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## Abstracts

# South African Association of Botanists - Annual Meeting 2006

### Abstracts of papers and posters presented at the 32nd Annual Congress of the South African Association of Botanists held at the Nelson Mandela Metropolitan University, Port Elizabeth, 16–19 January 2006

*The presenter of multi-authored papers is underlined*

★ Awards made to students

#### Plenary Lectures

#### Botany and pharmacy — the child is father of the man

PJ Houghton

*Pharmacognosy Research Laboratories, Research Division of Pharmaceutical Sciences, King's College London, 150 Stamford Street, London SE1 9NH, U.K.*

The interplay between the medicinal uses of plants and the need to classify them botanically is probably found in some form in every human culture. The attraction for human beings of the beauty and usefulness of plants is evidenced in pictorial and written form from many ancient civilizations and still continues to fascinate today. The botanical gardens of Europe arose out of a need to correctly identify useful plants, especially as the colonial expansions from the fifteenth century onwards resulted in the 'discovery' of many new species, some of which have become global crops of immense importance.

The need for a botanical identification which is globally recognized is not appreciated today by many scientists carrying out research into the chemical, biological and clinical properties of plants. The science of taxonomy is vital in grouping plant species so that distinctions can be made between useful, useless and toxic species and also so that predictions can be made about the likely chemical basis for a reputed activity. The use of vernacular names can be confusing so the worldwide acceptance of a common scientific nomenclature, following the work of Linnaeus in the late eighteenth century, has been of immense importance.

There are still situations where botanical identity, traditional uses and current chemical knowledge do not appear to completely interconnect with each other. Studies on the chemical basis of the cytotoxic properties of *Kigelia pinnata* need further development to solve the controversy of whether it is one variable species, or actually consists of several species. This has been complicated by recent work which suggests that endophytic fungi in the ripe fruit may be responsible for at least some of the reputed anticancer effects.

*Cryptolepis* is an interesting genus which displays considerable chemical variety, and this is reflected in the variation of traditional use in different geographical areas. The pharmacognosist might argue for the creation of at least two novel genera because of this.

Examples from Thailand and China have shown that members of different families, as well as genera, can carry the same name and be used for a similar purpose but scientific investigations have shown that the biological effects are much better with some species than others. In contrast, studies on *Alpinia officinarum* have shown that two chemovars appear to exist which are impossible to distinguish botanically, but which have widely different abilities in

suppressing both the growth of cancer cell lines, and the ability of normal cell lines to resist oxidative damage that might result in malignancy.

Botanists and those investigating the chemistry and biological activity of medicinal plants need to develop, maintain and deepen their dialogue so that the scientist can be assured of using the correct material, a skill which perhaps is retained by traditional healers but which is under threat of being lost for ever.

#### Conservation cowboys: Perils and promise of privately owned protected areas

JA Langholz

*Graduate School of International Policy Studies, Monterey Institute of International Studies, Monterey CA 93940, United States of America*

Although governments have traditionally assumed the leading role in establishing national parks and other protected areas, a powerful new trend has emerged in the last decade — the dramatic rise of privately owned protected areas. Many of these areas conserve as much land as nearby national parks, if not more. On one hand, private sector willingness to create protected natural areas comes as welcome relief, as most of the world's land and biodiversity remain unprotected in any formal manner. Conventional approaches have left many countries, including South Africa, well below the international standard of protecting 10% of the terrestrial surface area. On the other hand, the trend brings an entirely new suite of concerns. Who are these actors? What motivates them? Why are their numbers growing? Will they protect the resources well and into the future?

This presentation takes a critical look at the private protected area phenomenon worldwide. It captures the history of private protected areas, explains why they are proliferating, and proposes a typology for the forms they take. It also analyzes their financial aspects (e.g., are they profitable ventures?), social implications (e.g., what is their track record with local communities?) and ecological issues (e.g., are they doing effective conservation?). The race to save biodiversity will be won or lost where most of it occurs — on non-state lands. This presentation describes an important tool for winning that race.

#### Have botanists become the dinosaurs of Biology in the 21st century?

DW Woodland

*Department of Biology, Andrews University, Berrien Springs, Michigan 49104, U.S.A.*

Many individuals have written in recent years that natural history, and more specifically classical botany has fallen out of favour in the major academic institutions of Europe and North America. The discovery of the structure of the DNA molecule by Watson and Crick, initiated a new wave of thought that classic botany–biology was doomed. Reflecting upon the scientific, political and academic developments of the mid-20th century to the present is necessary to understand these philosophical changes. Evidence that botany and other natural history science courses are being replaced by molecular–chemical science based courses can be found by looking at the

class offerings and majors offered by many academic institutions. Because these older disciplines are being replaced, does not mean they do not or will not contribute greatly to contemporary science. Instead, it is likely these sciences can play a role that is being overlooked. The value of classic botany needs to be recognized, strongly promoted and included in biological curricula to provide a needed balance—a holistic approach to the new areas of biology. Understanding the current situation is important to enhance the value of classic botany in the lives of researchers, students, and the public. If this is not done, classical botany, and natural history for that matter, will become a dinosaur, a thing of the past.

## Papers

### Isolation of compounds with antimalarial and antibacterial activity from *Croton steenkampianus*

AM Adelekan, JJM Meyer and AA Hussein

Department of Botany, University of Pretoria, Pretoria 0002, South Africa

Since ancient times humans have been using plants to prevent or cure diseases. Modern antimalarial drugs (artemisinin and its derivatives) have recently been isolated and characterized from ancient herbal remedies of *Artemisia annua*. Malaria infection is increasing by the day as the disease is spreading to new areas. It is also becoming more difficult to treat as the malaria parasite (*Plasmodium falciparum*) is developing resistance against antimalarial drugs. This has generated a need to study other natural herbal resources for antimalarials with a new mode of action.

Acetone, ethanol and hexane extracts of *Croton steenkampianus* have shown good antimalarial activity. Using bioassay-guided fractionation, five compounds (**1**, **2**, **3**, **4** and **5**) were isolated. Compound **1** showed significant activity against Gram positive and negative bacteria while compounds **2** and **3** showed moderate activity. Compounds **4** and **5** showed good antiplasmodial activity. The five compounds were also investigated for cytotoxicity and were found not to be toxic.

### Cell division and in vitro regeneration are not correlated in *Watsonia lepidia* hypocotyls

GD Ascough<sup>1</sup>, JE Erwin<sup>2</sup> and J van Staden<sup>1</sup>

<sup>1</sup>Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

<sup>2</sup>Department of Horticultural Science, University of Minnesota, St Paul 55108, U.S.A.

Control of cell division is vital so that plants can maintain and direct growth and development. Hypocotyls from in vitro germinated *Watsonia lepidia* seedlings were subdivided into four regions and analysed for cell division using flow cytometry. Region 1, at the junction between the root and start of the hypocotyl, had significantly lower cell division activity compared to region 2, while regions 3 and 4 had very little activity. These regions were cultured in media containing benzyladenine (0, 0.5 and 5 mg l<sup>-1</sup>) and  $\alpha$ -naphthaleneacetic acid (0, 0.5 and 5 mg l<sup>-1</sup>) and the response characterised. Surprisingly, region 1 was nearly 500% more regenerative than region 2 despite containing proportionately fewer cells undergoing division. The effects of positional cues restricting pluripotentiality and implication for the totipotency of stem and meristematic cells are discussed.

### Flower abscission in excised inflorescences of three *Plectranthus* cultivars

GD Ascough and J van Staden

Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

Flower abscission in three *Plectranthus* cultivars was investigated to characterise their response with the aim of preventing abscission using ethylene antagonists. Inflorescences exposed to 100 ml l<sup>-1</sup> ethylene gas or placed in various concentrations of ethephon (277, 27.7, 2.77, 0.277 and 0.0277  $\mu$ M) revealed that abscission in *Plectranthus* is mediated by ethylene, but subsequent ethylene production is not autocatalytic. Compounds that compete for the ethylene receptor (100 and 500 ppb 1-methylcyclopropene or 100 and 500 ppm 2,5-norbornadiene) did not reduce abscission, while compounds that blocked protein production (100  $\mu$ M cycloheximide) and non-competitive inhibitors (2 mM silver thiosulfate) prevented ethylene-induced abscission. Preventing ethylene biosynthesis with 1 mM aminooxyacetic acid was ineffective, as was stopping transcription using 0.8 mM actinomycin-D or blocking cell division with 1 mM maleic hydrazide. Our results indicate that flower abscission in *Plectranthus* is mediated by ethylene and regulated at the translational level.

### Optimizing the micropropagation protocol for the endangered *Aloe polyphylla*: can meta-topolins be a replacement for BA and zeatin?

MW Bairu<sup>1</sup>, WA Stirk<sup>1</sup>, K Dolezal<sup>2</sup> and J van Staden<sup>1</sup>

<sup>1</sup>Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

<sup>2</sup>Laboratory of Growth Regulators, Palacky University, 779 00 Olomouc-Holice, Czech Republic

The need for agricultural land coupled with destructive harvesting of plants for traditional and modern medicine and associated illegal trade are imposing serious hazards of extinction in many species. Plant tissue culture offers novel approaches to plant production, propagation and conservation. So far different categories of plant growth regulators have been discovered and are available for use. The search for new plant growth regulatory molecules or compounds is, however, continuing owing to the limitations of the existing ones. Currently BA is the most widely used cytokinin in the micropropagation industry due to its effectiveness and affordability. It, however, has disadvantages such as genetic alteration and abnormal growth in some plants. Naturally occurring zeatin on the other hand is not as widely used as BA and is far more expensive. The use of meta-topolins as an alternative to BA and zeatin, both of which frequently have negative effects in tissue culture was, therefore, investigated. In vitro grown plants of *Aloe polyphylla* were cultured on full strength Murashige and Skoog (1962) basal medium supplemented with sucrose (30 g l<sup>-1</sup>), myo-inositol (0.1 g l<sup>-1</sup>), IBA (0.5 mg l<sup>-1</sup>) and different concentrations of cytokinins (BA, mT, MemT, MemTR and zeatin) solidified with 1% Bacteriological Agar (Oxoid No. 1). Results showed that mT was the preferred cytokinin both in terms of multiplication rate and rooting. The optimum concentration that induced regeneration and rooting is 5.0  $\mu$ M. Also, the problem of hyperhydricity indicated in previous reports was totally controlled. Plants also rooted spontaneously in multiplication medium, thus avoiding the extra rooting step of the protocol. More than 91% of the plants transferred to ex vitro conditions were successfully acclimatized. This result necessitates the need to test meta-topolins in a wide range of plant families for comprehensive evaluation as a potential replacement for BA and zeatin in tissue culture protocols.

### Seasonal variation of naphthoquinones in *Euclea natalensis* subspecies *natalensis*

MJ Bapela, N Lall and JJM Meyer

Department of Botany, University of Pretoria, Pretoria 0002, South Africa

Eleven mature plants of *Euclea natalensis* subspecies *natalensis* (Ebenaceae) growing in natural populations of Tembe National Park, KZN, were selected for investigation. Their roots were harvested over four seasons and then extracted with chloroform to yield dry crude extracts. Quantitative analysis of bioactive antituberculosis naphthoquinones (shinanolone, 7-

methyljuglone, diospyrin, isodiospyrin and neodiospyrin), present in the root samples, was conducted using High Performance Liquid Chromatography (HPLC). Individual naphthoquinones were identified based on the retention time and UV spectrum of purified standards. Their integrated peaks were then quantified by standard curves generated for each compound using linear least-square regression. They were expressed in mg/kg of dry weight plant material.

The concentration of shinanolone and 7-methyljuglone was found to be uniform in all the seasons and no statistical correlation ( $P < 0.05$ ) was found between seasonal changes and their respective contents. Autumn proved to be favourable for both isodiospyrin and neodiospyrin, though a relatively trace amount was present in each sample. These two dimeric naphthoquinones were also not detected in winter. A statistically significant correlation ( $P > 0.05$ ) was found between the levels of diospyrin and seasonal fluctuations. Diospyrin was detected at the concentration of 3189.66 mg/kg during spring season, higher than the other quantified naphthoquinones at any other season. Among all the five naphthoquinones analysed, diospyrin was shown to be always present in every season and we therefore, suggest that this compound can be used as a chemotaxonomic marker of *E. natalensis* subspecies *natalensis*. This could also suggest its ecological significance in natural populations.

### Phylogeographic and taxonomic investigation of the *Arctotis arctotoides*–*microcephala* complex

NP Barker, SD Mitchell and RJ McKenzie  
*Molecular Ecology and Systematics Group, Department of Botany, Rhodes University, South Africa*

*Arctotis* comprises an estimated 60–70 species. Taxonomic relationships in the genus are imperfectly known, and there are a number of species complexes. The taxonomy of the *A. arctotoides*–*microcephala* species complex (predominantly found in the Eastern Cape) is particularly confused. A phenetic analysis based on morphological characters was undertaken using both living and herbarium material. A parallel phylogeographic study based on DNA sequence data of the Internal Transcribed Spacer (ITS) region was conducted to determine if there were any genetic discontinuities in the complex. In the course of this study, one new species has been discovered, and the limits of existing species defined. The results from these two studies are integrated to produce a new classification of species in the complex. The results are also subject to an ecological and biogeographical assessment in order to explain the observed patterns of morphological and genetic diversity.

### Impacts of invasive alien plant clearing on riparian vegetation recovery along riverine corridors in Mpumalanga, South Africa

MMT Beater and ETF Witkowski  
*Restoration and Conservation Biology Research Group, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits 2050, South Africa*

The Working for Water (WfW) programme focuses on controlling invading woody alien plants. The aim of this study was to determine if WfW has been successful in controlling these plants, and to investigate its impact on plant species composition, diversity and vegetation structure of riparian ecosystems on the Sabie River, Mpumalanga, which traverses through grassland and savanna biomes. The 40 Modified Whittaker nested plots that were surveyed in 1996 were resurveyed. Species richness and alpha diversity were higher in the grassland. Species evenness same in both biomes, while beta diversity was high. Invasion intensity was higher in the savanna. Irrespective of time period, soil, litter and grass cover were higher in the savanna, while rock and herb cover were higher in the grassland. Soil organic matter was higher in the grassland, while pH was similar in both biomes. 282 species were found in 2005, compared to 164 species found in 1996.

Although alpha diversity was higher in 2005, beta diversity over time (from 1996 to 2005) was low. Invasion intensity and vegetation structure were similar between the years, indicating that clearing efforts are not succeeding in controlling aliens. Soil, rock and litter cover were higher in 2005 (probably a response to the February 2000 flood event, which cleared much of the vegetation), whereas herb and grass cover were higher in 1996. It is concluded that the grassland was inherently more rich and diverse in terms of species, and had more fertile soils than the savanna, which in turn maintained a higher degree of invasion intensity (possibly due to its position lower in the catchment). With clearing of exotic plants, overall species richness increased, i.e. from 29 tree species in 1996 to 46 in 2005, from 44 shrub species to 82, from 74 herbaceous species to 121, and from 19 grass species to 33. The greatest relative increase was for category 1, 2 and 3 weed species, namely 15 in 1996 to 51 in 2005, a  $>3\times$  increase.

### Identification and halotolerance of an unknown flagellate from an estuary

TG Bell and SD Sym  
*School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits 2050, South Africa*

Microflagellates are largely ignored in ecological considerations simply because of their size and the difficulty that arises when trying to determine their identity. Those that exist in estuarine environments are no exception, so it is largely unknown whether the flora of an estuary is incidental (i.e. present only at the time of sampling) or whether they are adapted to this variable environment.

A photosynthetic, yellow-green, unicellular biflagellate isolated from the Piesangs River estuary in Plettenberg Bay was identified via light and electron microscopy as *Imantonia rotunda* Reynolds (Haptophyta). Cultures of *I. rotunda* were subjected to acclimation and shock halotolerance experiments, at salinities of 35%, 30%, 25%, 20%, 15% and 5%. Experimental data suggest that *I. rotunda* is halotolerant within the range 25% to 35%, but is tolerant of shocks down to 25%. Acclimated cultures at all salinities produced higher yields than shock cultures. Salinities between 20% and 25% are detrimental, but not fatal, and those at or below 20% are lethal. This suggests that the organism is an inshore species, yet capable of growing and reproducing in the marine environment, rather than being an estuarine species. However, it would be able to survive the moderate salinity fluctuations in estuaries that do not experience significant freshwater input. Studies along these lines with other species and used in conjunction with average flow rate data for our estuaries may provide us with a better grasp of the relative importance of this rather neglected group of organisms.

### Not your common or garden seeds! Plant germplasm conservation research at the University of KwaZulu-Natal in Durban

P Berjak  
*School of Biological and Conservation Sciences, University of KwaZulu-Natal, Howard College Campus, Durban 4041, South Africa*

Non-orthodox seeds undergo little or no drying during pre-shedding development, and those falling into the recalcitrant category sustain lethal injury under dehydrating conditions and might also be chilling-sensitive. Hence they are unstorable by 'conventional' means, and hydrated storage of recalcitrant seeds of most species is useful only in the short- to medium-term. Nevertheless, in terms of Target 8 of the Global Strategy for Plant Conservation of the CBD, 60% of threatened species should be available in ex situ collections by 2010: thus alternative strategies are necessitated for species producing non-orthodox seeds — many of which are threatened. We screen and categorise seeds of a wide spectrum of species — particularly red-data species. If non-orthodox, then development of a multi-step handling protocol aimed at cryopreservation of excised zygotic embryonic axes in liquid nitrogen is undertaken. This involves optimising in vitro methodology for plant production from excised axes; treatment to eliminate internally borne mycoflora; responses (paradoxically) to

flash drying; effects of cooling rate to  $-196$  or  $-210^{\circ}\text{C}$ ; optimisation of thawing and rehydration parameters after cryostorage; manufacture of synseeds for germplasm dissemination; and assessment of quality (phenotypically and in terms of DNA integrity) of resultant plants, and the requirements that will ensure field success. Sometimes alternative explants, e.g. apical and axillary buds, or somatic embryos, must be developed. However, the research programme is far from being merely technological: Seed responses—which are many and varied across species – are analysed at each stage of the procedure, using a range of physiological, biophysical, biochemical, microscopical and molecular biological techniques. The ultimate objective of our research programme—which is supported by the Darwin Initiative (UK) in collaboration with the Millennium Seed Bank, Kew—is to establish a cryoconservation centre for the continent, as well as to promote in-depth understanding of cryobiology in theory and practice.

### **Making spatial data (SURGEO) and data management (PIMS) easy and effective**

N Berjak and J Eales

*S3 Technologies, 1 Wembley Terrace, Wembley, Pietermaritzburg 3201, South Africa*

The Surveyed Geography (SURGEO) system allows the user to extract natural resource data, e.g. geology, rainfall, temperatures, soils etc, for South Africa, by entering a coordinate value, in either decimal degrees or degrees/minutes/seconds, into a graphic user interface. The aim of the program is to make GIS data available in a usable format that is easy to extract—no more technical requirements; no more expensive software packages; no need to purchase newer, more expensive hardware. The software operates through the standard Internet Explorer (IE), from any PC and generates an HTML report documenting the requested information. Once retrieved, the output can be ported to any text editing program for further use. As with any information, what you put in is what you get out. With the SURGEO system we ensure that only reputable data is accessible. Through rigorous testing and advice from professionals in the respective fields, we have compiled an initial body of data from which to search and as more information becomes accessible, we are able to update and add datasets. We can even personalise data packages to suit specific user requirements. SURGEO can be set up to run on an individual PC or through an intranet server, providing maximum end user access.

The Plant Information Management System (PIMS) enables supervisors, researchers and students to store, manage, access and modify data regarding past and current 'project' information and their associated data files (\*.xls, \*.doc, \*.jpg, \*.txt). PIMS is an online storage mechanism, which runs through an intranet server and is viewable on any connected PC via their preferred IE, with varying user access rights in terms of overall 'project' management. PIMS will provide supervisors and students the ability to minimise administrative tasks and maximise the time they are able to spend on actual work.

### **To what extent are the alien plant clearing methods currently used in the Western Cape resulting in riparian ecosystem recovery?**

R Blanchard

*Leslie Hill Institute of Plant Conservation, Botany Department, University of Cape Town 7701, South Africa*

Invasive alien clearing has been ongoing for a number of years within South Africa. Clearing has been brought to the fore by the Working for Water project. A major focus has been clearing riparian zones believed to improve surface runoff to rivers. Their main aims are to remove alien vegetation and promote natural vegetation cover, which uses less water than their alien counterparts. Various clearing methods are employed by project managers in charge of alien removal, based on experience and knowledge. Past studies have given managers some more information to work with. However little is known about which methods best promote natural vegetation recovery after alien clearing. This study focuses on the removal of dense alien stands cleared more than two years

prior to sampling to highlight which methods best promote natural vegetation recovery. Since only invaded and cleared sites were sampled another database dealing with pristine riparian zones will be used for comparisons. Preliminary results are to be presented comparing three clearing treatments (fell only, fell and remove and fell and burn) and pristine plots to indicate which method best facilitates natural vegetation recovery.

### **What keeps trees out of grassland? In search of the real keystone herbivore**

W Bond, H Hill and C Staver

*Botany Department, University of Cape Town, Private Bag, Rondebosch 7701, South Africa*

Tree densities are increasing in many grassy ecosystems world-wide. A common explanation for the phenomenon is that heavy grazing reduces competition from grasses promoting tree seedling establishment. In addition, grazing reduces grass fuel loads reducing fire frequencies which also then favours tree seedlings. In the African context, it has also been argued that replacement of the indigenous megafauna, especially elephants and rhinos, by domestic livestock, promoted trees at the expense of grass. We discuss the results of a six year enclosure experiment in a Zululand savanna and what it reveals about controls on woody plant recruitment. The results are surprising and at variance with some long-held assumptions.

### **Estuarine vegetation of the Benguela Current Large Marine Ecosystem**

TG Bornman and JB Adams

*Department of Botany, Nelson Mandela Metropolitan University, P.O. Box 77000, Port Elizabeth 6031, South Africa*

The Benguela Current Large Marine Ecosystem (BCLME) is situated along the coast of south western Africa, stretching from east of the Cape of Good Hope in the south to near the northern geopolitical boundary of Angola. The region is characterized by an arid and semi-arid climate with few large permanently open estuaries. Although the estuaries themselves generally have a low species diversity, they make a large contribution to the diversity of the dry coastal region. Salt marsh and *Phragmites* are the dominant estuarine plant communities from South Africa to southern Angola ( $13^{\circ}\text{S}$  latitude). Further north the higher river flow maintains freshwater sedge communities and from the Longa Estuary northwards mangrove forests of the Central African mangrove eco-region dominate. The activities of human populations in the region have had, and continue to have, a negative impact on the environment of the BCLME. Key threats to estuarine biodiversity and ecosystem health are unmanaged development, abstraction of freshwater and over exploitation of resources. An overview of the biodiversity and ecological functioning of selected estuarine ecosystems as well as key threats will be discussed.

### **Illuminating the xylem to phloem retrieval pathway in *Oryza sativa***

CEJ Botha<sup>1</sup>, N Aoki<sup>2</sup>, GN Scofield<sup>2</sup>, RT Furbank<sup>2</sup> and R White<sup>2</sup>

<sup>1</sup>*Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa*

<sup>2</sup>*Division of Plant Industry CSIRO, Black Mountain, Canberra ACT, Australia*

The uptake of 5,6-carboxyfluorescein diacetate (5,6-CFDA) via the transpiration stream was studied using laser scanning confocal (LSCM) microscopy. The fluorophore 5,6-CFDA was taken up by xylem parenchyma cells adjacent to the metaxylem vessels and tracheary elements, thereafter rapidly moving towards the phloem parenchyma, as well as companion cell thin-walled cell complexes and thick-walled sieve tubes. Upon arrival in the xylem parenchyma, 5,6-CFDA is rapidly cleaved—the resultant 5,6-carboxyfluorescein (5,6-CF), a fluorescent and highly polar molecule, is trafficked cell to cell

via functional plasmodesmata. The retrieval pathway from the apoplast to the phloem using an *Oryza sativa* Os-SU 1-GUS clone *O. sativa* sucrose transporter (OsSUT-1) was explored using Imogene Green and GUS reactions, to specifically localize the promoter within vascular bundles of leaves. We show that the promoter co-localized with vascular parenchyma cells in all bundle classes. Presence of 5,6-CF within thin- and thick-walled sieve tubes confirms the functional links between these phloem elements and adjacent parenchymatic elements in the case of the thick-walled sieve tubes, and between the companion cells and phloem parenchyma cells, in the case of the thin-walled sieve tubes for the first time, and we comment on the significance of the xylem to phloem retrieval pathway.

### Gradients between equilibrium and non-equilibrium dynamics in southern African savannas

GJ Breidenkamp<sup>1</sup>, LR Brown<sup>2</sup> and F Siebert<sup>3</sup>

<sup>1</sup>African Vegetation and Plant Diversity Research Centre, Department of Botany, University of Pretoria, Pretoria 0002, South Africa

<sup>2</sup>Applied Behavioural Ecology and Ecosystem Research Unit, Department of Environmental Sciences, UNISA, Private Bag X6, Florida 1710, South Africa

<sup>3</sup>Department of Botany, University of Zululand, Private Bag X1001, KwaDlangezwa 3886, South Africa

From semi-arid to arid southern African savannas examples of vegetation were described that represent different positions on an equilibrium–non-equilibrium vegetation dynamics gradient. The main determining factor for savanna dynamics is rainfall, which provides plant-available soil moisture. The equilibrium–non-equilibrium gradient is essentially the response of the vegetation along a rainfall gradient, with the moister savanna towards the equilibrium side of the gradient, and arid savanna towards the arid side. Soil texture modifies the position of the different savanna types on the gradient, where the droughty fine-textured soils tend to push the dynamic process towards the non-equilibrium side of the gradient. It is also shown that the woody and herbaceous layers of a particular savanna can occupy different positions on the gradient, implying that, in spite of a very specific tree–grass interaction, these layers may have different dynamic processes. The interplay of soil moisture, soil nutrients, herbivory and fire in savanna dynamics is discussed.

### Fire and vegetation relationships under two fire management systems in Pilansberg National Park

BH Brockett<sup>1</sup>, S MacFadyan<sup>2</sup>, WS Seithlamo<sup>1</sup> and HC Biggs<sup>2</sup>

<sup>1</sup>Pilansberg National Park, P.O. Box 1201, Mogwase 0314, South Africa

<sup>2</sup>Scientific Services, Kruger National Park, Private Bag X402, Skukuza 1350, South Africa

We explored relationships between fire regime descriptors (frequency (or number of fires) and fire interval), and vegetation types, under two fire management systems in Pilansberg National Park each over a nine year period. Pilansberg National Park is a semi-arid savanna (mean annual rainfall 620 mm) and 50,000 ha in size. The two systems were prescribed block burning, and patch mosaic burning. Under the prescribed block burning system the area was subdivided into units of treatment (blocks), which are burnt (using perimeter ignitions) under selected weather and fuel conditions. Criteria of range condition, and grass–fuel loads guided decision-making. If unplanned fires occurred then subsequent planned block-burns were adjusted. The primary goal of a patch mosaic burning system was to produce landscape-scale heterogeneity as a result of applying a variable fire regime in space and in time. Fires were point-ignited (under a range of fuel and weather conditions) and generally allowed to burn out by themselves, and unplanned fires were managed according to a set of rules. The vegetation was classified into 9 types, and mapped at a scale of 6.25 ha. A majority rule was applied and the data was resampled to 25 ha. Fires were mapped onto a 1:50,000 scale map of PNP. Applying a criteria that if  $\geq 50\%$  of a 25 ha grid square burnt then the grid was classified as burnt. Prescribed block burning was applied from 1980 to 1988, and patch mosaic burning from 1989 to 1997. For each nine year period the number of fires per

grid square was calculated, as well as overall for both periods. A relationship was developed between the proportion of area, and fire frequency for the two fire management systems, and for the periods combined. A logistic regression was fitted between the probability of a fire, and the time-since-fire for each vegetation type.

### An integrative in silico strategy for further insight into ripening-related gene regulation in grapevine

AL Burger<sup>1,2</sup>, M Venter<sup>1</sup> and FC Botha<sup>1,3</sup>

<sup>1</sup>Institute for Plant Biotechnology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa

<sup>2</sup>Biotechnology Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa

<sup>3</sup>Current address: South African Sugarcane Research Institute, Private Bag X02, Mount Edgecombe 4300, South Africa

To remain internationally competitive the wine industry is increasingly emphasising the importance of fruit quality for the production of quality wines. The quality of the grape berry is therefore one of the main targets for the genetic manipulation. Two major obstacles in the genetic manipulation of fruit ripening in (*Vitis vinifera* L.), and other non-climacteric fruit, are the lack of understanding of the biochemical and molecular basis of fruit development and ripening, and promoters to regulate transgene expression in the ripening berry.

In this study a fruit- and ripening-specific promoter from grapevine (*mrip1*) was subjected to an integrative in silico analysis using a combination of plant transcription factor (TF) databases and predictive promoter software. A comparative analysis with 19 other promoters, six from grapevine and nine from climacteric fruit, was conducted. The only two other ripening-specific promoters reported to date, viz. the tomato *E4* and *E8* promoters, were also included in the analysis. This study forms a basis for future comparative analysis and the identification of *cis*-regulatory elements involved in ripening-specific gene regulation. It illustrates how probabilistic software combined with experimental data (expression analysis data) can be used for a refined analysis of promoter architecture and to provide a more comprehensive insight in the regulatory mechanisms involved in grapevine fruit ripening. The identification of such regulatory mechanisms is key for strategic modifications in the tailoring of transgene expression and for the development of synthetic promoters.

