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

## The incorporation of a world renowned collection into the National Collection of Fungi

The British mycologist I.B. Pole-Evans was appointed as the first South African government mycologist in 1905, and in 1908 the Onderstepoort Veterinary Research Institute was founded with Arnold Theiler, a Swiss veterinarian, as the first Director. This signalled the start of mycotoxicological research in South Africa. The first accounts of this pioneering research appeared in the 'Seventh and Eight reports of the Director of Veterinary Research, Union of South Africa, 1918' in which D.T. Mitchell reported on the experimental reproduction of diplodiosis, a neurotoxic syndrome in cattle, with pure cultures of *Stenocarpella maydis* (= *Diplodia zea*). These cultures were isolated by P.A. Van der Bijl and grown on sterile maize kernels, and represent the first report of the experimental reproduction of a veterinary mycotoxicosis with a pure culture of a fungus in South Africa.

Eighty years later, another major breakthrough marked South African mycotoxicological research following the isolation and chemical characterisation of the carcinogenic fumonisins produced by *Fusarium verticillioides* in maize, by researchers at the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC) Unit of the South African Medical Research Council



Boxes of living fungal cultures being unpack from a refrigeration truck.



Drs Rong and Jacobs overseeing the unpacking of the PROMEC fungal collection.

Research at the PROMEC Unit centred on risk assessment of fumonisins and intervention methods to reduce fumonisin intake by rural populations living on a maize staple diet. Fungal strains representing the above research, as well as many subsequent surveys conducted at the PROMEC Unit, have been consolidated into the PROMEC fungal collection. This collection of 9000 fungal isolates was under the curatorship of Prof Wally Marasas, a renowned expert on fungi and toxins. In 2002, the Institute for Scientific Information in 2002 rated Prof Marasas as one of the most cited researchers world-wide in two categories.

After his retirement, the PROMEC collection continued to grow until the Medical Research Council (MRC) decided to close down the unit in 2013. This decision formed the basis of negotiations between the MRC and the ARC to relocate the collection to Roodeplaat and thus form part of the National Collection of Fungi (NCF).

The curation of the PROMEC collection will form part of the NCF's mandate in future, and will secure the long-term storage and care of this irreplaceable collection. The incorporation of the PROMEC collection also forms part of the NCF's mandate under the National Research Strategy, the Nagoya protocol, the Convention on biodiversity (<http://www.cbd.int/>), the National Environmental Management: Biodiversity Act, and also its mandate under the Department of Agriculture, Forestry and Fisheries. The Memorandum of Understanding (MoU)

between the ARC and the MRC was signed in October 2014.

In January 2016, Sandton Office Removals, the same company that was responsible for the successful relocation of the Biosystematics Division to Roodeplaat in 2009, moved the PROMEC collection from Tygerberg Hospital in Cape Town to the NCF at Roodeplaat. The entire collection, comprising 9000 fungal cultures, was marked, repacked and transported overnight in a refrigeration truck.

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Team from Sandton Office Removers involved in the successful relocation of the Medical Research Council fungal collection to the National Collection of Fungi.



Steel racks used to house the MRC fungal collection as part of the NCF's living culture collection.

## '*Candidatus Liberibacter africanus* subsp. *tecleae*' associated with a native *Teclea gerrardii* from South Africa

The phloem limited bacterium, '*Candidatus Liberibacter africanus*' (Laf), is associated with citrus greening disease in South Africa. This bacterium has thus far been solely identified from commercial citrus in Africa and the Mascarene islands, and it has been suggested that its origin lies within an indigenous citrus host from Africa. Recently, in determining whether alternative hosts of Laf exists amongst the indigenous rutaceous hosts of its triozid vector, *Trioza erytrae*, three novel subspecies of Laf were identified i.e. '*Candidatus Liberibacter africanus* subsp. *clausenae*' (LafCl), '*Candidatus Liberibacter africanus* subsp. *vepridis*' (LafV) and '*Candidatus Liberibacter africanus* subsp. *zanthoxyli*' (LafZ) in addition to the previously identified '*Candidatus Liberibacter africanus* subsp. *capensis*' (LafC) (Roberts *et al.*, 2015).

The study described herein expands upon the range of indigenous rutaceous tree species tested for liberibacters closely related to Laf and its subspecies. A collective 122 samples of *Teclea* and *Orcia* species were sampled from Oribi Gorge and Umtamvunu nature reserves in KwaZulu-Natal. Total DNA was extracted and the presence of liberibacters from these samples were determined by a generic liberibacter TaqMan real-time PCR assay. Liberibacters from positive samples were further characterized through amplification and sequencing of the 16S rRNA, outer-membrane protein (*omp*) and 50S ribosomal protein L10 (*rpLJ*) genes. A single *Teclea gerrardii* specimen tested positive for a liberibacter, and through phylogenetic analyses of the three genes sequenced, was shown to be unique,

albeit closely related to Laf and LafZ. This newly identified liberibacter species is proposed to be named '*Candidatus Liberibacter africanus* subsp. *tecleae*' (LafT).

