University of Pretoria

Department of Microbiology and Plant Pathology

Annual Report 2000
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The new millennium announced itself with spectacular fireworks all over the world, with no millennium bug glitches or global disasters, as was predicted and anticipated by many. For our Department, it was all too soon back to the basics and the challenges of the day.

Another exciting year has passed and the outputs of the Department have surpassed all previous years, in every respect. The number of final year students, tallied 80, and 24 students graduated at the honours level. Furthermore, 4 MSc and 4 PhD students completed their degrees. Staff members contributed 27 science citation index publications, 89 papers presented at local conferences and 44 presentations at international meetings. Two patents were also registered.

Dr. Jacques Theron was honoured for his contribution to water research by being awarded the prestigious Water Institute of South Africa award for young scientists. Dr. Teresa Coutinho was promoted to associate professor and new two members of staff were appointed i.e. Mr. Julian Jaftha as junior lecturer and Prof. Terry Aveling as associate professor.

The introduction of the new course work MSc degree in Water Resource Management was a great success. Ten students enrolled on a full time basis and three part-time. A further 3 students enrolled for the PhD in Water Resource Management. This has given the Centre for Water Biotechnology in the Department a tremendous boost in terms of the education programme. The UP/CSTR Alliance also came into full swing during 2000. Three new joint research projects were approved and the appointment of Prof. P. Ashton as an extraordinary professor in the Department paid many dividends. We are looking forward to the expansion of the alliance during 2001.

The introduction of the School system at the UP has also had a positive impact on our Department. We became fully integrated into the School for Biological Sciences in 2000. A revolutionary step was taken, when it was decided that the curriculum for the first two years, for students in Biological Sciences would be the same as from 2001. Specialisation will only happen in the third year. I have no doubt that we will reap the benefits of this strategic move in the near future. Research has also been re-organised into focus areas (programmes), which are multi-disciplinary, rather than projects run by individuals or small groups in a particular department. This will give impetus to our research strengths across all disciplines in the Biological Sciences.

FABI has again made a major contribution to our Department. Prof. Mike Wingfield and the “Fabians” continue to do research and publish scientific papers of a very high standard and provide excellent opportunities for postgraduate students in the Department. We are also experiencing an unprecedented growth in the Institute and are already in the planning stages of expanding the facilities to accommodate this.

For me, it remains a honour and a privilege to head such a wonderful Department and I would like to pay tribute to the highly motivated and committed members of staff who contribute to making this a first class institution in higher education.

Prof. T.E. Cloete
New Appointments

?? Mr. Julian Jaftha was appointed a junior lecturer in the Department for 1 July 2000.

?? Professor Terry Aveling was appointed an associate professor in the Department from 1 January 2000.

Awards & other Academic Achievements

?? US Medical Education for South African Blacks (MESAB) Scholarship was awarded to D. Masoabi (MSc Water Resource Management).

?? Mellon Mentoring Programme award to J.B. Weyers and V.S. Brözel; B.O.Z. Maseko and M.J. Wingfield; and B. Slippers and M.J. Wingfield.

?? NRF prestigious PhD scholarships to J.B. Weyers, R.M. MacDonald and M. Venter.

?? NRF Aaron Klug Scholarship to J. de Wet.

?? Wirsam Scientific Prize for best student paper at the MMSA Conference – N. van den Berg.

?? Pannar Prize for the best student paper presentation at the SASPP conference – I. Barnes.

?? Water Institute of Southern Africa (WISA) Award to Dr. J. Theron for contributions made to water research.

?? Merck Award to Lucinda Paulsen for the best honours students in the Department.

MSc degrees awarded

Riana Jacobs
Supervisor: Prof. M.J. Wingfield
Co-supervisors: Prof. B.D. Wingfield & Dr. K. Jacobs
Christi Burger
Thesis title: “Tolerance of citrus rootstocks to root pathogens”
Supervisor: Dr. N. Labuschagne

Marieka Venter
Supervisor: Prof. M.J. Wingfield
Co-supervisors: Dr. T.A. Coutinho & Prof. B.D. Wingfield

Juanita van Heerden
Thesis title: “The determination of microbial species diversity and evenness in activated sludge systems using different Biolog systems”
Supervisor: Prof. E. Cloete

PhD degrees awarded

Karin Jacobs
Supervisor: Prof. M.J. Wingfield

Emma Steenkamp
Thesis title: “Molecular taxonomic studies of selected species in the Gibberella fujikuroi complex”
Supervisor: Prof. B.D. Wingfield
Co-supervisors: Prof. M.J. Wingfield, Dr. T.A. Coutinho & W.F.O. Marasas

Baomi Kwerepe
Thesis title: “Integrated pest management Meloidogyne incognita on bambara groundnut (Vigna subterania)”
Supervisor: Dr. N. Labuschagne

Gina Swart
Thesis title: “Comparative study of Colletotrichum gloeosporioides from avocado and mango”
Supervisor: Prof. L. Korsten
Co-supervisor: Prof. F. Wehner
Visitors to the Department

Dr. Vittorio Venturi, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy. 29 September – 3 October 2000.

Prof. Roberto Kolter, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, USA. 19 – 31 January 2000.

Dr. Ahmed El Ghaouth, USDA, January 2000.

Prof. James Dale, Director of Research, Queensland University of Technology, Brisbane, Australia.

Dr. Suzy Bentley, Research Officer, CRC for Tropical Plant Protection, Brisbane, Australia.

Dr. Julio Hernandez, Project Director, ICIA, Tenerife, Canary Islands.

Prof. Shin-Chuan Hwang, Director, Taiwan Banana Research Institute, Pingtung, Taiwan.

Dr. Winfred Hammond, Biological Control Center for Africa, International Institute of Tropical Agriculture, Cotonou, Republic of Benin.

Dr. Jennifer Clancy, Clancy Environmental Consultants, St. Albans, Vermont, USA.

Prof. R. Kolter

Prof. Brözel (right) with Prof. Kolter and Dr. Maria Zambrano
Prof. Louis Nel

Period: August 1999 to August 2000

Host Institution: Centers for Disease Control and Prevention
National Center for Infectious Diseases
DVRD, VRZB, Rabies section
1600 Clifton road
Atlanta, GA 30333
USA

Laboratory director: Dr C E Rupprecht

Lyssavirus research: Vaccine development and evaluation

Background

Rabies is one of the oldest infectious diseases known. Despite its fatal and fearful nature, the disease still takes its toll in dogs and wildlife across the world, with regular spillover to humans. Approximately 50,000 people succumb to this disease annually, with the greatest epidemiological threat in the developing world. One of the major economic costs of rabies is the post-exposure prophylaxis of the disease: each potential human exposure requires treatment at a cost of around $200.00 (up to $1500.00, if available in the case of the developing world). In addition, Mokola virus, one of a number of rabies-related lyssaviruses which have to date only been reported from the African continent, appears to present an emerging public health concern. This is due to a recent increase in the number of cases diagnosed in domestic animals, and evidence that rabies vaccines do not protect against lethal Mokola infection.

