

SCIENCE FOR SOUTH AFRICA Quest

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mystery of DNA*

*International Year
of Plant Health*

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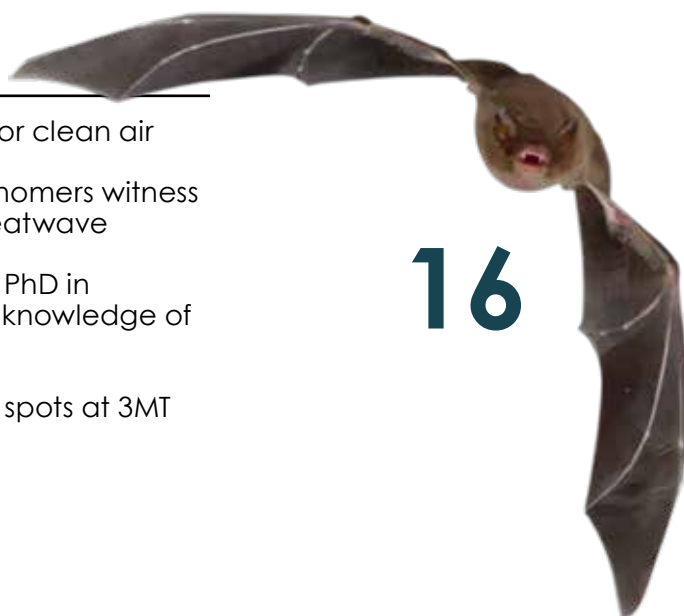
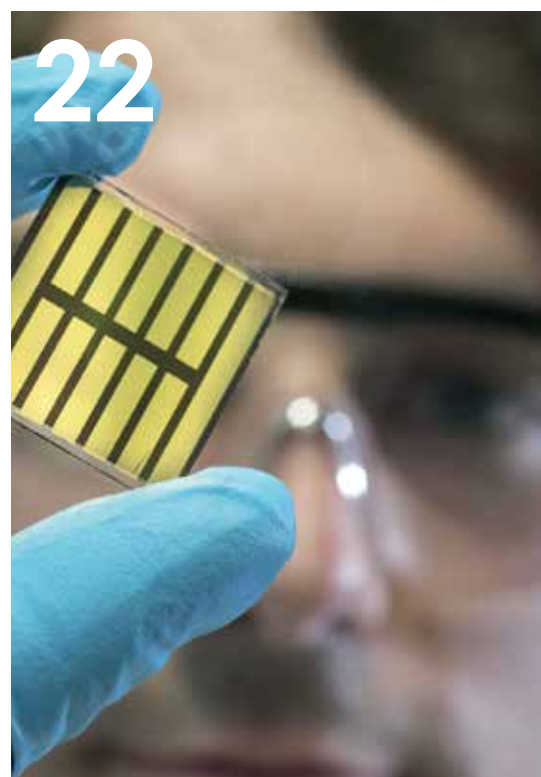
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Editorial enquiries

The Editor | e-mail: Quest-Editor@assaf.org.za

Advertising enquiries

Barbara Spence | Avenue Advertising
PO Box 71308, Bryanston 2021
Tel: (011) 463 7940 | Cell: 082 881 3454
e-mail: barbara@avenue.co.za

Subscription enquiries and back issues

Tsepo Majake | Tel: (012) 349 6645
e-mail: tsepo@assaf.org.za

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EDITOR'S NOTE

Protecting our plants

This year has been declared the International Year of Plant Health (IYPH) by the United Nations (UN) General Assembly, the idea being to raise awareness on how protecting plant health can help end hunger, reduce poverty, protect the environment, and boost economic development.

IYPH activities are being spear-headed by the UN Food and Agriculture Organisation (FAO) and the secretariat of the International Plant Protection Convention (IPPC) – a global treaty to protect the world's plant resources from the harm caused by pests and diseases. South Africa is one of the 184 contracting parties to the IPPC, and abides by phytosanitary standards designed to prevent the spread of pests and disease-causing pathogens through cross-border trade activities.

Other human activities that have ultimately resulted in altered ecosystems and climate change have also allowed pests and pathogens to thrive in areas where they may have been manageable before. It is estimated that pests and diseases now cause up to 40% of global food crops to be lost annually, which not only threatens food security, but pushes up the price of food. Plants also provide raw materials for our buildings, clothes, medicines and biofuels, so losses in agricultural products due to pests and diseases amount to some US\$220 billion per year. Rural poor communities are the worst affected, because they depend

more on agricultural resources for subsistence and income.

Of course, plants also have considerable environmental and aesthetic value – they produce the oxygen we breathe, promote biodiversity by providing habitat, play an important role in climate regulation, and increase our enjoyment of gardens, parks and streets. Yet over the past two years, city dwellers in South Africa have witnessed the destruction wreaked by a tiny beetle from South East Asia, the polyphagous shothole borer, which has killed thousands of urban trees by tunnelling into the wood and introducing a symbiotic fungus that causes dieback. The pest also poses a potential threat to some commercial crops, such as avocado and pecan.

In this issue of *Quest* we show some ways in which scientific research is helping to protect plants from pests and diseases, manage the risks and mitigate the impacts.

Sue Matthews
QUEST Editor



Lesisqephu se *Quest* sibheka izinambuzane, nezifo ezihlasela izitshalo. Ngokulimaza kwazo izitshalo zehlisa ukutholakala kokudla bese zenyusa intengo yakho. Ukuvikela izitshalo ezinambuzaneni nasezifweni kungasiza futhi kweseke nemizamo yokuqeda indlala, kwehlise inhlupheko, kuvikele imvelo, kwenyuse namandla omnotho.

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IPM

Quest explores Integrated Pest Management as a means of ensuring healthy plants



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Promoting plant health by controlling pests and diseases cannot be achieved purely by spraying pesticides. Apart from the threat to non-target organisms and human health, many pests and disease-causing pathogens have developed resistance to commonly used pesticides, rendering them ineffective.

Integrated Pest Management (IPM) uses a combination of methods to prevent disease and the development of pest populations above a particular 'economic threshold' level. It is an ecosystem approach that requires careful consideration of all available options, and encourages natural control mechanisms as far as possible, with pesticides used in a manner that minimises the risk to people and the environment. The options are typically grouped into the following categories.



Kay Ledbetter, Texas A&M AgriLife Research, CC BY-NC-ND 2.0

Cultural control

These methods require an understanding of the environmental factors and agricultural practices that allow a pest or disease to thrive, followed by actions to make its conditions suboptimal. This may entail, for example, changing the timing of planting or harvesting, altering irrigation or pruning practices, rotating crops more frequently, as well as planting crop cultivars that were selectively bred for their resistance to pests and diseases. Good farm sanitation, such as removing plant debris and cleaning farm machinery and equipment after use, can also help prevent the spread of pests and diseases.

Biological control

Biological control is the use of natural enemies, such as predators, parasites and pathogens, to control pests, weeds and diseases. In the case of invasive alien pests, the biocontrol agents are introduced from the pest's natural distribution range or country of origin after careful screening to ensure they won't attack indigenous species. For indigenous pests, the natural enemies that attack or outcompete the problem species can be released in the area, or their populations can be promoted through habitat management and other means of optimising their environment. This is known as natural control or conservation biological control.



Scott Bauer, USDA



Vine Nets Australia

Mechanical and physical control

These methods either kill the pest or pathogen, or prevent pests from reaching the plant. They include handpicking, traps of various kinds, barriers such as fences, nets and screens, as well as soil sterilisation using steam, gamma irradiation, or plastic sheeting to trap the sun's heat.

Chemical control

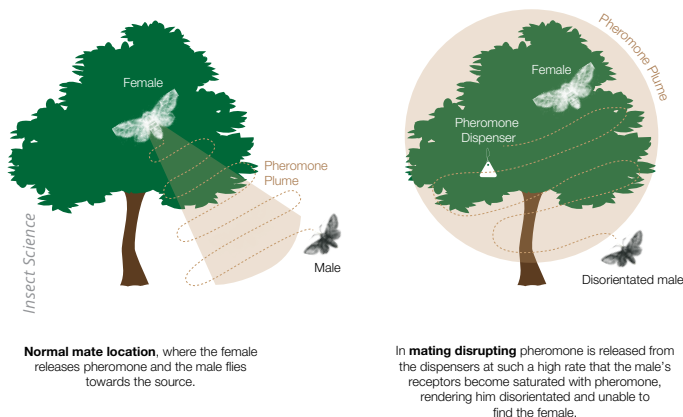
Pesticides should be used according to established guidelines, and only when other methods have failed to control the pest or disease. Selective pesticides that kill the target pest or disease-causing pathogen with minimal damage to populations of beneficial natural enemies should be chosen.

Sterile insect technique

This technique is used as part of an area-wide IPM programme for specific production regions, rather than at the farmer's field level. It involves mass-rearing the insect pest in a breeding facility and then using ionising radiation with X-rays or gamma rays to sterilise the males. These are released in the area on an ongoing basis in great numbers – typically millions per week. The sterile males mate with 'wild' females, which will produce no offspring. Over time the population will decline, and eventually be reduced to low levels or eradicated. Only a few pests in South Africa are controlled this way, among them the Mediterranean fruit fly and the false codling moth.



Whale Coast Conservation



Mating disruption

Mating disruption is a pest-management technique that uses artificial stimuli to prevent male insects from finding females and mating. It is mostly used for moth pests, as the females release chemical signals known as sex pheromones to attract a mate. Synthetic sex pheromones released from dispensers placed among crops will confuse males and limit their ability to locate the 'calling' females. This reduces the likelihood of successful mating, helping to control the pest population. The technique is most effective early in the season, when the pest's numbers are low. It has the advantage of being highly selective, with no control of non-target insects.



Eugene E. Nelson, Bugwood.org

Semios

Pheromone traps used to monitor pest populations rely on a similar approach, but in this case the insects are lured by the synthetic pheromone into the trap, where they are caught on a sticky surface.



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Fall armyworm in maize

Managing the local risk of infestation

By Hannalene du Plessis, Johnnie van den Berg and Annemie Erasmus

The fall armyworm (*Spodoptera frugiperda*) was first reported in South Africa in January 2017. This pest has its origin in the tropical and subtropical areas of South America and the most southern states of the USA. In the past three years it has also spread to India, the Far East and most recently Egypt, from where it threatens maize crop production in Europe.

Although the fall armyworm is capable of feeding on approximately 350 different plant species, the African population of this pest is largely only important on maize and sorghum. Limited damage has been reported on rice, sugarcane, soybean, millet and cotton.

Crop loss due to fall armyworm damage in African countries with warmer climates, where pest populations persist throughout the year, is significantly higher than in South Africa. Fall armyworm has been reported to

potentially cause combined maize yield losses of 8–27 million tons per annum in 12 Central and East African countries.

Status in South Africa

The status of a migratory and tropical pest such as fall armyworm is largely determined by the ability of pest populations to persist in maize production regions throughout



John C. French Sr, Bugwood.org, CC BY-NC 3.0

the year, and to survive the winter. Pest status outside of the areas where fall armyworm occurs permanently is determined by the migration patterns of moths and climate suitability for survival.

Fall armyworm moths have both a long-range migratory habit and a more localised dispersal habit. In the migratory habit, moths can migrate over 500 km before oviposition. Depending on wind patterns, moths can travel much larger distances – up to 1 000 km. It is therefore important to distinguish between areas where environmental conditions will always be suitable for fall armyworm development and survival, and areas where fall armyworm will migrate to during times when environmental conditions change and become temporarily suitable for their development and survival.

The risk of pest infestation and management strategies differ in different scenarios, such as areas where sporadic infestations occur, plant growth stage at time of infestation, and whether the infestation is on seed production or commercial fields.



G. Goergen, IITA, CC BY-NC-SA 2.0

Effect of temperature

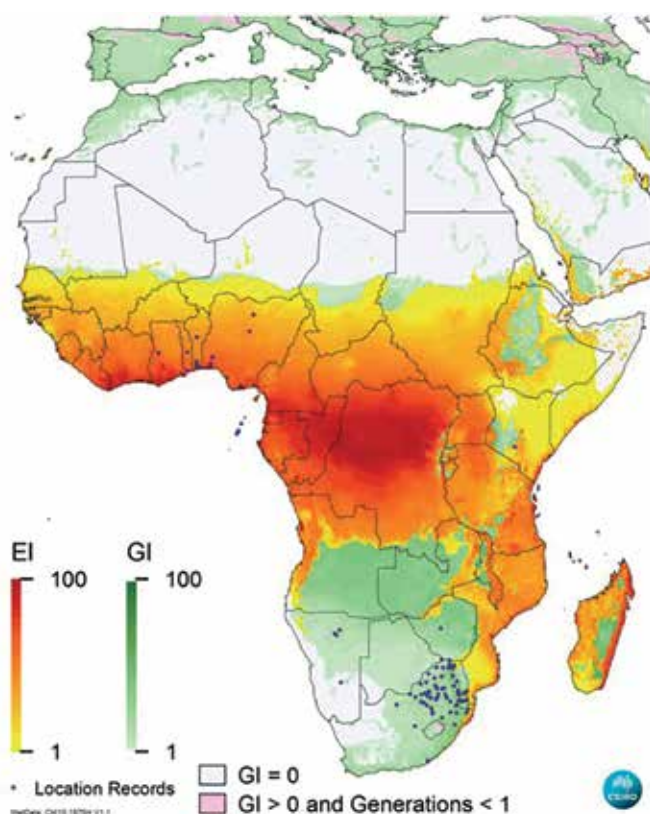
Since it is known that fall armyworm does not have a diapause stage by means of which it can survive the harsh winters in the South African Highveld region, research was conducted to determine the effect of different temperatures on fall armyworm development and survival. This is used to predict areas where it may establish in South Africa and those where it may only be of sporadic occurrence.

At high summer temperatures of 30–32°C, fall armyworm eggs hatch within two days and larvae develop into pupae in 10 to 12 days. At 18°C eggs take six to seven days to hatch and the larval development period is extended to between 28 and 37 days. In general, the fall armyworm completes its lifecycle in about 30 days during the summer, 60 days in early spring and autumn, and 80 to 90 days during the winter.

The number of generations that can attack a specific crop (up to eight generations in tropical areas) therefore varies between regions. The pest status of fall armyworm on maize in South Africa differs between the Lowveld region, Highveld region and other areas such as the Eastern and Western Cape provinces, where sporadic infestations are expected late in the cropping season.

Predicting potential distribution

Since it is possible for fall armyworm to survive mild winters, its pest status may in future become higher in temperate areas. The regular occurrence of fall armyworm on maize in the East London area, and reports of minor infestations in maize in the Clanwilliam area of the Western Cape, indicate that although pest attacks in these regions may be sporadic, maize producers need to stay alert during the cropping season and regularly monitor their maize fields for the presence of this pest.



To predict the areas that are suitable for fall armyworm populations to persist in South Africa and to identify areas to which moths may migrate and cause sporadic pest outbreaks, a CLIMEX programme was used, based on research on pest development in relation to climate variables.

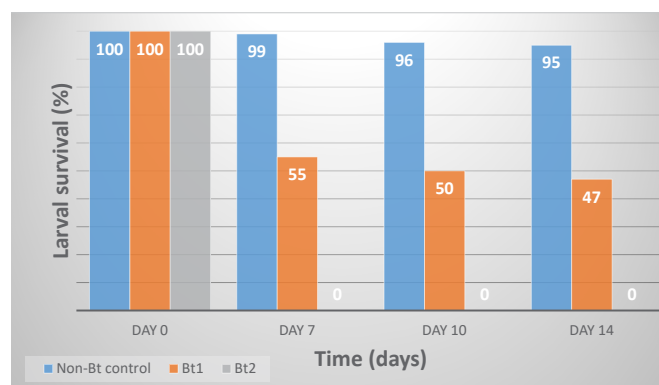
Areas where permanent populations can occur are indicated in yellow to red, while areas where sporadic and seasonal infestations may occur are indicated in green. Field observations indicated that permanent populations of fall armyworm, as predicted with the CLIMEX model, can establish in the Lowveld region of South Africa, northern parts of Limpopo, as well as the coastal areas of KwaZulu-Natal and the Eastern Cape. Infestation in other areas will therefore be as a result of migrating moths, and will be sporadic.

Current studies on temperature thresholds for survival of this pest suggest that it may be more tolerant to lower temperatures than expected, which will mean that the overwintering sites may be more expanded than indicated with this model. The increased risk of pest attacks by populations that establish in other areas in South Africa is currently being investigated, and will result in more accurate predictions of where the pest may reach pest status, and areas that may be more regularly invaded by migrating moths.

