# Southern African Society for Plant Pathology

The following are selected abstracts of keynote addresses, oral papers and posters presented at the 44th Annual Congress of the Southern African Society for Plant Pathology held at Magalies Park Country Club, Gauteng, 22–25 January 2006.

## Keynote Addresses

### Recent advances and novel concepts in managing Sclerotinia diseases on sunflower and other crops

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*Sclerotinia sclerotiorum,* with a host range of more than 500 dicot genera and its long-lived sclerotia, is a pathogen that is becoming a major concern on many crops worldwide. Total immunity has not been found in any host, nor have fungicides proved an economical, effective control. *Sclerotinia* is thus a poster-child example of the need for an integrated package of genetic, chemical, biological and cultural controls. The impact of *Sclerotinia* upon U.S. agriculture is evidenced by the creation of a federally supported 'Sclerotinia Initiative,' which currently funds 30 research projects on five major *Sclerotinia* hosts in the U.S. (www.whitemoldresearch.com). Advances made in the arena of genetic resistance within the last decade, on sunflower or other crops, include improved and mechanized field inoculation methods, marker-assisted selection, and introgression of genes from wild *Helianthus.* Sunflower is an unusual host in that it is prone to both head rot and to stalk rot, and since resistance to each phase is independent, this doubles the breeding efforts. The most resistant breeding lines and commercial hybrids currently exhibit as low as 10–15% head rot or stalk rot compared to 90–100% on susceptible material. While transgenic resistance, via the *ox-ox* gene, has been incorporated into sunflower, this gene is currently not used in commercial hybrids. Near immunity to stalk rot is observed in most perennial *Helianthus* species, and less so in annual species. Chemical control of sunflower head rot is minimally successful, in contrast to some other hosts, while chemical control of stalk rot is impractical. In crops, such as canola, where fungicides are effective, disease forecasting, aided by web-based information (www.northerncanola.com/maps/index.asp), is helping growers economically to control *Sclerotinia.* Research to determine the effect of root exudates on sclerotial germination and varietal susceptibility is under way. Commercial biocontrol products, principally *Coniothyrium minitans,* have been registered worldwide, and do aid in shortening rotations, but must be used as a curative treatment. While crop rotation is a passive management tool, current research suggests a profound effect, based not only on *Sclerotinia* susceptibility but also on sclerotial production by the previous crop. A more novel approach to *Sclerotinia* management is biofumigation, in which a cover crop (most often a *Brassica* species) is incorporated into the soil and allowed to release isothiocyanates that kill *Sclerotinia.* With the current emphasis on minimum or no tillage, research is under way to determine which tillage system maximizes sclerotial degradation and minimizes disease in succeeding crops. Another novel approach is to use an early-planted cereal crop to induce apothecial development. This cover crop is turned under or killed, and a susceptible crop is planted, with a greatly decreased inoculum potential. In summary, a wide range of diverse research projects are under way, on sunflower and other hosts, which, when combined, should ultimately keep losses from *Sclerotinia* to a minimum.

### Virulence dissociation and pyramiding genes for resistance to *Puccinia helianthi* in sunflower

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The rust fungus, *Puccinia helianthi* Schw., is a serious pathogen of sunflower in Australia. It survives on volunteer plants and wild sunflower populations scattered throughout the eastern and western seaboards, completing the sexual phase of the life-cycle in favourable seasons, and thereby generating diverse pathotypes. To date, 77 pathotypes have been identified which combine avirulence for more than 25 unique resistance genes. Initially, commercial hybrids in Australia generally deployed single resistance genes, which were easily overcome by pathotypes already present in the 'wild' pathogen population or by pathotypes that were introduced. Non-strategic attempts at combining resistance genes simply led to the selection of complex recombinant pathotypes with a corresponding proliferation of virulent pathotypes in the *P. helianthi* population. We attempted a strategic approach to combining resistance genes to confer durable resistance to sunflower rust, by identifying specific resistance gene combinations for which there were observed virulence dissociations in the rust population. Virulence dissociations were identified from 30 years of pathotype data where certain virulence combinations had never been observed. Resistance genes corresponding to several of these dissociations were combined as two-gene pyramids and inbred to fix both genes in a single pedigree. DNA markers were identified for specific resistance genes and were used to select double-resistant individuals at each cycle of selection. Gene pyramids can consist of defeated genes as well as durable and or novel undefeated resistance genes. The life of undefeated resistance genes may be prolonged by exposing them in combination with other genes rather than by themselves. Two such pyramids include the crosses HAR2 // MC29 ($R_5$ // $R_2$ locus genes) and $P_1$ // $P_2$ (putative $R_{4i}$ // $R_{4j}$). Although the genes in HAR2, $P_1$ and $P_2$ are susceptible to known pathotypes, positive dissociations have been identified for these specific combinations. The putative genes $R_{4i}$ and $R_{4j}$ are linked in repulsion at <10 cM, so a coupling linked recombinant was selected using both traditional linkage experiments and DNA markers. The genes in HAR2 and MC29 are independent. The power of pyramiding is amplified in hybrid crops where individual parents can carry multiple resistance genes that can then be combined in the resulting hybrid. The pyramid lines HAR2 // MC29 and $P_1$ // $P_2$ are currently being introgressed with elite male and female lines owned by Pacific Seeds Pty Ltd.

### Trends in plant disease management research

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Trends have been derived from over 150 manuscripts submitted to me, as principal editor, for consideration for publication in the journal *Crop Protection,* over the past 18 months. *Crop Protection* emphasizes research on the more practical applications of disease management in greenhouse and/or field situations. The manuscripts originated from many geographical areas covering most continents, but as it is a European-based periodical, more manuscripts came from Europe than any other continent, with Africa way down the list. Of the main topics, fungicides as tools for disease management dominated, closely followed by alternative compounds and novel methods of disease control and biocontrol. Cultivar resistance often formed part of the methods employed to reduce disease levels. It was gratifying to see that to manage diseases effectively, epidemiology studies still played a vital role. Many of the above topics were integrated on crops to present a comparison of standard and newer methods or compounds. Others presented ways in which different methods could be used complementarily either to enhance overall efficacy or to keep pesticide residues to acceptable limits. There were several studies concerned with boosting plant growth and resistance as a means of disease management, others tested accepted cultural practices for disease management under experimental conditions, often with surprising results. The role of biocomposts for soil and foliar pathogen control was explored in several studies. Besides the more familiar biocontrol agents, there was some interesting research on rhizobacteria and vesicular arbuscular mycorrhizal fungi being used as biocontrol agents. Applying some of the more exciting laboratory studies in a practical situation is very challenging, some techniques failed, but many showed promise for the future.

## Ethel Mary Doidge Memorial Address

### Construction, calibration and application of a comprehensive species to strain taxonomic framework for *Xanthomonas* based on rep-PCR genomic fingerprint typing

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The genus *Xanthomonas* is one of the largest groups of bacteria charac-

terized by DNA–DNA homology studies and genomic fingerprinting. This genus has also been well characterized by complementary phenotypic and genotypic methods. Presently, a 70% total genomic DNA–DNA homology value represents an internationally accepted criterion to define bacterial species levels. However, the complexity of DNA–DNA reassociation kinetics methods precludes the rapid analysis of large numbers of isolates, which is imperative in bacterial classification and typing. More facile PCR-based techniques, such as rep-PCR and AFLP genomic fingerprinting, were compared with DNA–DNA hybridization studies. Similarity values of rep-PCR (using three different primer sets) and AFLP genomic fingerprint analyses were calculated and used to determine the correlation with corresponding DNA–DNA homology values. A high correlation was observed, highlighting that genomic fingerprinting techniques reveal taxonomic and (phylo)genetic relationships between bacterial organisms. More than 5000 rep-PCR genomic fingerprint profiles of over 700 *Xanthomonas* collection strains have been analysed. Cluster analysis revealed distinct groups that correspond directly to the 20 DNA–DNA homology groups (genospecies) previously identified. Group 9 strains (*X. axonopodis*) were an exception and did not cluster together into a coherent group but comprised six subgroups. Hundreds of strains not previously characterized by DNA–DNA hybridization analysis, or not previously classified, were assigned to specific genospecies based on the classification framework developed. The rep-PCR delineated sub-specific groups within *X. hortorum, X. arboricola, X. axonopodis, X. oryzae, X. campestris* and X. *translucens.* The genomic fingerprint analysis disclosed that some strains classified as the same pathovar had different genotypes and conversely, several strains previously classified as different pathovars showed highly similar genotypes. The large database of rep-PCR fingerprints provided a detailed assessment of diversity and confirmed/refined the current classification of *Xanthomonas.* Numerous taxonomic issues with regard to the diversity, similarity, redundancy or misnaming were resolved. This classification framework enables rapid identification and classification of new, novel, or unknown *Xanthomonas* strains that are pathogenic or are otherwise associated with plants. Novel strains pathogenic on ornamental asparagus from Florida and deviating isolates causing bacterial spot of tomato in the Caribbean and Central America as well as newly emerging lineages from the Midwest and Ontario could be characterized in detail. Genomic fingerprinting analysis proved to be effective to classify and type xanthomonad strains. The large database of rep-PCR fingerprints proved to be instrumental in the classification of xanthomonads.

## Dr J.E. Vanderplank Memorial Address

### Tackling the grapevine leafroll disease problem in South Africa

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Grapevine leafroll disease is a serious affliction of grapevines worldwide and a number of viruses are associated with it. These viruses are successfully eliminated from clonal material by heat therapy and meristem tip culture or somatic embryogenesis. Research initially revolved around preparing virus detection methods to monitor the efficacy of the virus elimination methods. Virus-free material remains susceptible to viruses and, when grown under field conditions where the mealybug vector of GLRaV-3 has access to them, they often become infected again. Subsequent research therefore focused on identifying the most important means of spread of leafroll disease, and the dispersal of viruliferous mealybugs. This was achieved by determining the temporal and spatial changes in distribution of grapevine leafroll disease infected vines in 70 mother block vineyards in the Western Cape, South Africa. Changes in the number of leafroll-infected vines in vineyards in which leafroll had occurred and where rouging was not applied (57 vineyards) were monitored from 2001 to 2005. The positive relationship between the number of infected vines present and the number of new infections, showed the importance of disease pressure. The most frequently encountered spatial distribution pattern of infected vines was significant ($P < 0.05$) runs of adjacent infected vines, mainly along rows, increasing from season to season. This is indicative of secondary spread within vineyards after establishment, and constitutes the most important pattern of leafroll spread in local vineyards. Two mother blocks, newly established on soil not previously planted to vines, had randomly occurring leafroll infections within the first or second season after establishment. These infected vines were also spatially associated with specific rootstocks or scion clones only. This distribution provides strong circumstantial evidence of primary spread of leafroll by infected planting material. Evidence of leafroll spread from a preceding vineyard is presented, based on the association of greater numbers of infected vines in one half of a mother block, correlated spatially with a severely leafroll-infected Cabernet franc vineyard previously planted in that half. Gradients of leafroll-infected vines, often associated with proximal leafroll-infected vineyards, were also often observed. In view of the four common distribution patterns observed, and the inferred means of spread, several control strategies were proposed and are currently being evaluated. These include the control of secondary spread by roguing, combined with systemic insecticide in certain circumstances; the planting of certified material with systemic insecticide treatment at establishment, followed by subsequent removal of infected sources; the use of fallow periods, and removal of volunteer vines at sites where prior plantings of *Vitis* spp. occurred. To reduce leafroll spread from adjacent infected vineyards, changes in cultivation practices to reduce movement from infected vineyards to healthy ones are proposed, or, where this is not practical, sanitation of equipment and clothing between vineyards is recommended.

## Oral papers

### Dynamics and molecular characterization of citrus tristeza virus (CTV) strains within the South African GFMS 12 cross-protecting population

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The Citrus tristeza virus (CTV) is one of the most important causes of viral diseases of citrus worldwide. It is transmitted by aphids and infected propagative material. Citrus plants are infected with multiple CTV strains, causing varying degrees of severity. The dynamics of the strains are affected by the aphid species involved, citrus cultivar, environmental conditions and strain competitiveness. Mild strain cross-protection is used to protect plants against severe CTV strains; however, breakdown of this protection sometimes occurs. Samples tested were infected via single aphid transmissions from the GFMS 12 population kept in Nelspruit. Genotyping of the CTV genome of each source was conducted using 24 multiple molecular marker primer sets targeting 4 specific genotypes (T3, T36, T30 and VT) with optimized RT-PCRs. The samples had products amplified with the T30 genotype specific primers. This indicated that the samples were generally of a mild nature, which corresponded to the biological symptoms obtained. However, more extensive sampling needs to be done to characterize all the strains within the GFMS 12 cross-protecting population and to shed light on possible cross-protection breakdown.

### Evaluation of the efficiency of four mealybug species to transmit activated-episomal *Banana streak badnavirus*

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Nineteen species of mealybug have been recorded on Musaceae (mainly banana and plantain) in Africa. Mealybugs (Hemiptera: Coccoidea: Pseudococcidae) have been shown to be vectors of various plant viruses including species of the *Closterovirus, Trichovirus* and *Badnavirus* genera. In this study, four mealybug species were experimentally evaluated for their efficiency to transmit activated-episomal *Banana streak badnavirus* (BSV) to Williams banana (Cavendish subgroup, AAA genome). BSV is a member of the sub-order *Pararetrovirineae.* The virus shares a common characteristic with some other members in the *Pararetrovirineae,* which have endogenous viral sequences, integrated into the host genome. To obtain activated-episomal BSV, tissue culture stress was applied to virus-free FHIA-21 (AAAB genome) material, as tissue culture is known to trigger the expression of integrated BSV.

FHIA-21 progeny infected with activated-episomal BSV were used as virus donors under controlled transmission studies. Two control donor sources were also included. A 72-h virus acquisition access period, followed by a 120-h inoculation period was used. Immunocapture (IC)-PCR and triple antibody sandwich (TAS)-ELISA results showed that *Planococcus citri* (Risso) transmitted BSV to 100% of the receptor plants, whereas *Dysmicoccus brevipes* (Cockerell) transmitted BSV to only 20%. Transmission of activated-episomal BSV was demonstrated for the first time with *P. ficus* (Signoret). This vector transmitted BSV to 80% of the receptor plants. BSV was not transmitted by *Pseudococcus longispinus* (Targioni-Tozzetti). Furthermore, transmission of episomal BSV from asymptomatic FHIA-21 leaf-tissue was also demonstrated.

