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ORAL PRESENTATIONS

Keynote: Management and stewardship – Nematodes in the soil ecosystem

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Carbon and energy, which dictate the size and activity of the soil food web, are depleted in the metabolic and growth processes of successive consumers. They fuel the contribution to ecosystem services and disservices by nematodes and other soil organisms. Important services include the mineralisation of organic molecules and regulation of opportunistic pest species; disservices include the reduction of carbon inflow into the food web by plant-damaging herbivory. The nematode assemblage provides useful bioindicators of ecosystem structure and function. Indices based on relative proportions of structural and functional guilds indicate the framework of ecosystem structure and the potential for ecosystem services. The magnitude of services depends on the biomass and activity of the organisms. Estimates of carbon utilisation by various functional guilds provide metrics of nematode contributions to services. Management to ameliorate nematode disservices often results in unintended, but long-lasting, collateral disruption of higher trophic levels. The challenge in sustainable systems is to manage the disservices of soil nematodes within the context of stewardship of their services. Functional complementarity and continuity of nematode services may be enhanced by resource subsidy to increase organism abundance and diversity and by mitigation of environmental constraints to enhance food web connectance.

Soil nematode diversity and community composition analysis as a basis for biosafety assessment of transgenic banana in the field

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Soil nematodes are considered to be good indicators of soil health because nematodes constitute a diverse group within the soil fauna, feeding on bacteria, fungi, other nematodes and plant material. This intimate feeding relationship implies that any disturbance which influences their food source or environment is likely to be reflected in the abundance and composition of the nematode communities. Thus, differences in nematode diversity and community composition were used to assess the potential effect of a transgenic banana on non-target organisms, and thus an indication on their effect on the overall environment. Soil nematodes were sampled from a confined field trial site planted to transgenic banana lines RCG3.21, RCG3.31, RCG3.15 and RCC2.02 and a non-transgenic control line CO^3. The transgenic banana lines were developed for resistance to Black sigatoka diseases (*Mycosphaerella fijiensis*) by over expression of the antifungal chitinase gene from rice. Using ecological indices such as trophic diversity (proportion of the trophic group), Shannon-Weiner Diversity, Simpson's diversity and structure indices, the effect of the transgenic banana on nematode community composition and diversity was not apparent. This is a preliminary analysis over a single season and therefore, continued assessment is necessary to observe if heterogeneity in nematode community composition and diversity on a field scale occurs over time.

Nematode community structures as bio-indicators of agricultural soil health under local conditions

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Soil is a fundamental, non-renewable resource in any ecosystem. To uphold food production for increasing global human populations, it is imperative to develop ways in which to sustain healthy biological productivity of agricultural soils. This has prompted research in the use of different biological soil organisms as indicators of soil health. Nematodes are one of the most abundant groups of the Metazoa occurring in all soils and form an integral part of the soil food web. T hey respond rapidly to changes within their environments, can easily be extracted from soil, identified and be characterised into functional guilds. Therefore, nematodes act as good bio-indicators of soil health. To determine the practical use of nematode community structures as a tool for measurement, soil samples were analysed from natural habitats as well as conventional and organic deciduous fruit orchards. The total number of nematodes in each soil sample was counted and the percentage of nematodes within a family determined. The results were used to evaluate nematode food webs for trophic group distribution and enumerated by the Maturity Index, Enrichment Index (EI), Structure Index (SI) and Channel Index (CI) based on the weighted abundance of coloniser-persister guilds.

Assessment of nematode community structures in potato-based cropping systems

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Potato, ranked as the third biggest staple food crop in South Africa during 2008 in terms of production (2 098 581Mt), is parasitised by plant-parasitic nematodes (PPN). Root-knot nematodes (RKN; *Meloidogyne* spp.) are in particular the economically most important genus that cause significant quality losses to this crop in local production areas. Subsequently a tool to implement environmentally-sound strategies to manage RKN in potato-based cropping systems was initiated on request of seed potato producers from the Western Free State Potato Seed Association. The status of plant- as well as non-parasitic nematodes in terms of their nematode community structures and species composition in 31 fields where potato was planted once during an eight-year cycle was conducted during 2010 and 2011. For soil samples, the Seinhorst elutriation and sugar-flotation methods resulted in 11 and five different PPN genera being identified, respectively. For root samples, five genera were identified using the sugar-flotation method and six using the mist-chamber method. Calculation of prominence values (PV) indicated that *Meloidogyne* spp. was predominant in soil samples, followed by *Pratylenchus* and *Tylenchorynchus* spp. In root samples, *Meloidogyne* spp. dominated, followed by *Pratylenchus* spp. Second-stage juveniles (J2) from an unknown cyst (Heteroderidae) were

also identified from two of the 31 fields. Non-parasitic nematodes present in soil and root samples mainly consisted of bacteriovores.

The nematode complex in greens on South African golf courses

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Only a limited number of nematodes surveys have been conducted on turf on golf courses in South Africa. In addition, those surveys only focused on estimating the population levels of plant-parasitic nematodes that infect roots of such grasses. No information is thus recorded on the presence and abundance of non-parasitic nematodes that form part of the total nematode complex with the soil-root interface of turf grasses. Therefore, during the present study, both plant-parasitic and non-parasitic nematodes that include free-living, predator and omnivore individuals were studied, when greens of 11 golf courses situated across South Africa were sampled. In terms of plant parasitic nematodes, nine genera including *Hemicycliophora, Helicotylenchus, Rotylenchus, Hemicriconemoides, Paratrichodorus, Xiphinema, Paratylenchus, Pratylenchus* and *Meloidogyne* were identified while a wide variation of functional guilds including fungivore, bacterivore, omnivore and carnivore guilds were recorded from soil samples. Enrichment and structure indices indicated that most of the nematode-based soil food webs of local golf courses were situated in the A and B quadrants. The nematode community structures differed for the 11 golf courses although non-parasitic nematodes generally dominated, particularly enrichment families belonging to Ba1. Nematode complexes seemed to be area-related. Plant-parasitic nematodes were related to the poorest turf patches and especially *Hemicycliophora, Meloidogyne* and *Pratylenchus* were the most important indicators of turf damage followed by *Xiphinema, Criconematidae* and *Hoplolaimidae. Paratrichodorus* seemed less important in terms of turf damage as a result of this survey.

New nematode records from the Bakwena Cave, South Africa

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Surveys forming part of the Bakwena-cave project were carried out from January 2009 to February 2010 at Bakwena Cave, South Africa. The main aim of these nematode surveys were: 1) to contribute to the biodiversity of the Bakwena Cave and 2) to increase our current knowledge and understanding of non-parasitic nematodes from South Africa, since these non-parasitic nematodes have received little attention in recent years. Nematodes were collected from six localities, representing different habitats, which were situated between the cave entrance and a pool 30 m underground. A total of 27 nematode genera belonging to 23 families were collected, with 19 genera being reported for the first time from cave environments. The underground pool showed the highest species diversity of the six localities, with lowest diversity associated with fresh and dry guano deposits. Bacteriovores were most abundant being found from four of the sampling localities, the remaining two localities comprised of fungal feeders, obligate and facultative plant feeders and omnivores. A number of species were taxonomically described during the study including *Diploscapter coronatus* and *Panagrolaimus n*. sp. This nematological study represents the first of its kind from South Africa and also provides additional information to the study of nematodes from cave environments worldwide.