### Conserving thicket clumps in mined Bontveld

EE Campbell

Department of Botany, Terrestrial Ecology Research Unit, Nelson Mandela Metropolitan University, P O Box 77000, Port Elizabeth 6031, South Africa

Bontveld is thicket–grassveld mosaic vegetation found in the Eastern Cape. It occurs in semi-arid regions, apparently confined to a radius of 200 km from Port Elizabeth. Bontveld landscapes develop where karst geomorphology occurs (i.e. calcrete pavements are interspersed with dolines or sinkholes). The grassveld covers the pavements while the deeper soils of the dolines support thicket clumps. The economically valuable calcrete beneath Bontveld makes this vegetation type highly endangered and mining activities are constrained by environmental remediation. The grassveld is relatively simple to restore, however the thicket clumps will not restore easily. Four environmental management options have been attempted: thicket clump conservation (resulting in a thicket clump perched 2 to 3 m above the mined landscape); thicket clump translocation (moving of a thicket clump with its soil onto the mined landscape); generation of thicket clumps (construction of an artificial doline in the mined landscape followed by filling of this with thicket soil) and generation of thicket clumps by translocation of termitaria onto the mined landscape. After four years of investigation, it is evident that clump conservation is the only successful option; however generation of thicket clumps appears promising. Clump translocation is highly destructive, but may prove to be a long-term solution. Termitaria contribute little to the restoration of Bontveld landscapes.

## Environmental correlates of tree species richness in the indigenous forests of Transkei, South Africa

SG Cawe

Botany Department, Walter Sisulu University, Private Bag X1, UNITRA 5117, South Africa

The importance of past and present climatic conditions in determining the species richness of various biota has long been debated. Whilst past climate (history) has long been regarded as the more important factor, it has been increasingly recognized that current climate is as important, if not more so. In general, climatic factors act at large scale ( $\geq 10\,000\text{ km}^2$  or at distances of 100 km). This allows for a test of whether the coastal and inland forests of the Transkei region are differentiated by climatic factors. Species lists of 85 forest patches (31 coastal and 54 inland) were used for this test. Synthetic climatic data including temperature, precipitation and evaporation as well as altitude, latitude, longitude and forest size were used in the correlation studies. Predictably, altitude differentiated the coastal forests from the inland forests. Within the coastal forests, tree species richness was most strongly correlated with forest size, whilst in the inland forests the mean temperature of the coldest month was the strongest environmental correlate. Significantly, no measure of temperature correlated with tree species richness in the coastal forests, and in the inland region forest size was not a good predictor of tree species richness. The lack of correlation between forest size and tree species richness in the inland forests suggests that these forests are not in equilibrium with their environment. Ambient energy does not play a role in determining tree species richness in the relatively warm coastal region.

## The influence of root chilling on the hydraulic characteristics and photosynthetic ability of three *Eucalyptus* taxa

MD Cheek and NW Pammenter

School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban 4041, South Africa

The Hydraulic Limitation Hypothesis suggests that the ability of a plant to supply water to its leaves can have a significant effect on leaf physiology, including  $\text{CO}_2$  assimilation, and hence growth. To test this hypothesis requires that hydraulic conductivity be manipulated in the long-term. Root chilling is, potentially, one way of doing this. Root chilling has been used in the past to elucidate mechanisms of biomass allocation, low temperature tolerance, root specific activity, water transport and more recently the gating of aquaporins. A number of short term (1–4 weeks) root chilling studies have shown low temperatures to cause a significant decrease in root hydraulic conductance; the most likely reasons for this reduction is the stronger suberisation of the endodermis and the inactivation of aquaporins. In this study the roots of three commercially important forestry taxa – one cold tolerant, one cold sensitive species and their hybrid – were sealed in an airconditioned box and chilled to  $10^\circ\text{C}$  for approximately four months. Growth, a number of hydraulic parameters, gas exchange characteristics as well as root anatomy were then assessed and compared among the taxa.

## Double-glazing, gas reservoirs or corrugated cardboard; which analogue appropriately describes *Brunsvigia orientalis* leaves?

MD Cramer, C Kleizen, IP Newton and C Morrow

Botany Department, University of Cape Town, Private Bag, Rondebosch 7701, South Africa

Leaves of the geophyte *Brunsvigia orientalis* grow adpressed to the soil and have large air-filled channels running the length of the leaf. This prostrate growth habit may facilitate  $\text{CO}_2$  uptake from below the leaf, allow cultivation of a moist micro-climate below the leaf and/or provide temperature

advantages. We tested the hypothesis that prostrate leaves might enable utilisation of soil-derived  $\text{CO}_2$  and attempted to explain the functional significance of the intra-leaf lacunae. Photosynthetic rates of the prostrate leaves were up to  $31\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$  whereas soil  $\text{CO}_2$  efflux rates were up to  $5.4\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ . Approximately 31% of  $\text{CO}_2$  assimilated was through the lower surface of the leaf and we concluded that ca. 1/4 of photosynthesis could depend on  $\text{CO}_2$  released from the soil. The utilisation of this  $\text{CO}_2$  source was expected to alter the  $\delta^{13}\text{C}$  isotope abundance of the prostrate leaves relative to that of leaves held erect by surrounding vegetation, but evidence for this was weak. We separated the upper and lower surfaces of the leaves and found that the lower surface of the leaves did have more negative  $\delta^{15}\text{N}$  than the upper surface, possibly indicating the of increased re-assimilation of photorespiratory  $\text{NH}_4^+$  in the air-space between the prostrate leaves and soil. We determined that the intra-leaf lacunae provided no temperature benefits as a consequence of insulation of the upper — from the lower surface or reduction of transmission of heat to the soil ('double-glazing'). The volume of the lacunae was calculated to be too small to support more than a few seconds of photosynthesis ('gas reservoirs'). The structural arrangement, however, provides the leaf with strength in the longitudinal axis ('corrugated cardboard'), possibly facilitating growth across the surface of the soil and over surrounding small plants.

## A phylogenetic study of *Cineraria* (Senecioneae, Asteraceae)

GV Cron<sup>1</sup>, K Balkwill<sup>1</sup> and EB Knox<sup>2</sup>

<sup>1</sup>School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits 2050, South Africa

<sup>2</sup>Department of Biology, Indiana University, Bloomington, IN 47405, USA

*Cineraria* (Senecioneae, Asteraceae) has long been in need of revision. Phylogenetic studies using molecular and morphological evidence revealed that 11 species should be removed from *Cineraria*. Two new genera (*Bolandia* Cron and *Oresbia* Cron and B.Nord.) have been established to accommodate three of the previously misplaced species. Two species have been reassigned to and another reinstated in *Senecio*; a fourth species has been matched to the recently reinstated monotypic genus *Mesogramma*. The affinities of four species remain unresolved. Species relationships within the genus have yet to be fully resolved, but it is evident that reticulate evolution has played a major role in speciation in *Cineraria*, as seen by the lack of congruence between the chloroplast and nuclear DNA sequences. The ancestral condition of *Cineraria* is proposed as herbaceous with long peduncles and large capitula, deltoid–reniform upper leaves and reniform lower leaves with a cobwebby to tomentose indumentum. A southern African origin for *Cineraria* in the Western and/or Eastern Cape is postulated, based on current distributions of the putative sister genus, *Bolandia* and *C. mollis*.

## Red Listing of threatened species in the Eastern Cape

F Daniels

Threatened Species Programme, South African National Biodiversity Institute, South Africa

The Eastern Cape has a remarkable array of botanical diversity as it houses six of South Africa's nine biomes. Furthermore over 16% of the taxa in the current Red List Database of Threatened Plants occur in the Eastern Cape. The threatened species of the Eastern Cape are of especial concern as the flora of the region is facing continual pressure from habitat loss and degradation through urbanisation and development, invasive alien plants and agricultural activities. Of the 762 Eastern Cape taxa of special concern in the Red List database of plants, 1% are Extinct, 3% Critically Endangered, 4% Endangered, 16% are Vulnerable and 8% are Data Deficient. Those not meeting the IUCN criteria for Threatened status include 13% Near Threatened and 55% Least Concern. All taxa were assessed using the IUCN's criteria (Version 3.1) so that the assessments are compliant with world standards.

Listing the Eastern Cape taxa on the Red Data List and other national and provincial protected species lists will help to ensure their protection through the development and implementation of adequate land conservation and species management plans.

### A review of geography and morphology of the genus *Cussonia* (Araliaceae)

BJ de Villiers and B-E van Wyk

*Department of Botany and Plant Biotechnology, University of Johannesburg, P. O. Box 524, Auckland Park 2006, Johannesburg, South Africa*

The African genus *Cussonia* Thunb. (Family Araliaceae) comprises ca. 20 species and is endemic to Africa, Arabia and the Comores. A previous synoptic revision by Bamps (1974) is now outdated, while a revision of the South African species by Reyneke (1981) has remained unpublished. *Cussonia* and the related genus *Seemannaralia* R. Vig., together with *Schefflera*, J.R. and G. Forst., are critical to an understanding of the evolution and diversity of Araliaceae in the Old World. No molecular systematic study has yet been undertaken, but a preliminary investigation of the family as a whole indicated that *Cussonia* is a monophyletic group sister to the monotypic *Seemannaralia* (Plunkett et al., 1997, 2004; Lowry et al., 2004). Previous studies of *Cussonia* and the monotypic *Seemannaralia* (Burt and Dickinson, 1975; Reyneke, 1981; Strey, 1981) are listed in a recent world checklist and bibliography (Frodin and Govaerts, 2003) but no rigorous comparisons of the morphology of *Cussonia* and *Seemannaralia* species are available. A study of geographical patterns is presented here which already gives some clues as to the morphological diversification of the genus *Cussonia* in Africa.

### Pollinator-mediated selection of *Pelargonium reniforme* as described by inter-simple sequence repeat markers

LR de Wet, NP Barker and CI Peter

*Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa*

Populations of *Pelargonium reniforme* in the Grahamstown area show variation in floral form and two distinct types have been recognized: bright pink flowers with long tubes (up to 50mm), and pale pink flowers with shorter tubes (as little as 8mm). These two forms have been found in a single population, where they both flower simultaneously. This wide range of tube lengths is thought to be attributable to pollinator-mediated selection on the plants. Visitors to the flowers of *P. reniforme* include both butterflies and long-tongued Nemesiine flies to long-tubed plants and Bombyliid flies and Anthrophorid bees to the short-tubed plants. Inter-Simple Sequence Repeat (ISSR) markers are being used in a study to determine whether long- and short-tubed flowering plants from one population are genetically distinct, indicating that there may indeed be reproductive isolation between the two forms as a result of different pollinators.

### Who wants to study plants? Lecturers', teachers' and learners' perceptions

M Doidge

*Marang Centre for Maths and Science Education, University of the Witwatersrand, Private Bag 3, Johannesburg 2050, South Africa*

Who wants to study plants? Is Botany a popular or an outdated science on the road to extinction in South African schools and universities? In the USA and UK, there is concern about the future of Botany in secondary and tertiary institutions. Botany has been seen as boring and irrelevant and is thus underemphasized and neglected in these institutions, with fewer tertiary students choosing to study Botany. Is there a similar decline in interest in South African schools and universities? This paper reports on a study amongst biology teachers and grade 12 learners in 14 Gauteng schools concerning their level of interest in

botany, and amongst 17 SAAB members from universities and research institutions concerning their perceptions of interest in botany at secondary and tertiary institutions. The results are contradictory and at times unexpected as perceptions differ widely. Thus while teachers and lecturers do not believe that learners are interested in botany, learners indicate they are; and whilst lecturers say that teachers are not qualified and not interested, the reverse seems to be true in this study. With regard to enrolment in botany, some universities report on increasing numbers, whilst others report on a decline in numbers. Changing enrolment figures for Botany and the possible causes of an increase or decrease of interest in Botany in different universities will be reported on. The extent to which perceptions of Botany, plant blindness, zoochauvinism, bias in the media, poor teaching and lecturing, and dull botanical content contribute, in these lecturer's opinions, to a lack of interest in Botany will be discussed. Lecturers' views on whether the new curriculum can improve botanical literacy will also be reported on.

### Fire stimulated carbon allocation during re-growth of C3 and C4 subspecies of *Alloteropsis semialata*

GM Donald and BS Ripley

*Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa*

Fire-prone grasslands are generally dominated by C4 grasses. Whether or not the successful recovery of C4 grasses following burning treatments is linked to the presence of the C4 photosynthetic pathway is unclear. The re-growth of the C3 and C4 subspecies of *Alloteropsis semialata* was compared to investigate the possibility that carbon allocation in C4 grasses facilitates C4 domination in fire prone grasslands. Potted C3 and C4 *A. semialata* were frosted and burned in a controlled fire during early spring. Tiller recovery and initiation, leaf elongation rates, leaf number per tiller, time to flowering and biomass allocation were measured. Carbohydrate allocation to tiller organs will be determined. Preliminary results indicated that tiller initiation following frost in burned C3 pots was reduced compared to unburned C3, however, in burned C4 pots, tiller initiation was enhanced compared to unburned C4. Growth of dark control C3 and C4 pots suggests that recoverable underground reserves in C4 pots, but not in C3 pots, contributed to the recovery of tiller growth following frosting and burning.

### Acclimation of adaxial and abaxial stomatal structure and function to increased CO<sub>2</sub> availability in maize leaves

SP Driscoll<sup>1</sup>, A Prins<sup>1,2</sup>, E Olmos<sup>1,3</sup>, DW Lawlor<sup>1</sup>, KJ Kunert<sup>2</sup> and CH Foyer<sup>1</sup>  
<sup>1</sup>*Crop Performance and Improvement Division, Rothamsted Research, Harpenden, Herts AL5 2 JQ, U.K.*

<sup>2</sup>*FABI, Botany Department, University of Pretoria, Pretoria 0002, South Africa*

<sup>3</sup>*CEBAS-CSIC, Department of Plant Physiology, P.O. Box 164, 30080-Murcia, Campus de Espinardo, Spain*

CO<sub>2</sub> is not only a passive substrate in gas uptake processes but it is also involved in signal transduction processes that influence leaf structure and function. We studied the acclimation of abaxial/adaxial morphology and photosynthetic function to CO<sub>2</sub> enrichment in maize leaves by growing them in either 350 or 700 ppm CO<sub>2</sub>, or by growing them in air until the leaf 5 stage and then transferring them to the high CO<sub>2</sub>. High CO<sub>2</sub> availability led to a decrease in whole leaf chlorophyll and protein and also altered epidermal cell size leading to fewer, larger cells on both leaf surfaces. CO<sub>2</sub> uptake rates increased as atmospheric CO<sub>2</sub> was increased up to the growth concentrations on both leaf surfaces. Above these values, CO<sub>2</sub> uptake on the abaxial surface was either stable or increased. In contrast, CO<sub>2</sub> uptake rates on the adaxial surface were progressively inhibited at concentrations above the growth CO<sub>2</sub> value. This suggests that maize leaves adjust their stomatal densities through changes in epidermal cell numbers rather than stomatal numbers and that the response curve of photosynthesis on the adaxial surface is determined by growth CO<sub>2</sub> abundance. Conversely, stomatal function on the abaxial surface is largely independent of CO<sub>2</sub> availability.



## An approach to develop commercially useful products from plant extracts

JN Eloff

*Phytomedicine Programme, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa*

Because plant extracts contain thousands of compounds and many of these compounds have useful biological activities, it is reasonable that one should be able to develop commercially useful products from some of these extracts.

There are several positive factors in developing products from plants: many people are willing to pay a premium for products that can be marketed as “natural” or “organically produced” when using plant extracts e.g. in the production of food, our diverse floral heritage is under exploited in many areas, several companies are interested in this field possibly due to the free for all mentality where products without any real proof of safety is sold if marketed well, our government and funding agencies readily this type of work if the standard is acceptable, many students are interested in this field.

There are also several negative factors: difficulties in changing the academic mindset of many researchers, the requirements for proving safety before registration, the irresponsible people in this field that use legal loopholes to make a quick profit, cost of doing animal experiments for proving efficacy, complications in developing products from plants that have been used by rural communities, difficulties in ensuring quality control of the products, the variability in chemical composition of plants, getting an industrial partner willing and able to commercialize the product, protection of the intellectual property.

Approaches that we have followed to minimize the negative factors and build on the positive factors in successfully developing several commercial products will be discussed.

## Interrogating gradients from sub-continental to site-specific scales: have southern African studies yielded answers to global change challenges?

KJ Esler

*Dept of Conservation Ecology, University of Stellenbosch, Private Bag XI, Matieland 7602, South Africa*

The southern African sub-continent is a biodiversity rich region facing significant global change challenges. Of major concern in the drylands of this region are issues relating to habitat transformation, climate change and invasive aliens. To appreciate the implications of these pressures and to grapple with solutions, we need to understand the selection pressures and drivers that vary over space and time. Gradient based research provides an heuristic tool for hypothesis generation and allows the identification of relationships between ecosystem variables and the drivers of change across all spatial scales. Using examples of field-based gradient studies of plant–environment relationships, the value and limitations of this approach in addressing environmental challenges in southern Africa will be discussed. Examples will be drawn from the Karoo and Fynbos biomes. These studies have focused on a variety of scales ranging from large scale ecological transects in southern Africa to landscape to single sites. All have attempted to infer process from pattern and in doing so to address key management questions. Lessons learnt include the value of combining top–down and bottom–up approaches and the insights gained when linking empirical and experimental approaches to the study of gradients.

## Mechanisms of desiccation tolerance vary among the angiosperm resurrection plants

JM Farrant

*Department of Molecular and Cell Biology, University of Cape Town, Private Bag, Rondebosch 7701, South Africa*

The vegetative tissues of a small group of angiosperms, termed resurrection plants, are able to survive desiccation to 5% relative water

content (RWC), resuming full metabolism on rehydration. We use these plants as models to gain insight into the mechanisms of water deficit tolerance. Research conducted at the physiological, biochemical and molecular levels has shown that there are differences among the various resurrection plants with respect to the mechanisms of protection accumulated during drying. For example, protection against mechanical stress associated with turgor loss is effected by regulated wall folding in the *Craterostima* spp. In the *Xerophyta* spp. and in *Myrothamnus flabellifolius*, stabilisation is achieved by vacuolation (and water replacement within vacuoles) and organelle packing, whereas in the grasses, *Eragrostis nindensis* and *Sporobolus stapfianus*, both mechanisms are used. Wall folding occurs in the mesophyll and organelle packing in the bundle sheath cells. The nature of protection against reactive oxygen species (ROS) also varies among species. Morphological mechanisms used to avoid light-associated ROS include poikilochlorophyllly (e.g. *Xerophyta* spp.) and homoiochlorophyllly (*Craterostigma* spp. and *M. flabellifolius*) and variations in antioxidant protection include the use of, inter alia, 3, 4, 5 tri-*O*-galloylquinic acid in *M. flabellifolius* and 1-Cys peroxiredoxin in the *Xerophyta* spp. Our presentation will discuss these and other protection mechanisms employed in response to desiccation by a range of angiosperm resurrection plants.

## An overview of Red Listing in South Africa

W Foden

*Threatened Species Programme, South African National Biodiversity Institute, South Africa*

The World Conservation Union’s (IUCN) system of Red Listing was developed to provide objective criteria whereby the extinction risk of species from any habitat and taxonomic group can be assessed. Species identified as “Threatened” (i.e. with statuses of Critically Endangered, Endangered and Vulnerable) are therefore those that face the greatest risk of extinction, though at present, the system is not able to evaluate the threat of climate change. Six key Red List user groups have been identified, including conservation agencies; conservation planners; policy-makers; law enforcers; researchers and the public and each of these groups has specific uses of and needs from Red Lists. Over 20 projects and programmes have produced Red List assessments over the past 30 years and current programmes include assessments for plants, reptiles, freshwater organisms and butterflies. Invaluable lessons and best practices can be distilled from the programmes’ experiences and these include guidelines on setting up and running programmes, collecting and managing data and involving experts in conducting and reviewing assessments.

Future Red Listing priorities include initiating assessments for important groups such as invertebrates and marine organisms, as well as repeating and ideally supporting ongoing assessments for other groups. While Red Lists help to inform conservation decisions, they are not directly translatable into conservation priorities and debates about their use in developing the national lists of threatened and protected species and our new National Environmental Management: Biodiversity Act (2004) have been heated, prolonged and are yet to be resolved. Many useful museum and herbarium specimen data remain undigitised and therefore unavailable for Red Listing and the development of data management systems to collect and serve this data and relevant products to Red List users is a further priority. This will enable Red Lists to play a justifiably important role in the proposed national biodiversity monitoring strategy.

## The composition and distribution of the soil seed bank after clearing of alien invasive vegetation in the Albany district, Eastern Cape

S Fourie

*Department of Environmental Science, Rhodes University, Grahamstown 6140, South Africa*

The negative impacts of alien invasive plants have been well documented and have given rise to the National Working for Water programme, which is aimed at eradicating alien invasive plants. However, where ecological thresholds

have been exceeded, ecosystems do not recover after removing alien vegetation, and active intervention in the form of manipulation or modification of the physical environment and/or the vegetation is required to prevent re-invasion or further degradation.

The degree of disturbance, in terms of initial invasion density and age, clearing methods, number of follow-up treatments and fire cycles, will determine whether an ecological threshold has been exceeded. Where areas have been densely invaded for a long period of time, the soil seed bank may have become impoverished, resulting in poor indigenous vegetation recovery after clearing of alien vegetation. In these cases, additional intervention by means of active rehabilitation may be necessary to re-establish a self-sustaining, functional ecosystem.

The recovery potential based on the soil seed bank of an area cleared of alien invasive plants in the Albany District was investigated by means of a seedling emergence study. This indicated that the highest numbers of seeds are found in the upper 0–4 cm of soil, mainly belonging to the genera *Senecio*, *Conyza* and *Helichrysum*. The genera *Anthospermum*, *Chironia* and *Hypoxis* were also well represented. Most of the seedlings emerged from soil taken from the riparian zone.

The number of viable *Acacia* seeds was found to be highest in the zone directly adjacent to the riparian zone, with up to 3000 seeds per square meter and over 2000 seeds per square meter in the riparian zone.

### Waterlogging and oxygen deficiency: possible factors influencing die-back of potted *Chromolaena odorata* plants at Cedara in the KZN midlands

M Gareeb<sup>1,2</sup>, NW Pammenter<sup>2</sup> and JP Bower<sup>3</sup>

<sup>1</sup>ARC-Plant Protection Research Institute, Private Bag X6006, Hilton 3245, South Africa

<sup>2</sup>School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban 4041, South Africa

<sup>3</sup>School of Agricultural Sciences and Agribusiness, University of KwaZulu-Natal Pietermaritzburg, Scottsville 3209, South Africa

The insect biocontrol unit at Cedara in the KwaZulu-Natal midlands conducts research on the highly invasive shrub *Chromolaena odorata*, (L.) King and Robinson (Asteraceae: Eupatorieae). However, over the past few years there has been high mortality of potted *C. odorata* plants even when grown in tunnels, thereby reducing the number of healthy plants available, placing a constraint on biocontrol research. In an attempt to identify possible causes of the problem, plants were grown on elevated grilles in two trials; (winter–spring 2003) and (autumn–winter 2004) in Durban (subtropical region) and Cedara (temperate region). In neither trial did plants grown at either site display any of the die-back associated with mortality. However, results from the 2003 trial indicated that plants in the Cedara tunnel were stressed due to higher vapour pressure deficits and lower transpiration rates compared with the Durban tunnel. In addition, although the total non-structural carbohydrate (TNC) content of plants at both sites was similar, Cedara plants required twice the leaf area to produce the same amount of carbohydrate as those in Durban. A spacing and drainage trial further indicated that plants placed on elevated grilles grew better, and this could be attributed to better drainage, aeration of soil and air-nipping of roots. *C. odorata* plants at Cedara have been placed on elevated grilles for the past 18 months and plant mortality has been reduced by 50%. Die-back of roots due to poor aeration leads to roots being unable to accumulate sufficient carbohydrates to supplement depleted reserves required for overcoming high levels of stress.

### In search of plant functional types in the Kalahari: A response to grazing pressure

C Geldenhuys and MW van Rooyen

Department of Botany, University of Pretoria, Pretoria 0002, South Africa

The vegetation of South Africa has been mapped through the invaluable work of leading plant ecologists. The natural and anthropogenic pressures

exerted on our flora and vegetation necessitate an understanding of the mechanisms of plant responses to those pressures. Overgrazing and desertification are examples of the stressors that affect plant community composition, on both large and small scales. In order to investigate the plant community response to these stressors it has been shown that it is more practical and informative to group plant species according to their functional characteristics into functional types than to try and predict plant response purely based on present and previous range distribution data. The present study involved a survey of the duneveld vegetation of several privately owned farms as well as a communal farm in the southern Kalahari. The farms were subjectively chosen to represent a gradient from under-utilized to over-utilized. Plant community surveys were conducted on the farms and the dominant plants species were sampled for selected functional traits such as seed mass, specific leaf area, plant height, growth form and leaf dry matter content amongst others. Ordination techniques were used to identify plant functional grazing response types. The identification of plant functional types through easy measurable traits will be a great aid to modeling and long-term environmental monitoring approaches.

### A quantitative analysis of plant-growth strategies enabling survival after burial in a low-nutrient beach environment

M Gilbert<sup>1</sup>, B Ripley<sup>1</sup> and NW Pammenter<sup>2</sup>

<sup>1</sup>Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

<sup>2</sup>School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban 4041, South Africa

Few studies have considered the quantitative contribution that a wide range of hypothesized growth strategies may have on the survival of plants in a harsh beach environment. Firstly, the majority of studies investigate the contribution of single plant strategies to growth, and secondly few studies quantify these contributions in the terms that apply to plant survival. This study quantifies the importance of a range of growth strategies, for *Scaevola plumieri*'s survival of burial. In this nutrient limited environment we find that: 1) the primary growth response to burial is an increase in stem production mediated by an increased internode length, not via an increase in leaf production rate, 2) the reallocation of nutrients and/or carbon from senescing leaves plays a major role in the burial response, 3) under normal burial conditions having leaves at the top of a bare stem allows this species to avoid burial of photosynthetic tissue, 4) a stem's burial response is affected by local nutrient supply from the sand that it is buried in, 5) the carbon and nitrogen costs of stem height and leaf area are not altered to allow reduced construction costs when responding to burial and, 6) the magnitude of these responses are sufficient to account for the increased stem growth in response to burial. However, the quantitative role of inter-stem transport and stored carbon allocation are currently being investigated.

### Cell wall beta-glucosidase in rye is involved in plant defence

T Gradin, J Nikus, F Michoux, G Delp and LMV Jonsson

Department of Life Sciences, Södertörn University College, SE-141 89 Huddinge, Sweden

A defence system in rye (*Secale cereale*) and other grasses such as maize (*Zea mays*) and wheat (*Triticum aestivum*) consists of a hydroxamic acid glucoside, 2-O-beta-D-glucopyranosyl-4-dihydroxy-1,4-benzoxazin-3-one (DIBOA-Glc), and beta-glucosidases. The substrate and enzyme are compartmentalized in the intact tissue, but when the tissue is damaged, they come together. The final hydrolysis product, hydroxamic acid aglucone, is toxic and is proposed to be involved in defence against insects and microorganisms. DIBOA has also been shown to be exuded from roots and to inhibit growth of weeds. Here, we investigate a putative role of a cell wall beta-glucosidase in this defence system. The expression on RNA-level and distribution on protein-level in different tissues were investigated by Northern Blot and activity measurements of purified cell wall fractions, respectively. Amounts of mRNA were found to be higher in the basal parts of the plant shoot and slightly higher in the root tip than in the whole root. The enzyme showed slightly higher relative

enzyme activity in the basal part of the shoot. The cell wall enzyme was purified and different substrates were tested. The enzyme showed activity against DIBOA-Glc and typical cell wall substrates. The activity against DIBOA-Glc indicates that the enzyme takes part in defence reactions. The activity against cell wall substrates suggests that the enzyme may be involved in cell elongation processes. The presence of the enzyme in root tips can be explained by the proposed allelopathic properties of rye. Results from cloning, expression in *E. coli* and studies of the recombinant cell wall beta-glucosidase will be discussed. In conclusion, the cell wall beta-glucosidase may have several important effects in rye.

### **Photosynthetic characteristics of *Arthrospira (Spirulina) platensis* grown in commercial scale open outdoor photo-bioreactors: What do the organisms tell us?**

JU Grobbelaar

Department of Plant Sciences, University of the Free State, Bloemfontein 9321, South Africa

The health food market is particularly lucrative and world-wide annual sales are approaching US\$100 billion. *Spirulina* taken as a food supplement has many benefits and although wholesale prices have steadily declined the market for biomass is growing at more than 15% per year. Algal biotechnology is facing many exiting challenges and major developments have taken place in photo-bioreactor design and operation. The crux is to capture solar radiation and to convert it as efficiently as possible into biomass. Using chlorophyll fluorescent transient analyses (JIP) major photosynthetic variations take place over short time intervals, depending on the optical cross section of the cultures, diurnal and seasonal variations in irradiance. Photo-inactivation of PSII and the formation of "Silent Reaction Centres" are central to photo-acclimation and energy capture. Large open outdoor cultures at Musina, Limpopo Province were used to quantify short-term photosynthetic variations and acclimation.

