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Newsletter of the Plant Protection Research Institute (PPRI), an institute in the Crop Sciences Programme of the Agricultural Research Council (ARC)

ARC-PPRI SCIENTIST RECEIVES AWARD

Dr Sandra Lamprecht was elected as a fellow of the Southern African Society of Plant Pathology (SASPP) during the 49th congress in January 2015. She was awarded this fellowship in recognition of her outstanding accomplishments in Plant Pathology, specifically soilborne diseases, as well as support for and service to the Society and to Plant Pathology in southern Africa.

For the past 24 years Dr. Lamprecht has actively promoted the field of soilborne diseases in South Africa by hosting annual symposia of the Soilborne Plant Diseases Interest Group. The soilborne symposia have acted as a focal point to bring together plant pathologists and scientists from other disciplines in South Africa who focus on soilborne plant diseases and plant health. One of the outstanding features of these symposia is that multidisciplinary collaboration is actively promoted as the symposia are attended by researchers from disciplines such as agronomy, botany, economy, entomology, genetics, horticulture, microbiology, soil microbiology, mycology, nematology, plant pathology, plant physiology, soil science and zoology. The symposia are attended by international experts and representatives of research councils, national and provincial Departments of Agriculture, private companies, universities and farmers and are supported by many role players in South Africa who regard this as one of the major annual events. This event also promotes the image of plant pathology in South Africa and thereby indirectly, also the SASPP.



From left to right: Drs Gert Marais, Sandra Lamprecht and John Purchase after the JE Vanderplank Memorial Address Sandra presented during the 49th SASPP congress.



Dr Sandra Lamprecht (left) receiving the award from Prof Teresa Coutinho, President of the Southern African Society of Plant Pathology (right).

During her career as a soilborne plant pathologist, Dr Lamprecht has published 57 peer-reviewed articles, and regularly reviews articles for international journals. She has made 42 presentations at international conferences and 96 presentations at local conferences or symposia. Dr. Lamprecht is also very active in communicating her research to the industry with 26 semi-scientific publications and 43 presentations at farmer's days or information days. She has supervised one MSc student and co-supervised nine MSc students and one PhD student. She is currently the cosupervisor of three PhD students.

The relevance and success of the disease management programmes she has developed have also been acknowledged by the SASPP with the Applied Plant Pathology award she received in 2006. It is evident that Dr Lamprecht has made a highly significant contribution to the field of soilborne diseases in South Africa.

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Biosystematics

Red Listing of South African Spider Species

The Convention on Biological Diversity (CBD) was inspired by the world community's growing commitment to sustainable development. It represents a dramatic step forward in the conservation of biological diversity, the sustainable use of its components, and the fair and equitable sharing of benefits arising from the use of genetic resources. The IUCN Red Listing activity is the world's most comprehensive information source on the global conservation status of wild species and their links to livelihoods. Far more than a list of species and their status, it is a powerful tool to inform and catalyse action for biodiversity conservation, critical to protecting the natural resources we need to survive.

The Arachnida Unit in Biosystematics, along with the Threatened Species Programme (TSP) of the South African National Biodiversity Institute (SANBI), has been working over the last few years to prepare information that can be used to evaluate the South African spider species against the ICUN Categories and Criteria. It has been possible to evaluate the spiders because for each of the 2170



Participants of the Red Listing workshop. From left to right: Robin Lyle, Ansie Dippenaar, Domitilla Raimondo and Theresa Sethusa.

species an endemicity index (EI) has been calculated based on their distribution patterns. The EI was calculated using data on current distribution which included six categories, ranging from species known only from the type locality, to cosmopolitan species (Table 1). All the species with an EI value of 3-6 are endemic to South Africa and will be accessed. All the species with EI values of 1-2 can be listed as being of less concern because of their wide distribution patterns.

Armed with this knowledge, Dr Theresa Sethusa and Mrs Domitilla Raimondo from TSP joined Dr Ansie Dippenaar and Ms Robin Lyle at the Biosystematics building from the 23rd to 27th of March 2015 to evaluate 76 species of spiders for placement on the Red List, and to determine their status nationally. These 76 species will add to the 120 species that were done at the 11th Colloquium of the African Arachnological Society in Bloemfontein in January 2014. A second workshop for the year will be held in June 2015. After completing all the species, a book with the evaluations will be produced.

The information will also feed into the species pages being generated by SANBI for the Encyclopaedia of Life.

Table 1. Level of endemicity used to determine the endemicity index for each species

- 6 = endemic to South Africa, known only from the type locality
- 5 = endemic to South Africa known only from one province
- 4 = endemic to South Africa known only from two adjoining provinces
- 3 = endemic to South Africa known from >2 provinces
- 2 = endemic to southern Africa
- 1 = endemic to Africa;
- 0 = world-wide

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Spider diversity of the iSimangaliso Wetland Park: uMkhuze Game Reserve

The Biosystematics Arachnida Unit has been participating in an atlas field research programme hosted by the iSimangaliso Wetland Park Threatened Species Project. The surveys started in 2007 and were conducted in the uMkhuze section of the iSimangaliso (formerly known as the Greater St Lucia) Wetland Park under the leadership of Xander Combrinck. Arachnids were collected as part of the iSimangaliso Wetland Park Threatened Species Project, Operation Wallacea and Wildlife & Ecological Investments. Sampling included the use of 250 pitfall traps at 10 different sample stations, as well as active searches including sweep netting, bush beating, and searching under rocks for arachnids. Operation Wallacea (www.opwall.com) makes use of volunteers, mainly undergraduate students, from UK universities but also school children. Carol Smith of the Spider Club led the arachnid collecting part of the survey in 2008.