## Epidemiology of Cassava mosaic disease and its whitefly (*Bemisia tabaci*) vector in the lowveld region of South Africa

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Cassava mosaic disease (CMD) is caused by whitefly-transmitted geminiviruses and poses a serious threat to cassava production in many subtropical and tropical regions. The disease is almost exclusively transmitted by *Bemisia tabaci* (whitefly) and disseminated in stem cuttings used for propagation. To investigate the incidence and severity of cmD and its whitefly vector, surveys were conducted in January and March 2005 in the lowveld regions of Mpumalanga and Limpopo, in the subregions of Bushbuckridge, Marite and Tonga. From each region, 10–15 farms were randomly selected within a minimum proximity of about 5 km. On each farm 30 plants were assessed along a diagonal or 'Z' configuration. Plants were assessed for cmD incidence, severity (scale: 1 = no symptoms to 5 = severe symptoms), infection type (i.e. cutting or whitefly) and whitefly counts (on top 5 leaves). Results were analysed by one-way ANOVA *t*-test. Geminivirus infection was confirmed by PCR using core coat protein (CCP) primers. The average disease incidence during January was lower in Bushbuckridge (27.4%) than in Tonga (53.6%) and Marite (39.8%); however, the overall differences were insignificant due to large variances between farms within the same regions. During March, the incidence in Bushbuckridge (14.9%) was significantly lower than that of both Tonga (43.5%, $t = 3.45$; $P < 0.05$) and Marite (42.0%, $t = 3.22$; $P < 0.05$). The proportion of plants infected as cuttings did not vary significantly in all three regions from January to March, probably owing to a single planting season between July and October 2004. There was no significant increase in disease incidence due to whiteflies in each region from January to March. However, the Bushbuckridge region (January 0.86%; March 0.96%) showed the lowest disease incidence caused by whitefly transmission in both surveys and the incidence was significantly lower than that for Marite (January 12.3%; March 10.0%, $P < 0.05$) and Tonga (January 15.1%; March 10.9%; $P < 0.05$). Whitefly numbers increased significantly from January to March in all regions, with Bushbuckridge having the lowest numbers. There was little variation in disease severity for all regions during both surveys. Since a preliminary survey (October 2004) indicated no whitefly yet cmD symptoms (incidence: 20–30%), these indicate that initial infections come from cuttings and therefore are of concern in all regions. Whitefly infection is additionally important because in the Tonga and Marite regions high disease incidence was accompanied by greater whitefly numbers, thus resulting in superinfection. Infection by both whitefly and cutting therefore contributes to serious disease incidence and spread.

## Influence of ergot on grain mould of sorghum (*Sorghum bicolor*)

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Understanding the role of both biotic and abiotic factors in grain mould development is important in designing efficient and economic management practices. A greenhouse experiment was conducted to determine the influence of sorghum ergot on the incidence of grain mould pathogens and on damage to sorghum grain. Grain mould pathogens used were *Curvularia lunata, Fusarium subglutinans* and *Phoma sorghina.* Sorghum heads were inoculated with the ergot pathogen (*Claviceps africana*) at anthesis and with the grain mould pathogens at the soft dough growth stage. In all four sorghum cultivars tested, grains from panicles inoculated with grain mould pathogens following inoculation with *C. africana* revealed a significantly higher ($P \leq 0.05$) incidence of pathogens than those inoculated with only the ergot or grain mould pathogens. Significantly more marked grain discolouration and lower grain weight and germination were found where both ergot and grain mould pathogens were inoculated on panicles. Depending on sorghum variety and fungus species, inoculation with the ergot pathogen increased incidence of grain mould fungi and grain discolouration by over 47% and 46%, respectively. In contrast, grain weight and percentage germination decreased by 10%. The incidence of grain mould pathogens and ergot severity were positively correlated, the relationship being strongest in *P. sorghina* ($r = 0.82$–$0.97$, $P \leq 0.05$). Ergot severity correlated positively with grain discolouration ($P \leq 0.05$) and negatively with grain weight and percentage germination. Results indicate that ergot can significantly influence grain mould development and hence the yield and quality of sorghum grains. Management of ergot may thus help to reduce the severity of grain mould, thereby reducing the resulting damage to grain.

## The role of weather on sorghum grain mould severity and grain quality

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Grain mould of sorghum (*Sorghum bicolor* (L.) Moench) is a complex disease caused by fungi from various genera. Disease symptoms are often manifested on kernels as pink, grey, white, or black discolourations of the grain surface. Most sorghum is grown in semi-arid regions under rain-fed conditions, where environmental conditions are conducive to the development of grain moulds. Grain mould is one of the most important biotic constraints affecting grain yield and quality. The disease reduces grain size, mass and seed germination. It can also result in the complete destruction of the grain. The objectives of this study were to determine the incidence of grain mould fungi in sorghum grains harvested from different locations and planted on different plating dates, to assess the effects of wetness duration on grain mould development under a controlled environment and to evaluate the effect of mould fungi on grain quality and yield. Five cultivars from three localities were used in the assessment. Fungal frequency varied across localities and flowering dates. In all cultivars, higher grain mould incidence was recorded on grains from Potchefstroom. *Alternaria alternata* was the most dominant mould fungus at all localities and all flowering dates. An increase in panicle wetness and temperature was positively correlated ($P \leq 0.05$) with fungal invasion. The milling quality indicated by abrasive hardness and dehulling index of grains as well as malting quality indicated by germination, diastatic power, malt loss and water uptake were affected by grain mould fungi. There was a negative correlation between germination energy and incidence of grain mould fungi, as follows: *Fusarium* spp. ($r = –0.85$), *Curvularia lunata* ($r = –0.70$), *Phoma sorghina* ($r = –0.68$) and *A. alternata* ($r = –0.21$). Grain mould fungi had similar effects on threshed grain mould score, kernel mass and germination. *A. alternata, C. lunata, F. graminearum, F. proliferatum, F. thapsinum* and *P. sorghina* were used to determine the effects of wetness duration on grain mould development under controlled environment. Panicles of a greenhouse-grown white sorghum cultivar (PAN 8706W) were spray inoculated with each fungus at soft dough stage and incubated in a dew chamber for 0, 24, 48, 72 and 96 hours. Increasing wetness duration increased grain infection by the respective grain mould fungi.

## Resistance to rust caused by *Phakopsora pachyrhizi* in soybean cultivars

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Soybean rust was first recorded in South Africa in February 2001 in the Vryheid area of KwaZulu-Natal, whence it spread to most production areas east of the escarpment. Producers have had to rely on two or three fungicide applications to manage the disease. Trials were conducted at Cedara during 2003/04 and 2004/05 to quantify rust resistance in commercial soybean cultivars and to determine their value in disease control. Field trials consisted of 34 entries replicated three times in a randomized

block design. Each plot consisted of 4 rows, 8 m in length and spaced 45 cm apart. A population density of 360 plants $ha^{-1}$ was used. Planting of replicates was spaced from mid-November to late December during each season. Ratings were conducted at weekly (2003/04) or two-weekly (2004/05) intervals and expressed as affected leaf area based on rust severity and the degree of defoliation. Onset of disease, expressed as relative lifetime (RLT), differed significantly ($P > 0.05$) between cultivars with the earliest onset at RLT = 67.7 in SNK500 and RLT = 55.7 in Stork during 2003/04 and 2004/05, respectively. The greatest delay in onset was in LS678 (RLT = 82.3 and RLT = 75.0, respectively) during both seasons. AUDPC was significantly ($P > 0.05$) related to onset, with higher values with earlier onset ($R^2 = 0.92$ and $R^2 = 0.71$ during 2003/04 and 2004/05, respectively). There were significant differences in AUDPC between cultivars. Yield loss across cultivars was poorly related to AUDPC. Yield losses were, however, related to days to maturity, with lower losses in shorter-season varieties than in longer-season varieties. This, in turn, was related to premature defoliation, which was 17 to 21 days earlier in the longer-maturity group, unsprayed plots. Reductions in yield losses were, however, not sufficient to be of economic value and none of the cultivars showed significant rust tolerance levels.

## Breeding for stripe rust resistance in wheat: are we progressing?

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* Westend, was introduced to South Africa in 1996. During the past 10 years, this disease has often been epidemic, either on a regional or more widespread scale, causing significant losses in bread wheat (*Triticum aestivum* L.) production. Areas most at risk are the Western Cape, eastern Free State and KwaZulu-Natal. Since no breeding for stripe resistance was carried out in South Africa prior to 1996, a considerable proportion of wheat germ plasm in local breeding programmes was susceptible at the time of the introduction. It has been estimated that between 30–60% of early generation breeding lines had to be discarded due to stripe rust susceptibility. To determine if breeding progress has been made in levels of resistance to *P. striiformis* f. sp. *tritici,* field data on a collection of cultivars, varying in date of release, were analysed. Data were collected over several seasons from stripe rust nurseries at Greytown, KwaZulu-Natal. Cultivars were grouped as released prior to 1996, from 1997-99, and since 2000. Stripe rust assessment was based on the percentage foliage damaged per plot, irrespective of the reaction type. Cultivar responses were expressed relative to Palmiet, a dominant yet susceptible spring wheat cultivar in 1996. Based on this comparison, the highly susceptible Morocco control entry was rated as 147.9. For those cultivars released before 1996 ($n = 54$), a mean stripe rust score of 57.4, ranging from 9.1 to 115, was obtained. Cultivars released from 1997 to 1999 ($n = 8$) had a mean rating of 45.8, whereas those released from 2000 onwards ($n = 27$) displayed a mean stripe rust score of 41.9. In the most recent group, stripe rust ratings of 75% of the cultivars varied between 7.5 and 56.6. Although susceptible varieties are still being released, the majority of cultivars have improved resistance to *P. striiformis* f. sp. *tritici.*

## Bacterial resistance improvement in dry beans

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Common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) and halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*) are serious seedborne diseases of dry beans. These diseases are widely distributed throughout the bean producing areas of South Africa and can cause severe yield and seed quality loss. The use of resistant cultivars is the most effective and economical control strategy for bacterial diseases. Backcross breeding was used to improve common- and halo blight resistance in two major seed types (red speckled sugar beans and small white canning beans) using resistance in XAN 159, Vax 4 and Edmund. High resistance levels in near-isogenic lines, developed in the two independent breeding programmes, indicated successful transfer of resistance from the sources used. The presence of available SCAR-markers for common blight resistance confirmed resistance in these lines. Two cultivars, Kranskop-HR1 (halo blight resistant) and Teebus RCR2 (common blight resistant), have been released from this programme. Results from the National Cultivar Trials indicated a yield increase of 17.8% in Kranskop-HR1 compared to Kranskop and an increase of 21.8% in Teebus RCR2 compared to Teebus.

## Infection structures of *Puccinia striiformis* f. sp. *tritici* in flag leaves of resistant (Kariega) and susceptible (Avocet S) wheat cultivars

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Amongst the biotrophic fungi, stripe rust (*Puccinia striiformis* f. sp. *tritici*) causes one of the most important diseases of wheat in northern Europe and many other parts of the world. In South Africa, it was first observed in the Western Cape in 1996 and has subsequently spread to all wheat production areas of the country. Kariega, a South African wheat cultivar with excellent baking quality, exhibits complete adult plant resistance to stripe rust, without sustaining yield losses. To gain insight into the adult plant resistance mechanism of Kariega, Prins *et al.* (2005) developed a doubled haploid mapping population derived from a Kariega × Avocet S cross, and identified two major QTL, located on chromosomes 7D (*QYr.sgi-7D*) and 2B (*QYr.sgi-2B.1*) and two minor QTL. For further molecular biological and biochemical studies on the four QTL, we compared stripe rust-infected flag leaves of Kariega and Avocet S by means of fluorescence and confocal laser scanning microscopy. Initial fungal penetration into flag leaves was identical in both cultivars. During the following four days, fungal development occurred faster in flag leaves of Kariega than in Avoce S. Seven days post inoculation, however, the situation changed and exponential growth of the fungus was observed in the susceptible cultivar only. Induced cellular lignification was observed in Kariega four days post inoculation, while in Avocet S, a necrotic response did not occur earlier than six days post inoculation. Although Kariega tissue reacted to invasion by a more rapid lignification response, the lignified host cells did not totally enclose the fungal structures. Long, unbranched hyphae with few or no haustorium mother cells were observed, obviously outgrowing the resistance reaction of the host tissue. However, seven days post inoculation the fungus was completely surrounded by lignified mesophyll cells.

## Physiology of growth stage-related susceptibility in soybean to rust

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Investigation of effective control of soybean rust, caused by *Phakopsora pachyrhizi,* has concentrated on fungicide application and breeding for resistance. The study reported here is aimed at relating changes in host susceptibility to host physiology. Identification of antifungal substances and induction of defence mechanisms could lead to their exploitation in partial resistance to soybean rust. Field experiments were conducted during 2003/04 and 2004/04 at Cedara, consisting of seven sequential plantings of LS 666 (a commercial variety of medium maturity), to create variation in developmental stages. During the season, leaves were collected from plants at six growth stages, namely, at one month, mid-vegetative, flowering, early podform, early podfill, and advanced podfill. The youngest and oldest leaves of each plant were collected and treated as separate samples. Intercellular wash fluids (IWF) were extracted and frozen, together with fresh plant material, at –74°C. Activities of three pathogenesis-related proteins, i.e. $\beta$-1,3-glucanase, chitinase and peroxidase, as well as total phenolics, were determined for each of the samples. Significant differences ($P < 0.05$) were found in enzyme activities between growth stages. Early growth stages, prior to podformation (onset of the susceptible growth stage), were characterized by low concentrations of peroxidase and an increase in $\beta$-1,3-glucanase and chitinase activity, whereas growth stages after flowering were characterized by an increase in peroxidase activity and decrease in $\beta$-1,3-glucanase and chitinase activities. $\beta$-1,3-glucanase activity ranged

between 4.71 (podform stage) and 13.21 (mid-vegetative) mg glucose $min^{-1}$ $mg^{-1}$ prot, chitinase ranged from 0.27 (one month old) to 1.61 (flowering) $\Delta A_{550nm}$ $mg^{-1}$ prot $h^{-1}$, whereas peroxidase activity ranged from 2.55 (mid-vegetative) to 929.1 (advanced podfill) mol tetraguaicol $min^{-1}$ $mg^{-1}$ prot. These findings suggest that $\beta$-1,3-glucanase and chitinase are developmentally regulated and not induced by infection, as in the case of peroxidase. Significant differences ($P < 0.05$) were observed in phenolic content in leaves between growth stages, with increased levels after the podform stage and in general higher levels in younger leaves.