### **Biogeographic trends in the genus *Hermannia***

DC Gwynne-Evans

Department of Botany, University of Cape Town, Private Bag X1, Cape Town 7701, South Africa

*Hermannia* §*Mahernia* (Malvaceae) is currently being revised, and contains almost 100 species, all confined to South Africa. Patterns of distribution are examined, with particular attention to habitat preference and geological specificity. This work provides strong support for the concept of "regions of floristic endemism" and explores affinities between these regions using *Hermannia* distribution data. Broad molecular patterns emerge from the phylogenetic assessment of 100 species based on the nuclear region ITS 4–5. Suggested reasons for these patterns include dispersal method and pollination syndrome. As a genus that has undergone a radiation in arid areas, the possibility of further expansion under climate change is theorised, together with possible threats to the future persistence of many *Hermannia* species.

### **Mycothione reductase inhibition by anti-mycobacterial naphthoquinones isolated from *Euclea natalensis*: Possible mode(s) of biological activity**

CJ Hamilton<sup>1</sup> and N Lall<sup>2</sup>

<sup>1</sup>School of Chemistry and Chemical Engineering, Queens University of Belfast, Stranmillis Road, Belfast BT9 5AG, U.K.

<sup>2</sup>Department of Botany, University of Pretoria, Pretoria 0002, South Africa

The roots of *Euclea natalensis* are used by the indigenous people of southern Africa against various bacterial infections. The naphthoquinones diospyrin, isodyospyrin, and 7-methyljuglone have recently been isolated from root extracts of this plant and been shown to exhibit antimycobacterial activities against *M. tuberculosis*. The activity of 7-methyljuglone is

comparable to that of current antimycobacterial drugs. In *M. tuberculosis*, millimolar concentrations of the low molecular weight thiol mycothiol (MSH) play an important role in oxidative stress management. It is oxidised to the symmetrical disulfide mycothione (MSSM) in the process. The NADPH-dependent enzyme mycothione reductase (MyR) helps to maintain an intracellular reducing environment by reducing MSSM back to MSH. MSH-deficient *M. tuberculosis* show a significant increase in sensitivity to oxidative stress making this redox pathway an attractive biological target for novel antitubercular therapies. Naphthoquinones can potentially perturb this MSH/MSSM redox balance by chemical reaction with MSH and/or oxidation of MSH to MSSM. Furthermore, they are also known to behave as subversive disulfide reductase substrates which result in the generation of toxic superoxide. Herein we describe some mechanistic studies on the interaction (s) of naphthoquinones, isolated from *E. natalensis*, against MSH and MyR and the likelihood of MyR and other disulfide reductases being plausible biological targets for these compounds in *M. tuberculosis*.

### **Lowland sand fynbos restoration: current insights and remaining questions**

PM Holmes

Cape Ecological Services, 23 Dreyersdal Road, Bergvliet 7945, Western Cape, South Africa

Lowland sand fynbos vegetation types are poorly conserved and many remnants are degraded. The two most urgent management objectives in sand fynbos are alien plant control and fynbos restoration. Re-introduction of fynbos species by seed is an order of magnitude cheaper than planting and offers the potential for restoring greater biodiversity. However, establishment from seed will only succeed in a relatively competition-free environment. Compared to mountain fynbos, sand fynbos vegetation is rapidly colonized by herbaceous weeds when disturbed by over-grazing, tilling or alien tree invasion. Competitive species, particularly grasses, become dominant where soil nitrogen is enhanced, either following crop cultivation or invasion by nitrogen-fixing acacias. Thus a key step in restoring degraded sand fynbos is to investigate methods of lowering the competitive weed cover and soil available N. A replicated split-plot field trial was set up in an old cultivated field at Riverlands Nature Reserve in the west coast lowlands. Vegetation was dominated by the perennial grass *Cynodon dactylon* and alien annuals. The entire site was burnt prior to a tilling treatment and subsequent herbicide and C-rich mulch applications. Two indigenous bioassay species were planted and monitored together with weed cover, soil available N and N mineralization rates. Initial results indicate that weed cover was best retarded by herbicide, as tilling alone had a relatively short-lived effect. Mulch addition had no effect on weed cover. Soil available N was low (365 µg/g soil) and changed little following treatment. N mineralization rates were very low (7.3 mg/m<sup>2</sup>/mo) and no significant treatment effects were detected. Bioassay plant establishment was higher in plots herbicided, owing to an indirect effect of reducing mole rat activity and mounding rate via a reduction in weed cover. This resulted in the burial of fewer bioassay seedlings. Pot experiments indicated that the bioassay species were sensitive to mulch addition.

### **Phylogeographic analysis suggests a recent radiation of the southern African endemic genus *Gazania* (Gorteriinae; Asteraceae)**

S Howis and NP Barker

Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

★ **Awarded van Staden Prize for best oral presentation by a PhD student, and best overall student talk**

*Gazania* is a small genus of 16 species endemic to South Africa and Namibia. The genus was last revised in 1959 by Roessler, who noted that

“the formation and delimitation of the species [of *Gazania* can be] extraordinarily difficult”. These small herbs and subshrubs are popular with horticulturalists and gardeners due to the large size and bright colour of their flowers, as well as the ease with which they hybridize to generate novel character combinations. Two nuclear spacer regions (ITS and ETS) and multiple chloroplast noncoding regions were sequenced to investigate correlations between genetically delimited units and morphologically defined taxa. These genetically defined clades are correlated with geographical distributions in an effort to elucidate the climatic or environmental factors that may have shaped the phylogeographic structure of the genus. The current species descriptions are problematically vague for some taxa. ITS data has elucidated a split between Namibian and South African taxa. Low levels of genetic variability in cpDNA data were found, but ITS and ETS sequence data proved more variable. This lack of variability, in conjunction with limited morphological diversity, points to a recent radiation.

### AFLP's and Aizoaceae systematics: *Delosperma* as a case study

L-L Janse van Rensburg<sup>1</sup>, C Mienie<sup>2</sup>, MH Buys<sup>1</sup>, L Mills<sup>3</sup>, P Burgoyne<sup>4</sup>, H Hartmann<sup>5</sup>, NP Barker<sup>6</sup> and L van Rensburg<sup>7</sup>

<sup>1</sup>AP Goossens Herbarium, School of Environmental Sciences and Development, North-West University, Potchefstroom 2520, South Africa

<sup>2</sup>Agricultural Research Council, Grain Crops Institute, Potchefstroom 2520, South Africa

<sup>3</sup>Department of Agriculture, Conservation and Environment, Gauteng Provincial Government, South Africa

<sup>4</sup>SANBI, Pretoria, South Africa

<sup>5</sup>Molecular Ecology and Systematics Group, Department of Botany, Rhodes University, Grahamstown, South Africa

<sup>6</sup>Universität Hamburg, Biozentrum Klein Flottbek, 22609 Hamburg, Germany

<sup>7</sup>Director Research, Focus Area: Environmental Science and Management, North-West University, Potchefstroom 2520, South Africa

The Aizoaceae is enigmatic in terms of species delimitation. Morphological and gene sequence data have been investigated in search of the “holy grail” — all to no avail. We here report results from a study utilizing AFLPs (amplified fragment length polymorphism) in *Delosperma herbeum* and additional seven named species from Gauteng and the adjacent parts of North West, Free State and Limpopo Provinces. For comparison purposes, garden material of the type species, *Delosperma echinatum*, was also included. A total of 103 individuals, representing 33 populations belonging to eight taxa (*D. herbeum*, *D. leendertziae*, *D. davyi*, *D. gautengense*, *D. macellum*, *D. echinatum*, *D. purpureum*, *D. vogtsii*) were analysed using 3 primer combinations, which produced 158 scorable bands. A cluster analysis of the obtained bands clearly demarcates the majority of species. Within *D. herbeum*, clusters correlate broadly with distribution.

### Biological activities, essential oils composition and leaf trichome morphology of four selected *Salvia* species indigenous to southern Africa

GPP Kamatou<sup>1</sup>, AM Viljoen<sup>2</sup>, PM Tilney<sup>3</sup>, RL van Zyl<sup>1</sup>, DH Davids<sup>1</sup>, T Seaman<sup>4</sup> and AC Figueiredo

<sup>1</sup>Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, South Africa

<sup>2</sup>School of Pharmacy, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa

<sup>3</sup>Department of Botany, University of Johannesburg, P.O. Box 524, Auckland Park 2006, South Africa

<sup>4</sup>Division of Pharmacology, Faculty of Health Sciences, University of Cape Town, Observatory 7925, Cape Town, South Africa

<sup>5</sup>Universidade de Lisboa, Faculdade de Ciências de Lisboa, Departamento de Biologia Vegetal, Centro de Biotecnologia Vegetal, C2, Campo Grande, 1749-016 Lisbon, Portugal

*Salvia* species are widely used in traditional medicine to treat various ailments. Twenty-three species are indigenous to southern Africa and have been poorly investigated. The biological activities of the essential oils and the solvent extracts of four related species namely *S. africana-caerulea*, *S. africana-lutea*, *S. chamelaeagnea* and *S. lanceolata* were studied. The essential oils were extracted by hydrodistillation and analyzed by GC and GC/MS. The essential oils displayed good antimalarial ( $IC_{50} < 8.6 \mu\text{g/ml}$ ) and promising anti-inflammatory properties ( $IC_{50} < 77 \mu\text{g/ml}$ ) in comparison to respective controls. The solvent extracts also exhibited potent antimalarial ( $IC_{50} < 26 \mu\text{g/ml}$ ), anticancer ( $IC_{50} < 50 \mu\text{g/ml}$ ), antituberculosis ( $MIC \leq 0.5 \text{mg/ml}$ ) activities but poor anti-inflammatory properties ( $IC_{50} > 100 \mu\text{g/ml}$ ). The toxicity of the essential oils was higher compared to that of solvent extracts ( $P < 0.05$ ) with the  $IC_{50}$  values ranging from 1.87 to 7.24 and from 14.38 to 25.01  $\mu\text{g/ml}$  respectively. The analysis of the leaf trichome morphology with SEM and LM revealed the presence of the non-glandular and glandular trichomes in all four species. The non-glandular trichome, unbranched, composed of 2 to 3 stalk cells, was common to all the species investigated. The glandular trichomes, were single or multicelled in the head, 2–4 short/medium stalk cells were also common to the four species. The composition of the essential oils analysis is also reported and the taxonomical relationship using the essential oils data discussed.

### The potential application of smoke-technology for priming maize seeds

MG Kulkarni, SG Sparg, ME Light and J van Staden  
Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

The stimulatory effects of smoke on seed germination are widely known and utilized in various ways. Smoke has the potential to be used for a variety of applications related to seed-technology. This study highlights the effects of aerosol smoke, smoke–water and butenolide (a compound isolated from plant-derived smoke that stimulates germination) on maize seed germination and seedling vigour. In this study, aerosol smoke, smoke–water and butenolide treatments significantly ( $P < 0.05$ ) improved the germination and seedling vigour of maize. Results from this investigation showed that smoke treatments had a positive response on the short-term growth performance of maize plants. Maize seeds soaked in a solution of butenolide ( $10^{-7} \text{M}$ ) for an hour, followed by surface-drying, and grown for 30 days showed a significant ( $P < 0.05$ ) increase in root and shoot fresh weight compared to that of the control. The plants from the butenolide-treated maize seeds and plants in smoke–water (1:500) drenched pots developed into significantly ( $P < 0.05$ ) taller plants. Additionally, the smoke-treated maize seeds had a higher percentage survival. The best survival percentage was recorded for the drench treatment, which resulted in over 90% survival of maize plants. This study has revealed that the effects of the butenolide and smoke–water extend beyond the germination stimulation of maize seeds and that it can also act to enhance seedling vigour and accelerate plant growth. Findings from the study suggest a possible use of aerosol smoke, smoke–water or butenolide for better growth of field grown maize plants.

### A taxonomic revision of the *Lebeckia sepiaria* species complex

MM le Roux and B-E van Wyk  
Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, South Africa

*Lebeckia* Thunb. (tribe Crotalariaeae, Fabaceae) is a genus of some 33 species endemic to southern Africa. The genus was last revised by Harvey in 1862. The type section (sect. *Lebeckia*) comprises about 12 fynbos-endemic species with needle-like leaves. As a first step towards a complete revision of the section *Lebeckia*, a detailed study was made of the *Lebeckia sepiaria* complex (*L. sepiaria* is the type species of the genus). A detailed study of morphological characters showed that four species should be recognized: *L. sepiaria* (L.) Thunb., *L. ambigua* E. Mey., *L. sarcophylloides* E. Mey. (hitherto known by the

illegitimate later homonym *L. simsiana* Eckl. and Zeyh.), and a new species, *L. brevicarpa* M. le Roux and B-E van Wyk *ined.* Last-mentioned has been confused with *L. sarcophylloides* but is easily distinguished by the ovoid, few-seeded fruits and the localized distribution in the Cedarberg Mountains. Descriptions of the species, diagnostic characters, nomenclature, typification and distribution maps of the four species are presented.

### Developing sustainable propagation and conservation strategies for *Pelargonium sidoides*

FB Lewu, DS Grierson and AJ Afolayan

Department of Botany, University of Fort Hare, Alice 5700, South Africa

*Pelargonium sidoides* is a medicinal plant used by the people of the Eastern Cape for the treatment of various infections including tuberculosis and bronchitis. Traditionally, only the roots of the plant are used for medicinal formulations. In addition to traditional uses, there is a great demand for the plant for export, therefore, the rate of its harvesting and the number of its collectors have increased in recent years. This has led to the drastic drop in the population of the species in the wild. In order to develop sustainable conservation program for the species, various studies were conducted on its general growth and biology. These include the collection of ethnobotanical information, validation of medicinal efficacy of the species, clonal and in vitro propagation methods, germination study of its seeds and its morphological characterization. The results revealed that the roots of the species are used mainly for the treatment of cold related diseases in man and livestock. The rate and methods of harvesting are not sustainable. A comparative study of the roots and shoots of the species suggested that the shoot could as well substitute for the roots in the treatment of bacterial diseases. Clonal propagation of *P. sidoides* was achieved via the vine and petiole. The result of the antimicrobial actions of the species revealed a broad spectrum activity which further validates its uses for the treatment of various infectious diseases by the people of the Eastern Cape.

### The response of juvenile plants to clipping

MH Ligavha-Mbelengwa

Biological Sciences Department, University of Venda for Science and Technology, Private Bag X5050, Thohoyandou 0950, Limpopo Province, South Africa

The study of the effect of disturbance through cutting was carried out on two savanna plants; the plants are *Dichrostachys cinerea* and *Terminalia sericea*. The former plant species is known to be non-lignotuberous whilst the latter species is lignotuberous. We found that both species responded in a similar way to cutting; that is, the two species regardless of whether they were cut once yearly or on a monthly basis for a period of one year recovered from such form of injury. Generally any period of the year proved to be suitable to disturb both the two plant species for the current study. It was only in winter where the plant species were naturally found to stay dormant. Frequency of disturbance did not affect the two plant species in any significant deleterious manner. Except for the winter period both singly and multiply clipped individual plant species were found regenerating within a period of one month. The plant species showed a regeneration waiting period of more than a month when clipped in April, May, June and July. Our study revealed that savanna plants are generally adapted to survive disturbances in their various forms.

### Formation of a germination promoter, 3-methyl-2H-furo [2,3-c]pyran-2-one, from a Maillard reaction

ME Light<sup>1</sup>, BV Burger<sup>2</sup> and J van Staden<sup>1</sup>

<sup>1</sup>Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

<sup>2</sup>Department of Chemistry, University of Stellenbosch, Stellenbosch 7600, South Africa

The ability of plant-derived smoke to act as a germination cue in many species has led to widespread interest in this aspect of seed biology. Recently, 3-methyl-2H-furo[2,3-c]pyran-2-one was identified as the main germination cue from smoke. Heating proteins or amino acids with sugars at 180°C for 30 min produces water-soluble extracts that promote germination. High-performance liquid chromatography (HPLC) indicated that the active compound(s) derived from these reactions co-eluted with the active fraction from a plant-derived smoke solution. Gas chromatograph-mass spectroscopy (GC-MS) showed that the active constituent is identical to the germination cue from plant-derived smoke. The results presented in this paper provide evidence for the formation of the major germination cue found in smoke from ubiquitously occurring organic compounds.

### A revision of the genus *Arctopus* (Apiaceae)

AR Magee, B-E van Wyk, PM Tilney and M van der Bank

Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, South Africa

*Arctopus* is a genus of three dioecious geophytes endemic to the Cape Floristic Region of South Africa. The Khoi-San and subsequently the early Cape settlers held *Arctopus echinatus* in great esteem as a comfort to the sick, hence the Afrikaans vernacular name *sieketroos*. This anomalous genus has several very unusual morphological characters, such as the flat spiny leaves, thick resinous tubers, sessile uniserial female flowers and pseudanthia surrounded by distinctive bracteoles. *Arctopus* shares many morphological and anatomical characters with both of Drude's subfamilies Saniculoideae and Hydrocotyloideae. The generic position of the genus was explored using DNA sequence data (ITS), confirming a taxonomic position within the Saniculoideae and sister to the African endemic Saniculoid *Alepidea*. The unusual vegetative and reproductive morphologies of the three species of *Arctopus*, together with their correct nomenclature, typification and geographical distributions are discussed.

### Seasonal and inter-annual photosynthetic response of representative C<sub>4</sub> species to soil water content and leaf nitrogen availability across a tropical seasonal floodplain

B Mantlana

Global Climate Change Research Group, South African National Biodiversity Institute, Kirstenbosch Research Centre, Cape Town, South Africa

Seasonal wetlands in semi-arid regions witness surface flooding as well as very dry soils, exposure to sometimes very high temperatures and air saturation deficits, and rapid switches between short, sometimes heavy, rainfalls and dry spells during the rainy season—all with a seasonal, often unpredictable, spatial and temporal pattern. As a result very distinct microhabitats develop across an aquatic–terrestrial landscape, which change in aerial extend very rapidly and dynamically within and between years, and which are occupied by a range of accordingly adapted species. We studied gas exchange parameters of three C<sub>4</sub> perennial species, *Cyperus articulatus*, *Panicum repens* and *Imperata cylindrica* to environmental variables that regulate the seasonal leaf CO<sub>2</sub> and water fluxes, to determine the comparative physiological responses of plants that represent a particular microhabitat in a seasonal tropical floodplain in the Okavango River Delta in northern Botswana. These three are the typical C<sub>4</sub> species when moving along a hydrological gradient from the wettest to the driest part across the floodplain.

In all the species, light-saturated net photosynthetic rates ( $A_{\text{sat}}$ ) and stomatal conductance ( $g_{\text{sat}}$ ) decreased with decreasing soil water content encountered over the year, with a seasonal range in  $A_{\text{sat}}$  of approximately 5–45  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and in  $g_{\text{sat}}$  of 0.03–0.35  $\text{mol m}^{-2} \text{s}^{-1}$ . Each species displayed high correlations between  $A_{\text{sat}}$  and  $g_{\text{sat}}$  with a slope that was clearly higher in the two grass species growing in the drier parts of the floodplain, when compared to the sedge representing the moist parts. On diurnal basis did all the species depict the typical decline of  $g_s$  with increasing leaf to air vapour pressure deficit ( $D_l$ ) but both, maximum  $g_s$  at low  $D_l$ , and the rate of change with increasing  $D_l$ , differed between the microhabitats. The species representing the wettest microhabitat (*Cyperus*) had comparatively highest  $g_s$  at low  $D_l$ , highest rate of change as well

as highest ratios of intercellular to ambient CO<sub>2</sub> concentration ( $C_i/C_a$ ) indicative for its adaptation to a moist growth environment that allows for non-conservative water use strategies. This microhabitat calls for high nitrogen use efficiency (NUE) when compared to the drier parts.

In general the lower floodplain community, represented here by a C<sub>4</sub> sedge, differed in many respects in its response from the grasses of the drier floodplain parts and it remains to be evaluated whether across the Okavango Delta, or indeed tropical wetlands and seasonally flooded grasslands species with similar growth forms (grasses when compared with sedges) respond in a similar manner to prevailing environmental conditions.

## The ecology and population biology of several threatened *Delosperma* species in Gauteng Province, South Africa

DN Marom<sup>1</sup>, ETF Witkowski<sup>1</sup> and M Pfab<sup>2</sup>

<sup>1</sup>Restoration and Conservation Biology Research Group, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, P.O. Box WITS, Private Bag 3, Johannesburg 2050, South Africa

<sup>2</sup>Gauteng Department of Agriculture, Conservation, Environment and Land Affairs, Johannesburg, South Africa

Gauteng is the smallest province in South Africa, yet has the highest human density, and also contains high levels of biodiversity. The ecology and population biology of all known subpopulations of threatened Gauteng endemics, *Delosperma macellum*, *D. purpureum*, *D. gautengense* and *D. davyi*, and South African endemic *D. leendertziae*, were studied. *D. herbeum*, a common *Delosperma* species, was also investigated for comparison. *D. davyi* and *D. herbeum* were the most vigorous species with the highest capsule and seed production and fastest growth rates. Seed mass and seed germination were not significantly different between the species and subpopulations, with germination of 91–100%. Germination success was generally positively related to parent plant size and percentage germination was positively related to seed mass. *Delosperma* is predominantly self-fertilizing and at least short-term persistent seed banks exist for all the species. *D. macellum*, *D. purpureum* and *D. gautengense* allocated more to the shoot than *D. davyi* and *D. herbeum*. Reproductive structure allocation was highest in *D. herbeum*, *D. davyi* and *D. leendertziae*. Urbanisation and in turn habitat loss are the main factors threatening all these species, and protection from urbanisation is the key to preserving these species.

## Photodegradation and carryover among mesic and semi-arid grasslands

ML Masubelele and WJ Bond

Botany Department, University of Cape Town, Rondebosch 7700, Cape Town, South Africa

Grasslands vary in the amount of dry matter ('carryover') persisting from one growing season to the next. Grasslands with large carryover accumulate fuel over several growing seasons and grassland productivity can decline unless the litter is burnt off. Here, we explore factors influencing variation in grass litter carryover between mesic and arid savannas. Photodegradation is likely to be more important than microbial decomposition of standing dead litter in the dry season. We compared decomposition rates of different grass species under different shading conditions in a field experiment. The study showed that there were differences in litter disappearance between mesic and semi-arid grasslands with the former more resistant to photodegradation and the latter breaking down more readily in the sun. Thus mesic grasslands accumulate proportionately more carry-over than semi-arid grasslands partly because of the properties of the grass species in each type.

## Sink-strength regulates photosynthesis in sugarcane

AJ McCormick<sup>1,2</sup>, MD Cramer<sup>3</sup> and DA Watt<sup>1,2</sup>

<sup>1</sup>South African Sugarcane Research Institute, Crop Biology Resource Centre, Private Bag X02, Mount Edgecombe 4300, South Africa

<sup>2</sup>School of Biological and Conservation Sciences, University of KwaZulu Natal, Howard College, Durban 4041, South Africa

<sup>3</sup>Department of Botany, University of Cape Town, Private Bag, Rondebosch 7701, South Africa

It has been suggested that sucrose accumulation by sugarcane is regulated at the level of the sink and strong evidence exists that sink tissues exert an influence on the net photosynthetic rates and carbon status of source organs. A role of sugar molecules as effectors of this relationship has also been established. The existence of a robust sugar-dependent relationship between source and sink tissues in sugarcane could represent a potentially fundamental limiting factor for sucrose accumulation in the stalk and consequently plays a major role in overall sucrose crop yield. In this study, the relationship in sugarcane between photosynthetic source tissue and sink material was examined through manipulation of the sink: source ratio of field grown *Saccharum* spp. hybrid cv. N19 (N19). To enhance sink strength, all leaves, except for the third fully expanded leaf, were enclosed in 90% shade cloth for varying time periods. In this way, leaves that served as source were converted to sink, producing an overall increase in plant sink strength. Variations in sucrose, glucose and fructose levels were then measured and the effects on gas exchange characteristics and leaf fluorescence recorded. Following a decline in sucrose concentrations in young internodal tissue and shaded leaves, significant increases in the CO<sub>2</sub> saturated photosynthetic rate ( $J_{max}$ ), carboxylation efficiency ( $V_{Tubisco}$ ) and electron transport rate were observed in non-shaded leaves after 8 days of shading treatment. Changes in carbon partitioning due to shading were examined based on the uptake and translocation of <sup>14</sup>CO<sub>2</sub>. It was concluded that up-regulation of source leaf photosynthetic capacity is correlated with a decrease in assimilate availability to acropetal culm sink tissue. Furthermore, a significant relationship was revealed between source hexose levels and photosynthetic activity.

## Evolution and biogeography of subtribe Arctotidinae (Compositae–Arctotideae)

RJ McKenzie and NP Barker

Molecular Ecology and Systematics Group, Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

Arctotidinae is a small subtribe in Asteraceae comprising approximately 80–90 species in five genera. The majority of the species (c. 60–70) belong to *Arctotis*. The other genera comprise *Arctotheca* (5 spp.), *Cymbonotus* (2 spp.), *Dymondia* (1 sp.) and *Haplocarpha* (9 spp.). The majority of the species occur in South Africa, but three afro-montane species extend northwards to Ethiopia and three species are endemic to Australia. The biogeography of *Arctotis* is particularly interesting as it has centres of diversity in the semi-arid Nama Karoo and Succulent Karoo biomes, as well as the Fynbos and Grassland biomes. However, delimitation of genera in the subtribe has long been problematic and the taxonomy of *Arctotis* is extremely confused. Morphological and molecular phylogenetic methods are being utilised with the aim of producing a species-level phylogeny, resolving the taxonomy and elucidating the group's evolution. Five chloroplast DNA regions (*ndhF*, *psbA-trnH*, *trnT-trnL-trnF*, *rps16* and *trnS-trnM*) and one nuclear DNA region (the Internal Transcribed Spacers [ITS] region) have been sequenced. The data indicate generic concepts in the subtribe need revising. The afro-montane species are indicated to be the earliest-diverging lineage. The Australian species are nested within the subtribe, indicating at least one long-distance dispersal event from South Africa has occurred.

## Genetic structure and evolution of *Ziziphus mucronata* in South Africa

BJ Melson and JM Lamb

School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban 4041, South Africa

*Ziziphus mucronata*, (Family Rhamnaceae) occurs in a wide range of habitats in South Africa. The plant is adapted to grow in most soil types and is easily distinguished by its shiny leaves and sharp thorns. *Z. mucronata* exhibits a

variety of different morphologies in terms of height, and bark, leaf and thorn structure in different regions in South Africa. The aim of this project was to examine the genetic diversity of *Z. mucronata* from different regions, in order to establish whether the plants were genetically different, or whether the differences were environmentally based. If the plants were genetically different, measures could then be taken to ensure the conservation of the different genetic forms. In order to assess genetic differences, the *trnL* intron and the *trnL-F* intergenic spacer (non-coding regions of chloroplast DNA) were sequenced in samples of *Z. mucronata* selected from five different sites across South Africa. The *trnL-F* intergenic spacer was found to be genetically variable within the samples, whereas the *trnL* intron showed no variation. *Z. mucronata* samples from KZN (Hawaan and Shongweni) formed a well-supported monophyletic clade, separate from Pilansburg, Pongola and Augrabies. The samples from Hawaan, in KZN, formed a monophyletic clade, derived from the other KZN samples. Nested clade analysis of the dataset revealed a significant association between the genetic haplotype of the Hawaan sample and its geographical location ( $p=0.008$ ). Maximum parsimony analysis shows that *Ziziphus mucronata* forms a well-supported monophyletic clade, and is distinct from the most closely related outgroup, *Ziziphus ornata*.

### Identifying priority areas and future pressures for threatened plant species in South Africa

B Mohamed, Z Jonas, M Rouget and W Foden  
*South African National Biodiversity Institute, Private Bag X101, Pretoria 0001, South Africa*

This paper focuses on identifying the priority areas for threatened plant species conservation based on species distribution and areas of high pressure (crop potential, afforestation, mining potential, population density, climate change, alien plants, habitat fragmentation and harvesting). GIS was used to integrate spatial analysis and statistical analysis for identifying geographical priority areas where conservation actions are needed. We used measures of irreplaceability for achieving threatened plant species targets to identify priority areas for threatened plant species. This analysis highlights eight regions of maximum irreplaceability (i.e. priority areas) for threatened plant conservation in South Africa. The priority areas were ranked based on their area of high potential for crop potential, mining potential, afforestation, harvesting potential, climate change, population density increase, alien plant invasion potential and habitat fragmentation. This was compared with the number of threatened plant species per priority area.

