The effect of *Beauveria bassiana* as biological control for sweetpotato weevil

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*Beauveria bassiana* is a fungus which causes a disease known as the white muscadine disease in insects. When spores of this fungus come in contact with the cuticle of suspected insect they germinate and grow directly through the cuticle of the inner body of their host. Here the fungus proliferates throughout the insect body and produce toxins, draining the insect of nutrients, eventually killing it. The aim of the study was to test the use of *B. bassiana* for controlling weevil of sweetpotato. An experiment was conducted at ARC-VOP with four treatments namely: 1) dipping sweet potato cuttings in a solution of *B. bassiana* before planting at 1g/litre of water, 2) spray with registered chemical decis at 50ml/100litre of water, 3) spraying the leaves every 2 weeks with the fungus for four months after planting; and 4) control which is sprayed with distilled water. The experiment layout was a Latin square design with the pest management strategy as the main plot factor and the cultivars (Bophelo, Ndou and Blesbok) as subplot factors (4x3 Latin square). Data was collected on number of tubers that had insect damage per treatment. The data was analysed using Genstat statistical package 12\(^{th}\) edition. The results revealed that spraying the fungus on a forth nightly basis significantly (p<0.05) reduced the number of sweet potato tubers damaged by insect as compared to the control. Dipping the cuttings in a solution of the fungus did not significantly reduce the number of sweetpotato tubers damaged by the weevils. There was no significant difference between the chemical spray and the spraying with the fungus. *B bassiana* can be sprayed on the sweetpotato leaves every 2 weeks with the fungus for four months after planting as alternative control method for the sweet potato weevil.
Screening *Busseola fusca* populations for resistance to Bt maize events in south Africa

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Transgenic maize expressing Bt proteins to manage lepidopteran pests such as *Busseola fusca* (Lepidoptera: Noctuidae) have been commercialized in South Africa. *Busseola fusca* has been reported to be resistant to Bt maize (Cry1Ab protein) at several localities in South Africa. However reports of pest infestation in Cry1Ab Bt maize are regularly made in several regions, resistance has only been confirmed in controlled laboratory experiments with larvae collected from a few of these regions. There is an urgent need to evaluate *B. fusca* populations in South Africa for their susceptibility to Bt maize. The aim of the study was to screen different populations of *B. fusca* for resistance to Bt maize and to generate baseline data regarding pest susceptibility for South Africa. Results will provide an indication of the resistance status of *B. fusca* populations across the maize production area. Stem borer larvae were collected from different field sites. Laboratory feeding studies with maize events expressing Cry1Ab and Cry1A.105+Cry2Ab2 (pyramid), were conducted to compare pest fitness to that on non-Bt iso-hybrids as control. The following life-history parameters were recorded: larval survival and mass, LT50, larval duration, pupation percentage, mass of male and female pupae, pupal duration and sex ratio. Larval survival of up to 51% on Cry1Ab maize have been observed. No survival was recorded on the pyramid event. Larval mass for some populations was significantly higher on the non-Bt iso-hybrid compared to the single-gene event. This study provides a background on pest susceptibility that can be used in other African countries where Bt maize will be introduced in future. This study documented the levels of resistance of maize stem borer in SA and will allow us to be able to give early-warning if this pest also evolves resistance to the pyramided events which have been cultivated in SA from 2013 onwards. It is foreseen that these resistance screenings will be an on-going activity.
Survival of *Busseola fusca* migrating between Bt and non-Bt maize plants: mimicking a seed mixture scenario

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The high-dose/refuge IRM strategy is used globally to manage the development of resistance in Bt crops. Seed mixture strategies are being considered for a number of pest species, especially after the reported cases of resistance development. *Busseola fusca* are one of the most important stem borer species in Southern Africa and have evolved resistance to Bt maize expressing Cry1Ab proteins. A possible disadvantage with deployment of the seed mixture strategy is that high levels of larval migration can occur between non-Bt and Bt plants. This could lead to exposure to sub-lethal dosages of Bt proteins. The high-dose/refuge strategy was set in place in order to delay resistance development in insects. Sub-lethal dosage expression levels of the Cry protein in maize plants could also play a role together with farmers that do not scout and monitor their fields for pests as they assumed the technology controlled the pests effectively. Evolution of resistance by pests threatens the continued efficacy of GM crops. This study was conducted to determine the efficacy of Bt maize to control larvae of different growth stages that migrate from non-Bt to Bt plants. A laboratory study was conducted in which Cry1Ab-resistant *B. fusca* larvae were reared on maize cultivars (non-Bt, single-gene and pyramid) for three, nine and 21 days, after which larvae were transferred to similar treatments, in various combinations. Larval survival and mass were determined seven days after transferring the larvae of the different ages to the other maize cultivar treatments. Results proved that older larvae survived when moved from non-Bt to Bt maize. The “pyramid” gene event indicted to be more effective in controlling larvae than the single gene event, especially very young larvae. Results indicate that the probability of larvae migrating from Bt to non-Bt plants increase with age.
Insight into three putative *Cercospora zeina* effector genes and the role they play in virulence.