Vaccine development and evaluation

The following vaccine research was carried out during the sabbatical period:

1. Virus isolates: Seven Mokola virus isolates were selected and adapted to different types of cells in culture, including Mouse Neuroblastoma cells.

2. Animal models: The neuro-invasiveness and virulence of the above viruses in different animal models was investigated. In a series of experiments aimed at determining the potential of the different virus isolates for use as vaccine challenge strains, batches of mice were infected with all seven Mokola viruses, using different routes of infection. Mice were found to have a very high survival rate when infected peripherally, regardless of Mokola virus strain. However, hamsters were found to be more susceptible to periferal exposure and this model allowed for the selection of three Mokola virus isolates to be considered as virus challenge strains for use in vaccine trials. Studies on the pathogenesis of Mokola in domestic dogs are in progress.

3. Cloning: Towards the construction of different recombinant vaccines, fourteen Mokola virus-specific clones were generated. Specific primers and a PCR assay for the amplification of full-length Mokola-
specific glycoprotein and nucleoprotein encoding genes have been developed. Specific fragments of the glycoprotein gene as well as the full-length glycoprotein genes and nucleoprotein genes of three Mokola virus isolates have been cloned and sequenced in full.

4. Recombinant vaccines: Three different plasmids have been identified and prepared for use in the DNA vaccine experiments. These are pSG5, pBUD4 and pCIneo and they differ, among others, in terms of the nature of their promoters, intron sequences, other regions important in eliciting immune response or gene expression level and dual expressing capability. The glycoprotein encoding gene of Mokola virus 7 were cloned and expressed in each of the three different plasmids pSG5, pBUD4 and pCIneo. Plasmid pBUD4 has dual expression capability and thus a fourth construct was generated in which the nucleoprotein gene of Mokola virus 7 was inserted under control of the second promoter in the pBUD/MOK7G construct. The expression of authentic glycoprotein and nucleoprotein products in mammalian cells were verified by calcium-phosphate induced transfection of cultured cells, followed by immunofluorescence of permeabilized cells with rabies-specific antibodies.

5. Vaccine trials: Batches of mice were vaccinated intramuscularly using each of the four recombinant vaccine constructs and with appropriate controls. After 4 weeks, half of each group received a booster vaccination and all animals were challenged 14 days later. Sera were collected from all animals at 2-weekly intervals and were analyzed for the presence and titre of virus neutralizing antibodies. Much variation in vaccine efficacy was found among the different constructs, and full protection against lethal challenge was obtained with two of the vaccines. Details are published in Vaccine.

Field and laboratory work (rabies) - oral vaccination of free-ranging species

1. With Dr Cathleen Hanlon, participation in the Ohio State Oral vaccination project (raccoons): week of 25 - 29 October.
2. With Dr Gayne Fearnhough, participation in the Texas oral vaccination program, January 15 - 20, 2000. In this program various species of wildlife are vaccinated by air distribution of baits containing a recombinant rabies vaccine.
3. With Dr Charles Rupprecht and Lillian Orciari, participation in the Cape Cod (Massachusetts) raccoon oral vaccination project, April 9 - 15, 2000.
4. Further sequence analysis towards elucidating the molecular epidemiology of viverrid rabies (70 virus isolates) in southern Africa.

Professional interaction:

Conference participation, workshops and official visits

3. Rabies laboratory workshop organized by the APHL, CDC and Texas State Department of Health in San Antonio, Texas (January 2000).
4. Poster exhibition in recognition of work from this sabbatical, April 2000, Division of Viral and Ricke tetsial Diseases, NCID, CDC.

5. 100th American Society for Microbiology meeting, Los Angeles, CA (21 - 25 May 2000). Poster: “Molecular analysis of rabies and rabies-related viruses in southern Africa”.


8. Invited guest scientist: Rabies Centre of Expertise, Ottawa, Canada (2 - 4 August 2000). Seminar: “The APHL fellowship year at CDC, advances in research on rabies and rabies-related viruses from southern Africa”.

Prof. Nel & daughter, Kayla

Prof. Nel, his wife Karen and daughter Kayla

Prof. Nel at a poster exhibition at CDC
Prof. Terry Aveling

Period: October 1999 to April 2000

Host Institution: University of Aberdeen
Scotland
UK

Laboratory director: Dr Alison Powell

Prof. Aveling was awarded a British Commonwealth Research Fellowship for 1999/2000.

Research involved an integration of seed pathology and seed physiology. We looked at the effect of seed coat colour of cowpeas on seed vigour and susceptibility to damping-off. Seed vigour tests included imbibition, tetrazolium and conductivity tests. Percentage germination was also determined in the laboratory according to the International Seed Testing Association and percentage emergence in the greenhouse. The effect of three damping-off pathogens, *Fusarium solani*, *Pythium ultimum* and *Rhizoctonia solani*, on emergence of different coloured seeds was recorded. It was found that light coloured seeds were more susceptible to imbibition damage, had lower vigour and were more susceptible to damping-off than dark coloured seeds. The results of the various experiments have been compiled into two articles that have been submitted for publication in accredited international journals. The results have also been presented at national and international conferences.

Aberdeen is found in the north-east of Scotland (slightly closer to the North Pole than Denmark!). It is a wonderful fairyland during the middle of winter. Many weekends were spent scouting the surrounding mountains, playing in the snow and warming up on hot chocolate. I managed to drive my tiny Vauxhall all the way to the most northern part of the UK at John O’Groats and also visited the Isles of Mull and Skye. I now call myself qualified to drive in rain, snow, sleet and ice! I also attempted some dry-suit diving in the North Sea in the middle of winter but after two dives decided that the Scottish divers were crazy. Apart from the water temperature of around 4 degrees, there was nothing noteworthy to ooh and aah at. At the end of the fellowship I visited Ireland and also went down to Land’s End and the most southern point of the UK before flying home to experience the South African winter. This seemed much colder than Scotland’s as I really missed the central heating! It was a wonderful experience both intellectually and socially. The Scottish people are really great and I had to leave a few very good friends behind.
Prof. Lise Korsten visited Hanoi in April 2000 on invitation by the Australian Centre for International Agricultural Research (ACIAR) and presented a keynote address "Status of citrus greening in South Africa. She visited America in August 2000 and attended the 7th International Symposium on the Microbiology of Aerial Plant Surfaces, University of California, Berkeley USA and also visited Microflow.