All sampled arachnid material, comprising more than 3500 specimens, has been donated to the National Collection of Arachnida (NCA) in Pretoria. To date, 42 spider families represented by 247 species have been identified and accessioned into the NCA. A final report on the diversity of the reserve was prepared in February 2015. Several species are new to science, and another three species have recently been described with uMkhuze as their type locality. A paper on the arachnid diversity of iSimangaliso Wetland Park: uMkhuze is currently in preparation.

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Crab spider<u>.</u> *Mystaria savannensis* Lewis & Dippenaar-Schoeman, 2014 new from uMkuze Game Reserve

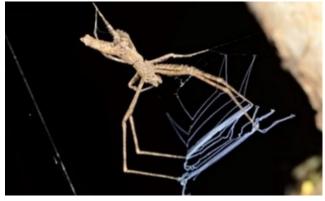


Crab spider_ Sylligma ndumi Lewis & Dippenaar-Schoeman, 2011 new from uMkuze Game Reserve

Arachnid Bio-Blitz at Lephahlale

A second bio-blitz was recently undertaken at the farm Zandriver in the Limpopo Province. During the first bio-blitz survey in March 2014, a total of 71 spider species were sampled and photographed by Peter Webb. The specimens were identified at the Spider Unit and accessioned in the National Collection of Arachnida (NCA). All the photographs were loaded onto the SANSA virtual museum. The second bio-blitz took place during the weekend of 14-15 March 2015. The arachnid collection now includes members of four arachnid orders: pseudoscorpions, solifugids, scorpions, spiders, and a whip spider. They were all sampled and photographed by Mr Peter Webb. The checklist of spiders present at Lephahlale has increased by 46 species to a total of 117 species. All the material was deposited in the NCA and the photographs have been loaded onto the virtual museum.

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A net-throwing spider Deinopidae *Menneus camelus* from Zandriver

Course on urban insects presented to Coopers Environmental Science

The middle of March saw the Entomology Unit abuzz with activity, preparing and presenting a course for Coopers Environmental Science. The course was specifically developed for Coopers, who sent two very keen delegates, eager to learn more about the pests they commonly encounter. Coopers Environmental Science is a supplier of pest control products throughout South Africa and various other African countries. Staff at the Entomology Unit enthusiastically imparted their knowledge, while covering the following topics: general insect morphology; collecting methods; killing and temporary storage; labelling, accessioning and dispatching of specimens; identification of mites and insects in an urban environment including grasshoppers, cockroaches, bugs, lice, fleas, flies, stored grain pests and termites. A very full two days were concluded with a tour of the insect collections at the Biosystematics Division, followed by the presentation of certificates.

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Dr Cornel du Toit demonstrating material to course participants

VISIT OF ISRAELI COLLEAGUE

Dr Eric Palevsky of the Department of Entomology, Newe-Ya'ar Research Centre, Israel visited the ARC-PPRI Biosystematics Division from 8-13 March 2015. This visit resulted from a taxonomy course on soil mites that Prof. Eddie Ueckermann presented in Israel from 27 January to 13 February 2014. Prof. Ueckermann assisted Dr Palevsky with the identification of soil mites that were collected during surveys in Israel. This collaboration is part of a project that involves searching for potential biological control agents for root knot nematodes (*Meloidogyne* spp.) in pepper and tomato and for the control of bulb mites, *Rhizoglyphus robini*, gnats and thrips pupae, or overwintering stages of pests, in soils. Data based on this material will also contribute towards identification keys to the species of some of these soil mite families that are being prepared for publication.

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Left to right: Dr Pholoshi Maake, Dr Eric Palevsky, Prof Eddie Ueckermann, and Ms Tshidi Makutoane

49th Congress of the Southern African Society for Plant Pathology

Several staff members from Biosystematics, Plant Microbiology and Weed Research divisions attended the biennial conference of the 49th congress of the Southern African Society for Plant Pathology (SASPP) that were held at Bains Game Lodge near Bloemfontein, Free State Province from 18-21 January 2015. The programme included a variety of sessions including ecology and epidemiology; pathogen genetics; disease management (biological control, chemical control and host plant resistance); pathogen characterisation and identification; and mycotoxins. Two workshops were held during the congress, one on fungicide application technology and plant disease control and the second on GenBank and other online resources to identify unknown fungi. It was an excellent opportunity to present research findings not only to the Plant Pathology community of South Africa but also various attendees from overseas and the industry. Dr Sandra Lamprecht presented the JE Vanderplank Memorial Address on invitation. The title of her presentation was Confessions of a serial Soilborne Plant Pathologist - 30 years of digging holes, and finding my way out of them. The congress will be remembered for the high quality applied research presentations delivered by researchers and students.