Response to genetically modified maize

In the Americas, fall armyworm was exposed to and effectively controlled with genetically modified maize – known as Bt maize – but it developed resistance to some products. These resistance traits could have come with the fall armyworm populations that invaded Africa. A pilot trial was therefore conducted to determine larval susceptibility to Bt maize. Larvae were inoculated onto maize whorls of a non-Bt maize plant (control), as well as the first generation Bt maize commercialised in South Africa (Bt1) and the second generation Bt maize (Bt2).

Larval survival on the control non-Bt maize plants was 95% after feeding for 14 days, 47% on Bt1 and 0% on



Above: Fall armyworm larval survival on conventional and genetically modified maize over time.

Left: Climate suitability for fall armyworm in Africa, modelled with CLIMEX. The EI describes the areas that are potentially suited for permanent establishment of fall armyworm, while the GI indicates areas that are suited for establishment on a seasonal basis. The model is being refined.



M Shindler, CIMMYT, CC BY-NC-SA 2.0

Bt2. From these results, it can be concluded that Bt1 will suppress the fall armyworm and Bt2 has the potential to control larval infestation. Nevertheless, the species is known to have the potential to develop resistance to Bt maize, which makes insect resistance management (IRM) of critical importance.

Biological control

The local natural enemies were found to attack the invasive pest, as evidenced by some parasitoids that were reared from fall armyworm larvae sampled from the field. The most promising discovery was an egg parasitoid – a species that parasitises the eggs of its host.

This species, an alien wasp known as *Telenomus remus*, is used in the Americas as a biological control agent for fall armyworm, where it is reared and then released in the field to control the pest. Since this species is already in South Africa, it could help control fall armyworm here if chemical control is applied with care to protect it. There is thus potential for biological control to form part of an integrated pest management (IPM) system for the fall armyworm in South Africa.

Chemical control

The rapid and unexpected nature of fall armyworm infestations resulting from migrating moths provides challenges to effective chemical control applications. The window of opportunity for effective control is very short, since infestations are often not observed before plants exhibit advanced levels of leaf damage, caused by large larvae that are difficult to control.

This is due to the initial cryptic feeding behaviour of larvae inside plant whorls – the damage caused by small larvae (instars one to three) during the first seven-day period is largely hidden inside the whorl. From the fourth instar onwards, however, the growth rate and feeding of larvae increase rapidly and severely damaged leaves can be observed.

The rapid appearance of damage symptoms frequently leads to indiscriminate spraying of pesticides, often without regard to whether chemical control is necessary or effective. Assessing fall armyworm infestation levels by means of scouting for damage symptoms prior to insecticide application is important.

The number of moths captured in pheromone traps cannot be used as a threshold level for decision-making on whether or not to apply insecticides. It can, however, indicate the presence of fall armyworm moths in an area. Once moths are recorded in these traps, scouting should commence to provide an indication of the level of pest infestation in a particular maize field. Without information generated through monitoring, surveillance and scouting, no informed decision can be made by either producers or extension officers regarding pesticide application.

Insecticide application should not be done when the first symptoms of borer damage are observed. Guidelines in terms of action thresholds indicate that control action should be taken when 20% of plants during the mid-whorl stage and 40% of plants during the late vegetative growth stages show symptoms of damage.

The application of pesticides for fall armyworm control should be guided by IPM principles. The misuse and indiscriminate spraying of pesticides can have serious adverse effects on the environment, human health and the natural enemies of this pest. Fall armyworm is also known to develop resistance to insecticides, which is often also a result of misuse and indiscriminate spraying.

Keeping fall armyworm populations below economically important levels in areas where the pest persists may require frequent insecticide applications. In such cases it is important to make use of insecticides with different modes of action, in order to delay resistance development.

Prof. Hannalene du Plessis and Prof. Johnnie van den Berg are members of the IPM programme at North-West University: Potchefstroom. Dr Annemie Erasmus is an entomology researcher at ARC-Grain Crops in Potchefstroom.

This article is based on the content of two articles originally published in SAGraan/Grain and reproduced with permission.



Andy Reago & Chrissy McClarren

Genetics to the fore

Kirsty Botha tells us about research to improve disease resistance in wheat

What is *Fusarium* crown rot?

Fusarium crown rot (FCR) is a soil-borne plant disease caused by *Fusarium pseudograminearum*. This is a fungal pathogen that can infect many cereal crops and is particularly severe in bread wheat. The symptoms include lower stem browning and crown necrosis (cell death). Pink fungal growth can sometimes be seen in the stems, while white or dead heads containing shrivelled or no grain is a common feature.



Honey brown discoloration of the bases of wheat tillers caused by *Fusarium pseudograminearum*.

Why should we be concerned?

FCR is one of the most important soil-borne diseases of wheat in South Africa, as well as many other countries. It is known to have the greatest impact on yield in water-scarce wheat-cropping regions. Early rains followed by drought conditions have also been shown to increase FCR incidence. The amount of *F. pseudograminearum* in the field is increased by planting susceptible cultivars and practising conservation farming (e.g. no-tillage).

In South Africa, wheat-producing areas of the Western Cape have the largest occurrence of FCR and this is also where adoption of conservation farming is widely expanding. South Africa is a semi-arid country where water is a precious resource, and the Western Cape is still recovering from the recent drought. With climate change, this region may receive increasingly unpredictable rainfall, creating perfect conditions for *F. pseudograminearum* to thrive. This would cause increased yield losses for farmers, and reduced food security for consumers.

What can we do?

An integrated approach is the most effective way to control the disease. FCR management strategies include crop rotation, controlling grass weeds, stubble burning (not recommended for conservation farming), maintaining nutrient levels, seed treatment, and disease resistance.

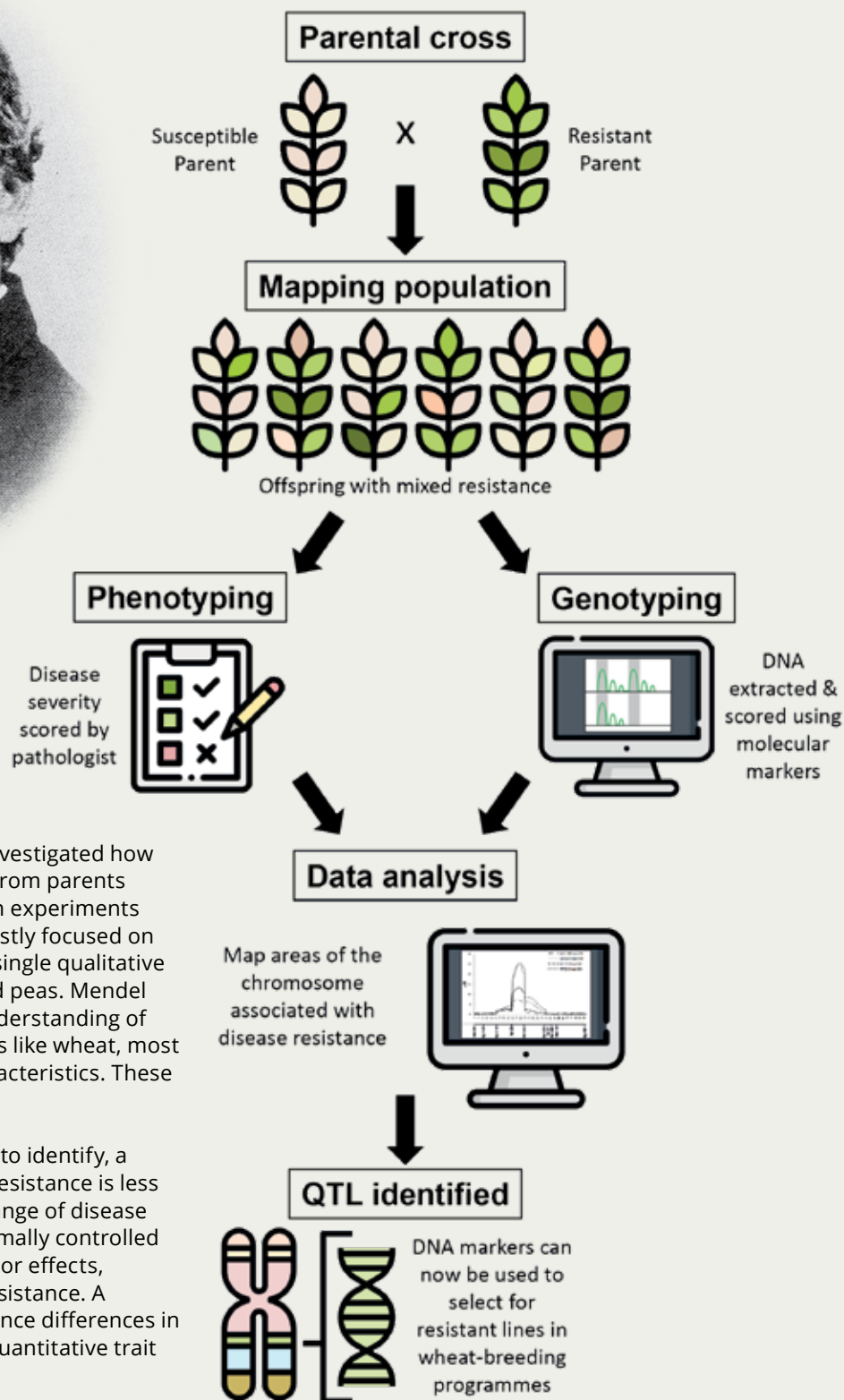
Most of the research on disease resistance has been conducted in Australia, and information for wheat cultivars in South Africa is still very limited. Improving

Identifying a QTL

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Gregor Mendel, the 'father of genetics'.



In the 1800s, Gregor Mendel investigated how specific traits are transmitted from parents to offspring in cross-pollination experiments with garden peas. His work mostly focused on how one gene can influence a single qualitative trait, such as round or wrinkled peas. Mendel undoubtedly facilitated our understanding of genetic heritability, but in crops like wheat, most traits have more complex characteristics. These are called quantitative traits.

While a qualitative trait is easy to identify, a quantitative trait such as FCR resistance is less distinct, as plants will have a range of disease severities. These traits are normally controlled by a number of genes with minor effects, working together to provide resistance. A section of DNA shown to influence differences in a quantitative trait is called a quantitative trait locus (QTL).

The process of identifying a QTL is shown here.



CSIRO

White or dead heads of wheat are symptomatic of *Fusarium* crown rot.

FCR resistance of South African wheat cultivars is an important goal and will contribute greatly to the effective management of the disease. Combining plant breeding with DNA-based tools can speed up this process considerably.

To date only partially resistant or tolerant wheat cultivars have been identified, and DNA regions contributing towards resistance have only had minor effects. It is vital to identify new sources of resistance and to breed cultivars with durable resistance if future yield losses to this disease are to be prevented.

How are we doing this?

A high-yielding South African bread-wheat cultivar produced by Sensako in 2008 was found to have partial resistance against FCR. We then set out to identify the genetic components responsible for FCR resistance in this cultivar by using a quantitative trait locus (QTL) mapping approach (see box).

A population was created using the resistant source as one parent, and an FCR-susceptible cultivar as the other parent. The population was then screened with thousands of DNA markers, which act as DNA 'road signs', allowing regions along the chromosome to be linked to the resistance trait.

In order to link this genetic information to FCR resistance, the disease scores for each plant in the population are needed. This is referred to as phenotypic data, and is obtained by conducting very labour-intensive field trials in which individual plants are grown in FCR-infected soil. After harvest, individual plants are rated for disease severity by measuring the amount of browning present on the stems of the plants.

Once all the data is collected, it is analysed using specialised software, and the QTL contributing towards disease resistance is identified. DNA markers are developed in order to incorporate these QTL regions into new bread-wheat cultivars.

Outcome

This project is making use of current genetic resources in South Africa to gain a better understanding of FCR resistance, and to help wheat breeders produce cultivars that are resistant to FCR, which will be to the benefit of farmers and consumers in the future.

Kirsty Botha completed her master's degree, specialising in plant physiology and biotechnology, at the University of Pretoria in 2017. She now works at CenGen, a private plant genetics company based in Worcester in the Western Cape. One of the major research focuses at CenGen is characterising new sources of pathogen resistance in bread wheat, and helping breeders to incorporate these genes into new cultivars.

The FCR project is funded by The Winter Cereal Trust, and is jointly led by Dr Renée Prins (CenGen) and Dr Anke Martin (University of Southern Queensland), in collaboration with Dr Sandra Lamprecht (ARC-Plant Health and Protection) and Mr Driecus Lesch (Sensako). <http://www.cengen.co.za>



Driecus Lesch



The Sensako team planting wheat for an FCR resistance field trial in Napier; two months after planting; established field trial.

Viruses as biopesticides

By Michael Jukes and Marcel van der Merwe

Viruses are generally perceived as an enemy of the people, and for good reason, given the often devastating outcomes of viruses such as the COVID-19 coronavirus, Ebola and HIV, and the nuisance of rhinoviruses, responsible for the common cold. But as with many things in life, viruses can be manipulated to our benefit. The tight evolutionary bond between viruses and their hosts often means there is a very narrow range of organisms that they can successfully infect. Entomopathogenic viruses, for example, primarily infect insects, and have become a valuable and highly effective tool in the fight against insect pests of agricultural crops.

Viruses used in biological control have several advantages over other forms of pest control. They are highly diverse, with many distinct species classified to date. These species have themselves been shown to vary genetically, with unique isolates often found in geographically separated regions across the globe. This provides us with a rich library of biological agents that can be developed into biopesticides. Viruses are also self-replicating organisms, which enables them to spread within host populations and persist in the environment for long periods of time. Compared to most chemical insecticides, the application of virus-based biopesticides in the field is far less detrimental to our beneficial insects, such as bees and parasitoids, due to their narrow host range. This results in a more environmentally friendly approach.

The Virus Research Group (VRG) and the Centre for Biological Control (CBC) at Rhodes University have been working to harness the benefits of entomopathogenic viruses for many years. Pests such as codling moth, cotton bollworm, diamondback moth and false codling moth cause immense damage to important crops such as apple, tomato, potato, rice and citrus, among many others. For each of these pests, projects were initiated to isolate, identify and evaluate indigenous strains of viruses that can be utilised on farms as pest control agents.

The basic process involves the establishment of an insect colony, which is regularly checked for any

larvae exhibiting symptoms of a viral infection, such as becoming lethargic and pale in appearance. As the infection progresses, larvae begin to climb upwards in a somewhat 'zombified' state. In a natural environment, the virus triggers cells to rupture, releasing viral particles back into the environment and contaminating the surrounding plant material to be ingested by its next host. However, if collected at this stage, it can be purified, studied and developed into a biopesticide.

To date, the CBC and VRG have identified many South African virus isolates, including *Cryptophlebia peltastica* nucleopolyhedrovirus (CrpeNPV), *Cryptophlebia leucotreta* granulovirus-SA (CrleGV-SA) and *Cydia pomonella* granulovirus-SA (CpGV), to name a few. All these viruses are grouped under a single family, the Baculoviridae, with many known to only infect lepidopteran insects (moths and butterflies), making them ideal candidates as biocontrol agents.

Of all the viruses worked on at the CBC and VRG, CrleGV-SA serves as a prime example of how these viruses have been taken from the field and developed into effective



Culturing yeast isolated from false codling moth larvae for volatile testing.

biopesticides to control agricultural pests. Once isolated and identified, an extensive range of laboratory tests and field trials were conducted to determine the optimal concentration and how best to apply the virus. CrleGV-SA was formulated into a liquid spray that can be easily applied to citrus crops, using standardised farming equipment. For nearly two decades now, this virus has been successfully applied in the field to control false codling moth in South Africa. However, our work is far from complete. A number of challenges arise with the use of viruses as control agents. This includes their slow speed of kill, their susceptibility to UV light and the development of host resistance.

These challenges led to the initiation of several novel projects, each aiming to improve aspects of this virus to ensure its continued use in the field. One of these projects is exploring the beneficial effects of combining the virus with yeast. While this may sound like a strange idea, certain yeasts have been shown to have a synergistic relationship with lepidopteran insects. Experiments conducted with the false codling moth and yeasts isolated from the field have already shown that they prefer fruit sprayed with yeast over those without. Furthermore, combining the virus with yeast has resulted in higher levels of pest control than when used on its own. The project is now moving towards identifying the volatiles produced by the yeast, which are responsible for attracting the moths. Identification of these compounds may lead to the formulation of highly attractive biopesticides, or the development of novel lures for the monitoring of pests in the field.

One of the more recent developments at the CBC and VRG was the discovery of the virus species, CrpeNPV. This virus was isolated from the litchi moth, a major pest of litchi in South Africa. The true importance of this virus was stumbled upon while testing its host range. Experiments soon revealed that this virus also infects false codling moth and codling moth. This discovery will enable the virus to be utilised in multiple markets around the world against three major pests, protecting a variety of different crops. The discovery is even more important given the recent rise of resistance in codling moth populations in Europe to its homologous virus, CpGV, which has been in use for several decades as a biocontrol agent. The strategic application of CrpeNPV, in combination with viruses already in use in Europe and South Africa, could serve as a resistance management and prevention tool.