With the addition of LafT, there are now five recognized subspecies to Laf which have been identified from South Africa. For the sake of the South African citrus industry, it will be important to fully characterize various biological properties of these liberibacter subspecies i.e. vector and host range to help fully understand the possible impact these liberibacters may have on commercial citrus crops. Additional sequence information LafC, LafCl, LafV, LafT and LafZ could potentially help clarify the exact taxonomic position of the various subspecies in relation to Laf and give further insight into the divergence of these liberibacters.

### Reference:

Roberts, R., Steenkamp, E. T. and Pietersen, G. 2015. Three novel lineages of '*Candidatus Liberibacter africanus*' associated with native rutaceous hosts of *Trioza erytrae* in South Africa. *International Journal of Systematic and Evolutionary Microbiology*. **65**: 723-731

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Leaves of *Teclea gerrardii* showing Triozid marks, caused by the vector of '*Candidatus Liberibacter africanus* subsp. *tecleae*', *Trioza erytrae*.

## Digital mobilization of the type specimens in the South African National Collection of Insects

The Biosystematics Division houses the largest collection of insects in South Africa, and is officially known as the South African National Collection of Insects (SANC). The emphasis of the collection has mainly been on local species of agricultural importance, but specimens from natural ecosystems are also included. Over 2800 insect primary type specimens are housed in the SANC. They include mainly Coleoptera (beetles), Hemiptera (bugs), Diptera (flies), Hymenoptera (bees, wasps) and Thysanoptera (thrips).

Recommendation 72F of article 72 of the International

Code of Zoological Nomenclature (1999) states that institutions should publish lists of name-bearing types in their possession or custody. A type list is beneficial to the global research community when revisions of taxa are undertaken and taxonomic problems need to be resolved. Several lists of the type specimens housed in the SANC have previously been published, but they are now well out of date and mostly exclude specimen collection data.

Last year, a grant from the National Research Foundation (NRF) Foundational Biodiversity Information Programme was obtained to further catalogue the type specimens in the SANC. The project aims to update and complete the current database of these type specimens, and to make the information available in the form of a basic catalogue/inventory which can be accessed through the internet

and downloaded as a PDF document. In addition, digital images are to be produced of the primary type specimens of a key agriculturally important group of insects, viz. the scale insects, which belong to the order Hemiptera. These images will be made available on the internet through the Encyclopaedia of Life.

The NRF funding for the project was specifically granted to employ a technician on contract for about four months to capture type specimen images and manage data. Work on the project began in earnest in February with the employment of Ms Liezl Krüger to digitize the required specimens and to assist with the upgrading of the type database. Liezl obtained a B.Sc. (Hons.) degree in Zoology at the University of Pretoria last year. So far, she has almost completed the task of capturing the required array of digital images of the morphological features for each scale insect primary type specimen in the SANC, involving over 3000 images of 240 species. This procedure is being done with the Zeiss micro-imaging system at Biosystematics Division which was purchased during 2014 through a NRF National Equipment Programme grant. The project continues for the rest of this year, and is providing a wonderful opportunity to begin making these scientifically valuable specimens publicly accessible in digital form.

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Liezl Krüger at work capturing digital images of scale insects on the Zeiss micro-imaging system.

## A study towards the management of *Verticillium* wilt of tomatoes in the Limpopo Province

*Verticillium* wilt is a vascular disease which threatens the sustainable production of tomatoes in South Africa. It is caused by the soilborne fungal pathogens *Verticillium dahliae* and *Verticillium albo-atrum*, which are the most aggressive, and *Verticillium tricorpus*, which is known to be a less virulent pathogen. In South Africa, most tomato cultivars have obtained resistance to *V. albo-atrum* and *V. dahliae* race 1, through resistance breeding, but cultivars have no resistance to *V. dahliae* race 2 (Ferreira *et al.*, 1990; Grogan *et al.*, 1979).

Symptom development of *Verticillium* wilt starts with interveinal chlorosis of leaves, which is followed by wilting and necrosis and symptom development correlated with the systemic movement of the fungal infection from the base to the apex of the plant. Foliar symptoms are sometimes restricted to only one side of the plant. Entire stems may become necrotic although they remain upright. Dissections of stems reveal a brown discoloration of the vascular tissues. *Verticillium* wilt is known to lead to premature plant death and a decline in yield (Fradin and Thomma, 2006). In the USA, tomato yields have been reduced by *Verticillium* wilt by between 25-67%.