ARC-PPRI presented 8 oral presentations and 11 posters as listed below:

Oral presentations:

- Dube JP, **Truter M** & Van der Waals JE. 2015. Reduced sensitivity to Qol in *Alternaria alternata* isolates from potato in South Africa. [Oral]
- Moloto VM, Goszczynska T & Coutinho TA. 2015. New pathovar of *Pseudomonas syringae* from onion plants. [Oral]
- Moloto VM, Coutinho TA & Goszczynska T. 2015. Characterisation and identification of Agrobacterium species from South Africa. [Oral]
- Muzhinji N, Woodhall J, **Truter M** & Van der Waals J.2015. A survey of genetic diversity of *Rhizoctonia solani* anastomosis groups associated with potatoes in South Africa. [Oral]
- Truter M, Kandolo D, Thompson A, Cloete M, Nkosi Z, Dau N & Oelofse D. 2015. Re-evaluation of *Alternaria* species causing Alternaria blight on sweet potato in South Africa. [Oral]
- Tewoldemedhin YT & Lamprecht SC. 2015. Detection and distribution of sudden death syndrome of soybean in South Africa. [Oral]
- Van Coller GJ, Lamprecht SC & Viljoen A. 2015. Resistance of South African wheat cultivars and test lines to *Fusarium* head blight caused by *F. graminearum* s.s. and *F. pseudograminearum*. [Oral]
- **Wood AR**. 2015. The life cycle of *Uromycladium tepperianum*: things are not what they seem. [Oral]

Poster presentations:

- Bopape FL & Hassen Al. 2015. Investigating the tolerance to extreme environmental conditions by commercial strains of Rhizobia from the South African Rhizobium Culture Collection (SARCC). [Poster]
- Kgatle MG, Ramusi TM, Flett B, **Truter M** & Aveling TAS. 2015. A survey of *Alternaria* species on sunflower in South African production fields. [Poster]
- Goszczynska T, Moloto VM, Coutinho TA, Botha WJ & Jacobs-Venter A. 2015. A new disease of banana, scaly black spot of fruit, caused by *Xanthomonas* species. [Poster]
- Moloto VM, Goszczynska T & Coutinho TA. 2015. Bacterial pathogens in onion seed. [Poster]
- Ncedana C, **Pavlic-Zupanc D** & Roux J. 2015. Botryosphaeriaceae associated with *Acacia erioloba* (camel thorn) die-off in the Kathu area, Northern Cape. [Poster]
- Nkosi BZ, **Jacobs-Venter A**, Cloete M, Thompson AH, Oelofse D & Rees J. 2015. Characterization of *Fusarium oxysporum* species complex associated with *Fusarium* wilt of sweetpotato in South

Africa. [Poster]

- Pavlic-Zupanc D, B. Piškur, B. Slippers, M.J. Wingfield, D. Jurc. 2015. Molecular and morphological characterization of *Dothiorella* species associated with dieback of *Ostrya carpinifolia* in Slovenia and Italy, and a host and geographic range extension for *D. parva*. [Poster]
- Pennells S, le Roux PAL, **Jacobs-Venter A** & Gryzenhout M. 2015. Selected *Fusarium* species isolated from grazing lawn soils in the Kruger National Park. [Poster]
- Pieterse Z, Jacobs-Venter A & Aveling TAS. 2015. Culturable fungi associated with mesembs in the Namaqua National Park of the succulent Karoo biome. [Poster]
- Retief E, Lamprecht SC & McLeod A. 2015. Characterization of Verticillium and nematodes associated with tomatoes in the Limpopo province in South Africa. [Poster]
- Tewoldemedhin YT & Lamprecht SC. 2015. Pythium species associated with soilborne diseases of soybean in South Africa. [Poster]

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Australian visitor to the National Collection of Fungi

Prof Brett Summerell, the Deputy Executive Director of Science and Conservation Botanic Gardens and Centennial Parklands in Australia, visited the National Collection of Fungi (NCF) on an NRF-ARC funded visit during the first weeks of March. Brett is a worldrenowned Fusarium expert, author of more than 100 peer-reviewed publications and co-author of the Fusarium Manual, a book very well-known to mycologists. During his visit, he spent time in the laboratory with students involved in the Fusarium grasslands project at both the ARC and the University of Johannesburg. He demonstrated Fusarium-specific laboratory techniques and discussed preliminary results, as he will act as co-supervisor of these studies. His visit also included a sampling trip to Melville koppies during which soil samples were collected for analyses. Brett's inputs also extend to other collaborative projects currently under way between ARC-PPRI and ARC-VOPI, such as Fusairum wilt of sweet potato. One of the highlights of his visit was the seminar he presented at the University of Pretoria on the current species concepts in the genus Fusarium. As Brett will be co-presenting the International Fusarium workshop in South Africa in 2016, we hope to host him again at the NCF.

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Prof Brett Summerell during his visit

New appointments in Biosystematics

Nematology: Chantelle Jansen



Chantelle started as a Ph.D. student in the Professional Development Programme at the National Collection of Nematodes. She has an MSc in Environmental Sciences from the North West University where she was introduced to the world of nematodes. Her project will be on the nematodes of the Groenkloof Nature

Reserve, with ample opportunity to develop her skill as a nematode biosystematist, under the supervision of Prof. Driekie Fourie, North West University and Dr Mariette Marais, ARC-PPRI. Chantelle says she is very excited to be a part of the biosystematics team, is very humbled by the opportunity that the ARC has given her, and intends grabbing it with both hands.