The CBC and VRG at Rhodes University will continue to advance the use of entomopathogenic viruses for the control of important agricultural pests in South Africa. Through innovative projects, strategic industry partnerships and responsible use of chemical insecticides and biological pesticides, a stronger agricultural industry can be developed, which can simultaneously reduce our impact on the environment.

The authors are based at Rhodes University's Centre for Biological Control and Virus Research Group, where Dr Michael Jukes is a postdoctoral researcher and Marcel van der Merwe is completing his PhD.



Bugwood.org

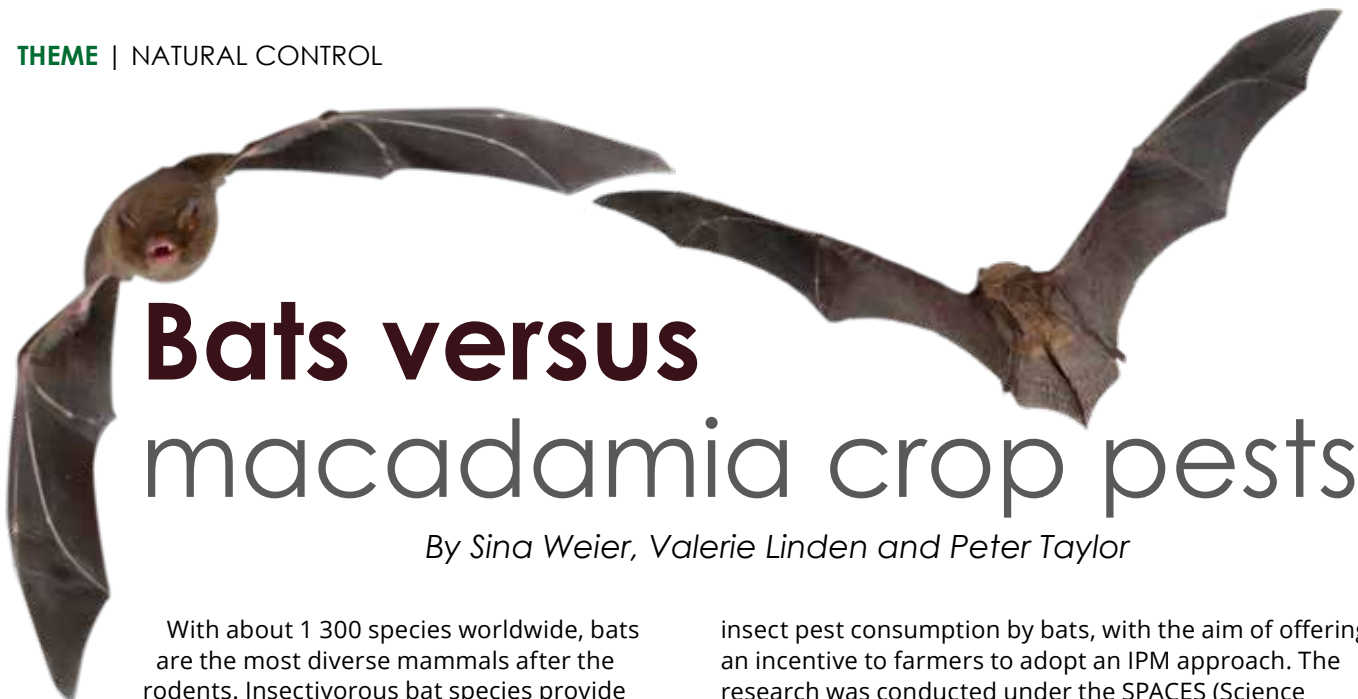
Marcel van der Merwe

The false codling moth

The false codling moth, *Thaumatotibia* (formerly *Cryptophlebia*) *leucotreta*, is indigenous to sub-Saharan Africa, but has spread throughout the continent and has also become established in parts of Israel. As well as being a major problem in the citrus industry, it is a pest of many other crops, including macadamia, avocado, peaches, apples, cotton and sorghum. Its economic impact is not limited to direct losses of produce – biosecurity officials at international borders may prevent entry of entire consignments if infected produce is detected, or even put a ban on imports from certain regions.

In oranges, the female moth lays her eggs on the surface of the fruit, and the larvae burrow through the rind after hatching to feed on the soft flesh inside. This not only spoils the fruit, but also provides an entry point for other pests and pathogens. The final larval instar emerges from the fruit and drops to the ground on a silken thread, before burrowing below the soil surface to pupate inside a cocoon.

Various methods are used to control false codling moth in South Africa, including chemical insecticides, pheromone traps, mating disruption, sterile insect technique, and biological control by parasitoid wasps. Entomopathogenic nematodes (EPN) and fungi (EPF) that control the soil-dwelling stages of the pest have also shown promise as biocontrol agents.



Bats versus macadamia crop pests

By Sina Weier, Valerie Linden and Peter Taylor

With about 1 300 species worldwide, bats are the most diverse mammals after the rodents. Insectivorous bat species provide an important ecosystem service to farmers around the world by reducing numbers of insect pests in agricultural areas. Promoting high bat activity in agricultural landscapes as part of an integrated pest management (IPM) approach could not only improve the livelihood of farmers, but also potentially decrease the use of pesticides without losing crop. Unfortunately, bat populations keep declining at an alarming rate and about one quarter of all bat species are currently threatened with extinction. This decline is mainly attributed to the loss and fragmentation of habitats, roost sites and feeding opportunities, caused primarily by agricultural intensification – more and more natural areas being turned into farmland.

South Africa has been the world's largest producer of macadamia nuts since 2014, and the loss from insect pest damage to the macadamia crop is estimated at about US\$15.23 million per year. In 2015 the University of Venda started several projects to provide evidence of

insect pest consumption by bats, with the aim of offering an incentive to farmers to adopt an IPM approach. The research was conducted under the SPACES (Science Partnership for the Assessment of Complex Earth Systems) consortium, with funding received from the German Federal Government and collaboration with the University of Goettingen in Germany. The study sites were in macadamia orchards in Levubu, Limpopo, where an estimated 25 different insectivorous bat species occur.

Initial research focused on investigating the diet of insectivorous bats in more detail. Given that bats hunt at night and in flight, it is very difficult to study their diet and nearly impossible to study their feeding behaviour by observing them directly. But studying their faecal pellets – either under a microscope or through molecular work in a laboratory – provides a non-invasive way of gaining information on the foraging preferences of these predators. The research team at the University of Venda decided to use fluorescently labelled primers to show the presence of four of the main insect pests in bat faecal pellets: the twin-spot stinkbug (*Bathycoelia distincta*), the green vegetable bug (*Nezara viridula*), the macadamia



Macadamia nuts are enclosed in a green husk that splits open as the nut ripens.

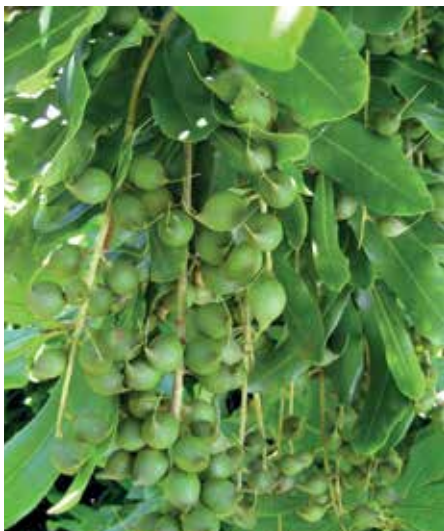


Initial research into the installation of bat houses to provide additional roosting opportunities showed that insectivorous bat species prefer houses that are warm and well insulated.



Frank Vossen, CC BY 2.0

Mauritian tomb bat and pup.



young macadamia fruit, into which the larvae bore after hatching. Apart from leading to a decrease in nut quality, the feeding damage can cause nuts to drop immaturely, germinate or grow mould.

Interestingly, all the species or families of bats from which pellets were collected foraged on at least one of the four main insect pests, and pests were found in more than half of the samples. Nearly all species and families of bats analysed foraged on both the moth and the stinkbug insect pest species. Bats consumed insect pests throughout the macadamia growing season, and are much more generalist – and presumably opportunistic – feeders than previously assumed. The research conclusion was that bats appear to be of utmost importance for insect pest control.

nut borer (*Thaumatotibia batrachopa*) and the litchi moth (*Cryptophlebia peltastica*). The first two are stinkbugs that feed directly on macadamia nuts, while the second two are moths that lay their eggs on the

Other studies conducted by the University of Venda, looking into habitat use by bats, showed that the overall activity of bats increased with the abundance of true bugs (which include the main macadamia pests, the stinkbugs) and the amount of natural vegetation close to the macadamia plantation. The study further showed that semi-natural vegetation, such as fallow land, increased the activity of a certain group of bats, which preferably forage above vegetation and in open areas. As part of the project, bats and birds were also excluded from macadamia trees by putting up cages around the trees. The researchers found that the biocontrol provided by both insectivorous bats and birds saves macadamia farmers around US\$5 000 per hectare each year, which is economically more important than the losses through crop-raiding by monkeys (~US\$1 600 ha/year). Both the biocontrol provided by bats and birds and the crop-raiding by monkeys were linked to the vicinity of natural vegetation patches.

The research team therefore suggests that farmers should maintain or restore (semi-) natural vegetation inside and adjacent to their farms. Adding water sources and roosting opportunities, as well as minimising pesticide treatments, may also help promote bat activity and the biocontrol provided by them.

Dr Sina Weier and Dr Valerie Linden were awarded their PhDs in zoology for this research in May 2019 by the University of Venda (Univen), where they are both now postdoctoral fellows. Prof. Peter Taylor supervised their research, and holds the SARCHi Chair in Biodiversity Value and Change in the Vhembe Biosphere Reserve.



Remote sensing to the rescue

Christine Cuénod explains how researchers used satellite imagery to develop a quick method of assessing pest infestations in forestry plantations

The province of Mpumalanga is home to 40% of South Africa's 1.3 million hectares of forested landscape, but an indigenous, wood-boring moth species is emerging as a significant threat to its eucalypt plantations.

The cossid moth, *Coryphodema tristis*, harms *Eucalyptus nitens* gum trees as its larvae – after hatching from eggs laid on the bark by adult female moths – feed on the bark, damaging the cambium and blocking the movement of water, which causes blackening of the trunk and branches. The larvae also bore into the tree, creating tunnels that reduce the value of the wood and weaken the tree, increasing the probability of early death or wind breakage. The tunnelling triggers the release of resin by the tree and this, together with sawdust around the base and pupal casings protruding from the bark, are typically the first signs of infestation.

The cossid moth has a wide host range of both indigenous and exotic trees in South Africa, and was a well-known pest of fruit trees and grapevines in the south-western Cape before it was first detected in the exotic *E. nitens* plantations in Mpumalanga in 2004. The shift in distribution and target species is mainly attributed to the absence of natural predators, but has also prompted questioning of the role that climate change may be playing.



Feeding damage by the cossid moth larvae causes blackening and weakening of the tree trunk.

It is estimated that the moth affects up to 80% of some *E. nitens* compartments, raising concerns about the consequences on yield quality and quantity, and the resulting economic impact. Researchers from the

University of KwaZulu-Natal and Sappi identified the need for methods allowing swifter detection of cossid moth infestations, given that by the time sawdust, resin and pupal casings on and around the trees are discovered in more traditional field surveys, which are both time-consuming and labour-intensive, most trees are beyond salvageable. They set out to fill a gap in research on monitoring and mapping the occurrence of this insect pest, building on previous work that identified the environmental variables predicting its spatial distribution, including the age of trees affected, elevation, rainfall and temperature conditions of infestation sites.

Their investigation used a multispectral image from the European Space Agency's Sentinel-2 satellite, which measures the solar radiation reflected from the Earth's surface in 13 spectral bands

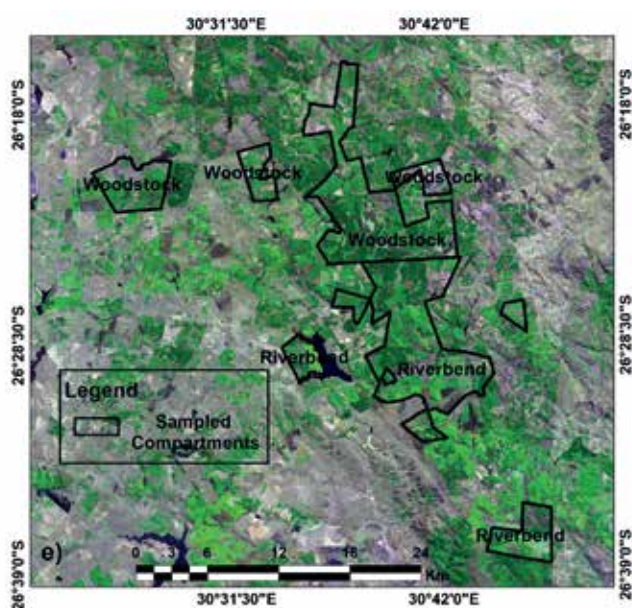
ranging from visible to shortwave infrared regions of the electromagnetic spectrum. Reflectance from vegetation is known to change with plant type, leaf pigments, water content and other factors, so by measuring variations in reflectance from the plantations, the researchers hoped to be able to detect trees that were unhealthy or stressed



Cossid moth larvae tunnel through the wood as they feed, reducing its value.

because of attack by the pest. For example, the Sentinel-2 multispectral instrument's red-edge bands are known to be useful for assessing canopy chlorophyll content, which can be used as an indicator of forest defoliation.

The multispectral image selected for the study was acquired by the satellite in the same week that the researchers visited two plantations in the target area to conduct traditional field surveys. Working with Sappi plantation management teams, they set up pheromone traps in 878 *E. nitens* compartments and inspected trees for resin and sawdust indicating the pest's presence. In this way, 371 compartments were positively identified as infested, and their locations used to generate cossid moth presence data. This, together with spectral bands and vegetation indices derived from the multispectral data, was fed into the Maxent algorithm for analysis. Maxent is freely available, open-source software that uses a machine-learning technique for modelling species distribution. The algorithm was 'trained' using 70% of the presence data, with the remaining 30% used to test the model's performance.



The compartments sampled during the field survey are shown on a false-colour composite of the Sentinel-2 multispectral image, using bands 4 (red), 8 (near-infrared) and 2 (blue).

The results allowed the researchers to ascertain which variables performed best in matching the pest's presence as recorded in the field survey, and hence would have the best potential to predict moth infestations elsewhere. Of the spectral bands, the red-edge band 5 was the most influential in the modelling process, while Photosynthetic Vigour Ratio (PVR) was most important amongst the vegetation indices. The researchers found, however, that spectral bands and vegetation indices combined together in the model provided more accurate predictions of cossid moth occurrence than using them on their own.

The model output revealed some areas with a high probability of the pest's occurrence where it had not been detected during the field survey, confirming that the method presents a quicker and more accurate way of improving pest monitoring in plantations, and can play an important role in supporting forestry management strategies. There are currently no effective biological or chemical control solutions for cossid moth infestations, so plantation managers' present approach to mitigating losses is to harvest trees earlier to minimise the extent of the damage, which they can do more easily if infestations are detected early.

This method of identifying infestations to support forest protection interventions has applicability beyond just *E. nitens*, with researchers saying that the method would be effective for any eucalypt species, with the appropriate amendment of vegetation indices.

"The application of remote sensing and GIS technology is important for the protection of plant health," said Mr Samuel Kumbula, who wrote up the research as part of his master's thesis. "The use of reflectance to indicate plant health and identify any changes improves decision-making capabilities, and can be used for crop monitoring where biological and chemical agents are available to remediate the problem."

Kumbula remarked that while GIS and remote-sensing divisions for monitoring and evaluation are being established in government departments, the private sector has adopted this technology much more quickly.

In the context of a changing climate, with pests and diseases likely to undergo shifts in target and distribution, crop management is in need of detection methods with quick results. This research highlights a tool that could support much of Africa in the monitoring of pest infestations, with the aim of ensuring better crop protection.

- Kumbula ST, Mafongoya P, Peerbhay KY, Lottering RT and Ismail R, 2019. Using Sentinel-2 multispectral images to map the occurrence of the cossid moth (*Coryphodema tristis*) in *Eucalyptus nitens* plantations of Mpumalanga, South Africa. *Remote Sens.* 11(3): 278. <https://doi.org/10.3390/rs11030278>

Christine Cuénod is the primary journalist for the College of Agriculture, Engineering and Science at the University of KwaZulu-Natal, and also acts as the networking facilitator for the College's agricultural alumni association, Friends of UKZN Agriculture.