### Pectic oligomers suppress chitosan-induced resistance reaction in wheat

J Moldenhauer<sup>1</sup>, N Wiethölter<sup>1</sup>, S Mark<sup>1</sup>, I Ortmann<sup>1</sup>, BM Moerschbacher<sup>1</sup> and AJ van der Westhuizen<sup>2</sup>

<sup>1</sup>*Department of Plant Biochemistry and Biotechnology, Westphalian Wilhelms-University Münster, Hindenburgplatz 55, 48143 Münster, Germany*

<sup>2</sup>*Department of Plant Sciences, University of the Free State, Bloemfontein 9300, South Africa*

The interaction between a fungal pathogen and its host plant is initiated by a surface interaction between the fungal and plant cell, most likely an interaction between fungal and plant cell wall or plasma membrane components. The high degree of structural complexity of plant cell wall polysaccharides led to the suggestion that some components might function as latent signal molecules that are enzymically released during pathogen infection and can either elicit or suppress active defense responses by the plant cell. In dicot plants, pectic oligogalacturonides can act as elicitors triggering disease resistance reactions, whereas no endogenous elicitors have so far been found in monocot plants. In contrast, the elicitor activity of a fungal glycoproteogalactan in wheat leaves was suppressed by pectic oligogalacturonides. In this study, we compare the suppressor activities of three different types of pectic oligomers, namely the products of pectin lyase (non-saturated, methylesterified), pectate lyase (non-saturated, non-methylesterified), and endopolygalacturonase enzymes (saturat-

ed, non-methylesterified). None of the pectic oligomers tested exhibited elicitor activities when injected into wheat leaves alone. When the oligomers were co-injected with chitosan as an elicitor into wheat leaves, they all exhibited suppressor activity towards different aspects of induced cellular lignification involved in the hypersensitive resistance reaction. The saturated and non-saturated, non-methylesterified pectate oligomers suppressed the chitosan-induced resistance reactions slightly better than the non-saturated, methylesterified pectin oligomers. The suppressor activity of purified non-saturated pectate oligogalacturonides increased with increasing chain length. Clearly, the suppressor activity of pectic oligogalacturonides in wheat is strongly dependent on the degree of polymerization of the oligomers, but only weakly so on the character of the oligogalacturonides.

### A phytosociological study in the Kruger National Park, south of the Sabie River

RE Mostert<sup>1</sup>, GJ Bredenkamp<sup>2</sup>, F Siebert<sup>3</sup> and T Mostert<sup>2</sup>

<sup>1</sup>*Data Department, South African National Biodiversity Institute, Private Bag X101, Pretoria 0001, South Africa*

<sup>2</sup>*African Vegetation and Plant Diversity Research Centre, Department of Botany, University of Pretoria, Pretoria 0001, South Africa*

<sup>3</sup>*Department of Botany, University of Zululand, Private Bag X1001, KwaDlangezwa 3886, South Africa*

This is a phytosociological study with a difference. Instead of solely relying on data generated by the student, the study aims to draw conclusions from data collected by former KNP officer, Piet Van Wyk, in the southern district of the KNP. This existing data set was collected about thirty years ago, and was never classified or analysed. The original data sheets containing the *environmental* data were lost, however the *floristic* data were captured electronically. Initially this project was aimed at locating the original sample plots marked on aerial photographs, in order to collect environmental data at these sample plots. Since the original aerial photographs could not be traced, *new data* were collected to supplement the existing database. At the same time, environmental data were collected for meaningful plant community classification. This vegetation classification will add to understanding ecosystems in the southern district of the KNP on a finer scale than landscape level. Information sprouting from this study will have a direct significance towards maintaining biodiversity as stipulated in the terrestrial ecosystem management objective of the KNP.

### A review of the taxonomy of the genus *Argyrolobium* (Fabaceae)

AN Moteetee and B-E van Wyk

*Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, South Africa*

*Argyrolobium* is one of six genistoid genera in Africa and is closely related to *Polhillia*, *Melolobium* and *Dichilus*. The 15 tropical African species were revised by Polhill (1968), the three Madagascar species by Du Puy (2000) and the 45 South African species by Edwards (2005). The aim of this paper is to present information about the remaining 11 species, which are distributed in North Africa, the Mediterranean region and western Asia. The correct names, typifications and diagnostic characters of these species are discussed and illustrated and their geographical distributions are recorded for the first time. *Argyrolobium*, together with the other African genera, appears to be sister to the rest of the tribe Genisteae.

### Preliminary investigation of genetic diversity study of *Stangeria* sp. using ISSR fingerprinting

M Mukherjee and JM Lamb

*School of Biological and Conservation Sciences, Howard College Campus, University of KwaZulu-Natal, Durban 4041, South Africa*

The Natal Grass Cycad, *Stangeria* sp., exclusively found in South Africa, exists in scattered populations in grassland and forest ecosystems in a narrow coastal belt from KwaZulu-Natal to Eastern Cape. *Stangeria* is under increasing threat from habitat loss and unsustainable harvesting for traditional medicinal purposes and has been Red Data Listed. The University of KwaZulu-Natal is collaborating with Durban Botanical Gardens, the South African National Biodiversity Institute (SANBI) and other organizations to improve the conservation status of *Stangeria* sp.

*Stangeria* grows in different ecosystems and exhibits markedly variable leaf morphology. The appropriate taxonomic status of the cycad has long been debated. The purpose of the study is to investigate the genetic diversity and molecular systematics of *Stangeria*. The project will explore the genetic distances between *Stangeria* and its nearest cycad relatives, and within populations of *Stangeria* along the extent of its range. Another aim is to identify genetic markers, diagnostic of populations, which might contribute towards the elucidation of evolutionary relationships — essential if the cycad is to be conserved efficiently.

Inter-Simple Sequence Repeat (ISSR) fingerprinting has been reported to be reliable, reproducible and can lead to highly polymorphic multilocus patterns. ISSRs are semi-arbitrary markers amplified by Polymerase Chain Reaction (PCR) in the presence of one primer complementary to a target microsatellite. ISSR fingerprinting was carried out in *Stangeria* samples using anchored and non-anchored primers in various combinations: (CCTA)<sub>4</sub>, (TAG)<sub>4</sub>, (GGAT)<sub>4</sub>, (CAA)<sub>5</sub>, (GA)<sub>9</sub>T, (CAG)<sub>5</sub>, (GACA)<sub>4</sub>, (GATA)<sub>4</sub>. A presence-absence matrix, reflecting the banding patterns, was constructed and neighbour joining analyses was carried out. The unrooted tree showed one cluster specific to the Eshowe region in northern KwaZulu-Natal, several intermediate clusters containing samples from the north, middle and south of the range, and another cluster specific to the southern extent of the range of *Stangeria* in Eastern Cape.

### Sprouting of woody species following cutting and tree/branch-fall in a lowland semi-deciduous tropical rainforest, north-western Uganda

EN Mwavu<sup>1,2</sup> and ETF Witkowski<sup>1</sup>

<sup>1</sup>Restoration and Conservation Biology Research Group, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, PO WITS 2050, Johannesburg, South Africa

<sup>2</sup>Department of Forest Biology and Ecosystems Management, Makerere University, P.O. Box 7062 Kampala, Uganda

Little is known of woody species sprouting in African tropical rainforests where anthropogenic and natural disturbances are less severe. Yet effective management, conservation and restoration of the vegetation require an understanding of responses to disturbances. We report on sprouting among tree and shrub species in 32, 0.5ha plots within Budongo Forest Reserve (BFR), in response to (a) human harvesting for poles and saplings, and (b) disturbances from tree and branch fall. Sprouting ability is generally common among the woody species, with both canopy and sub-canopy trees sprouting prolifically. Of the 121 species affected by these disturbances, 119 from 31 families sprouted from stems, with only *Caloncoba crepiniana* exhibiting root sprouting too. The number of sprouts/stump ranged between 1 and 35 (*Celtis mildbraedii*). Generally the highest mean ( $\pm$ SE) number of sprouts/stump recorded was  $16.3 \pm 1.8$  ( $n=3$ ) sprouts/stem for *Rawsonia lucida*, while the lowest was 1 for a number of species. Number of sprouts/stump differed significantly among families (Kruskal-Wallis  $H=182.63$ ,  $P<0.0001$ ) and species ( $H=256.26$ ,  $P<0.0001$ ). There were also species-specific significant differences in the height ( $H=39.92$ ,  $P=0.0297$ ) and basal diameter (BD) ( $H=52.34$ ,  $P=0.0011$ ) of the leading sprout. BD of stump ( $\chi^2_1=6.62$ ,  $P=0.0101$ ), height of stump ( $\chi^2_1=38.52$ ,  $P<0.0001$ ) and height at emergence of first sprout ( $\chi^2_1=74.42$ ,  $P<0.0001$ ) were significant predictors of sprouting ability for the species. However, BD of stump was not a significant predictor ( $\chi^2_1=2.44$ ,  $P=0.1179$ ) when only species with  $\geq 10$  stumps were included in the regression model. Overall there were very few dead sprouts on the sprouting stumps. Our study reveals that semi-deciduous tropical rainforests not only have a high proportion of sprouting species, but also a greater incidence of sprouting stems, further advancing our understanding of their

vegetation dynamics. With the ever increasing human population, and loss of woodlands outside BFR, sprouting will be an important factor in maintaining the highly sought after woody species for saplings, poles and timber. However further information is needed on the success of sprouting as a means of persistence, and the external management and site factors that influence sprout growth for these and others species of silvicultural importance.

### Factors contributing to dwarfing in the mangrove *Avicennia marina*

G Naidoo and A Kaliden

School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban 4041, South Africa

In Richards Bay, South Africa, *Avicennia marina* frequently exhibits a distinct productivity gradient, with tree-height decreasing markedly from 6 to 10m in the fringe zone to less than 1.5m in the dwarf zone within an elevational distance of 120m. In this investigation, we compared soil physico-chemical conditions between fringe and dwarf *A. marina* and the constraints imposed by these differences on mangrove ecophysiology and productivity. In the dwarf site, soil salinity, total cations, electrical conductivity and soil concentrations of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Cu}^{2+}$  were significantly higher than those in the fringe zone. Soil water potential and the concentration of soil P, however, were significantly lower in the dwarf site by 142% and 29% respectively. In the leaves,  $\text{Na}^+$  was the predominant ion and its concentration was 24% higher in dwarf than fringe mangroves. Leaf concentrations of  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$  and P, however, were significantly lower in dwarf mangroves. Carbon dioxide exchange, quantum yield of Photosystem II (PS II) electron transport and electron transport rate through PS II were significantly reduced in dwarf plants by 48%, 39% and 37% respectively. The results suggested that high soil salinities, low soil water potential and P limitation contribute to dwarfing in *A. marina*.

### Assessing the beauties: Red Listing of the Iridaceae and Orchidaceae

K Naidoo

Threatened Species Programme, South African National Biodiversity Institute, South Africa

The Iridaceae comprise about 1800 species with 65 genera recognised worldwide, and just over half of them, 38 genera, occur in southern Africa. There are about 27 genera, 707 species recorded in the Cape Floral Region alone. The Iridaceae was one of the major families assessed within the Red Data List of Threatened Plants of Southern Africa. South Africa boasts a large variety of endemic members of the Iridaceae families, but a significant number of these however have become threatened with extinction. The Irids are attractive plants with striking flowers which make them a favourite among collectors, though not all genera do well under cultivation. The Iridaceae is one of the largest and most challenging families assessed within the Red Data List of Threatened Plants of Southern Africa. The Irids are more particularly difficult to assess as some of them are fire-dependent and some taxa may remain dormant as bulbs for long periods not all plants come up at once. However with the expertise and extensive field knowledge of Peter Goldblatt and John Manning, the Iridaceae have been assessed according to the IUCN's categories and criteria resulting in 56.3% least concerned, 12.1% near threatened, 20.8% vulnerable, 5.1% endangered, 3.1% critically endangered, 2% data deficient, 0.2% extinct in the wild and 0.2% extinct.

Another major family that attracts attention because of the beauty and rarity of its members is the Orchidaceae. The orchid family includes over 20000 species worldwide and is therefore one of the largest families of flowering plants. Orchids, like the Irids, are well represented in southern Africa where 467 species in 53 indigenous genera occur, of which 65% are endemic. The contention in this family comes from the fact that orchids are very widely distributed throughout the tropics. Those that are endemic to South Africa however are top priority and those that get over our borders from



elsewhere and have a restricted population in South Africa on which other populations elsewhere of the same species are dependent are also given priority. Orchid tubers are a source of food throughout Africa and this has caused significant declines in the populations of some species, which are on the brink of extinction due to this trade. Orchids are also used medicinally and this is a further pressure on the survival of those species. Conducting Red List Assessments of the family is challenging because orchids tend to be very widely distributed throughout the tropics, but those that are endemic to South Africa, as well as those with restricted populations in South Africa but on which populations in neighbouring countries are dependent, are also given priority. The Irids and Orchids of South Africa face a number of pressures such as development, urban sprawl, harvesting, and alien invasion. Furthermore, orchid tubers are a source of food throughout Africa and this has caused significant declines in the populations of some species, which are on the brink of extinction due to this trade. Many orchids are also used medicinally and this is a further pressure on the survival of those species. Many experts were contacted in order that consulted while conducting the final assessments and workshops were held to further assess and review this family. The Orchids have been assessed according to the IUCN's categories and criteria resulting in 60.1% least concerned, 13.5% near threatened, 10.4% vulnerable, 5.7% endangered, 4.1% critically endangered, 4.1% data deficient, and 2.1% extinct.

### Genetic diversity of *Warburgia* across Africa

P Naidoo and JM Lamb

*School of Biological and Conservation Science, University of KwaZulu-Natal, Durban 4041, South Africa*

The level of genetic diversity of *Warburgia*, Africa's most widely utilised medicinal plant, has not been explored. *Warburgia* is the only genus of the Canellaceae family to be found in Africa, where it is endemic. Five species of *Warburgia* have been reported, whilst other sources have reported a single species that occurs across this range. As a result of predation of developing fruits by unidentified insect larvae and overexploitation by local communities, *Warburgia* is classified as endangered in the southern part of Africa, where it faces extinction. This research project utilised the techniques of DNA sequencing and fingerprinting to determine the genetic diversity of *Warburgia* populations across Africa. The regions sequenced were the *trnL* (UAA) intron and the *trnL/trnF* intergenic spacer. These non-coding regions of the chloroplast genome were amplified and sequenced. A DNA fingerprinting procedure namely inter-simple-sequence-repeats (ISSR) was utilised since it has been reported to be highly sensitive for the detection of polymorphisms at population and individual levels. Analysis of the intron sequences of all *Warburgia* samples was identical. Thus, this region did not provide information about genetic variability amongst the *Warburgia* populations. Neighbour joining, maximum parsimony, maximum likelihood and Bayesian analysis of the intergenic spacer revealed congruent trees. All *Warburgia* samples formed a clade with 100% bootstrap support. Nested within this clade was a sub-clade comprising the Kenyan and Tanzanian samples which formed a single cluster with two discrete sub-clusters (Kenya vs Tanzania). ISSR data revealed similar information with that of the sequencing data. Overall, the results indicate a gradient of genetic change in *Warburgia* from the south to the north of its range in Africa, with the possibility of one taxonomic unit in the south, and two in the north (Kenya and north-east Tanzania) respectively.

### Is there value in investigating seed germplasm conservation on the basis of families rather than individual species?

#### A case study on the family Amaryllidaceae

S Naidoo, NW Pammenter and P Berjak

*School of Life and Environmental Sciences, University of KwaZulu-Natal, Durban 4041 South Africa*

This study attempted to establish a short- to medium-term seed storage regime, and cryopreservation protocol for the seeds of *S. punicus*, *A.*

*belladonna*, *N. huttoniae*, *N. humulus*, *H. humulus humulus*, *H. coccineus*, *B. gregaria*, *B. orientalis*, *Boophane disticha*, *S. discifera*, *N. filifolia*, *H. deformis*, *N. bowdenii*, *H. bakerae*, *Crinum macowanii* and *C. bulbispermum*, all of which belong to the family Amaryllidaceae. The seeds of all species exhibited non-orthodox post-harvest characteristics. Moist-storage at 6°C significantly extended seed storage longevity but there was a progressive decline in seed viability over storage time. Excision and subsequent encapsulation of embryonic axes in calcium alginate beads were more effective in limiting fungal infection and lengthening moist-storage longevity (at 6°C) than storage of whole seeds. Typical of recalcitrant seed behaviour, embryonic axes lost viability on rapid dehydration to water contents as high as, and in some cases in excess of, 0.4 g g<sup>-1</sup>. Desiccation-sensitivity was species-specific, differed with developmental status and was not correlated with shedding water content or drying time. Inter-species differences in desiccation-sensitivity were not related to habitat-specific environmental characteristics but parent plants were not confined to any particular biome or vegetation type. Maximum viabilities after cryopreservation, across all species, were recorded for embryonic axes that were rapidly cooled, by direct immersion in nitrogen slush (-210°C). Maximum post-thaw viability was not correlated with embryonic axis water content and ranged between 30% and 75% for embryonic axes partially dehydrated to water contents between 0.14 ± 0.08 and 0.31 ± 0.09 g g<sup>-1</sup>. In eleven of the fifteen species investigated here maximum post-thaw viability was obtained only when axes were cryoprotected with glycerol or sucrose. Results of the current contribution suggest the value, certainly for the Amaryllidaceae, of investigating post-harvest seed behaviour and germplasm conservation on the basis of families rather than individual species.

### Carbon allocation in transgenic sugarcane with altered sucrose metabolism through expression of 1-SST

T Nicholson, BI Hockett and FC Botha

*South African Sugarcane Research Institute, Private Bag X02, Mount Edgecombe 4300, South Africa*

Two genetically modified sugarcane (*Saccharum* spp. hybrids) lines were used as a tool for investigating carbon partitioning. The genetic modification involved the expression of the gene encoding sucrose:sucrose 1-fructosyl transferase (1-SST) which facilitates the transfer of a fructosyl moiety from one sucrose molecule to another to produce the trisaccharide 1-kestose. Transcription of 1-SST and production of 1-kestose in these lines were confirmed by cDNA gel blot hybridisation and high pressure liquid chromatography (HPLC) respectively. HPLC confirmed that both transgenic lines produced significantly ( $p < 0.05$ ,  $N = 72$ ) more total sugar than the control line. This implied that 1-kestose was produced as an additive to glucose, fructose and sucrose in the transgenics. To determine whether carbon allocation had been altered, <sup>14</sup>C whole-plant radiolabelling experiments were conducted. A portion of the leaf subtending internode five was enclosed in a sealed polyethylene bag and fed with radiolabel (9.25 GBq NaH<sup>14</sup>C<sub>3</sub>O<sub>3</sub>) for one hour. Thereafter, the bag was removed and six selected internodes spanning the culm were harvested in quadruplicate from plants of the two transgenic and one non-transgenic (control) lines after one, seven and twenty-one days. The amount of radiolabel in the insoluble and soluble fractions in each internode was determined by liquid scintillation counting. There was no significant difference ( $p > 0.05$ ,  $N = 72$ ) between the lines in the distribution of total radiolabel down the culm. However, in one transgenic line, the relative proportion of radiolabel in the soluble fraction per internode was significantly ( $p < 0.01$ ,  $N = 72$ ) more than that in the insoluble fraction. This suggested that carbon allocation had been altered differentially in this novel genotype. The implications of this are being explored and will be reported.

### Quality control variables for *Aloe* gel

C O'Brien and B-E van Wyk

*Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, South Africa*

There is a need for uncomplicated analytical methods to determine the quality of aloe gel in commercially important species. The *Aloe vera* industry is a well-established, multi-billion dollar industry. This industry has developed many standards for determining a good quality gel. A local *A. ferox* gel industry has developed recently but no attention has yet been given to quality control variables. In this study, the following variables and methods were explored for their potential use in standardizing aloe gel:

#### 1. Gel discolouration

Anthraquinone presence in gel results in unwanted colour changes (from clear in fresh gel to pink in non-stabilized gel). Colour change potential of a gel can be determined by subjecting the gel to a treatment with sodium hydroxide.

#### 2. Organic acids and acidity (pH)

Organic acids such as malic acid and lactic acid show potential as markers for gel 'freshness'. These acids can be recorded using a specialized high performance liquid chromatographic method.

#### 3. Conductivity

In the fruit juice industry, it is known that conductivity levels increase in aged juice. This variable appears to be species-specific.

#### 4. Free and hydrolyzed sugars

Sugars are derivatized into alditol acetates and analyzed using gas chromatography. This method is of some diagnostic value in identifying the source species of a gel.

### Chemical variation in three African tonic plants (*Sutherlandia frutescens*, *Ziziphus mucronata* and *Arctopus echinatus*)

D Olivier<sup>1</sup>, FR van Heerden<sup>2</sup>, CF Albrecht<sup>3</sup> and B-E van Wyk<sup>3</sup>

<sup>1</sup>Department of Chemistry, University of Johannesburg, P.O. Box 524, Auckland Park 2006, South Africa

<sup>2</sup>Department of Chemistry, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

<sup>3</sup>Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, South Africa

*Sutherlandia frutescens* (Fabaceae), *Ziziphus mucronata* (Rhamnaceae) and *Arctopus echinatus* (Apiaceae) are three well known medicinal plants that have been described as tonics because of the large number of different ailments for which they are traditionally used. *Sutherlandia* infusions are known to contain free amino acids (L-canavanine, GABA, arginine, asparagine and several others), a cyclitol (pinitol), hitherto unidentified flavonoids and bitter substances (triterpenoid glycosides). As part of an ongoing research project, a chemical variation study was done on the genus *Sutherlandia* in order to explore the pattern of major triterpenoids and flavonoids. Extracts of selected plants were subjected to isolation and structure elucidation techniques and two novel triterpenoids were characterized. *Arctopus* species (*A. echinatus*, *A. dregei* and *A. monacanthus*) are rather invariable and contain kaurenoic acids, sugars, triterpenoids and unusual phenolic compounds. The alkaloid pattern in *Z. mucronata* was investigated by means of GC-MS analysis. The root, bark and leaves of several trees from different localities were also compared in terms of triterpenoid, flavonoid, amino acid and sugar content. The three genera are similar in their extreme chemical diversity and this seems to agree with the wide diversity of ailments for which they are used in traditional medicine.

### On the hydraulic characteristics of vascular plants

NW Pammenter

School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban 4041, South Africa

During transpiration water moves in the liquid phase from the soil, through the plant to the leaf. This flow of water is driven by the hydrostatic pressure difference (estimated as water potential) between the leaf and the soil and encounters resistances in the pathway. The study of plant hydraulics concerns itself with the driving forces, the magnitude and location of the

resistances, and the impact these can have on leaf physiology. The Hydraulic Limitation Hypothesis suggests that a high resistance will either give rise to a low flow, or require a large driving force, implying a low leaf water potential. This can lead to stomatal closure and so potentially to low rates of photosynthesis and hence growth. Furthermore, the water flowing through the xylem conduits is under tension, the magnitude of which is influenced by the resistance to flow. Excessive tensions can lead to water column rupture (cavitation events) which will increase resistance to flow, and plants differ in their vulnerability to cavitation events. Thus hydraulic characteristics may influence growth rates, size of large trees, and plant response to water stress. This paper will review some of the current concepts and controversies concerning the hydraulic characteristics of vascular plants and offer some thoughts on their significance.

### Physicochemical and phytochemical evaluation of *Cephalandra indica* fruits

MB Patil and HJ Pramod

KLES College of Pharmacy, JNMC Campus, Nehrunagar, Belgaum-590010, Karnataka, India

Initially the physicochemical evaluation is done by considering the parameters like organoleptic characters, extractive values, loss on drying, ash values etc and further the herb is investigated for chemical constituents like triterpenoids, alkaloids, flavonoids, lipids and proteins as per standard procedures. Extraction of crude powdered drug is done with ethanol and later the ethanolic extract was subjected to quality chemical investigation and was found to be compliant with the WHO standards.

### Different approaches for the cryopreservation of embryonic axes of *Ekebergia capensis*, a species producing recalcitrant seeds

R Perán, P Berjak, NW Pammenter and JI Kioko

School of Life and Environmental Sciences, University of KwaZulu-Natal, Durban 4041, South Africa

Cryopreservation is an ideal genetic-resource conservation technology which facilitates long-term preservation of plant material. Because of the specific characteristics of recalcitrant seeds, i.e. desiccation sensitivity which eliminates quiescence (as occurs in orthodox seeds), long-term storage by conventional methods cannot be used. Hence, cryopreservation constitutes the only tool for long-term germplasm conservation of species producing recalcitrant seeds. Despite the availability of appropriate explants and refinement of technique, other factors are also important in establishing a successful cryopreservation protocol which requires that normal plants will be developed. These include: determination of the optimal development stage (for seeds) and manipulation of post-cryopreservation conditions. It was found that embryonic axes excised from red *Ekebergia capensis* fruits consistently afforded material that withstood the stresses of rapid dehydration and liquid nitrogen ( $-196^{\circ}\text{C}$ ) and sub-cooled nitrogen ( $-210^{\circ}\text{C}$ ) immersion. In this study, embryonic axes from mature seeds excised with and without part of the cotyledon attached were used as explants. Desiccation/cooling and encapsulation–dehydration/cooling techniques were applied to both types of explants (noting that the term 'cooling' is used in preference to 'freezing'). High survival values were obtained by both methods (80–100%), and germination was consistently followed by root production, but a maximum of only 20% of shoot production from axes with part of cotyledon attached, following encapsulated–dehydrated/cooling. However this percentage was improved to 54% when axes that had been processed via desiccation/cooling protocol were cultured on the recovery medium supplemented with  $4.44\mu\text{M}$  BA. The inclusion of this plant growth regulator promoted vigorous multiple adventitious shoot formation from the wound sites left by cotyledon excision, overcoming the lack of shoot production from the apical meristem.

## Doing the twist: A test of Darwin's cross-pollination hypothesis for pollinarium reconfiguration

CI Peter<sup>1,2</sup> and SD Johnson<sup>1</sup>

<sup>1</sup>*School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa*

<sup>2</sup>*Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa*

Mating success in plants depends largely on the efficiency of pollen dispersal. For hermaphrodite plants, self-pollination, either within or among flowers, can reduce mating opportunities because of pollen and ovule discounting and inbreeding depression. Self-pollination may be particularly detrimental in plants such as orchids and asclepiads that package each flower's pollen into one or more pollinia which, together with accessory structures, comprise a pollinarium. Darwin proposed that physical reconfiguration of pollinaria serves as a mechanism for reducing the likelihood of self-pollination. To be effective, the time taken for pollinarium reconfiguration would need to exceed the time spent by a pollinator on a plant. We investigated pollinarium reconfiguration (including pollinarium bending, pollinium shrinking and anther cap retention) in 17 orchid and two asclepiad species. We found a strong positive relationship between reconfiguration time and the duration of pollinator visits. Reconfiguration times were also consistently longer than pollinator visit times. These results provide strong support for Darwin's idea that this mechanism promotes cross-pollination.

## The taxonomic value of fruit structure in African *Peucedanum* (Apiaceae)

N Phephu, B-E van Wyk and PM Tilley

*Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, South Africa*

*Peucedanum* as currently circumscribed is a genus of 120 to 140 species native to Africa, Eurasia and North America. At least 63 species are widespread in Africa of which 31 occur in southern Africa. The genus is characterized by an exceptionally variable habit (woody to herbaceous) and dorsally compressed, ribbed fruits that are conspicuously winged along the margins. A morphological and anatomical comparison was made between the fruits of eight African groups and also with *Peucedanum* species from other parts of the world. The fruit size, wing structure (narrow or broad) and rib oil ducts (present or absent and their position) proved to be the most taxonomically valuable and interesting characters.

## SA's succulents under threat: A Red Listing update

L Potter

*Threatened Species Programme, South African National Biodiversity Institute, South Africa*

Despite increasing awareness about the world's growing numbers of threatened species, the plight of succulent species often continues to be overlooked. This is exacerbated by a scarcity of basic taxonomic, ecological and distribution information of many succulent taxa, such as a number of members of the Mesembryanthemaceae and Euphorbiaceae families. The exceptional beauty of many succulent species has enchanted horticulturists and succulent enthusiasts for centuries and has led to increasing pressure by collection from the wild.

The Threatened Species Programme is currently compiling a Red List for South Africa's threatened plants, which includes 775 succulent taxa of special conservation concern. The taxa are assessed according to the IUCN's categories and criteria (Version 3.1) in order to ensure that the assessments comply with international standards. The Red List is a useful guideline, which documents the extinction rates of species and thereby highlights some priority areas for conservation.

## Phylogenetic analysis of *Encephalartos* species [(Zamiaceae) Cycadales] employing Random Amplified Polymorphic DNA (RAPD) markers

S Prakash and J van Staden

*Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa*

Cycads are the most primitive surviving gymnosperms. Today they are on the verge of extinction. They are unique as they are seed bearing and the seeds are naked. *Encephalartos* is the second largest genus of the order Cycadales comprising 65 species and 2 sub-species. Randomly amplified polymorphic DNA (RAPD) markers were employed to assess the genetic relatedness between 21 species of *Encephalartos* collected from the Botanical Gardens at Durban and Pietermaritzburg. Out of 120, 10-mer random primers used for initial screening, 35 were selected for studying the genetic relationship on the basis of their polymorphic nature and the reproducibility of results. A total of 378 bands were scored among which 336 bands (89%) were polymorphic and 42 (11%) were monomorphic. Primers OPA-11, OPA-12, OPB-20, OPC-08, OPC-10, OPC-15, OPD-02, OPD-17 and OPD-20 elicited 100% polymorphic bands. The polymorphism of the primers ranged from 0.8 to 4.2 with an average of 2.45. Primer OPB-09 had minimal polymorphism while OPC-10 showed maximum polymorphism. The resolving power of the primers ranged from 2.47 to 17.33. The similarity matrix values ranged from 0.516 to 0.738, while the genetic distance varied from 0.348 to 7.916. The dendrograms generated using these two parameters resulted in different hierarchical positions for the species. The one generated on the basis of similarity matrix grouped them into three major groups with three outliers. However, the dendrogram generated on the basis of genetic distance values grouped all 21 species into one major group with two sister groups. The polymorphism of primers observed in the RAPD analysis suggests the potentiality of this technique for studying phylogenetic relationship in *Encephalartos*.