## Seasonal variation in ascospore availability of *Guignardia* spp. under different climatic conditions

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The aim of this study was to determine the seasonal variation in inoculum potential, that is, the number of available ascospores of *Guignardia* spp. produced on citrus leaf litter on the orchard floor. The presence of ascospores was determined with an ascospore monitor, which was specifically developed for trapping ascospores of *Guignardia* spp. The effect of climatic conditions in geographically different areas was also studied. This project was begun in August 2002 in an unsprayed Navel orchard on Letaba Estates. In May 2004, two Valencia orchards, one at Mahela Citrus, Letsitele, and one at Richmond Estates, Hoedspruit, were included in the project in order to include different production areas. Results obtained to date show that leaves dropping at any stage throughout the year can serve as substrate for ascospore production but with the major portion of inoculum produced on leaves that dropped between June and January. The majority of asci were produced within 4 months during periods when rain often fell. During periods when conditions were drier, the process took longer (4–6 months). In most cases, leaves deteriorated completely after 6 months of exposure on the orchard floor. Seasonal differences in inoculum potential were observed. During the 2003/04 season, ascospore numbers were much higher than during the 2002/03 season. Results also showed that the peak for ascospore availability shifted from January and February in 2003 to January, February, and March in 2004. Climate, especially rainfall (number of days with rain) and temperature may play an important role, especially regarding the speed of the composting process, which appears to be directly correlated with the production of ascospores.

## Progress in modelling grey leaf spot (*Cercospora zeae-maydis*) on maize

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Grey leaf spot (GLS) on maize was first discovered in South Africa in 1989 near Greytown, KwaZulu-Natal. After its initial epidemic in the early 1990s, it is now relatively well managed with the use of resistant cultivars. However, there is still a need for fungicide control of GLS in certain circumstances, e.g. under favourable climatic conditions, where producers prefer the use of higher-yielding, susceptible cultivars and for seed production. A model to time fungicide applications was developed at the KwaZulu-Natal Department of Agriculture & Environmental Affairs (KZNDAEA), Cedara, in the 1993/94 season with good results, for instance, up to 98% accuracy under field conditions. However, differences between predicted and observed disease in the 1998/99 and 1999/00 seasons were noted. The hypothesis for the cause of this was that the model was developed during the early epidemics of GLS when inoculum levels were high. In recent years, however, with the advent of resistant cultivars, GLS severity is no longer as high as it used to be. Consequently, initial inoculum levels at the beginning of each new season are lower than when the model was first developed. As one of the model's input coefficients is residue at planting, and all residue is assumed to be infected, the model over-predicts disease severity. A correction factor has, therefore, been introduced into the model, by using only the percentage of infected residue for the calculation of the residue coefficient, instead of the total residue. This correction factor has improved the model's performance significantly. A predictive model, as already available for *Phytophthora infestans,* is proposed for GLS on maize. As residue cover and final disease severity for the different weather stations are unknown, multiple scenarios will be used to simulate farm conditions. Results from the simulations will be published on the KZNDAEA website and, in addition, will be distributed via e-mail to provincial farmers and consultants.

## Fungicide spray cover in grape bunches and control of *Botrytis cinerea*

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Poor control of fruit and foliar diseases in vineyards is often attributed to insufficient coverage of susceptible tissue. In order to study the optimization of spray cover in vineyards, a spray cover assessment protocol using fluorometry, photomicrography and digital image analyses was developed. This protocol was used in this study to determine the minimum spray coverage levels needed for effective *B. cinerea* control in grape bunches, specifically on the susceptible pedicels. Bunches of table grapes (Waltham Cross) and wine grapes (Chenin blanc) were cut into two-dimensional shapes (to minimize variation in conidium and spray deposition) and sprayed at set, pea size, bunch closure and preharvest with different volumes of a mixture of fenhexamid and a yellow fluorescent pigment. Photos of pedicels were taken with a digital camera under a stereomicroscope at 30× magnification. The total area of deposited pigment in selected areas of interest (AOI) was calculated and the percentage area covered was subsequently calculated for each AOI by means of digital image analyses. Unsprayed and sprayed bunches were inoculated with dry airborne conidia of *B. cinerea* in a settling tower and moist-incubated for 24 h. *B. cinerea* infection levels on pedicels were determined by means of isolations onto paraquat medium. Infection levels and coverage data were subjected to sigmoidal regression analyses. From these biological efficacy curves, fluorescent pigment coverage that effected 75% control of *B. cinerea* infection (FPC75 values) was calculated for each cultivar × stage combination. The highest FPC75 values were measured early in the season and decreased as the season progressed. This might be attributed to the increase of host resistance, and also to decrease in FPC measurements with bunch maturity. The latter can be explained by increasing roughness of pedicel surfaces with maturity, which influenced photomicrography and image analysis. Markedly higher FPC75 values were recorded on Chenin blanc than on Waltham Cross grapes, which might be attributed to differences in host resistance to *B. cinerea* infection and/or differences in the efficacy of the infection assay between these cultivars. It is therefore obvious that FPC75 values should be obtained for each test cultivar and growth stage. The FPC75 values obtained in this study were used as benchmarks to evaluate spray application in vineyards. Vineyard spray trials with commercial spray applicators showed similar pedicel coverage values obtained with the airblast (axial fan) and airshear (centrifugal pump) spray applicators. However, coverage values were markedly lower than FPC75 values for all stages in Waltham Cross, and substantially lower in Chenin blanc, which has characteristically more compact bunches. Sub-optimal spray coverage would most likely lead to loss of control during high disease pressure conditions.

## The efficacy of seed treatment fungicides on *Fusarium* crown and root rot of grain sorghum

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Seed rot, pre-emergence and post-emergence damping-off and seedling blights are responsible for stand loss of sorghum (*Sorghum bicolor* (L.) Moench). A greenhouse experiment was conducted to evaluate four fungicide and biocontrol seed treatments, namely, difenoconazole/metalaxyl-m, tebuconazole/triflumuron, *Trichoderma harzianum,* tebuconazole and carboxin/thiram for control of *Fusarium* spp. that infect sorghum seed and seedlings. Treated seeds of three sorghum genotypes, NK 283, SNK 3939 and PAN 8706W, were planted in soil inoculated with ground oat seeds colonized by *F. solani, F. thapsinum, F. proliferatum* and *F. equiseti.* The fungi reduced seed germination by more than 40%. There were significant interactions between the effects of fungicide treatments, *Fusarium* spp. and sorghum genotypes on root rot severity. Generally, results indicated that all four fungicides and the biocontrol agent significantly improved shoot and root mass and decreased crown and root rot

severity. *Trichoderma harzianum,* difenoconazole/metalaxyl-m and tebuconazole/triflumuron improved emergence, shoot and root mass, whereas carboxin/thiram and tebuconazole improved root health by decreasing crown and root rot severity to 0.83 and 0.92 (0–5 rating scale), respectively. Results indicated that all four fungicides and the biocontrol agent were effective against crown and root rot severity caused by *Fusarium* species. However, carboxin/thiram and tebuconazole were generally more effective than tebuconazole/triflumuron, difenoconazole/metalaxyl-m and *Trichoderma harzianum.* Crown and root rot severity were significantly lower in the resistant genotype PAN 8706W compared with the susceptible SNK 3939. *F. equiseti* consistently caused a reduction in fresh shoot and root mass, whereas shoot mass was highest in plants inoculated with *F. proliferatum.* Root mass was highest for plants inoculated with *F. solani. F. proliferatum* and *F. thapsinum* consistently caused the greatest reductions in root length and root rot. *F. equiseti* was the most aggressive isolate and resulted in the highest crown and root rot severity ratings. Plant mass and root rot severity were not always correlated.

## The gall rust fungus, *Uromycladium tepperianum*, is successfully controlling *Acacia saligna* in the Western Cape province, South Africa

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*Acacia saligna,* originating from Western Australia, is considered to be one of the most important alien invasive weeds of the Cape Floristic Region, South Africa. The gall-forming rust fungus *Uromycladium tepperianum* was introduced as a biocontrol agent against this tree in 1987. Ten sites were set-up in 1991 by M.J. Morris, to monitor the impact of *U. tepperianum* on populations of *A. saligna* in the Western Cape. In 2005, the density of plants at all surviving sites had been reduced by 73–99% compared to the initial density of 1991. This was despite fires at several sites, which saw a post-fire increase in plant density. Additional data were collected by destructive sampling of trees, at 18 sites during 2004 and 2005. Plant height, stem diameter, and plant age (number of tree rings) were recorded for each plant, and the total dry weight of all leaves determined. The data indicated that the impact of *U. tepperianum* on total leaf dry weight of *A. saligna* increases with age of the plant, and the older the stand (i.e. a longer time since the last major disturbance such as fire or felling), the earlier this impact. The average age of trees at these sites ranges from 3 to 6 years, despite it being 10 to 15 years at least since the last major disturbance at most of the sites. This indicates that the population dynamics of the plant has changed since introduction of the gall rust. The total number of pods per tree produced at four sites was determined in 2004 ($n$ = 120 trees). In comparison to equivalent data collected in 1989, 62% of trees had at least 50% fewer pods than expected, and 49% of trees had 80% fewer pods than expected. *Uromycladium tepperianum* should therefore be considered a successful biocontrol agent, having considerably reduced the negative impact of *A. saligna* in the Western Cape. In conjunction with the impact of the seed-feeding weevil *Melanterius compactus,* it is expected that populations of *A. saligna* will continue to decline.

## Host-specificity testing of rust fungus, *Puccinia conoclinii*, on *Campuloclinium macrocephalum*

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*Campuloclinium macrocephalum,* also known as pom pom weed, was introduced as an ornamental to South Africa from South America (Argentina and Brazil). Subsequently, this weed has rapidly established itself in many areas, is found mostly along roadsides, open savanna and wetlands and has become a serious threat to grasslands, especially in the Gauteng highveld. It can also be found in Mpumalanga, Limpopo, North West province, KwaZulu-Natal, Eastern Cape and Free State. Chemical control is very expensive and some areas might not be easily accessible for spraying. Mechanical control is labour-intensive and may even lead to denser stands, if done incorrectly. Two species of *Puccinia,* namely *Puccinia eupatorii* and *Puccinia conoclinii,* are recorded on *C. macrocephalum* in Brazil. This raised the possibility of controlling pom pom weed by introducing these two rusts as biocontrol agents. Leaves of pom pom weed infected with *Puccinia conoclinii* were therefore collected in northern Argentina and introduced into quarantine at ARC-PPRI in Stellenbosch. Locally collected *C. macrocephalum* plants were inoculated with the rust fungus and found to be susceptible to this isolate. A technique was developed for optimal inoculation, which established that the optimum temperature for urediniospore germination is 18°C and a minimum dew period of 6 hours is required for infection. To ensure the safety of local indigenous plants and crops, a list of plants most likely to be hosts was compiled and host-specificity testing has been initiated. The germination and infection of *P. conoclinii* was also studied on several of the test plants using light microscopy. Preliminary results show *P. conoclinii* to be host specific to *C. macrocephalum* and all of the other plants (15 plant species) tested so far to be immune to this fungus. A further 19 species of plants must still be tested. Pending these results, permission for release will be sought from the relevant authorities. *Puccinia conoclinii* is expected to make a valuable contribution to the biocontrol of this highly invasive weed.

## Grapevine trunk disease pathogens: symptomatology and distribution in climatically diverse table grape growing regions of South Africa

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*Phaeomoniella chlamydospora, Eutypa lata, Phomopsis, Phaeoacremonium,* and *Botryosphaeria* spp. are important trunk disease pathogens that cause premature decline and dieback of grapevine. Previous research has focused primarily on wine grapes and the incidence and symptomatology of these pathogens on table grapes were largely unknown. A survey was therefore conducted to determine the status and distribution of these pathogens and associated symptoms in climatically diverse table grape growing regions. Fifteen farms were identified in winter rainfall (De Doorns, Paarl and Trawal) and summer rainfall areas (Upington and Groblersdal). Samples were taken in Dan-ben-Hannah vineyards older than 8 years during July and August 2004. Distal ends of arms were removed from 20 randomly selected plants in each vineyard. These sections were dissected and isolations were made from each of the various symptom types observed: brown or black vascular streaking, brown internal necrosis, wedge-shaped necrosis, watery necrosis, esca-like brown and yellow soft wood rot, as well as asymptomatic wood. Fungal isolates were identified using molecular and morphological techniques. *Pa. chlamydospora* was most frequently isolated (46.0%), followed by *Phaeoacremonium aleophilum* (10.0%), *Phomopsis viticola* (3.0%), *Botryosphaeria obtusa* (3.0%), *B. rhodina* (2.2%), *B. parva* (2.0%), *Fusicoccum vitifusiforme* (0.6%), *B. australis, B. dothidea* and an undescribed *Diplodia* sp. (0.2% each), whereas *E. lata* was not found. Most of these pathogens were isolated from a variety of symptom types, indicating that disease diagnosis cannot be based on symptomatology alone. *Pa. chlamydospora* was isolated from all areas sampled, although most frequently from the winter rainfall region. *Pm. aleophilum* was found predominantly in Paarl, while *P. viticola* only occurred in this area. Although *B. obtusa* was not isolated from samples taken in De Doorns and Groblersdal, it was the most commonly isolated *Botryosphaeria* species, being dominant in Upington and Trawal. *B. rhodina* occurred most frequently in Groblersdal and *B. parva* in Paarl, while *B. australis* was isolated from Paarl only. Additionally, two unidentified basidiomycetes were isolated from five samples with yellow esca-like symptoms from the Paarl area. These findings clearly illustrate that grapevine trunk diseases are caused by a complex of fungal pathogens, which has serious implications for disease diagnosis and management.