Nanotechnology



State of the art nanomaterials production facility at the CSIR Nanotechnology Innovation Centre

Everything on Earth is made up of atoms - the food we eat, the clothes we wear, the buildings, the stationery we use at school and even our own bodies. The arrangement of atoms in something affects how strong or weak it is, if it has the ability to conduct electricity, if it is see-through, and even its texture. Atoms are extremely small to see with the naked eye or the typical microscope used at secondary schools' science classes and laboratories. They are a million times smaller than the thickest human hair. The diameter of an atom ranges from about 0.1 to 0.5 nanometres (1×10^{-10} m to 5×10^{-10} m). Nanoscientists and nanotechnologists can work with individual atoms and molecules to modify matter at a macroscale.

What is Nanotechnology?

Nanotechnology is science, engineering, and technology conducted at the nanoscale, which is about 1 to 100 nanometres. The fields of nanotechnology and nanoscience involve the application of extremely small things and involve many different disciplines of science, including chemistry, biology, medicine, physics, materials science, and engineering. The field of nanotechnology and nanoscience has grown due to improved understanding of the characteristics of nanomaterials, the relationship between nanostructures and properties, and how these are engineered.

Applications of Nanotechnology

In South Africa nanotechnology has been applied in several industries, including energy conversion, aero-space and automotive, storage and distribution, nano-biotechnology, defence, chemical applications. The technology is continuously used to improve current products and processes, e.g. chemical processing done by SASOL and other local manufacturing industries.

Nanotechnology is also applied in various products such as the application of nanoparticles in paints, cosmetics we use on a daily basis, sunscreens and membranes used in water purification. With nanotechnology, new ways of synthesising or improving already existing products and processes are possible. The technology is having a great impact on several sectors including energy, medicine, electronics, ICT and other associated sectors.

Nanotechnology in Energy

In the energy sector, hydrogen energy is having impact as an alternative energy, and as an energy-carrier, due to the abundance of hydrogen and the relative prevalence of platinum, a key catalytic material in the fuel cell, in South Africa. The energy in hydrogen is electrochemically converted directly to electrical energy using fuel cell technology. This fuel cell technology is considered to be environmentally friendly, since the only waste produced from pure hydrogen is water.



Fuel cell system installation at Poelano Secondary High School in Ventersdorp providing off-grid electricity for ICT and lighting to the school.

Nanotechnology in Medicine

In medicine, nanotechnology has a future in advancing drug delivery. The third generation of nanotechnology could make an entirely new product such as sensors and devices that can monitor body functions, detect pathogens and environmental conditions, clothes that can clean themselves or regulate temperature. Scientists and engineers are using nanotechnology to improve humanity by finding various ways to improve materials at the nanoscale for use at a macroscale.



Dr Phuti Chelopo holds a PhD in Pharmaceutical Chemistry and specialises in nanomedicine for drug delivery

Dr Phuti Chelopo - Nanomedicine Researcher

1. What influenced you to pursue this career path and why did you choose it?

I always had a great interest in science, which was the stream of subjects I chose in Grade 10, and my favourite science subject was biology. This interest led me to pursue my 1st degree in biochemistry and chemistry. I then pursued both my MSc and PhD degrees in pharmaceutical chemistry. My PhD project involved the use of nanotechnology to improve the current treatment regime for tuberculosis. Before my PhD studies, I had developed an interest in the impact of nanotechnology to solve societal problems and having had the opportunity to be involved in it made me feel fulfilled. My greatest influence when choosing the career I did was to be involved in projects that have the potential to improve our everyday lives through science-based solutions, such as nanotechnology.

2. What average marks in matric (science and mathematics) are required to study a degree at a university?

I would recommend that students work hard to attain marks that are above 65% for their core science and mathematics subjects. Anything below 60% will risk one not being considered at the university.

3. What qualification does one need to pursue nanotechnology as a career, and what courses should one major in at a university?

Nanotechnology merges concepts from a variety of science disciplines, from concepts of chemistry and technology. At the university one can do a Bachelor of Science degree majoring either in biotechnology, chemistry, biochemistry or biology. Having two majors (co-majoring) is a possibility and will offer a greater advantage due to diversified skills. More importantly, continuing further to pursue MSc and PhD research will provide one with highly specialised skills in nanotechnology.

4. Where can one work with a nanotechnology degree?

One can work as a researcher at science councils or in academia (universities). One can also lecture at the university, while doing research and contributing to the knowledge base. Another contribution one can make as a nanotechnologist would be to work as a science expert at manufacturing companies that manufacture nanotechnology-based products. It is also very critical for the future upcoming scientist to start thinking of possible innovation they could pursue in their area of interest, such as nanotechnology for water purification or medical applications. This approach could help them become entrepreneurs who will contribute to economic growth.

5. What area of nanotechnology do you specialise in and what does it entail?

I focus on the use of nanomedicine for drug delivery purposes. Nanomedicine is the application of nanotechnology to make devices or systems for medical application at the scale of nanometre (1 to 100 nm). It involves the delivery of drugs, targeting and diagnostic agents using nanotechnology-based devices. My project involves the design of a drug delivery system for transporting anti-TB drugs more efficiently so that we can reduce the current treatment period of TB from six months to about two months.

6. What would you say is fulfilling about your career and which three words describe your occupation?

What fulfills me as a scientist is the desire to solve problems through science, which I can apply in a variety of areas. I am currently applying the skills I have obtained during my research in nanotechnology and nanomedicine to contribute to some of the activities that drive the "Bio-economy Strategy" of the Department of Science and Innovation. The three words that describe my occupation is: Research; Innovation and Impact.

7. Take us through your job responsibilities briefly?

My day involves current research on innovations in the bio-science field and seeing how our economy can benefit or grow by correctly aligning itself. The activities ultimately lead to making an impact towards socio-economic developments.

8. What skills/talents does one need to excel in this field?

Dedication, willingness to learn, flexibility, communication and presentation skills, problem-solving and research and in-depth analytical skills.

9. What are the pros and cons of nanotechnology as a career?

The greatest advantage of this field is the enormous potential for growth. There are many great products that are yet to be discovered in this area with the potential to improve our everyday lives, such as improving health. Therefore with the right skills, there is great potential to grow in the field of nanotechnology. The cons of being involved in this field in our country are the low number of industries to offer employment. Therefore one has to work hard to pass tests and to prove your worth.

The Nanotechnology Public Engagement Programme (NPEP) is an initiative funded by DSI and implemented by the South African Agency for Science and Technology Advancement (SAASTA), a business unit of the National Research Foundation (NRF). NPEP aims to promote credible, fact-based understanding of nanotechnology through awareness, dialogue and education to enable informed decision-making on nanotechnology innovations to improve quality of life.

For more information, visit: info@npep.co.za or visit: www.npep.co.za or follow us on

Facebook: www.facebook.com/nanotechn/ or on

Twitter: [@npeptweet](https://twitter.com/npeptweet).

Small science for big innovations

Nicklaus Kruger reports from SA NanoSchool



Solar Shop Australia, CC BY-NC-SA 2.0

Meeting the world's energy needs is a big challenge, but the solution may just lie in some small science: nanoscience.

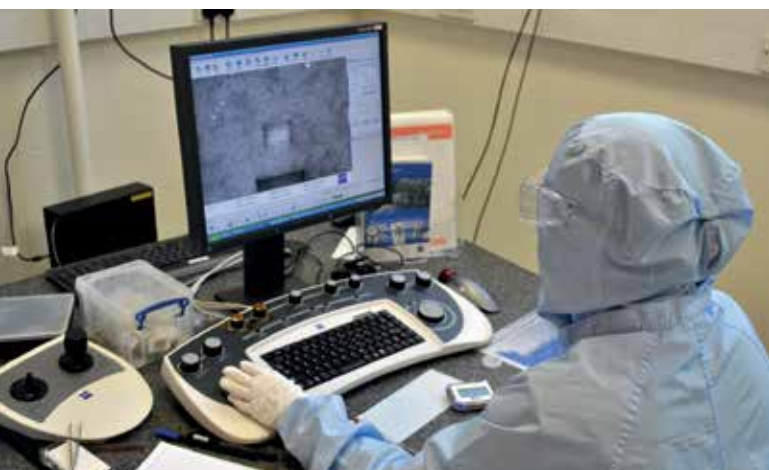
"Energy consumption is rapidly increasing worldwide – and that, along with extreme exponential growth in the world's population, is resulting in a variety of challenges: global climate change, slowing economic growth, a lack of access to electricity for many people," said Prof. Ruud Schropp, Extraordinary Professor in Physics at the

University of the Western Cape. "We need to meet our increasing energy needs in a sustainable way, and for that we need solar power. And for effective and efficient solar power, we need to employ nanoscience."

Prof. Schropp was speaking at the opening of the five-day South African Nanoscience and Nanotechnology Summer School, held in November 2019. In his talk 'Nanostructured thin films for multiband-gap silicon tandem and triple-stacked solar cells', Prof. Schropp noted that stabilising CO₂ emissions at a level that limits global warming to 2°C will require that at least a 14 TW renewable energy capacity be installed by the year 2050.

"If we were going to replace that amount of fossil energy by nuclear energy, we would need to build a power plant every two days, and that would come with some serious safety concerns," he said. "Other renewable power sources are promising, but don't scale as easily. It is clear that among the various renewable energy options, only solar energy offers ample resources to cover this demand."

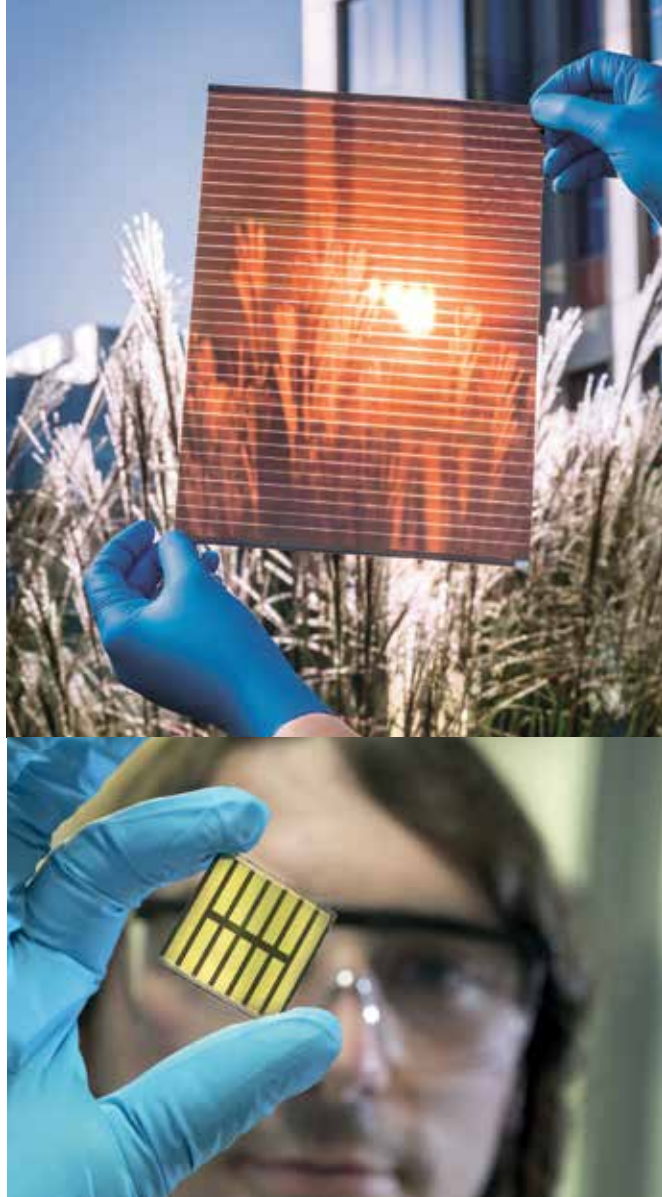
Given the large scale needed, solar technologies that need to be developed should use naturally abundant and preferably non-toxic materials. Among the various



UCL Mathematical and Physical Sciences, CC BY 2.0

Soule Technologies

KIT/Marcus Breig



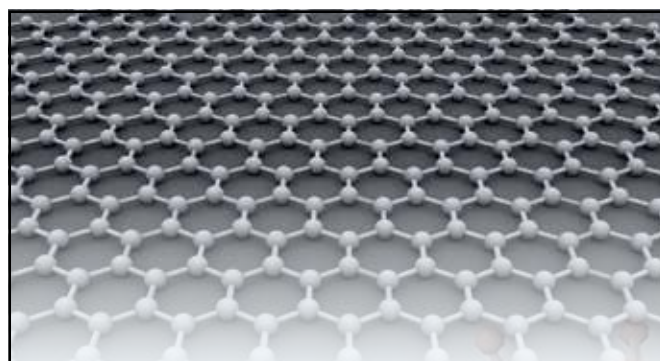
options available, silicon solar cells are dominating the market.

Even though silicon is the second most abundant element in the Earth's crust, the high purification that is needed and the indirect optical absorption make it an expensive source material. Therefore, further price reduction has to come from the use of thin films, the implementation of nanostructures, and the adoption of tandem solar cells utilising the full spectrum more efficiently.

"What we can do is try to make the best possible solar devices that will convince people to adopt solar power for their energy needs – devices that are cheap and efficient, and whose production is scalable," Prof. Schropp noted. "When it comes to solar panels, thinner is beautiful: the light doesn't have to travel as far, or through as much material, and that means less wasted energy."

As a source of energy, solar power is in ample supply. To meet the world's projected energy needs in 2050 using solar cells, we'd only need a few pieces of land: just 1.7% of the land area, globally speaking, would be sufficient.

"Renewable energy supply from solar cells can help build a sustainable society," said Prof. Schropp, "and further research can build technologies that are highly efficient and inexpensive, so that solar electricity will be abundantly available to everyone."



AlexanderAIUS, CC BY-SA 3.0

The 5th South African Nanoscience and Nanotechnology Summer School, held in Stellenbosch on 25–29 November 2019, brought together 120 local and international industry experts, academics and postgraduate students to explore nanoscience matters on the theme, 'From research to applications, innovation and commercialisation'. Dubbed SA NanoSchool 2019, it was run by the Nanoscience Hub at the University of the Western Cape, which forms part of the National Nanoscience Postgraduate Teaching and Training Platform (NNPTTP), funded by the Department of Science and Innovation (DSI).

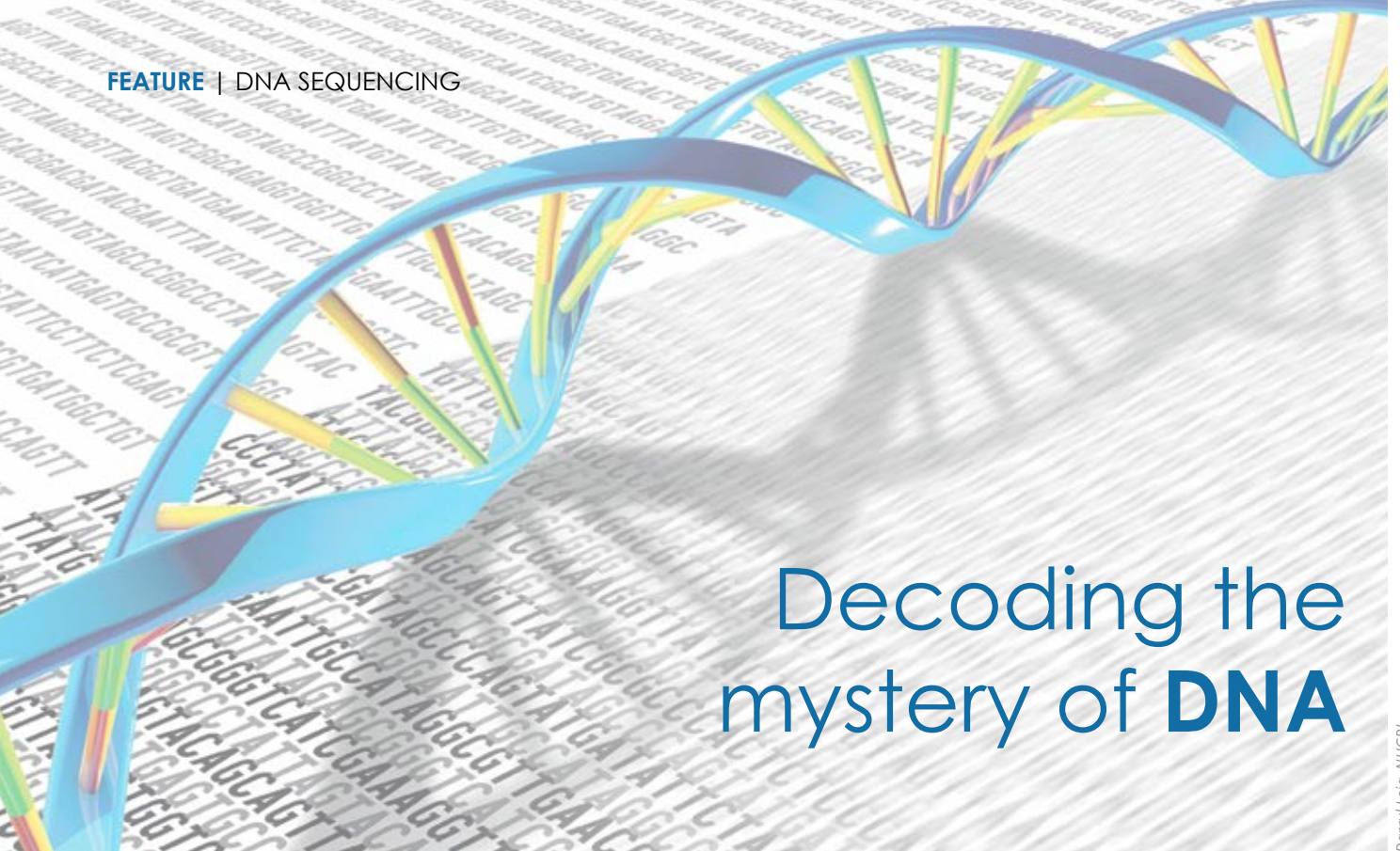
"Nanoscience can help us deal with many important issues: water purification, our energy and transport needs, biomedical and agriculture applications just waiting to be discovered and developed," says Prof. Dirk Knoesen, member of the SA NanoSchool 2019 organising committee and head of the NNPTTP.