## Screening of in vitro antifungal activity and quality control profiles of *Picrorhiza kurroa* (Scrophulariaceae)

HJ Pramod and MB Patil

*KLES College of Pharmacy, JNMC Campus, Nehrunagar, Belgaum-590010, Karnataka, India*

Initially the physicochemical evaluation is done by considering the parameters like Organoleptic characters, extractive values, loss on drying, ash values etc and further the herb is investigated for chemical constituents like alkaloids, flavonoids and glycosides as per standard procedures. Using different solvents, extraction of crude powdered drug is done and later these extracts were subjected to quality chemical investigations and in vitro antifungal studies of *Picrorhiza kurroa* extracts have been investigated against *Aspergillus niger*, *Rhizopus japonicum*, *Candida albicans*, *Candida tropicalis* and *Rhodotorula glutinis*. The extracts of *P. kurroa* showed high degree of activity against all test fungi. The inhibitory effects of the extracts are very close and identical in magnitude and are comparable with that of standard antibiotics used.

## Involving communities in the conservation of threatened plants in the Cape Floral Region, South Africa

DC Raimondo, I Ebrahim and JS Donaldson

*The Custodians of Rare and Endangered Wildflowers programme CREW, South African National Biodiversity Institute, Kirstenbosch, South Africa*

The Cape Floristic Region (CFR) has over 9000 species of plants. Recognized as a Global Biodiversity Hotspot, it currently has 526 threatened plant species.

Given the large number of threatened plants, and the limited capacity of conservation organizations, monitoring the status of populations of these species

is extremely challenging. The South African National Biodiversity Institute has set up a program that capitalises on botanical amateur expertise through recruiting volunteers from communities based in the Threatened Lowlands of the CFR. Volunteers survey fragments of threatened habitats for threatened plant populations, capturing abundance data, distribution data and threats affecting each species. These data are captured in a national database used for Red Listing and land-use planning. In addition, volunteers contribute to conserving critical sites for threatened plants through active management and awareness creation amongst landowners. This presentation will focus on examining the effectiveness of involving volunteers in monitoring, it will also examine how the status of certain threatened plant populations has changed over the past 20 years by comparing historical data collected in the 1980's with data collected as part of CREW.

### Population models to determine sustainable yields for harvesting in a mangrove forest

A Rajkaran and JB Adams

Department of Botany, P.O. Box 77000, Nelson Mandela Metropolitan University, Port Elizabeth 6031, South Africa

The Mngazana Estuary in the Eastern Cape Province has the third largest area (118 ha) of mangroves in South Africa. Harvesting has taken place in the mangrove forest for more than 60 years. Research over the last five years has shown that the present population structure is probably a consequence of the past harvesting history. Population modeling will be used to determine sustainable harvesting yields for the forest and to predict future changes in population structure caused by harvesting. Some of the important variables that are being measured include; growth rate, mortality, recruitment and propagule production. Early estimates show that *Rhizophora mucronata* seedlings and saplings are growing marginally faster than *Bruguiera gymnorhiza* and *Avicennia marina* at  $1.61 \text{ cm yr}^{-1}$ . This growth rate is much lower compared to that measured by other studies in this forest ( $20.92 \text{ cm yr}^{-1}$ ) and indicates large annual variability. Mortality rates for *Rhizophora* were highest in sites where the density of individuals was greater than  $16 \text{ m}^{-2}$ , while for *B. gymnorhiza* and *Avicennia* mortality was highest at a density of 4 individuals and 20 individuals  $\text{m}^{-2}$  respectively. This study will contribute to a management plan for the Mngazana mangrove forest. Recommendations will be made to ensure the viability of the mangrove forest and will range from harvesting practices and cutting limits to zonation of the forest for future or alternative uses.

### Phylogeographic studies in *Kniphofia* (Asphodelaceae)

S Ramdhani<sup>1</sup>, NP Barker<sup>1</sup> and H Baijnath<sup>2</sup>

<sup>1</sup>Molecular Ecology and Systematics Group, Department of Botany, Rhodes University, Grahamstown 6140, South Africa

<sup>2</sup>Department of Botany, University of KwaZulu-Natal, Westville Campus, Private Bag X54001, Durban 4000, South Africa

The genus *Kniphofia* contains approximately 72 species with an African–Malagasy distribution, including one species from Yemen. The centre of diversity is the Drakensberg Mountain Range. The genus is known for its complex taxonomy and species relationships are poorly understood. A number of species have considerable horticultural appeal. DNA sequence data from the nuclear ribosomal internal transcribed spacer (ITS) region and the *trnT-L* spacer region of the chloroplast genome were used to investigate the evolutionary history of *Kniphofia* and determine biogeographic patterns. Conventional phylogenetic analyses showed that the nuclear marker failed to resolve relationships, as many taxa and samples had identical sequences. The chloroplast marker revealed that multiple sequences of several species delimited on the basis of morphology were not monophyletic, but five clades were resolved. Furthermore, genetic lineages and geographic distributions showed a strong correlation. Thus a phylogeographic approach was adopted to explain the evolutionary history of the genus.

### Benefits of a virtual herbarium: Putting a face to a name. A contribution by the African Plants Initiative project

ET Rampho, M Koekemoer and RR Klopper

National Herbarium, SANBI, Private Bag X101, Pretoria 0001, South Africa

Having a collection of specimens in a herbarium cupboard, whether it is databased or not, does not ensure knowledge about the extent of the collection. Digitisation, databasing and georeferencing will facilitate the collection management process. It can make the collection widely known and easier to access. Through the African Plants Initiative (API) users can now obtain high-resolution images of specimens and its associated data. The API is a worldwide network of more than 40 institutions housing herbarium specimens of African plants. The project produces high-resolution images of type specimens, non-type specimens representing all taxa, non-specimen resources such as illustrations or artwork, slide images with all associated data, as well as multiple publications and checklists. All these images will be made available on-line by Aluka, as a fully searchable resource. This presentation highlights the achievements over the past two years of the South African National Biodiversity Institute (SANBI) as one of the major stakeholders of the API project, the challenges and potential payoff.

### The Red Data List of proteas

AG Rebelo

Protea Atlas Project, SANBI, South Africa

The Red Data List of African Proteaceae has been produced from the Protea Atlas data. Using the 2001 IUCN criteria to generate a computer-derived Red Data List provided the fuel for a two one-day workshop, to which all interested parties were invited, to refine and debate the status of our Protea taxa (species and subspecies). After many months of heated discussion the findings are ready for publication. The new IUCN criteria offer a standardised means of evaluated threatened status that are uniform worldwide. For the first time we are in a situation to compare this standardization with a plant family in the Cape Flora. The situation is bleak! Some 15% of our proteas are Critically Endangered, 20% are Endangered, 20% are Vulnerable: therefore 55% of our Proteaceae are “threatened”. And a further 21% are eligible for the “Orange List” of taxa that should be monitored because they are almost threatened. Only 31% of our Proteaceae are “safe.” The situation is dire compared to the first evaluation in 1982, when some 22% of our proteas were “threatened” and a further 22% were “naturally rare”, leaving 50% as “safe”.

### Is selfing advantageous for single colonising trees?

#### A comparison of two exotic *Acacia* species with contrasting breeding systems

J Rodger and SD Johnson

School of Biological and Conservation Sciences, University of KwaZulu Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

★ Awarded van Staden Prize for best oral presentation by a MSc student

It has been hypothesised that self-compatible plants are better colonisers than self-incompatible plants because individuals occur at low abundance during colonisation, resulting in their reproduction being severely pollen limited: that is limited by availability of mating partners and pollinators. Plants capable of self-fertilisation should therefore have higher reproductive success during colonisation. I tested this hypothesis by comparing fruit set of the self-incompatible *Acacia mearnsii* and the self-compatible, autogamous *Acacia dealbata* at two levels of abundance: isolated and non-isolated. If trees were separated from their nearest flowering conspecific neighbour by more than 50m they were regarded as isolated and if not as non-isolated. To assess whether isolation resulted in an increase in pollen limitation, inflorescences on isolated and non-isolated trees were artificially hand pollinated using

pollen from another population. If fruit set is higher under supplemental pollination than under natural pollination this demonstrates that fruit set is pollen limited. It was expected that isolated individuals would experience more severe pollen limitation of fruit set than non-isolated individuals in both species but that this effect would be greater in the self-incompatible *A. mearnsii* than in the self-compatible *A. dealbata*. As predicted isolated trees had lower fruit set than non-isolated trees in both species. Although fruit set was found to be pollen limited in the self-incompatible *A. mearnsii* isolated trees were not significantly more pollen limited than non-isolated trees so the lower fruit set in isolated trees cannot be ascribed to pollen limitation. Pollen limitation in non-isolated trees of *A. mearnsii* is probably a consequence of a high frequency of self-pollination between inflorescences. Display size is very large in this mass flowering tree, so pollinators probably seldom move between individuals and therefore the majority of pollen deposited on plants is likely to be self-pollen, resulting in fruit set being limited by supply of cross pollen as this tree is self-incompatible. These results suggest that breeding systems are not an important determinant of colonization by invasive *Acacia* trees.

### Comparison of structural damage caused by the Russian wheat aphid (*Diuraphis noxia*) and the Bird cherry-oat aphid (*Rhopalosiphum padi*) in a susceptible barley cultivar, *Hordeum vulgare* cv Clipper

SA Saheed, L Liu and CEJ Botha

Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

The Russian wheat aphid (RWA, *Diuraphis noxia* Mordvilko) and the Bird cherry-oat aphid (BCA, *Rhopalosiphum padi* L.) are known pests of many grain crops including barley (*Hordeum vulgare* L), causing severe damage and crop losses. An investigation of the comparative effects of these aphids on a susceptible cultivar, cv Clipper, was carried out at the electron microscope level to compare damage due to probing and feeding by these aphids.

After two weeks, BCA-infested plants remain healthy-looking, while RWA infested plants demonstrate stress and damage symptoms including chlorosis and necrosis of the leaves. The TEM study revealed that despite continued feeding, and the damage that was caused to the phloem parenchyma, companion cells and associated sieve elements (the site of sustained feeding) by BCA, it remains limited in comparison to that inflicted by RWA. Where cellular damage, specifically to the phloem, was substantial and non-functionality of the phloem ensues rapidly.

This paper explores the comparative damage visible at the TEM level and relates these findings to known effects of salivary components as well as other reactions to aphid feeding.

### Seed bank structure and the potential for rehabilitation of the Orange River mouth salt marsh

GA Shaw, TG Bornman and JB Adams

Department of Botany, Nelson Mandela Metropolitan University, Port Elizabeth 6031, South Africa

The Orange River Mouth is a transboundary RAMSAR site of South Africa and Namibia. It has high biodiversity and is a wetland of international importance providing an essential habitat for migratory birds. The salt marsh located at the mouth of the Orange River is degraded due to the influence of water abstraction upstream and mining activities. Attempts have been made to re-establish the desertified marsh with some success. Approximately 70 ha of salt marsh are presently still desertified. It was hypothesised that seed is important for the distribution and recruitment of vegetation and that if the physico-chemical properties are favourable the salt marsh will re-establish. Seed dynamics (production and seed bank) of the two dominant salt marsh species were investigated. Seeds produced by the plants are deposited along water driftlines where conditions are favourable for germination. The intertidal species

(*Cotula coronopifolia*) had a production of 325 792 seeds m<sup>-2</sup> of which approximately half were deposited in driftlines on the shoreline and 0.1% germinated into seedlings. The majority of seeds are probably lost through tidal flushing and wind. Similarly, the supratidal species, *Sarcocornia pillansii*, produced 293 000 seeds m<sup>-2</sup> of which 0.01% remained on the driftline. Only 0.1% germinated to seedlings. The two species have different life history strategies. The intertidal species has a higher seed production, lower seed loss and lower germination success. The supratidal species has the same germination success but a higher percentage of seed being lost probably through predation as a result of the larger seed size. Factors affecting the establishment and maintenance of plants in this area are the salinity of the sediment and groundwater, wind transport, erosion and deposition, mist (fog) and tidal exchange. It is expected that if these physical conditions become favourable with an improved hydrological regime, the salt marsh may re-establish itself within 10–15 years from the available seed bank.

### The distribution of micro-algae in the Mngazi and Mngazana Estuaries, eastern Cape Province

GC Snow and JB Adams

Department of Botany, Nelson Mandela Metropolitan University, PO Box 77000, Port Elizabeth 6031, South Africa

A growing demand for good quality fresh water in South Africa has led to this valuable resource becoming more limited and consumption is beginning to exceed supply in many areas. Not only has the high demand for freshwater resulted in decreased flow but modifications such as agricultural and wastewater return flow have led to a decrease in the quality of water too. The distribution of micro-algal communities in an estuary is the result of a complex series of interactions between hydrological, chemical and biotic factors. Biological communities integrate the physical and chemical effects and reflect the overall ecological integrity of an ecosystem over an extended period of time.

The Mngazi and Mngazana are neighbouring temporarily open-closed (TOCE) and permanently open (POE) estuaries located in the Eastern Cape Province. The catchments are relatively small and are still in a largely natural state and the Mngazana Estuary supports a large productive mangrove forest.

To determine the current state of micro-algae in the two estuaries and better understand the effects of mouth state (open versus closed) the aims of this project were; 1) to describe the distribution of intertidal benthic diatoms, based on relative abundance, along the length of the estuaries, 2) describe the vertical and longitudinal distribution of phytoplankton biomass, based on chlorophyll *a* concentration, in the two estuaries, and then to 3) briefly compare the micro-algal results from two estuaries.

There was a gradual change in benthic diatom species along the longitudinal axis of the estuaries. This could be in response to the gradual decrease in salinity from the mouth to the head of the estuary or as a result of slightly elevated nutrients (ammonium, soluble reactive phosphorus and silicate). However, organic matter, the percentage fine sediment (<125 μm) and water content of the sediment were strong driving factors for benthic micro-algal biomass and diatom community composition in the middle reaches of the estuaries. Phytoplankton biomass was well distributed throughout the estuaries with a possible weak river–estuarine interface zone (REI) in the Mngazi Estuary. The REI is the region of an estuary where river and estuary water mix resulting in a localised maximum in phytoplankton biomass. Vertically averaged salinity in the interface zone is usually <10 ppt. Highest water-column chlorophyll *a* concentrations were recorded in the middle–lower reaches of the Mngazana Estuary. This could have been the result of remineralisation of nutrients from organic matter in the fine sediments, the resuspension of benthic micro-algae or from nutrients entering the estuary at creek 2, a tributary dominated by dense mangrove forests.

An accumulation of fine sediments and organic matter in the middle reaches of both estuaries supports a high benthic micro-algal biomass in both estuaries. This may be typical of both estuaries in their natural states but it is essential that the sediment composition be closely monitored as further degradation from agriculture and forestry may result in the water column becoming more turbid, particularly during high flow periods. Increased abstraction of freshwater is

likely to make the water column more stable, increasing the amount of fine sediments and organic matter settling out in the middle reaches of the estuaries. The reduced freshwater flow is also likely to reduce phytoplankton biomass in the already weak REI zone, a possible food source for organisms in higher trophic levels.

### Validating the anti-cancer property of *Solanum aculeastrum*

K Srinivas<sup>1</sup>, DS Grierson<sup>1</sup>, M van de Venter<sup>2</sup> and AJ Afolayan<sup>1</sup>

<sup>1</sup>Department of Botany, University of Fort Hare, Alice 5700, South Africa

<sup>2</sup>Department of Microbiology and Biochemistry, Nelson Mandela Metropolitan University, Port Elizabeth 6031, South Africa

An ethnobotanical survey of plants used for the treatment of cancer was carried out around Alice, King William's town, Hogback and Umtata, all within the Eastern Cape. Seventeen plant species, belonging to 13 families, were reported as those used for the treatment of cancer in these areas. The roots, corms and bulbs are the most common parts of plants used, while decoctions and infusions are the main methods of preparation. Out of these species, *Solanum aculeastrum* was the most frequently used plant for the treatment of internal (breast, liver and stomach) cancers. The in vitro anti-proliferative activities of methanol and water extracts of the berries and leaves of this species were studied using three human tumour cell lines (HeLa, MCF7 and HT29). Methanol extracts of the berries had the highest anti-proliferative activity with IC<sub>50</sub> values between 17.1 and 41.9 µg/ml, while the activity of the aqueous extract of the berries ranged between 27.9 and 48.5 µg/ml. Leaf extracts showed no anti-cancer activity during the experimentation. Overall, the HeLa and MCF7 cell lines were more sensitive to both extracts than HT29 cells.

### Changes in the phytohormones of *Chlorella minutissima* on a daily cycle

WA Stirk<sup>1</sup>, D Drimalová<sup>2</sup>, M Strnad<sup>2</sup>, J van Staden<sup>1</sup>, V Ördög<sup>3</sup> and P Bálint<sup>3</sup>

<sup>1</sup>Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

<sup>2</sup>Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany AS CR, Slechtitelů 11, 783 71 Olomouc, Czech Republic

<sup>3</sup>Department of Plant Physiology and Plant Biotechnology, Faculty of Agricultural and Food Sciences, University of West Hungary, H-9200 Mosonmagyaróvár, Hungary

Two axenic strains of synchronized *Chlorella minutissima* cultures were grown in a 10h dark (0–10h) and 14h light (10–24h) cycle or in continuous low light. Samples were collected every 2h over a 24h period. Endogenous levels of cytokinin, auxin (IAA) and abscisic acid (ABA) were determined by direct specific ELISAs of the cell homogenates. No or very low cytokinin concentrations were detected during the dark phase and there was a gradual increase in concentrations during the light phase with levels peaking at 16–18h. Similarly, low ABA concentrations were detected during the dark phase with concentrations rapidly increasing once the algae were growing in the light with concentrations peaking at 18h. When the cultures were grown in continuous light, the cytokinin concentration immediately began increasing, peaking earlier at 12–14h. These cytokinins occurred at much lower concentrations than those growing in the light–dark cycle. There was a sharp decrease in the cytokinin concentrations just prior to cell division at 18h. IAA concentrations gradually increased peaking at 16 and 22h in the two respective strains. IAA levels were much higher than the cytokinin levels. ABA concentrations followed the same trend although occurring at much lower concentrations than IAA. These ABA concentrations were also much lower than the concentrations detected in the cultures grown in a light–dark cycle. The results clearly show that phytohormone production in synchronous cultures of *C. minutissima* follows a diurnal cycle.

### Aluminium speciation and micronutrient content in tea leaves (*Camellia sinensis*) and their infusions

R Street<sup>1</sup>, O Drabek<sup>1</sup>, J Szakova<sup>2</sup> and L Mladkova<sup>1</sup>

<sup>1</sup>Department of Soil Science and Geology, Czech University of Agriculture Prague, Czech Republic

<sup>2</sup>Department of Agrochemistry and Plant Nutrition, Czech University of Agriculture, Prague, Czech Republic

A total of 29 tea samples of different origin imported to the Czech Republic were collected and the tea leaves and their infusions analyzed for total content of aluminum (Al) and micronutrients (Cu, Fe, Mn, Zn). Al speciation in the infusions was performed using HPLC/IC. The influence of two additives (sugar and artificial lemon juice) and extraction time on Al speciation were tested. The addition of Al<sup>3+</sup> to the tea infusion showed that Al<sup>3+</sup>, Al(Y)<sup>2+</sup> and Al(X)<sup>1+</sup> species are detectable in tea infusions. Increased extraction time, or the addition of sugar, did not influence Al speciation. The addition of lemon juice, however, resulted in a change of Al speciation in one sample of black tea and five samples of green tea. These findings suggest that the addition of lemon juice can significantly influence Al speciation in tea infusions. Analysis of the micronutrient content showed that the total Mn content was higher compared to the total content of Cu, Fe, and Zn, and varied between 511 and 2220 mg kg<sup>-1</sup>. The proportion of element content in the infusion from the total content in the leaves was 30±16% Cu, 26±10% Zn, 18±10% Mn, and 1.5±0.8% Fe, respectively. The results indicate that tea infusions can be an important dietary source of Mn.

### Investigations of intra-organellar putative skeletal structures in *Azadirachta indica*

A Subbiah<sup>1</sup>, P Berjak<sup>1</sup> and VS Merhar<sup>2</sup>

<sup>1</sup>School of Biological and Conservation Sciences, University of KwaZulu-Natal, Howard College Campus, Durban 4041, South Africa

<sup>2</sup>Microscope Unit, University of KwaZulu-Natal, Howard College Campus, Durban 4041, South Africa

From an unrelated study on seed behaviour, electron micrographs of cells in the embryonic axis of an *Azadirachta indica* (neem) seed showed an array of parallel structures within the matrix of plastids. The initial conjecture was that they probably are an intra-organellar form of supporting structure, similar to those found in the cytoskeleton of eukaryotic cells taking into account the endosymbiont theory. The widely accepted endosymbiont theory was hypothesised, on the basis of mitochondria and chloroplasts having evolved from ancient bacteria. The theory uses the truly semiautonomous and prokaryote-like nature of these organelles as its basis. In the past it was believed that prokaryotes lack cytoskeletal elements but recent opinion has changed in light of questions pertaining to the maintenance of mitochondrial shape, as well as the transport of intra-organellar synthesised proteins to specific areas of function. This has led to the characterisation of a tubulin homologue, FstZ, and an actin homologue, MreB, in bacteria. In the present work the presence of these putative skeletal structures has been investigated by the use of TEM (transmission electron microscopy), aiming to provide confirmation of the nature of the structures. Fluorescence microscopy and TEM were used as a means of possible identification of these structures, by isolating mitochondrial and chloroplast fractions in pellet form using differential centrifugation and labelling the fractions with specific fluorescent tags, FITC-phalloidin for actin and anti-β-tubulin-Cy<sub>3</sub> for tubulin structures. Fluorescence microscopy of smears of isolated mitochondrial fractions showed the presence of structures, exhibiting the tubulin epitope as indicated by specific fluorescence with the anti-β-tubulin-Cy<sub>3</sub> tag under a green (G-2A) filter combination (excitation filter 510–560nm and barrier filter 590nm). As a result of these observations immuno-localisation of tubulin on TEM sections was carried out, established convincing proof of the presence of tubulin in these organelles.

### Population structure and distribution of *Oldenburgia grandis* (Asteraceae) in the Eastern Cape

C Swart and S Vetter

Botany Department, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

*Oldenburgia grandis* is a rare, endemic species restricted to Witteberg quartzite outcrops in the Eastern Cape. *O. grandis* is found in areas prone to fire disturbances, and responds by resprouting. The aim of this study was to assess the distribution of existing populations and to examine the stage class distribution of selected populations. The structure of a population and abundance of individuals are influenced by a number of environmental factors. Population structure will therefore give insight into the adaptations of plants in response to these factors. A detailed analysis of the spatial distribution including size and densities of existing populations across the range of the species was done. The majority of populations mapped were small (1–10 individuals) associated mostly with grassland and fynbos vegetation. Infestations of woody aliens were abundant, particularly in unconserved areas and on private farms. *O. grandis* often grows directly on rocky ridges whereas aliens tend to grow in the areas surrounding the outcrops, resulting in an apparent spatial segregation between the populations. An investigation into the population structure across various stages of recovery after fire and associated vegetation type was done. Similarities between populations based on stage class distributions were analysed using NMDS ordination. Fire occurrence was the major factor influencing the similarities between populations. Sites less recently affected by fire had higher percentages of the smaller stage classes (seedling, sapling and immature) whereas populations more recently affected by fire had the highest percentages of the suffrutice (resprout) class. Data from most populations recently affected by fire had low mortality as most damaged individuals resprouted. In sites where fires appeared to have been very intense, however, mortality was high. The findings of this study suggest that aliens may pose a risk to *O. grandis* populations by increasing fire intensities rather than via displacement.

### An ecosystem health index for the sandy beaches of the Eastern Cape

WB Todkill, EE Campbell and DR du Preez  
Department of Botany, Nelson Mandela Metropolitan University, P.O. Box 77000, Port Elizabeth 6031, South Africa

The coastal areas of the world are integral to the global economy and climate system. To achieve lasting and sustainable development, healthy coasts and oceans are essential. However, since the Earth Summit in 1992 most of the world's coastal resources have suffered from overuse and degradation. There is clearly a need to develop and implement more coastal management programs. The Eastern Cape has been the focus for research efforts on sandy beach biology and management in South Africa. Yet, the integration of this wealth of work into a beach ecosystem health index to aid management remains to be done. This study aims to develop an index that will be applied to the Eastern Cape beaches. This presentation will focus on the process of the development of such an ecosystem health index for sandy beaches, highlighting the features that are critical to ecosystem health.

### Matrix modeling of *Elaeodendron transvaalensis* tree species population

MP Tshisikhawe<sup>1</sup> and MW van Rooyen<sup>2</sup>  
<sup>1</sup>Department of Biological Sciences, University of Venda, Private Bag X5050, Thohoyandou 0950, South Africa  
<sup>2</sup>Department of Botany, University of Pretoria, Pretoria 0002, South Africa

Intense and frequent harvesting of bark from species with a high market demand often results in ring barking of trees. The trees subsequently die, and the species become rare over time. Matrix modeling is a powerful method used to determine harvest impacts on a species and can be used to assess the future of a population. *Elaeodendron transvaalensis* is a species in demand because of its medicinal value. Matrix modeling was done in order to determine the growth rate of the population as well as the stage that can be targeted for management purposes.

### Anti-HIV and toxicity of isolated compounds from *Elaeodendron transvaalense*

TE Tshikalange<sup>1</sup>, JJM Meyer<sup>1</sup> and F Ivars<sup>2</sup>  
<sup>1</sup>Department of Botany, University of Pretoria, Pretoria 0002, South Africa  
<sup>2</sup>Section for Immunology, Department of Cell and Molecular Biology, Lund University, SE-221 84, Sweden

*Elaeodendron transvaalense* (Celastraceae) is used traditionally in the treatment of a variety of diseases, including cancer and AIDS. An investigation of *E. transvaalense* stem bark for anti-HIV activity has led to the isolation of four known triterpenes and a flavonol. The antiviral activity of the isolated compounds was studied through a luciferase-based assay targeting the HIV-1 promoter activation induced by the cellular transcription factor (NF- $\kappa$ B), which plays a crucial role in HIV-1 replication. Only one terpenoid showed to be a potent NF- $\kappa$ B inhibitor. The compounds were further analyzed for cytotoxicity using the XTT assay on a MCF-7 breast cancer cell line. The active terpenoid demonstrated greater than 50% growth inhibition at 25 mg/ml concentration, and the crude extract and other isolated compounds showed very little or no toxicity at the same concentration. The results indicate that the anti-NF- $\kappa$ B activity of the terpenoid is partially caused by cellular toxicity.

### Inhibition of cysteine proteinase activity alters leaf senescence patterns and enhances abiotic stress tolerance in genetically modified tobacco

PDR van Heerden<sup>1</sup>, A Prins<sup>2</sup>, KJ Kunert<sup>2</sup> and CH Foyer<sup>3</sup>  
<sup>1</sup>School of Environmental Sciences and Development: Section Botany, North-West University, Potchefstroom 2522, South Africa  
<sup>2</sup>FABI, Department of Botany, University of Pretoria, Pretoria 0002, South Africa  
<sup>3</sup>Crop Performance and Improvement Division, Rothamsted Research, Harpenden AL5 2JQ, U.K.

Cysteine proteinases (CPs) play a crucial role in protein degradation during leaf senescence and are regulated by endogenous cysteine proteinase inhibitors (phytoecystatins). Constitutive expression of the phytoecystatin, oryzacystatin-1 (OC-1), on leaf senescence patterns and chilling tolerance were investigated in wild type and transformed tobacco. OC-1 expression resulted in a phenotype with altered growth and development, 75% lower extractable CP activity and 25% higher total soluble protein content. Under optimal growth conditions the photosynthetic capacities of fully expanded leaves of wild type and OC-1 plants were very similar. However, in contrast to the normal senescence-associated decline of photosynthetic capacity in leaves of the wild type, OC-1 plants maintained high photosynthetic rates for much longer. After 6 consecutive nights of chilling stress (5 °C) considerably higher photosynthetic rates were maintained in OC-1 than wild type plants. The content and activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in control and chilled plants were also quantified. Under optimal growth conditions, OC-1 plants had up to 69% higher maximal Rubisco activities than the wild type. Similar to the loss in photosynthetic capacity, senescence-induced declines in Rubisco protein content were observed much earlier in fully expanded leaves of the wild type. In addition, chilling stress-induced loss of Rubisco activity and content was prevented by OC-1 expression. These results indicate that phytoecystatins play a key role in the modulation of leaf senescence and that abiotic stress tolerance could be improved by modification of CP activity in planta. We propose a possible mechanism explaining how OC-1 prevents the degradation of chloroplast proteins such as Rubisco.