## *Botryosphaeria* spp. from native *Syzygium cordatum* and introduced *Eucalyptus* trees in South Africa

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*Botryosphaeria* spp. are among the most important canker pathogens on introduced *Eucalyptus* spp. (Myrtaceae) in South Africa. These fungi have also been reported from native South African trees such as

*Syzygium cordatum* (Myrtaceae). *Eucalyptus* and *S. cordatum* are sufficiently closely related that they may be expected to share pathogens. In this study, the potential of *Botryosphaeria* spp. from both *Eucalyptus* and *S. cordatum* to cross-infect each other in South Africa was considered. *Botryosphaeria* strains were isolated from stem cankers, dying twigs, as well as asymptomatic twigs and leaves. All isolates were induced to sporulate in culture and identified based on anamorph morphology and ITS rDNA sequence data. The cryptic species *B. parva* and *B. ribis* were separated by additional sequence data for the elongation factor 1-$\alpha$ and $\beta$-tubulin gene loci, as well as PCR-RFLP of an unidentified locus. Eight *Botryosphaeria* spp. were identified from *Syzygium* including *B. parva, B. ribis, B. lutea, B. australis, B. rhodina, B. dothidea, Fusicoccum mangiferum* and *Lasiodiplodia gonubiensis.* The species that commonly occurred on *Eucalyptus* include *B. parva, B. eucalyptorum* and *B. eucalypticola. Botryosphaeria rhodina,* which is also known to infect *Eucalyptus* in South Africa, was not isolated from this host in this study. Only two species, namely *B. parva* and *B. rhodina,* thus overlap on native *Syzygium* and introduced *Eucalyptus* in South Africa. Both of these cross-infecting species are potentially serious pathogens of their respective hosts. This potential movement of pathogens between native and introduced trees will impact on management of disease in commercial plantations and native tree conservation programmes alike.

## *Ophiostoma* and *Ceratocystis* species associated with wounds on native South African tree species

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*Ceratocystis* and *Ophiostoma* species are carried by insects and they infect wounds visited or made by these vectors. These fungi include well-known plant pathogens, such as the Dutch elm disease fungi *O. ulmi* and *O. novo-ulmi* and *Ceratocystis fagacearum,* the causal agent of oak wilt. In this study, *Ceratocystis* and *Ophiostoma* spp. were collected from wounds on native tree species in South Africa. Both morphological characteristics and DNA sequence comparisons were used to identify the fungi. Cultures were grown on malt extract agar supplemented with the antibiotic streptomycin. Slides bearing fruiting structures were examined using light microscopy. DNA sequence comparisons for parts of the ITS, $\beta$-tubulin and elongation factor 1-$\alpha$ gene regions were used to confirm morphological observations. *Ceratocystis* and *Ophiostoma* spp. were common on wounds and in some cases they were associated with discolouration of the xylem surrounding the wounds. *Pesotum quercus, Pesotum fragrans, Ceratocystis albifundus* as well as an undescribed *Ophiostoma* sp. and two *Ceratocystis* spp. were collected. This study expands on reports of *Ophiostoma* and *Ceratocystis* spp. from South Africa and supports the view that the diversity of these fungi is incompletely understood in the country. Surveys will now be extended to include additional geographical areas.

## *Paecilomyces* spp. from wooden utility poles in South Africa

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The genus *Paecilomyces* Section *Paecilomyces* includes species that are thermophilic or thermotolerant, which makes it possible for these fungi to survive in wood during kiln-drying. The type species of this Section, *P. variotii,* is tolerant to the common preservatives used on utility poles, including CCA, creosote and copper sulphate. It is also capable of producing soft rot cavities in wood. This type of decay can cause failure of the sapwood in poles and greatly decreases the lifespan of utility poles. A survey was recently conducted for Eskom to determine which fungal species are involved in decay processes in wooden utility poles in South Africa. The survey yielded more isolates from the genus *Paecilomyces* than from any of the other fungal genera obtained. The aim of this study was to identify the 165 isolates of *Paecilomyces* collected. The morphology of the cultures and sporulating structures was studied with light microscopy. In addition, the DNA sequences of the ITS and $\beta$-tubulin gene regions were determined for selected isolates representing all the morphological groups. From morphology and sequence data, we could distinguish at least five *Paecilomyces* spp. Comparisons with described species of *Paecilomyces* is ongoing and we aim to reveal the identity of these isolates.

## Bacterial blights of leek and onion caused by *Pseudomonas syringae*

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Between the 2001 and 2004 growing seasons, a new and damaging disease of leak and onion was observed on nursery-produced and field-grown plants in South Africa. Symptoms on leek were water-soaked lesions on leaves, which extended down the length of the leaf and resulted in brown, elongated strips with yellow margins. Lesions on onion leaves and seed stalks were irregular oval spots, 4–10 cm long with a necrotic centre and a dirty yellow or water-soaked margin. Small water-soaked lesions were also observed. Blue fluorescent pseudomonads were consistently recovered from lesions on King's B, KBC and MT media. In LOPAT tests, isolates were negative for oxidase, arginine dihydrolase and ability to rot potatoes, and positive for production of levan from sucrose and hypersensitive reaction on tobacco leaves. This indicated that they were *P. syringae.* In pathogenicity tests, five representative strains, two from leek and three from onion, induced disease symptoms in leek and onion similar to those observed in the nurseries and field. The isolates were identified as *P. syringae* based on carbon source utilization (Biolog GN2 micro-plates) and comparison with the Microlog 4.2 database (Biolog Inc., Hayward, CA). Biolog did not provide a clear pathovar designation. A BLAST search of the EMBL/GenBank database conducted with 16S rDNA sequences revealed a high degree of sequence identity (99%) with a previously determined sequence of *P. s.* pv. *atropurpurea* (accession number AB001440), belonging to genomspecies 4 of *P. syringae.* However, the sequences of the spacer region between 16S and 23 rDNA genes (ITS), clearly divided leek and onion isolates into separate groups. The ITS sequences of the two South African leek isolates and *P. s.* pv. *porri* (AF098251 and AF098252) were identical. The ITS sequences of three onion strains were identical to each other, but only 94.7% homologous to leek stain sequences. Phylogenetic relationships derived from a neighbour-joining analysis of the pairwise comparison among the ITS sequences of isolates from this study and sequences of described pathovars of *P. syringae* confirmed this division. Leek strains sequences grouped with *P. s.* pv. *porri, garcae, papulans* and *coronafaciens,* belonging to genomspecies 4. Onion strains sequences formed a separate cluster most similar to *P. s.* pv. *atrofaciens, phaseolicola, syringae* and *pisi* (97.1%), belonging to the genomspecies 1 of *P. syringae.* The results indicate that the leek and onion diseases are caused by two different pathovars of *P. syringae*; leek blight by *P. s.* pv. *porri* and onion blight by an undescribed agent. These pathogens were recovered from commercial seed lots, which suggests that they are seed-borne. Assaying seed lots for *P. syringae* and seed treatments to reduce or eradicate seed contamination would be practical measures for managing these diseases.

## Investigating resistance against the causal agent of bacterial wilt, *Ralstonia solanacearum*, in the model plant *Arabidopsis thaliana*

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Resistance against pathogens has become the focus of plant research, as other control measures are often ineffective. This is also true in the case of *Ralstonia solanacearum,* the causal agent of bacterial wilt, which affects several plant species worldwide including some tree species such as *Eucalyptus.* This soil-borne vascular pathogen also infects the model plant *Arabidopsis thaliana.* Previous work has revealed a pathosystem between a *Eucalyptus* isolate of *R. solanacearum* (CK) and the *Arabidopsis*

ecotypes Be-O and Kil-O. Isolate CK caused disease symptoms on Be-O three to five days after infection, while Kil-O remained healthy two weeks after infection, at which time Be-O was dead. Using whole-genome microarrays, transcriptome changes resulting from the resistant interaction between CK and Kil-O was investigated in order to identify candidate genes conferring resistance. Several genes were up-regulated one week after infection. These included some defence response genes such as PR3 and peroxidase, and several uncharacterized hypothetical and expressed proteins. These proteins, which may confer *R. solanacearum* resistance in Kil-O, are worthy of further investigation.

### Seeing the bigger picture: a multi-gene view of *Leptographium* phylogeny

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Species of *Leptographium* are typically recognized by their long, slender conidiophores that terminate in brush-like conidiogenous apparatuses. These conidiogenous apparatuses give rise to a series of branches that terminate in conidiogenous cells that produce ameroconidia through percurrent proliferation. Delayed secession of the conidia is responsible for the false impression of sympodial development often observed in *Leptographium* species. Most *Leptographium* spp. are associated with coniferous trees, with only a few species occurring on other trees or habitats. These fungi have a close association with insects, especially Scolytine bark beetles. A small number of species have serious consequences such as vascular wilt diseases, while most others are agents of sap stain in lumber or are saprophytes. Since the publication in 2000 of the first monograph treating *Leptographium*, the number of species has grown from 46 to over 50 and new species are regularly found. The aim of this study was to characterize *Leptographium* spp. based on a multiple gene phylogeny. For this purpose, sequences derived from the ITS2 and 28S region of the ribosomal DNA gene region as well the $\beta$-tubulin and elongation factor 1-$\alpha$ gene regions were used in comparisons. Previously, only the ITS region sequences were available for *Leptographium* spp. but results of this study showed that they are not ideal for comparisons of these fungi. Differences between well-recognized species were more apparent when comparing sequences from the protein-coding regions. Results also confirm the findings of a previous study showing that there is no correlation between the phylogeny and phenotypic characters considered to be important for the identification in this group. Likewise, there was no correlation between the geographic origin of species or host specificity and their phylogenetic relationships. Data from this study will be used to develop means for the rapid comparison and identification of known as well as undescribed *Leptographium* spp.

### Spore dispersal patterns of grapevine trunk disease pathogens

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Recent studies have identified several trunk disease pathogen complexes from grapevines in South Africa that cause decline and dieback of grapevines. These include *Eutypa lata, Phaeomoniella chlamydospora, Phaeoacremonium, Botryosphaeria* and *Phomopsis* spp. These pathogens are known to infect wounds, specifically pruning wounds. Airborne inocula should therefore play an important role in their epidemiology. However, little is known about the spore dispersal patterns of these pathogens in South Africa. A study was therefore conducted to address this issue, and also to correlate spore dispersal patterns with weather data. During the 2004 pruning season (June to mid-September), spore trapping was conducted in an 18-year-old Chenin blanc vineyard in the Stellenbosch area by means of a Quest volumetric spore trap for a period of 14 weeks. A weather station was erected in the vineyard row adjacent to the spore trap to record temperature, rain, relative humidity, wind direction and speed. The spore trap disc was covered with clear adhesive plastic and sprayed with petroleum jelly. After a period of 4–5 days, the disc was brought into the laboratory and the adhesive plastic cut into segments that each represented a specific 6-hour period. Each segment was placed in a small glass bottle containing 10 ml sterile water and heated for 2 min at 50°C to melt the petroleum jelly and suspend the trapped spores. This treatment did not adversely affect spore germination of the target pathogens. Each spore suspension was subsequently spread-inoculated onto five 65-mm Petri dishes containing water agar and allowed to dry in a laminar flow bench. After 36 hours, all germinating spores observed at ×50 magnification were transferred singly to 65-mm Petri dishes containing potato dextrose agar. These pure cultures were identified morphologically at least to genus level. Six-hourly spore counts were subsequently calculated for each genus and correlated with the climatic data obtained. Spores of *E. lata, Phomopsis* and *Botryosphaeria* spp. were trapped throughout the 14-week period, while spores of *Pa. chlamydospora* and *Phaeoacremonium* spp. were not trapped. Spore dispersal events for *E. lata, Botryosphaeria* and *Phomopsis* spp. were primarily driven by rain (as little as 0.03 mm) that occurred 6–18 hours preceding and during spore release periods. In the absence of rain, spore dispersal events were recorded for *Botryosphaeria* spp. only; in all cases following periods of high relative humidity (>80%). Moreover, periods of high relative humidity seemed to prolong *Botryosphaeria* spore dispersal after rain events. This trial is being repeated and spore release and climatic data will be subjected to statistical analyses in an attempt to develop models for spore dispersal patterns of these pathogens.

### Fungi on stone fruit trees – a threat to South African vineyards?

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Trunk diseases of grapevine (*Vitis vinifera*) are economically important in many grape-producing countries worldwide. Canker pathogens cause premature decline and dieback, as well as quantitative and qualitative yield losses. Several species of ascomycetes in genera such as *Eutypa, Phaeomoniella, Phaeoacremonium, Botryosphaeria* and *Phomopsis* have been implicated in this disease complex. Grapevine and stone fruits (*Prunus* spp.) are often grown in close proximity of each other, and frequently have the same pathogens, for example *Botryosphaeria obtusa* and *Eutypa lata*. A study was therefore undertaken to determine whether stone fruit trees in South Africa are inhabited by these and other known grapevine trunk disease pathogens and whether they could act as alternative hosts. Living wood of *Prunus* spp. with dieback, canker or necrotic symptoms as well as pruning debris were sampled in climatically different grapevine producing areas. Fungi were isolated from the samples, and characterized in terms of morphology and DNA phylogeny (ITS, $\beta$-tubulin, elongation factor 1-$\alpha$ and large subunit genes). Several species that were reported to be pathogenic on grapevine were identified: *Botryosphaeria obtusa, B. australis, B. stevensii, Fusicoccum vitifusiforme, Togninia minima* (anamorph: *Phaeoacremonium aleophilum*), *Pm. viticola, Phomopsis amygdali* and *Cryptovalsa ampelina*. Some taxa constitute first reports on *Prunus* or on single *Prunus* species, e.g. *B. australis, B. stevensii* and *Pm. aleophilum* on plum and peach. Other taxa such as *B. stevensii, Pm. australiense* and *Pm. griseorubrum* represent new records for South Africa. This is also the first record of *Pm. griseorubrum* from plants, as the fungus has hitherto been known only from humans. A selection of the fungi will be tested for their pathogenicity on grapevine, plum and peach. In addition to taxa known to be grapevine trunk disease pathogens, several other species were isolated from symptomatic *Prunus* wood. These include ascomycetes such as *Coniochaeta velutina, Sporormiella africana* and *Karstenula shepherdiae*. According to these results, stone fruit orchards should be considered as potential inoculum sources of grapevine trunk disease pathogens.