Report on the most important nematode species in the Czech Republic

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After the dissolution of Czechoslovakia in 1992, the Czech Republic lost the significant nematology centre that was located in Slovakia at that time. Along with the separation of nematological activity, any systematic survey of important plant-parasitic nematode species and their regulation was discontinued. The State Phytosanitary Administration (SPA), employing one qualified nematologist, was not able to monitor the occurrence of nematodes throughout the Czech Republic. Consequently, since 1998 monitoring and diagnosing significant nematode genera and species that cause yield and quality damages of crops in the country were conducted. During the last 11 years, the occurrence and economic importance of *Globodera rostochiensis, Meloidogyne hapla* and *Ditylenchus dipsaci* was proved and documented. These nematode species have become target organisms for the Czech nematological research. Respecting SPA and farmers' requirements, diagnostics methods have been optimised and also methods for alternative plant protection against these pests have been tested with the intention of ecological production of crop commodities. The research was supported by MEYS; project number MSM 6046070901 and by NAAR; project number QH81163.

The effect of crop rotation over time on two nematode species in an organic cotton-farming system

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The effect of antagonistic plants was evaluated for the control of nematodes in an organic cotton-farming system during a long-term crop rotation trial. Several crop cultivars that are known to perform well in terms of nematode resistance/tolerance were included. During the summers of 2008/2009 and 2009/2010 sesame, marigold (Bonita mix), sunnhemp (*Crotalaria juncea*) and oats (cv. Kompasberg) were planted in a field in the Jan Kempdorp region (South Africa) where both *M. incognita* race 4 and *Pratylenchus zeae* are present. Cotton cv. Delta OPAL was used as a susceptible control. During the winters of 2009 and 2010 the sesame, marigold, sunnhemp and one cotton rotation were followed by the oat cv. Kompasberg, while the summer oat was followed by Abyssinian crambe during the spring. The other cotton rotation was left fallow. During the third summer (2010/2011) the cotton cv. Delta OPAL was planted in all the rotations. Six- and 12-week counts in the cotton/fallow and the cotton/oat rotations had significantly higher *M. incognita* race 4 numbers during 2008/2009 and 2009/2010 than the rest of the seasons. Although the numbers were significantly reduced in the 2010/2011 season, cotton/oat had significantly more root-knot nematodes than cotton/fallow, marigold/oat and sesame/oat. Results showed that certain rotations could be beneficial in reducing root-knot nematode numbers. *P. zeae* numbers were significantly higher in the sunnhemp/oat and sesame/oat rotations, especially during the 2009/2010 season. This indicates that these crops might not be suitable options where *P. zeae* could be a problem.

Response of plant-parasitic nematodes to canola-based rotation systems under irrigation

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A prerequisite for effective crop rotation systems in terms of reducing plant-parasitic nematodes (PPN) is inclusion of poor or non-host crops. The effect of such rotation systems on nematode populations under irrigation were studied over a four-year period in the Vaalharts Irrigation Scheme (Northern Cape Province) and included canola, wheat or barley during winter rotated with maize or groundnut during summer. In addition, combinations of canola and wheat rotated with maize, groundnut and soybean were also evaluated. The diversity and population levels of PPN species increased with inclusion of a wider variety of crops. Root-knot nematodes (RKN) were present in root and soil samples throughout the trial period indicating their wide host

range. Inclusion of canola in rotation with maize or groundnut resulted in a substantial decrease in RKN. Monoculture of maize as well as maize/groundnut sequences with winter sequences of wheat/canola or canola/wheat resulted in a progressive build-up of lesion as well as spiral nematode numbers. All crops, except groundnut, did not affect pod nematode numbers.

Confirmation of presence of PTSL in South Africa

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Peach tree short life (PTSL) is a syndrome involving ring nematode (*Criconemoides xenopla*x), bacterial canker (*Pseudomonas syringae* pv. *syringae*) and typically an additional stressing factor, resulting in the death of peach and nectarine trees. PTSL is a serious problem in the USA states of Georgia and South Carolina. Although similar symptoms have occasionally been observed in South African orchards, it has not been extensively investigated because attention focused on the much more serious problem of die-back and death of plum trees. Previously evidence linking plum tree death with PTSL could not be demonstrated beyond reasonable doubt, as some symptoms differed from typical PTSL symptoms. A severe case of nectarine tree deaths in an orchard in Tulbagh during the spring of 2010 provided an opportunity to compare local symptoms of peach tree death with PTSL and also with plum tree death. The authors postulate that peach tree deaths observed in South Africa are identical to American PTSL, and that local plum tree deaths are another manifestation of PTSL.

Evaluation of vegetable crops for host suitability to the root-knot nematode *Meloidogyne incognita* race 2

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Root-knot nematodes (*Meloidogyne*) are a major constraint in vegetable production systems, particularly under subsistence-farming conditions in South Africa. The use of host plant resistance could provide farmers with a sustainable option for nematode control in these production systems. This study was conducted to establish whether *M. incognita* race 2 resistance is present in commercially available *Amaranthus* spp., Swiss Chard and Green Pepper varieties. Nematode parameters used to select for resistance were reproduction factors (Rf), egg-laying females (ELF), number of eggs and J2/r root system and gram of roots as well as the number of egg masses/root system. Although substantial variation existed among the relevant vegetable genotypes with regard to the parameters used, none of the genotypes were immune to *M. incognita* race 2. However, one *Amaranthus* spp. genotype had significant lower Rf-values than the others, indicating resistance to *M. incognita* race 2. Some of the green pepper varieties were also identified with some degree of resistance, but none of the Swiss Chard varieties had low Rf-values, indicating susceptibility to *M. incognita* race 2.

Plant-parasitic nematodes on United States turfgrasses of regulatory concern to southern Africa

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Major breeding programs developing high-quality, warm season turfgrasses are located in the southeastern United States. In particular, many of the bermudagrass/couch grass (*Cynodon* spp.) and seashore paspalum (*Paspalum vaginatum*) cultivars that

are commonly used on golf courses worldwide, including southern Africa, originate in this region. The majority of these grasses are vegetatively propagated from sod or sprigs, making movement of plant-parasitic nematodes in infested plant material common. Several plant-parasitic nematode species associated with sod in the southeastern U.S. that are potential invasive threats to agriculture in southern Africa are: *Belonolaimus longicaudatus, Trichodorus obtusus*, and *Hoplolaimus columbus*. The biology, host range, identification and diagnosis of these, and several other plant-parasitic nematodes, will be presented and discussed.

Molecular characterisation of potato root-knot nematodes in South Africa

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Root-knot nematodes (RKN) or *Meloidogyne* spp. are economically important pests of many crop plants, including potato, in South Africa. The use of nematicides is currently the most reliable method of control, however, pressure from environmentalists and health groups on the use of these products has resulted in their increasing withdrawal from the market. Thus, alternative methods of control such as the use of resistant cultivars, crop rotation and soil amendments need to be incorporated as part of an integrated pest management approach. Rapid and accurate identification of RKN is essential for the successful deployment of these alternative management strategies. The aim of this project is, therefore, to develop qualitative and quantitative PCR as a diagnostic tool for accurate identification and quantification of *Meloidogyne* spp. infecting potato crops in South Africa. Firstly, different methods of extracting nematodes from soil and potato tubers were established. Thereafter several methods of extracting DNA from nematode samples were investigated. Of the methods investigated, "worm lysis buffer" was chosen as the best method of DNA extraction. The following primer pairs have thus far been tested on nematode DNA samples, 1) 194/195 ribosomal primers amplifying the IGS region between 5S and 18S, 2) Fjav/Rjav and 3) MIF/MIR specific for *M. javanica* and *M. incognita* respectively.