Ms. Jacquie Smith attended the World Potato Congress 2000 in Amsterdam, Netherlands during September and presented a poster.

Prof. Lise Korsten and Dr. Gina Swart attended the International Symposium on Tropical and Subtropical Fruits in Cairns, Australia, in November 2000 and two papers were presented.

Prof. Lise Korsten attended the Ninth International Society of Citriculture, Walt Disney World's Coronado Springs Resort in Orlando, Florida, USA, in December 2000.

Mr. Bernard Slippers visited Switzerland and Italy in September 2000 in order to collect fungal isolates from specific tree species growing in these two countries.

Dr. Teresa Coutinho and Ms. Marieka Venter attended the annual American Phytopathological Society Meeting in August. They also attended a Forest Pathology field trip for three days prior to the meeting.

Dr. Nico Labuschagne visited Australia on invitation and presented an overview of research on citrus rootstock resistance at the Elizabeth MacArthur Agricultural Institute in Sydney.

Ms. Bridgitta Steyn working in the laboratory of Dr. Thierry Jouenne, University of Rouen, France, for a three week period during March. She standardised a method for the proteomic analysis of bacterial biofilms as part of a collaborative project.

Prof. Terry Aveling attended the 3rd International Cowpea Conference in Ibadan, Nigeria, in September 2000. Two papers and two posters were presented.

Mr. Tendani Nevondo attended the Euro Summer School on Decentralized Sanitation and Re-use at Wageningen University in the Netherlands.

Ms. Ankia Wagner spent three months in the laboratories of Prof. L. Blackhall at the University of Queensland, Australia.

Prof. Eugene Cloete attended the first International Water Association (IWA) meeting in Paris where he presented papers and was elected to the IWA Management and Policy Council and re-elected as the Chairperson of the IWA Specialist Group on Biofouling and Biocorrosion. In this capacity he was furthermore involved in the arrangement of an International Conference on Exopolymer substances (EPS) held in Germany.
The Water Biotechnology Group focused their research on the passive treatment of acid mine drainage, microbial community diversity studies in activated sludge, and on solar pasteurisation of water supplies in rural areas. A new dimension was added with the three PhD students in Water Resource Management focusing on a multi-criteria decision making model for integrated catchment management in the one instance, a national strategy for monitoring the microbial quality of water supplies in the other and the third project revolving around the development of a national strategy for water supply in rural areas.

Significant advances have been made in the development of a passive treatment system for acid mine drainage. The group working on activated sludge population dynamics, indicated for the first time, that the exopolymers produced by bacteria in activated sludge contained phosphorus and could play a major role in biological phosphorus removal from waste water. Research on solar pasteurisation indicated that this technique could be used successfully for treating groundwater to meet potable standards, hence providing an ideal cost effective method for treating contaminated water in rural areas.
The MSc Water Resources Management Students

Virology and Molecular Biology

Research team
Prof. Louis Nel, Dr. Jacques Theron and Mr. Fanus Venter

Co-workers: GJ Viljoen, D H du Plessis, B Dungu, W Vosloo, J Bingham, CE Rupprecht

MSc students
Johnathan Keytel
Wanda Markotter
Livio Heath (degree completed)
D Rachidi
Jennie Erasmus (degree completed)
M Khalata
Anel Espach
Cecilia Stipinovich (degree completed)
Willem Hechter
V Gayo Ortiz (degree completed)
C Goosen
P Kara
D Benguric (degree completed)

PhD students
D Barthie
E van Marie-Koster
C Sabela
H van Rensburg (degree completed)
Amanda Bastos-Slager
J Jacobs
J Wentzel (degree completed)

The virology programme focuses on animal viruses that are of major concern in agriculture and conservation or as zoonotic agents in southern Africa and elsewhere. These investigations may be directed at:

- development or improvement of vaccines,
- issues of molecular epidemiology and spreading of disease,
- elucidation of molecular mechanisms of virus replication and morphogenesis.

Specific viruses or virus groups currently under investigation are:

- Rhabdoviruses (including Bovine Ephemeral Disease Virus and the Lyssaviruses which include rabies and mokola viruses),
- Orbiviruses (including BTV and EHDV),
- Capripoxviruses (specifically lumpy skin virus),
- Apthoviruses (foot and mouth disease virus),
- Bunyaviruses (specifically Rift Valley Fever Virus).

Other research projects of molecular biological nature included:

- Generation of a recombinant botulism vaccine;
- Improving detection methods and study of Legionella in industrial waters;
- Population genetics: Genetic diversity of domestic chicken and the domestic antelope species Damaliscus pygargus through microsatellite analysis and sequence analysis of DRB and MHC gene complexes.
- Molecular evaluation of transovarial transmission of Babesia bovis and bigemina.
- Generation of antigenic mimics of a Mycoplasma capricolum epitope towards an improved diagnostics for contagious caprine pleuropneumonia.

Biofilm Physiology

Research team
Prof. Volker Brözel and Dr. Jacques Theron

Postdoctoral fellow
Dr Marinda Oosthuizen

BSc honours students
Annette van Schalkwyk
Mare Pretorious
Sunette Malan

MSc students
Christopher Cooper
Marietjie Meiring

PhD students
Bridgitta Steyn
Boet Weyers
Raynard MacDonald

Dr. J. Theron
The biofilm physiology group is studying mechanisms involved in the broad range of phenotypic alterations of cells upon attachment to non-living surfaces. The model organisms used are *Pseudomonas aeruginosa* because it is currently the best understood of the biofilm-forming bacteria, and *Bacillus cereus* due to its relevance to food and dairy processing. Furthermore we also study biofilms in their industrial context, focusing both on industrial waters and biofilms in food processing systems.