### *Leptographium* spp. associated with bark beetles on conifers in China

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*Leptographium* spp. are well known as the causal agents of blue-stain in the sapwood of conifers associated with bark beetle infestations. Very

little is known of the occurrence or distribution of *Leptographium* spp. on forest trees in China. A comprehensive survey on Ophiostomatoid fungi was thus conducted in China between 2001 and 2004. Based on morphology, approximately 18.5% of the large collection of fungi isolated were species of *Leptographium.* These strains were mainly isolated from beetle-infested *Pinus, Picea* and *Larix* species. The aim of this study was to identify the isolates and to determine the geographic distribution of *Leptographium* in China. Strains were initially grouped according to their morphological characteristics. Taxa defined in terms of morphology were then compared using DNA sequences of the ITS2 and part of the large subunit (28S gene) of the rDNA operon and partial $\beta$-tubulin gene. From morphology, the strains could be placed in 12 distinct morphological groups. Phylogenetic analysis of representatives of these groups indicated that they are closely related to *Leptographium terebrantis, L. yunnanense, L. bistatum, L. abietinum* and *L. pineti,* of which *L. abietinum, L. bistatum, L. pineti* and *L. terebrantis* represent first reports from China. Some isolates did not correspond to known *Leptographium* spp. They are currently being studied further and will be provided with appropriate taxonomic treatments.

### Epidemiological importance of *Plasmopara viticola* oosporic infections in South African vineyards

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Downy mildew, caused by *Plasmopara viticola,* is a devastating disease of grapevines worldwide. Recently, *P. viticola* population genetic studies in Europe have challenged some long-standing views on the epidemiology of the disease, which limit the contribution of oosporic infections to primary infection only. These studies have suggested that oosporic infections play a more prominent role during the course of epidemics than previously conceived. In South Africa, this aspect of the epidemiology of the disease is poorly understood and needs further investigation. Some epidemiological aspects of the disease were therefore investigated through population genetic studies in a chemically sprayed (fosetyl-Al + mancozeb, iprovalicarb + propineb, and potassium phosphate) and an organically grown vineyard (copper and compost tea). Additionally, the genotypes of *P. viticola* populations collected in different countries were compared with genotypes collected in South Africa. In the fungicide and organically treated vineyards, *P. viticola* lesions were collected at three sampling times during the 2004/05 season, and subsequently genotyped using four published microsatellite markers. Only a small section of each lesion was collected, allowing continued contribution of sampled genotypes to the epidemic. Genotypic analysis of populations collected at the start and end of the growing season did not reveal significantly different genotypic diversity in either of the vineyards. New genotypes were detected throughout the growing season within each vineyard, suggesting that oosporic infections contribute to the development of the epidemic throughout the growing season. Accordingly, all loci, except for locus BER that was fixed, were in Hardy-Weinberg equilibrium. Only a small number of genotypes were able successfully to reproduce asexually later in the season, with only one (fungicide vineyard) or three genotypes (organic vineyard) being the driving force of the epidemic. The dominant genotype in the fungicide vineyard showed clonal multiplication close to the source followed by plot-scale dispersion. In the organic vineyard the predominant genotype showed random plot-scale dispersion without previous clonal multiplication close to the source. Population differentiation (Fst) between the organic and fungicide-treated populations was only 0.004, indicating that fungicide sprays applied in the chemically treated vineyard did not cause selection. A comparison of *P. viticola* populations from different countries showed that South African *P. viticola* populations were genetically more similar to populations from Greece.

### Polymorphic microsatellites for studying the population genetics of *Cylindrocladium pauciramosum*

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*Cylindrocladium pauciramosum* has a wide host range and is a serious pathogen affecting *Eucalyptus* in South Africna forestry nurseries. On *Eucalyptus* cuttings and seedlings, it causes leaf spot, root rot, cutting rot and stem cankers. An understanding of the population genetics of *C. pauciramosum* will contribute to the management of this disease on *Eucalyptus* and will assist breeding strategies aimed at developing resistant *Eucalyptus* clones. The aim of this study was to develop microsatellite markers to assess the genetic diversity of *C. pauciramosum* populations. These markers are tandem repeats of short (2–6 bp) DNA sequences that are highly polymorphic due to variation in the number of repeats. They are polymorphic, co-dominant and occur throughout genomes, making them valuable for genetic studies. Fifteen polymorphic microsatellite markers were developed for *C. pauciramosum.* Thirty isolates from different hosts and geographic origins were used to characterize these markers. Analyses showed that isolates collected from Italy, Australia and the U.S. show less genetic variability than isolates from South America. Although the South African population of isolates showed a strong clonality, some isolates displayed high levels of genetic variability. The markers developed in this study will contribute to an improved understanding of the origin and genetic structure of *C. pauciramosum* populations in various parts of the world.

## Poster presentations

### Optimization of inoculation techniques for *Pectobacterium* spp. (*Erwinia*) on potatoes

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Bacterial tuber soft rot, aerial stem rot and blackleg are serious diseases of potatoes in the field and in storage. These diseases are caused by *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*), *Pectobacterium carotovorum* spp. *atrosepticum* (*Pca*) and *Pectobacterium chrysanthemi.* Infection of potato tubers begins at wounds, lenticels, or stolon ends. The type of wound plays a major role in bacterial infection, symptom succession and bacterial survival. Therefore, inoculation methods need to be optimized to reduce discrepancies in studies. The aim of this research was to compare five different inoculation methods in tubers using a bacterial suspension of $10^8$ cfu $ml^{-1}$. The best inoculation method was compared with soil inoculation. Potato tubers were inoculated using: a) vacuum infiltration in 1 l of bacterial suspension for 10 min before restoring atmospheric pressure; b) tubers dipped in 500 ml bacterial suspension for 10 min; c) tubers injured by rubbing against sandpaper and dipping in a 500 ml bacterial suspension for 10 min; d) wounds made with a thick injection needle and inoculated with 0.01 ml bacterial suspension by a micropipette; e) sterile toothpicks dipped into bacterial suspension and immediately stabbed into the tubers. Five repetitions were included. The inoculated tubers were randomly placed in tomato boxes and placed in a mist bed at 24°C for 7 days. The first symptoms appeared after the third day. The greatest degree of soft rot was produced by vacuum infiltration followed by sandpaper inoculation. In all treatments disease severity and incidence increased with a rise in humidity. Vacuum infiltration and soil inoculation were then compared. Soil inoculation was performed by adding ~160 ml bacterial suspension to each pot. Evaluation was done throughout the growing season. The disease incidence was low at the beginning of the growing season. Vacuum-infiltrated plants were symptomless at germination but these plants showed symptoms first. Disease in soil-inoculated plants began later in the growing period but symptoms developed quickly. The succession of symptoms progressed from chlorosis and/or wilting, to partial or total desiccation of the plants for both techniques. The severity of symptoms differed for each inoculation method. These results show that aerial stem rot does not originate only from seed piece infection but also from the soil. The water-soaked tissue on the stems is very soft and ranges from light green, yellow to light brown in colour. Plants exhibiting aerial soft rot symptoms eventually develop overall chlorosis. The seed piece is well recognized as the primary source of inoculum for blackleg. Greater efficiency of vacuum infiltration compared with soil inoculation may be the result of better access to susceptible tissue after penetration through lenticels and good survival of bacteria in lenticels.

## *Fusarium* species associated with *Fusarium* head blight of wheat in South Africa

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Wheat production in South Africa is an economically important industry. Fungal diseases cause significant damage to wheat annually. Fusarium head blight (FHB) is one of these diseases, reducing grain quality and yields. Wheat infected with head blight also contains a variety of mycotoxins harmful to both humans and livestock. Effective disease control depends on understanding the aetiology of the disease. The aim of this study was to identify, morphologically and genetically, *Fusarium* spp. associated with FHB. Samples were collected from FHB-infected wheat heads from the principal irrigation areas of South Africa and plated onto nutrient-poor agar (SNA). Resulting *Fusarium* spp. were isolated onto potato dextrose agar (PDA), single-spored onto half-plates with potato dextrose agar and carnation leaf agar, and finally screened and identified using morphological characteristics. *Fusarium graminearum* was the predominant pathogen, yielding 83.9% of isolates from the warmer irrigation regions6 – 6.7% in Brits (North West province), 82.4% in Orania (Free State) and 60% and 83% in Douglas and Prieska (Northern Cape), respectively. In the cooler regions such as the Bergville/Winterton district (KwaZulu-Natal) and the Bethlehem/Vrede district in the eastern Free State, *F. crookwellense* dominated. A number of other *Fusarium* spp. were isolated from irrigated wheat production fields, for instance, *F. equiseti* (9.6%), *F. chlamydosporum* (1.2%), *F. solani* (4.8%) *F. avenaceum* (3.6%), *F. oxysporum* (1.2%) and *F. culmorum* (2.4%). These species are of lesser importance. The increasing use of molecular methods in fungal identification has emerged as a possible solution to problems associated with the existing phenotypic identification in recent years. Amplified fragment length polymorphism (AFLP) analysis is highly discriminatory, suggesting its applicability in molecular characterization of *Fusarium.* AFLP analysis was done using different primer pair combinations on 15 strains of morphologically known *Fusarium* isolates, consisting of seven *Fusarium* spp. Standardization of the analysis showed a high percentage of variable genetic characteristics among and within some *Fusarium* spp. A dendrogram was constructed based on Dice similarity coefficients and statistically analysed with goodness of fit and shown to be a very good fit at $r = 0.99$. This indicates that AFLP analysis is a valuable tool for the molecular identification of *Fusarium* and can be useful in the determination of the genomic relationship among and within species.

## Characterization of *Xanthomonas campestris* pv. *musacearum* causing disease of *Ensete* and banana in Central Africa

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A serious bacterial wilt disease, caused by *Xanthomonas campestris* pv. *musacearum* (*Xcm*), was first reported from enset in Ethiopia in 1968. Six years later, the disease was discovered in banana plants in Ethiopia, causing only minor damage. However, when bacterial wilt was discovered in Uganda 26 years later, it resulted in substantial losses of banana plants, and rapidly spread to 28 districts within three years. The disease has subsequently been reported also from the Democratic Republic of Congo (DRC). The aetiology of bacterial wilt in Ethiopia, Uganda and the DRC differs substantially. The objectives of this study, therefore, were to determine whether bacterial wilt in the three African countries was caused by the same bacterium, and to determine their phylogenetic relationship. Bacterial strains were collected from diseased enset and bananas in Ethiopia, Uganda and the DRC and characterized. *Xcm* strains collected during the initial outbreak of bacterial wilt in Ethiopia in the 1970s and deposited in the BCCM/LMG culture collection (LMG 785 and 7431) in Belgium were included as reference strains. All strains were characterized both phenotypically (morphological and cultural characterization; API analysis) and genotypically (sequencing of the 16S rRNA gene). For phylogenetic comparison, amplified fragment length polymorphism analysis (AFLPs) was performed. Strains were all Gram-negative rods with a distinct colony colour and morphology on nutrient agar. API results confirmed that they belonged to the genus *Xanthomonas* and 16S rRNA sequences confirmed that they were *Xcm.* Preliminary AFLP analysis showed that the populations from Ethiopia, Uganda and the DRC are related. The introduction, dissemination and presence of *Xcm* in new plantations in central Africa should be further investigated.

## Phylogenetic relationships among *Armillaria* species based on partial elongation factor 1-$\alpha$ DNA sequence data

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*Armillaria* spp. are important root rot pathogens with a wide host range and a worldwide distribution. The taxonomy of these fungi has been problematic for many years but the understanding of relationships has been substantially improved through the application of DNA sequence comparisons. In this study, the relationships between different *Armillaria* spp. was, for the first time, determined using EF 1-$\alpha$ DNA sequence data. A total of 42 isolates, representing the majority of *Armillaria* spp., with diverse geographic distribution and hosts were included in this study. PCR amplification yielded products of 600 base pairs for all the isolates. Phylogenetic trees resulting from parsimony analysis showed that this gene region is useful for studying relationships among species. Generally, results were similar to those emerging from previous comparisons using ITS and IGS-1 sequence data. This is the first time a single copy gene has been used to study phylogenetic relationships in *Armillaria* and, overall, the data support previously held views regarding the relationships between species.

## Phylogenetic relationship between *Pantoea* spp. and the bacteria causing blight and die-back of *Eucalyptus*

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The genus *Pantoea* belongs to the family *Enterobacteriaceae.* There are currently seven known species of *Pantoea,* namely, *P. ananatis, P. dispersa, P. agglomerans, P. citrea, P. punctata, P. terrea,* and *P. stewartii.* There are two subspecies of *P. stewartii, P. stewartii* subsp. *stewartii* and *P. stewartii* subsp. *indologenes.* Species belonging to the genus *Pantoea* are typically plant-associated and are either pathogenic, saprophytic or epiphytic in nature. Of all of the seven species, *Pantoea ananatis* causes the widest range of diseases on a variety of plant hosts. *Pantoea ananatis* was first found to cause bacterial blight on *Eucalyptus* trees in South Africa in 1998. A similar disease has been noted on *Eucalyptus* species in South America and in Uganda. The aim of the study reported here was to investigate the genetic relatedness of different *P. ananatis* strains isolated from *Eucalyptus* from different locations. It is not possible to differentiate easily between *Pantoea* species based solely on phenotypic tests. The strains and species were thus compared using 16S rRNA sequencing and AFLP analysis. The *Pantoea* strains included in this study are representative of all seven species and subspecies as well as strains isolated from different locations and hosts. The 16S gene was amplified and sequenced using seven different internal primers. AFLP analysis was performed using fluorescently labelled Eco-C and Mse-GC selective primers to produce DNA fingerprints. A phylogenetic tree was constructed from aligned 16S rRNA sequences of the different strains used in this study. All the strains isolated from diseased *Eucalyptus* in South America showed 98–100% sequence similarity to either *P. ananatis* or *P. agglomerans.* In the phylogenetic tree constructed from the AFLP results, the isolates grouped with either *P. ananatis* or in a separate group with high similarity values. It would thus appear that bacterial blight and die-back of *Eucalyptus* is caused by more than one *Pantoea* spp.