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Mycology: Angel Maduke

Miss Angel Maduke joined the Mycology Unit at the ARC-PPRI in February 2015 as a postgraduate student in the Professional Development Programme. She obtained a BSc Agric degree from the University of the Free State, majoring in Plant Pathology and Soil Science. During her undergraduate study she has had the opportunity to work as a laboratory assistant for a well-known mycologist, Dr Marieka Gryzenhout, Senior Lecturer at the University of the Free State. That experience further stimulated her interest in



Plant Pathology and particularly in Mycology, and Miss Maduke desired to pursue her postgraduate degrees in these fields. She was accepted for her MSc degree at the Department of Microbiology and Plant Pathology at the University of Pretoria. In her research project, Miss Maduke will focus on the diversity, distribution and pathogenicity of Botryosphaeriaceae (Dothideomycetes, Ascomycota) on native and exotic Myrtaceae trees including *Syzygium, Heteropyxis* and *Eucalyptus* species in eastern and southern Africa. She will be doing her project under the supervision of Prof.Jolanda Roux of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, and Dr Draginja Pavlic-Zupanc at ARC-PPRI.

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Mycology: Maano Tshimange



Miss Maano Tshimange joined the Mycology unit in March 2015 in the Professional Development Programme as an MSc student in Microbiology at the Department of Microbiology and Plant Pathology, University of Pretoria under the supervision of Dr Mariette Truter (ARC-PPRI) and Dr Wilhelm de Beer (Forestry and Agricultural Biotechnology Institute, University of Pretoria). Maano is investigating the diversity of fungi in the Pleosporaceae (Dothideomycetes, Ascomycota) on agricultural important crops and other plants associated with agriculture. Important genera of the Pleosporaceae include *Alternaria, Bipolaris, Curvularia, Epicoccum, Phoma*-like and *Stemphylium*, of which some species are important plant pathogens that could cause significant economic losses if not controlled. Fungal strains collected during the study will be characterised based on morphological characteristics and on a multi-gene phylogeny.

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Nematology: Moloko Olga Motheketlela

Miss Moloko Olga Motheketlela was appointed as a support research assistant in plant pathology at ARC-Grain Crops Institute in November 2009. She was transferred to the Nematology unit at the Biosystematics Division in January 2015 as a research assistant. She has experience in screening of plant material for disease developments, plant measurements, sampling of plant material, preparation of research



material as well as planting trials. She is currently spending most of her time in the extraction laboratory fulfilling that essential part of nematode systematic research and diagnostic services, extracting the nematodes from soil and plant material.

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Biosystematics bids farewell to valuable staff

Entomology: Mr Simon Mamogale



Simon Mamogale started working for the Plant Protection Institute within the Department of Agriculture as a general assistant in November 1983, and ended up as a research assistant when he left over 31 years later, in February this year. Mr Mamogale played an important role as a

member of the insect collections management team. He was responsible for the sorting of material, checking for pest infestations within the collection as well as the preparation and labelling of specimens, which eventually led to him specialising in beetles. He was also highly skilled at making collections equipment, and his departure therefore leaves a big gap in the Entomology unit. Mr Mamogale will be greatly missed for his years of experience and contribution to the Institute. We wish him all the best with his early retirement.

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Mycology: Mr Prechers Dikhoba and Miss Lucia Maluleke

Lucia and Prechers spent the last 16 months at the Mycology Unit as contract research technicians. Although, they previously worked within the ARC and had diverse experience in laboratory techniques, mycology was not their main focus. At Mycology they were responsible for various activities within the research project soil Fusarium survey in



the grassland biome of South Africa under the supervision of Dr Riana Jacobs. Activities included sample collection, isolating and preserving almost 900 fungal cultures in the PPRI collection. They were also part of the collections team with various responsibilities pertaining to client requests, new accessions and maintenance of the collections, as well as the fungal identification service helping out with sample processing for fungal identifications. They were great team members, who played to each of their strengths. Their enthusiasm will be missed and we wish them all the best.

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Mycology: Mrs Grace Kwinda

In November 2014 the Mycology Unit bid farewell to a long -standing colleague and friend, Grace Kwinda. Grace was affiliated with the Mycology Unit for 13 years. She started as a Department of Science and Technology volunteer and contract worker on the National Karnal Bunt Survey for the Department of Agriculture, Forestry & Fisheries, and later became a junior researcher in the unit. Grace was primarily responsible for the curation and maintenance of fungal cultures in the PPRI living culture collection of the National Collection of Fungi. Her ef-



forts resulted in an addition and safeguarding of 10 353 living cultures. Grace not only curated the PPRI collection, but was also closely involved in lecturing aspects of the Introductory Mycology Course and all student orientation in the unit. She also served as our health and safety officer for a number of years. No task was ever too small or insignificant for Grace. Grace will be greatly missed by us all!

