Delving into the world of **isoprene**

Dr Tom Sharkey discusses just what isoprene means for atmosphere-plant gas exchanges and shares the importance of his work predicting isoprene emissions

Can you explain what isoprene emissions are?

Not all plants make isoprene, but plants from widely divergent phylogenetic positions – for example some flowering plants and mosses – emit a tremendous amount of it.

One advantage of this emission for plants appears to be tolerance of a particular type of heat stress. This is the kind of stress that occurs when leaves in full sunlight heat up and cool down rapidly from large heat loads of sunlight, the cooling effects of occasional breezes, and the small heat capacities of thin leaves. Plants that get hot and stay hot all day tend not to produce isoprene, as that type of stress appears to be better dealt with by other mechanisms. Plants that use a lot of water, for example most crop plants, stay cool by evaporative cooling, but for leaves at the top of an oak tree, much less evaporative cooling occurs relative to most crop plants, and they are exposed to full sunlight and occasional breezes. Leaves at the top make much more isoprene than leaves at the bottom of the same tree when put under identical conditions.

The disadvantage of emitting isoprene is the large energy and carbon cost to the plant. At the top of an oak tree, when little water is being lost because stomata are closed, there will be a significant resistance to CO_2 diffusion into the leaf. When combined with this resistance, the loss of carbon as isoprene is especially costly to the tree. Interestingly, phylogenetic analyses indicate that the ability to emit isoprene has been lost many times, for example in soybean, a close relative of the very prolific emitter kudzu (*Pureria lobata*). Soybean has genes or pseudogenes indicating that they probably once made isoprene synthase enzymes.

Could you share why you are working with isoprene emissions from plants?

In the late 1970s I read reports of isoprene emission from plants, especially trees. As a graduate student studying photosynthesis I found that this was a fascinating example of another gas exchanged between plants and the atmosphere. The early reports were from Professor Guivi Sanadze in Tblisi, Georgia, in the *Russian Journal of Plant Physiology*. He suggested that isoprene emission might be related to abiotic stress in plants, for example heat stress or lack of water. Together with Bill Hitz, we made a few measurements of isoprene emissions from velvet beans (*Mucuna puriens*) and found the lack of water stimulated isoprene emission. However, it wasn't until a decade later, in the late 1980s, that I was able to devote significant effort to studying isoprene emission.

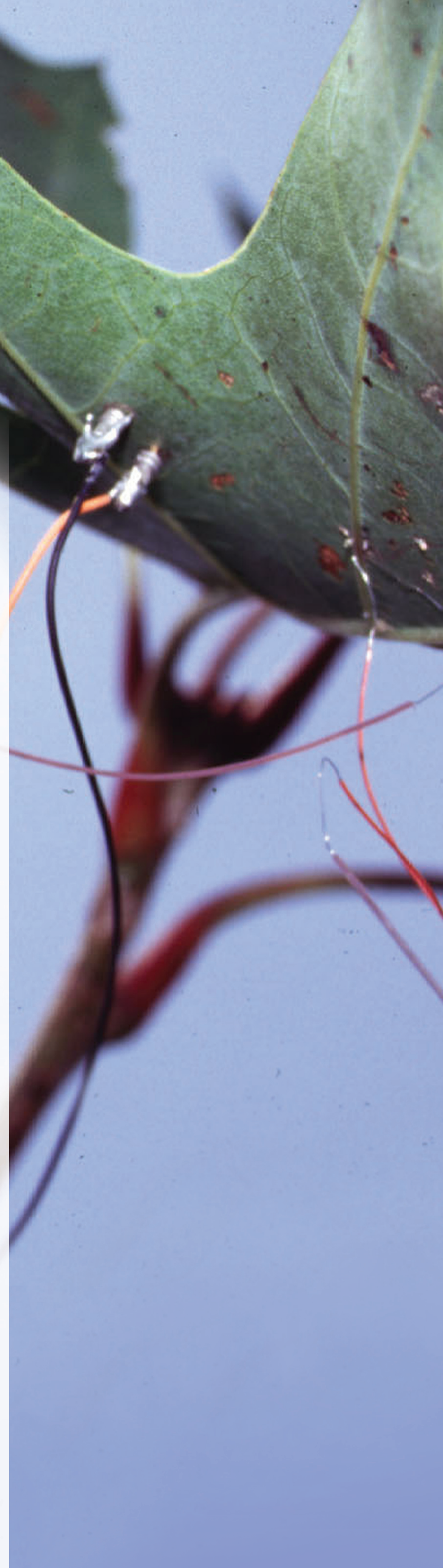
Could you outline the skills represented by your partners and research team?