The causal organisms of *Verticillium* wilt survive in the soil by means of dormant resting structures, of which microsclerotia are the most important since they have the ability to persist for several years under unfavourable conditions in the absence of the host plant. In tomatoes and several other crops, a strong correlation exists between the inoculum density of *Verticillium* species in the soil and disease incidence and severity. In California, 100% disease incidence was found on tomato plants (containing the resistant *Ve* gene) with an inoculum density of 6 microsclerotia per gram of soil. A single microsclerotium can lead to systemic symptom development in a plant, and even symptomless infections may lead to decreased yield in tomato crops (Ashworth *et al.*, 1979; Pegg and Brady, 2002).

*Verticillium* wilt is a vascular disease and is thus very difficult to control once the plants have been infected. Furthermore, *V. dahliae* has an extremely broad host range, including cauliflower, artichoke, eggplant, potatoes, lettuce, cotton, olives and peppers. It also has the ability to colonize non-hosts plants such as cereals and certain weed species, therefore maintaining inoculum populations (Fradin and Thomma, 2006). Management strategies should focus on three aspects, namely reducing the microsclerotia levels in the soil before planting, preventing secondary introduction of microsclerotia from external sources and preventing infection or spread of the fungus within the plant (Klosterman *et al.*, 2009). The latter can only be achieved through resistance breeding, which has proven to be a difficult task. The focus of this study is to target the first two aspects.

Traditionally, control has focused on reducing populations of microsclerotia in the soil at the beginning of the growth season by using chemical fumigants. However, several of the effective fumigants have been phased out worldwide due to their negative impact on health and the environment, and high costs.



Severe wilting of tomato plants in South Africa caused by *Verticillium* species.



Foliar symptoms of *Verticillium* wilt of tomato.

This increases the demand for alternative control methods, especially methods that are compatible with organic and sustainable agriculture. Other management strategies that can be used for this purpose are tillage, solarisation, crop rotation, organic amendments and biofumigation. Sanitation and weed control are strategies that can be used to prevent infection from external sources of microsclerotia (Klosterman *et al.*, 2009).

All of the above management strategies have been tested against *Verticillium* wilt with some degree of success, but

none of them have proven successful on its own and therefore a combination of some of these strategies is necessary. A strategy that shows great promise for the management of soilborne diseases in various crops, particularly Verticillium wilt of strawberries, is known as anaerobic soil disinfestation (ASD). It involves the incorporation of a carbon source into the soil, either as an organic amendment, or a green manure harvested from cover crops, or crops used in rotation with tomatoes. After the incorporation of the carbon source, the field is covered with airtight plastic and the top layers are irrigated to field water capacity, which creates anaerobic conditions in the soil. The processes that occur as a result of the anaerobic degradation of the carbon source can involve biofumigation or a shift in the microbial population of the soil where the natural enemies of *V. dahliae* increase, and thereby resulting in the management of *V. dahliae*. In the USA, ASD was found to consistently reduce the number of *V. dahliae* microsclerotia in strawberry field soils by 85 to 100% (Shennan *et al.*, 2014).

A study is currently under way to develop a molecular technique to quantify microsclerotia from soil samples. This technique is used to establish a threshold for deciding when management strategies should be implemented. The study will also investigate ASD as a method of controlling Verticillium wilt of tomatoes at various locations in the Limpopo Province. The efficacy of ASD is dependent on the particular carbon source that is used, as this will ultimately result in the release of certain volatiles and/or a shift in the microbial population. Our study will also involve testing different carbon sources that must not only be effective against *V. dahliae* race 2 but should also be practical for use by the farmers of the Limpopo Province.

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## ARC - Russian collaboration on eriophyoid mites continues

Dr Charnie Craemer, a researcher at ARC-PPR Biosystematics, and Dr Philipp Chetverikov of the Zoological Institute and Saint Petersburg State University are specialists on the systematics and morphology of eriophyoid mites and have been collaborating on joint research since 2013. All eriophyoid mites are microscopic and feed on plants, and some are economically important crop pests. There are pressing problems involved in the taxonomy of these mites, some of which are being addressed by Drs Craemer and Chetverikov through the study of their comparative morphology using technologically advanced techniques, improved descriptions and diagnostic characters, and exploring new morphological characters that may be useful in the systematics of these ultra-tiny mites.

Dr Chetverikov visited Dr Craemer during March 2013 (*Plant Protection News* 95: 5). One of the aims of his visit was to survey and collect eriophyoid mites in South Africa's KwaZulu-Natal coastal regions for systematic study. During this survey they focused on collecting mites from indigenous gymnospermae, palms and sedges. These plants in particular may host the more ancient eriophyoids of the putatively most plesiomorphic eriophyoid family, Phytoptidae. Research on this group of mites could assist in understanding the evolution of eriophyoid mites which consequently may facilitate their classification.