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Mycology: Miss Madira Manganyi

Madira left the Mycology unit in December 2014 after a short appointment as research technician in February 2013. As the only permanent research technician within the Mycology unit, Madira fulfilled essential tasks in the daily running of the fungal identification service, the living PPRI collection and dried PREM collection of the National Collection of Fungi, laboratory management, equipment maintenance and re-



search support. She was always willing to help and advice students with daily laboratory work. Her positive outlook on life and enthusiasm towards research contributed greatly to a dynamic and productive mycology team.

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Pesticide Science

ARC-PPRI host STOPRATS review workshop

The first annual review workshop of the StopRats project (Sustainable Technologies to Overcome Pest Rodents in Africa Through Science) was hosted by ARC-PPRI at the ARC Convention Centre in Hatfield, Pretoria, during March 2015 (Fig. 1).

StopRats is a project funded by the European Union (ACP-EU Cooperation Programme in Science and Technology) aimed at bringing together African rodent experts to strengthen science, technology and innovation on ecologically-based rodent management as a key enabler for poverty reduction, growth and socioeconomic development. The project is managed by the Natural Resources Institute, University Greenwich in the UK, and has seven project partners in six African countries: ARC-PPRI and the University of Venda in South Africa, the University of Namibia, University of Swaziland, Sokoine University of Agriculture in Tanzania, Association Vahatra in Madagascar and Concern Worldwide in Sierra Leone. The project has an External Advisory Board of three independent, internationally respected experts. The ARC -PPRI team consists of Emil von Maltitz, Phanuel Malebana and Frikkie Kirsten, all from the Pesticide Science Division.

All the project partners (excluding those from Sierra Leone due to the Ebola situation) and advisory board members, as well as guests from the Centre for Wildlife Management of the University of Pretoria and Rat-O-Pel in Port Elizabeth, attended the workshop in Pretoria from 10 to 13 March. Partners presented their progress and achievements over the first year, and discussed the planned activities going forward.

StopRats aims to link researchers across Africa and to develop their wider networks with civil society organisations, businesses and policy makers in order to evaluate relevant national/regional policies on rodent management, build capacity in staff and institutions, and increase awareness about rodents; the problems they cause and their ecologically-based management.

The ARC-PPRI team are founder members of Rodenticide Integrated Management (RIM) Group, which is a Gauteng-based rodent pest control manufacturer, operator and research discussion group that aims to improve standards in rodent control, to develop effective safe use of rodenticides and to look for the possibility of rodenticide resistance. Representatives of the group and the PPRI team (industry and research) participated in the StopRats northern-Limpopo regional stakeholder workshop, which included representatives of small-holder farmers from rural communities and government agricultural advisors in the province. The stakeholder's workshop was hosted by the StopRats partners of the University of Venda, at Polokwane during February 2015 (Fig. 2).

To raise awareness and visibility of the StopRats project, Emil presented an overview of the project "Sustainable technologies to overcome pest rodents in Africa through science: StopRats" at the ARC-PPRI Research Symposium at Roodeplaat in November 2014. The project and activities has also been introduced to a



Sustainable Technologies to Overcome Pest Rodents in Africa through Science

wider audience through papers presented at international symposia. Stoprats project leader Professor Steve Belmain provided overview talks about the project such as "The StopRats project: The next step in delivering ecologically based rodent management in Africa" at the 5th International Conference on Rodent Biology and Management in Zhengzhou, Henan in China during August 2014 and at the 12th African Small Mammal Symposium (ASMS) in Madagascar in April 2015.

Stoprats partners were well presented at the 12th ASMS held at Mantasoa in Madagascar (Fig. 3), contributing as presenters or coauthors of 33% of the papers and 24% of the posters at the symposium. Emil was funded by StopRats to attend and present a paper "Chemical control; short-term solution to manage gerbil damage to maize", results of a research project conducted in the maize producing areas of the western Free State, at the symposium.

Based on training models used in Madagascar (Fig. 4), regional field biology schools will be established in West, East and southern Africa to increase skills of post-graduate students and early career researchers in order to inspire a new generation of rodent experts. For this purpose, Phanuel attended a 10-day field school hosted by StopRats partner, Association Vahatra, in the forest of Ambohitantely in Madagascar during October 2014. He also attended the StopRats field school at the Lajuma Research Centre in the Luvhondo Nature Reserve in the Soutpansberg near Louis Trichardt (Fig. 5). This international field school was led by the University of Venda's South African Research Chair in Biodiversity and Change



Fig. 1. StopRats review workshop, Pretoria



Fig. 2. Farmer community stakeholder workshop, Limpopo

Pesticide Science (continued)

PPRI host STOPRATS review workshop (continued)



Fig. 3. Arrival of Stoprats partners at 12th African Small Mammal Symposium ASMS, Mantasoa in Madagascar.



Fig. 4 Field school, Madagascar.

in the Vhembe Biosphere Reserve (SARChI), during the first week of March 2015. Field activities and lectures were led by experts in zoology, trapping programmes, as well as in parasitology of birds and small mammals such as bats, rodents and shrews.

