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Euphorbia is a large genus including more than 2100 species having a wide global distribution. Several *Euphorbia* spp. occur in South Africa, of which *Euphorbia ingens* (naboom) is the largest. During the course of the last 10 years, increasing numbers of *E. ingens* trees in the Limpopo Province have become diseased, followed by rapid death. Initial studies conducted in 2006 and 2007 recorded various insects and fungi associated with the decline, but a clear cause of disease was not found. Symptoms observed included grayish discoloration, rotting and browning of the succulent branches, white and yellow spots on succulent branches, blue stain of the main woody stems and insect infestation. Various genera of fungi were isolated including several undescribed genera and species. Insects found included genera in the Curculionidae and Pyralidae. A more extensive study was initiated in 2009 in which multiple factors associated with the decline of *E. ingens* were considered. These factors included symptom and disease development as well as animal damage, elevation, fire damage, grazing levels, rainfall, site aspect, size of each tree, slope, temperature and the vegetation surrounding the trees. Sites in the Limpopo and North West Provinces were monitored in 2009. Damage caused by a moth was found to be devastating in both provinces with over 64% incidence. Differences in the occurrence of graying symptoms were observed in the Limpopo and North West Province with 74 and 4% incidence respectively. It is hoped that the knowledge emerging from this extensive study will shed light on the factors responsible for the decline and death of *E. ingens* in the Limpopo province in the future.

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The role of cytokinins in seed germination

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Endogenous cytokinin levels were monitored during germination and early seedling establishment in monocotyledonous (oats and maize), dicotyledonous (lettuce and lucerne) species and thermoinhibited *Tagetes minuta* achenes. Endogenous cytokinins were identified using UPLC-ESI-MS/MS. *Cis-zeatin* types (*cZ*) were the predominant cytokinins present in dry seeds of lettuce, lucerne and oats while dihydrozeatin (DHZ) derivatives and benzyladenine (BA) occurred in the highest

concentrations in maize. Following imbibition there was a transient peak in cytokinins prior to radicle emergence in lettuce due to an increase in BA and various topolins, in lucerne due to an increase in *cZ*- and topolin-forms and in oats due to an increase in *trans-zeatin* (*tZ*) forms. Another transient cytokinin peak was detected following radicle emergence due to an increase in *cZ*-, BA- and topolin-forms in lettuce, *cZ*- and topolin-forms in lucerne, BA- and isopentenyladenine—(iP) forms in oats and a very large increase in *cZ* forms and smaller peaks in iP-, *tZ*- and BA-forms in maize. DHZ-type cytokinins occurred in the highest concentrations in dry *T. minuta* achenes. Following imbibition at 25 °C, there was a three-fold increase in the *cZ*-type cytokinins. Aromatic BA-type cytokinins showed the largest fluctuation, increasing between 26 and 40 h and dropping to low concentrations by 48 h. The thermoinhibited achenes imbibed at 36 °C had a similar cytokinin profile to the achenes germinated at 25 °C even though germination was arrested. In view of the high concentrations of *cZ* isomers and aromatic cytokinins detected in these seeds, it is necessary to re-examine the origin and physiological functions of these cytokinins during germination and seedling establishment.

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Freshwater algae (excluding diatoms) of the Sub-Antarctic Marion Island

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Marion Island is a unique, isolated natural freshwater ecosystem. Algae play an important role in the island's freshwater ecosystem, because the aquatic food web is dominated by plankton species. This represents the first taxonomical survey on cyanobacteria and eukaryotic algae (excluding diatoms) present in the freshwaters of Marion Island. The composition of freshwater algal communities, in relation to different types of freshwater bodies, was also studied. A total of 85 algal genera from 7 major divisions were present in the freshwaters of Marion Island. These divisions include Chlorophyta, Chrysophyta, Craspedophyta, Cyanophyta, Euglenophyta, Pyrrophyta and Tribophyta. *Lyngbya*, *Tribonema* and *Ulothrix* were present in all water bodies. Chlorophyta was dominant in terms of diversity, followed by the Cyanophyta. Environmental variables such as pH and PO₄-P, and to a lesser extent conductivity and NH₄-N influenced the algal composition of the freshwaters of Marion Island. Different types of freshwater bodies are inhabited by specific algal assemblages due to the extreme variability in chemical composition of the freshwaters. The main ecological factors that influence the algal community include enrichment, caused by sea spray, and biotic fertilization. An in-depth study on the algal species composition of the freshwater bodies of Marion Island will provide further