### Stimulation of tomato seed germination by 3-methyl-2H-furo[2,3-c] pyran-2-one: Some physiological aspects

J van Staden and N Jain  
Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

The role of smoke and smoke solutions in seed germination has invoked an immense interest in the scientific community for almost a decade. Recently, 3-methyl-2H-furo[2,3-c]pyran-2-one, a butenolide compound, was identified as the main germination cue from smoke. This step forward in research on smoke-stimulated seed germination now allows for more detailed studies on the physiological and molecular basis of the role of butenolide, in the complex process of seed germination. The present work reports on experiments conducted with tomato seeds (*Lycopersicon esculentum* cv. HEINZ 1370). The imbibition curves of the seeds imbibed with either butenolide or water followed the same trend, with no significant differences. In the butenolide-treated seeds, emergence of the radical was enhanced by four hours, compared to control seeds. This could be correlated with the early induction of DNA synthesis in the butenolide-treated seeds. Seedlings raised in the presence of butenolide had longer roots, compared to the control. Other growth parameters for the butenolide-treated seedlings were significantly greater. Analysis of the sugars in the cotyledons supported the notion of differential rates of mobilization of seed reserves, accounting for differences in the seedling growth parameters. Furthermore, the butenolide promoted germination at sub- and supra-optimal temperatures. Butenolide-treated seeds developed into phenotypically normal seedlings at 10 and 40 °C, when imbibed with butenolide. In comparison, control seeds imbibed with water failed to do so. Furthermore, priming the seeds with butenolide proved to be as effective as imbibing the seeds in the continuous presence of butenolide.

### Phyto-synergy: Examples from indigenous aromatic medicinal plants used in antimicrobial therapy

SF van Vuuren<sup>1</sup> and AM Viljoen<sup>2</sup>

<sup>1</sup>Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, South Africa

<sup>2</sup>School of Pharmacy, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa

Many traditional healers rely not only on a single plant extract for therapeutic regimens but often combine various plant parts and even different species in the belief that efficacy may be enhanced. A number of in vitro antimicrobial experimental procedures on South African medicinal plants have been undertaken which validate the role of synergism in phytotherapy. Using time-kill methodology the synergistic interaction is demonstrated for the combined use of *Lippia javanica* with *Artemisia afra* for the treatment of respiratory infections associated with *Klebsiella pneumoniae*. Antimicrobial studies on *Croton gratissimus* show synergistic, additive, antagonistic or non-interactive action between plant parts depending on the specific ratio in which the plant parts are combined. Higher synergistic sensitivities have been noted for root/leaf combinations. The antimicrobial activity of the non-volatile and volatile fractions singularly and in combination is demonstrated with *Tarhonanthus camphoratus* indicating pathogen specific selectivity. The interaction on a molecular level is demonstrated for major oil constituents of *Osmitopsis asteriscoides*, where camphor and 1,8-cineole in combination enhances antimicrobial efficacy. Conversely, the major volatile constituents of *Artemisia afra* in various combinations have no significant role on the antimicrobial activity of the plant. These examples detailing microbial interactions whether synergistically, antagonistically or on an additive level play an integral role in phytotherapy which remains poorly explored.

### Taxonomic studies of the Cape endemic genera *Glia*, *Itasina* and *Stenosemis* (Apiaceae)

B-E van Wyk<sup>1</sup>, PM Tilney<sup>1</sup>, CI Calviño<sup>2</sup> and SR Downie<sup>2</sup>

<sup>1</sup>Department of Botany and Plant Biotechnology, University of Johannesburg, P. O. Box 524, Auckland Park 2006, South Africa

<sup>2</sup>Department of Plant Biology, University of Illinois at Urbana-Champaign, 265 Morrill Hall, 505 South Goodwin Avenue, Urbana, Illinois 61801, U.S.A.

The vegetative and reproductive morphology, fruit structure, geographical distribution and possible phylogenetic relationships of three poorly known Cape

genera of the family Apiaceae are explored in this paper. The genus *Itasina* is a monotypic but variable plant with hysterothous, filiform leaves and peculiar spiny sepals. Recent (as yet unpublished) DNA studies have shown a close relationship with the genera *Annesorhiza* and *Chamarea*. *Stenosemis* comprises two species of suffrutices with strongly ribbed fruits (*S. angustifolia* and *S. caffra*). This genus appears to be closely related to Cape *Peucedanum* species. *Glia* comprises the widely distributed and well known *G. prolifera* and an undescribed species with smaller fruits and a less woody habit. Genetic evidence from DNA sequencing suggests that *Glia* is very closely related to the genus *Anginon*, despite distinct differences in the fruit structure. The remarkable diversity of the southern African genera of Apiaceae and their importance in understanding the early evolution of the subfamily Apioideae are becoming more evident.

### Bioinformatical analysis of horizontal gene transfer in rice

BJ Vorster<sup>1</sup> and CA Cullis<sup>2</sup>

<sup>1</sup>Department of Botany, University of Pretoria, Pretoria 0002, South Africa

<sup>2</sup>Department of Biology, Case Western Reserve University, Cleveland 44106 OH, U.S.A.

Plastid DNA fragments are often found in the nuclear genome and DNA transfer from the plastids to the nuclear genome is ongoing. The release of whole plant genome sequences provides the opportunity to undertake genome wide analysis of integration of DNA on non-nuclear origin into the plant genome from either plastids or microbes and fungi. During endosymbiotic evolution, eukaryotic nuclear genomes have acquired numerous genes from the endosymbiotic organelles, which later evolved into the present chloroplasts and mitochondria and although most of this gene transfer occurred at an early stage of organelle evolution, functional gene transfer continues to occur in plants.

### Natural rehabilitation potential of riparian zones after alien clearing in the Fynbos Biome—Phase 1: A reference study of indigenous seed banks

S Vosse<sup>1</sup>, KJ Esler<sup>2</sup>, PM Holmes<sup>3</sup> and DM Richardson<sup>4</sup>

<sup>1</sup>Department of Botany and Zoology; and Centre for Invasion Biology, University of Stellenbosch, Private Bag XI, Matieland 7602, South Africa

<sup>2</sup>Department of Conservation Ecology; and Centre for Invasion Biology, University of Stellenbosch, Private Bag XI, Matieland 7602, South Africa

<sup>3</sup>Cape Ecological Services, 23 Dreyersdal Road, Bergvliet 7945, South Africa

<sup>4</sup>Centre for Invasion Biology, University of Stellenbosch, Private Bag XI, Matieland 7602, South Africa

Riparian areas contribute significantly to the spread of many invasive species which alter the ecosystem and bring about significant water loss to river systems. These areas have therefore been targeted by many alien clearing programmes. Little attention, however, has been paid to rehabilitating these areas after clearing and the role that soil seed banks play in this process. This study aimed to assess the composition and viability of reference seed banks, to be used as a bench mark for conservation managers and landowners, when assessing post disturbance rehabilitation techniques. The focus was on soil seed bank composition of the riparian vegetation in four river systems within the fynbos biome of the south-western part of the Cape. Both mountain and foothill sections of the Eerste, Molenaars, Berg and Wit Rivers were sampled. Plots were located in relatively undisturbed fynbos/riparian areas, with less than 25% invasion. Two factors were taken into consideration when setting up the sampling plots, namely lateral (wet and dry bank) and longitudinal (mountain stream and foothill) zones. Vegetation data were collected on site and contrasted with the seedlings that emerged from the soil samples to compare diversity and vegetation groupings. The seed bank composition was found to have little overlap with the current aboveground vegetation. The mountain stream slopes of all rivers were found to have a greater diversity within the seed bank, particularly so under the influence of past intermittent fires. On a lateral scale, the transitional zone between wet and dry banks was



found to have a higher seed bank diversity with representatives of both riparian and fynbos species.

### Ultrastructure of wood fibres: Obtaining reliable information

J Wesley-Smith

Forestry and Forest Products Research Centre, CSIR–UKZN, Durban 4041, South Africa

During recent years there has been growing interest in visualising and quantifying the surface characteristics of wood pulp fibres. The removal of cell wall constituents such as lignin during chemical pulping, and the plasticizing effect induced by refining, bring about changes in the chemical and structural characteristics of fibres. These changes influence inter-fibre bonding, and thus the strength properties of paper end-products. As fibres collapse from their initial cylinder-like shape, the area of contact between these in the three dimensional paper network increases. Bonding between fibres is further aided by the development of threadlike cellulose fibrils at fibre surfaces during refining. Ultrastructural examination of fibre surfaces using conventional techniques is hampered by cell wall distortions occurring during air drying or solvent-extraction prior to critical point drying. The present study assessed the reliability of various preparative methods and microscopy modes to examine never-dried pulp fibres of *Eucalyptus*. Environmental SEM revealed the presence of fibril bridges between fibres, although thorough examination of fibre surfaces was hindered by fibres progressing rapidly from being covered by a film of water to becoming visibly dehydrated. Conventional SEM of samples cooled in nitrogen slush and freeze-dried showed greater surface detail and extensive fibrillation, in agreement with light microscopy observations. Present results suggest that nitrogen slush cooling and freeze-drying of sufficiently small samples reveal extensive surface structure comparable, if not more detailed, than that of hydrated samples. Freeze-dried samples have the added convenience of being able to be viewed using conventional high vacuum systems, and are not susceptible to undesirable drying during viewing.

### Investigating cellulose fibril aggregation in dissolving pulp of *Eucalyptus* using atomic force microscopy

J Wesley-Smith, T Bush, M Madikane and A Bissessur

Forestry and Forest Products Research Centre, CSIR–UKZN, Durban 4041, South Africa

Dissolving pulp is the end-product of sulphite pulping and bleaching processes, and constitutes the raw material for the production of cellulose derivatives such as viscose, rayon and cellulose acetate. Only limited information is available on the desirable characteristics of dissolving pulp, and it is almost entirely based on softwood species. At a cell wall level, cellulose chains form fibrils of about 4nm diameter and these, in turn, form fibril aggregates of varying diameters. While the exterior of these aggregates are immediately available to react with chemicals during the production of cellulose derivatives, crystalline and para-crystalline domains within the centre are not. The proportion of accessible and inaccessible surfaces is thought to influence the reactivity characteristics of the pulp.

Hemicelluloses bound to cellulose fibrils are removed during pulping and bleaching, allowing closer association between fibrils. Recent studies on Norway spruce pulp using atomic force microscopy have reported increased cellulose aggregation during fibre processing, and a concomitant decrease in surface area available for chemical reaction. It is therefore important to monitor changes in the ultrastructural arrangements of cellulose fibril aggregates during pulping and bleaching in order to understand how this is influenced by industrial processing conditions, and how these changes affect the reactivity of the pulp.

The work presented will report on the use of atomic force microscopy to investigate the fibril aggregate characteristics of dissolving pulp of a clone of *Eucalyptus*, grown in compartments with contrasting site qualities (low and high), at different stages of the process (raw pulp and fully bleached) to different end-product purities (96 and 92 alpha-cellulose content).

### Quantifying stress induced bioactive umckalin content in wild harvested and greenhouse cultivated *Pelargonium sidoides* and evaluating tuber re-growth subsequent to harvests

AG White<sup>1</sup>, MT Davies-Coleman<sup>2</sup> and B Ripley<sup>1</sup>

<sup>1</sup>Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

<sup>2</sup>Department of Chemistry, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

Extracts from the tubers of *Pelargonium sidoides* (Geraniaceae) are used in the Eastern Cape as a traditional medicine for the treatment of respiratory tract and gastro-intestinal infections and globally as herbal treatments for bronchitis, asthma and as an immune system booster. Despite documented exploitation of wild populations by illegal harvesters this species has not been awarded a protected status. The level of exploitation has raised questions about the sustainability of wild harvesting and the need to develop cultivated material with high bioactivity. This study developed a novel method for the purification of umckalin such that tuber umckalin content could be quantified by HPLC. HPLC analyses showed a correlation between umckalin content and geographical region, soil pH and rainfall. Umckalin content increased with decreasing rainfall and increasing soil pH. The content of newly produced tubers from cultivated plants was similar to the low content found in wild-harvested tubers from a high rainfall and low soil pH area. Thus the possibility of inducing high umckalin content in greenhouse-cultivated plants was investigated by subjecting plants to water stress and soil pH treatments. The re-growth of shoots from which a standard proportion of the tuber was harvested showed that water availability to the post harvest shoot affected shoot survival but not tuber re-growth rate. Re-growth rates were low questioning the viability of wild harvesting.

### Generic positions of platyspermous Apiaceae in tropical and subtropical Africa

PJD Winter

National Herbarium, South African National Biodiversity Institute, Private Bag X101, Pretoria 0001, South Africa

This paper compares 45 tropical and subtropical African *Peucedanum* species with *Peucedanum* L. s.s. (Europe), and other platyspermous genera. The remaining 19 'Cape' and Madagascar species are sufficiently distinct to be dealt with in a separate paper. Several species allied to *P. petitianum* A. Rich. appear to have an affinity with *Afroligusticum* C. Norman, suggesting an expansion of that genus. In the same way it is investigated whether *Lefebvrea* A. Rich. or *Erythroselinum* Chiov. can be expanded. If the remainder can be satisfactorily delimited from other Laurasian *Peucedanoid* genera, they will be circumscribed and described as a new genus. A re-analysis of characters from the literature in a more focused way is presented as a search for taxonomically informative patterns of character states or combinations previously obscured either by broad circumscriptions of taxa, or by artificial grouping of states into character suites. The neglected character of habit shows taxonomic potential when evaluated with habitat.

### Conserving Africa's succulent euphorbias

ETF Witkowski

Restoration and Conservation Biology Research Group, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, P.O. Wits 2050, South Africa

Many succulent Euphorbias (Euphorbiaceae) are listed as threatened in southern African country Red Lists. Euphorbias are popular with horticultural collectors, but typically don't feature on medicinal plant lists, although they have various uses. Threats include fungal diseases, plant collectors, habitat loss and fragmentation. Succulent Euphorbias are often prevalent in more arid

tropical climes, often on rocky hill-slopes, where human land pressure is typically less intense. We have a poor understanding of the effects of fire on this group in moister regions. Climate change is likely to have profound impacts on Euphorbias and particularly those in drier regions. Due to generally poor dispersal abilities, responses to climate change may depend largely on in situ tolerance. Furthermore bush-encroachment (woody plant densification) may inhibit establishment of Euphorbias due to increased shading. Surprisingly, mammalian herbivory is a considerable threat to a number of threatened Euphorbias, and becomes particularly prevalent during droughts. Plants are often damaged by cattle, and the impacts of goats can be locally severe. Increased precipitation may also increase disease prevalence. Case studies of South African species, *E. perangusta*, *E. clivicola*, and *E. barnardii*, suggest that threats and regeneration requirements are highly species and site-specific, so familial generalizations helpful for conserving a wider range of species are probably illusive.

### Assessment of the post-shedding development, desiccation sensitivity and storage behaviour of *Encephalartos gratus* and *E. natalensis* (Zamiaceae) seeds

W Woodenberg, D Erdey and P Berjak

School of Biological and Conservation Sciences, University of KwaZulu-Natal, Howard College Campus, Durban 4041, South Africa

Cycads are ancient plants, having been in existence for at least 250 million years. Currently, cycads are one of the most prized, sought after plants. However, as their popularity has increased, natural populations of cycads have declined to virtually unsustainably low levels due to poaching. Seeds are arguably the most significant propagatory unit of plants, and potentially serve as ideal source material for the development of long-term conservation strategies of plant genetic resources. However, very little is known about the post-shedding storage behaviour of cycad seeds. In the current investigation, newly shed *Encephalartos gratus* and *E. natalensis* seeds were placed into storage at 16 °C. Seed component water contents and germination were assessed at two-monthly intervals. When seeds were shed the developing sporophyte consisted primarily of the coiled, elongated suspensor bearing the pro-embryo at its tip. This structure was characterised by a water content much higher than that of the surrounding megagametophyte tissue (i.e. 2.5 to 4.0 g H<sub>2</sub>O g<sup>-1</sup> dry mass [g g<sup>-1</sup>] and 0.5 g g<sup>-1</sup>, respectively). Embryo development continued during storage at 16 °C and the seeds attained full germination potential six to eight months after shedding. During this period, the water content of the developing embryos decreased to the range 1.75–1.25 g g<sup>-1</sup>, with little or no change in the water content of the megagametophyte tissue or in the overall fresh mass of the seeds. This suggests that the reallocation of resources from the megagametophyte to the developing embryo is a post-shedding event, and that the sclerotesta ('seed coat') is a substantial barrier to water loss by the seed. Preliminary results, however, indicated that the seeds are desiccation sensitive, as the germination of mature *E. gratus* seeds was greatly reduced when dried below an embryo water content of 0.49 g g<sup>-1</sup>, suggesting recalcitrant behaviour.

### Elicitation of 7-methyljuglone in *Drosera capensis*

SM Ziaratnia, N Lall, KJ Kunert and JJM Meyer

Department of Botany, University of Pretoria, Pretoria 0005, South Africa

A compound belonging to the naphthoquinones group, "7-methyljuglone" (7-MJ) is one of the major constituents of *Drosera capensis*. 7-MJ has been shown to possess potent antituberculosis activity. An in vitro culture of *D. capensis* was established by using germinated seeds on a hormone-free Murashige and Skoog (Full MS, 1/2 MS, 1/3 MS) and Gomborg (B<sub>3</sub>) medium. It was found that B<sub>3</sub> and full MS medium were favourable for the production of 7-MJ when compared to the concentration of 7-MJ in naturally grown *D. capensis*. The favourable medium for roots and shoots were found to be B<sub>3</sub> and half MS respectively as far as length is concerned. Germinated seeds were grown on MS medium with two different levels of total nitrogen (30 and 60 μM) and ratios of nitrate to ammonium. The concentration of 7-MJ was found to be the highest at 30 μM total nitrogen and 75% to 25% nitrate to ammonium ratio. Elicitation of 7-MJ on intact plants was achieved

by using different concentrations of salicylic acid (SA) and jasmonic acid (JA). The roots and shoots were harvested separately at 0.5 to 48 h after treatment. The concentration of 7-MJ in roots was found to be higher than shoots in the presence of both elicitors. JA did not have a positive influence on 7-MJ accumulation as compared to the control. The highest amount of 7-MJ (24 mg/kg) was detected when plants were exposed to 100 μM of SA after 48 h. Those elicitors are currently being added to cell suspension culture of *D. capensis* for the analysis of 7-MJ.

### An assessment of the reproductive biology and ecology of selected rare and endangered *Oxalis* species

J Zietsman<sup>1</sup>, LL Dreyer<sup>1</sup> and KJ Esler<sup>2</sup>

<sup>1</sup>Department of Botany and Zoology, University of Stellenbosch, Private Bag XI, Matieland 7602, South Africa

<sup>2</sup>Department of Conservation Ecology, University of Stellenbosch, Private Bag XI, Matieland 7602, South Africa

*Oxalis* is the seventh largest genus in the Cape Floristic Region, yet an estimated 53 of the 211 southern African *Oxalis* species are rare/endangered and highly localized. Small populations are especially vulnerable to extinction (inbreeding, low genetic variation, stochastic events, disrupted biological interactions, etc.). Moreover *Oxalis* displays tristylous, which is a restrictive breeding system that includes a strong self-incompatibility component that promotes out-crossing between different floral morphs within populations. As it requires equal availability of three floral morphs as well as effective pollinators for seed production, this breeding system can be a further threat to small populations when fully expressed. Breakdown of the tristylous breeding system may significantly alter reproductive assurance and thus future survival of rare species.

Potential factors that may contribute towards rarity in *Oxalis* are being investigated by focusing on the reproductive biology and ecology of two widespread and eight rare/highly localized *Oxalis* species. The three aspects of tristylous (self-incompatibility, population structure and flower morphology) are assessed. Natural seed production, pollinator availability and extent of clonality are also assessed for the selected rare and common species. In addition, several abiotic features of the selected species are explored, including habitat preference, specificity and availability, geographical distribution, extent of fragmentation/disturbance, recent climate fluctuations, and population size and density. Comparisons of results of different rare species together with comparisons between rare and related common species are presented to reveal the most likely threats to specific populations. Identification of current and possible future threats will aid future conservation planning for each of the selected rare/endangered species.

### Macrophytes role in winery wastewater treatment using constructed wetlands

NS Zingelwa

Environmental Management: Soil Science, ARC Infruitec-Nietvoorbij, Stellenbosch 7599, South Africa

Constructed wetlands have long been recognised for their role in wastewater treatment, but have just recently been used in wineries. Sodium is a major element in winery wastewater and that can have a detrimental impact on the environment if not treated. Treatment processes occurring in constructed wetlands include phytoremediation by macrophytes. These have the ability to take up or remove nutrients and incorporate them into their tissues at high concentrations and plant species used in the trial were *Typha latifolia* (cattail) and *Phragmites australis* (bulrush). Element uptake/removal was affected by the following factors: the source of effluent (distillery or winery), wetland retention period (4.5, 9, and 18 days) and position of plant in the wetland, plant species comparison, season of growth and organs of concentration. Results have shown that plants growing on distillery effluent have higher element concentrations than those in winery effluent. *T. latifolia* accumulates higher element concentrations than *P. australis* though the latter is more tolerant to effluent. Most nutrients were accumulated in below-ground tissues, whilst cations accumulated mostly in harvestable above-ground tissues. Over the growing season it

was found that plants accumulation increased due to exposure and that significant differences were found between newly growing shoots and adult plants. No significant differences were found within plant concentrations in wetlands of different retention periods and also in different wetland positions.

### Traditional knowledge transfer of activities practiced by Zulu women to manage medicinal and food plant gardens

AM Zobolo and QN Mkabela

Department of Botany, University of Zululand, Private Bag X1001, KwaDlangezwa 3886, South Africa

A survey of plants grown in home gardens was conducted through questionnaires among 80 homesteads in rural areas of northern KwaZulu-Natal in South Africa. The areas surveyed were located at Mbonambi, Kwadlangezwa, Ntambanana and Eshowe. Elderly women ( $\geq 55$  years) and girls ( $\leq 21$  years) were questioned on names and uses of plants grown in the home garden. Various medicinal and food plants were found in the home gardens. The well known plants reported to be used around the homestead were *Citrus limon*, *Citrus sinensis*, *Cucurbita pepo*, *Musa davyae*, *Zea mays*, *Bidens pilosa*, *Encephalartos villosus*, *Momordica foetida*, *Plectranthus ambiguus* and *Sansevieria hyacinthoides*. The elderly women possessed more knowledge (64.4%) than girls (7.5%) on plant uses. Knowledge on rituals pertaining to harvesting and field protection was 28.6% for elderly women and 1.79% for girls.

### POSTERS

#### The effect of nitrogen supply on the photosynthesis and growth in C<sub>3</sub> and C<sub>4</sub> subspecies of *Alloterosis semialata*

T Abraham<sup>1</sup>, C Osborne<sup>2</sup>, and B Ripley<sup>1</sup>

<sup>1</sup>Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

<sup>2</sup>Department of Animal and Plant Sciences, University of Sheffield, United Kingdom

C<sub>4</sub> plants have a greater efficiency of CO<sub>2</sub>-fixation and therefore require less nitrogen investment in photosynthetic enzymes and hence have higher photosynthetic nitrogen use efficiency (PNUE), compared with C<sub>3</sub> plants. Such a mechanism may explain the relative success of C<sub>4</sub> and C<sub>3</sub> grasses in response to soil nutrient status. This study compared the responses in photosynthetic parameters, leaf nitrogen and biomass between C<sub>4</sub> *Alloterosis semialata* subsp. *semialata* and C<sub>3</sub> *A. semialata* subsp. *eckloniana* supplied soil nitrogen at a range of levels chosen to represent nutrient rich to poor savannah soils. Subspecies were grown separately in 20L pots and were supplied three levels of nitrogen via a Long Ashton's growth solution: 0mg g<sup>-1</sup> (LN), 3.5mg g<sup>-1</sup> (MN) and 7mg g<sup>-1</sup> (HN). Photosynthesis was assessed at 12 weeks after tiller planting by means of A/Ci curves and showed a significant difference in carboxylation efficiencies between subspecies, in response to nitrogen supply and for the interaction of both. C<sub>4</sub> subspecies and higher nitrogen supply increased carboxylation efficiency, while C<sub>3</sub> photosynthesis and lower nitrogen supply decreased it. Stomatal limitation and CO<sub>2</sub> compensation points were significantly different between subspecies but not between treatments. Rates of RuBP regeneration were not significantly different between subspecies or treatments. Total leaf area was significantly affected by treatment and the C<sub>3</sub>-LN plants, relative to the HN plants, had reduced leaf areas that were similar to the leaf areas of all C<sub>4</sub> plants, irrespective of nitrogen supply. C<sub>3</sub> root biomass, sampled by means of root cores, showed a small but significant increase in response to nitrogen supply for the MN and HN plants, relative to the LN plants. Nitrogen supply did not affect C<sub>4</sub> root biomass, which was similar to the biomass produced by the C<sub>3</sub>-LN plants. Results confirm the prediction that the growth of the C<sub>4</sub> subtype is less affected by the availability of nitrogen than the C<sub>3</sub> subtype and suggests C<sub>4</sub> dominance in nitrogen poor soils.

### Gamma rays induced an early wrinkled seeded mutant in chickpea

JD Barshile<sup>1</sup>, BJ Apparao<sup>2</sup>, SC Dalve<sup>2</sup> and SG Auti<sup>3</sup>

<sup>1</sup>Department of Botany, Shri Anand College, Pathardi 414 102, M.S. India

<sup>2</sup>P.G. Department of Botany, P.V.P. College, Pravaranagar, M.S. India

<sup>3</sup>Department of Botany, RNC Arts JDB Comm. and NSC Science College Nashik Road, M.S. India

Among the pulses chickpea (*Cicer arietinum* L.) is an important source of protein. It is an important source of protein in the diets of the SAT and WANA regions (semi-arid tropics and West Asian-North Africa). In last decade the production of chickpea remains static in India due to limited genetic variability in it for breeding. So mutation techniques are the best methods to enhance genetically induced variability of a species and have played role in development of crop varieties. In present investigation seeds of chickpea variety Vishwas (PG-5) were procured from Mahatma Phule Agriculture University, Rahuri (India) and treated with 8, 12 and 16 mM EMS and 400, 500 and 600 Gy of gamma rays, to raise the M<sub>1</sub> generation. Seeds of M<sub>1</sub> plants were harvested individually to raise M<sub>2</sub> generation. The early wrinkled seeded variant was isolated from 500 Gy gamma rays treatment in M<sub>2</sub> generation. The mutant was confirmed as true breeding in M<sub>3</sub> generation. The mutant was characterized morphologically and biochemically in M<sub>3</sub> generation. The flowering and maturity occurred 10 days earlier and seeds were wrinkled against smooth in the parental variety, which could be improved for milling. The mutant had significantly higher number of pod plant<sup>-1</sup>, seeds plant<sup>-1</sup>, yield plant<sup>-1</sup> and harvest index that makes it an economic mutant. Based on biochemical study, the mutant showed significantly enhanced total chlorophyll, protein, globulin and free amino acids contents. The mutant has shown increased nitrate reductase activity. However, the nitrate reductase activity reflected the yield potential and protein content of the mutant. SDS polyacrylamide gel electrophoresis protein profile of the early wrinkled mutant in M<sub>3</sub> generation revealed 13 bands against 12 in the parental cultivar. The early mutant induced from the variety Vishwas could cope better with the late seasons moisture stress that is usually encountered in the chickpea growing area in the Mediterranean region especially in the state of Maharashtra (India).

### Floristic composition of remaining natural grassland in the Pretoria National Botanical Garden

SP Bester

South African National Biodiversity Institute, Private Bag X101, Pretoria 0001, South Africa

Since the foundation of the Pretoria National Botanical Garden (PNBG) in 1946, the garden has been developed to the detriment of much of its natural grassland areas, which had to make way for the layout of formal lawns and flowerbeds. Today only about 6.4 ha (8.4%) of the garden still exists as natural grassland. The grassland offers visitors to the garden and learners attending educational programmes an ideal opportunity to be informed and educated on the grassland ecosystem, its ecology and biodiversity.

Since 2003 the grassland has been surveyed continuously throughout the year to compile a dynamic checklist (to be regularly updated). This checklist will serve as a basis for further studies and for the compilation and implementation of management plans and will also be used in educational programmes at PNBG.

The checklist was compiled through: (1) collecting of specimens over three years (and continuing); (2) sight records and (3) interrogation of PRECIS records (Pretoria [PRE] Computerized Information System).

The floristic checklist revealed the following current composition:

- Taxa: 230. Families: 55. Genera: 159.
- Bryophytes (0.4%), pteridophytes (0.4%), dicotyledons (66.1%) and monocotyledons (33.1%).
- Exotic (5.2%) and indigenous (94.8%).
- Annuals (11.8%), biennials (0.9%) and perennials (87.3%).
- Life form (predominating): Graminoids and cyperoids (18.7%), herbs (37.4%), geophytes (10.0%), shrubs (4.8%) and small trees/shrubs (4.8%).