Following Dr Chetverikov's 2013 South African visit, the two researchers described a new eriophyoid species, *Mackiella reclinata* Chetverikov and Craemer, 2014 (Chetverikov *et al.* 2014). They collected this mite with Dr. Stefan Nesper (retired from ARC-PPR) on a South African indigenous palm tree, the Senegal or Wild date palm (*Phoenix reclinata*). The description is very detailed and is an improvement on the usual standard of eriophyoid species descriptions. Apart from morphology studied with the traditional light microscope, they included results obtained using Confocal Laser Scanning Microscopy (CLSM) and Low Temperature Scanning Electron Microscopy (LT SEM). The latter two techniques are relatively newly employed for the study of Eriophyoidea. The article includes advanced study of the internal genitalia, a research focus of Dr Chetverikov. It has previously been demonstrated that the morphology of the internal genitalia, which could not be adequately studied in the past, is potentially appropriate for taxonomic revisions of eriophyoid mites. In the article they revised the mite tribe Mackiellini



Confocal Laser Scanning Microscope image of a new eriophyoid genus on *Hyphaene coriacea* (Ilala palm) from South Africa (P. Chetverikov and C. Craemer).

to which the new species belongs.

Collaborative research between Drs Chetverikov and Craemer has also resulted in the publication of an article on the gnathosoma (mouth parts) of the Eriophyoidea, describing a hitherto unknown structure for which they coined the term "gnathosomal interlocking apparatus", and remarking on the functional morphology of the frontal lobe of Eriophyoidea (Chetverikov and Craemer, 2015). Part of these results were from Dr. Craemer's research presented for her PhD degree, which she obtained in 2010.

Dr Craemer visited Dr Chetverikov in St. Petersburg, Russia during 6-22 March 2015 to continue their research. They worked on a paper on eriophyoid gnathosomal morphology in collaboration with other researchers from Italy and the USA, which will soon be submitted for publication.

Dr Craemer further studied the morphology of primarily Northern Hemisphere eriophyoid taxa. She also undertook various collecting trips with Dr Chetverikov and collected eriophyoid pest species that are not yet present in South Africa, but which are of quarantine



From left to right: Drs Philipp Chetverikov, Charnie Craemer, and Prof Andrey Granovitch, Head of the Department of Invertebrate Zoology at the Saint-Petersburg State University

concern. These included the juniper gall mite (*Trisetacus quadrisetus*), which causes enlarged and damaged seeds on common juniper (*Juniperus communis*), and hazelnut gall mite (*Phytoptus avellanae*) and filbert bud mite (*Cecidophyopsis vermiformis*), which both cause damaging bud galls on hazelnut (*Corylus avellana*) from which no nuts develop. Slide-mounted specimens of these mites were deposited in the ARC-PPR National Collection of Arachnida: Mites, and will be valuable diagnostic reference material should these mites be found on plant material imported into South Africa.

Together they also studied several undescribed species of mites that are indigenous to South Africa. These include a new species from common karee (*Searsia lancea*), a new *Aberoptus* species spinning nests on *huilboerboon* or weeping boer-bean (*Schotia brachypetala*), and a unique new species and genus from Ilala palm (*Hyphaene coriacea*). Samples were studied using CLSM instruments available at the University of St. Petersburg. These new species will be described in a joint publication.

The collaboration between Drs Craemer and Chetverikov has been most productive and is expected to continue, and to result in several further projects. A joint paper describing a new *Sierraphytoptus* species from the most plesiomorphic eriophyoid family Phytoptidae has recently been accepted for publication. Dr Chetverikov plans to visit Dr Craemer again in November 2016 when they will undertake a collecting trip to the Western Cape, and will continue to work on collaborative papers.

#### References:

Chetverikov, P.E. and Craemer, C. 2015. Gnathosomal interlocking



Top: Enlarged and damaged seeds on *Juniperus communis* caused by the eriophyoid mite, *Trisetacus quadrisetus*. Thousands of mites are overwintering in these seeds. Seed production can be entirely destroyed by these mites.

Bottom: Two enlarged damaged buds of hazel nut. The bud galls are still very young, and especially the gall on the left, caused by the eriophyoid mite *Phytoptus avellanae*, will become much larger later in the season. The bud on the right is galled by *Cecidophyopsis vermiformis*. No nuts will develop from these damaged buds. These pests are not present in South Africa and can be considered serious quarantine pests.

apparatus and remarks on functional morphology of frontal lobes of eriophyoid mites (Acariformes, Eriophyoidea). *Experimental and Applied Acarology* 66: 187-202.