Up until now, my laboratory has focused on the isoprene synthases. There are two major platforms currently available; the kudzu isoprene synthase sequenced in my laboratory and the isoprene synthases from poplars and aspens, which are very close in sequence, and have been reported by several laboratories, including mine. These are being modified to perform better in commercial production. My graduate students are also studying the regulation of the pathway that makes the precursor for isoprene.

How extensive is the field of isoprene research today?

Science is a social process and there are a number of people who have played important roles in isoprene research. Guivi Sanadze certainly deserves credit as the discoverer of this phenomenon. Reinhold Rasmussen discovered it independently, but slightly later and made many contributions. David Tingey in Oregon did some of the best work on physiology of the process during the 1970s and early 1980s. Moreover, Russ Monson and Ray Fall and their colleagues at the University of Colorado started what might be called the modern era of isoprene research with their 1989 paper, and they have made many contributions since.

At the time I was happy to have a postdoctoral associate, Francesco Loreto, who came to the U.S. to study photosynthesis, but was willing to be side-tracked into the then almost unknown field of isoprene research, and he has been a major contributor since returning from Italy. Wolfgang Zimmer was the first to clone isoprene synthase and his colleague Jörg-Peter Schnitzler has made many contributions since. Nick Hewitt in Lancaster, UK and his colleagues, especially Malcolm Possell and Claudia Vickers (now in Australia), have also contributed significantly. Once bitten by the isoprene bug many scientists find the topic an irresistible line of research. I am most certainly among those.



Developing efficient commercial isoprene applications

Studies being undertaken at **Michigan State University** will help build a platform to better understand how isoprene production in microbes can be made more commercially viable

A RESEARCH TEAM based at the Department of Biochemistry and Molecular Biology at Michigan State University is looking at the function and regulation of isoprene synthesis in leaves. Professor Thomas D Sharkey, who is heading up the group of scientists, describes how the evolutionary origin of the biochemical capacity for isoprene emission can be surmised for some flowering plants. He observes that the gene that encodes the isoprene synthase looks very similar to the genes that encode enzymes, which are responsible for generating plant odours such as pine scent (monoterpene synthases): "I have hypothesised, and subsequent crystal structures reported by others have confirmed, that two specific changes in monoterpene synthases may be sufficient to convert a monoterpene synthase gene into an isoprene synthase gene".

This gene family can be traced back through evolution. Evolutionary pressure caused by short episodes of high temperature suffered by some leaves may explain why some plants make isoprene and others do not. Simply put, plants whose leaves suffer these heat flecks are more likely to survive if they have an isoprene synthase gene, keeping isoprene synthase in the gene pool. Plants that do not suffer such heat flecks, but lose a lot of carbon as isoprene, are thought to be at a disadvantage, and thus isoprene emission has died out in these species.

USING GAS TO MEASURE EMISSIONS

The methodology employed by the research team is based on measurements of gas exchange between leaves and air. Typically, a leaf is enclosed in a chamber and air, or synthetic air mixed from nitrogen, oxygen and carbon dioxide, is passed over the leaf. The air entering the chamber will have no isoprene, but the air leaving the chamber will have some amount, sometimes 100 parts per billion of isoprene. Sharkey explains that this is easily measured by gas chromatography, or by chemiluminescent methods in a machine designed just for isoprene measurements and now sold commercially by Hills Scientific in Colorado. In addition, Sharkey's team has made high-speed measurements of leaf temperature to test some of their theories about isoprene and leaf temperature using a specially-designed fine wire thermocouple.

For trees, there are techniques for measuring a great range of scales, from parts of a leaf up to the scale of a forest. Beyond that, atmospheric scientists can use modelling up to the global scale. This, says Sharkey, is how a number can be estimated for the global production of isoprene. Despite U.S. Presidential candidate Ronald Reagan's warning in 1980 that plants pollute more than people, isoprene emission is a natural process and not one Sharkey believes we can control globally: "To date, I know of no decision about which species of tree to plant that has been affected by whether the chosen tree emits isoprene". The fact that there is so much isoprene coming from trees means that we need to address atmospheric pollution problems differently than if this emission source were lower.