For more information on StopRats visit our website at http://projects.nri.org/stoprats/

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Fig. 5 StopRats field school, Limpopo

Plant Microbiology

Characterization of novel lineages of 'Candidatus Liberibacter africanus' from indigenous rutaceous hosts of *Trioza erytreae*

Greening disease in South Africa is associated with a phloem-limited member of the Alphaproteobacteria known as 'Candidatus Liberibacter africanus' (Laf). Spread of this bacterium is assisted by the flight and feeding activities of its triozid vector, *Trioza erytreae*. In addition to Laf, 'Candidatus Liberibacter africanus subsp. capensis' (LafC) has been described from this country. LafC is widely associated with *Calodendrum capense* (Cape Chestnut), an indigenous Rutaceous species. Despite the implementation of control strategies, citrus greening remains problematic suggesting the existence of reservoir hosts to Laf. In a CRI funded study, Prof. Gerhard Pietersen and Mrs. Ronel Roberts set out determine the existence of such reservoirs amongst the indigenous hosts (Fig. 1) of *T. erytreae*.

During various field trips, samples from 234 *Clausena anisata* (horsewood tree), 289 *Vepris lanceolata* (white ironwood) and 231 *Zanthoxylum capense* (small forest knobwood) were collected throughout the natural distribution of these trees in South Africa. Total DNA was extracted from samples and tested for the presence of

liberibacters by a generic Liberibacter TaqMan real-time PCR assay. Liberibacters present in positive samples were characterised by amplifying and sequencing rpIJ, omp and 16S rRNA gene regions. The identity of tree host species from which liberibacter sequences were obtained was verified by sequencing host rbcL genes.



Fig. 1 Native hosts of *Trioza erytreae* a) healthy and b) triozid infested *Clausena* anisata; c) healthy growing *Vepris lanceolata* in Knysna, Western Cape; d) Leaves of *V. lanceolata* showing characteristic *T. erytreae* infestation; e) Bark of *Zanthoxylum capense* found in KwaZulu-Natal; f) *Z. capense* leaf with evidence of previous triozid infestation.

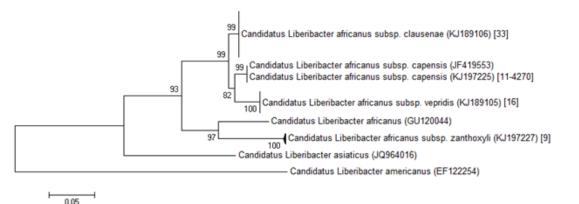


Fig. 2 Maximum likelihood phylogeny of liberibacter based on the *rplJ* sequences obtained from the *Clausena, Vepris* and *Zanthox-ylum* samples examined in this study, as well as for Laf, Las and Lam. Bootstrap support values based on 1000 replicates are indicated at the branches. The number of sequenced liberibacter-positive samples per tree host is indicated in square brackets.

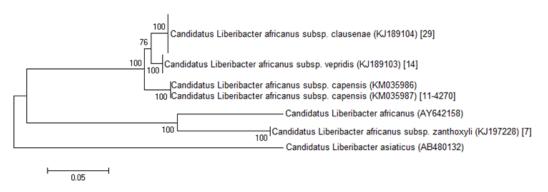


Fig. 3 Maximum likelihood phylogeny of Liberibacter based on the *omp* sequences obtained from the *Clausena, Vepris* and *Zan-thoxylum* samples examined in this study, as well as for Laf, Las and Lam. Bootstrap support values based on 1000 replicates are indicated at the branches. The number of sequenced liberibacter-positive samples per tree host is indicated in square brackets.

Plant Microbiology (continued)

Characterization of 'Candidatus Liberibacter africanus' (continued)

Of the trees tested, 33 Clausena, 17 Vepris, and 10 Zanthoxylum tested positive for liberibacter. Phylogenetic analysis of the rplJ (Fig. 2) and omp (Fig. 3) gene regions revealed unique clusters for liberibacters associated per tree species. Phylogenetic analysis of 16S rRNA (Fig. 4) sequences indicated that liberibacters obtained from Vepris and Clausena were identical to 16S rRNA sequences for LafC, whereas those from Zanthoxylum species grouped separately. Due to the homology of the liberibacters identified from this study to Laf, it was proposed that these libeibacters are granted subspecies status. Names corresponding to the hosts from which these liberibacters were identified from were given for these new Liberibacter subspecies as follow; 'Candidatus Liberibacter africanus subsp. clausenae' (LafCl), 'Candidatus Liberibacter africanus subsp. vepridis' (LafV) and 'Candidatus Liberibacter africanus subsp. zanthoxyli' (LafZ).

Initially we set out to determine whether reservoir hosts exist for Laf. However, typical citrus-infecting Laf were not detected in any of the indigenous rutaceous specimens tested, hence these native Rutaceae hosts of *T. erytreae* do not appear to play a role in the epidemiology of Laf on citrus. We were_however, able to identify novel liberibacters from all three tree hosts tested. Further studies are needed to determine whether these liberibacters are transmitted to commercial citrus and whether any of these Liberibacter subspecies are capable of causing disease on commercial citrus species. Additionally, the association of various Laf subspecies with native Rutaceae trees presents researchers with a unique opportunity to explore the possible evolution of Laf on citrus from a liberibacter source indigenous to the African continent.