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## StopRats bag Stinkwater

Airtight or hermetic storage systems eliminate the exchange of gases between the inside and the outside of a storage container. Living organisms, such as insects within the container, will deplete oxygen until they die and this eliminates the need for insecticides. Fungi that are problems in grain storage systems can also be controlled, if for example maize is dried to below 14% moisture before storage in an air tight system.

Hermetic storage bags (transportable flexible plastic crop storage bags) are currently targeted at high value crops e.g. coffee and cocoa beans and to protect seed material for the next growing season. However there is a growing international drive to use hermetic bags for small-holder storage to reduce post-harvest losses and to increase food security and nutrition. Storing crops hermetically also enables farmers to wait for better market prices instead of selling immediately after harvest.

Grain can be stored for up to two years in a hermetic storage bag, the bags are reusable and with careful use may last for longer. However, the hermetic seal is destroyed once it is punctured. Rodent damage is recognised as a potential problem, as rodents can easily gnaw through the bags, but damage remains unquantified. In theory, rodents will not recognise the hermetically sealed bags as a

potential food source if they cannot smell the grain inside the bag, thus damage should be limited particularly if there are no other food resources nearby the bags. If this hypothesis holds, these bags could be an excellent means of protecting against post-harvest loss but could also reducing rodent-human proximity as rodents will not be drawn to people's houses if they don't find food inside. StopRats provides an opportunity to provide evidence.

The Sustainable Technologies to Overcome Pest Rodents in Africa Through Science (StopRats) project (see *Plant Protection News* 103: 7-8) provides an opportunity to provide evidence on the effectiveness of hermetic storage bags to reduce grain losses during storage. StopRats has put hermetic bags on trial in southern African communities. Trials are being conducted by StopRats partners with farming communities in South Africa, Tanzania and Namibia. Two types of hermetic grain storage bags are compared with ordinary woven polypropylene bags (polybags) which are currently used by many subsistence farmers for maize storage. The hermetic bags are the *Purdue Improved Crop Storage (PICS) Triple bag* and the *IRRI Super Bag*.



PICS bags were developed in the 1980's by Purdue University, West Lafayette, Indiana in the U.S.A., initially to protect cowpeas in storage from destruction by weevils, and then extended to control insect pests in stored grains and other seeds in West and Central Africa. A *PICS Triple bag* is composed of three layers: two inner layers of high density polyethylene enveloped in a woven polypropylene bag.

The *IRRI Super Bag* was developed by the International Rice Research Institute (IRRI) in collaboration with GrainPro Inc. of Concord, Massachusetts. The product is manufactured by GrainPro Inc. and marketed as SuperGrainbag™. The Super bag is a thicker multilayer polyethylene bag, placed in an outer protective layer, such as an ordinary woven polypropylene bag. The PICS Triple bags used for the trial were manufactured in Tanga, Tanzania, while the IRRI Super Bags were sourced from IRRI in the Philippines. Note that the above manufacturers have never claimed that their hermetic bags are rodent proof.

Two parameters were initially chosen for testing: 1. Households with one of each type of storage bag, stored together in normal location on premises (rodents cannot smell the grain inside the hermetic bag). 2. Households where all bags are of the same type, stored in normal location on premises (hermetic bags work best when there are no other food sources to attract rodents). Due to drought and the poor grain crop harvest, all maize used for the trial was sourced from a commercial maize provider, as clean, untreated and undamaged kernels, and only the first parameter was put on trial.

Twelve volunteer households of the “Maize Producer’s Club” of Block Mokone in Stinkwater, a peri-urban community near Hammanskraal in the north-western corner of Gauteng, were randomly selected for the storage trial (Fig. 1). Details of the project were explained during community meetings (Fig. 2). A questionnaire was completed by each volunteer household to provide background on farmer’s knowledge and practices. In October 2015, each household was provided with one bag of each treatment, containing clean and insect-free maize of equal and known weight, closed and sealed with cable ties. The three bags were placed together in ran-



Fig. 1. Members of the ‘Maize Produce Club’, Block Mokone, Stinkwater.



Fig. 2. Phanuel explaining details of the project trial in Block Mokone.

dom order on the floor where the household would store their maize harvest on their premises (Fig. 3).

Bags are currently being inspected monthly for damage and data collected, for a period of at least six months. A single hole in the bag, whether from accidental puncture or rodent gnawing (Fig. 4), accounts for damage, as the hermetic seal is broken. Holes are patched up with adhesive tape, the bags are closed and the hermetic seal is restored as well as possible. At the end of the trial period, the bags will be weighed to determine loss due to rodent damage and the maize will be sampled for insects and damage level.



Fig. 3. Mrs. Maseko with maize stored in the kitchen.



Fig. 4. Rodent damage and spillage .