ENGINEERING BIO-ISOPRENE PRODUCTION

Today, most common automobile tyres are produced from rubber or latex-bearing trees, but this is not considered to be particularly eco-friendly. Sharkey's team are investigating an isoprene-inspired alternative to this more traditional form of rubber: "Once it was clear just how much isoprene plants make, and how important polyisoprene (rubber) is for making tyres, it was natural to wonder if a

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The enzymes he has cloned have been used by several companies to develop methods of synthesising isoprene either in bacteria or yeast. His ultimate dream is to have photosynthetic bacteria making isoprene, where carbon dioxide would be pumped in one end of a reactor and isoprene would be collected at the other end: "Before this dream is realised, however, it is likely that a simpler system in which sugars from some source – hopefully not in competition with the food supply – would be converted to isoprene by bacteria or yeast using my enzymes".

Over the past few years, the price of rubber has increased dramatically, so Sharkey's team are working with a company (ZuvaChem, Inc.) to develop methods for efficient commercial isoprene production. He notes that two other companies, Genencor and Amyris, are also important in this arena, as well as several smaller companies who all are working toward this goal. It is hoped that competition among these companies will lead to effective new ways to engineer bio-isoprene production, ultimately keeping costs low using this renewable rubber alternative. It appears that there is now a growing interest in engineering bacteria to make isoprene as a chemical feedstock for the global tyre industry.

The researchers are also investigating the regulation of the rate of isoprene emission. They are studying both isoprene synthase regulation and regulation of the pathway that makes the precursor to isoprene. Sharkey says that this same pathway is responsible for the synthesis of carotenoids in plants (the orange of carrots and the red of tomatoes, etc.). The information that will come from these studies will help predict isoprene emission and the effects of global change on future isoprene emissions, and will also help to design bacteria for efficient production of isoprene.

THE IMPORTANCE OF THERMOTOLERANCE

It is also thought that isoprene could affect membrane stability or could quench (detoxify) reactive oxygen species (ROS). Sharkey says it is possible that increased membrane stability accounts for thermotolerance and quenching accounts for ROS protection. On the other

hand, both heat damage and ozone damage could have mechanisms in common: "It has been proposed that heat causes damage by inducing ROS and therefore ROS quenching could underlie both thermotolerance and ozone protection," Sharkey notes. However, because both high temperature and ozone can cause membrane breakdown, he thinks that membrane stability effects, rather than ROS quenching, could underlie both thermotolerance and ozone tolerance. This is now an active area of the group's research.

The main challenge that the research project has encountered during the investigations into isoprene emissions and the creation of renewable tyres is that the commercial production of isoprene depends on achieving consistent and high yields of isoprene from bacteria or yeast. From Sharkey's perspective the system works well at the laboratory scale, but a great deal of work remains.

On a positive note, Sharkey considers the most significant achievement of his laboratory colleagues to be the thermotolerance hypothesis for why plants emit isoprene. Over time, this has come to be accepted as the most likely explanation for why isoprene emission persists in some species and it has significant explanatory power: "Isoprene results in other effects, including protecting against ozone, but thermotolerance is likely a better explanation for why we see the particular distribution of emitting and non-emitting species". This theory also gives rise to hypotheses about the mechanism of isoprene action. These hypotheses are currently under intense investigation and Sharkey has high hopes for significant breakthroughs in the near future.

INTELLIGENCE

FUNCTION AND REGULATION OF ISOPRENE SYNTHESIS IN LEAVES

OBJECTIVES

To understand the function and regulation of isoprene emission from plants, experiments will be undertaken to test and determine how plants that emit isoprene are affected by this emission, utilising novel optical methods to measure the leakiness of photosynthetic membranes and determine how isoprene affects this leakiness.

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CONTACT

Dr Thomas D Sharkey
Professor and Chair

Michigan State University
Dept Biochemistry and Molecular Biology
410 Biochemistry Building East Lansing
MI 48824
USA

T +1 517 353 3257
E tsharkey@msu.edu

DR THOMAS D SHARKEY received both his BS and PhD degrees from Michigan State University. Following a postdoctoral fellowship at the Australian National University, he moved to the Desert Research Institute in Reno Nevada as a Research Professor. After five years in Reno, Sharkey spent 20 years in the Department of Botany at the University of Wisconsin-Madison. He returned to MSU as the Chair of the Department of Biochemistry and Molecular Biology because of the strong plant biology community at MSU.

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