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Grey leaf spot (GLS) disease is an economically important foliar disease of maize caused by *Cercospora zeina* in southern Africa. However, little is known about the molecular mechanisms underlying *C. zeina* infection. Fungal effectors are a pathogen’s strategic method to evade host detection or to be able to suppress or interfere with the host defence mechanisms for successful infection. Most fungal effector proteins identified have been species-specific, and shared no sequence similarity with any other known protein sequences. Recently, fungal effectors Avr4, Ecp2, and Ecp6 from *Cladosporium fulvum* have been shown to have homologs in other fungal species belonging to the Dothideomycete class. The aim of this study was to identify whether *Avr4*, *Ecp2*, and *Ecp6* effector homologs were present in *C. zeina*. Homologs of the effector genes *Avr4*, *Ecp2*, and *Ecp6* were identified and annotated in the draft *C. zeina* genome. The presence of the *Avr4*, *Ecp2*, and *Ecp6* effectors in the *C. zeina* genome, together with their putative conserved domains, provide insight into the possible roles that these proteins might play during maize infection. The *in planta* expression profiles of *C. zeina* *Avr4*, *Ecp2*, and *Ecp6* were analysed by reverse transcriptase quantitative PCR (RT-qPCR). The study identified two *C. zeina* reference genes suitable for *in planta* gene expression normalisation, namely glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and cytochrome c oxidase subunit III (*Cyt III*). *GAPDH* and *Cyt III* showed constant expression across all inoculation time points analysed making them suitable reference genes for expression normalisation. It was shown that *C. zeina Avr4* and *Ecp6* were expressed at constant levels during infection, while *Ecp2* was expressed at very low levels at all time points analysed. Determination of *C. zeina* genomic DNA content by means of an optimised qPCR method at each time point enabled correlation studies between fungal quantity and effector gene expression. *Avr4* and *Ecp6* expression showed a weak positive correlation to fungal quantity. Phytotron inoculations of maize with *C. zeina* were established in this study which facilitated experiments independent of the maize growing season. *In planta* expression analysis of the effector genes *Avr4*, *Ecp2*, and *Ecp6* therefore has yielded further insight into the molecular mechanisms of maize infection by *C. zeina*. 
In planta expression of novel Antarctic bacterial stress-response protein

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A novel bacterial gene, homologous to Water Hypersensitive domain (WHy) which is a typical component of Late Embryogenesis Abundant (LEA) proteins, was identified in an Antarctic desert metagenomic library. The LEA proteins occur widely in prokaryotes as well as in eukaryotes (e.g. bacteria, archaea and plants) and are expressed under stress conditions. A previous study showed significant protection of an E. coli recombinant, expressing the novel WHy protein, against freeze and cold stress. The aim of this study is to address the question of whether this novel WHy protein can be functionally expressed in Arabidopsis and whether it will confer cold- and freeze-protection in planta. Materials/Method: Two different WHy gene constructs were created; one carrying an additional signal peptide sequence that is thought to be beneficial for protein expression in Arabidopsis and a truncated construct without this sequence (WHy and ΔWHy). WHy gene expression in Arabidopsis thaliana used Agrobacterium-mediated transformation of the host plant. First and second generation (T0 and T1) recombinant plants were screened for the successful integration of the WHy gene sequence in the host genome and WHy protein expression. Both WHy and ΔWHy recombinant plants have showed a successful integration of the novel gene into the Arabidopsis genome, and positive WHy protein expression was observed in both recombinants. For stress tolerance tests, plant candidates showing (I) a successful integration of the WHy gene into the Arabidopsis genome, (II) constitutive expression of the WHy protein and (III) the capacity to develop seeds to produce healthy T1 offspring have been selected. Stress tolerance tests are now underway.
Transcriptome sequencing of the *Exserohilum turcicum-Zea mays* interaction

