed chambers providing high containment. We've done a production run in a mine 30 m down in Pittsburgh, Pennsylvania," he says.

"They are also amazingly economical because they grow on industrial waste products. You can obtain pounds of mushroom tissue, which is loaded with the protein of interest, for a couple of U.S. dollars," he adds.

The most valuable market for the transgenic mushroom is in biologic drugs aimed at the medical market. But it's likely that the first commercial application of the mushroom technology will be in the production of a specific industrial enzyme. Intrexon, a U.S.-based synthetic biology company, is currently providing field test quantities of this enzyme prior to commercial supply.

LIKE THE PLANT scientists, Romaine agrees that alternatives to animal cell production platforms have been a long time coming and that industry is slow to change. "Pharma is resistant to change because the chicken-egg system is a rubber stamp. Regulatory agencies are also familiar with the old system."

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Alexander Krichevsky, an ex-professor of biochemistry and cell biology at Stony Brook University in New York State who has recently set up his own biotechnology company, says plants offer many benefits over 'traditional' systems such as bacteria or mammalian cells.

"Production of recombinant proteins in plants means that you are allowing plants to effectively do the pharmaceutical work. They are the factories."

Within the plant-drug 'factory', gene encoding for the therapeutic protein is cloned and expressed in the plant, then plants are grown and the therapeutic product is extracted, he elaborates. "This approach is significantly more cost-effective, as it does not require fermenters, and plants can simply be grown in the field. Also, it's possible that some therapeutic proteins might not require extraction and can be ingested with food when produced in feed crops such as potato or lettuce, thus reducing cost of production even further," he adds (see "Malaria tea break" above).

Caution in the drug industry is warranted and expected, but evidence in favour of plant-based platforms is reaching a critical mass and becoming harder to ignore. "Every concern over drug manufacture you might have can be dealt with in a rational way – it's just a matter of time until regulatory agencies and the pharma industry become comfortable with the production of human drugs in new non-conventional platforms," says Romaine.

In commercial terms, the significant costs incurred by contamination incidents, loss of supply and customer confidence in animal pharma might just tip the balance in favour of plants. Industry is waking up to find that glorious landscapes of 1.8-m-high sunflowers are more than just a pretty sight; they really are fields of gold.

Becky McCall is a science writer and TV presenter based in London and a regular *Cosmos* contributor.

Malaria tea break

THE EXPENSE OF drug manufacturing becomes an even greater concern when making drugs for the developing world. Pharmaceutical companies have a tendency to develop drugs for wealthier countries to ensure investment return, while diseases affecting people in poorer countries are neglected. Malaria is a case in point.

Artemisinin is a drug active against the malaria parasite, extracted from the *Artemisia annua* plant, also known as a type of wormwood. It's been used for centuries and is probably one of the best-known plant-derived medical products. Currently the drug is extracted and purified from field-grown plants; however, the plants' yield of active ingredient is around 1–2% of dry weight – which isn't enough to treat malaria.

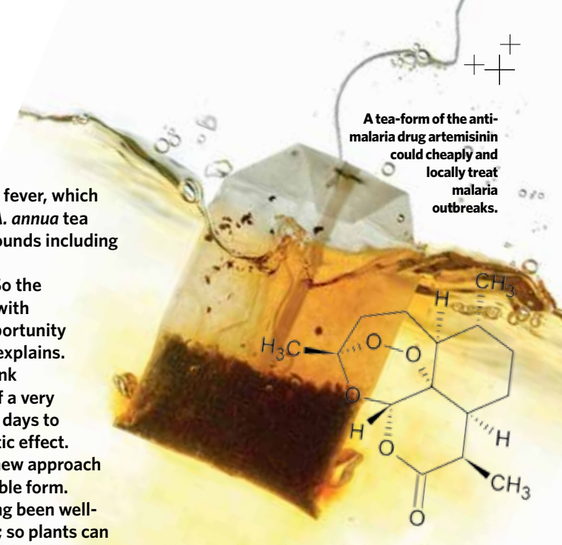
Plant biotechnologist Pam Weathers and her colleagues have suggested an alternative low-technology method for increasing the drug's accessibility, and maybe even combatting recently emerging artemisinin-resistant forms of the disease. "Artemisinin was used by the Chinese 2,000 years ago as a tea for fever, which was probably malaria. The *A. annua* tea contains a mixture of compounds including flavonoids, which also have some antimalarial activity. So the artemisinin in combination with flavonoids provides less opportunity to develop resistance," she explains.

Patients would have to drink excessively large amounts of a very bitter-tasting tea for several days to obtain the desired therapeutic effect. So researchers are trying a new approach that involves creating an edible form.

"Because the plant has long been well-tolerated as a tea, it's edible; so plants can be dried, the leaves pulverised, and drug content measured and dosing controlled through use of different-sized capsules to accommodate different body weights," explains Weathers. "Also, since artemisinin is relatively benign it can be given to pregnant women and children. It would also be dirt-cheap and could be produced locally, thereby empowering poor communities, which are the main populations suffering from malaria outbreaks.

"Our animal studies have shown that the drug readily passes from the plant material into the gut and then into the blood."

The scientists are waiting to see if the pulverised tea leaves work against malaria. Eventually, they hope to use a higher-yield strain of *A. annua* to make the edible form.



A tea-form of the anti-malaria drug artemisinin could cheaply and locally treat malaria outbreaks.

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