The program is driven along three foci with the goal of developing an improved understanding of the biofilm modulon, transmembrane signalling and regulatory mechanisms involved in various stages of biofilm development.

i) The phenotypical changes occurring directly following attachment and during development of mature biofilms are characterized by studying biofilm proteomes by 2-D electrophoresis of whole-cell proteins. This is performed in collaboration with Dr Thierry Jouenne, University of Rouen, France and Prof A. van Holy, University of the Witwatersrand.

ii) The regulation of the biofilm phenotype of *P. aeruginosa* is under intense investigation from two angles. Firstly, a number of biofilm-specific regulatory elements of *P. aeruginosa* isolated previously are being studied. Secondly, the role of various extra-exponential sigma factors in biofilm development is under investigation. We have also begun to search for biofilm-regulated genes of *B. cereus* by transposon mutagenesis.

iii) In a collaborative project with Dr A. van Holy at the University of the Witwatersrand we have recently found that *Bacillus cereus* isolated from dairy processing systems are extremely alkaline tolerant. They survive at pH 12 by forming biofilms and decreasing the surrounding pH before reverting to planktonic mode of growth. This is investigated from both a molecular and physiological angle.

### Biotechnology

**Research team**
Prof Volker Brözel and Dr Jacques Theron

**MSc students**
Francois du Plessis
Jacques Smith

**PhD student**
Joseph Hawumba

**Prof. V. Brözel**

The biotechnology group is working towards the production of proteases and biosurfactants using biofilm-grown bacteria. The expression of two enzymes in *P. aeruginosa* biofilms, viz. an alkaline protease and rhamnosyl transferase is
currently under investigation. Secondly a number of thermophilic *Bacillus* from Ugandan hot springs are being investigated for their potential to produce thermo-stable and cold-active alkaline proteases.

**Citrus Diseases**

**Research Team:**
Prof. Lise Korsten and Dr. Gina Swart

**Postdoctoral fellow**
Dr. Linda Swart

**MSc students**
E Auret  
M Juckers

**PhD student**
Joseph Obagwa

The integrated disease control program has been extremely successful and has resulted in the semi-commercial evaluation of new biocontrol products. These biocontrol products will be tested on a commercial scale during 2001 and 2002 and results will be used for product registration. If successful, these products will be commercialised and distributed by Anchor Yeast, SA. The successful evaluation of biocontrol products has led to the development of a new innovation fund application focusing on developing organic production systems for South African agriculturalists. The organic market has grown globally beyond all expectations and it is now anticipated that producers delivering an organic product can demand premium prices internationally. The organic production of fruit and vegetables is a particularly lucrative market for small scale farmers who can easily implement organic production systems and utilise biocontrol strategies. Through this citrus integrated project we have now developed expertise in alternative disease control systems for small scale and commercial growers that will ensure a specific niche market for SA fresh produce.

**In vitro and in vivo techniques** were used to screen natural epiphytes for antagonism against pre- and postharvest pathogens. Studies of mode of action of antagonists against pre- and postharvest citrus pathogens including *Guignardia citricarpa*, *Alternaria alternata* and *Penicillium* spp. are currently still in progress. Studies will continue during 2001 and will include in vitro and in vivo mode of action studies and fruit colonisation work using the antagonist. Extraction of inhibitory substances from antagonists and characterisation in collaboration with the Depts of Chemistry and Biochemistry are in progress.

**Alternative natural plant products:**
Plant extractions from indigenous bulbous plants was initiated during 2000. Five indigenous bulbous plant spp were selected and natural inhibitory plant extracts obtained that inhibited in vitro growth of citrus pathogens. During 2001 we will continue to evaluate plant extracts for in vivo control of citrus pathogens. The extracts will also be identified and characterised.
Bioactive and Biocure products:
The different Bioactive coatings were prepared by suspending the different yeasts in a solution of chitosan dissolved in acetic acid containing CaCl$_2$ $10^8$ cfu.ml$^{-1}$. The bioactive and biocure products have now been formulated in commercial products and are being tested on a semi-commercial scale.

Packhouse experiments:
Packhouse trials will continue on a semi-commercial scale at three production areas throughout the season. Different antagonistic yeasts, bacteria and new natural plant products will be tested on line. Simulated export conditions will be used and fruit will be evaluated as described by De Villiers et al. (1998). Antagonist survival will be monitored over time as well as mode of action of the biocontrol agents.

Citrus black spot control
*Guignardia citricarpa* isolates were obtained from different citrus production areas in SA. Isolates were compared phylogenetically using RAPD and RFLP techniques. In this study it was found that avirulent and virulent isolates can be distinguished.

Greenhouse studies are still in progress. Young, mature leaves and litter from an orchard with a history of high incidence of black spot, and where no chemicals have previously been sprayed (Letaba Estate) were collected. Leaves and litter were placed on soil in asbestos experimental trays. Samples were kept moist throughout the duration of the experiment. Five selected antagonists were applied weekly over litter. The experiment included relevant controls and enough replicates. Litter degradation and pathogen inoculum development on leaves was visually monitored.

A commercial antagonist formulation (B. subtilis) was mixed with citrus peel compost and applied to soils under tree canopies. Additional antagonist on its own or mixed with compost have also been tested successfully in field applications.

Orchards with a history of a high incidence of black spot disease were used. Antagonist survival are being monitored over time using the ELISA (monoclonal antibodies have previously been developed in our laboratory specifically for the antagonistic B. subtilis isolate). Disease development are being monitored over a three year period - up to 2001.

Bee dissemination experiment
Bees were used to disseminate natural antagonists to citrus flowers for control of Alternaria. This project was initiated during 1998. An MInstAgrar student, Mr M P Mphahlele has already completed all aspects of this project.

Complete field trial. Two field experiments were set up in orchards with a history of high Alternaria infections - La Motte farm, Northern Province (Minneola orchard) and Croc Valley, Mpumalanga (Navel orchard). Bees were marked with special paint to monitor effective antagonist dissemination and determine the range of foraging. Effective deposition of antagonists on flowers was determined. Fruit development, number and size as well as disease incidence were successfully monitored. The experiment was repeated over three years.

Postharvest diseases
Semi-commercial packhouse experiments were done with existing local and overseas commercial biocontrol products (also include second generation antagonists in all experiments). Different application methods such as dip and ULV, combining antagonists with natural wax and applying it ULV, integrate biocontrol with other control options such as reduced chemical concentrations; hot water and disinfectant combinations were evaluated.