- Largest families: Poaceae (17.4%), Asteraceae (15.7%), Fabaceae (9.1%), Hyacinthaceae (4.3%), Apocynaceae (3.5%) and Orchidaceae (3.0%).
- Largest genera: *Senecio* (3.0%), *Eragrostis* (2.2%), *Hypoxis* (2.2%), *Eulophia* (1.7%) and *Rhynchosia* (1.7%).

### The effect of chilling on the ultrastructure of recalcitrant *Ekebergia capensis* seeds of Port Elizabeth provenance

V Bharuth<sup>1</sup>, JI Kioko<sup>1</sup>, P Berjak<sup>1</sup> and VS Merhar<sup>2</sup>

<sup>1</sup>School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban 4041, South Africa

<sup>2</sup>Electron Microscope Unit, University of KwaZulu-Natal, Durban 4041, South Africa

Recalcitrant seeds of tropical origin may be chilling-sensitive, probably depending on the provenance. Preliminary studies have indicated that seeds of *Ekebergia capensis* of Durban provenance are chilling-sensitive, while those of Port Elizabeth provenance may not be. In this study, the effect of chilling on viability and ultrastructure of Port Elizabeth seeds was investigated after storage under hydrated and dehydrating conditions at chilling (6 and 16 °C) and non-chilling (25 °C) temperatures. Axes of fresh seeds had a water content of approximately 0.7 g g<sup>-1</sup> dry mass. After 8 weeks, the axis water content of the seeds in dry storage had dropped to approximately 0.1 g g<sup>-1</sup> at all storage temperatures, and viability was reduced to 45% at 6 °C and to 20% at both 16 and 25 °C. Fresh seeds showed well organised ultrastructure with numerous well-developed mitochondria evenly distributed throughout the cytomatrix, which was moderately vacuolated. Following the dehydrating storage treatment, deleterious ultrastructural changes such as electron translucent mitochondria and nuclei were noted, as well as a high degree of vesiculation. The seeds that were stored in the hydrated condition remained 100% viable at all storage temperatures after 8 weeks, during which time the axis water content increased to approximately 2 g g<sup>-1</sup> at all storage temperatures, presumably due to respiration during germination-associated metabolism. Organelles such as mitochondria, ER and nuclei in the ultrastructure were well developed. It is apparent that seeds of *E. capensis* of P.E. provenance are not sensitive to chilling, at least at temperatures of 6 °C or higher. The seeds should, however, be stored hydrated to remain viable.

### A case for leakage and retrieval of solutes via the xylem to phloem in monocotyledons

CEJ Botha<sup>1</sup>, N Aoki<sup>2</sup>, GN Scofield<sup>2</sup>, RT Furbank<sup>2</sup> and L Liu<sup>1</sup>

<sup>1</sup>Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

<sup>2</sup>Division of Plant Industry CSIRO, Black Mountain, Canberra ACT Australia

The vascular system in plants is composed of a system of conduits that conduct water and solutes in opposite directions. Water moves via a passive solute transport pathway. In source regions, nutrients are remobilized along with assimilate and are transported passively in the phloem in the opposite direction. Phloem transport is driven by increased solute concentration at the source, which, in turn, drives the transport system in a classic source to sink direction. Our study focuses on the recycling processes that occur in transpirationally driven sink regions of the plant. We demonstrate that offloading occurs at points along the transpiration stream, and that this offloading process is patchy. Once offloaded the membrane-permeable xenobiotic 5,6-carboxyfluorescein diacetate (5,6-CFDA) is cleaved to 5,6-carboxyfluorescein (5,6-CF), within nucleate xylem parenchyma and is rapidly transported to the phloem. Transport and movement of 5,6-CF require access to plasmodesmata to enable cell to cell transport of the fluorophore. This paper focuses attention to a little-understood, but physiologically vital process, involving retrieval from the xylem and reloading via the phloem. We suggest that the retrieval process is not confined to re-entry of nutrients that normally translocate passively in the xylem, but to remobilization of excess carbon lost along the transport pathway and their retrieval, and remobilization via the phloem. This would ensure reallocation to symplasmically compartmentalized sinks in the developing root–shoot system.

### Structural damage resultant from aphid feeding on resistant and susceptible wheat and barley cultivars

CEJ Botha, H van Schalkwyk, SA Saheed and L Liu

Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

Aphid feeding was studied using resistant wheat (*Triticum aestivum*); Betta and Betta Dn and the control susceptible cultivar SST825, colonized by the Russian wheat aphid (*Diuraphis noxia*), the grass aphid (*Stobion yakini*), or the bird cherry aphid (*Rhopalosiphum padi*) and with non-resistant barley (*Hordeum vulgare* var Clipper) which served as an additional control. Leaf material was examined specifically for the formation of phloem-related wound callose, using aniline blue fluorochrome. Our study confirms that aphid feeding quickly stimulated the formation of wound callose in susceptible varieties, but not in resistant strains. Using the phloem mobile fluorophore, 5,6-carboxyfluorescein (5,6-CF), we have shown that phloem-transported 5,6-CF was not located downstream of aphid-probed sieve tubes in control or susceptible plants. Low levels or absence of 5,6-CF suggests that either the aphids successfully redirected longitudinal sieve tube sap to themselves, or that the phloem in the probed areas was no longer functional. As callose deposition is reduced in the Dn variety, we surmise that transport through sieve tubes may not be as adversely affected by aphid feeding in the resistant wheat varieties and that the 'resistance' may in fact be tolerance of aphid feeding, possibly due to down-regulation of  $\beta$ -1,3 glucan synthase and related callose synthesis. In control, non-resistant barley and wheat plants, wounding may well be a long-term response to feeding, in which wound callose deposition prevents recovery of transport capacity as evidenced by reduced 5,6-CF movement. In contrast, the resistant Betta-Dn showed no reduction in 5,6-CF transport below sites of aphid probing, suggesting that the phloem was still capable of long-distance transport even though examination of resistant as well as susceptible plants by transmission electron microscopy revealed that probing of the vascular tissue by the aphids induced noticeable subcellular damage, more especially so to phloem parenchyma and their associated companion cells and sieve tubes. We explore the functional state of sieve tubes using fluorescence and transmission electron microscopy.

### Flora of the Eastern Cape

CL Bredenkamp and GF Smith

South African National Biodiversity Institute, Private Bag X101, Pretoria 0001, South Africa

One of the important goals of the South African National Biodiversity Institute (SANBI) is to provide a Flora for southern Africa. A useful approach in achieving this goal is to ultimately compile an incremental series of regional Floras. Building on the success of Floras for the Cape Floristic Kingdom and Northern Provinces, good progress has been made with the Flora of the Eastern Cape. This Flora is coordinated from the National Herbarium. Many botanists have already been approached to contribute in their fields of expertise and further inputs by contributors will be appreciated. Guidelines concerning the delimitation, format, contents, taxonomic expertise, possible contributors, herbarium work, field trips and funding of the Flora were compiled by botanists of the four Eastern Cape Universities. Further discussions with the authors of the other regional Floras, KwaZulu-Natal, Free State and Succulent Karoo take place twice per annum.

### The use of structural species size classes for woody vegetation description

LR Brown<sup>1</sup> and GJ Bredenkamp<sup>2</sup>

<sup>1</sup>Applied Behavioural Ecology and Ecosystem Research Unit, Department of Environmental Sciences, UNISA, Private Bag X6, Florida 1710, South Africa

<sup>2</sup>African Vegetation and Plant Diversity Research Centre, Department of Botany, University of Pretoria, Pretoria 0002, South Africa

The need for a scientifically based wildlife management plan and more knowledge on the vegetation ecology of the Borakalalo Nature Reserve led to an ecological investigation of the Reserve. One of the aims was to develop a structural classification of the woody component using species size classes (SPIZE's). A further aim was to compare the various structural classes identified with the recognised floristically derived plant communities of the Reserve. The frequency, density, percentage crown cover and importance value for each woody species were calculated. A classification of the woody component was done using a TWINSPAN classification algorithm on this structural density data. Fifteen structural SPIZE classes were identified, described and compared with the described plant communities. The results of this study indicate that a specific SPIZE class may sometimes occur over a range of related plant communities. Different plant communities may have similar structure, even if they have very different species compositions. It is therefore argued that Braun–Blanquet descriptions of plant communities should form the basis of any ecological management plan, as these plant communities represent sound vegetation-cum-habitat units that form the basis for the compilation of veld management plans.

### ***Lobostemon*: Barking up the right tree?**

MH Buys

*AP Goossens Herbarium, School of Environmental Sciences and Development, North-West University, Potchefstroom 2520, South Africa*

A cladistic analysis of *Lobostemon* (Boraginaceae) based on morphological data is reported on. The analysis utilises *Echiostachys incanus* and *Pontechium maculatum* as the outgroup and includes 28 ingroup taxa. A strict consensus tree hypothesises 4 monophyletic groups within *Lobostemon*, corresponding to four sections within the genus. Section *Grandiflori* Levyns is paraphyletic. Medium sized flowers, and the presence of staminal scales and stigma branches are synapomorphies for *Lobostemon*. New hypotheses include actinomorphic flowers to be derived and Section *Argentei* to be sister to the rest of the genus.

### **Surf diatom community associations**

EE Campbell

*Department of Botany, Nelson Mandela Metropolitan University, P.O. Box 77000, Port Elizabeth 6031, South Africa*

Six diatom species (*Anaulus australis*, *Asterionellopsis glacialis*, *Asterionellopsis socialis*, *Attheya armata*, *Aulacodiscus africanus* and *Aulacodiscus kittonii*) are known to form semi-permanent brown discoloration in surf-zones at some high-energy sandy beaches. Generally, only one or two species dominate the surf-zone at a particular beach. The exception to this rule is a beach on the north island of New Zealand. Five of the six species were recorded at this surf-zone in the patches on more than one occasion. Finding all these species at one beach offers a unique opportunity to compare their diel and long-term dynamics. Some species appear to be permanent features of the beaches where they are recorded (e.g. *A. armata*), others may be episodic (e.g. *A. glacialis*), while others appear or disappear depending on the surf conditions (e.g. *A. australis*). A seventh surf diatom species was found in the patches of New Zealand and the Washington coast of North America. In addition, the regular presence of the freshwater diatom, *Asterionella formosa*, at these two surf-zones raises interesting issues relating to the importance of river water for surf diatoms in contrast to groundwater known to be a source of nutrients to the South African and American surf-zone communities.

### **Biogeography, rarity and endemism in *Cineraria* (Asteraceae)**

GV Cron<sup>1</sup>, K Balkwill<sup>1</sup> and EB Knox<sup>2</sup>

<sup>1</sup>*School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits 2050, South Africa*

<sup>2</sup>*Department of Biology, Indiana University, Bloomington, IN 47405, U.S.A.*

The biogeography of the afro-montane genus *Cineraria* (Asteraceae) is assessed and discussed with reference to its distribution range and centres of diversity and endemism. Plotting the numbers of species per degree square for southern Africa reveals that the centre of diversity for *Cineraria* is in the KwaZulu-Natal Midlands, part of the Maputaland–Pondoland Centre of Endemism, with a secondary centre of diversity in the Albany Centre of the Eastern Cape. The main affinity of *Cineraria* is afro-montane (to afroalpine) within various centres of diversity and endemism. Fifteen species endemic to specific mountains or regions of endemism and five near-endemics are identified. Rare species are identified and classified/categorised according to Rabinowitz's criteria of geographic range, habitat specificity and local population size. Rare species (in the sense of low abundance, restricted range and high habitat specificity) number at eleven, however only five are considered under threat as indicated by IUCN Red Data Criteria — but a number are data deficient. Causes of rarity in *Cineraria* are linked to narrow habitat specificity, particularly soil or rock type and/or altitudinal range.

### **Genetic diversity of the mangrove *Avicennia marina*, in South Africa**

D Dheopursad and JM Lamb

*School of Biological and Conservation, University of KwaZulu-Natal, Durban 4001, South Africa*

The South African black mangrove, *Avicennia marina*, plays a significant role in the reduction of coastal erosion, and, in coastal stabilisation, the dissipation of winds and cycling of carbon dioxide, nitrogen and sulphur. Activities such as ecotourism, aquaculture, mining, chemical waste disposal and non-sustainable exploitation by local communities have impacted negatively on the survival of *A. marina* in local estuaries. The characterisation of genetic variation among *A. marina* populations is of importance to the conservation of genetic diversity in this plant. The present study implemented molecular methods for the analysis of genetic diversity and structure of *A. marina* across its range in South Africa. Two methods of assessing genetic diversity, with differing sensitivities, were used in order to assess genetic structure on two different scales. The techniques used were (1) sequencing of the chloroplast trnL/F intergenic spacer and trnL (UAA) intron and (2) ISSR fingerprinting, a technique involving PCR amplification of inter-simple sequence repeat regions. Sequencing techniques provide an overall picture of genetic variability in comparison with similar taxa from around the World. ISSR fingerprinting is capable of revealing genetic variation on a fine scale, at population and individual level. The sample populations were from estuaries at Kosi Bay, St Lucia, Richards Bay, Mtunzini, Beachwood, Durban Bay, Isipingo, Umkomaas, and Mngazana. Sequencing results indicated no variation in the trnL (UAA) intron and in the less conservative trnL/F intergenic spacer. South African samples were found to differ by an indel of one nucleotide from the Australian populations of *A. marina*. Thus these sequences provide little information on genetic diversity in this slowly evolving species. ISSR fingerprinting, a more sensitive indicator of overall genetic diversity, reveals largely identical fingerprints from samples within each estuary, and a low level of variation between estuaries. Detailed analysis of results is incomplete at the time of this submission.

### **The role of mucilage in the biology of surf-diatoms**

DR du Preez

*Department of Botany, Nelson Mandela Metropolitan University, P.O. Box 77000, Port Elizabeth 6031, South Africa*

Surf-zone diatoms are a small group of microalgae that dominate in some surf-zones of the world. These surf-zones tend to be associated with long, dissipative beaches. The species involved are *Anaulus australis*, *Attheya armatus*, *Asterionellopsis glacialis*, *Asterionellopsis socialis*, *Aulacodiscus africanus* and *Aulacodiscus kittonii*. Each of these species produces mucilage to some or other degree. *A. armatus* produces copious amounts of mucilage that is permanently found on the outside of the frustule, while the *Aulacodiscus* species produce localised mucilage associated with the labiate processes. *A. australis* has the most complex pattern of mucilage production, the mucilage being absent in the early morning and becoming quite abundant and coating the cell by mid-

afternoon. Daughter cells of *A. australis* are held together after cell division by a mucilage strand, which persists until shortly before the next cell division. The role of mucilage in these organisms is complex and is not a feature that can be used to provide a common feature of these diatoms and influence their abundance in surf-zones. The different roles that these mucilages play in surf diatoms will be discussed.

### Hydraulic differences between C<sub>3</sub> and C<sub>4</sub> subspecies of *Alloteropsis semialata*

K Frole, M Gilbert and B Ripley

Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

C<sub>4</sub> plants have higher water use efficiencies (WUE) compared to C<sub>3</sub> plants due to their greater CO<sub>2</sub>-fixation rate. This higher WUE lowers the C<sub>4</sub> plant's hydraulic demand and has been used as an explanation for shorter xylem vessels with increased hydraulic safety from cavitation in C<sub>4</sub> plants found in arid environments. In mesic environments some C<sub>4</sub> plants produce similarly sized xylem vessels as C<sub>3</sub> plants but increase leaf area. Both of these xeric and mesic C<sub>4</sub> scenarios have been shown in dicotyledonous plants and lead to a decreased leaf specific hydraulic conductivity. This study explored the hydraulic differences in monocotyledonous C<sub>4</sub> *Alloteropsis semialata* subsp. *semialata* and C<sub>3</sub> *A. semialata* subsp. *eckloniana*. Results showed that the C<sub>4</sub> subspecies had twice the whole plant hydraulic conductivity of the C<sub>3</sub> subspecies and maintained higher transpiration and photosynthetic rates, relative water contents, and leaf water potentials throughout the day. The C<sub>4</sub> subspecies produced longer xylem vessels, which constituted a greater proportion of total leaf-xylem length when compared to the C<sub>3</sub> subspecies. Other hydraulic parameters such as leaf specific conductivity, xylem vulnerability and soil to root conductivity were investigated.

### The effect of developmental status on successful cryopreservation of recalcitrant seed germplasm

M Goveia, K Kioko and P Berjak

School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban 4041, South Africa

The zygotic germplasm of plant species producing desiccation-sensitive seeds can be conserved in the long-term only via cryopreservation, usually of the excised embryonic axes. The success of this intricate process is affected by many factors, including in vitro regeneration techniques, the size of the explant, physical damage induced upon excision, and the developmental status of the seed. The current study investigated the effect of the above factors, with particular attention to the developmental status of the seeds, on the successful cryopreservation of the embryonic axes of *Trichilia dregeana*, a tropical plant producing desiccation-sensitive seeds. Thus, investigations were conducted on embryonic axes excised from mature seeds either immediately after harvesting or following hydrated storage for three or five months. The results showed that in vitro axis germination was optimal when activated charcoal was included in the germination medium. Seeds stored for five months had developed sufficiently that, even with complete excision of the cotyledons, normal axis development occurred on in vitro germination, providing the smallest possible germinable explant for cryopreservation. Subsequent cryopreservation was best achieved using rapid cooling in nitrogen slush, with axes from three month stored seeds showing the highest survival. However, the surviving embryonic axes regenerated only as non-embryogenic callus, and ongoing studies are aimed at inducing embryonicity of the callus.

### Ultrastructural variation in leaf salt glands of fringe and dwarf *Avicennia marina*

O Hiralal<sup>1</sup>, G Naidoo<sup>1</sup> and Y Naidoo<sup>2</sup>

<sup>1</sup>School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban 4041, South Africa

<sup>2</sup>Electron Microscope Unit, University of KwaZulu-Natal, Durban 4041, South Africa

In many *Avicennia marina* stands natural productivity gradients occur with trees in the fringe zone being tall and large (6–10m) while those in the dwarf zone are less than 1.5m. We hypothesized that there are structural variations in leaves of fringe and dwarf trees. Typically, leaf salt glands consist of 2–4 basal collecting cells, 1–2 disk-like stalk cells and 8–12 radially arranged secretory cells which are covered with a thin cuticle. In both fringe and dwarf glands, nuclei of secretory and stalk cells are relatively large and the side walls of stalk cells appear heavily cutinised. There were many vesicles of varying sizes commonly observed in secretory cells of both leaf types. In leaves from fringe trees, the vacuoles in secretory cells of salt glands are smaller than those from dwarf leaves. Additionally, the cytoplasm in one of the secretory cells appeared to be retracted and the region between it and the cell wall was filled with amorphous electron dense matter. Within the cytoplasm of secretory cells, elongated, narrow and vacuole-like membranous structures near and surrounding the small vacuoles were characteristic in secretory cells but were lacking in stalk cells. In these vacuole-like structures several electron-dense areas with electron-dense margins were observed in secretory cells. Many of the structures enclosed fibrillar-like material. The nuclei were large and numerous mitochondria were clustered and some evenly dispersed in the cytoplasm of the secretory cells. In dwarf mangrove salt glands, however, mitochondria were scattered in the cytoplasm. In all three cell types of the glands, multivesicular structures featured within the vacuoles and also between the cytoplasm and the cell wall. Withdrawal of the cytoplasm of the stalk cell from the common horizontal walls between secretory and stalk cells was evident, suggesting plasmolysis. The results suggested that there are distinct variations in ultrastructure of salt glands of fringe and dwarf leaves.

### Responses of *Avicennia marina* to selected heavy metals

T Hiralal and G Naidoo

School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban 4041, South Africa

Heavy metal contamination in mangroves is of critical concern due to their accumulation and adverse effects in aquatic ecosystems. This study was undertaken to investigate the effects of Hg, Pb, Cu and Zn on plant responses in *Avicennia marina*. *A. marina* plants were grown for eleven months in pots contaminated with Hg, Pb, Cu and Zn at concentrations of 0, 40, 80, 120 and 160ppm. We hypothesized that heavy metals have deleterious effects on plant growth and that they are secreted from salt glands present on the leaves. Measurements were made of CO<sub>2</sub> exchange and chlorophyll fluorescence on leaves using the LICOR 6400 and a Fluorometer (PAM 2100) respectively. Carbon dioxide exchange, transpiration and leaf conductance generally decreased with increasing metal concentration. CO<sub>2</sub> exchange at a concentration of 160ppm was significantly lower than the control for all metals. Percentage decreases in CO<sub>2</sub> exchange at 160ppm for Hg, Pb, Cu and Zn were 49.6%, 37.7%, 47.7% and 63.7% respectively compared to control values. Photosystem II (PS II) quantum yield, photochemical efficiency (Fv/Fm) and electron transport rate (ETR) through PSII generally decreased with increasing concentration for all heavy metals. In addition, plant height and dry biomass accumulation were significantly lower at 160ppm than the control values for all heavy metals. This study has shown that *A. marina* experiences dose-dependent stress responses to the selected heavy metals in leaf tissue, evidenced by decreases in growth and photosynthetic performance.

### Viability and ultrastructural responses of seeds and embryonic axes of *Trichilia emetica* to different dehydration and storage conditions

JJ Kioko, P Berjak and NW Pammenter

School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban 4041, South Africa

The seeds of *Trichilia emetica*, a multi-purpose tropical forest species, displayed typical recalcitrant behaviour, being shed at an average axis water

concentration of 2.82 g per g dry matter ( $\text{g g}^{-1}$ ), and losing viability when dehydrated to axis water concentrations below 0.42 and  $0.26 \text{ g g}^{-1}$ , when dried slowly or rapidly, respectively. The ultrastructure at shedding was indicative of active metabolism, as would be expected of mature recalcitrant seeds which grade into germinative metabolism after shedding. Rapid dehydration enabled the maintenance of ultrastructural integrity to water concentrations as low as  $0.3 \text{ g g}^{-1}$ , while cells of axes dried slowly to similar water concentrations displayed total subcellular destruction. In the fully hydrated state, the storage lifespan of the seeds was limited to 60 days at  $16^\circ\text{C}$ , after which all the seeds had germinated in storage. Ultrastructural examination, however, indicated that prolonged mild water stress had occurred, which the seeds are suggested to have suffered as germination proceeded in storage. When stored at  $6^\circ\text{C}$ , the seeds showed extensive ultrastructural derangement, which was accompanied by loss of viability after 20 days, presumably as a result of chilling injury, while storage at  $25^\circ\text{C}$  resulted in all seeds germinating in storage in 35–40 days. Even though the seed coat has been shown to inhibit germination, it did not appear to affect seed longevity or germination in storage at any of the temperatures used.

### The African Plant Checklist and Database Project

RR Klopper<sup>1</sup>, GF Smith<sup>2</sup>, L Gautier<sup>3</sup>, C Chatelain<sup>3</sup> and R Spichiger<sup>3</sup>

<sup>1</sup>National Herbarium, South African National Biodiversity Institute, Private Bag X101, Pretoria 0001, South Africa

<sup>2</sup>Office of the Chief Director: Research and Scientific Services, South African National Biodiversity Institute, Private Bag X101, Pretoria 0001, South Africa

<sup>3</sup>Conservatoire et Jardin botaniques de la Ville de Genève, case postale 60, CH-1292 Chambésy, Switzerland

The African Plant Checklist and Database Project (APCD) is a collaboration between the South African National Biodiversity Institute (SANBI) and Conservatoire et Jardin Botaniques de la Ville de Genève (CJB), Switzerland. It provides botanists with the first-ever angiosperm checklist and database for Sub-Saharan Africa. This was achieved by merging two existing datasets for Southern and Tropical Africa. The end products of the APCD project will be a once-off hardcopy publication of the checklist, and a database with additional information, the latter already freely accessible through the internet. The database will be regularly updated. The APCD, for the first time, gives accurate statistics for the angiosperm flora of Sub-Saharan Africa. There are 50 136 current taxa in the database, plus an additional 393 taxa where there are discrepancies in genus concept between the two original datasets. Taxa are arranged into 274 families and 3802 current genera. Several other important statistics can also be drawn from the database. The APCD does not aim to solve all taxonomic problems between the two original datasets, but rather to highlight discrepancies, thereby pointing out taxa where further research is necessary. The APCD will be an invaluable tool to botanists working on the African flora and it has already emerged as a nomenclatural standard for the African Plants Initiative.

### The effect of elevated atmospheric carbon dioxide on the growth and physiology of *Chromolaena odorata*

R Lalla and NW Pammenter

School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban 4041, South Africa

The amount of  $\text{CO}_2$  in the atmosphere ( $C_a$ ) is increasing rapidly. As  $\text{CO}_2$  is a primary substrate for photosynthesis, this has prompted an array of studies on plant responses to elevated  $C_a$ . Weeds in particular are of great concern because they are inherently fast growing, and may respond considerably to increasing  $C_a$ . *Chromolaena odorata* is a weed that is a serious threat to indigenous vegetation. In this study *C. odorata* was grown in competition with two grass species: *Eragrostis curvula* and *Themeda triandra*. All three species were grown in large pots in open-top chambers. Each pot contained four plants of each species, and half the pots were exposed to elevated  $C_a$  (~700 ppm), the remainder being exposed to ambient  $C_a$  (~370 ppm). The pots at each  $C_a$  treatment were further divided, with half receiving high nutrients, the other half receiving low nutrients.

Studies on growth and photosynthetic characteristics (particularly A:C<sub>i</sub> curves) were undertaken. Results showed that plants responded positively to high nutrient treatments, but, in contrast, elevated  $C_a$  did not affect growth or photosynthesis of *C. odorata*. In a subsequent experimental trial, *C. odorata* plants were grown monospecifically to assess whether there was a 'chamber effect' associated with planting density. Pots at both  $C_a$  treatments contained either two or four *C. odorata* plants. Results from this experimental trial are currently being analyzed. However, recent studies on *C. odorata* have shown that, generally, this species does not respond to elevated  $C_a$ . Future studies should be directed towards understanding this unexpected response, and could include short-term down-regulation and cellular-level changes, when exposed to increased  $C_a$ .

### Floral biology of *Struthiola ciliata* (Thymelaeaceae)

T Makholela and JC Manning

Compton Herbarium, South African National Biodiversity Institute, Private Bag X7, Claremont 7735, South Africa

*Struthiola ciliata* is widespread through fynbos vegetation in the southwestern Cape. Flowers may be produced throughout the year and are creamy white in colour with a slender floral tube  $\pm 20$  mm long. The mouth of the tube is surrounded by eight, hairy petaloid scales. The four, subsessile stamens are included within the tube just below the mouth. Anthesis occurs in the evening at  $\pm 18\text{h}00$ , at which time the flowers begin to emit a strong, sweet, spicy scent from the petaloid scales. The compounds thujone, isothujone, verbenone, alpha-terpineol, benzyl acetate, eugenol and vanilline contribute largely to the scent profile detectable by the human nose. Small volumes ( $0.025\text{--}0.18 \mu\text{l}$ ) of moderately concentrated nectar (20–34% sucrose equivalents,  $x = 27.15 \pm 0.91\%$ ) accumulate in the floral tube. The only floral visitors observed were the noctuid moths *Syngrapha circumflexa* and *Cucullia terensis* (Lepidoptera: Noctuidae), which visited the flowers at dusk and early evening. Anthesis of the entire floral crop of each plant is closely synchronised, taking place over a short period of a few days. Individual flowers last for 9 to 11 days, with nectar accumulating at the base of the hypanthium only during the first 3 to 4 days. Thereafter the nectar disappears and is presumably resorbed.

### The extent of woody plant invasion in a selected site in the Molopo Magisterial District

PW Malan

Department of Biology, North West University, Mafikeng Campus, Private Bag X2046, Mmabatho 2735, South Africa

Woody plant invasion in the Molopo Magisterial District was investigated. Woody plants especially *Dichrostachys cinerea* and *Acacia mellifera* are invading in natural grasslands in rangelands. Communal farming practices contribute to the declining of natural grasses, thus giving the invasion of woody species a competitive advantage. The tree equivalents per unit area were determined for Disaneng Area and compared with a benchmark site nearby. Bush densities in the communal areas of Disaneng were substantially higher than the control area. This will eventually lead to the lowering in grazing capacity of the grasslands in the rangelands of Disaneng Area.

### Biological screening of some plants used in the treatment of gonorrhoea in Limpopo, South Africa

NA Masevhe and DEN Mabogo

Biological Science Department, University of Venda, Private Bag X5050, Thohoyandou 0950, South Africa

Africa is rich in indigenous plants which can serve as sources of medicine. *Datura stramonium* (Solanaceae), *Senna petersiana* (Fabaceae), *Albizia versicolor* (Fabaceae), *Lannea edulis* (Anacardiaceae) and *Bridelia micrantha* (Euphorbiaceae) were selected and collected on the basis of their use by local

traditional practitioners for treating gonorrhoea. They were extracted using acetone, methanol and water respectively.