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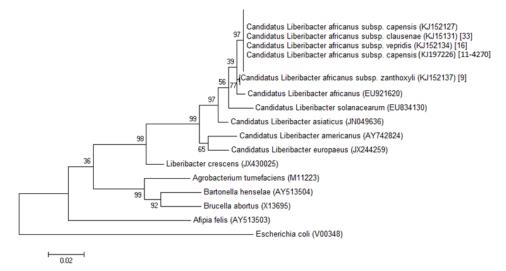


Fig. 4 Maximum Likelihood phylogeny of the genus Liberibacter based on the 16S rRNA sequences obtained from the *Clause-na, Vepris* and *Zanthoxylum* samples examined in this study, as well as for all known liberibacters and related Proteobacteria. Bootstrap support values based on 1000 replicates are indicated at the branches. The number of sequenced liberibacter-positive samples per tree host is indicated in square brackets. *Escherichia coli* (V0038) was used as the outgroup.

Sudden death syndrome of soybean

Sudden death syndrome (SDS) (Fig. 1) is responsible for economically devastating reductions in yields of soybean in North and South America, including in Argentina, Brazil, Canada and United States. Since its initial detection in Arkansas in the early 1970s, SDS of soybean has been reported in all major production areas within North and South America. From 1996 to 2005, SDS was listed as one of the most important diseases of soybean in the United States, and it was described as a premature dying of soybeans after flowering and before maturity.

The fungus

SDS is caused by four closely related soilborne fusaria that are morphologically and phylogenetically distinct species *Fusarium brasiliense*, *F. crassistipitatum*, *F. tucumaniae* and *F. virguliforme*. All species are found in Argentina, while *F. virguliforme* is the only species present in North America and the three species except *F. virguliforme* have been reported in Brazil. Recently in South Africa (in 2013), SDS was spotted in a commercial soybean field (Lydenburg) in Mpumalanga and *F. virguliforme* (Fig. 2) was identified as the causal agent. After the first detection of SDS in Lydenburg, it has been found in other areas of South Africa including



Fig. 1 Sudden death syndrome in a commercial soybean field in Mpumalanga at pod filling stage of the plant.

Plant Microbiology (continued)

Sudden death syndrome of soybean (continued)

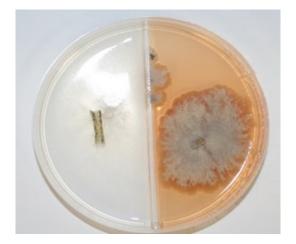


Fig. 2 Culture of *Fusarium virguliforme* on divided (water agar and potato dextrose agar) plate that was isolated from a commercial soybean field in Mpumalanga, South Africa.

Kinross (2013), Bethlehem (2014), Brits (2014), Potchefstroom (2014) and Vredefort (2014).

Symptoms

Leaf symptoms of SDS first appear as inter-veinal chlorotic blotches that become necrotic, while the mid-vein and major lateral veins remain green (Figs 1, 3). These symptoms appear throughout the plant but are most severe on the top leaves and they usually first appear during early reproductive stages of the plant. Foliar symptoms may be caused by phytotoxins translocated from roots to leaves. Severely affected plants defoliate prematurely, and pod abortion may occur. The abscission of pods is most pronounced at the top of the plant. Plants that develop symptoms later produce smaller, lighter seed. In very severe cases, the plant will lose all leaves and most, if not all, pods, leaving only a bare stem with the petioles remaining. The vascular tissue in the upper taproot and lower stem turns grey-brown, but the pitch remains white. Roots of affected plants show extensive root and basal stem rot (Fig. 4).

Fungal root colonisation and severity of foliar symptoms seem to depend on environmental as well as soil physical, chemical, and biological factors. Severity of SDS varies among years, which suggests that variations in environmental conditions such as soil moisture and temperature affect disease development. Reports indicate that high levels of soil moisture and low soil temperatures, especially in early reproductive stages of the soybean, increase severity of SDS. Soil compaction that inhibits soil profile drainage and increases duration of soil saturation also can increase SDS due to its relationship with soil moisture.

Management

- 1. Plant cultivars with light tolerance reaction to SDS.
- 2. Plant an infested field with cultivars of different maturity groups, or plant portions of the field at different times.
- Cultural practices to reduce plant stress, control of the soybean cyst nematode, and improvements in drainage may help plants tolerate SDS.

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Fig. 3 Sudden death syndrome symptoms artificially induced in five-weekold soybean seedlings under greenhouse conditions by inoculating the planting medium with *Fusarium virguliforme*.



Fig. 4 Roots of five-week-old soybean seedlings infected with *Fusarium virguliforme* showing extensive root rot compared to the uninfected control on the far left.

Weeds Research

Green light for release of the tiny seed-feeding weevil on *Parthenium hysterophorus* in South Africa

The rampant spread of the herbaceous annual plant Parthenium hysterophorus L. (Asteraceae), commonly known as parthenium or more recently, 'famine weed', in South Africa and further north on the continent, has raised concern in recent years. This invasive plant, originating from Central and South America, is an adept coloniser of disturbed environments and is detrimental to crop and animal production, biodiversity conservation, as well as human and animal health, causing considerable economic losses. Poor land management practices such as incorrect stocking densities, which lead to overgrazing, exacerbate the spread of parthenium by creating empty niches that the plant rapidly exploits, forming dense, mon-ospecific stands. Roadsides, productive and fallow crop lands, grazing land, water courses, rail sides, areas protected for the conservation of biodiversity, and the surrounds of homesteads in rural communities of southern Africa, are readily invaded by parthenium. Infestations in KwaZulu-Natal, Mpumalanga provinces, Northwest and Limpopo provinces in South Africa require management interventions. Neighbouring countries of Swaziland, Mozambique, Zimbabwe and beyond into East Africa are also invaded by this plant. Parthenium is a severe invader in Asia, and for some decades has been under successful management in Australia, using a combination of interventions. Biological control has long been recognised as the most sustainable solution to reduce infestations of parthenium to a manageable level, particularly in the most intensively invaded areas as it is not feasible to chemically treat all of the large tracts of land invaded



Fig. 1 Adults of *Smicronyx lutulentus*, the seed-feeding weevil, on shoot tips with young flower buds of *Parthenium hysterophorus*.