Preliminary results indicate that the hermetic bags are insect free. Insect infestations, such as *Sitophilus zeamais* weevils were observed on the normal woven polypropylene bags (polybags), even those which had no rodent damage. Results also indicate that hermetic bags were better in protecting maize from rodent damage, compared to maize in the polybags, in the same location. Fig. 5 shows the number of bags (in percentage) for each bag category (treatment) damaged by rodent activity during March 2016. However, the hermetic bags are not rodent proof, as holes were gnawed through the layers of polyethylene, in locations where polybags were damaged. These are initial observations and conclusions will be made after the final data collection.

This trial also demonstrated the importance of “social science” in an on-farm participatory approach “natural science” trial. Soon after late summer rains, maize had disappeared, without any visible signs of entry, from the storage bags on trial. This was explained as “exceptionally large clever rats” who knew their way into maize bags.

For more information on Stoprats hermetic bag trials, please see our website <http://projects.nri.org/stoprats/>

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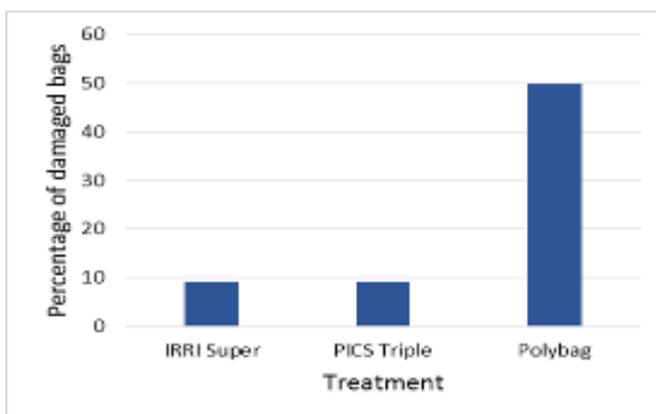


Fig. 5. Rodent damage to three types of storage bags during March 2016.

## Bacterial pathogens of onion seed

South Africa is one of the biggest producers of onion seed in the world. During the 2014/2015 growing season, 368 tons of onion seed were produced, of which 282 tons were for the export market. New bacterial diseases of onions have recently been reported from South Africa (Brady *et al.*, 2011; Coutinho *et al.*, 2009; Goszczynska *et al.*, 2006). Many of these bacteria are seed borne or seed transmitted. Different bacteria may cause similar symptoms, for example, leaf and seed stalk necrosis is caused by *Pseudomonas syringae* and *Pantoea* species (Coutinho *et al.*, 2009; Goszczynska *et al.*, 2006). Several pathovars of *Pseudomonas syringae* pathogenic to onion were found in South Africa and at least one of them belong to a possibly new. Bulb rot diseases are caused by *Enterobacter cloacae*, *Pantoea ananatis*, *Pantoea allii*, *Pectobacterium carotovorum* and *Bulkholderia* species. The correct diagnosis is essential for farmers to apply disease management tactics, and to ensure that seed is pathogen-free.

During 2014-2016, 65 samples of seed produced in the country were evaluated for the presence of six bacterial pathogens, *Pantoea allii*, *Pantoea ananatis*, *Pantoea agglomerans*, *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *porri* and *Xanthomonas axonopodis* pv. *allii*. Isolations were done on four selective media, namely PA 20, Kings B medium, Milk-Tween, Tween A and one general medium, Tryptone glucose extract agar (TGA). A variety of saprophytic bacteria were isolated from all seed samples. Yellow colonies resembling *Pantoea* species were recovered from 41 seed samples on TGA (Fig. 1). Yellow colonies with a clear zone on Milk-Tween agar, characteristic of *Xanthomonas* species, were recovered from four seed samples. At least 30 yellow colonies per seed sample were purified, resulting in 1342 isolates in total.

Pathogenicity was determined on three week-old onion seedlings in the greenhouse using the stab inoculation method. A sterile needle was dipped into the bacterial colony on TGA (24-hours growth) and then the needle was inserted under the epidermis of a leaf. Inoculated plants were maintained in a greenhouse with 27/23°C day/night temperatures and observed daily for the development of symptoms. Of the 1342 isolates evaluated, only two isolates from two different seed samples were pathogenic when inoculated into onion seedlings. The two isolates produced similar symptoms to the *Pantoea agglomerans* strain isolated by Hatting and Walters (1981) (Fig. 2). Two pathogenic strains were Gram negative rods, oxidase negative, facultatively anaerobic and indole negative. Furthermore, API20E profiles of the two pathogenic strains were identical to those of the strain isolated in 1981 and identified as *Pantoea agglomerans*. The seed lots from which the pathogenic *Pantoea agglomerans* were recovered, were destroyed.