## *Fusarium* species associated with *Syzygium cordatum* floral malformation

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Waterberry (*Syzygium cordatum* Hochst.) is a native southern African tree that occurs along stream banks from KwaZulu-Natal northwards to Mozambique. Recently, it has been noticed that waterberry trees are affected by abnormal development of inflorescences. The affected inflorescences are characterized by abnormally enlarged and branched panicles that are sterile and do not produce fruit. A similar floral malformation disease is known on mango, *Mangifera indica,* and it is associated with a number of *Fusarium* species belonging to the *Gibberella fujikuroi* species complex. The objective of this study was to identify the *Fusarium* spp. that are associated with waterberry malformation. Fungi were isolated from diseased flowers using *Fusarium* selective media and identified based on their morphology as well as DNA sequence comparisons. The gene encoding the translation elongation factor 1-$\alpha$ was PCR amplified from each isolate and subjected to restriction digestion with four base-pair cutters. The digested products were separated by electrophoresis, after which the profiles were compared. An isolate representing each of the unique profile was subsequently sequenced and compared with sequences in the *Fusarium* identification database using BLAST. Our preliminary results show that several known *Fusarium* species such as *F. oxysporum* and *F. equiseti* are associated with malformed *S. cordatum* flowers. Several novel species also appear to be present. The relationship between these fungi and their role in the development of waterberry floral malformation remains to be determined.

## Identification of *Botryosphaeria* spp. from *Eucalyptus* planted to feed koala bears at the Pretoria Zoo

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*Botryosphaeria* spp. are endophytes and latent pathogens of numerous woody hosts, including *Eucalyptus.* These fungi cause canker and die-back diseases in exotic *Eucalyptus* plantations in South Africa. Such disease symptoms were observed on the *Eucalyptus* trees grown in Pretoria for feeding koala bears at the Pretoria Zoo. The aim of this study was to identify and characterize *Botryosphaeria* spp. from 20 *Eucalyptus* species maintained by the zoo. Isolations were made from diseased and asymptomatic twigs and leaves. All isolates resembling *Botryosphaeria* spp. were induced to sporulate in culture. Two groups of isolates were distinguished based on anamorph morphology and their identity was confirmed using comparison of ITS rDNA sequence data with known *Botryosphaeria* spp. Preliminary results indicate that isolates belong to the *B. parva/B. ribis* complex and *B. dothidea.* These species were identified on *E. saligna, E. terreticornis, E. microcorys, E. maculata* and *E. ovata.* To further distinguish isolates of *B. parva* and *B. ribis,* sequences of the $\beta$-tubulin and translation elongation factor 1-$\alpha$ gene regions will be used. A hierarchical sampling and isolation strategy is now being followed, to characterize the population and community structure, origin and relative contribution to diseases of these and other *Botryosphaeria* spp.

## First report of oomycete diseases on petunia, sweet basil and *Osteospermum* in South African nurseries

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The genera *Phytophthora* and *Peronospora,* which are classified within the diploid, algae-like oomycetes in the Stramenopile clade of the Chromista, are important plant pathogens of many plant species worldwide. These pathogens are especially problematic in nursery-grown plants where humidity and temperature levels are often conducive to oomycete disease development. Diseased samples of three nursery-grown plants including *Osteospermum, Petunia* × *hybrida* and *Ocimum basilicum* (sweet basil) were received at the Stellenbosch University Disease Clinic. Symptoms on *Osteospermum*s included black stem lesions at the soil level, which expanded into leaf petioles and bases. The *Petunia x hybrida* and *Ocimum basilicum* samples both showed foliar blight symptoms accompanied by either white or brown sporulation, respectively. Isolations from two independently submitted *Osteospermum* samples, as well as two *Petunia* × *hybrida* leave samples revealed the presence of *Phytophthora.* The isolated *Phytophthora* species were identified as *Phytophthora infestans* and *Phytophthora cryptogea* on *Petunia* × *hybrida* and *Osteospermum,* respectively, using internal transcribed spacer region sequence data as well as morphological characteristics. The pathogens causing leaf blight and yellowing on *O. basilicum* leaves were identified as a *Peronospora* species. Pathogenicity tests using all three oomycete species were conducted twice to confirm that the isolated organisms were the causative disease agents. Inoculation of *P. cryptogea* onto *Osteospermum jacundum* and *O. eckloni* both resulted in the development of symptoms similar to the submitted samples. Inoculation of mixed varieties of *Petunia x hybrida* with *P. infestans* showed initial black necrotic spots, followed by the appearance of white sporulation, mostly on the upper side of leaves. The mixed *Petunia* × *hybrida* varieties showed different levels of susceptibility, with susceptible varieties dying 10–14 days after inoculation. Inoculation of *O. basilicum* showed symptoms similar to the submitted samples, including brown sporulation on the lower side of leaves, necrotic lesions and yellowing.

## Characterization of *Fusarium oxysporum* isolates associated with onion basal plates

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*Fusarium* bulb rot, caused by *Fusarium oxysporum* f. sp. *cepae,* is an important post-harvest disease affecting long-term storage of onion bulbs and exports from South Africa. Early detection of latent infection is critical in order to implement preventative control measures. However, these detections are currently impossible because pathogen identification can only be conducted using long, labour-intensive pathogenicity tests. A further complication is that both pathogenic and saprophytic *F. oxysporum* isolates are associated with onions. A study was therefore started to characterize *F. oxysporum* isolates associated with onion tissue as a first step in the development of a molecular detection system of this pathogen. Onion bulbs were collected in a survey conducted in nine geographical regions of South Africa. Seventy-two *F. oxysporum* isolates were collected from the basal plate region of onions and subsequently characterized using pathogenicity tests, as well as partial sequence data of the translation elongation factor 1-$\alpha$ (TEF) and mitochondrial small-subunit (mtSSU) ribosomal DNA genes. The Fusarium Database v. 1.0 (http://fusarium.cbio.psu.edu/cgi-bin/david/blast.cgi) was used to confirm morphological species identification of all isolates as being *F. oxysporum.* A combined phylogeny of the TEF and mtSSU genes was used to investigate the diversity and relatedness among pathogenic and saprophytic *F. oxysporum* isolates associated with onion. Several different pathogenicity testing methods were evaluated in order to identify a reliable pathogenicity test. The studies showed that inoculation of mature onion bulbs from a susceptible variety were the most reliable and consistent method. *Fusarium oxysporum* isolates identified as *Fusarium oxysporum* f. sp. *cepae* all caused typical dry rot symptoms in and around the onion basal plate after incubation periods of 3–4 weeks. In pathogenicity studies, negative controls included inoculations with water, other *Fusarium oxysporum forma speciales* isolates, and known non-pathogenic isolates of *F. oxysporum.* None of these treatments resulted in any basal rot symptoms.

## Morphological and molecular characterization of *Pythium* species in South Africa

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The genus *Pythium* contains many important plant pathogens of several field-grown crops including deciduous fruit trees, vegetables, cereals and ornamentals. *Pythium* species are furthermore important pathogens of crops grown in hydroponic systems. Despite the impor-

tance of this group of straminipilous fungal-like pathogens, little is known about the diversity of *Pythium* species in South Africa. A literature review revealed that only 32 *Pythium* species have thus far been reported in South Africa. This is a small number considering that there are more than 120 accepted *Pythium* species. One factor contributing to the few *Pythium* species reported in South Africa is difficulty in identifying *Pythium* isolates to the species level. *Pythium* species are difficult to identify using only morphological characteristics, which are few in number, show high variability and considerable overlap among species. Furthermore, heterothallic isolates for which a compatible mating type isolate cannot be found, as well as sporangial forms G, T and P groups, are difficult to identify in the absence of oogonia. Molecular techniques can greatly aid in the identification of *Pythium* species that are difficult to identify using only morphological characteristics. Recently, a molecular phylogeny of 116 *Pythium* species was published by Levesque & De Cock (2004), which greatly assists the identification of these species. In *Pythium* the internal transcribed spacers 1 and 2 are valuable species markers and are approximately twice the length of genera in the Mycota, increasing the probability for polymorphisms, although 100% ITS similarity does not always mean conspecificity in all *Pythium* species. The aim of our study was to characterize *Pythium* isolates that have been collected between 1990 and 2005 in South Africa, using ITS sequence data as well as morphological characteristics. We showed that most of the morphological studies could be confirmed using ITS sequence data. However, a few isolates could be identified with more certainty using ITS sequence data, especially in species of which the morphological characteristics are poorly described, or contain overlapping characteristics. *Pythium* species that have not previously been reported in South Africa were identified in our study include *P. graminicola, P. periilum, P. oedochilum, P. nunn, P. vanterpoolii, P. helicoides, P. chamaehyphon* and *P. heterothallicum.* Additionally, two *Pythium* isolates were found that had no high ITS sequence homology with any known species, and thus could possibly represent new species.

## Characterization of *Pythium* species associated with grapevine decline

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Replant and decline diseases of grapevine cause economic losses due to a reduction in quantity and quality of grapes, as well as costs incurred when vineyards have to be replanted prematurely. The specific factors contributing to replant and decline diseases have been poorly addressed, except for the contribution of trunk diseases. *Pythium* species are often isolated from diseased vines, suggesting that they could be involved in the destruction of feeder roots, and thus contribute to decline. The aim of this study was therefore to identify which *Pythium* spp. are associated with grapevines exhibiting decline symptoms. Additionally, the possibility that pathogenic *Pythium* species are distributed through nursery grapevines was also investigated by conducting surveys in grapevine nurseries. Surveys were conducted in five grapevine nurseries (4 climatic regions) in February/March 2005, as well as in 30 established vineyards (10 climatic regions) in February/March, July and September/October 2005. In the nurseries, *Pythium* species were recovered from the roots as well as the crown region of plants, whereas in older vineyards isolates were obtained only from fine feeder roots. Baiting of *Pythium* was performed on some of the soils. *Pythium* species that were obtained through isolations were identified to the species level through sequence analyses of the internal transcribed spacer 1 and 2 regions. *Pythium* species that were identified among 44 nursery isolates included *P. vexans, P. ultimum* var. *ultimum* and *P. irregulare.* Among the *Pythium* species recovered from nursery plants, some were isolated from both crowns and roots (*P. vexans*), whereas other species were isolated only from crowns (*P. ultimum* var. *ultimum*) or roots (*P. irregulare*). The *Pythium* isolates collected from roots of established vines included *P. irregulare* and *P. heterothallicum.* The selection of isolates from the soil included known pathogens such as *P. ultimum* var. *ultimum, P. irregulare, P. sylvaticum* and *P. vexans.* In previous studies, *P. ultimum, P. irregulare* and *P. sylvaticum* were shown to cause substantial root rot of grapevines in South Africa. There are no previous reports of *P. vexans* and *P. heterothallicum* isolated from grapevines in this country, and their pathogenicity therefore needs further investigation.

## Identification of *Botryosphaeria* spp. occurring on *Schizolobium parahybum* in South Africa

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*Botryosphaeria* spp. are endophytes and latent pathogens of many woody plants including *Schizolobium parahybum,* commonly known as the yellow jacaranda or Brazilian fern tree. *Botryosphaeria* spp. give rise to symptoms such as die-back and cankers on susceptible hosts. *S. parahybum* is native to the Amazon region including parts of Ecuador, where it is extensively used as a source of timber and for reforestation. *Botryosphaeria rhodina* has been identified as a component of a serious disease problem in that country. *S. parahybum* is commonly planted as an ornamental in South Africa and these trees also suffer from die-back symptoms similar to those found in Equador. The objective of this study was to determine whether *Botryosphaeria* spp. are present in *S. parahybum* in South Africa and to consider their possible role in disease development. *Botryosphaeria* spp. were isolated from leaves and twigs of asymptomatic and diseased tissue. Isolates were induced to sporulate in culture and identified based on conidial morphology and DNA sequence data for the rDNA internal transcribed spacers, ITS 1 and ITS 2. Selected isolates were inoculated onto one-year-old *S.* parahybum plants. Lesions were measured after six weeks. Based on conidial morphology, three species, *B. parva/ribis, B. rhodina* and *Fusicoccum vitifusiforme,* were identified. Phylogenetic analyses confirmed this result. Isolates belonging to *B. parva/B. ribis* group were morphologically and phylogenetically indistinguishable and this was the most abundant species present. *Botryosphaeria rhodina* was represented by only one isolate and it was the most pathogenic of the fungi considered. Isolates of *B. parva/ B. ribis* and *F. vitifusiforme* were also pathogenic but to a lesser extent than *B. rhodina.* Other than identifying possible causal agents of *S. parahybum* die-back in South Africa, results of this study suggest that locally occurring *Botryosphaeria* spp. colonize trees and that host specificity is relatively unimportant.