Citrus greening
Greening is caused by a highly fastidious, Gram negative phloem-limited bacterium. Based on 16S rDNA sequence data, two distinct species were identified viz. Liberibacter
africanus and Liberibacter asiaticus. Due to dual infections and cultivar differences it is difficult to identify this disease based on symptomology alone. The objectives of this study were to clarify symptomology, use PCR to answer epidemiological questions, detect Liberibacter in alternative hosts and citrus endophytes. Four hundred and fifty samples of greening infected and healthy citrus from different cultivars and 92 samples from potential alternative hosts (botanical gardens/areas adjacent to greening infected orchards) were collected. A range of previously isolated citrus endophytes were also tested. DNA extractions done using Wizard DNA purification kit using primers directed to the rplKAJL-rpoBC operon, which produced an amplicon of 667 bp (L.africanus), and PCR conditions conducted according to previously described protocols. Asymptomatic samples from healthy sectors often tested positive and all samples with typical greening symptoms tested positive and all healthy trees tested negative. None of the alternative hosts tested positive except for Calodendrum capense from Stellenbosch. Thusfar only C. capense from Stellenbosch tested positive using new Cal 1 primers (specific to Liberobacter africanum subspecies capensis). No positive reactions were obtained from cryopreserved endophytes tested thusfar. Mottling followed by yellow vein symptoms were found to be the most reliable indicators of greening (P=0.001). Green islands, rabbit ears and mineral deficiencies were the least reliable (P=0.0012). There were differences in the number of positive reactions from different cultivars, with Valencia (most susceptible) having the most and Satsuma, the least. These results are in accordance with previous findings of varietal susceptibility. There were also different levels of positive reactions from specific areas, with the most from Croc Valley followed by Letaba.

A second project entails the monitoring of psylla populations at a greening infected orchard in Mooinooi. Bimonthly visits were made and psylla traps replaced. This continues until March 2001 when populations will determined and incidence of greening infected psylla determined by PCR.

### Mango Diseases

**Research team**
Prof. Lise Korsten and Dr. Gina Swart

**MSc student**
N Gantsho  
Rene Scherman

**MinstAgrar student**
M Silimela

**PhD student**
Marius Boshoff

The Department of Microbiology and Plant Pathology has been involved with mango research since the early 1970s. However, it was only until June 2000 that the Department through FABI formed a formal alliance with Du Roi and SAMGA (South African Mango Growers’ Association) to
manage the industry's total mango pathology research budget. Through this alliance major focus areas have been identified and include:

Epidemiology of soft brown rot
Under this project theme, the taxonomic status of the pathogen previously known as *Nattrassia mangiferae* is being investigated through morphological and molecular comparative studies. The *Botryosphaeria* anamorph of mango is being studied by René Scherman in close collaboration with Bernard Slippers. The endophytic nature of the pathogen is also being investigated as well as its relationship with sudden tree dieback and the blossom malformation complex. An extensive collection of over 300 isolates have been collected from various hosts and particularly soft brown rot infections obtained during a market survey the previous year. Thus far the endophytic status of the organism has been confirmed in nursery trees and mature healthy trees and trees showing dieback symptoms. The role of other fungi in the dieback syndrome is further being investigated as well as the distribution of the pathogen in healthy and stressed orchards with a recorded history of high and low incidences of soft brown rot. Phylogenetic studies of the pathogen continue. These studies are crucial for the clarification of the taxonomic status of the pathogen and will have an international impact on mango research.

Copper resistance in *Xanthomonas pv. mangiferaeindicae*
Studies of the genetic diversity of the mango black spot pathogen, *Xanthomonas pv. mangiferaeindicae* was initiated during 1999 and has since focused on the build-up of copper resistance in the population. Orchards with a known history of mango black spot which follows an extensive copper spray program and no spray program has been compared in terms of copper resistance being expressed in the pathogen population. This is the first study where copper resistance in this pathogen has been recorded and will undoubtedly cause major international concern. Understanding virulence and copper resistance, which seems to be linked is currently being investigated. This project formed part of Ms N. Gantsho's MSc studies, and is being continued by Dr G. Swart.

Studies of *Colletotrichum gloeosporioides* studies on mango
A market survey was conducted to determine the incidence of selected post-harvest diseases in mango fruit from major production areas in South Africa. Isolates of *Colletotrichum gloeosporioides* collected during a three-year survey were compared in terms of virulence, fungicide resistance, morphology, physiology, soluble proteins and molecular characteristics. The study revealed the following: The incidence of SE, anthracnose and soft brown rot (SBR) on Sensation mango fruit varied for all production regions evaluated, and was higher in 1996 than 1995. *C. gloeosporioides* could be isolated readily throughout both seasons, with the highest isolation frequency from the Hazyview areas. *C. gloeosporioides* isolates collected during the market survey could be categorised according to lesion size in fruit inoculation studies. All mango isolates produced larger lesions when inoculated onto their own hosts and produced lesions on strawberries, peppers, guavas and papayas, but not on citrus. A total of 17.7 % of all *C. gloeosporioides* isolates tested in vitro were resistant to benomyl, half of them highly resistant and the other half, moderately resistant. No isolates showed resistance to thiabendazole or prochloraz. Morphologically, South African isolates of *C. gloeosporioides* were as variable as isolates from over the world. However, isolates from mango were generally less diverse than those from avocado. No correlation was found between the length:width ratio of conidia and virulence of isolates when inoculated into avocado and mango. Significant differences were found between growth response at 15, 25 and 37 °C and geographic origin of isolates. *C. gloeosporioides* isolates from avocado, mango, citrus, papaya and overseas
sources were closely related in terms of soluble proteins. At 60% similarity, isolates could be grouped into two clusters, but no distinction could be made between avocado or mango isolates or geographic origin of these isolates. PCR-RFLP could not distinguish test isolates of *C. gloeosporioides*. RAPD analysis separated isolates clearly and was used for further comparisons. The OPC02 primer proved to be the most discriminatory, and could distinguish isolates according to host and symptoms from which isolations were made. Differences were also observed when representative band profiles from different hosts, viz. avocado, mango, papaya and citrus, were compared. Sequence analysis showed that mango and citrus isolates were the most closely related, with papaya isolates being more distant, but closer to isolates of mango and citrus than avocado. This study formed part of Ms G. Swart’s PhD studies.

**Biological control**

Preharvest biological control trials have been established during 2000 and both pre- and postharvest diseases will be evaluated during January, February 2001. The preharvest trials focuses on evaluating a previously developed biocontrol agent, *Bacillus licheniformis* (emanating from our biocontrol program). The antagonist is applied on its own or in combination with currently used fungicides. Three different strategies have been evaluated: preharvest sprays during flowering to control the endophytic soft brown rot pathogen and during fruit development to control preharvest black spot or anthracnose or postharvest anthracnose. Application of the antagonist or copper by means of sun shield and plastic caps with a woolly base is another innovative approach to disease control evaluated in this study. The sun protective effect linked to disease control by means of a "slow release" action through treated caps is being evaluated to control mango black spot, anthracnose and soft brown rot. The cap treatment concept has been patented. The study forms part of Mr M. Silimela’s MInstAgrar studies.