Nanoscience is the study and development of materials at the nanometre size level (about 1/1000th the diameter of a human hair). This knowledge is applied in nanotechnology and the development of nanomaterials – materials with at least one external dimension in the size range from approximately 1–100 nanometres.

"The famed Fourth Industrial Revolution may be a digital revolution, but it has a physical basis in the materials that make a variety of products possible," says Prof. Knoesen. "For example, the very high speed computers of today, the huge increase in data storage and the extremely small devices (just about everything inside cellphones is nano-related), the fast biosensors that take a minute amount of blood to detect a variety of bio-based properties or viruses, etc. All of this is possible because of advancements in materials science, and especially nanoscience."

Funded by the DSI, the South African NanoSchools are designed to equip master's and PhD students with the necessary skills for conducting research in nanoscience and nanotechnology. They complement existing human capital development programmes in the field, and form one of many platforms for the implementation of the 2005 National Nanotechnology Strategy.

Nicklaus Kruger is a writer focusing on education, health and science in the University of the Western Cape's media section.



Darryl Leja, NHGRI

Decoding the mystery of DNA

By René Veikondis and Alvera Vorster

At the start of this century, on 26th June 2000, the completion of the first draft of the human genome sequence was announced by President Bill Clinton of the United States and Prime Minister Tony Blair of the United Kingdom. This announcement ushered in an era that has been an exciting time to be a molecular biologist. The first human genome, consisting of three billion nucleotide base-pairs, was sequenced by researchers from six countries over a period of 13 years and at a cost of US\$3 billion. Today, we have the technology to read the entire genetic code of almost any living organism on Earth, in less than a week and at a cost of no more than US\$1 000.

The DNA Sequencing Unit at Stellenbosch University's Central Analytical Facilities (CAF) makes use of the rapid DNA sequencing method that was used to sequence the first human genome. The method, known as Sanger dideoxy sequencing, was introduced in 1975 to sequence the human mitochondrial genome, and this work was published in 1977 by a group of scientists in Frederick Sanger's laboratory. For this effort Frederick Sanger – a British scientist – received his second Nobel Prize in chemistry, which he shared with two American scientists in 1980.

Though the Sanger method is still considered the most accurate way to sequence a single DNA fragment, next-generation sequencing methods are the most cost-effective to read the entire genetic code of an organism.

Staff at the DNA Sequencing Unit at Stellenbosch University's Central Analytical Facilities celebrated the 21st anniversary of its inception in 2018. Back row: René Veikondis, Carel van Heerden, Alvera Vorster. Front row: Sinead Robberts, Annette Laten, Marianna Retief.

At the CAF we offer massively parallel sequencing on the Ion Torrent platforms. With this method, a genome is broken up into millions of fragments, which are all sequenced at the same time. The data that is produced in parallel from all the fragments are pieced together by high-performance computing clusters, with the aim of delivering a single piece of DNA that encodes the instructions for the organism's life.

With these methods the staff at the CAF provide an affordable, cutting-edge sequencing service, delivering the highest amount of quality data to South African researchers in the shortest possible time frame. The



success of this service relies on three stakeholder groups: the Department of Science and Innovation, which funds our sequencing instruments, the manufacturers and distributors of sequencing reagents, and the researchers, who hail primarily from the agricultural and medical fraternities. Here we celebrate the researchers who support DNA sequencing in the local context.

Mitochondrial DNA (mtDNA) sequencing

While it took Frederick Sanger's laboratory more than two years to determine the genetic code for one human mitochondrial genome, the advances in sequencing methodologies over the past two decades have enabled us to produce the DNA sequence for 59 of these genomes in less than two weeks on the Ion Torrent S5 next-generation sequencing platform.

The genetic determinants of Parkinson's disease

The sequencing data mentioned above forms part of the research that a doctoral candidate, Amica Muller-Nedebock, is conducting at Tygerberg Hospital. Her research is focused on South Africans with Parkinson's disease, an incurable movement disorder characterised by the loss of dopamine-producing and highly energy-dependant neurons.

Amica explains that mitochondria are the powerhouses of cells and their genomes encode critical components that are required for energy production. She theorises that small variations in the mtDNA may account for a decrease in energy production of dopamine-producing neurons, and in turn cause deterioration of these highly energy-dependent cells. With this sequencing data, the Parkinson's disease research group at Stellenbosch University is analysing the mtDNA of individuals living with the disease to identify whether several of the mtDNA changes may collectively contribute to its development. Finding the underlying cause for this debilitating disease

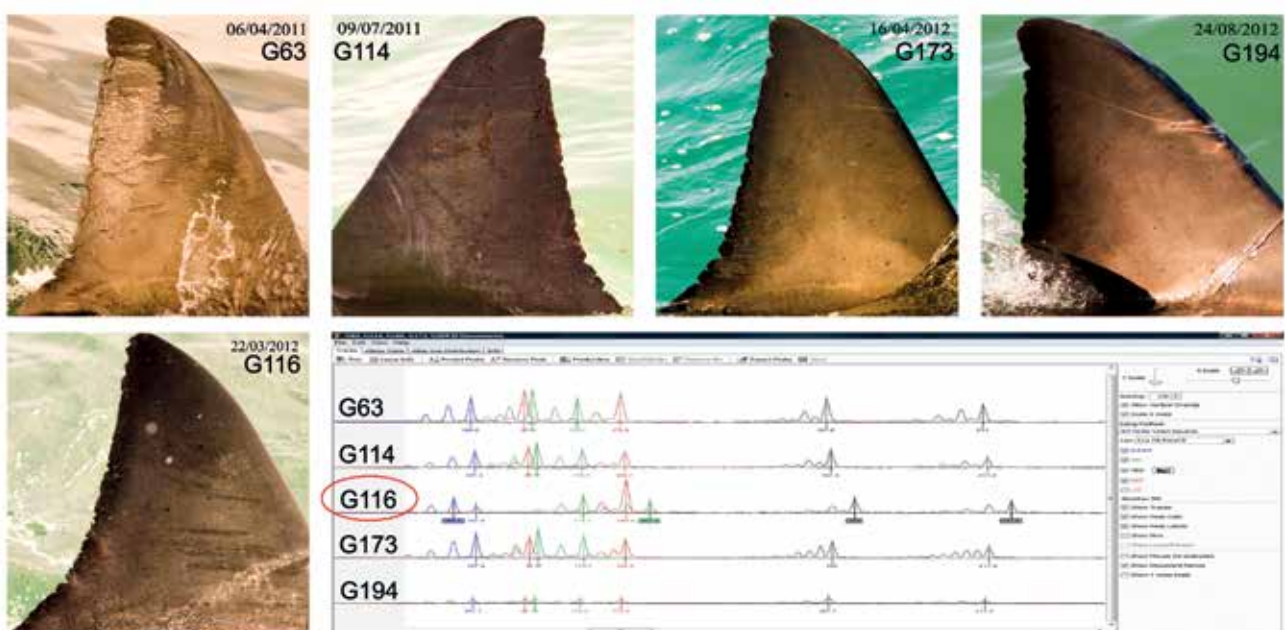
provides an opportunity to develop new treatment and disease-management strategies.

Molecular research on great white sharks

Great white sharks are magnificent creatures, but their elusive nature means very little is known about them. Questions exist about their lifespan, their numbers, and the location of their nurseries. To address these questions, Dr Sara Andreotti is investigating the population size of great white sharks along the South African coastline, with specific emphasis on the level of genetic diversity amongst the counted individuals. High genetic diversity confers some protection to a population in the event of a catastrophic change in the environment or disease outbreak, helping to ensure the continuity of the species.

Dr Andreotti started this study with a photographic database of dorsal fin notch patterns from more than 5 000 photographs. Analysis of this data identified 426 individuals, but to verify this photographic evidence, biopsy samples were collected from 233 sharks for genetic analysis. The molecular sizes of 14 genetic markers called microsatellites were determined at the CAF's DNA sequencing unit for all biopsy samples. The markers, which generate a unique genetic fingerprint for each great white shark, indicated a South African population of 333 individuals.

Dr Andreotti also used the Sanger sequencing method for mtDNA analysis. The results showed that the South African great white shark population has low genetic diversity, with only four maternally inherited lineages and 89% of sharks belonging to one of these, indicating significant inbreeding. This finding is concerning, as it implies that the species could become extinct in a very short period of time if faced with environmental changes or disease.



Correspondence between photo identification and genetic fingerprint of a great white shark (C_050606B) sampled on four different occasions over two years. Each genetic sample has been uniquely coded (G63, G114, G173 and G194) to allow for blind scoring of duplicates using a genetic fingerprint. The sample G116 belongs to a different individual (C_040705), as confirmed by the genetic profile and the different notch pattern on the dorsal fin (Andreotti et al. 2016, <https://doi.org/10.3354/meps11744>).

16S ribosomal RNA (rRNA) gene sequencing

At the turn of this century, 16S rRNA gene sequencing revolutionised bacterial isolate identification. Sections of the 16S rRNA gene are identical between bacterial species and are used as a common target to amplify the signal for all bacteria in one reaction tube. Subtle differences in the rRNA gene serve as a fingerprint, allowing us to distinguish the individual bacteria in a sample. With this approach, tedious bacterial culturing and costly biochemical identification techniques are avoided. Instead, DNA is extracted from bacteria in a sample and then sequenced to produce data that can be compared to existing public databases. One such database, GreenGenes, contains the DNA sequences serving as fingerprints for specific bacteria.

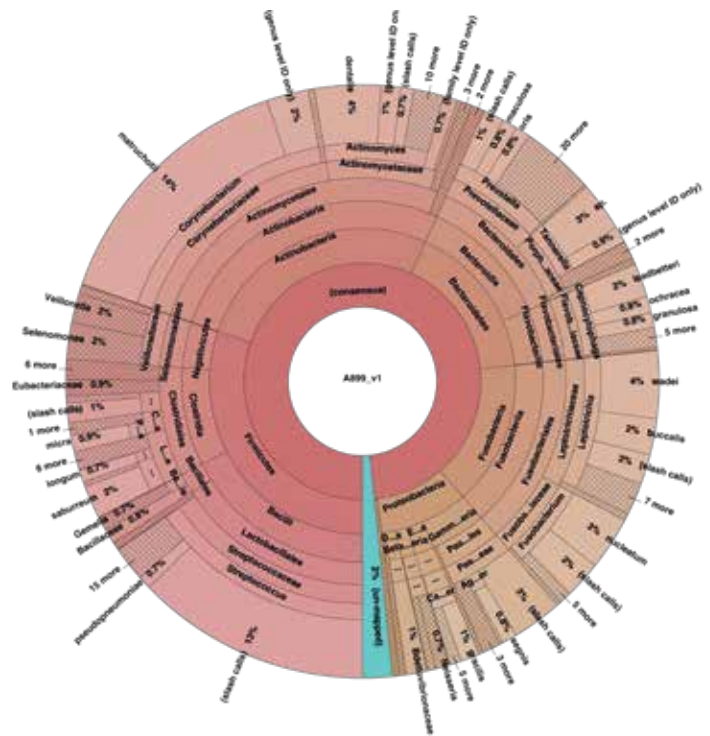
A probiotic for broiler chickens

There is a high demand for animal protein worldwide, and the poultry industry is ranked amongst the largest suppliers, but it incurs substantial losses due to bacterial and fungal infections. The main reason for this is because newly hatched chicks are not exposed to mature birds for long enough to obtain the beneficial bacteria from the gastrointestinal tract of hens. It takes two to four weeks for a chick to develop a balanced and stable gut microbiome, while a well-developed immune system takes even longer. Chicks raised in broiler houses are therefore more susceptible to infections that lead to weight loss, poor meat quality and even death.

As part of his doctoral research, Dr Deon Neveling focused his study on the development of a multi-species probiotic that can be added to feed, with the aim of improving growth performance and health. Bacteria were isolated from different sections of the gastrointestinal tract of healthy free-range broilers, identified to species level using Sanger sequencing at the CAF, and then screened for probiotic properties. Those that exhibited the desired probiotic effect were used by Dr Neveling to develop the probiotic, which was patented after being successfully tested as a feed additive.



Sanger sequencing was used to develop a probiotic feed additive for broiler chickens.



A Korona plot representing the bacterial diversity (alpha diversity) within a single oral plaque DNA sample. The relative abundance of a bacterial species is shown as a percentage of the total sequencing data that was generated for the poly-bacterial DNA sample.

Diabetes and cardiovascular risk

In April 2019, 128 poly-bacterial DNA samples were sequenced at the CAF, using the 16S rRNA gene sequencing approach. This work forms part of Yvonne Prince's doctoral degree, which focuses on the effects of smoking and alcohol on the oral microbiome in persons with cardiometabolic risk factors. Her research is conducted under the leadership of Prof. Tandi Matsha and Prof. Glenda Davison at the South African Medical Research Council's Cardiometabolic Health Research Unit, based at Cape Peninsula University of Technology. Yvonne recognises that the incidence of diabetes mellitus, obesity and cardiovascular disease is becoming a growing problem in South Africa. Internationally, research has shown evidence of a bi-directional relationship between diabetes and oral disease – the presence and management of one influences the presence and management of the other – so individuals with diabetes are more likely to have an increased severity of oral disease.

Because the oral microbiome is such a complex system, harbouring a rich and diverse population of more than 600 microflora that includes bacteria, viruses, fungi and protozoa, it makes it extremely difficult to identify and isolate the microorganisms present. With the help of independent molecular methods such as 16S rRNA sequencing, developed to easily group and identify the uncultivable microbiota that are present, this analysis has become possible. The data generated from the sequencing was used to search the Greengenes and MicroSEQ databases for comparison to 16S sequences from closely related species. The results are listed as highest or closest relatives to the species present within the sample. Finding signatures in the bacterial diversity that are specific to persons with diabetes would enable

early diagnosis and allow opportunities to promote health and wellness in pre-symptomatic persons.

The genetics, distribution and diversity of cat fleas

Fleas are a pesky problem for pet owners. Worldwide, there are about 2 500 species or subspecies of these tiny bloodsucking insects. They cause discomfort due to itching, and can transmit tapeworms and pathogens that may cause disease in humans and pets. Dr Luther van der Mescht is a postdoctoral researcher in the evolutionary ecology of parasites at Stellenbosch University. He is interested in the genetic and morphological differences within cat-flea species, particularly those that are found on the African continent. Genetic analyses of the fleas are completed by performing Sanger sequencing of similar regions

in the cat-flea genome, allowing Dr van der Mescht to draw phylogenetic trees that assist with species classification.

The aim of the study is to develop an accurate classification system that can be used to develop a pest control programme. Genetic analyses will also include 16S rRNA gene sequencing to determine the diversity of the bacterial population on the various cat-flea species.



Andrei Savitsky, CC BY 4.0

Whole genome sequencing

During the 13 years that it took to complete the first draft of the human genome, one of the main challenges facing researchers was computational power. To sequence an entire genome, the DNA is broken into pieces that are less than 600bp (base-pairs) in length. The sequence of each fragment is determined individually and the resulting data is pieced together by finding identical, overlapping regions. Today the computers that can piece together this genome puzzle are cheaper, smaller and faster – allowing a laboratory like ours to complete genome assembly in weeks instead of years.