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*Exserohilum turcicum* is the causal agent of Northern corn leaf blight (NCLB), a yield-limiting foliar disease of maize, sorghum and related grass species. The resistance of maize to NCLB is mediated by four major resistance (*R*)-genes; namely *Ht1, Ht2, Ht3* and *HtN*. *Exserohilum turcicum* is classified into races based on the ability of the pathogen to overcome these *R*-genes. Fungal effectors play an important role in mediating susceptibility of the host to disease. The aim of this study is therefore to identify specific effectors interacting with the *Ht*-genes in maize. Maize seedlings at the trifoliate leaf stage were infected with either a race 13N or a race 23N *E. turcicum* isolate. Plants were collected prior to inoculation as well as at two, five, seven and thirteen days post inoculation. RNA was extracted from infected leaf material and sent to the Beijing Genomics Institute in Hong Kong for paired-end, strand-specific transcriptome sequencing. Transcripts were assessed for quality and will subsequently be mapped to the available *E. turcicum* and *Zea mays* genomes. The results from this study will be used to identify the effectors that play an important role in the pathogenicity of *E. turcicum* on maize.
Functional characterisation of the putative pathogenicity factor, czk3 in _Cercospora zeina_

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Grey leaf spot is a destructive foliar disease of maize that occurs in most regions in the world. It is caused by _Cercospora zeina_ and _Cercospora zeae-maydis_, with _C. zeina_ being the causal agent of grey leaf spot in South Africa. Little is known about the biochemical and molecular events of _C. zeina_ pathogenesis. In this study we characterised the _C. zeina czk3_ gene, which has been shown to play a role in the pathogenicity of _C. zeae-maydis_. The _czk3_ gene was identified in the genome sequence of an African _C. zeina_ isolate. Thereafter, single _C. zeina_ conidial cultures were recovered from maize leaves with severe disease symptoms, DNA was isolated from these cultures and PCR amplification and sequencing were used to verify the _czk3_ gene sequence from _C. zeina_. The African _C. zeina czk3_ gene has a 4119bp open reading frame which encodes 1,373 amino acids which is similar to the _czk3_ gene from _C. zeae-maydis_. However, comparison between _czk3_ from _C. zeina_ and _C. zeae-maydis_ confirmed differences in the gene sequence. We prepared split marker constructs containing 5’ and 3’ _czk3_ flanking regions re-joined to a hygromycin marker gene. The split marker constructs have been transformed onto _C. zeina_ through protoplast transformation to knockout the _czk3_ gene. The presence of the hygromycin construct has been verified in a putative _czk3_ knockout mutant. Future studies will involve further screening to ensure that the hygromycin gene has replaced the _czk3_ gene in _C. zeina_. Additionally, a maize infection trial will be undertaken with wild type and knockout _czk3_ strains to assess the role of _czk3_ in pathogenicity of _C. zeina_.

Genetic analysis of yield and flesh colour in sweetpotato [Ipomoea batatas (L.) LAM]

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Sweetpotato is an important root crop that combines high edible energy and vital micronutrients to combat micronutrient deficiencies and food insecurity in the developing countries. Breeding of sweetpotato aims at both high yielding and high in β-carotene content to meet needs of farmers and consumers. Although superior cultivars have been released, pre-breeding information on the inheritance mechanism of the essential traits such as β-carotene, yield and yield related traits is limited. This study aimed at assessing the performance of the diallel progenies, establishing the genetic control of root yield and flesh colour of five selected cultivars. A 5 x 5 full diallel analysis was performed and F1 progenies evaluated in two environments alongside the parents. Data on yield parameters and flesh colour was collected and analysed using Genstat and Agrobase. Significant differences (P<0.01) were found between the performance of the genotypes for the marketable fresh root yield (MFRY), marketable number of roots (MNR), total fresh root yield (TFRY), total number of roots (TNR) and root β-carotene content (RBCC). There was significant interaction between the environment and the performance of the progenies for MFRY and RBCC. Significantly higher MFRY and RBCC were observed in Roodeplaat and Empangeni, respectively when results were compared across two locations. Progenies from crosses W-119 x Khano, Ndou x Khano, Monate x W-119, Monate x Resisto showed significant improvement in the total fresh root yield and root β-carotene content across the two environments compared to the parental lines. Significant GCA and SCA (P<0.01) were observed for all the traits. Additive gene action was involved in the inheritance of yield parameters and flesh colour. Low to moderate narrow heritability values were observed for the yield parameters and very high narrow sense heritability for RBCC across the two locations. These results are useful for future selection and breeding of elite high yielding and β-carotene rich cultivars.
Alexin™ treatment boosts defence responses in wheat against Russian wheat aphid (Diuraphis noxia) infestation