## First report of watermelon soft rot caused by *Pectobacterium carotovorum* subsp. *carotovorum* in South Africa

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In 2002 and 2003, a watermelon fruit disease was observed in commercial fields of watermelon in Gauteng and KwaZulu-Natal. Initial symptoms consisted of water-soaked, oval, 3–5 cm lesions on the surface of fruits of 50–80% of the crop. The lesions enlarged in later stages of infection, resulting in severe soft rot. Bacterial streaming from the edges of freshly cut young lesions was clearly visible in a droplet of water under ×100 magnification. Isolations were made from small sections of tissue cut aseptically from the lesion margins. Plant extract was streaked on the following agar media: King's B, Milk-Tween and CVP, and plates were incubated at 26°° for five days. From all lesions, non-fluorescent, non-pigmented, pectolytic bacteria were isolated. Six representative isolates were purified and characterized. All were Gram-negative rods, oxidase and indole negative, strongly fermentative, produced acetoin and $H_2S$ from cysteine, liquefied gelatin and caused soft rot of potato. The isolates were identified as *P.c. carotovorum* based on carbon source utilization (Biolog GN2 micro-plates) and comparison with the Microlog 4.2 database (Biolog Inc., Hayward, CA). Similarity indices ranged from 0.89 to 0.96. Pathogenicity was confirmed on mature, healthy watermelon fruits (cv. Miki Lee). For each isolate, a single fruit was injected subepidermally with 0.1 ml bacterial suspension ($10^7$ cfu $ml^{-1}$ in sterile distilled water). Two control fruits were injected with sterile distilled water. Inoculated fruits were placed in a humidity chamber at 26°C and inspected daily for development of symptoms. Water-soaking, soft, expanding lesions appeared after three days on fruits inoculated with the bacterial isolates. After five days, soft rot developed in all six fruits. The symptoms were identical to those found in the field. No symptoms were observed in control fruits. Bacteria re-isolated from inoculated

fruits were identical to the original isolates. This confirmed that *P.c. carotovorum* was responsible for soft rot of watermelon observed in South Africa. Watermelon is widely grown in South Africa by both commercial and subsistence farmers. Early diagnosis of bacterial soft rot may prevent spread of the disease by introduction of resistant cultivars and correct control measures.

### Characterization and pathogenicity of *Cylindrocladiella* spp. associated with root and cutting rot symptoms of grapevines in nurseries

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Species of *Cylindrocladiella* occur on a variety of hosts, where they are known to act as pathogens or saprobes. Eight species from this genus are currently recognized, of which five have been reported previously in South Africa: *C. camelliae, C. elegans, C. lageniformis, C. parva,* and *C. peruviana.* Isolates of *Cylindrocladiella* were obtained from a newly established 99-Richter grapevine mother vine block exhibiting decline symptoms. The DNA phylogeny of these and additional isolates, also obtained from declining grapevines, was determined by sequencing the internal transcribed spacers (ITS1, ITS2 and 5.8S) as well as $\beta$-tubulin and histone H3 gene regions. This identified four species of *Cylindrocladiella* on grapevines in South Africa: *C. lageniformis, C. parva, C. peruviana,* and a new species described here as *C. viticola,* which forms part of the *C. infestans* species complex. Pathogenicity trials, using wounded and unwounded stem inoculations on green and one-year-old 99-Richter cuttings to determine the pathogenicity of selected isolates of these species, gave inconclusive results.

### Phylogeny of the pine pitch canker fungus, *Fusarium circinatum:* an emerging global view

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Pitch canker, caused by the ascomycete *Fusarium circinatum,* is one of the most serious diseases of *Pinus* species. The disease manifests itself as large resinous cankers accompanied with pitch-soaked wood, crown die-back and stunted growth of mature trees. In nursery stock, this fungus can cause serious root and root collar disease, resulting in large-scale seedling mortality. Globally, pitch canker or the pitch canker fungus is known in the U.S.A., Mexico, Chile, Haiti, South Africa, Spain and Japan, with unconfirmed reports from Italy, Iraq, South Korea and China. In the majority of these locations, *F. circinatum* represents a significant threat to native forests or commercial pine-based forestry. Therefore, our main objective is to gain a better understanding of the global evolution and population biology of *F. circinatum.* In this study we conducted a phylogenetic analysis that included representatives from *F. circinatum* populations from California, Florida, Mexico, Chile and South Africa. DNA was extracted from these isolates and several housekeeping genes were PCR amplified and sequenced. These sequences were then aligned and subjected to various phylogenetic analyses. Analyses of the sequences revealed several nucleotide polymorphisms between the five populations, with fewer differences within populations. These data also allowed us to identify sequence signatures which differentiate among the various populations. Preliminary data suggest that the Chilean populations are more closely related to the isolates from Mexico than those representing the other populations examined.

### Establishing synteny relationships among *Fusarium circinatum, F. subglutinans* and *F. graminarium*

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*Fusarium circinatum, F. subglutinans* and *F. graminarium* are all important plant pathogens. The complete *F. graminarium* sequence provides the basis for a novel method of establishing synteny between the genomes of these three organisms. Twenty-five degenerate primer pairs were designed using putative open reading frames spread over the three largest linkage groups of the *F. graminarium* genome sequence, in order to amplify specific gene regions from all three fungal species. The open reading frames chosen all had significant homology with known highly conserved genes across the plant and animal kingdoms. The designing was done using PrimerExpress 1.0 (ABI) and Light Cycler Probe Design Software (Roche). The 25 primer pairs were used in degenerate PCRs and primers that resulted in amplicons from all three species were selected for further investigation. The amplicons were cloned into the p-GEMT vector and subsequently sequenced. Sequences were subjected to BLAST analysis to determine if the expected gene region had been cloned. Positive sequences were then examined for useful restriction enzyme site polymorphisms between *F. circinatum* and *F. subglutinans* isolates in order to map these gene regions onto the already established AFLP linkage maps for *F. circinatum* and *F. subglutinans.*

### A low-cost, rapid DNA extraction method of microbial DNA from soil for PCR-based applications

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The interaction between soil microorganisms and above-ground organisms is complex and often very fragile. In recent years, it has been shown that the diversity and concentration of microorganisms in soil can have a profound effect on the establishment and survival of above-ground plants. However, our knowledge regarding the different soil communities in South African soils is relatively limited and this is especially true for fungal populations. Previously, studies on the microbial diversity of soils relied on the isolation of especially fungi in pure culture using various growth media. This method is, however, flawed as it favours only culturable and often only fast-growing species. A large number of non-cultureable organisms are therefore not taken into account. Recently, PCR methods have been shown to improve this picture greatly, as it is culture independent as well as sensitive to small amounts of DNA. However, the first step in this process is the extraction of high quality DNA. Difficulties arise when impurities like humic and fumeric acids are present in the samples. Even small amounts of these substances can inhibit PCR reactions. The aim of this study, therefore, was to develop an easy, rapid DNA extraction method for soil samples that can reliably be used in PCR-based methods. DNA was extracted using a modified extraction procedure published for the extraction of fungal DNA. The resultant DNA was found to contain relatively high concentrations of impurities but also high concentrations of DNA. Two purification methods were compared. In the first instance, samples were purified by running them on a 2% agarose gel and the desired band was excised. In a second method impurities were removed by filtering the sample through a commercial resin column. The samples were tested for their applicability for PCR by amplifying the mixed DNA with a number of primer pairs specific to fungi, bacteria, arthropods and plants. Results showed that DNA could be isolated consistently from environmental samples and could subsequently be used reliably in PCR-based methods.

### Fungal biodiversity of indigenous trees and plants in the Northern Cape

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This project focuses on endemic trees of South Africa, specifically in

the Northern Cape, and the biodiversity of the fungi associated with them. Little is known of indigenous trees and their fungal biodiversity, probably because of the limited economic importance of these groups of plants. Because of the unique diversity of plants in South Africa, it is also likely that there is a unique diversity among the mycoflora associated with these plants. To determine the fungal biodiversity of the prominent indigenous flora of the Northern Cape, a representative collection of samples was taken in the Prieska area, situated on the south bank of the Orange River at the foot of the Doringberg. Moisture chambers were set up at the University of Pretoria and plant material was placed on growth medium. Fungal isolations were made and identifications were performed based on morphological characteristics. From the 30 samples collected, 903 fungi were isolated from which 96 different species were identified and 64 identified to genus level. In total, 29 unknown fungi were isolated, of which some included various coelomycetous and basidiomycetous fungi. These results indicate that a wide variety of fungi are associated with indigenous trees and plants in the Northern Cape.

## Reporter gene transformation of the grapevine pathogen, *Phomopsis viticola*, and the biocontrol agent, *Trichoderma harzianum*

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Trunk diseases cause major economic losses in the grapevine industry due to decline and premature dieback as well as reductions in quantity and quality of yields. Recently, *Phomopsis viticola,* the causal organism of cane and leaf blight, was also implicated in the grapevine trunk disease complex. As pruning wounds are the main entry portals, wound protection with chemical, biological or physical barriers is central to effective disease prevention. However, pruning wounds remain susceptible for prolonged periods of time and sustained protection is therefore required. Strains of *Trichoderma harzianum* have shown the ability to form a living barrier in grapevine pruning wounds against pathogen invasion and might also induce systemic host resistance. Little is known about the extent to which these organisms colonize the wound site, their survival in the plant and the *in vivo* biological control mechanisms of *T. harzianum.* To facilitate the histopathological study of host–pathogen–biocontrol agent interactions in grapevine pruning wounds, a study was conducted to transform selected *P. viticola* and *T. harzianum* strains with reporter genes. The latter will allow the visualization and discrimination of these organisms within host tissue among endophytic and pathogenic fungi. A protoplast-based polyethyleneglycol–calcium chloride system was optimized for transformation of *P. viticola* with the green fluorescent protein (GFP) and *T. harzianum* with the red fluorescent protein (DsRed-Express). *Trichoderma harzianum* transformants expressing DsRed-Express were obtained by co-transformation with a plasmid containing the hygromycin B resistance gene as a selectable marker and a plasmid containing the reporter gene DsRed-Express. *Phomopsis viticola* was transformed with a plasmid containing both the GFP and hygromycin B genes. Stable transformants as well as transgene copy number of transformants were investigated through polymerase chain reaction and Southern analysis.

## Sources of variation in the quantification of fumonisin concentrations in maize samples

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Maize is a major component of the diet of millions of people, but fungal contamination and concomitant mycotoxin production are a threat to their food security. *Fusarium* species are the most important mycotoxin-producing fungi that infect maize worldwide. Fumonisins are toxins produced by *F. verticillioides* and *F. proliferatum* and cause leucoencephalomalacia in horses, pulmonary oedema in swine and are hepatoxic and carcinogenic to rats. *F. verticillioides* and concomitant fumonisin production have been statistically associated with oesophageal cancer in humans in South Africa, China, Italy and Iran. Accurate measurement of mycotoxins is essential for determining the safety of grain and its products for consumption. Which grain sampling technique is used is critical in ensuring accurate estimates of mycotoxin contamination. Ninety per cent of variation in toxin levels results from sampling variability. A variety of detection methods exist and their results may differ. Variation is increased by small grain samples and inaccurate evaluation techniques, which may produce false positive and/or negative results. Five maize samples of 50 kg each were collected from various silos in the local maize production areas. Three sources of variation were studied, namely number of replicates, subsample size, and different detection techniques by independent laboratories. Number-of-replication studies, samples and subsample size were analysed for fumonisins using the ELISA technique at ARC-GCI. The number of replicates was determined with 25-g samples using 5-replicate increments from 5 to 25. The size of subsamples was increased by increments of 25 g, starting from 25 g and ending at 1000 g. Three replicates of the five samples were submitted to each of three independent laboratories, namely, the ARC-GCI (ELISA), the Medical Research Council (MRC, HPLC) and SGS Laboratory Services (HPLC) for quantification of toxin levels. The number of replicates and sample size were analysed using ANOVA to obtain coefficients of variance and standard deviations, which were subsequently subjected to a regression analysis. Results from the three laboratories were compared using a correlation analysis. Number of replicates had no significant effect on reducing variation. Sample size, however, significantly reduced the coefficient of variation but had no effect on standard deviation. Laboratory analyses indicated that ELISA reactions correlated significantly with HPLC results of the MRC, but not with those of SGS. Neither did the HPLC results of the MRC correlate with those of SGS. The technique used, sample size, as well as the laboratory where analyses are conducted, appear to be a major source of variation for quantification of fumonisins.

## Innovative technology to improve the storage potential of South African export litchi fruit

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Litchis become unmarketable soon after harvest as a result of browning, decay and mass loss. Although excellent control of browning is achieved through sulphur fumigation, fungal growth still remains a problem. *Penicillium* spp. are among the most common fungi affecting litchis, but a few other fungi have also been associated with this fruit. A study was conducted with HLH Mauritius and McLean's Red cultivars, over three consecutive seasons, to improve the storage potential. Slow-release Grapetek $SO_2$ sheets were placed in three configurations in 2-kg boxes (bottom, top of fruit or both positions). During the 2003/04 and 2004/05 seasons, studies were also conducted to quantify the effect that fruit maturity has on the procedure. Appropriate cut-off points were calculated using the TSS/TA ratio as a maturity parameter. Results clearly showed that $SO_2$ sheets have a beneficial effect when used in combination with commercially fumigated HLH Mauritius and McLean's Red fruit. On day 7 of the shelf-life period, a top sheet reduced fungal growth by 40%, while a bottom sheet and sheets on both sides reduced it by 70% and 80%, respectively. The $SO_2$ residue measured in the aril of HLH Mauritius fruit was within the 10 ppm limit in all three seasons. However, considerable variation occurred with McLean's Red fruit and the residue limits were transgressed whether sheets were used or not. Slow-release fumigation sheets are recommended for both HLH Mauritius and McLean's Red fruit. For HLH Mauritius fruit grown in the Nelspruit area, a maturity cut-off point of 30:1 (TSS/TA) is recommended, whereas a ratio of 40:1 is advised for fruit of the Onderberg area.

