Ensuring Peanut Safety by Harnessing Plant Defences

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Scientia

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Fungal toxins that may accumulate in peanuts pose a hidden threat to people globally. Whereas European countries and the USA have controls to prevent contaminated seed from entering the market, this is not available in many developing countries, where peanuts are a vital source of protein and nutrients. However, detecting and controlling these toxins has posed significant scientific and economic challenges. **Dr Renée Arias**, **Dr Victor Sobolev** and **Dr Marshall Lamb** of the USDA National Peanut Research Laboratory have pioneered methods for inhibiting toxin production using RNAi technology and enhancing natural peanut defences.

A Versatile Superfood

Whether you love them or hate them, the fact remains that peanuts are a powerhouse in the food world. This superfood is high in vitamins, minerals, fibre, healthy oils and protein. Peanuts also offer protection against some types of cancer. An amazingly versatile food with a rich, distinct flavour, peanuts can be eaten straight out of their soft shells after roasting, boiled, turned into peanut oil, peanut butter or peanut flour, and are used as ingredients in an array of snacks and meals.

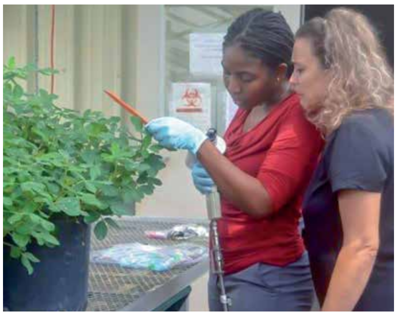
Although grouped with other nuts for culinary purposes, peanuts are actually a type of legume and are therefore more closely related to peas and beans than tree nuts such as almonds or cashews. They form underground inside soft, fibrous shells, and like other legumes, can turn nitrogen in the soil into amino acids and proteins.

Peanuts use a small fraction of the water required to grow tree nuts, making them a vital food resource in dry climates. Currently, peanuts are the main source of protein in Sub-Saharan Africa and for lower-income families in the USA. With droughts and water shortages predicted to escalate due to climate change, peanuts are likely to become an increasingly important resource in the future.

A Toxic Interloper

But what if eating your most important food was like playing a game of Russian Roulette? What if you had to make the choice between eating potentially contaminated food or going hungry? In some developing countries this is not merely hypothetical, but a reality faced every day.

Aspergillus is a genus of fungi found abundantly in many different environments across the globe. Perhaps more recognisable as the mould that thrives on damp household surfaces and can cause lung infections, some species of *Aspergillus* contaminate a range of crops including peanuts, rice, cereals, and beans.



Dr Mohammed performing DNA extractions from Aspergillus *isolates from Ethiopia.*

Peanuts are transformed in the laboratory and grown in tissue culture until the plants are large enough to transfer to greenhouse. CREDIT: Dr Dang

Once in the crop, the fungus proceeds to digest nutrients, create spores for reproduction and release metabolites – simple molecules that help the fungus to compete with other fungi. Some of these *Aspergillus* metabolites, called 'aflatoxins', are extremely toxic to humans and other mammals. The aflatoxins produced by *Aspergillus* are arguably some of the most dangerous toxins found in nature, being some of the most powerful cancer-causing substances, and potentially causing immune suppression, growth impairment in children, and severe toxicosis.

Dr Renée Arias, Dr Victor Sobolev and Dr Marshall Lamb of the United States Department of Agriculture (USDA) – National Peanut Research Laboratory, investigate the *Aspergillus* species commonly found in peanut crops across the globe and the natural resistance of peanut plants to fungal infections. Their aim is to develop methods of preventing *Aspergillus* infection in peanut crops. In addition to making peanuts safer to eat and preventing huge annual crop losses due to *Aspergillus* contamination, their work could be extended to other commonly-affected crops. Eventually, this could remove the risk of eating these foods in areas where appropriate government regulations and adequate laboratory testing are too costly.

Silencing *Aspergillus* Genes

The discovery of the ability of plants to shut down the genes within invading fungal cells opened a whole new world of possibility for controlling harmful fungi. The mechanism behind this is called RNAi – or 'Ribonucleic Acid interference'. RNA forms the basis of converting the information in DNA into cellular functions; therefore, if a plant produces a segment of RNA that matches and therefore interferes with a segment of RNA produced by a fungus, it can effectively neutralise the biological functions within the fungal cells.

Dr Arias and her team were the first to apply this concept to *Aspergillus* fungi in peanuts. Initially, the team conducted extensive surveys of the predominant *Aspergillus* species invading peanuts in Georgia, the state producing most of the peanuts within the USA, and in Eastern Ethiopia and Uganda, where peanuts form a vital part of the diet.

'For RNAi to be effective, the DNA sequence of the genes targeted needs to be known,' notes Dr Arias. Thus, the team followed up their surveys by investigating which *Aspergillus* genes are the most appropriate for targeting using RNAi technology. They achieved this by sequencing the entire genome of the most common *Aspergillus* strains they detected. Using advanced molecular techniques, Dr Arias and her colleague Dr Phat M. Dang then developed peanut plants that have the ability to produce aflatoxin-fighting RNA within their own cells. By preventing the genes in *Aspergillus* fungus from making aflatoxins, the accumulation of these toxins within the peanuts could also be avoided.

Dr Arias was keen to build on this success, by investigating the behaviour of the aflatoxin-fighting RNA within peanut crops. Her research team traced the RNA movement within the plants, and discovered that it moves towards the peanuts forming underground – ideal for targeting *Aspergillus* in the most important invasion area. Their findings also showed that the RNA multiplies within the plant over time, potentially offering increasing levels of protection.