The FABI - Du Roi - SAMGA alliance has been extremely successful with Dr Fanus Swart (Du Roi) being responsible for grower linkage and field trials.

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**Postharvest Pathology & Biological Control**

**Research team**
Prof. Lise Korsten, Dr. Gina Swart and Ms. Ida Paul

<table>
<thead>
<tr>
<th>MSc students</th>
<th>MinstAgrar students</th>
<th>PhD students</th>
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<tbody>
<tr>
<td>Wilma Havenga</td>
<td>M Mphahlele</td>
<td>Elizabeth de Jager</td>
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<td>N Schoeman</td>
<td>Joseph Obagwu</td>
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<td>Karin Louw</td>
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The postharvest pathology and biological control research groups have focused mainly on developing new biocontrol products for control of postharvest diseases. Current biocontrol agents such as *Bacillus subtilis* have now been registered for avocado Cercospora spot control. This is the first biocontrol agent for preharvest applications to control fruit diseases that has been registered in South Africa. The product Avogreen is
currently being marketed by Ocean Agriculture. The antagonist's mode of action has been studied by Ms W. Havenga as part of her MSc studies.

Karin Louw started her MInstAgrar on isolating natural plant products from indigenous bulbous plants that can control plant diseases. The project in collaboration with Prof Marion Meyer at Dept of Botany and Dr Thierry Regnier from FABI has been very successful. Several inhibitory plant extracts are currently being identified/characterised. The eventual use of these natural plant extracts in postharvest biological control programs will be further tested during 2001.

Microbial populations on litchi fruit surfaces have been studied during 2000, from harvest to marketing. Effect of different chemical treatments on the fruit microflora have been investigated. Food safety aspects have been monitored and a HACCP program developed for the litchi industry. Twenty litchi packhouses were monitored for hygiene standards, foodborne pathogens and critical control points established. Several important food borne pathogens have been identified from either the fruit or packhouse surfaces. Various chemical and biological products have been evaluated for postharvest disease control. This study is the first of its kind in South Africa focusing almost exclusively on food safety issues. This study formed part of Ms E. de Jager's PhD.

Microbial populations on litchi fruit surfaces have been studied during 2000, from harvest to marketing. Effect of different chemical treatments on the fruit microflora have been investigated. Food safety aspects have been monitored and a HACCP program developed for the litchi industry. Twenty litchi packhouses were monitored for hygiene standards, foodborne pathogens and critical control points established. Several important food borne pathogens have been identified from either the fruit or packhouse surfaces. Various chemical and biological products have been evaluated for postharvest disease control. This study is the first of its kind in South Africa focusing almost exclusively on food safety issues. This study formed part of Ms E. de Jager's PhD.

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**Potato Diseases**

**Research team**
Prof. Lise Korsten

MSc student        MInstAgrar student        PhD student
Allan Hall          Kgabo Matlala            Jacquie van der Waals
Wilma van Broekhuizen

Potato research has traditionally formed a small project within our department and was established on request by the South African potato industry. The research focused on three major projects:

**Disease forecasting of early blight**
This project focuses on the epidemiology of early blight and the development of an early blight forecasting system. Ms Jacquie van der Waals is currently doing her PhD study on this project.

Disease development of early blight can be affected by several extrinsic factors including weather conditions and N-fertilisation. These and other factors make early blight more difficult and complex to manage. A disease prediction model, FAST (Forecast of *A. solani* on Tomato) was developed as a predictive system for initiating and timing fungicide spray in Pennsylvania, USA. Four environmental parameters (leaf wetness, air temperature, relative humidity and rainfall) combinations are used to depict the relationship between *A. solani* and its micro-environment and to determine periods when environmental conditions are favourable for early blight disease development. Modified versions of the original FAST models are currently being used to time fungicide sprays and subsequently control early blight of potatoes in Wisconsin and Pennsylvania, USA. These models are not designed for South African potato growing conditions or cultivars and should be modified to suit South African conditions. The development
of a disease forecaster will minimise excessive use of fungicides while maintaining disease control and yield. In addition, environmentally sound alternatives to standard chemicals will be replaced with biocontrol products and other products, which are generally regarded as safe.

**Soft rot Erwinia**
The soft rot project was finalised during 2000 with Kgabo Matlala completing his MInstAgrar. In this study, Erwinia isolates were compared using PAGE, ERIC and BOX-PCR and standard physiological tests. A new rapid isolation technique was evaluated. Disease control measures using disinfectants were evaluated and cultivar resistance tested.

**Ralstonia wilt**
Ralstonia isolates collected from various production areas are being compared using different molecular techniques. Survival of the pathogen in soil and in crop rotation systems are further evaluated. Mr A. Hall and Ms W. van Broekhuizen are currently completing their MSc studies on this project.

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## Cowpea Diseases

**Research team**
Prof. Terry Aveling

**MSc students**
Quenton Kritzinger
Noelani van den Berg
Tessa Bandounas
Appollinaire Adandonon
Luisa Tombolane
Buks Henning

**PhD student**
Yolisa Pakela

The focus of this research is on the pathogens of southern African indigenous food crops. We concentrate on those crops grown by subsistence farmers in areas of low agricultural potential. To date most research has been done on cowpeas which is a drought resistant crop that grows well on poor soils. Cowpea seeds provide an important protein food source. Mycotoxin studies and identification of storage fungi of cowpeas are underway in collaboration with the MRC and Prof W.F.O. Marasas.