Keynote: Use of entomopathogenic nematodes in biological control

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Entomopathogenic nematode (EPN) species used in biological control of insect pests are within the genera *Steinernema* and *Heterorhabditis.* These rhabditid nematodes have a free-living, infective third juvenile stage, the dauer juvenile (DJ). It is resistant to shear forces and can thus be applied with conventional application techniques. The DJ transmits cells of its bacterial symbiont (*Xenorhabdus* or *Photorhabdus*, respectively) into the haemocoel of target insects and kills the host within 24-36 hours. Since the discovery of the symbiotic relation between EPN and their enteric associates, EPN biotechnology has rapidly progressed. Today EPN are produced in cubic meter bioreactors at costs little higher than chemical insecticides. For improvement of beneficial traits of EPN genetic selection has been successfully applied. The advantages of EPN over chemical control measures are their ability to actively seek host insects in cryptic environments, their compatibility with biological and chemical control agents, the low probability of resistance development against EPN and the possibility to produce residue-free agriculture produce. Major disadvantage is the limited shelf-life of nematode-based control products at ambient temperature. Some examples of the successful use of EPN in IPM systems will be presented.

Identification of entomopathogenic nematodes from citrus orchards and their biocontrol potential against false codling moth

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A survey was conducted to determine the diversity and abundance of endemic entomopathogenic nematodes in citrus orchards of South Africa. The main aim of the survey was to obtain nematodes as biological control agents against false codling moth (FCM), *Thaumatotibia leucotreta*, a key pest of citrus in South Africa. Sequencing and characterisation of the internal transcribed spacer (ITS) region was used to identify all nematode isolates to species level and the D2D3 region to confirm new records or new species. Morphometrics, morphology and biology of the infective juvenile (IJ) and the first-generation male were used to support molecular identification and characterisation. Laboratory bioassays, using 24-well bioassay disks, have shown isolates of six nematode species found during the survey, to be highly virulent against the last instar of FCM larvae. *Steinernema yirgalemense*, at a concentration of 50 IJs/larva caused 100% mortality and 74% at a concentration of 200 IJs/pupa. Using a sand bioassay, *S. yirgalemense* caused 93% control of cocooned pupae and emerging moths at a concentration of 20 IJs/ cm^2. This is the first report on the potential use of nematodes to control the soil stages of FCM, which include larvae, pupae and emerging moths.

Biological control of fruit flies using entomopathogenic nematodes in South Africa - current research status and future prospects

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Fruit flies (*Diptera: Tephritidae*) are important pests of commercial fruit worldwide. Currently in South Africa, control methods for these pests focus only on the airborne post-teneral adult life stage since other stages are concealed, occurring either inside the fruit or soil. The potential of EPN to infect soil borne life stages of two local fruit fly pests - *Ceratitis capitata* (Wiedemann) and *Ceratitis rosa* (Karsch), was recently explored. The susceptibility of prepupating larvae, pupae and adults of *C. capitata* and *C. rosa* to EPN was determined. Efficacy of three in vivo cultured endemic EPN isolates: *Heterorhabditis bacteriophora, Heterorhabditis zealandica* and *Steinernema yirgalemense* against the two fly species was evaluated. Prepupating larvae and adults were found to be susceptible to EPN, with variation in infectivity among the fly species. High infection rates (above 70%) were recorded for *C. capitata* when tested with *H. zealandica* and for *C. rosa* when tested with *S. yirgalemense*. The assays demonstrated a potential for the use of EPN against two important fruit fly pests in South Africa. Future research should focus on determining the field efficacy of selected EPN isolates and integration of a biological control method using EPN into a fruit fly management programme.

Biocontrol potential of South African entomopathogenic nematodes for the control of codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae)

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The biocontrol potential of South African isolates of *Heterorhabditis zealandica, Steinernema citrae, S. khoisanae, S. yirgalemense* and *Steinernema* sp., was evaluated against codling moth, *Cydia pomonella*, in laboratory bioassays and field experiments. Codling moth was susceptible to all six nematode isolates tested under ideal conditions, with mortality ranging from 78-100%. Low temperatures, which can be expected during field applications, had a strong negative effect on larvacidal activity (3%), as did low levels of humidity (19%). Regarding host-seeking ability, no positive attraction to host cues could be detected amongst isolates, except for *H. zealandica*. Based on cumulative laboratory results, *H. zealandica* was selected as the most promising test isolate and was subsequently evaluated in the field together with isolates of *S. khoisanae* and a *Steinernema* sp., with satisfactory mortality levels of > 50% recorded for all three species. Insect containment methods for host deployment were shown to influence larvacidal activity. As pear tree logs were impractical, predictive equations were subsequently developed.

This enabled future field trials to be conducted using either planks or cages, predicting the expected level of control on tree logs using any of the three tested nematode isolates.

Bioassays with South African isolates of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) against *Pseudococcus viburni* (Hemiptera: Pseudococcidae)

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Pseudococcus viburni (Signoret) (Pseudococcidae), mealybug, is a common and serious pest of apples and pears in South Africa. The potential use of entomopathogenic nematodes against *P. viburni* in laboratory bioassays was assessed. Through screening, one isolate was selected to use against *P. viburni*. The development of nematodes in infected mealybugs and the effect of mealybug size on infectivity were assessed. The ability of nematodes to locate and infect mealybugs in the ovary and calyx of infested apples was investigated. To determine the lethal time and dose, *P. viburni* adult females were exposed to different nematode concentrations. The isolate J34 of *Heterorhabditis. zealandica* was the most effective and caused 80% mortality of *P. viburni*, 48 hours after inoculation at 25°C. *H. zealandica* (J34) and *Steinernema yirgalemense* (157-C) successfully reproduced in *P. viburni.* Mealybug size affected infectivity and crawlers were less susceptible than adults and intermediates. *H. zealandica* (J34) applied to *P. viburni* field-infested apples were able to enter the apple core where they infected *P. viburni*. After 48 hours at a concentration of 200 IJ/insect, the LD50 and LD90 values were 54 and 330 nematodes/insect, respectively, and LT50 was 30 hours, while LT90 was 62.5 hours.

The potential of genetic selection to improve beneficial traits of entomopathogenic nematodes within the genera *Steinernema* and *Heterorhabditis*

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Genetic selection can be a powerful tool to increase beneficial traits in entomopathogenic nematodes (EPN). Heritability of heat and desiccation tolerance in *Heterorhabditis bacteriophora* was sufficiently high to justify improvement by genetic selection. Significantly increased tolerance was recorded as a result of cross breeding tolerant parental strains and successive genetic selection. The strains originated from a prior screening among 60 strains for increased stress tolerance. An increase in mean heat tolerance of 5.5°C was achieved when nematodes had been adapted to heat stress. For non-adapted tolerance an increase of 3.0°C from 40.1° to 43.1°C was recorded. For comparison, a commercial strain had a mean tolerated temperature after adaptation of 38.2°C and of 36.5°C without adaptation. For assessment of the desiccation tolerance the mean tolerated water activity (aw-value) of a population was measured. Cross-breeding tolerant strains reduced the aw-value from 0.67 to 0.65 after adaptation and from 0.9 to 0.7 without prior adaptation. The following six selection steps could not increase the tolerance. In comparison, the commercial strain tolerated a mean aw-value of 0.985 after adaptation and 0.951 without adaptation. Similar progress is reported for the desiccation tolerance of *Steinernema feltiae*. Future research is targeting at stabilisation of the improved traits.