insight into the algal community composition and the relationship between algae and water quality.

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A review of ethnobotanical studies in the Cape region of South Africa

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Published and unpublished information on indigenous plant use in the Western, Northern and Eastern Cape Provinces of South Africa will be reviewed. Despite several studies, Khoi-San ethnobotany remains poorly documented and almost every recent survey has revealed important and interesting new records. These include new useful plant species, new uses and new vernacular names. Several examples of such new records from ongoing studies in the Swartland, Cederberg and Hantam regions will be presented. It can be concluded that there is an urgent need to systematically capture indigenous plant use knowledge in the Cape region in order to preserve this rich and unique cultural heritage for future generations.

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Interaction of cysteine protease inhibitor mutants with cysteine proteases

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The importance of specific amino acid residues in the structure of cysteine protease inhibitors is assessed in the project. Non-synonymous amino acid replacements are introduced in the structure of the wild-type inhibitors, with the intended effect of enhancing the inhibitory activity on targeted cysteine proteases. By “inhibitor engineering” these inhibitors might become more potent in their activity against gut cysteine proteases of targeted coleopteran insect pests. Using bioinformatics’ software packages, which predict structures based on the position and changes in the amino acid chain’s tertiary structure, structural changes of the inhibitor by altering specific amino acids have been already determined. However, the actual effect that these alterations will have on activity of targeted cysteine proteases is not yet known. So far, several mutated inhibitor sequences using both the rice and papaya cysteine protease inhibitor sequence have been produced through site-directed mutagenesis of various inhibitor sequence regions. Mutant coding sequences have been cloned into a bacterial expression vector allowing production of a GST-fusion protein in an *E. coli* system. Applying affinity chromatography using

GSH-sepharose to bind the GST fusion and treatment with Factor Xa protease to release the cysteine protease inhibitor from GST, several purified mutated inhibitors have been already produced. These purified inhibitors will be used for *in vitro* enzymatic assays to determine the influence mutations have on the inhibitory activity.

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Modeling plant cystatins for targeted activity in a multi-trophic context

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Plant cystatins represent an attractive target for the improvement of pest-resistance in transgenic crops intended for human use, given the absence of cysteine proteases in the human gut and the widespread occurrence of these target enzymes among herbivorous *Coleoptera*. In plants, cystatins are involved in the regulation of several physiological and cellular processes such as organogenesis and storage protein turnover. They also play an important role in the regulation programmed cell death, tolerance to abiotic stresses, and defense against herbivorous and various pathogens. Cystatins act as pseudo-substrates that enter the active site cleft of target cysteine proteases to form tight, reversible complexes that inhibit protease activity. It has been shown that selected mutations of plant cystatins can alter their inhibitory potential against a range of cysteine proteases. Ideally cystatins used in a transgenic approach would be active only against the digestive proteases of target insects while showing little or no activity against the endogenous proteases of the plant or against non-target insects. It has been shown that selected mutations of plant cystatins can alter their inhibitory potential against a range of cysteine proteases. Using a computational biology approach to predict the interaction of candidate cystatins against a range of proteases from plants as well as target and non-target insects we aim to modulate the activity of the cystatin by rational mutation of selected residues in order to achieve targeted activity.

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The evaluation of the effect of seed coating on the germination of three perennial grass seed types in different growth mediums

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