Crude extracts of *D. stramonium* leaves, *S. petersiana* and *L. edulis* root barks, *A. versicolor* and *B. micrantha* stem barks were tested for antibacterial activities using disk diffusion and cup plate techniques. In general the results obtained showed that *D. stramonium* had no activity, *B. micrantha* and *S. petersiana* had little activity while *L. edulis* and *A. versicolor* had significant activity against *Neisseria gonorrhoeae*. The results of this study support the traditional use of these plant species by local traditional practitioners.

## Evaluation of melanin synthesis from South African plants

LA Mashamba, AE Basson and N Lall

*Department of Botany, University of Pretoria, Pretoria 0002, South Africa*

Tyrosinase is an enzyme which is involved in melanin biosynthesis, a pigment that is responsible for the color of mammalian skin and hair. Various dermatological disorders, such as melasma and age spot etc, arise from the accumulation of an excessive level of epidermal pigmentation. The inadequacy of current therapies to treat these conditions as well as high cytotoxicity and mutagenicity, poor skin penetration, and low stability of existing cosmetic formulations has led us to seek new whitening agents to meet the medical requirement for depigmenting agents.

Three south African plants belonging to Rhizophoraceae (E1), Bignoniaceae (E1) and Sapotaceae (E3) families are extensively being used by Zulus, Venda and Xhosa tribes of South Africa for skin lightening purpose. Acetone extracts of all three plants were investigated for the inhibition of tyrosinase and melanin. E1 and E3 exhibited 80% and 60% inhibition of the monophenolase and diphenolase activities of tyrosinase respectively. E3 demonstrated a significant reduction on melanin production in vitro at 0.270 µg/ml, without being significantly ( $P > 0.05$ ) toxic to melanocyte cells. Fractionation of this extract is currently being carried on, in order to determine the active principle.

## Mechanistic studies of disulfide reductase inhibition by diospyrin, 7-methyljuglone and related anti-mycobacterial naphthoquinones isolated from *Euclea natalensis*

P Mativandlela<sup>1</sup>, CJ Hamilton<sup>2</sup> and N Lall<sup>1</sup>

<sup>1</sup>*Department of Botany, University of Pretoria, Pretoria 0002, South Africa*

<sup>2</sup>*School of Chemistry and Chemical Engineering, Queens University of Belfast, Stranmillis Road, Belfast BT9 5AG, U.K.*

The roots of *Euclea natalensis* are used by the indigenous people of southern Africa against various bacterial infections. The naphthoquinones diospyrin, isodiospyrin, and 7-methyljuglone have recently been isolated from root extracts of this plant and been shown to exhibit antimycobacterial activity against *Mycobacterium tuberculosis*. The activity of 7-methyljuglone is comparable to that of current antimycobacterial drugs. Herein we describe mechanistic studies of some potential modes of antimycobacterial activity for these naphthoquinones. In particular, their interactions with the low molecular weight thiol mycothiol (MSH) and its respective disulfide reductase (mycothione reductase) which are both important mediators of thiol/disulfide redox regulation and antibiotic resistance.

## Legume improvement for environmental stress protection: The LEGIM project

PW Mokwala<sup>1</sup>, RM Mangoale<sup>1</sup>, MC Mathabe<sup>2</sup>, CH Foyer<sup>3</sup> and KJ Kunert<sup>2</sup>

<sup>1</sup>*Biodiversity Department, University of Limpopo, Private Bag X1106, Sovenga 0727, South Africa*

<sup>2</sup>*Botany Department, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa*

<sup>3</sup>*Rothamsted Research, CPI Division, Harpenden, U.K.*

It is now understood that environmental abiotic stresses restrict plant vigour and create a "yield gap". This "yield gap" can occur in legumes whose

sustainability and predictability can be severely restricted by environmental stresses, such as drought, temperature extremes and also by abiotic factors such as nematode infestation. It is further established that environmental stresses trigger early senescence in legume nodules which are involved in the nitrogen supply for legume plants. However, mechanisms of nodule senescence are still unknown and unexplored. The LEGIM project which is an initiative between the University of Limpopo, University of Pretoria and CSIR supported by the Royal Society of London and NRF addresses the problem of early senescence induced by stressful environmental conditions using soybean, which is an important legume food and feed crop in South Africa. In this project, we focus in particular on the understanding of the function of senescence-related cysteine proteinases and their inhibitors, the cysteine proteinase inhibitors or cystatins, in nodule senescence. Among the different actions involved in the project are cysteine proteinase profiling and the engineering of soybean nodules with an endogenous cystatin to prevent cysteine proteinase activity in senescent nodules. First results about successful soybean transformation with an endogenous cystatin gene and results about cysteine proteinase profiling in senescent nodules will be presented.

## The impacts of invasive alien plant clearing on riparian vegetation recovery along the Sabie River in the Kruger National Park

T Morris, J Coetzee and ETF Witkowski

*School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, WITS 2050, South Africa*

Riparian zones represent a dynamic ecotone between aquatic and terrestrial ecosystems and are among the most diverse and complex habitats of all landscape features. Riparian vegetation fulfils a number of key ecological functions and is imperative in maintaining the healthy functioning of an ecosystem. Riparian areas are regarded as being highly prone to invasion by alien plants due to the efficient ability of water to disperse propagules, the increased nutrient levels and the frequency of disturbances in these systems. The impacts of invasive alien species (IAS) are widespread and the replacement of indigenous vegetation with alien stands (often as monocultures) can lead to the loss of both animal and plant species, cause alterations in abiotic factors such as soil chemistry and can disrupt ecosystem processes and disturbance regimes such as fire. Consequently, clearing stands of invasive alien plants (IAP's) in riparian areas have become a primary concern in order to maintain both terrestrial and aquatic ecosystems' health and biodiversity. Working for water has been undertaking extensive alien plant control projects across the country, focusing mainly on riparian areas. Some cleared riparian corridors appear to have returned to states approximating preinvasion conditions without further management intervention. Other sites, however, have often failed to revegetate, resulting in secondary problems and further degradation in many areas. Thus information on the state of the environment after clearing and the management and abiotic factors responsible for this state is urgently needed. In the Kruger National Park (KNP) and surrounding areas, very little post-clearance monitoring has taken place. Thus this project aims to determine the level of recovery of riparian vegetation after clearing of IAP's along the Sabie River in and around the KNP. Additionally it seeks to understand factors responsible for varying levels of vegetation recovery in cleared areas including an assessment of the effectiveness of clearing methods and follow up protocols. This research will have a multi-faceted approach. Data will be obtained from existing reports and literature, KNP archives, Working for Water archives, and from field research.

## The use of coarse resolution satellite data to measure vegetation cover in South Africa

TL Morgenthal and TS Newby

*Agricultural Research Council-Institute for Soil, Climate and Water, Private Bag X79, Pretoria 0001, South Africa*

Previous research indicated the potential of using of satellite remote sensing information as a measure of vegetation parameters such as cover, biomass and



leaf area index. This study investigated the suitability of NOAA–AVHRR, MODIS and SPOT-4 products to quantitatively determine herbaceous vegetation cover at a national scale using a regression model approach.

Field data was collected at 143 locations in three study areas, namely in the regions of Marble Hall, Queenstown and Beaufort West. Survey plots were selected a priori to present a gradient of Normalized Difference Vegetation Index (NDVI) values derived from MODIS. Vegetation cover was sampled along a 200m transect using a 1m<sup>2</sup> quadrat and the canopy spread cover methods. Vegetation indices, derived from coarse resolution satellite data, were compared with field data using regression models.

A high correlation ( $R^2=0.76$ ) existed between canopy spread cover, determined with the descending point method, and crown cover, estimated by the quadrat method. Canopy spread cover was approximately 11% higher than crown cover and related better to coarse resolution remote sensing data ( $R^2>0.7$ ) than vegetation cover determined with the quadrat method ( $R^2<0.6$ ). The highest correlation was found with the 1km SPOT VEGETATION and the 500m Enhanced Vegetation Index (EVI) MODIS products ( $R^2>0.78$ ).

A vegetation cover product (canopy spread cover) for the summer rainfall region was derived using MODIS EVI, with a natural logarithmic model. The regression coefficient for the model was 0.90 ( $R^2=0.81$ ) and the standard error 10.86. Results, therefore, indicate the potential use of coarse resolution satellite products as a measure of vegetation cover. Although both SPOT VEGETATION and the MODIS EVI products relate well to vegetation cover, the MODIS EVI product is recommended due to its higher spatial resolution providing more detailed information.

### The effect of soil and climate on the growth and survival of *A. saligna* and *A. cyclops* seedlings on a mountain gradient in Villiersdorp, South Africa

K Neethling<sup>1</sup>, KJ Esler<sup>1</sup> and GF Midgley<sup>2</sup>

<sup>1</sup>Department of Conservation Ecology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa

<sup>2</sup>Global Change Research Group, South African National Biodiversity Institute Private Bag X7, Claremont 7735, South Africa

Very few studies have simultaneously considered the effect of climate change and invasive species on ecosystems, and there is a great need for research in this field. Considered in isolation, climate change, invasive species and habitat destruction are the greatest threats to ecosystem functioning. It is believed that by causing disturbances in ecosystems (i.e. creating niches and lowering community resistance), climate change may increase the invasive ability of alien species. To determine the possible success of invasive alien species, we need to determine which factors (i.e. soil; climate; dispersal ability) could work together to facilitate or hinder their spread in a changing climate. Using two invasive alien species, *Acacia cyclops* and *Acacia saligna*, this study attempts to determine which factors (i.e. soil and climate) affect their survival and growth. This was done by means of reciprocal transplants using an environmental gradient as a proxy for different climatic conditions. *A. saligna* showed no significant response to soil type, whilst *A. cyclops* showed increased survival and growth on fynbos soil. Survival of *A. saligna* was affected by its position on the gradient, i.e. climate, but its growth once established was not affected. *A. cyclops* showed significant differences in both survival and growth due to climate. It is suggested that *A. saligna* is more likely to invade mountain Fynbos under future climate change scenarios: soil type will not be a barrier to its spread, and decreased rainfall may cause lowland conditions to become too dry. *A. cyclops* is more drought tolerant and will most likely be able to maintain its range in the lowlands. Since many other factors not included in this study are likely to be synergistic with climate change, further studies of this nature are encouraged.

### Adaptation of trees to the urban environment: *Acacia karroo* in Potchefstroom, South Africa

AY Pelsler, SS Cilliers, PDR van Heerden and GHJ Krüger  
Department of Botany, School of Environmental Science and Development, North-West University, Potchefstroom Campus, Private Bag X6001, Potchefstroom 2520, South Africa

Urban open spaces are of strategic importance for the quality of life of our increasingly urbanized society. Trees and related vegetation within the communities and cities are planted and managed to create or add value to the busy lives of the city dwellers.

Trees in towns and cities form an important part of complex urban ecosystems and provide significant ecosystem services and benefits for urban dwellers, for example; reducing particulate pollution, carbon sequestration, decrease air temperature, decrease water runoff, aesthetic value and an increase in human health. Trees are a solar-powered technology that can help restore balance to dysfunctional urban ecosystems. Trees form strands in the urban fabric that connect people to nature and to each other.

The urban environment put a tremendous strain on trees by trenching, limited root growth and emission of pollutants into the atmosphere, water and soil. The problem is we don't always know what the impact of the urban environment is on the trees within our community.

The aim of this investigation was to assess the anthropogenic and environmental impacts on urban trees by measuring the tree vitality of *Acacia karroo* using chlorophyll fluorescence kinetics (JIP-test) and leaf water potential using a pressure chamber. Tree vitality measurements were correlated with soil and vegetation information following an urbanization gradient approach. Additionally a model to determine the monetary value of trees in urban environments (SATAM) was tested. All this information would eventually contribute to develop an urban tree management program for Potchefstroom.

### Anther cap retention prevents self-pollination in *Eulophia foliosa*

CI Peter<sup>1,2</sup> and SD Johnson<sup>2</sup>

<sup>1</sup>Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

<sup>2</sup>School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

Pollination by insects that spend long periods visiting many flowers and inflorescence comes with an increased risk of facilitated self-pollination. Orchids and asclepiads may be at higher risk as their pollen is packaged as pollinia and so can be deposited on self-stigmas en masse. Many orchids and asclepiads have adaptations to limit self-deposition of pollinia, including gradual reconfiguration of pollinaria following removal. Here we examine an unusual mechanism – anther cap retention – that appears to prevent self-pollination in the South African orchid *Eulophia foliosa*. We observed and timed visits by pollinators to the inflorescence. Anther cap retention times were determined for removed pollinaria and atmospheric vapour pressure deficit recorded concurrently. Anther cap anatomy was examined using light microscopy. *E. foliosa* is pollinated almost exclusively by *Cardiophorus obliquemaculatus* (Elateridae) beetles, which remain on the deceptive inflorescences for an average of 301s ( $n=18$ ). The anther cap that covers the pollinarium is retained for an average of 512s ( $n=24$ ) after removal by the pollinator. In all populations measured, anther cap dimensions are greater than those of the stigmatic cavity, thus precluding the deposition of self-pollinia until after the anther cap has dropped. An anatomical investigation of this mechanism suggests that differential water loss from regions of the anther cap result in the opening of the anther cap flaps. This is supported by observations that as atmospheric vapour pressure deficits increased, the duration of anther cap retention was reduced. Retention of anther caps for a period exceeding average visit times by beetles to inflorescences appears to effectively prevent facilitated self-pollination in *E. foliosa*.

### Pollinator driven divergence in the *Eulophia parviflora* complex

CI Peter<sup>1,2</sup> and SD Johnson<sup>1</sup>

<sup>1</sup>School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

<sup>2</sup>Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

Intraspecific variation in floral morphology and phenology may reflect adaptations to local pollinator assemblages. *Eulophia parviflora* has been described as a “rather variable species” occurring in the south-eastern and eastern parts of South Africa. A phenetic analysis of floral and vegetative characters suggests that there are at least two distinct taxa included in *E. parviflora* as presently delimited. These include a later flowering short-spurred taxon with dense inflorescences occurring at moderate to high altitude and an earlier flowering, long spurred taxon with lax inflorescences occurring at lower altitudes. The short-spurred form (spur  $c$  3.4mm) is pollinated by a beetle, *Cyrtothyrea marginalis* (Cetoniinae, Scarabaeidae) while the long-spurred (spur  $c$  7.1 mm) form is pollinated by a bee, *Amegilla fallax* (Anthophorinae, Apidae), which has a proboscis  $c$  7.4mm long. Besides having distinct floral morphologies, the scents of the two forms are different. The scent of the short-spurred form is dominated by Geraniol which is known to attract some species of Cetoniinae to traps. Experiments using a Y-maze show that *C. marginalis* beetles discriminate between the scents of the two forms and are attracted only to the scent of the short spurred form. The phenology of the two forms may also be adapted to the pollinator activity. The short-spurred form begins flowering in mid-September corresponding with the emergence of *C. marginalis* after the first rains. The long-spurred form flowers even earlier, even in areas where the two forms are sympatric, possibly deceiving newly emerged and possibly naïve *Amegilla* bees at a time when few other plants are in flower.

### Differences in structural damage caused by Russian wheat aphid (*Diuraphis noxia*) in susceptible and resistant wheat cultivars

SA Saheed, L Liu and CEJ Botha

Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

Investigation of comparative effects of feeding damage by Russian wheat aphid (RWA, *Diuraphis noxia* Mordvilko) on both susceptible and resistant wheat cultivars (*Triticum aestivum* L var Betta and Betta-Dn respectively) was carried out to establish ultrastructural damage caused by this aphid and the possible influence of resistance genes over the susceptible type. Damage symptoms which include chlorosis and necrosis were first observed in the susceptible cultivar within two weeks of infestation while the resistant type showed little effect of aphid feeding during the same time frame. Our study shows diverse differences in damages due to probing and feeding activities. Deposition of salivary materials and subsequent cellular destruction were observed within mesophyll and bundle sheath cells, treachery elements, thick- and thin-walled sieve elements, including their associated companion and parenchyma cells.

### Effects of trifluoroacetic acid on photosynthesis of C<sub>3</sub> and C<sub>4</sub> crop plants

MF Smit<sup>1</sup>, GHJ Krüger<sup>1</sup>, PDR van Heerden<sup>1</sup> and RJ Strasser<sup>2</sup>

<sup>1</sup>School of Environmental Sciences and Development: Section Botany, North-West University, Potchefstroom 2522, South Africa

<sup>2</sup>Bioenergetics Laboratory, University of Geneva, CH-1254 Jussy, Switzerland

The aim of this study was to determine the physiological and biochemical basis of the inhibition of photosynthesis by trifluoroacetic acid (TFA) in *Phaseolus vulgaris* and *Zea mays*, representing plants with C<sub>3</sub> and C<sub>4</sub> photosynthetic pathways respectively. TFA is a pollutant generated in the atmosphere by the degradation of hydrofluorocarbons (HFCs), as well as other anthropogenic sources such as teflon. Photosynthetic gas exchange and fast phase fluorescence kinetics were measured in parallel over a 12-day period in plants cultivated in a hydroponic system under controlled conditions. TFA concentrations ranged from 0.625 to 160mg Na-TFA dm<sup>-3</sup>. Photosynthetic stimulation was observed at low TFA concentrations with inhibition at higher concentrations. The stimulating effect was reflected both by parameters of primary photochemistry and photosynthetic gas exchange. New information was obtained with regard to the site of action of TFA in photosystem II. Analysis of photosynthetic gas exchange revealed that, besides constraints on mesophyll

processes, stomatal limitation increased with increasing TFA concentration. Though the mechanism of inhibition was similar, in general the C<sub>4</sub> species were affected more than the C<sub>3</sub> species.

### Differences in the intertidal seaweed communities of wave exposed and sheltered rocky shores

P Steyn and CA Ray

Department of Botany, Nelson Mandela Metropolitan University, P.O. Box 7700, Port Elizabeth 6031, South Africa

Stephenson and Stephenson (1972) were some of the first researchers to record the apparent difference between the intertidal communities at wave-exposed sites, and those at sheltered sites. One of the areas cited as an example of this phenomenon was the intertidal rocky shore along the coast of Port Elizabeth. This anecdotal account of such a community change was investigated in more detail. Species abundance data was gathered using 0.25m<sup>2</sup> quadrats placed along a transect line at sites that were visually estimated to be either exposed to wave action, or sheltered. These data were analysed using kite diagrams and multivariate techniques. The results show a distinct difference in the macrophyte community structure between these different rocky shore habitat types. Coralline turf algae seem to dominate sheltered areas, with species like *Gelidium pristoides* being more characteristic of wave-exposed sites. These differences are important considerations when selecting sites for baseline monitoring (to be compared with impacted areas) in environmental studies.

### Mechanistic basis for chilling-inhibition of PS II photochemistry in soybean genotypes of contrasting tolerance

PDR van Heerden<sup>1</sup>, AJ Strauss<sup>1</sup>, GHJ Krüger<sup>1</sup> and RJ Strasser<sup>2</sup>

<sup>1</sup>School of Environmental Sciences and Development: Section Botany, North-West University, Potchefstroom 2522, South Africa

<sup>2</sup>Bioenergetics Laboratory, University of Geneva, CH-1254 Jussy, Switzerland

Low night temperatures (dark chilling) have dramatic effects on phenology, photosynthesis and yield of soybean. The mechanisms involved in the dark chilling-inhibition of photosystem II (PS II) function were determined in a chilling sensitive (PAN 809) and tolerant (Highveld Top) South African soybean genotype. Plants were grown under controlled environmental conditions and dark chilled at 6°C for up to 12 consecutive nights. The effects on PS II function were quantified by means of chlorophyll *a* fluorescence. Kautsky (O-J-I-P) transients were analysed with the JIP-test in order to translate stress-induced changes in the kinetics of these transients to changes in energy flux through PS II. The performance index (PI<sub>ABS</sub>), an expression that takes into account the three main functional steps of PS II photochemistry (light absorption, excitation energy trapping and electron transport), decreased on average by 65% in PAN 809, but only by 18% in Highveld Top. Recorded O-J-I-P transients revealed a substantial increase in fluorescence intensity at the J-step in PAN 809, which is symptomatic of over-reduction of the primary electron acceptor (Q<sub>A</sub>) pool due to chilling-induced limitations on PS II electron transport. Energy-flow models revealed large-scale deactivation of PS II reaction centres, reductions in electron transport and increases in heat dissipation in dark chilled plants of PAN 809 but not in Highveld Top. The changes in PS II photochemistry correlated with decreases in CO<sub>2</sub>-saturated rates of photosynthesis. More importantly, above differences between the genotypes correlated with their yield response long after the actual dark chilling stress episode.

### Physiological and biochemical response of the succulent *Augea capensis* to drought stress

PDR van Heerden, JW Swanepoel and GHJ Krüger

School of Environmental Sciences and Development: Section Botany, North-West University, Potchefstroom 2522, South Africa

Skorpion Zinc mine (Namibia) is situated in an area known as the *Spergebiet* (restricted diamond area). The unique vegetation in the area is ecologically sensitive and falls within the northern boundaries of the succulent Karoo biome, which is regarded as a global biodiversity hotspot. Concern was expressed about the possible impacts of mining activity (SO<sub>2</sub> pollution, excessive dust deposition etc.) on the vegetation. A long-term ecophysiological monitoring program was established to investigate this possibility. The first step, however, was to characterize the effects of drought stress on the vegetation. This information was required to enable distinction between effects caused by frequent drought stress episodes on the one hand, and mining activity on the other hand. The effects of drought stress were investigated in the succulent *Augea capensis*. Fifteen measuring sites were selected in the vicinity of Skorpion Zinc mine. During each of four visits, chlorophyll *a* fluorescence measurements were conducted on plants at each measuring site. For laboratory investigations, plants were transplanted from the desert environment to pots and transferred to a growth chamber. Potted plants were exposed to drought stress for a period of up to twenty days. Besides chlorophyll *a* fluorescence, CO<sub>2</sub> assimilation and Rubisco activity were also measured in these plants. Drought stress resulted in down-regulation of photosystem II (PS II) function. Although this phenomenon was more pronounced in the field-grown plants, both drought stress scenarios resulted in similar effects, namely deactivation of PS II reaction centres and decreased electron transport. Water availability modulated overall plant vitality to a very large extent under field conditions. Drought stress in potted plants led to decreased carboxylation efficiencies, CO<sub>2</sub> saturated rates of photosynthesis and extractable Rubisco activity. Rapid recovery of photosynthesis following rewatering, however, indicated that even severe drought stress effects induced in pots were easily reversible.

### Analysis of radiation-induced genome variation in cowpea

BJ Vorster<sup>1</sup>, C van der Vyver<sup>2</sup> and CA Cullis<sup>3</sup>

<sup>1</sup>Department of Botany, University of Pretoria, Pretoria 0002, South Africa

<sup>2</sup>Institute for Plant Biotechnology, University of Stellenbosch, Stellenbosch 7599, South Africa

<sup>3</sup>Biology Department, Case Western Reserve University, Cleveland, Ohio, U.S.A.

Cowpea (*Vigna unguiculata* L. Walp.) is an important food legume and an essential component of cropping systems in the drier regions of the tropics. Cowpea seeds (IT93K129-4) were exposed to irradiation dosages of 180 Gy. These seeds were then grown up and the plants screened and selected for drought tolerance through several generations. The mutagenic effects of radiation were first reported in the 1920's and are frequently used in plant breeding. A survey of mutations induced in irradiated *Arabidopsis* seeds suggests that ionizing radiation is a fairly reliable source of chromosomal rearrangements (Shirley et al., 1992). In this study we used representational difference analysis (RDA) as well as RAPDs to identify and isolate differences in the genomic make-up of irradiated vs. non-irradiated cowpea plants showing drought tolerance. Variable DNA regions possibly resulting from DNA methylation changes, genomic rearrangements and point mutations after exposure to gamma irradiation have been isolated for further analysis. The goal of the study is to gain an understanding of the response of the cowpea genome to gamma radiation and its DNA repair mechanisms by tracking the various mutations through several generations and possibly correlating a specific mutation to the increased drought tolerance.

### Secondary succession of Thicket at a limestone quarry in the Gamtoos River Valley, South Africa

CR Weatherall-Thomas and EE Campbell

Botany Department, Nelson Mandela Metropolitan University, P.O. Box 77000, Port Elizabeth 6031, South Africa

The method of revegetation implemented at the PPC Cement (Pty) Ltd Loerie limestone quarry 21 years ago was to cover mined sites with topsoil from other cleared areas and then allow the vegetation to return through secondary

succession. The quarrying of limestone resulted in the clearance of large areas of Thicket with two types of Thicket occurring in the surrounding area. In 1999 five different aged sites (1, 2, 6, 10 and 16 years) were assessed as a chronosequence. All revegetation sites were situated on north-facing slopes where Mesic Succulent Thicket occurs. The vegetation had not returned to Mesic Succulent Thicket but remained at a sub-climax stage, dominated by *Acacia karoo* Hayne, *Polygala myrtifolia* L., *Rhus incisa* L.f. var. *incisa* and *Clutia daphnoides* Lam. The same sites were reassessed in 2005 using a method identical to that of 1999. There was an increase in percentage canopy cover and species richness for most sites. While the oldest sites remained dominated by the sub-climax species, there were a few new Thicket species recorded as well as the geophytes *Dietes iridioides* (L.) Sweet ex Klatt and *Ledebouria ensifolia* (Eckl.) S. Venter. However succulent species such as *Aloe africana* Mill., *A. pluridens* Haw., *Crassula ovata* (Mill.) Druce, *C. tetragona* L. and *Euphorbia fimbriata* Scop. remained absent. This, together with an ordination separation between the revegetated stands and Thicket, indicates that Mesic Succulent Thicket had still not returned to the site.

### Some field observations of the infection of *Welwitschia mirabilis* seeds by *Aspergillus niger*

C Whitaker, NW Pammenter and P Berjak

School of Biological and Conservation Sciences, University of KwaZulu-Natal, Howard College Campus, Durban 4041, South Africa

The seeds of the rare desert gymnosperm *Welwitschia mirabilis* are orthodox and thus easily stored by conventional means at low temperature and relative humidity. However, the seeds are often infected by the fungus *Aspergillus niger* var. *phoenicis*, which kills the developing seedling. This seed-associated fungus could pose a serious problem in terms of propagation of the species, both in its natural habitat and in planting programs. It is therefore important that the course and mechanisms of infection are elucidated. Presented here are some field observations of the infection of the seeds of a population of plants at Hope Mine in the Namib-Naukluft Park, Namibia. The course of the infection of the seed-bearing cones was monitored between November 2004 and April 2005, and was found to reach a peak (100% of cones infected with *A. niger*) in February 2005. This was coincident with the appearance of the pollination drops on the cones and with weather conditions favouring the growth and dispersal of fungal spores. Spores of *A. niger* were found embedded in the dehydrating pollination drop of cones examined using the scanning electron microscope. *A. niger* was found to be carried by insects common in the area such as ants and *Probergrothius sexpunctatis*, and spores were present in the air and soil. Surrounding vegetation was also found to harbour the fungus. It is proposed that spores gain access to the developing ovule through the micropyle, whereupon the spores germinate and the fungus proliferates within the developing seed. *A. niger* infection was total in this population of plants, and it is doubtful whether any seedling recruitment will occur while the fungus is present to such devastating levels in the local environment.

### Responses of *Phragmites australis* to salinity under field conditions

T Xuma, G Naidoo and Y Naidoo

School of Biology and Conservation Science, University of KwaZulu-Natal, Private Bag X54001, Durban 4000, South Africa

*Phragmites australis* is a perennial aquatic grass species that is widely distributed in fresh water wetlands. This species also occurs in saline areas such as estuaries, salt marshes and fringes of mangrove swamps probably due to clonal variation. In this field investigation we compared soil physiochemical conditions, ion and water relations and photosynthetic performance of *P. australis* growing under saline and non-saline conditions in the Beachwood Nature reserve, Durban. Leaf and soil samples from both sites were analyzed for inorganic ions. Gas exchange was measured with a LICOR 6400, portable, photosynthesis system and chlorophyll fluorescence parameters with a fluorometer (PAM-2001, Walz, Germany). Soil concentrations of Na, K, and Mg were significantly higher

in the saline than in the non-saline site by 88%, 65% and 33% respectively. Ca ion concentrations were 30% higher in the non-saline site. CO<sub>2</sub> exchange in the non-saline site was 41% higher than that in the saline site. Conductance and transpiration rates showed trends similar to those for CO<sub>2</sub> exchange. Yield and ETR were 6.7% and 8.51% higher, respectively, in the non-saline site. The results in this study indicate that although *P. australis* is able to grow and spread in saline areas, growth and productivity are compromised.

### **Salt accumulation as means of secondary winery wastewater treatment by plants**

NS Zingelwa

*Environmental Management: Soil Science, ARC Infruitec-Nietvoorbij, Stellenbosch 7599, South Africa*

There could be a need for secondary treatment of winery wastewater flowing from wetlands as there could still be high element concentrations in the wastewater even after treatment. Using halophytes and salt accumulating plants is beneficial mainly by the accumulation of inorganic ions that can be taken up from the soil. Through irrigating the plants with treated wastewater, sodium and salts accumulate in the plants and the possible leaching of these into the soil will be greatly reduced. The harvestable tissues of accumulation can be used in animal fodder. Plant species used in the trial are *Atriplex nummularia*, Vetiver, covercrops such as Kikuyu, Lucerne and Flanker grass have been included. Effects on plant vigour, percentage productivity, and accumulation will be discussed.