Maize stalk borer (*Busseola fusca*) as potential target for control with entomopathogenic nematodes

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The development and use of biological pest control products is fuelled by not only environmental and health concerns, but also the development of resistance against chemical insecticides. By 2010, resistance against 333 chemical compounds (involving 557 insect species) was noted by the Insect Resistance Action Committee. The efficacy of two entomopathogenic nematodes (*Heterorhabditis bacteriophora* strain SGI22 and *Steinernema* sp. strain SGI35) was tested against larvae and pupae of the maize stalk borer, *Busseola fusca*. Exposure of *B. fusca* larvae (100 IJs/insect in a sand-well bioassay system) reared on artificial diet versus actual plant material indicated high susceptibility to SGI35, ranging from 43 to 57% and 87 to 95% after 24 and 72 hours, respectively. Likewise, dissection of insect larvae showed positive recycling with >85% of both cadaver types yielding EPN's. Additional assays were conducted also against pupae; a life stage expected to be less susceptible to infection by an entomopathogen. Using EPN's to target neonates in the whorl and/or pupae overwintering in maize stubble could present an opportunity for control of this pest if the formulation and mode of delivery facilitate contact with the pest. Bioassay results and potential field application strategies are discussed.

Development of mass production technology for entomopathogenic nematodes (*Steinernema* and *Heterorhabditis*)

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Since the discovery of the symbiotic bacteria of entomopathogenic nematodes (EPN) and their significance for the development of the nematodes by Poinar and Thomas in 1966, the development of in vitro mass production systems progressed rapidly. The first attempt to produce EPN in industrial scale bioreactors was by the company Biosys in the late 1980's. Since the use of this technology production costs have considerably decreased. Today EPN-based products are no longer only used in niche markets but are applied also in out-door horticulture crops. The development of large-scale liquid culture was possible due to scientific progress in the field of EPN biology as well as adaptation of bioreactor technology to the needs of EPN and improvement of downstream and storage technology.

In vitro culturing of *Heterorhabditis zealandica*

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In Europe the first steps towards outdoor commercial application have been taken using entomopathogenic nematodes. Various nematode formulations against a wide range of insect pests are commercially available. To culture these nematodes in quantities needed for field application, *in vitro* liquid culturing methods need to be developed. An endemic isolate of *H. zealandica* has been selected as an effective biological control agent against a wide range of key pests associated with deciduous fruit and citrus. To culture *H. zealandica*, the associated bacteria had to be characterised, sub-cultured and stored in a viable state. The isolated bacteria were found to be aerobic and catalase-positive and growth in broth is enhanced under aerobic conditions. Physiological and biochemical tests were performed at 28C using Biolog GN microplates and API tests. Monoxenic cultures of nematodes and associated bacteria were established on lipid agar plates. The Erlenmeyer flask method was used to evaluate three different liquid media. For evaluation of nematode reproduction, 100 l aliquots were diluted with Ringer's solution and counted. The identification of the associated bacteria and the difference in mass production of *H. zealandica* on the three different liquid media are discussed.

In vivo production of entomopathogenic nematodes

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Entomopathogenic nematodes as formulated biological pest control products are not yet available on the South African market. *In vivo* culturing is an important stepping stone towards future, *in vitro* mass rearing and formulation of nematodes on a commercial scale. Although labour intensive, *in vivo* production delivers high quality nematodes at low cost and technological input. Host insects, wax moth larvae (*Galleria mellonella*) and mealworms (*Tenebrio molitor*), were reared on five different diets at optimal rearing conditions. The superior diet for each host was selected according to the weight accumulation rate of the hosts and cost-effectiveness of the diet. Successful host inoculation is the key to effective nematode production. Therefore three inoculating techniques; using a pipette, host immersion and shaking of hosts with the nematode inoculum, were compared using *Heterorhabditis zealandica* (SF41) and *H. bacteriophora* (SF351). Each insect host was inoculated with nematodes at a concentration of 200/insect. Mortality percentage was determined after two days and infectivity, confirmed by colour change and dissection of the host, seven days after inoculation. For each host, the number of nematodes produced per gram was determined, as well as the nematode production/host, comparing frozen and non-frozen hosts.

The role of slug parasitic nematodes in the invasive Iberian slug *Arion lusitanicus*

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The invasive form of *Arion lusitanicus* probably originated from the Iberian Peninsula hence the common name the Iberian slug. The species has spread in many parts of Europe including Scandinavia in the last 30 years. In its native range, the slug species is relatively rare, due to drier conditions and high abundance of natural enemies (e.g. slug-parasitic nematodes). The Iberian slug was first recorded in Norway in 1988, and became a major pest during the early 1990's, causing damage to private gardens, horticultural fields and agricultural crops. In some areas large numbers of slugs have deterred sheep from feeding on pasture grass. Contamination of grass harvested as cow fodder has also been reported. Another impact of the Iberian slug is the decline of the native slug species, *Arion ater*. Management of this pestiferous slug is difficult once it has established in an area. Molluscicides are commonly used as a control measure, as well as cultural techniques. Biological control using the slug parasitic nematode *Phasmarhabditis hermaphrodita* is also an option. Results so far indicate that *P. hermaphrodita* is only effective against young stages of the slugs (<1 g), however field applications of the nematode appear not to reduce *A. lucitanicus* numbers. Ongoing studies mixing *P. hermaphrodita* in animal feed slug baits show some promise against slugs over 1 g in laboratory studies. Studies are currently conducted to elucidate the host-parasite relationship between *P. hermaphrodita*, associated bacteria and *A. lusitanicus* including effects of plant feeding.

Nematodes associated with terrestrial slugs in the Western Cape province of South Africa

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A survey of nematodes associated with native and introduced species of terrestrial slugs was conducted in the Western Cape Province of South Africa, in order to gather new data regarding their diversity and distribution. A total of 521 terrestrial slugs were collected from 35 localities throughout the Western Cape. All slugs were dissected and examined for the presence of internal nematodes. Extracted nematodes were identified using a combination of molecular (18S rRNA gene sequencing) and morphological techniques. Nematodes were found parasitising slugs at 14 of the 35 sites examined, amounting to 40% of sample sites. Of the 521 slugs collected, 6% were infected with nematodes. A total of seven nematode spp. were identified including; *Agfa flexilis, Angiostoma* sp., *Phasmarhabditis* sp. SA1, *Phasmarhabditis* sp. SA2, *Caenorhabditis elegans, Panagrolaimid* sp. and *Rhabditis* sp. Of these species, four were considered to be parasitic to slugs (*A. flexilis, Angiostoma* sp., *Phasmarhabditis* sp. SA1 and *Phasmarhabditis* sp. SA2), as opposed to those forming necromenic or phoretic associations. Three new species of slug-parasitic nematodes were identified during this study viz. *Angiostoma* sp., *Phasmarhabditis* sp. SA1 and *Phasmarhabditis* sp. SA2.

Resistance of two *Cucumis* species to *Meloidogyne* species and mechanisms of resistance

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Wild watermelon (*Cucumis africanus*) and wild cucumber (*Cucumis myriocarpus*) have the potential for being used as seedling rootstocks for watermelon (*Citrullus lanatus*) cultivars that are highly susceptible to root-knot nematodes. These two plant species, were subsequently investigated for resistance to *Meloidogyne incognita* races 2 and 4 and *M. javanica* in various trials. Treatments, comprising different levels of initial nematode densities (Pi) of the respective root-knot nematode spp. mentioned were applied on roots of seedlings of *C. africanus* and *C. myriocarpus*, respectively. The trial layout consisted of a randomised complete block design, with 10 replicates. At harvest, 56 days after nematode inoculation, the RF values on all test plants were less than one, whereas nematode infection had no effect on yield components of the two plant species. Penetration indices were greater than one in *C. myriocarpus* and less than one in *C. africanus*, suggesting post-infectional and pre-infectinal resistance, respectively. In most cases, third and fourth stages of root-knot nematode juveniles that were inside roots of these two plant spp. were identified as being male specimens. In conclusion, the two *Cucumis* spp. have the potential for serving as rootstocks in watermelon production for managing population densities of *M. incognita* race 2 and 4, as well as *M. javanica*, which are widely distributed in South African soils.