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Alexin™ is a nutrient complex with salicylic acid derivatives that increases the inherent resistance of plants. It acts as a priming agent providing long-term protection against a broad range of pathogens and pests. The effect of Alexin™ treatment on the defence responses (β-1,3-glucanase and peroxidase activity) of wheat during Russian wheat aphid (RWA, Diuraphis noxia) infestation was investigated. Wheat cultivars susceptible (SST 387) or resistant (Elands, PAN 3379) to the South African RWA biotype 1 were treated with different Alexin™ concentrations (0.25%, 0.375%, 0.5%) before RWA infestation (20 aphids per plant). Apoplastic enzyme activities of β-1,3-glucanase and peroxidase were determined immediately after infestation, and 48 h later. For both susceptible and resistant cultivars, Alexin™ treatment at a concentration of 0.25% did not induce any significant increase in activity compared to control. Infestation of Alexin™ treated (0.375% and 0.5%) susceptible plants induced almost similar responses in both enzyme activities, which were significantly higher than control; a concentration of 0.375% caused a 2.5-fold increase in β-1,3-glucanase and a 2.3-fold increase in peroxidase activity. In the resistant plants, RWA infestation of Alexin™-treated (0.375%) plants activated specific changes in enzyme activities; a significant increase was noted only in peroxidase activity of Elands but not PAN 3379. Furthermore, Alexin™ treatment and infestation did not induce any significant increase in β-1,3-glucanase activity of either Elands or PAN 3379. These results suggest that Alexin™ has a specific priming effect that is sensitive to concentration and depends on plant genotype. Future studies should indicate if Alexin™ treatment is also biotype specific in the protection of wheat against the RWA.
The morphological, molecular and protein (MALDI-TOF) identification of *Alternaria* species on sunflower (*Helianthus annuus* L.) in South Africa.

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In South Africa, the sunflower crop is the third most important field crop after maize and wheat. The quantity of the sunflower oil represents about 82% of all edible oil produced in South Africa. Seed health tests have shown that up to 98% of certain seed-lots sampled from South African sunflower farms have been found contain various *Alternaria* species. *Alternaria* species are known to cause spots on all plant parts. *Alternaria* eventually leads to the premature defoliation of leaves and causes poor seed germination. The aim of this study is to determine the distribution of Alternaria blight by surveying the major sunflower growing areas in South Africa and identifying the causal agents of Alternaria blight based on morphology, protein profiling (MALDI-TOF) and molecular techniques. A survey was conducted in over 15 major sunflower farms in South Africa; these farms were screened and rated for diseases. During the survey, infected sunflower leaves, stems and seed samples were collected for isolation and identification of disease-causing pathogens. The *Alternaria* isolates were grown on PCA at 25°C under 12 hours alternating-light/darkness. Three gene regions (RBP2, TEF and GADPH genes) were used for molecular identification. The results obtained from the surveys indicate that disease severity caused by *Alternaria* spp. on the sunflower crops ranged between 44-81% from research farms and also farmers/producers fields. Most isolates were recorded as *A. tenuissima* based on the morphological characteristics of conidia, conidiophores and the three-dimensional sporulation pattern. Previous surveys have shown *A. helianthicola* to be persistant on sunflower farms. There is a strong consensus between molecular and morphological identification. Identification of the *Alternaria* species using the MALDI-TOF to confirm the morphological and molecular results is currently underway.*Alternaria tenuissima* and *A. helianthicola* has not previously been recorded in South Africa and the information gathered in this study may help with control strategies.
The role of water stress on *Fusarium verticillioides* infection and fumonisin synthesis in maize kernels.