The aim of our programme is to improve the quality of indigenous food crops by:

- Surveying southern Africa for plant pathogens on indigenous food crops
- Collecting, isolating and identifying those pathogens reducing yields of indigenous crops
- Studying the biology and seed transmission and mycotoxins of these pathogens
- Collecting epidemiological information on pathogen survival, infection and spread
- Studying alternative control measures both in the field and during seed storage
- Training and educating students with the relevant expertise to do extension work in disadvantaged communities
Panama Wilt of Bananas

Research team
Dr. Altus Viljoen

B.Sc. honours students
Christine Burgess
Karen Surridge

MSc students
Edzard Grimbeek
Tessa Bandounas

PhD students
Marinda Visser
Anita Severn-Ellis

Fusarium wilt (Panama disease) of banana, caused by the soil-borne fungus Fusarium oxysporum f.sp. cubense (Foc), is the most important disease of bananas in South Africa. During 2000, the disease was discovered for the first time in two new banana growing areas, Tzaneen and Komatipoort. The epidemiology and ways of spread of this disease were, therefore, studied in detail. Isolations were made from water, soil, and possible alternative hosts, and the effect of fertilization, horticultural practices and temperature on disease development was investigated. The local population was analyzed by means of genetic (vegetative compatibility and mating type) and molecular tools (sequencing of the β-tubulin gene, δ-elongation factor and regions 5 and 7 of the mitochondrial DNA). Furthermore, a green fluorescent protein (GFP) gene was cloned into several isolates of the fungus. A greenhouse inoculation technique in a hydroponics system was develop to do preliminary studies on the infection process and control of Foc. Isolates of Foc were also screened for mycoviruses, and several field trials were conducted to investigate the effect of chemicals inducing systemically acquired resistance in plants, and the effect of nutrition on the development of disease.

Tree Pathology

Research team
Prof. Mike Wingfield and Dr. Teresa Coutinho

BSc honours students
Hein van Greuns
Charity Ramasodi

MSc students
Busisewe Tshabalala
Irene Barnes
Gavin Hunter
Riana Jacobs

Prof. M. Wingfield
Research conducted on tree diseases falls under the umbrella of the Tree Pathology Co-operative Programme. Staff and postgraduate students work on economically important diseases of mainly eucalypts, pine and wattle. Research topics include population diversity of fungal pathogens, mycoviruses, fungal taxonomy, phylogeny of fungal pathogens and diagnostics.

Some of the goals/highlights achieved in this programme during 2000 include the following:

Canker caused by *Endothia gyrosa* was discovered in South Africa in the early 1990's, although it might have been present in the country for much longer. The pathogen is well known in Australia on eucalypts and it was thought to be the same as that causing stem cankers on a wide range of trees in the United States. Our view has been that this fungus is rather different to the fungus in the United States and this impacts both on control strategies and on issues pertaining to quarantine. After intensive study, we have concluded that this fungus is a species of *Cryphonectria*, which makes it closely related to the Chestnut Blight pathogen. The fungus is the same as that occurring in Australia and we will refer to both as *Cryphonectria eucalypti* in the future. This fungus is very different to the American fungus known as *E. gyrosa*, but it remains an important pathogen of eucalypts, which is now being subjected to intensive pathogenicity tests.

The shoot tip and canker pathogen, *Sphaeropsis sapinea*, remains one of the most important causes of loss in pine plantations. Some findings relating to this fungus in recent years have been intriguing and have been discussed in previous reports. Perhaps most significant have been data to suggest that *S. sapinea* has been introduced into South Africa many hundreds if not thousands of times. Some data in support of this hypothesis have been presented in previous reports and these will not be repeated here. If *S. sapinea* has indeed been introduced into South Africa many times, this has serious implications for quarantine as well as for resistance breeding. Our initial findings have been based on so-called vegetative compatibility tests, which are reasonably reliable. However, what is needed to be certain of these results are molecular markers that are sufficiently robust to allow for population genetics comparisons. We have thus prepared such molecular markers in the form of microsatellite markers. Using these we will now be able to test our hypothesis regarding the origin of *S. sapinea* in Southern Hemisphere plantations.

One potential strategy to reduce the impact of *S. sapinea* in South Africa is through the deployment of dsRNA viruses that reduce the virulence of this fungus. We are actively pursuing this opportunity. However, various steps in the process are necessary. A key factor is to test currently occurring isolates for the presence of these factors. This is a time consuming and difficult task due to high numbers of isolates, and a complex technique. We have, however, now completed this study and found a wide range of dsRNA
elements in isolates. These must still be characterised, but none appear to result is obvious hypovirulence. One source of hypovirulence in fungi is the hypovirus of *Cryphonectria parasitica*. Unfortunately, this virus has been characterised by a group in the United States and its use in South Africa would be restricted by patents. We have thus been seeking alternative sources of hypovirulence and believe that we have found such a source in an important canker pathogen of pome and stone fruits known as *Diaporthe ambigua*. We have thus recently completed the characterization of the dsRNA virus in *D. ambigua* and work is currently underway to transform this into *S. sapinea* and other pathogens of interest to South African forestry. The worldwide concern regarding pitch canker is growing steadily. This is primarily amongst forestry companies growing *Pinus radiata* or *Pinus patula* that are known to be amongst the most susceptible species. South Africa remains in a unique position of having the pathogen well established in the country, but as yet not on mature trees. Of very significant concern in recent times has been emerging evidence to suggest that the pitch canker fungus has moved to plantations. Team members of this programme have thus spent considerable time in plantations examining apparent outbreaks of the pitch canker pathogen. This is a relatively difficult task given the fact that isolation of *Fusarium* spp. and particularly identification to the species level is beset with problems. Nonetheless, at this point we are convinced that the fungus is causing very significant problems in newly planted stands. There is also good evidence to suggest that it has developed an association with insects such as the root feeding bark beetle, *Hylastes*. During the coming year, a student will begin to work on this question and this study will be conducted in concert with the industry-established working group on pitch canker. It is hoped that a field forester might also become involved in this project such that field evaluations of loss can be made.

Until recently, we have believed that *Phytophthora cinnamomi* is the most important species of *Phytophthora* killing young cold tolerant *Eucalyptus* in South Africa. The Programme continues its ongoing research on Phytophthora root rot, which is now focussed on field avoidance and selection of disease tolerant planting stock. An intriguing discovery that has formed part of this process of investigation is that *P. nicotianae* appears to be the more important cause of the disease in South Africa. This discovery will have an impact on previous work to select disease tolerant trees, and more intensive field work will now be needed to extend our understanding of this interesting situation. The Programme has made significant progress in gaining knowledge regarding Botryosphaeria canker. There is, however, much to learn and many unanswered questions remain. Symptoms are variable on different hosts and we have long believed that more than one species of Botryosphaeria causes cankers in South Africa. Resolving this issue has been particularly difficult due to the fact that the taxonomy of this group of fungi is confused at best. During the course of the past year, we have conclusively shown that two species of Botryosphaeria are associated with cankers on eucalypts in South Africa. For the present, the one fungus retains the name that we have used for many years, ie *B. dothidea*. The second fungus will henceforth be known as *Botryosphaeria eucalyptorum*. Recognising these distinct taxa of eucalypts in South Africa will now enable us to evaluate their relative importance and to treat them with the separate status that will make breeding and selection efforts more logical.