Response of *Meloidogyne*-resistance in *Cucumis africanus* to infection by the greenhouse whitefly

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The greenhouse whitefly (*Trialeurodes vaporariorum*) – a vector for criniviruses, is emerging as an economically important pest to crops to Cucurbitaceae crops. Watermelon (*Citrullus lanatus*) is for example highly susceptible to *Meloidogyne* spp., with no resistant genotypes being commercially available. Wild *Cucumis* spp. (*C. africanus, C. myriocarpus*), with high resistance levels to *Meloidogyne* spp., however, have the potential for serving as nematode-resistant rootstocks. The effect of the greenhouse whitefly (GHWF) infection on *M. javanica*-resistance in *C. africanus* was subsequently tested under greenhouse conditions. The greenhouse was partitioned into 12 compartments using the greenhouse whitefly-proof nets. Uniform three-week old seedlings were each transplanted into a 20-cm-diameter pot contain 3:1 pasteurised sand: Hygromix per compartment. Treatments, viz. 0, 200, 600, 1000, 1400, 1800 and 2200 *M. javanica* J2 were inoculated on roots of *C. africanus* plants. Approximately 600-1000 GHWFs were introduced into randomly selected compartments containing nematode-inoculated plants. The other half of the experiment contained nematode-inocluated *C. africanus* plants, without addition of the GHWFs to compartments containing such plants. The trial was arranged in a randomised complete block design with six replicates. Fifty-six days after nematode inoculation, the GHWFs had eliminated resistance to *M. javanica* in *C. africanus*, probably due to the GHWF's deleterious effect on photosynthates. Consequently, there is need to suppress the GHWF densities where nematode resistance is used to manage *M. javanica* densities.

Response of endemic nematode-resistant *Cucumis* species to multi-nematode infections

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Wild watermelon (*Cucumis africanus*) and wild cucumber (*C. myriocarpus*) seedlings are highly resistant to *Meloidogyne* species. A field trial was carried out to validate *Meloidogyne*-resistance in *Cucumis* spp. under multi-nematode infections. In the *C. africanus* trial, the Pi values comprised 103 *Meloidogyne* and 23 *Criconema mutabile*, whereas the *C. myriocarpus* trial had 158 *Meloidogyne*, 27 *C. mutabile* and 38 *H. dihystera* nematodes. At 56 days after transplanting, *Meloidogyne* species had RF values of less than one, whereas *C. mutabile* and *H. dihystera* had RF values of greater than one. Infection by the test nematodes had no effect on yield components, except for *C. mutabile* which increased fresh fruit yield in *C. africanus*. Using nematode-plant interaction principles, the two *Cucumis* species were resistant to the test *Meloidogyne* species, but tolerant to *C. mutabile* and *H. dihystera*. Results demonstrated that the three nematode species had either stimulatory or inhibitory effects on one another, probably due to infecting similar or different sites. In conclusion, *Meloidogyne*-resistance in *Cucumis* species holds under multi-nematode infections, with the two *Cucumis* species having the potential for suppressing *Meloidogyne* species under field conditions. However, the two plant species should be used with circumspection in areas with *C. mutabile* and *H. dihystera* nematodes.

Response of nematode resistance to grafting nematode-susceptible *Citrullus lanatus* onto nematode-resistant wild *Cucumis* species

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Various studies demonstrated that wild watermelon (*Cucumis africanus*) and wild cucumber (*Cucumis myriocarpus*) seedlings are highly resistant to *Meloidogyne* species. The *Cucumis* genus, along with watermelons (*Citrullus lanatus*) - which are highly susceptible to *Meloidogyne* species, are members of the *Cucurbitaceae* family. An investigation was conducted to determine the inter-generic potential of grafting *Citrullus* cultivars onto *Cucumis* seedling rootstocks with respect to mortality of grafts, retaining of nematode resistance capabilities of rootstocks and the dry matter productivity of watermelon scions. Six treatments, viz. watermelon cv. 'Congo' alone, watermelon cv. 'Charleston Gray' alone, cv. 'Congo' onto *C. africanus*, cv. 'Congo' onto *C. myriocarpus*, cv. 'Charleston Gray' onto *C. africanus* and cv. 'Charleston Gray' onto *C. myriocarpus*, were arranged in a randomised complete block design, with 10 replicates. At 56 days after inoculation with 1000 nematodes, grafts had zero mortality. In addition, seedling rootstocks retained their capabilities to suppress *M. incognita* race 2, without nematode infection affecting crop yield of the grafts. In conclusion, the two *Cucumis* seedling rootstocks have the potential for use as nematode-resistant seedling rootstocks in watermelon production.

Productivity of watermelon cultivars when grafted onto *Cucumis* seedling rootstocks in a nematode-infested field

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Inter-generic compatibility of watermelon (*Citrullus lanatus*) cv. 'Congo' and cv. 'Charleston Gray' grafted onto *Meloidogyne*-resistant wild watermelon (*Cucumis africanus*) and wild cucumber (*C. myriocarpus*) seedling rootstocks was investigated with respect to flowering, fruit yield production and accumulation of essential nutrient elements in a nematode-infested field. Six treatments, viz. watermelon cv. 'Congo' alone, watermelon cv. 'Charleston Gray' alone, cv. 'Congo' onto *C. africanus*, cv. 'Congo' onto *C. myriocarpus*, cv. 'Charleston Gray' onto *C. africanus* and cv. 'Charleston Gray' onto *C. myriocarpus*, were arranged in a randomised complete block design, with 6 replicates. At week four, grafts had more flowers than the controls. Sixty-six days after transplanting, grafts had lower RF values, higher fresh fruit yield and dry shoot weight than control plants.

Grafts and controls had similar concentrations of essential nutrient elements in leaves except for Mn and Zn, which were higher in grafts than in controls. In conclusion, results of this study suggested that the inter-generic compatibility of *Citrullus* scions and *Cucumis* seedling rootstocks may have beneficial attributes. Consequently, wild *Cucumis* have the potential for serving as *Meloidogyne*-resistant seedling rootstocks in watermelon production.

Keynote: The potential of biological control for plant parasitic nematodes in African agriculture systems

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The cryptic nature of plant-parasitic nematodes presents numerous difficulties for their control. With an increasing awareness of the negative effects of synthetic pesticides, in tandem with the advent of many pesticides being removed from use, the quest for alternative pest and disease management options, especially nematodes, has become a major objective. The use of biologically-based products and biocontrol agents has many attractions in terms of their often high level of specificity and their limited environmental impact. However, they are often less than 100% effective, can be difficult to apply, are sensitive to climatic changes or dependant on specific environmental conditions. They also need to be strictly evaluated for their affect on non-target organisms, and their release or registration often follows lengthy and costly assessment to ensure their non-damaging nature. Difficulties regarding patenting and ownership issues of living organisms complicate matters, while limited regionalisation of standards and policies for biologically-based products in Africa discourages commercial investment. However, a need to identify alternative nematode management options is becoming increasingly urgent, which desperately needs greater harmonisation between the public and private sector. Recent developments and promise for biologically-based nematode management are presented, using comparisons from Central America.