## Fungi and their mycotoxins associated with animal feed in South Africa

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The total gross value of South Africa's agricultural production for 2004 is estimated at R71 billion, of which animal products contributed 43.6%. Animal products therefore comprise a major component of the agricultural industry in South Africa. It is estimated that 8 million tonnes of animal feed is produced annually in the country and commodities not fit for human consumption normally end up in the animal food chain. Although regulations against levels of aflatoxins only in animal feed are

in force, it is known that other mycotoxins are also associated with these commodities. Mycotoxins can have a detrimental effect on animals, thereby causing economic losses to farmers. These include poor performance, loss in yields, reduced quality, and even losses of livestock. There is also a chance that the mycotoxins can end up in the human food chain. The aim of this study was to determine the levels of mycotoxigenic fungi associated with various animal feed products available in South Africa. Twenty-four animal feed samples representing five companies, including feed destined for chickens, pigeons, pigs, horses, cattle, dairy cows and sheep, were collected. Fungal enumerations were done and fungi were identified in terms of their morphology. A wide variety of fungi were found in significant numbers, of which the most noteworthy included *Aspergillus flavus, A. versicolor, A. fumigatus, A. candidus, A. clavatus, Cladosporium* spp., *Euroteum chevalieri, E. rubrum, E. repens, Fusarium nygamai, F. subglutinans, F. scirpi, Penicillium citrinum, P. brevicompactum, P. aurantiogriseum, P. chrysogenum, P. griseofulvum, Phoma sorghina,* and *Rhizopus* spp. The presence of these fungi indicates that mycotoxins such as aflatoxins, moniliformin, sterigmatocystin, tenuazonic acid, rhizonin A and tremorgens could be present in the animal feed.

## The successful application of the microsatellite marker *gwm 533* for *Sr2* resistance in a conventional breeding programme in South Africa

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The stem rust resistance gene *Sr2* in wheat (*Triticum aestivum* L.) has remained effective against *Puccinia graminis* worldwide for more than fifty years. *Sr2* resistance is associated with variable levels of disease symptoms and is expressed primarily during the adult plant stage. The *Sr2* resistance phenotype is difficult to score in breeding programmes. Rust infection is not accompanied by a hypersensitive response, often observed in race-specific interactions. It is, however, associated with variable levels of disease symptoms influenced by genetic background and environmental effects. Because the resistance is expressed only during the adult plant stage, classification of lines is usually delayed until they are near maturity. Selection for *Sr2* in breeding programmes is further complicated by the recessive inheritance and the potential effect on the phenotype of other stem rust resistance genes present in the genetic background. Previous studies identified pseudo-black chaff (PBC), a dark pigmentation developing around stem internodes and glumes, to be closely associated with *Sr2.* Because the level of PBC expression is dependent on environmental conditions and genetic backgrounds, PBC can facilitate selection for *Sr2,* but is not a reliable marker across a range of environments. High levels of PBC are also thought to result in yield reduction. It was therefore necessary to test potential markers for *Sr2* in order to exploit this gene to its full potential in the breeding programmes of the ARC-Small Grain Institute. Several markers were tested, but *gwm 533* gave the best results, a 100% hit average in the local genetic backgrounds tested for the presence of *Sr2.*

## Chemical control of seedborne *Fusarium* species in wheat

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Several *Fusarium* species are associated with wheat diseases, ranging from seedling blight and damping-off, to crown rot and Fusarium head blight (FHB). A wide variety of chemical seed treatments are available for the control of various seedborne diseases. Six of these chemicals were tested for their efficacy to reduce survival of *Fusarium* spp. in infected seeds. Naturally infected wheat seeds were treated with three different dosages of seed treatment chemicals, that is, registered dosage, half registered dosage, double registered dosage, and a non-treated control. These chemicals included two concentrations of tebuconazole (15 g $l^{-1}$ and 60 g $l^{-1}$), two different formulations of carboxin/thiram (200/200 g $l^{-1}$), difenoconazole (30 g $l^{-1}$) and guazatine/tebuconazole (300/15 g $l^{-1}$). Seeds were plated onto Van Wyk agar and inspected for fungal growth after 7 days. Fungi were re-isolated onto potato dextrose agar, grouped and identified. Results indicated a reduction of *Fusarium* colonization of the seed from 77.5% in the control to an average of 12.3% in the treated seeds. Guazatine/tebuconazole (double dosage) was most effective, reducing *Fusarium* colonization to 6.5%. Tebuconazole (15 g $l^{-1}$ a.i., half dosage) was the least effective, reducing colonization to 22.5%. The reduction in *Fusarium* survival in treated seed will improve stands and seedling survival. Identification of the remaining fusaria revealed the treatments to be effective against *Fusarium* spp. in the Liseola section, including *F. proliferatum* and *F. verticillioides.* These seed treatments were ineffective against the Discolor section of *Fusarium,* which include *F. graminearum* and *F. culmorum.* Several Fusariums from the Discolor section are known to cause seedling blight of wheat and the majority of farmers still believe that seedborne *F. graminearum* can cause a systemic infection resulting in head blight.

## Antifungal activity of methanol and water extracts from *Tulbaghia violacea* and *Tulbaghia alliacea* against *Fusarium verticillioides*

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*Fusarium verticillioides* (Sacc. Nirenberg) is a fungus of significant economic importance because of its deleterious effects on plant and animal health and the quality of their products. Maize is the primary host of *F. verticillioides,* which produces the fumonisin mycotoxins. Fumonisins have been shown to be carcinogenic in horses and laboratory rats and mice. Naturally derived compounds with antimicrobial properties to reduce fungal growth or mycotoxin production are required for the agro-food industry. This study was undertaken to evaluate the methanolic and water extracts of two South African wild garlic species (*Tulbaghia*) against the mycelial growth of *F. verticillioides* (MRC826) *in vitro*. Methanolic and water extracts from the corms of *T. alliacea* and whole plant of *T. violacea* were prepared at concentrations of 10, 25, 40 and 75% (g/v). For *T. violacea* the leaves were separated from the bulbs and extracted with methanol and water at concentrations of 2, 5, 8, 10, 25, 50 and 75% (g/v). Similar extract concentrations of whole *T. violacea* plants were also included. The synthetic fungicides, fluconazole and itraconazole were prepared at 15, 18, 20, 25, 35 and 40% (g/v) as standard comparisons. The disc diffusion method was used, by loading 50 l of the extract concentrations onto 8-mm-diameter discs, which was then placed on spore ($1 \times 10^6$ spores $ml^{-1}$)-inoculated potato dextrose agar plates. It was observed that that the methanolic extracts of both plant species exhibited more antifungal activity than the water extracts at the same concentrations. *Tulbaghia violacea* showed greater inhibitory effects than *T. alliacea* in methanol and water extracts with the 10% extract. Furthermore, the high fumonisin-producing MRC 826 strain showed greater sensitivity to the bulb than the leave extracts, while the whole plant extract showed the least inhibitory effects. These findings suggest that compounds from the two *Tulbaghia* species might be useful in the development of antimicrobial strategies against *F. verticillioides.*

## Stable transformation of two endophytic *Fusarium oxysporum* isolates with green (GFP) and red (DsRed-Express) fluorescent protein genes

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*Fusarium oxysporum* is a species complex containing well-known soilborne pathogens, as well as non-pathogenic isolates capable of colonizing plants as endophytes. Recent studies have shown that the non-pathogenic endophytes are able to protect agricultural crops against diseases caused by pathogenic members of *F. oxysporum.* In banana, non-pathogenic *Fusarium oxysporum* endophytes have shown some potential as biocontrol agents of the Fusarium wilt (Panama disease) pathogen *Fusarium oxysporum* f. sp. *cubense* (*Foc*), the banana weevil (*Cosmpolites sordidus*), and the root-lesion nematode (*Radopholus similis*). However, the mechanism of biocontrol and the ecology of endophytic *F. oxysporum* isolates are not well understood, and need further investigation. Labelling fungi with different fluorescent reporter genes can greatly aid ecological studies, since they allow visualization of fungi within soil and host tissue. Therefore, a protoplast-based polyeth-

ylene glycol transformation method was used for transformation of two endophytic *F. oxysporum* isolates (CAV553 and Emb2-40). Protoplasts could be produced only from swollen spores using a complex cell wall-degrading enzyme mixture (driselase, cellulase, chitinase and B-glucanase). The transformation efficiency obtained for both isolates was low (0.5–2 transformants $\mu g^{-1}$ vector DNA), using the hygromycin resistance gene as a selectable marker. Nine transformants of each isolate, either expressing the green fluorescent protein (GFP) gene or red fluorescent protein (DsRed-Express) gene, were characterized further. Stable transformation of these isolates, as well as transgene copy number in each transformant, was investigated using Southern analysis. Epifluorescence microscopy was used to select transformants exhibiting the highest level of fluorescence, since isolates varied in their level of fluorescence. Subsequently, only two transformants of each isolate, either expressing GFP or DsRed-Express, were characterized further through growth studies to establish whether the transformation process has altered their fitness.

### Sexual compatibility in *Amylostereum areolatum*

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The homobasidiomycete *Amylostereum areolatum,* together with its vector *Sirex noctilio,* seriously threatens pine forestry in the southern hemisphere. In these areas, the fungus is spread via asexual arthrospores carried by the woodwasp. This is in contrast to its native environments, where *A. areolatum* also reproduces sexually, having a tetrapolar heterothallic mating system. In the model homobasidiomycetes, *Coprinus cinereus* and *Schizophyllum commune,* with this type of mating system, compatibility is determined by two unlinked mating type loci (loci A and B), each with a number of sub-loci. Since nothing is known about these loci in *A. areolatum,* information from the model homobasidiomycetes was used to characterize the mating type loci of *A. areolatum.* For this purpose, degenerate PCR primers based on the mitochondrial intermediate peptidase (MIP) and the pheromone receptor (PR) genes of *S. commune* were used. Resulting PCR products were cloned and sequenced. Subsequently, allele-specific primers for MIP were designed. Amplification using these primers showed that MIP and mating type locus A are tightly linked, because the amplified products co-segregated with one of the mating type locus A alleles (based on hyphal morphology during mating interactions). Sequences from products obtained using the PR gene primers were extended using PCR-based genome walking. These sequenced PR genes most likely represent a sub-locus of mating type locus B, since mating type B phenotypes co-segregate with diagnostic restriction sites in the sequences.

### Microscopy of bioassays to evaluate infectivity of *Tetranychus urticae* Koch by *Beauveria bassiana*

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The economic importance of mite pests in the horticultural industry has focused attention on the development of acaropathogens as commercial acaricides. The cosmopolitan entomopathogenic fungus (EPF) *Beauveria bassiana,* which has been used successfully as a biocontrol agent against many crop pests, was tested in this study as a biocontrol agent of mites. Although its infectivity against mites has been described previously, the objective in the present laboratory study was to evaluate an indigenous isolate for its potential to control the two-spotted spider mite (*Tetranychus urticae* Koch). Adult spider mites were placed on young, sterile detached haricot bean leaves in 1% water agar plates. Conidial suspensions of $10^7$ cells $ml^{-1}$ harvested in bulk from submerged phase cultures were sprayed directly onto the mites. The progression of the fungal infection of *T. urticae* was monitored by means of four different microscopic methods: scanning electron microscopy, light microscopy, phase contrast microscopy, and confocal microscopy on days 0, 1, and 4, mummified mites and finally when the mites showed fungal outgrowth. The mycotic infection of the spider mites by *B. bassiana* displayed a significant degree of virulence. This indigenous EPF can thus be considered to be an effective, low-cost candidate mycoacaricide in an integrated pest management scheme in the South African horticultural industry.

### Comparison of South African entomopathogenic fungus *Beauveria bassiana* with exotic strains for control of the maize pest *Sitophilus zeamais*

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Conventional control measures employed to contain the effects of the maize weevil *Sitophilus zeamais* (Coleoptera) include integrated approaches that combine the use of fumigants, residual chemical insecticides as well as methods of good agricultural practice. Even so, the maize weevil persists for such reasons as its ability to infest grain *in situ* and development of resistance to synthetic pesticides. To this end, biological means to mitigate the deleterious effects of the pest have been sought and evaluated *in vitro* for control of *S. zeamais.* Indigenous strains of the broad-spectrum entomopathogenic fungus *Beauveria bassiana* were isolated from agricultural soils in and around the town of Rustenburg (Limpopo province) using the *Galleria* bait method and compared with exotic strains in bioassays designed to evaluate pathogenicity of both wet and dry infectious propagules. In bioassays to test wet formulations, 8 isolates (3 local and 5 exotic) were evaluated at inundative rates of inoculum adjusted to a concentration of $10^8$ spores $ml^{-1}$. Experiments were laid out in a randomized design, in which 20 adults per replicate were inoculated using four replicates for each isolate by immersing in 0.25 ml of the inoculum for 20 seconds. To evaluate the infectivity of dry spores, 3 isolates were evaluated where mycosed cadavers were used as the carrier to attempt to infect live adults on a food matrix of sterilized maize seed. After a four-day incubation period, all isolates tested with the wet formulations were capable of infecting and killing the target pest, with no statistical variation detected between isolates. The same was evident from the dry spore evaluation, with significant variations ($P < 0.001$) being observed only between control and treated plots after a one month incubation period, with treated plots having mortality levels of >70%. Results showed no difference in infectivity between locally isolated strains and exotic ones, opening the door for further evaluation of indigenous strains in the exploration of biological control agents.

### The effect of irradiation on mycotoxigenic fungi in commercially produced maize cultivars

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Maize is the staple food of people living in South Africa and can be invaded by both storage and field fungi. It is estimated that 25% of grain crops worldwide are contaminated with mycotoxins every year. The cumulative effect that mycotoxins can have on the immune system, combined with the high incidence of HIV/AIDS, places a considerable burden on medical costs in our country. The purpose of this study was to find a way to elimate all viable fungi in 49 commercially produced cultivars in order to conduct resistance trials in a separate investigation. We concentrated on the effect of radiation on the viability of mycotoxigenic fungi in 49 maize cultivars. Two cultivars, representing white and yellow maize, were initially exposed to various levels of gamma-radiation (2, 4, 6, 8 and 12 kGy). Exposure to 8 kGy resulted in a dramatic decrease in the levels of viable fungi without significantly affecting the germination of the maize kernels. All 49 maize cultivars were eventually exposed to 8 kGy gamma-radiation and showed similar results. The levels of *Fusarium verticillioides* were extremely high in the initial samples and were almost non-existent after irradiation. In addition, viable levels of *Fusarium oxysporum* and members of *Cladosporium, Aspergillus, Penicillium, Rhizopus* and *Mucor* were reduced to insignificant numbers. Gamma-radiation proved to reduce the viability of both field and storage fungi, thereby also lowering the potential of further mycotoxin formation.