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Hydroponic Pathology & Resistance of Citrus Rootstocks to Root Pathogens
Research team
Dr. Nico Labuschagne

Research assistant
Roger Bagnal

B.Sc. honours students
Andries Fourie

MSc students
Fanie Verwey
Cornelia Gull
Christie Burger

Hydroponic Pathology
This program has been focusing on the etiology and control of *Pythium* wilt and root rot in hydroponic crops. One of the major discoveries of this study has been evidence of the important role of temperature and allelopathic stress in *Pythium* wilt of lettuce. Seven *Pythium* spp have been isolated from various hydroponic crops and evaluated for their pathogenicity. *Pythium spinosum* and *Pythium* type F were the most pathogenic spp on lettuce. A range of disease control measures were evaluated and a disease management strategy designed for control of *Pythium* in hydroponic systems. In future, the focus will be on biological control measures.

Resistance of citrus rootstocks against root pathogens
At the present time this study is focusing on biochemical mechanisms involved in rootstock resistance. Several phytoalexins are being implicated as contributors to resistance. A rapid bioassay technique has been developed to screen citrus roots for phytoalexin activity.

Emerging Pathogens

Research team
Mr. Fanus Venter, Dr. Jacques Theron and Prof. Volker Brözel

BSc honours students
Wilma Penzhorn
L Paulsen
Elaine Wolmarans

MSc students
Sonya Diergaardt
A growing number of emerging human pathogens are suspected of being transmitted by water. The focus of our study was *Campylobacter* and the microsporidia. The project entails the detection and quantification of these emerging pathogens in drinking, surface and treated water in four regions in South Africa. Collaborators include the University of the Western Cape, Umgeni and Rand Water Boards and the Free State Technikon.

**Rhizobium Research**

**Research team**
Prof. P.L. Steyn and Mr. Julian Jaftha

**MSc students**
Carinne Joubert

**PhD students**
Julian Jaftha
Marleen Kock

The main objective of this study is to increase our understanding of biological nitrogen fixation, focussing particularly on the diversity of rhizobia nodulating various leguminous plant species. To establish a true identity of all putative rhizobial isolates, morphological, genetic and phylogenetic traits are analysed. These results showed that most of the indigenous strains had two major generic affiliations: the first group being related to the slow-growing *Bradyrhizobium* genus, while the second was related to the fast-growing *Mesorhizobium* genus. Representatives of the other rhizobial genera were also found, but to a lesser extent. Isolates, showing no apparent relatedness to the rhizobial genera were also obtained. Detailed analyses of one such group indicated their ability to grow facultatively on methanol, a trait uncommon to rhizobia. This was illustrative of the extent of the diversity of symbiotic nitrogen fixating bacteria.

More recently, the existing rhizobia culture was extended with the inclusion of isolates from exotic wattle species and *Cyclopia* (honeybush). Wattle species are speculated to be promiscuously nodulated, however, initial results from our group indicate the contrary.
Publications


SASPP Congress. 23-28 January 2000. Rhodes University, Grahamstown. [poster]


Faculty of Agriculture, University of Aberdeen, Scotland. [Invited guest speaker]


Lindsay, D., Brözel, V.S. & Von Holy, A. (2000) Cytotoxicity and buffering capacity of an alkaline tolerant *Bacillus* isolate. 87th annual meeting of the International Association for Food Protection, 6 – 9 August 2000, Atlanta, Georgia, USA.


Old Faithfull hot spring erupting at Yellowstone National Park, USA

Marinda Oosthuizen, Boet Weyers, Volker Brözel, Raynard MacDonald, Chris Cooper and Marietjie Meiring relaxing after the Biofilms 2000 conference near Big Sky, Montana, USA. General Steyn is absent, having been ordered to take the photograph.
**Dr Nico Labuschagne**

Nico is married to an IT specialist and has one daughter and two step-daughters.

**Interests & Hobbies:**  
- Birdwatching (the type with real feathers!)  
- Mountain biking  
- Trout fishing  
- Backpacking (hiking)

Nico, therefore, has an insatiable love for nature, but apart from that reads whenever there is a minute to spare - on topics such as biographies, motivational & semi-psychological stuff and global trends.

**Favourite Actor and actress:** Anthony Hopkins; Gwyneth Paltrow

**Favourite quote:** I measure “success” in life by the degree to which one enjoys peace, love and health.

**Biggest problem:** Interested in too many things, and having to little time to explore it all!

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**Mr. Fanus Venter**

Fanus is married to a paediatrician and has a son and a daughter.

**Interests & Hobbies:**  
- Reading  
- Music  
- Weaving

Fanus loves to read fiction, both in English and Afrikaans. Recently read authors: Garbiel Garcia Marquez, André P. Brink and of course all the Harry Potter books by J.K. Rowling.

**Favourite actress:** Cathrine Deneuve

**Favourite quote:** “Dit is makliker om ekskuus te vra as toestemming”

**Biggest problem:** TIME!

---

**Ms. Kerien van Dyk**

Kerien is engaged to Andrew, an area manager in the footwear industry, and has a boerboel, great dane x Labrador and two moggies.

**Interests & Hobbies:** Outdoor activities such as game watching, camping, boating, reading and target shooting
Kerien’s first love is definitely nature, especially animals. Her mother has an exceptional gift of working with animals and she aspires to be like her. She also likes to read and her favourite books are psychological thrillers and great mysteries such as the pyramids.

Favourite actor/actress: Anthony Hopkins and Julia Roberts

Favourite quote: A smooth sea never made a skilful mariner

Biggest problem: Wanting to learn and to do many more things but lacking the time

Congratulations

To Annegret and Volker Brözel on the birth of their second son Nicholas Marc on the 9th of March 2000.

To Fanus and Izelle Venter on the birth of their second child Christelle on the 9th of July 2000.

To Madelien and Jandré du Preez on the birth of their first child, Chanelle, 2nd of December 2000.

To Colette and Johan Cronje on the birth of their first child, Simone on 26th of October 2000.

To Prof. Eugene Cloete and his wife, Heloïse, with their daughter, Marguerite, who passed matric with six distinctions. Marguerite intends studying graphic design at TUKS in 2001.
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**Extraordinary Professors**

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Prof. R.M. Atlas (USA)  
Prof. HansCurt Flemming (Germany)  
Prof. P. Jooste (ARC)  
Prof. P.C. Steyn  
Prof. B. Strydom