This study showed that the commercial seed tested contained mainly saprophytic bacteria including non-pathogenic *Pantoea agglomerans* and xanthomonads. However, evaluation of commercial onion seed for the presence of bacterial pathogens should be done routinely to avoid transmission of seed-borne pathogens worldwide.

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Fig. 1. Yellow colonies were recovered from 41 seed samples on Tryptone glucose extract agar.



Fig. 2. Symptoms produced by the *Pantoea agglomerans* strain isolated by Hatting and Walters reported in 1981.

## *Workshop and training*

# Biosystematics closely involved in training of quarantine Acarologists

Plant-feeding mites are regularly intercepted on plant material imported to South Africa, and an increase in the number and diversity of these mites has occurred in recent years. Some phytophagous mite species, belonging particularly to the Tetranychidae (spider mites), Eriophyoidea (eriophyoid mites) and Tenuipalpidae (flat mites) families are economically important pests of crops worldwide. Some are directly harmful to their host plants, while others have an indirect economic impact due to their ability to vector viruses and other plant pathogens.

Plant-feeding mites are particularly predisposed to becoming invasive aliens. They have a significant capacity to survive adverse conditions and can also reproduce parthenogenetically (they can start a new population from a single unfertilized female). Mites are usually microscopically small, and often occur hidden in tiny crevices on their host plants, and thus they are typically very difficult to detect on plant material. Moreover, symptoms caused by mites are usually not present on the host material, since these only become apparent when mite numbers are high. Worldwide, there have been many inadvertent introductions of phytophagous mites to new areas, sometimes resulting in significant crop damage and economic impact. The risk of introductions through the importation of plant material continues to increase, especially as international trade has intensified.

The South African Government's Department of Agriculture, Forestry and Fisheries (DAFF) is responsible for the biosecurity of imported plant material. After the initial point of entry, microscopic inspection and testing of imported plant material and identification of organisms found takes place at the Plant Health Diagnostic Services (PHDS) Laboratories at the DAFF Quarantine Facility in Stellenbosch, Western Cape Province. Due to the increased mite numbers found on imported material, a team particularly focused on acarological inspections, identifications and interceptions was formed in 2011, led by the acarologist, Ms Davina Saccaggi, DAFF-PHDS.

Appropriate training of quarantine officials is essential to conduct acarological inspections, especially due to the usual difficulty in detecting mites, and to undertake other tasks necessary for the interception process. Recently the PHDS Acarology team appointed a new technician, Ms Isabel Collett. As part of her training, Ms Collett attended a week-long detailed and custom-made course, An introduction to phytophagous mites, at the Mite Section of the Biosystematics Arachnology Unit. The course was presented by Ms Matseliso (Tshidi) Makutoane from 15 to 19 February 2016. It included field and microscopic identification of the most important plant-feeding families, and also covered their morphology and biology. The identification of mite damage and behaviour, especially pertaining to quarantine inspections, were incorporated. Techniques to find, collect and prepare mites for study were theoretically and practically dealt with.



Isabel Collett (left) from DAFF-PHDS attended a course on plant-feeding mites at ARC-PPR Biosystematics presented by Tshidi Makutoane (right).

The training of Ms Collett by ARC-PPR Biosystematic personnel formed part of an agreement and contract between ARC and DAFF. This binding agreement, "The rendering, through biosystematic research, of diagnostic assistance to DAFF regarding plant pests and diseases, and the maintenance of quarantine facilities for the introduction of biological control agents" has been in existence for more than 20 years and is renewed on an annual basis. The aim of the agreement is to provide DAFF with biological information and diagnoses of pests and diseases associated with plants and plant products during import and export of these commodities. The project also supports the identification of these organisms, which is important in compiling a comprehensive assessment of possible risks associated with agricultural trade. The activities of the project further serve as the basis for adequate facilitation of trade through the pest risk analysis process. Importantly, it also provides for the training of plant inspectors and other scientific quarantine personnel employed by DAFF. The permanent collaboration with mite taxonomists and the availability of quarantine reference collections are fundamental to give support to biosecurity procedures.

In addition to the official ARC-DAFF agreement, the taxonomist on plant-feeding mites at ARC-PPRI Biosystematics, Dr Charnie Craemer, and the acarologist at DAFF-PHDS, Davina Saccaggi, work in close collaboration on certain aspects and projects regarding mites in quarantine.

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## Technology Transfer

### Scientific publications

BJØRNSTAD, A., GROBBELAAR, E. & PERISSINOTTO, R. 2016. Review of *Afraustraloderes rassei* Bouyer, 2012: description of its female and a new species of *Pixodarus* Fairmaire, 1887 (Coleoptera, Cerambycidae, Prioninae). *ZooKeys* 558: 77-93.