Fungal resistance genes in grapevine

The CAF is situated in the Stellenbosch winelands region, which has some of the oldest vineyards in South Africa. The market demand for *Vitis vinifera*, the most common type of grapevine, places constant pressure on plant breeders to develop varieties that have improved characteristics, such as disease resistance. Using conventional breeding for disease resistance is a long and costly process, due to the relatively long growth cycle of grapevine. However, the introduction of molecular markers has made it possible to identify genes or genomic regions related to specific disease resistance much earlier in the breeding cycle. Extensive research has identified the disease-resistance genes in known resistant varieties, as well as microsatellite markers that are linked to these genes to enable marker-assisted selection (MAS). In other words, the progeny in a breeding experiment can be screened for microsatellite



DNA sequencing is used to study viruses affecting grapevines in the Stellenbosch winelands.

markers linked to desired traits, and those that do not carry the desired genes may be eliminated from the process, significantly reducing the costs involved in plant breeding.

Prof. Gerhard Petersen uses both Sanger and next-generation sequencing methods as fundamental tools in his research on plant viruses. Amongst these are plant pathogens of *V. vinifera*. The data generated from the sequencing techniques are used in diagnosis, characterisation, phylogenetic analysis, population analysis and discovery of various plant viruses that are new to South Africa, or to science.

Poultry disease research

Prof. Celia Abolnik, the Research Chair for Poultry Health and Production at the University of Pretoria, applies next-generation sequencing in the diagnosis and study of avian diseases. A diverse group of bacterial and viral pathogens cause diseases in the national chicken flock, which is important for regional food security. Pathogens that are regularly investigated through next-generation sequencing at the CAF include *Mycoplasma* species, influenza A virus, avian avulavirus type 1, infectious bronchitis virus, fowl adenovirus and infectious laryngotracheitis virus. The next-generation sequencing data for *Mycoplasma gallinarum* and *M. gallinaceum* have been assembled and published as the first complete annotated genomes of the species.

For enquiries about DNA sequencing services offered by the Central Analytical Facilities at Stellenbosch University, please contact Ms René Veikondis (renev@sun.ac.za) for Sanger sequencing services or Ms Alvera Vorster (vorster@sun.ac.za) for next-generation sequencing services.

CURRICULUM CORNER

LIFE SCIENCES: GRADE 11

DNA: the code of life; Genetics and inheritance

LIFE SCIENCES: GRADE 12

Evolution by natural selection: artificial selection

People and plants in the Stone Age

May Murungi tells us about archaeobotany at Bushman Rock Shelter

Ancient plants for archaeology

At the mention of the word archaeology, for most people what comes to mind are stone tools, bones and pottery, rather than plant remains. Yet ancient plants can be preserved as macrofossils, which can be observed with the naked eye (e.g. woody parts, leaves and seeds), or as microfossils, which have to be processed from deposited archaeological material, such as sediments and stone tools, before being studied under the microscope (e.g. pollen and phytoliths).

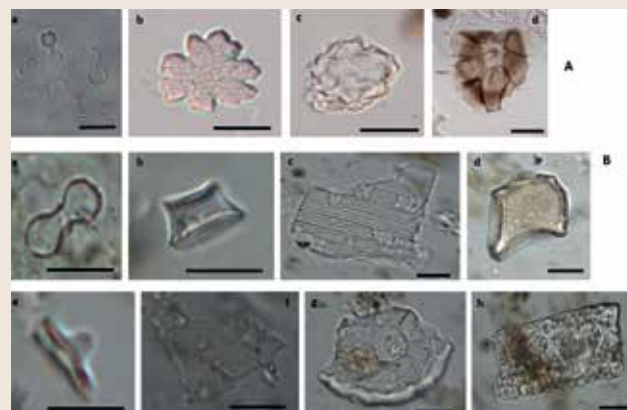
Ancient plant remains in the form of fossil charcoal, carbonised (charred) seeds, pollen and phytoliths provide information on the role of plants utilised by early modern humans for various purposes – including fire, food, medicine and bedding – helping us to understand cognitive and behavioural evolution, such as the origin of plant cultivation and the use of fire. Importantly, they can also provide insights into past vegetation and environments, indirectly telling us about the climate in which early humans thrived.

It is only in recent years, however, that archaeologists in South Africa have come to appreciate the wealth of information that can be provided by ancient plant remains studied by archaeobotanists. To this end, there is a growing body of archaeobotanical research in South Africa that started with the study of charcoal (Diepkloof Rock Shelter and Sibudu Cave) and carbonised seeds (Sibudu Cave), as well as phytolith analysis at Sibudu Cave and Pinnacle Point. Phytolith analysis is now taking shape in South Africa, with several ongoing studies at other sites, such as Bushman Rock Shelter, Border Cave and Umhlatuzana Rock Shelter.

It is often necessary to use a combination of archaeobotanical remains as proxies to provide a more complete picture of plant use and plants that were available in the vegetation at the time. For example, charcoal will give a good indication of woody plants, while phytoliths have the added advantage of providing insight into the use of non-woody plants such as grasses and sedges. Pollen is often poorly preserved at archaeological sites, as has been the case for Bushman Rock Shelter and Sibudu Cave, and phytolith analysis has proved to be more effective. In addition, while pollen tends to be blown into archaeological sites from far-off places, phytoliths are often produced on site from their

What are phytoliths?

Phytoliths are microscopic silica bodies that are formed within and between cells in many plants, as a result of absorption of silica from soil during the uptake of water and mineral salts. When plants die and decompose, or are burnt, the phytoliths are released directly into the soil, where – being hard and resistant to destruction – they can be preserved for thousands of years. Because phytoliths tend to take on the shape of living cells, they can be differentiated in many plant families, allowing for identification of taxa, sometimes up to genus level.



A - Phytoliths from modern plants:

a) bilobate-shaped grass short cells from the leaves of the grass *Melinis repens*, b) decorated bodies from the fruit of the tree *Celtis africana*, c) cystoliths from the leaves of a *Ficus* sp. tree next to the rock shelter d) hair base with polyhedral cells from the leaves of *Ficus* sp. next to the rock shelter.

B - Phytoliths from the Later Stone Age layers:

a) bilobate-shaped grass short cell, b) rondel-shaped grass short cell, c) grass articulated phytoliths composed of long cells and short cells, d) bulliform grass phytolith, e) cone-shaped sedge type phytolith, f) sedge type articulated phytoliths, g) hair base with polyhedral cells (from dicots), h) a weathered blocky phytolith associated with various plant groups.



Guillaume Porraz

parent plant, allowing for a very local reconstruction of the use of plant resources and space at a given site. For example, a phytolith study at Sibudu Cave in KwaZulu-Natal provided evidence for the presence of hearths (fireplaces) that are no longer visible at the site, because the phytolith content of the sediments taken from non-hearth layers was similar to those taken from visible hearths. Furthermore, unlike grass pollen which cannot be taxonomically identified beyond the grass family level, phytoliths can generally be associated with grass sub-families, corresponding to grasses that are adapted to different ecological niches and environmental conditions.

It is this very characteristic that puts phytoliths ahead of other archaeobotanical proxies, since grasses are highly sensitive to environmental change. Although caution needs to be taken when interpreting such data from archaeological sites for past environmental construction – as they often represent human choices of plant use – it is assumed that plants found in the archaeological record are generally representative of the type of plants that were available in the surrounding vegetation.



Dr Aurore Val collects modern soil samples for phytolith analysis.

Phytoliths at Bushman Rock Shelter

Phytolith analyses at Bushman Rock Shelter are currently in progress as part of a postdoctoral DST-NRF Centre of Excellence fellowship (2018/19) held at the Evolutionary Studies Institute at Wits University.

Our understanding of the archaeological phytolith record is based on analogies with phytoliths found in modern plants. Phytoliths from modern surface soils under different vegetation communities are studied to determine which phytolith assemblages are representative of a given vegetation type and which phytolith types do not survive long enough in soils. It is often recommended to create region-specific modern phytolith reference collections, as phytolith assemblages from one region may differ in application or interpretation from another region.

While it is understood that ancient landscapes may not have resembled those of today, it is important to make such present-day calibrations between modern plant vegetation types and the soils beneath them to fully understand the ancient phytolith assemblages at a given site. As a first step to the fossil phytolith study at Bushman Rock Shelter, Dr Aurore Val and I therefore collected modern plant specimens (wood and leaves of trees) and soils occurring in the area in the late winter of 2017. In the summer of 2018 more plant samples from both grasses and trees were collected with the help of Dr Christine Sievers. Sediments representing the Middle Stone Age (>45 000 years ago) and Later Stone Age (16 000–10 000 years ago) periods at Bushman Rock Shelter were collected from archaeological sediment layers during different field campaigns in 2014, 2017 and 2018.

Phytoliths were extracted from each of the modern plant parts (wood, leaves and fruit) using standard methods that involve burning the plant material at 450°C in a furnace, followed by boiling the ash in hydrochloric acid to remove any carbonates, and then in nitric acid to

remove the remaining organic matter. Fossil phytoliths have been extracted from several of the archaeological sediments by treating them with acids. For both modern and fossil phytoliths, the final samples are mounted on glass slides and viewed under the microscope at 400x magnification to identify and classify the phytolith morphotypes into plant types, such as grasses and sedges (monocot plants), trees, or dicot plants in general. Phytoliths from the archaeological sediments of Bushman Rock Shelter are generally of good preservation and – although their surfaces may be weathered – they are often still identifiable.

What we hope to achieve

Through phytolith analyses, we hope to supplement other ongoing botanical analyses (charcoal and seeds) at Bushman Rock Shelter by providing key information on mainly the grasses and sedges that were utilised at the site. This will in turn provide one of the best proxies to infer past climate, necessary for interpreting other studies that seek to understand how climate may have influenced technological adaptations during the Stone Age. Publication of the modern phytolith reference collection being developed will contribute to the few that currently exist for South Africa, and will provide a basis on which to interpret archaeological phytolith analyses from sites that occur within the country's Bushveld vegetation types. We hope in the future to apply this information to other sites in the area, such as the Heuningneskrans and Oliemboomspoor rock shelters.

With the increasing interest by archaeologists to understand how life of ancient populations may have been influenced by environmental change, there is growing recognition of the value of including archaeobotanists in archaeological projects in South Africa. Since there is a paucity of inland natural deposits in South Africa that span periods as long as the Stone Age, archaeologists are now depending on the environmental data that archaeobotanical studies are providing. It will therefore be up to archaeobotanists to provide quality scientific data that can be used for this kind of environmental interpretation. We will have to think of ways in which our data can contribute to archaeology beyond human subsistence, and clearly explain its limitations, given its inherent bias of human selection and the potential for misinterpretation.



Dr May Murungi completed a master's degree in biology, focusing on natural resources, ecology and conservation, at Mbarara University in Uganda in 2013. She attained a PhD in archaeobotany from Wits University in 2018 for her research on phytoliths in modern plants and Middle Stone Age sediments from Sibudu Cave. She is currently a postdoctoral fellow at Wits University's Evolutionary Studies Institute, funded by the Centre of Excellence in Palaeosciences. She joined the Bushman Rock Shelter project in 2014, and received a grant from IFAS-Recherche in 2017.

*Republished from the online journal of IFAS-Recherche, Lesedi #21
<http://www.ifas.org.za/research/2019/lesedi-21-july-2019/>*



Bushman Rock Shelter

The Bushman Rock Shelter site is located in the district of Ohrigstad in Limpopo, in the northern foothills of the Drakensberg range. Excavated in the 1970s by the team of Prof. Hannes Eloff from the University of Pretoria, the site revealed archaeological strata dating from the Middle and Later Stone Age. The mineral and organic remains, perfectly preserved over 7 meters depth, are key indicators for tracking the changes in the hunter-gatherer populations spanning the last 200 000 years.

In 2014 the French National Centre for Scientific Research (CNRS) and the French Institute in South Africa (IFAS) initiated a new project at the Bushman Rock Shelter, with the aim of clarifying the stratigraphy and getting new archaeological samples. The project is funded by the French Ministry of Foreign Affairs' Commission on Archaeological Excavations and supported by Wits University and the South African Heritage Resources Agency (SAHRA).

In 2018 the project came to the end of its first four-year term, but was extended to at least 2021 and now includes the nearby site of Heuningneskrans too. Excavations at the sites are conducted as part of a field-school involving 10–15 students from France, South Africa, Zimbabwe, Zambia and Mozambique each year.

Bushman Rock Shelter is open to visitors all year round, as it houses the Museum of Man, which forms part of the Echo Caves complex – a tourist attraction offering guided tours.

For more information, see <http://www.ifas.org.za/research/2019/bushman-rock-shelter-2014-2021/> or <https://www.echocaves.co.za/>



Chemistry for clean air



Amanda Mahlangu, a master's student in chemistry at the University of Pretoria (UP), won an award for the best student paper presented at the 2019 National Association for Clean Air Conference, held in Stellenbosch in October.

Her presentation was titled 'Characterisation of semi-volatile hydrocarbon emissions from diesel engines', and was based on a study in which she analysed diesel exhaust emissions. The results of the study will contribute to our understanding of ground-level ozone and secondary organic aerosol (SOA) – both being pollutants that affect human health and ecosystems.

"Receiving the award is a great honour as it meant recognition of the hard work that I have put into my project, by professionals with great knowledge in my field and in the scientific community," an elated Mahlangu said about the award. "It motivated me to continue to work hard and strive to grow within my field."

She said while choosing a career path can be very difficult for most young people, it wasn't for her.

"I have always loved chemistry from a young age and was quite good at it in high school, so the choice to do it at a degree level was almost an obvious one. Completing the degree was not an easy task, however winning this award felt like validation that I made the correct decision, and that – despite the challenges – following my passion was the best choice I could have made for myself."

Mahlangu completed a bachelor's degree in biochemistry, majoring in both biochemistry and chemistry, and it was during these years that she developed her passion for research and analytical chemistry. After her undergraduate degree, she went on to complete an honours degree in chemistry in 2017. The following year she started her master's degree, and joined the Environmental Monitoring and Sensing Research Group led by Prof. Patricia Forbes.

Mahlangu also won the Nico Nibbering Travel Award to attend the 4th International Mass Spectrometry School in Spain in 2019, and was awarded a UP postgraduate master's research bursary as well as a Sasol bursary in 2018 and 2019. Her MSc project, co-supervised by Prof. Forbes and Paul Schaberg from Sasol, focuses on characterising exhaust emissions from diesel engines using different fuels.

"I use portable sampling devices called denuders to collect diluted diesel exhaust emissions and analyse them using a thermal desorption-comprehensive 2D gas chromatography-time of flight mass spectrometry instrument, which uses a high-temperature thermal desorber to transfer the collected analytes into the instrument, where they are separated into different chemical classes," she explains. "My aim is to identify and quantify alkyl-benzene and n-alkane hydrocarbons, which are known to play a role in the formation of ground-level ozone, and to determine the ozone formation potential of these compounds."

Mahlangu also investigates whether there are discernible differences in their emission as a result of fuel composition, fuel property, engine operating conditions, and exhaust after-treatment technology.

"The study addresses a major analytical limitation in characterising these emissions, and studies like it are important to help understand the ground-level ozone levels and SOA formation in South Africa," she said.

Issued by Martie Meyer, First Communication Practitioner for the University of Pretoria's Faculty of Natural and Agricultural Sciences.



The Royal Observatory, Cape of Good Hope

Beyond 200 years of Astronomy

This year, 2020, marks the bicentenary of the South African Astronomical Observatory (SAAO). Africa has a long and rich relationship with astronomy, dating back millennia and the unique geographical importance of Africa in global astronomy was recognised two centuries ago with the establishment of the Royal Observatory, Cape of Good Hope in 1820.



Founded in on 20 October 1820, the Royal Observatory is the oldest scientific institution in the country and perhaps in Africa. Formally, it was controlled by the Royal Navy and was intended for the improvement of navigation. Its main duty was to chart the southern skies and provide a time service for passing ships.

It is now a national facility of the National Research Foundation and the national centre for optical and infrared astronomy in South Africa. As part of the South African Astronomical Observatory, it is known for the rich history it holds in the contributions in science as well as for its architecture. Consequently, it was formally declared a National Heritage Site by the South African Heritage and Resource Agency (SAHRA) in December 2018.



The main building around 1842(top), photographed by Charles Piazzzi Smyth and today(bottom) as visualised by Neal Katz and Daniel Cunnama(SAAO)

Local astronomers witness starburst heatwave



Katharina Immer

Artist's impression

Astronomers from the Hartebeesthoek Radio Astronomy Observatory (HartRAO) and the Centre for Space Research at North-West University (NWU) were part of an international collaboration monitoring a 'heatwave' of thermal energy that radiated outward from a massive young star, or protostar.

Nobody knows how some stars grow to have a mass that is tens to hundreds of times greater than the sun's. Protostars can't be observed with optical telescopes because they're shrouded by a molecular cloud composed of gas and dust, which over time collapses inwards under gravitational and frictional forces, building up the central star body in a process called disc-aided accretion. One theory proposes that high-mass protostars gain most of their mass in short intense bursts of accretion, or 'episodic accretion', followed by long periods of inactivity, possibly lasting hundreds to thousands of years.