To confirm the success of the technology, the team created and refined a highly sensitive aflatoxin testing technique, which requires only a quarter of a peanut per sample to obtain an accurate measurement of the amount of aflatoxin present. Previously, aflatoxin testing was labour and time intensive, and required a large sample of peanuts to get an accurate measurement. Using their new technique, Dr Arias and her team confirmed that aflatoxin accumulation in peanuts was 70% to 100% lower in their improved peanut plants.

The team aims to use these findings to develop broad-spectrum RNAi technology that will allow control of a wider range of *Aspergillus* species with a single method, and potentially extend to other food crops vulnerable to the fungus.

'We believe that its application in breeding of peanut and other crops will bring rapid advancement in this important area of science, medicine and human nutrition,' says Dr Arias, 'and will significantly contribute to the international effort to control aflatoxins.'

Searching for Naturally Resistant Peanuts

The development of the team's reliable and accurate aflatoxin testing techniques also offered the opportunity to revisit the potential natural resistance of wild peanuts to *Aspergillus* fungi. As with all living organisms, wild peanuts exhibit different individual characteristics, including some that make them less vulnerable to attack by *Aspergillus* fungi. This means that some wild peanut varieties stored in seed collections could hold the 'genetic key' to unlocking additional natural resistance in cultivated peanut plants.

By accounting for differences in viability of aged peanuts, Dr Sobolev was able to reliably investigate the wild peanut 'germplasm' – genetic material of the plant contained within the peanuts themselves – in stored collections, with the aim of identifying individuals exhibiting natural *Aspergillus* resistance. 'We identified several wild species of peanuts that did not accumulate aflatoxins,' says Dr Sobolev. 'Some germplasm identified as resistant has been incorporated in our breeding program.'

A fundamental component of the natural defence against *Aspergillus* invasion is a group of relatively simple molecules, called stilbenoids, which exhibit antifungal properties. Peanut plants begin producing these defensive substances in response to invasion by *Aspergillus*. Dr Sobolev and the team demonstrated for the first time that stilbenoids not only effectively impede fungal growth, but also inhibit aflatoxin production by the fungus when it interacts with damaged peanuts. This could be especially important for alleviating some of the vulnerability of damaged areas of the plant to fungal invasion.

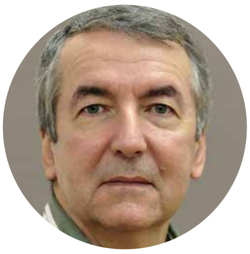
Dr Sobolev and his team conducted a comprehensive study of the properties of previously known and newly discovered stilbenoids, revealing the additional health benefits of these molecules to humans and other mammals. Their work indicates that stilbenoids have anti-inflammatory, cancer-fighting, and antioxidant properties, warranting further medical research.

The Future of Aflatoxin Eradication

Currently, the team is conducting extensive field-based experiments with their improved peanut cultivars, to ensure complete aflatoxin inhibition when the plants are exposed to *Aspergillus* under the variable and sometimes unpredictable environmental conditions found in nature. Additionally, they are continuing to develop peanut plants resistant to a wider range of *Aspergillus* species, with the aim of developing plants resistant to all the potential *Aspergillus* invaders.

In developing targeted RNAi technology and *Aspergillus*-resistant peanut plants, Dr Arias and Dr Sobolev, with the support of research leader Dr Marshall Lamb, have succeeded in inhibiting the accumulation of dangerous aflatoxins in peanuts. Their work has the potential to dramatically increase the safety of consuming peanuts across the globe, including in developing countries and lower-income areas, where government regulations and expensive testing may not be available. Also, by reducing the vast quantity of peanuts lost annually to aflatoxin contamination, this has the added benefit of improving access to a healthy and nutritious food source, even with predicted human population growth in the future.

By using multiple complementary approaches to reducing aflatoxin accumulation in peanuts, such as RNAi technology along with stilbenoid production, the team hopes to reach a global solution faster. 'Eliminating aflatoxins from the food chain is a world race in which scientists from different disciplines using multiple strategies should participate,' concludes Dr Arias, 'but no matter who gets to the finish line first, we all win.'



Meet the researchers

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Dr Renée Arias earned her PhD at the University of Hawaii, after which she took a postdoctoral position in the Department of Biosystems Engineering at the same University. Currently, Dr Arias is a Research Pathologist at the USDA Agricultural Research Service (USDA-ARS) National Peanut Research Laboratory, in Dawson, Georgia. Here, her research focuses on fungal diseases of peanuts, and particularly on methods of inhibiting the accumulation of aflatoxins produced by *Aspergillus* species. Dr Arias collaborates with researchers from around the world, and has received multiple awards for scientific merit, including the Presidential Early Career Scientist of the Year Award for USDA in 2014.

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Dr Victor Sobolev earned his PhD at the Russian Academy of Medical Sciences, Moscow, Russia. His chemistry research has led him to the USDA-ARS National Peanut Research Laboratory, where he currently researches the chemistry of aflatoxins and stilbenoids in peanut-*Aspergillus* interactions. Dr Sobolev has dedicated much of his time to teach many specialist courses on the analysis of aflatoxins and other mycotoxins in food and agricultural products.

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Dr Marshall Lamb achieved his PhD at Auburn University in 1995. He currently serves as Research Leader at the USDA/ARS National Peanut Research Laboratory and Lead Scientist on the USDA-ARS research project titled 'Enhancing the Competitiveness of US Peanuts and Peanut-based Cropping Systems'. In addition to his research, Dr Lamb has developed an expert system for farm management and marketing risk management (WholeFarm), and several systems for crop irrigation.

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