Efficacy of *Pochonia chlamydospori*a and *Paecilomyces lilacinus* in the control of root-knot nematodes

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Management of root-knot nematodes, *Meloidogyne* spp., using fungi that parasitise eggs and females, has been gaining popularity as the need for alternatives or supplements to chemical nematicides increases. This study was conducted with the aim of screening isolates of *Pochonia chlamydosporia* and *Paecilomyces lilacinus* in managing root-knot nematodes. Two experiments were conducted to assess the efficacy of six isolates (10, 126, 144, 147, 177 and 392) of *P. chlamydosporia* and two isolates (Pl-Rothamsted and Pl-plus™) of *P. lilacinus*. Isolates 10 and 392 were significantly ($P<0.001$) more effective in parasitising more eggs compared to other isolates. A significant ($P<0.001$) reduction in the numbers of second-stage juveniles compared to the untreated control was achieved with Pl-plus in sterilised and non-sterilised soil and with isolate 10 in non-sterilised soil. Shoot weight was significantly higher in plants treated with isolate 392 followed by isolates 10 and *P. lilacinus* from UK compared to the control in non-sterilised soil. This study concluded that overall, isolates 10 and 392 were most effective at controlling root-knot nematodes compared to the other fungal isolates.

Development of alternative control strategies for the citrus nematode, *Tylenchulus semipenetrans*, in South African citrus orchards

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Chemical control of the citrus nematode forms part of an integrated pest management approach that is currently being utilised effectively by the South African citrus producers. International market pressure and individual concerns about the use of extremely toxic nematicides resulted in the search for safer, more cost effective control measures. For several years, the use of post-plant nematicides has been a standard practice for the South African citrus grower. Increased awareness among citrus growers currently exists concerning the use of toxic compounds. Presently there are unregistered compounds and products that have not been scientifically evaluated for efficacy against soilborne pathogens. Less toxic, biological and GRAS (generally regarded as safe) chemicals were therefore re-evaluated. During the 2009/10 season the following average reduction in nematode female counts were recorded when compared to the untreated control treatment: Abamectin 41%, BioNeem 54%, Silica 64%, egg stimulants 61%' combined egg stimulants and nematicide product 82% and standard nematicides 81%. Future research will incorporate these and other approaches in an integrated root and soil health approach.

Effective, environmentally-friendly management strategies for root-knot nematodes in resource-poor agriculture

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Crop production for home consumption had been abandoned at the Morokweng village (North-West Province) during 2007/2008 due to the presence of abnormally high *Meloidogyne javanica* infestations. Consequently on-farm, nematode management trials were conducted at this locality from 2008 to 2010. Prior to planting tomato (Rodade var.), during the summer of 2008/2009, solarisation alone, a combination of solarisation and kraal manure (50 kg ha^{-1}), kraal manure (50 kg ha^{-1}) alone, a combination of solarisation and compost (30 kg ha^{-1}), compost (30 kg ha^{-1}) alone and untreated controls were applied and replicated six times each. During the following winter two Brassica vars. Nemat and Conquistador were grown in the plots where the abovementioned treatments had been during the preceding season. During the 2009/2010 summer the tomato var. Hytec 36 was grown in the same plots to evaluate the long term effect of the abovementioned treatments on *M. javanica* populations. In the first season significant differences occurred among the respective treatments, with the combination of solarisation and compost resulting in the lowest nematode egg and J2/50g roots counts. *M. javanica* population levels were also substantially reduced during the following winter where the Brassica vars. Nemat and Conquistador were grown. Solarisation and compost combined again had the lowest egg and J2/50g roots numbers as well as best yield in the final trial of 2009/2010. Crop production at this village improved from self-sufficiency for local consumption to supporting a feeding scheme for scholars at the Maiketso Primary School. A number of households in the community without income now also benefit from this intervention as a direct result of this research.

The role of cover crops in suppressing plant-parasitic nematodes in vineyards

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The negative impact of plant-parasitic nematodes on the production of grapevines in South Africa should not be underestimated. With most of the effective, synthetic nematicide control options under pressure of being phased out, an ever growing need exists for a more biological approach. The use of annual cover crops in vineyards is an established soil cultivation practice in South Africa which is environmentally friendly and financially sustainable in the long-term. Crops used for biofumigation mainly consist of plants from the Brassicaceae family and in this study, *Sinapis alba, Brassica napus, Brassica juncea, Eruca sativa* and *Avena sativa* were used as cover crops in vineyards during the 2009/2010 and 2010/2011 seasons to determine their effect on plant-parasitic nematode populations. Two different cultivation practices, namely mechanical incorporation into the top soil and chemical control, were applied to the cover crops. Soil samples were taken, prior to the start of the trial (April/May), just before

the start of the cover crop season, and thereafter on (0, 15, 30 and 60 days), after the management practice, to determine the effect of the different treatments on the plant-parasitic nematode populations in the soil. Laboratory soil bioassays and glasshouse trials, using *Meloidogyne javanica* and *Criconemoides xenoplax*, were also conducted to support results obtained from the field trial.

Resistance inducing chemicals – their effect on nematodes in naturally-infested soil, sugarcane yield and pathogenesis-related proteins

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Six potential resistance inducing chemicals were applied to two sugarcane varieties (N12 and N27) in a pot trial with the aim of inducing resistance to nematodes in naturally-infested soil. BION® (benzothiadizole), methyl jasmonate, cis-jasmone and 2,6-dichloroisonicotinic acid (INA) were applied as a foliar spray and SuSCon Maxi (imidacloprid) applied to the soil. All chemicals were tested at two rates. SuSCon Maxi (at the high rate) reduced *Pratylenchus* infestation of roots of N27 by 55%, 35% and 78% at three, five and eight weeks, respectively. SuSCon Maxi (at both rates) reduced infestation by *Meloidogyne* in both varieties. INA (at the high rate) reduced *Pratylenchus* infestation of roots of N12 by 39%, 58% and 78%, respectively. Chitinase, -1,3-Glucanse and peroxidase enzyme activity in the leaves of sprayed sugarcane was increased for all treatments. Conversely, polyphenol oxidase activity was lower in the treatments compared to the control. Sugarcane shoot and root biomass was highest in both varieties when treated with SuSCon Maxi.

Scientific review of the application of Crop Guard® via irrigation systems

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Crop Guard® (ai furfural) is registered on the major commercial crops in South Africa for the control of plant-parasitic nematodes. Crop Guard®'s attributes support ease of application via irrigation systems. Scientific studies have shown that the distribution profile of furfural supports nematicidal efficacy within the key root zone area in the ground. The presentation will review furfural movement within the soil and water profiles during commercial applications. Greenhouse and laboratory studies have indicated that when applied through a drip irrigation system, furfural is effectively distributed within the top 15 cm of the soil profile. The results also indicate that in order for efficient distribution to be achieved, the soil needs to be irrigated to field capacity prior to product application.

Furfural (a.i.) nematicide trials in the European union

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Illovo Sugar Limited has registered agricultural-based products with furfural as the active ingredient in Africa, the Middle East (Crop Guard®) and the USA (Multiguard Protect®EC). The company plans to expand registrations into Europe which requires that trials are carried out in Europe. Using experience gained in South Africa and the USA, field trials on potatoes commenced in 2009 with further trials on potatoes, carrots, ornamentals and turf grass in 2010. The intended pre-plant rate of the products evaluated varied from 50 to 75 ha^{-1} with post plant in season rates ranging from 25 to 75 ha^{-1}. The ½ x and 2 x rates were included in certain trials. Results from the field trials will be discussed in the presentation.