Fortunately, protostars can be investigated by studying their masers – an acronym for 'microwave amplification by stimulated emission of radiation'. These spectral line emissions arise from molecules such as water (H_2O), methanol (CH_3OH), hydroxyl (OH), formaldehyde (CH_2O) and ammonia (NH_3) in the protostars' molecular clouds.

In 2006 a high-mass protostar named G358 was discovered during a galactic plane survey of 6.7 GHz methanol maser emissions. The find was unremarkable, however, as it was just one of almost a thousand sources observed, and it warranted one of the briefest entries in the notes published on the survey results. But in January 2019, astronomers from Ibaraki University in Japan noticed a flaring of G358's methanol masers, indicative of a potential accretion burst. They alerted the Maser Monitoring Organisation (M20), a global community of scientists involved in maser-driven astronomy, which mobilised quickly to study the emissions.

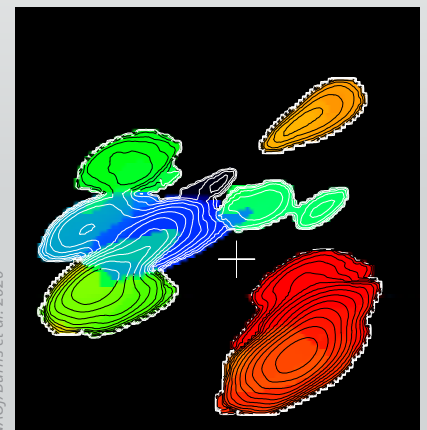
Dr Ross Burns of Japan's National Astronomical Observatory leads M20, and explains the benefits of the collaborative approach.

"Each radio observatory is different and each has its advantages and limitations. Typically, each will have a few receivers that detect radio emission at various frequency ranges. The 6.7 GHz methanol maser is a reliable and bright maser, but there are hundreds of masers of different molecules and different frequencies. So each observatory will be able to observe some, but not all masers. By establishing a communication network between radio observatories, the M20 can coordinate efforts so that as many maser lines as possible are observed when a source is bursting."

Indeed, over the next few months many new maser lines were discovered. The main maser spot, dubbed G358-MM1, was imaged using very-long-baseline interferometry (VLBI).

"In short, a collection of radio observatories record data at very high time resolution for the same source at the same time. Those data sets are synchronised and

Data image showing a methanol maser emission, which traces the heatwave as it propagates outward from the position of the high-mass protostar, represented by the white cross. The colours indicate the velocity of the gas motion in the direction of the observer's line of sight.



NAOJ/Burns et al. 2020

combined such that the signals interfere with each other, hence the name interferometry,” explains Dr Ross. “In this way we can establish the shape of the emission that produces the signal.”

The data image shown here was made using several radio telescopes in Australia and New Zealand, as well as South Africa’s HartRAO. The team compared multiple images spaced a few weeks apart to reveal the ‘heatwave’, which was later confirmed by SOFIA to originate in an accretion event. SOFIA – the Stratospheric Observatory for Infrared Astronomy – is a Boeing 747SP modified to carry a 2.7 m reflecting telescope. Flying the plane almost 14 km above the Earth’s surface puts it above 99% of the infrared-blocking atmosphere, allowing astronomers to study the solar system and beyond in ways that are not possible with ground-based telescopes.

“The M20 observations are the first to witness the immediate aftermath of an accretion burst in a high-mass protostar in such detail, which provide evidence in support of the ‘episodic accretion’ theory of high-mass star formation,” says Dr Burns. “Our team greatly benefits from close communication between a diverse, global community of observers, astrophysicists and theorists in planning, executing and interpreting transient maser events.”

The input of these specialists is reflected in the authorship of the paper describing the heatwave, which was published in *Nature Astronomy* in January 2020. There are 23 authors representing 15 countries, and three of them are from South Africa – Dr Gordon MacLeod, previously affiliated to HartRAO but now at the University of Western Ontario in Canada, Dr Fanie van den Heever, a postdoctoral fellow at HartRAO, and Associate Professor James Chibueze of the NWU’s Centre for Space Research. All three were also among the numerous co-authors on another paper, with Dr MacLeod as lead author, focusing largely on observations conducted with HartRAO’s 26 m telescope to confirm the flaring of the 6.7 GHz methanol masers, and to search for hydroxyl, formaldehyde, water and other methanol masers.

“It is a privilege to be at the cutting edge of humanity’s efforts to understand space and celestial bodies, and to have witnessed star behaviour that is entirely new,” says Prof. Chibueze. “The team’s discoveries are hugely exciting and we are hopeful that further investigation will reveal more about the physical processes taking place within the G358 star. New horizons are opening up in space and it is wonderful that NWU and South Africa are part of it.”

- Burns RA, Sugiyama K, Hirota T et al. A heatwave of accretion energy traced by masers in the G358-MM1 high-mass protostar. *Nat. Astron.* (2020) <https://doi.org/10.1038/s41550-019-0989-3>

CURRICULUM CORNER

PHYSICAL SCIENCES: GRADE 12

Emission and absorption spectra

Africa’s first PhD in indigenous knowledge of astronomy



Dr Motheo Koitsiwe at the graduation ceremony.

For centuries, people in Africa have measured time, seasons and direction by the stars, and now the North-West University (NWU) has conferred the continent’s first PhD in Indigenous Knowledge Systems (IKS) focusing on African indigenous astronomy. Dr Motheo Koitsiwe received his degree in October at the NWU campus in Mahikeng.

In his doctoral research, Dr Koitsiwe investigated African indigenous astronomy of the Batswana in Botswana and South Africa. The study revealed that the Batswana use their indigenous knowledge of celestial bodies for agriculture, natural disaster management, reproductive health, navigation, time calculation, calendar-making, as well as rainmaking and thanksgiving ceremonies. Traditional songs, poems and indigenous games are also used to transmit knowledge of celestial bodies to younger members of the community, to preserve it for posterity.

Dr Koitsiwe developed his passion for IKS research, and especially for indigenous astronomy, early in his academic career. “This passion was ignited by my late grandmother, Mmamodiagane Tladinyane, when she narrated stories, poems, riddles, songs of African night skies and cosmologies around the fireplace,” he explains.

The NWU’s campus in Mahikeng is the pioneer of IKS in South Africa, because in 2001 it was the first higher education institution in the country to have a registered teaching, learning and research programme in IKS, accredited by the South African Qualification Authority (SAQA).

According to Prof. Mogomme Masoga, who co-supervised Dr Koitsiwe’s doctoral studies, his student’s thesis had originality and novelty.

Dr Koitsiwe also holds a BA degree in social sciences, as well as an honours and a master’s degree in IKS from the NWU. He plans to translate his thesis into Setswana so that it not only reflects the aspirations of academia, but the Bakgatla-Ba-Kgafela and Batswana in general.

BIOPHOTONICS

Shedding light on point-of-care diagnostics

The CSIR biophotonics group share their research



Biophotonics is a field of research combining biology and photonics, which is the science and technology of generating, controlling and detecting photons (particles of light). It focuses on the development and use of light-based methods to study biological substances such as atoms, molecules, cell organelles, whole cells and tissues.

The link between biological substances and light is beneficial because it can be used for both therapeutic and diagnostic purposes. In most applications, the light-

Lolucwaningo lisebenzisa ukukhanya njengendlela yokucwaningwa izicubu zomzimba zifana nama cell nedlela akhiwe ngayo, bese kwakhiwa imishini engasiza ukubona izifo ezahlukahlukeni kanye nokuzelapha.

Translation by Zamantimande Kunene

The seven members of the CSIR's biophotonics group, from left to right: Dr Patience Mthunzi-Kufa (group leader), Dr Saturnin Ombinda-Lemboumba, Lebogang Thobakgale, Rudzani Malabi, Charles Maphanga, Masixole Lugongolo and Dr Sello Manoto.

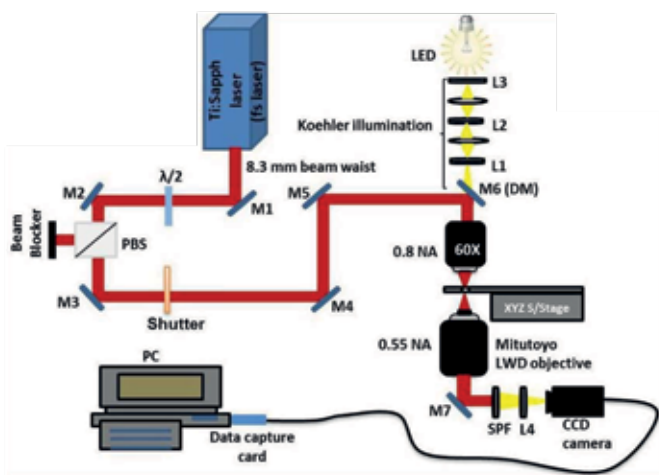
based methods used in the field of biophotonics are non-invasive as they do not require the use of dyes and do not affect organelles and cells outside the region of interest. Their non-invasiveness, sensitivity and specificity mean that light-based technologies can help improve the tools available for the study of various diseases, as they make it possible to detect changes in cells and organelles during different stages of infection.

The CSIR's biophotonics group is made up of researchers from various scientific disciplines, including biomedical sciences, biochemistry, biotechnology, chemistry, physical science and virology. The group is conducting

research towards the development of light/laser-based point-of-care devices for diagnostic and therapeutic purposes for various medical conditions, such as human immunodeficiency virus (HIV) and cancer. The group also conducts research in optical micromanipulation of embryonic stem cells for tissue engineering investigations, as well as quality screening of medicinal drugs. The research is intended to facilitate early disease diagnosis and to expand healthcare in resource-limited populations. The envisaged medical tools will be designed to be used outside a formal clinic environment, without the need for specialised training. So far, most of this research is still being conducted *in vitro*. What follows is a summary of research projects conducted within the biophotonics group.

Targeted drug delivery within HIV-infected cells

Nearly 40 years after the discovery of HIV, there is still no cure, although antiretroviral treatment can control the virus, meaning that HIV-infected people can live long and healthy lives. One of the major reasons the current treatment cannot cure HIV infection is because there are sites in the human body where the virus 'hides'. As such, when the infected individual stops taking treatment, the virus in those sites multiplies and spreads throughout the body again. Because of this challenge, the biophotonics group is conducting research with light in the form of femtosecond laser to deliver drugs to specific cells. In this work, HIV antiretroviral drugs have been successfully delivered to target cells *in vitro* using a custom-built photo-translocation (laser-induced drug delivery) system, and a reduction in infection has been achieved.



A schematic representation of the photo-translocation system used to introduce drugs into cells. From the titanium-sapphire laser (Ti:Sapph), the beam (in red) is directed via mirrors (M1–7) to the sample stage (xyz stage), where the culture dish containing cells is placed. The Koehler illumination lights the sample, and the camera captures cell images for display on the computer (PC).

The femtosecond laser uses ultrafast laser pulses that gently disrupt the cell membrane, allowing the drug to enter the poked cell without compromising the integrity of the membrane, which would lead to cell death. The ability to deliver drugs in this manner reduces side effects as the meds are directed to the diseased site/cell without interfering with other healthy sites that have no need of the drug. The study showed that there is a way



Charles Maphanga using the femtosecond laser photo-translocation system, which was built in-house, to introduce drugs into mammalian cells.

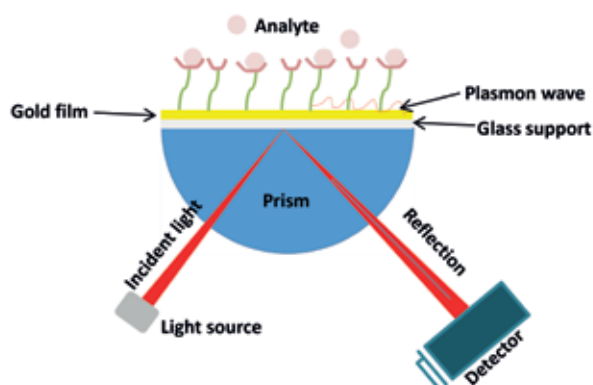
to target specific sites, so future studies will focus on delivering antiretroviral drugs to the latent sites – where the virus is hiding – with the expectation that the virus will completely die.

Photo-transfection of stem cells

In tissue engineering research, stem cells have been used as starting material in the synthesis of mammalian cells for the treatment of various cell-based diseases. This is performed by manipulating the DNA content of the cells to bring about a specific effect, such as developing a new cell type through the process of differentiation. This controlled gene expression of stem cells is achieved by transfection, where DNA is introduced into a stem cell using chemical, viral or physical methods. In biophotonics, instead of introducing the DNA with these conventional methods, a femtosecond laser home-built microscope system is used to prick the cellular membrane and allow entry of DNA to change the behaviour of stem cells. Photo-transfection improves transfection efficiency when compared with standard transfection methods. So far the CSIR team have been able to introduce green fluorescent protein DNA into stem cells using this system.

Surface plasmon resonance for quantitative studies

Surface plasmon resonance (SPR) is a label-free light-based detection method used in clinical analysis. It is commonly used for quantitative purposes to determine concentrations. The analyte is loaded onto a sensor chip that is coated with a metal, such as gold, in order to enhance the signal produced by the interaction of light and the analyte. The signal generated originates from the changes in the refractive index at the surface of the sensor chip.



The surface plasmon resonance system used for measuring the analyte concentration in biological samples.

The work that has been conducted to date in the biophotonics group shows that the custom-built SPR system can detect biological samples, and coating the sensor chip with gold improves the signal. The ultimate goal of conducting this research is to develop a point-of-care device that can be used to measure viral particles in a given specimen.

A similar technology that is studied in the group is the photonic crystal-based biosensor, which is based on homogeneous sensing. It senses the presence of an analyte by means of refractive index variations – there is a shift in the detection wavelength, which forms the basis of label-free detection by light-based technologies.

Photodynamic therapy for cancer

Photodynamic therapy (PDT) is a treatment that uses a photosensitiser and a specific type of light to activate the photosensitiser. Generally, during a PDT treatment, a photosensitiser is introduced into the bloodstream and is distributed all over the body, absorbed in cancer cells and stays longer in them. Roughly 24 to 72 hours after injection, by which time the photosensitiser has exited normal cells but remains in cancer cells, the site of cancer is exposed to light. The photosensitiser absorbs light and produces a toxic form of oxygen that kills cancer cells. In addition, PDT destroys cancer cells by damaging the cells' blood vessels, preventing them from receiving the nutrients required for survival, and also stimulates the immune system to attack cancer cells. The CSIR's biophotonics group has demonstrated the efficiency of PDT in their *in vitro* studies on melanoma cells. When

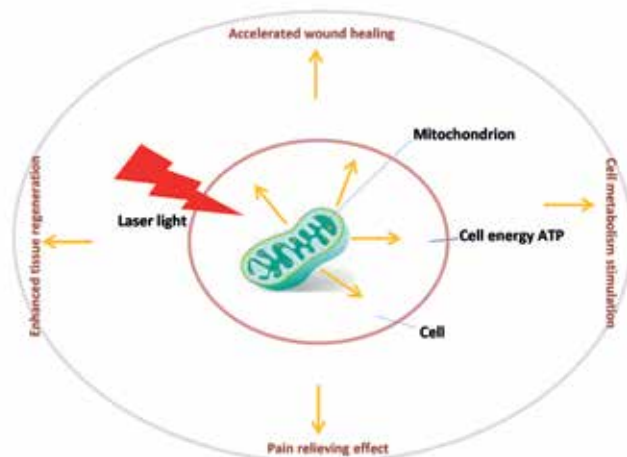
exposed to a photosensitiser, these cancer cells were destroyed, whereas the untreated cells continued to grow without any changes in their morphology and viability.