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Maize (*Zea mays* L.) is one of the most important crops planted worldwide, it is a host of many plant pathogens, amongst them *Fusarium verticillioides*, which is distributed worldwide. This fungus produces secondary metabolites, fumonisins, which can cause mycotoxicoses in animals and humans. The WHO for cancer research classified fumonisins as group 2B which means is can possibly cause cancer in humans. In a glasshouse trial two cultivars, PAN6P-110 and CRN3505, were planted in 80 ℓ black bags and treated with five watering regimes (35 ℓ, 30 ℓ, 25 ℓ, 20 ℓ- and 15 ℓ per week). Maize ears were inoculated at silking stage with *F. verticillioides* (MRC826) to investigate the effect of water stress on fungal biomass and fumonisin production. After harvest, fumonisins and *F. verticillioides* fungal biomass were quantified with HPLC and qRT-PCR respectively. Chlorophyll fluorescence as indication of plant stress was measured at five different growth stages namely 8-leaf, silking, after inoculation and soft dough. Data was analysed with GenStat (14th Edition) using an ANOVA to determine the effect of water stress on fungal biomass and fumonisin production. After harvest, fumonisins and *F. verticillioides* fungal biomass were quantified with HPLC and qRT-PCR respectively. Chlorophyll fluorescence as indication of plant stress was measured at five different growth stages namely 8-leaf, silking, after inoculation and soft dough. Data was analysed with GenStat (14th Edition) using an ANOVA to determine the effect of water stress on fungal biomass and fumonisin synthesis. Chlorophyll fluorescence data was analysed using PEA plus software version p1.30 from Hansatech. ANOVA indicated significant treatment (P=0.01) effect for fungal biomass and cultivar (P=0.00) effects for fungal biomass and fumonisins. Mean fungal biomass was 25.25 pg at 30 ℓ and 45.12 pg at 15 ℓ-per week. Mean fungal biomass and fumonisins for PAN6P-110 was 20.49 pg and 4.04 ppm, for CRN3505, 38.33 pg and 7.72 ppm respectively. Chlorophyll fluorescence data indicated PAN6P-110 to withstand water stress better than CRN3505 at certain growth stages. This trial was inoculated, which could have attributed to the significant water regime x cultivar interaction regarding fungal biomass. Previous studies confirmed fungal biomass is not a direct reflection of fumonisin synthesis which may explain the significant treatment effect on fungal biomass, but not fumonisin synthesis.
Incidence of plant-parasitic nematode infections and aflatoxin production in groundnut kernels

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Groundnut (*Arachis hypogaea*) is a nutritious cash crop for subsistence farmers. Such crops are often damaged by plant-parasitic nematodes, hence impacting adversely on quality and yield. Consumption of aflatoxin-contaminated groundnut pods/kernels can lead to acute or chronic aflatoxicosis in humans and animals. The aims of this study were to i) identify nematode pests that parasitise groundnut hulls and kernels and ii) quantify aflatoxin production in groundnut kernels. Groundnut pod samples were collected from the Jozini, Manguzi and Mbazwana districts in the KwaZulu-Natal Province during the 2012/13 and 2013/14 growing seasons at harvesting. Plant-parasitic nematodes were extracted from groundnut hulls and kernels by soaking such plant parts in tap water for 24 h at 25 °C. Nematodes obtained were subsequently counted and identified using a stereomicroscope. The LC-MS/MS technique was used to quantify aflatoxin levels in groundnut kernels. *Ditylenchus africanus* (peanut-pod nematode) as well as individuals belonging to the genera *Pratylenchus* (lesion nematode), *Helicotylenchus* (spiral nematode) and *Meloidogyne* (root-knot nematode) spp. were identified from hulls and kernel samples. The peanut-pod nematode was the predominant nematode pest in both hull and kernel samples. During the 2012/13 season, none of the groundnut-kernel samples obtained from all three districts were contaminated with aflatoxins at harvest. There was significant aflatoxin contamination (above 500 ppb per 1 g sample) in kernels at harvest and at storage from Manguzi and Mbazwana during the 2013/14 season. Groundnut kernels from Jozini had less nematode numbers and were least contaminated with aflatoxins, whereas those from Manguzi had high nematode numbers and the highest aflatoxin contamination. The extent of variation in this study necessitates further investigations on nematode survival under certain environmental conditions.