The development of Devguard® (iprodione) as a nematicide

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The nematicidal properties of Devguard® when applied as a soil treatment against *Meloidogyne* spp. have been demonstrated in field trials and confirmed in commercial practice on a range of crops. The active ingredient, iprodione, is listed as a nematicide (patents filed) under the EU's Annex I (91/414). Regulatory approval has been granted in the USA (peanuts) and in Turkey (protected vegetable crops). Submissions have been filed in Spain, Italy, Greece, Morocco and South Africa. Key features of Devguard® include proven efficacy and crop safety, reduced risk to the environment, residue and worker exposure. Attributes make it ideal for integration into nematode management programmes. Activity is through contact. Application can be either a soil spray (incorporated) or drip chemigation. Laboratory studies with RKN inoculated at periods up to 35 days after transplanting tomatoes, indicated residual activity of 14 days at 1kg ai ha^{-1} increased to 28 days at 3kg ai ha^{-1} and greater than 35 days at 6kg ai ha^{-1}. In Southern Europe, applications via drip chemigation at 1kg ai ha^{-1} (fortnightly up to six times) resulted in improved yields in protected vegetable crops. South African trials in potato and tomato have confirmed 6kg ai ha^{-1} sprayed broadcast and mechanically incorporated prior to planting as an effective treatment.

The effect of plant-derived products on the motility and survival of *Meloidogyne javanica*

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Since Class 1 synthetic nematicides are increasingly being withdrawn from local and international markets, a demand exists for alternative, environmentally-friendlier products with nematicidal/nematostatic effects. Although a wide range of plant-derived products have been studied extensively to determine their effect on the biology and survival of plant-parasitic nematodes, newly developed ones are continuously being released. The effect of three such test products at a dosage rate of 500 ppm was evaluated *in vitro* at 24, 48, 72 and 96 hours after onset of the trial on the motility and survival of second-stage juveniles (J2) of *M. javanica*. Three commercially-available products (standard treatments), salicylic acid and two untreated controls (tap and distilled water, respectively) were also included. The trial consisted of a randomised complete block design with four replicates for each treatment. For the untreated control treatments, 92% to 97% of J2's were motile at all of the respective time intervals. Data for J2's suspended in the three commercial standards, salicylic acid and the three test products varied substantially in term of their motility for the duration of the trial. Salicylic acid was the only product with a 100% mortality rate for J2's when stained with Trypan Blue after 96 hours.

Screening extracts of *Maerua angolensis* and *Tabernaemontana elegens* for egg–hatching inhibition and J2 motility of *Meloidogyne incognita* race 2

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Organic and freeze dried water extracts of powdered ground leaves of *M. angolensis* and *T. elegens* were tested for nematicidal activity in terms of second-stage juvenile (J2) motility and egg hatch-inhibition at five concentrations (200, 400, 600, 800, 1000

g ml^{-1}) each in a 96-well plate. Controls consisted of a standard salicylic acid and two negative controls viz. methanol and a medium of pluronic gel plus de-ionized water. Aliquots of 100 to 150 juveniles of *M. incognita* were inoculated in 90 l pluronic gel in each well prior to the addition of the abovementioned compound concentrations. Plates were incubated at 22°C in the dark in a climacabinet for 15 and 30 days, respectively, for evaluation of J2 motility and egg-hatch inhibition. Ten microliters of each concentration of *M. angolensis* and *T. elegens* extracts were added to each well and the numbers of immotile juveniles were counted every three days. Nematicidal effects were based on the number of moving and stolid J2 and the number of hatched eggs in each treatment compared to the controls at each observation interval. Salicylic acid significantly reduced egg hatching inhibition by 83-98% and increased motility of *M. incognita* J2 by 100% at all concentrations of both plant materials. Freeze dried extracts of *M. angolensis*, *T. elegens* and methanol /dichloromethane extracts reduced egg-hatching inhibition by 56% at a rate of at 200 g ml^{-1}, respectively. Egg-hatching inhibition rate was reduced with increasing exposure time for most of the extracts.

Influence of fermented fruit extracts of *Cucumis africanus* and *Cucumis myriocarpus* on nematode numbers and tomato productivity

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Responses of *Meloidogyne incognita* race 2 population densities and the productivity of tomato to fermented extracts of *Cucumis africanus* and *C. myriocarpus* fruits were investigated under greenhouse conditions. *Floradade* tomato seedlings in 20-cm-diameter pots were each inoculated with 1 130 J2s of *M. incognita* race 2. Approximately 500 g dried fruits/20 l water was fermented in EM using industry standard for 14 days until the pH was below 3.7. Fermented crude extracts (0, 10, 20, 30, 40, 50, 60 70, 80 and 90% dilutions), were arranged in a randomised complete block design, with 10 replications. The materials were applied once weekly. At harvest, 56 days after inoculation, the reduction in nematode numbers due to the fermented extracts of *C. africanus* and *C. myriocarpus* were 89 % (range 80 – 100%) and 69% (range 52 – 79%), respectively, with Rf values being below one. At low dilutions, both fermented extracts had fertiliser effect on tomato plant growth, whereas at high dilutions they were phytotoxic. Results of this study suggested that the two materials could serve as potent bio-nematicides at low dilutions.

Using a computer-based model to quantify ground *Cucumis myriocarpus* fruit for serving as pre-emergent bio-nematicide in tomato production

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Crude extracts of *Cucumis myriocarpus* fruit had been successfully used as post-emergent bio-nematicide in suppression of *Meloidogyne* species. The objective of this study was to determine the quantity of the material required for use as pre-emergent bio-nematicide in tomato (*Solanum lycopersicum*) production using the Curve-Fitting Allelochemical Response Data (CARD) programme. Ten different dosages of crude extracts of *C. myriocarpus* fruit were evaluated for density-dependent growth pattern of tomato plants in a greenhouse trial. The trial layout randomised complete block design trial, with five replicates. Eighteen days after planting, the CARD programme indicated that growth stimulation on tomato plants by the material occurred from 0.10 g to 0.44 g dosages, with the average growth stimulation quantity of 0.27 g crude extracts. This quantity was validated in pot trials for tomato plant growth and for suppression of ca. 10 600 juveniles of *Meloidogyne incognita* race 2. The material had no effect on emergence of tomato seedlings but suppressed nematode juveniles within 18 days after nematode inoculation. Results suggested that at the CARD-generated average stimulation point, crude extracts of *C. myriocarpus* fruit could serve as a pre-emergent bio-nematicide.

Responses of *Vernonia galamensis* accessions to *Meloidogyne incognita* race-2 infection

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Vernonia galamensis, an alternative crop in arid areas of Limpopo Province, is greatly valued for its seed oil. The latter is important in the reduction of greenhouse gases and the removal of carcinogenetic acrylic formulations in paints and plasticisers. However, the host-suitability and host-sensitivity of *V. galamensis* to root-knot nematodes have not been documented. The objectives of this study were to determine the host-suitability and host-sensitivity of three accessions of *V. galamensis* to *M. incognita* race 2 under greenhouse conditions. Treatments consisted of five Pi levels of *M. incognita* race 2 that was included in a randomised complete block design with nine replicates. Sixty-two days after nematode inoculation, the reproductive factor (Rf = Pf/Pi) of *M. incognita* race 2 at a low initial population density (Pi) was greater than one. On the other hand the Rf value was less than one for the higher Pi levels used. Results of this study suggested that the three accessions of *V. galamensis* tested were sensitive to *M. incognita* race 2.

Penetration indices of *Meloidogyne incognita* race 2 and *Meloidogyne javanica* in 32 maize genotypes

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Penetration indices (PIs) of *Meloidogyne incognita* race 2 and *M. javanica*, respectively, in 32 commercial maize (*Zea mays*) genotypes were investigated under greenhouse conditions in separate trials. For each trial, 32 maize genotypes, comprising 16 hybrids, 15 open-pollinated varieties and one exotic nematode-resistant standard, were arranged in a randomised complete block design, with six replicates. Fifty-six days after inoculating the roots of each seedling with 10 000 juveniles, the PIs (nematodes in roots/nematodes in soil) were subjected to analysis of variance and means separation achieved using the Duncan's multiple-range test. Penetration indices suggested that one genotype (OBATAMPA) had post-infectional host-status against both nematode species, whereas two other genotypes (QPM-SR and QSOBA) had pre-infectional non-host status. In conclusion, results of this study demonstrated that certain commercially used maize varieties in South Africa have the capacity to be used in excisting maize breeding programmes for the introgression of root-knot nematode resistance into popular, high-yielding genotypes.