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DAVIS, A.L., STALS, R. & DESCHODT, C.M. 2015. New replacement name for the species *Scarabaeus (Scarabaeolus) nitidus* Davis & Deschodt, 2015 (Coleoptera: Scarabaeidae: Scarabaeinae) - not an American pest. *Zootaxa* 4057(4): 582.

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### Chapters in Book

HASSEN, A. 2016. Microbial inoculants as agents of growth promotion and abiotic stress tolerance in plants. In: Singh, D.P., Singh, H.B. & Prabha, R. (eds.), *Microbial Inoculants in Sustainable Agricultural Productivity*. Vol. 1: Research Perspectives. pp. 23-36. ISBN 978-81-322-2645-1.

### Scientific meetings

JACOBS, A. et al., 2016. Soil *Fusarium* survey in the grassland biome of South Africa Joint conference of the South African Association of Botanists (SAAB) and the Southern African Society for Systematic Biology (SASSB). Bloemfontein. 10-13 January 2016.

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### Popular publications

ANDERSON, J., SCHOLTZ, C., EARDLEY, C., BONNER, M., MAPODI, D. 2016. Biodiversity and Extinction. Part 5. Insects. *Super Nova* 5.3:36-37.

GOUS, A., DE BRUIN, J.J., EARDLEY, C.D. SWANEVELDER, D. & WILLOWS-MUNRO, S. 2015. Metabarcoding of pollen from a historic bee collection. *iBOL-Barcode Bulletin* 6: 12 -13.

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VON MALTITZ, E. & KIRSTEN, F. 2016. Gerbils: Ecologically based rodent management in maize. *Grain SA*. March 2016, pp.22-25. E-book <http://www.grainsa.co.za/sagrain/march2016>.

## Workshops

**TRUTER, M., JACOBS-VENTER, A., KWINDA, G. & BHIYA, T.** Introductory Mycology workshop, Biosystematics building, Roodeplaats, 9-12 February 2016 (6 delegates).

**TRUTER, M.** Introductory Fungal Taxonomy workshop, Biosystematics building, Roodeplaats, 25 February 2016 (12 delegates).

**JACOBS-VENTER, A.** Introductory Molecular Biology Course, Biosystematics building, Roodeplaats, 15-17 March 2016 (9 delegates).

**STRATHIE, L.** Training at the International Workshop on Biological Control of *Parthenium hysterophorus* in East Africa, Ethiopia, 17-18 Dec 2015. (25 participants).

**STRATHIE, L.** Farmer's Days training given on the biological control of *Parthenium hysterophorus* in Phumelela Farmers' Cooperative, Pongola, KZN. 19 January 2016. (31 learners).

**STRATHIE, L.** Farmer's Days training given on the biological control of *Parthenium hysterophorus* in Makhathini Flats near Jozini, KZN. 20 January 2016. (33 learners).

**STRATHIE, L.** Farmer's Days training given on the biological control of *Parthenium hysterophorus* in KwaShukela community near Makhathini Flats, KZN. 21 January 2016. (93 learners).

**KOCH, S., NKGAU, T., HLEREMA, I., VAN DER LINDE, E. & MARTMARI VAN GREUNING, M.** Oyster Mushroom Production Training for City of Joburg Blue Economy Programme, 22-26 February 2016, ARC-PPRI. (17 participants)

**SWART, A., MARAIS, M. SHUBANE, A., GIRGAN, C. & RAV-ELE, N.** Nematodes in Agriculture. Training of pre-graduate plant pathology students at University of Pretoria, 17 February 2016. (24 students).

**SWART, A., MARAIS, M. SHUBANE, A., GIRGAN, C. & RAV-ELE, N.** How to identify and characterise nematodes. Training of pre-graduate plant pathology students at University of Pretoria, 18 February 2016. (26 students).

## Fact sheets

A range of fact sheets on popular garden plants that have become invasive in South Africa are available here: <http://www.arc.agric.za/arc-ppri/Pages/Terrestrial-plants.aspx>:

**SZTAB, L AND HENDERSON, L.** Technical Fact Sheets:

- Wandering Jew *Tradescantia fluminensis*.
- Yellow Water Iris *Iris pseudacorus*.
- Fountain grass *Pennisetum setaceum*.
- Tick seed *Coreopsis lanceolata*.
- Blue periwinkle *Vinca major*.
- Butterfly bush *Oenothera lindheimeri*.
- Queensland umbrella tree *Schefflera actinophylla*.
- Singapore daisy *Sphagneticola trilobata*.
- Madagascar periwinkle *Catharanthus roseus*.
- Goosefoot vine *Syngonium podophyllum*.
- Mother-of-millions *Bryophyllum delagoense*.