Optical trapping and spectroscopy for detection of HIV infection

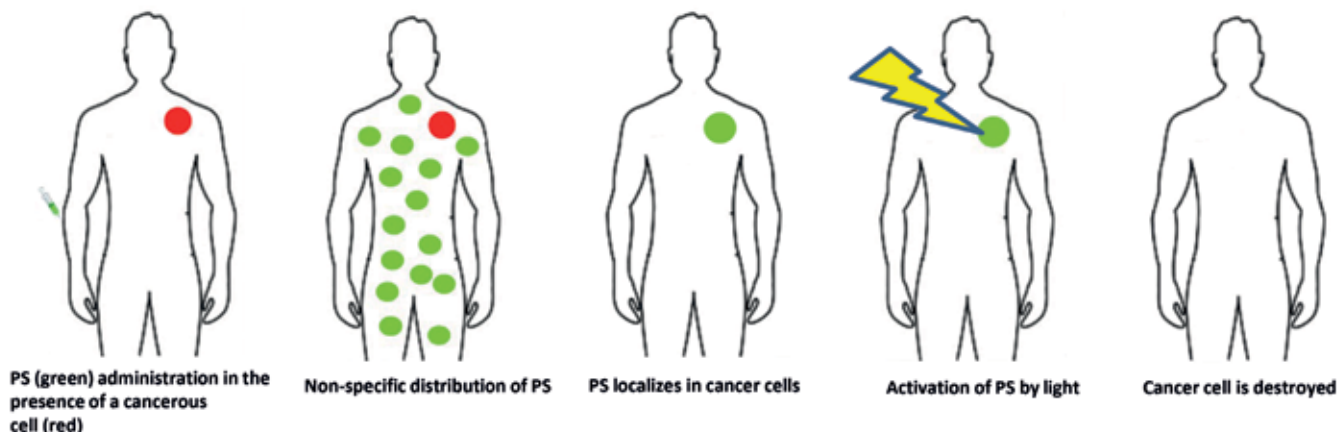
Optical trapping is a process that occurs when a highly focused laser beam creates a force attracting particles to the centre of the beam. This force, typically in the order of piconewtons, can be used to hold and manipulate microscopic particles, such as cells and subcellular components, and is particularly useful for single cell manipulation. The trapped particle can then be further analysed using various spectroscopic techniques. The biophotonics group has been able to distinguish between optically trapped HIV-infected and uninfected TZM-bl cells using a custom-built optical trapping system coupled with a transmission spectroscopy system. The results obtained showed that the infected cells transmitted less light compared to the uninfected cells, making it possible to differentiate between healthy and unhealthy cells.

Low-level laser therapy

Low-level laser therapy (LLLT) is the application of light in biological systems for therapeutic purposes such as wound healing, tissue regeneration, cell metabolism and



Low-level laser therapy (LLLT) mode of action. The cell mitochondrion absorbs laser light, leading to the production of adenosine triphosphate (ATP), which is associated with beneficial processes such as pain relief, accelerated wound healing, enhanced tissue generation and stimulation of cell metabolism.



Photodynamic therapy relies on the administration of the photosensitiser (PS), which binds to the cancer cells. Upon exposure to light, cancer cells are destroyed.



Dr Sello Manoto working on the reflectance spectroscopy platform built in-house for HIV detection and viral load quantification.

pain relief. LLLT uses light within the red (visible light) and near-infrared regions of the electromagnetic spectrum, in the wavelength range 600–1100 nm.

As the light beam comes into contact with the skin, photon energy penetrates tissues and interacts with various cell organelles such as mitochondria, stimulating biological processes that restore normal cell function and enhancing the healing process in the body. Researchers in the biophotonics group are evaluating its effect on HIV infection. So far, an *in vitro* study has shown that LLLT has the ability to reduce infection levels in TZM-bl cells. The use of LLLT is attractive as it has lesser side effects than drugs and it can be delivered via small point-of-care gadgets. Currently, however, there is no evidence that the findings from test tubes can be translated to the human body.

Raman spectroscopy for disease detection and screening of medicinal drugs

Raman spectroscopy is a light-based analytical tool generally used in chemistry to present detailed information about a particular material, such as its chemical structure, phase and any contamination. A Raman spectrum serves as a distinct chemical fingerprint that can be used to identify the material or distinguish it from others. In the biophotonics group, Raman spectroscopy research focuses on both disease diagnosis and screening of medicinal drugs as a means of conducting quality assurance. In one of the studies, a custom-built Raman spectroscopy system was used to investigate a group of non-essential amino acids through comparison of peak area versus concentration. Non-essential amino acids are the building blocks for producing proteins of various functionalities within living systems. Understanding the chemical properties of non-essential amino acids allows a thorough investigation of biological processes when there are irregularities.

Outcomes

With this research, the biophotonics group hopes to help uplift the quality of life of people in South Africa, especially those in resource-limited settings, by providing efficient laser-based, point-of-care tools for improved healthcare services. The group also contributes to human capital development, as it offers training to university students and interns as well as studentships at master's and doctoral levels.

Point-of-care is a medical procedure done close to the patient, at the time and place of care.

In vitro studies refer to experiments that are performed outside a living organism, typically taking place in a test tube or culture dish.

Stem cells are basic cells that can become almost any type of cell in the body.

Label-free detection methods do not require labels such as fluorescent dyes or radioactive isotopes to be attached to the molecule of interest to determine its presence or activity.

Analyte is a substance that is being studied or analysed.

Refractive index is the measure of the bending of a ray of light when passing from one medium into another.

Photosensitisers are molecules or drugs that become activated by exposure to light of a particular wavelength and cause changes to other molecules or cells.

TZM-bl cells are mammalian cells widely used in the field of HIV research for *in vitro* experiments because of their ability to mimic the body cells that HIV infects.

Masixole Lugongolo was the lead author of this article, which was based on contributions from the other members of the CSIR biophotonics group. Masixole joined the group in 2015 as a PhD student, and is registered with the University of South Africa. She has been involved in HIV-related research for more than a decade, having previously worked at the Centre for HIV and STIs at the National Institute for Communicable Diseases.

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Winner Martin Bekker with Pertunia Mashile (left) and Keafon Jumbam (right), who came second and third.

Charl Devenish

UJ take top spots at 3MT

Two postgraduate students from the University of Johannesburg (UJ) made it a one-two finish at the national Three Minute Thesis (3MT) competition in October, taking first and second place.

Martin Bekker, from UJ's Centre for Sociological Research, was declared the overall winner for his presentation titled 'Everything I thought about protest was wrong', and was awarded R16 000 in prize money. The video of his presentation was then submitted for the international Universitas 21 3MT competition, which was ultimately won by a student from the University of Queensland.

"For my PhD I was interested to see why people protest," Martin says in his presentation. "I looked at the largest dataset of its kind in the world, which happens to be from the South African Police Service attending every crowd incident, and I used machine learning processes to read 150 000 records."

His findings about the level of violence at protests and the key contributing factors can be viewed in the video, available at <https://universitas21.com/get-involved/student-competitions/three-minute-thesis-competition>.

Pertunia Mashile, from UJ's Department of Chemical Sciences, was awarded R13 000 as her second place prize, but also won the People's Choice Award for her presentation titled 'Can what is useful be harmful?' Pertunia is studying emerging pollutants such as cosmetic and pharmaceutical compounds in river-, waste-

and tap-water for her PhD, with a focus on developing analytical techniques. At the National Research Foundation (NRF) Awards ceremony in September, she was the recipient of one of two 'research excellence by next generation researchers' awards for outstanding academic performance by final-year doctoral students.

Third place and the R10 000 prize went to Keafon Jumbam, a zoology student from the University of the Free State (UFS), which hosted the 3MT national competition. Keafon's PhD research is on the social, ecological and personality factors influencing bat-eared fox foraging behaviour.

There were eight participants in the event, comprising both the winners and runner-ups from heats held at UJ, UFS and University of the Witwatersrand (Wits), as well as the winners from the Central University of Technology (CUT) and Durban University of Technology (DUT) heats. The three judges were from UFS, CUT and Wits.

- The 3MT concept originates from the University of Queensland in Australia, where the first competition was held in 2008. Participants must explain their PhD research in three minutes, in language appropriate to a non-specialist audience, and may use only one static PowerPoint slide, with no other resources or props. Today, 3MT competitions are held in more than 600 universities and institutions in 65 countries around the world.

Books

Cradle of life:

The story of the Magaliesberg and the Cradle of Humankind

By Vincent Carruthers. 256 pp. Struik Nature. R300.

This book is a truly impressive undertaking. Between its covers is an enormous amount of information, and it's all presented in a highly accessible form. The text is broken up into short sections and interesting boxes, which make it easy to dip in and out, and it's richly illustrated with photographs and colourful diagrams.

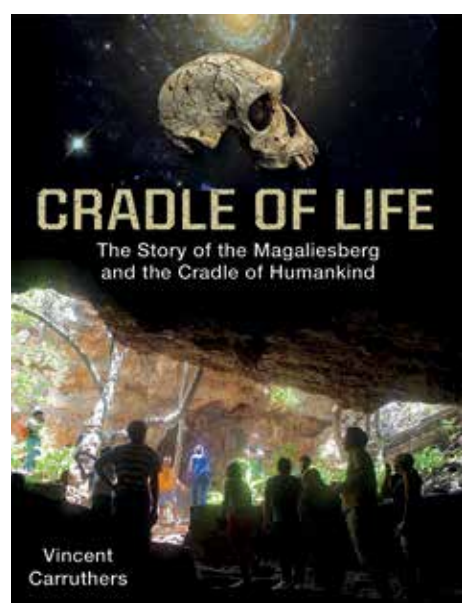
The author certainly knows his subject, as he was part of the teams that prepared plans for the declaration of the Cradle of Humankind World Heritage Site in 1999, and from 2006 led the campaign to have the Magaliesberg designated a UNESCO Biosphere Reserve, ultimately proclaimed in June 2015.

Here, he traces the history of the Magaliesberg landscape, following a timeline that begins with the origin of the universe in the Big Bang 13 800 million years ago

and the birth of our planet 4 600 million years ago, and ends with the anti-apartheid activity leading up to South Africa's first democratic election in 1994.

The book is divided into three parts – Life and Landscape, Human Evolution, and Archaeology and History – and it covers topics as diverse as astronomy and cosmology, geology and geomorphology, biology and ecology, palaeontology and anthropology, colonial history and the Boer War, as well as technological developments like the Hartbeespoort Dam, the Hartebeesthoek Radio Astronomy Observatory and the Pelindaba nuclear energy facility.

At the heart of the book, the Human Evolution chapters provide a good overview of the fossil discoveries – from Little Foot to *Homo naledi* – that have made the Cradle of Humankind world famous. Given that this topic forms part of the curriculum, the book would be a worthwhile addition to school libraries, but its attractive layout means it could also be displayed as a 'coffee-table book' in the home or office, or shelved as a keepsake from a visit to the area.



Ascent from the rough 10 to the top of the Magaliesberg into mountainous slopes.

The effect of the erosion carving the Magaliesberg landscape is a testament to its former self. The high quartzite craters had been greatly reduced and shaped up to a constant altitude along the entire length of the range. The shape of the mountain range had been smoothed to a rounded

280–180 million years ago: The Cradle-Magaliesberg in Gondwana

As Gondwana began to emerge from under the sea about 280 million years ago, the climate became warmer, and rainforest plants and animals began to develop and spread. However, in the Cape region, the elevated land in southern Gondwana, the landscape of the Magaliesberg and many of the other mountain ranges were still under the sea. The highlands across the Cradle-Magaliesberg area were still under the sea. The highlands across the Cradle-Magaliesberg area were still under the sea. The highlands across the Cradle-Magaliesberg area were still under the sea.

100 LIFE AND LANDSCAPE



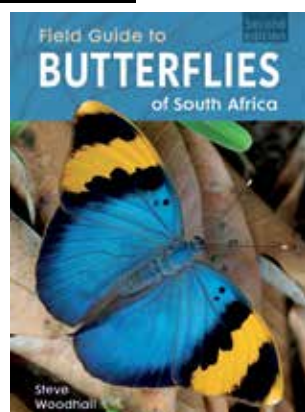
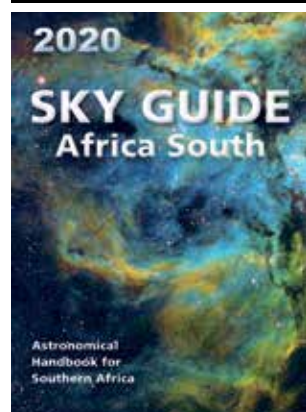
Below: The Cradle-Magaliesberg was once buried under shallow seas before volcanic eruptions triggered the disintegration of Gondwana.

Left: Specimens of hominid skulls similar to *Procrastin*, a fossil found in the Magaliesberg, may have once inhabited the Magaliesberg-Cradle area.

with sediments in a succession of stages known as the Karoo Supergroup, and the sedimentary layers eventually covered the Cradle-Magaliesberg. These layers have subsequently been stripped away from the higher ground, leaving to date only the lower ground, but they still cover much of the area.

about 280 million years ago, the climate became warmer, and rainforest plants and animals began to develop and spread. However, in the Cape region, the elevated land in southern Gondwana, the landscape of the Magaliesberg and many of the other mountain ranges were still under the sea. The highlands across the Cradle-Magaliesberg area were still under the sea. The highlands across the Cradle-Magaliesberg area were still under the sea.

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Other recent releases by Struik Nature include the 2020 edition of the *Sky Guide*, published each year by the Astronomical Society of South Africa (R145), and a revised edition of *Field Guide to Butterflies of South Africa*, by Steve Woodhall (R390).

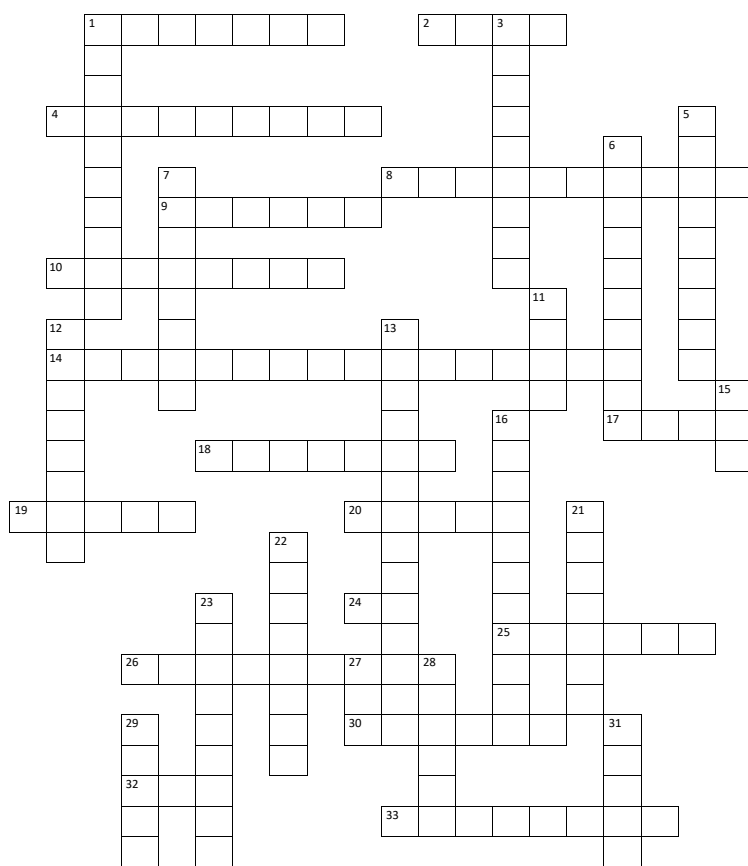
CURRICULUM CORNER

LIFE SCIENCES: GRADE 12

Human evolution: importance of the Cradle of Humankind

Test your knowledge

All of the answers can be found in this issue of *Quest*.



ACROSS

- The 3MT winner used machine learning to examine this
- Acronym for this International Year
- The main insect pests of macadamia nuts
- A photo ___ is a drug that is activated by light
- Satellite imagery is a form of ___ sensing
- This invasive alien borer beetle carries a fungus that kills our trees
- Describes microorganisms that are pathogens of insects
- Acronym for a type of light therapy
- An element used in solar panels
- Fusarium* ___ rot is a fungal disease of wheat
- Eucalypt trees release this in response to tunnelling larvae
- Refers to genetically modified maize
- Modelling software to predict the effect of climate on species distribution
- Science and technology related to light particles
- His experiments on peas made him the 'father of genetics'
- Acronym for a technique that involves sterilising insects
- These insect larvae cause severe feeding damage to maize

DOWN

- Microscopic silica bodies in plants
- A young star in an early stage of formation
- A chemical released by an organism as a communication signal
- A type of pest control that relies on natural enemies
- Single-celled organisms such as *Amoeba* and *Plasmodium*
- A prefix meaning very small, or one billionth of a metre as an SI unit
- Devices used to sample gases, such as diesel emissions
- A ___ tree shows evolutionary relationships that can be used for species classification
- A section of DNA associated with a quantitative trait
- A medical procedure done close to the patient, at the time and place of care
- The false ___ moth is a severe pest of citrus fruit
- This functional group has the symbol OH
- A substance containing beneficial microorganisms to promote intestinal health
- Acronym for an approach to controlling pests and diseases
- A type of DNA sequencing technique
- A spectral line emission arising from molecules during star formation
- Refers to studies conducted outside a living organism, in a test tube or culture dish

QUEST MATHS PUZZLE NO. 52

You have two identical cubes. You cut the first cube into smaller, equally sized cubes and leave the second cube as it is.

You have two options: to paint all the small cubes with silver or the remaining big cube with gold. Gold paint is five times more expensive than silver paint.

Which of the two options is cheaper, if the first cube is cut into

- a) 27, (b) 125, (c) 1000 small cubes?

Answer to Maths Puzzle no. 51: 28

2	3	5	4	1
3	2	4	3	4
5	1	3	5	2
3	3	2	3	1
1	4	2	4	4

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