Since the inception of the South African research programme on parthenium in 2003, three biological control agents that affect the leaves and stems of parthenium have been introduced, assessed, mass-reared and released since 2010 and 2013 at more than 200 sites in KZN and Mpumalanga provinces in South Africa. These agents are the summer rust fungus Puccinia xanthii var. partheniihysterophorae (Pucciniales: Pucciniaceae), the leaf-feeding beetle Zygogramma bicolorata (Coleoptera: Chrysomelidae), and the stem -boring weevil Listronotus setosipennis (Coleoptera: Curculionidae), with the winter rust fungus, Puccinia abrupta var. partheniicola (Pucciniales: Pucciniaceae) already previously found to be established in field. All agents have established, although with variable performance, and efforts are underway to ascertain their impact. Natural enemies that target various parts of parthenium are required to adequately suppress the plant under the different conditions that it invades. To this end, a seed-feeding weevil Smicronyx lutulentus

(Coleoptera: Curculionidae), originating from Mexico, was considered for introduction into South Africa in 2010, to complement the effects of the other agents. More than 1200 *S. lutulentus* weevils were imported into ARC-PPRI quarantine facilities at Cedara in April 2010 after field collection at sites west of Rockhampton in Queensland, Australia, where it had established after releases conducted during 1981 to 1983 in Australia. Field work by ARC-PPRI researchers (L. Strathie and A. McConnachie) was undertaken in collaboration with experienced entomologists (K. Dhileepan and M. Trevino) from the Queensland Department of Primary Industries.

Adults of *S. lutulentus* are tiny, about 2 mm in length, and are often observed sitting on the shoot tips of parthenium during the day, frequently in pairs. Their feeding creates numerous tiny shotholes in the leaves, with negligible impact. Females are highly selective regarding the age of flowers chosen for oviposition; eggs are insert-



Fig. 2 Shotholes on leaves created by adult feeding by *Smicronyx lutulentus* weevils.



Fig. 3 Young flower buds and flowers of *Parthenium hysterophorus* showing holes where *Smicronyx lutulentus* eggs have been inserted through the bracts of young buds.

Weeds Research (continued)

Green light for release of weevil (continued)

ed through the bracts surrounding unopened or newly opened flower buds only, and laid among the outer disc florets or more centrally in the disc. Newly hatched larvae bore into a young, green developing seed within the same flowerbud. Each larva completes its development within the developing achene, its feeding destroying that seed and also often affecting the development of other seeds (up to five) within that flower. As up to 25 000 viable seeds can be produced per parthenium plant and which, if buried a few centimetres below the soil surface, may survive for up to 6 years, it is beneficial to reduce seed set to curb spread of the weed. The mature larva makes a hole in the achene wall through which it exits when the mature flower breaks open and the achenes fall to the ground, before pupating in the soil. Rainfall stimulates adult emergence from the soil in the spring season, after the dry winter period.

Following research conducted at the ARC-PPRI Cedara quarantine facility, S. lutulentus was demonstrated to be highly host specific, laying eggs on only the target plant P. hysterophorus and not on any of the other 38 (and 11 sunflower cultivars) indigenous and economically important Asteraceae species that the adult weevils were exposed to during rigorous tests that examined oviposition and adult feeding. Based on this research, an application for approval to release S. lutulentus in South Africa, submitted to the Department of Agriculture, Forestry and Fisheries in October 2014, was approved in late December 2014. The first releases of about 670 S. lutulentus adult weevils were conducted by the ARC-PPRI Cedara parthenium biocontrol research team at sites around Hluhluwe and KwaJobe community in KZN on 14 January 2015. Mass-rearing is currently being undertaken at ARC-PPRI Cedara and, from January to early April 2015, about 5500 S. lutulentus weevils were released at 18 sites in KZN and Mpumalanga provinces. Mass-rearing and releases will continue, and monitoring and evaluation of establishment, spread and impact of the weevil will be undertaken at field sites. In the native range of Mexico, it was estimated that S. lutulentus likely contributed to at least 30% reduction in seed production. In the absence of native predators and parasitoids in countries of introduction, higher levels of damage may be



Fig 4: The parthenium biocontrol research team at ARC-PPRI Cedara conducted the first releases of *Smicronyx lutulentus* on *Parthenium hysterophorus* in South Africa on 14 January 2015, at field sites around Hluhluwe and KwaJobe community in KwaZulu-Natal Province.

expected. Therefore, considerable impact on the reduction of seed set is anticipated from this tiny weevil in years to come.

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Technology Transfer

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