POSTER PRESENTATIONS

Prospects for controlling plant-parasitic nematodes by improving soil health through conservation agriculture

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One of the primary objectives of conservation agriculture (CA) is to promote soil health by stimulating an increase in the numbers and diversity of beneficial soil organisms. Aspects that need particular consideration in terms of nematodes and CA include i) how CA practices benefit soil organisms [in this case non-parasitic nematodes (NPN)]; ii) what the importance of NPN is in crop production and iii) how soil health could be defined in terms of the ratio between different nematode trophic groups. Two trial sites are currently being monitored in a CA programme of the Agricultural Research Council's Grain Crops Institute on a

long-term, seasonal basis at Ventersdorp (North West Province) and Viljoenskroon (Free State Province) of South Africa. Both trials consist of four treatments with four replicates each *viz*. monoculture maize under conventional cultivation practices and three divergent treatments where minimum soil disturbance, different crop rotations and permanent soil cover are applied. For nematode extractions, counting and identification, soil is sampled from all plots before planting, while both soil and crop root samples are taken 60 and 100 days after planting. NPN are identified to trophic level according to leading literature sources in the field and plant-parasitic nematodes to species level. Early results indicate strong crop and cultivation tendencies in terms of nematode population and species diversity but the study should carry on for a considerable period before any conclusions can be drawn.

Nematodes associated with organic substrates in caves

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Nematodes inhabit a wide range of habitats and are abundant and omnipresent in both terrestrial and aquatic ecosystems. Therefore, these organisms play an important role as indicators of environmental disturbances and ecosystem health. The objective of this study was to determine which nematode genera dominate in guano droppings and soil substrates occurring in caves in South Africa. Two caves were included in the study, namely one in Gauteng and one in the North West Province. Nematodes were extracted using the sugar-flotation as well as the adapted Baerman-tray method. Results obtained showed that non-parasitic nematodes dominated both in guano droppings and soil samples obtained from both caves. High population levels of bacterial feeders, in particular *Panagrolaimus* spp., were identified as the predominant nematode group present in guano droppings. On the other hand, low population levels of bacterial feeders were associated with soil samples. Future research will focus on the interaction of nematodes and other organisms that form part of the food webs in these caves. This research will ultimately contribute to the conservation of biodiversity in these unique ecosystems.

Is mixture cultivation of *Musa* varieties a pertinent agroecological strategy for nematode management?

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Varietal mixtures is a cultural technique based on the introduction of genetic and functional diversity to manage pests and diseases commonly used on some grass crops to mitigate wind- and soil-borne pathogens. Reports of effect of varietal mixture still remain very scarce in other crops. In a field experiment conducted in Martinique, we evaluated the ability of the varietal mixture of bananas to manage plant-feeding nematodes. Three cultivars of banana (a Cavendish dessert banana, a new synthetic hybrid FB924 and a plantain French Horn) having different levels of susceptibility to nematodes were planted two by two forming six couples of both mono- and mixed varieties. As a result, banana varietal mixtures had significant effects both on nematodes abundance and community compositions. However, special attention has to be paid in the choice of varieties in plant mixtures. The introduction of the synthetic hybrid FB924 contrary to the plantain increased the relative abundance of the spiral nematode *Helicotylenchus multicinctus* while that of the most damaging burrowing nematode *Radopholus similis* decreased. This study provides the first discussion on the way to combine genotypes for an agroecological management of plant-feeding nematodes to increase profitability and yield stability.

Litchi dieback and litchi decline: How do they differ?

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Litchi dieback is becoming a serious problem in orchards where adult trees become sick and eventually die. Several organisms have been linked with the disease, but while a combination of fungi has been identified, none of them were directly linked with the disease and its symptoms. Factors like nematodes, drought stress and other abiotic factors were never really considered to play an important role. An intensive survey was conducted in all litchi producing areas. Sampling was done irrespective of presence of litchi tree dieback but, when it was present, a representative sample of both healthy and sick plants were taken. The amount of samples was determined by the size of the area. Besides nematodes and fungi, present on roots and in the soil, other data collected included irrigation schedule and type, soil nutrients, fertilisation, age, cultivar and size of trees. Fungi identified were *Pythium, Phytophthora*, while *Cyclindrocladium* and *Pythium* was present in all the provinces and in 88% of the samples taken from healthy as well as diseased trees. Although several nematodes species were present in samples the ring nematode *Hemicriconemoides mangiferae* was associated with all the orchards, confirming it as the most important nematode species on litchi in production areas of South African. Although nematodes are not the primary cause of litchi dieback they can play an important role in the development of the disease. Particularly when numbers are high they should be controlled to reduce the risk of litchi dieback.

A survey of plant-parasitic nematodes associated with cereals, lupin and canola in the Overberg, South Africa

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Wheat and barley are the main grain crops that are produced mostly under dry-land conditions in the Overberg area of the Western Cape Province. Next to maize, wheat is considered the second most important grain crop in South Africa. A large percentage of the barley grown in the Overberg region is used for brewing malt beverages such as beer. Monocropping is not common practice in this region and crop rotation commonly includes wheat, barley, lupin, oats, canola, medics, clovers and lucerne. A survey was conducted with the main objective to determine which plant-parasitic nematodes are associated with wheat, barley, oats, lupin and canola in the Overberg production area. Samples were collected from two farms, "Tussenbeide" and "Droëkloof". Three plant-parasitic nematode genera were identified i.e. *Pratylenchus, Tylenchorhynchus* and *Paratylenchus*. All three of these genera were associated with wheat and canola samples, while *Pratylenchus* and *Tylenchorhycnhus* were associated with barley and oats samples. Very low numbers of *Pratylenchus* spp. individuals were detected in soil samples from lupin, with none in the root samples. Beneficial nematodes were found in all samples and their population levels varied from low to moderately high.

Integrative taxonomic study of the virus vector family Trichodoridae from the Iberian Peninsula, an apparent centre of speciation

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Trichodoridae are polyphagous root ectoparasites occurring worldwide. Their major pest status is as virus vector of Tobravi-

ruses. Currently, the family has 102 species classified within six genera. Eighty-eight percent of the species belong to the didelphic genera (females with two well developed genital branches) with the genus *Trichodorus* being the largest in number of species (56). *Trichodorus* species predominantly occur in temperate regions. Surveys for trichodorids were carried out in cultivated and natural habitats in Spain and compared with the trichodorid fauna from Portugal. A comparative morphological study was carried out together with molecular analyses based on nuclear ribosomal RNA genes (D2-D3 expansion segments of 28S and partial 18S gene). Characteristic for the Iberian Peninsula is the high number of morphologically closely resembling species, but clearly separated molecularly (cryptic species), characterised in males by slightly ventrally curved spicules with a mid-blade constriction with bristles and females with relatively large vaginal sclerotised pieces, quadrangular to triangular in shape. Molecular analyses demonstrated that D2-D3 expansion segments are suitable diagnostic markers for *Trichodoridae*. Comparative morphology and molecular analyses provide support for the Iberian Peninsula as an apparent centre of speciation. South Africa is another known centre of speciation of *Trichodorus*.

Prospective extended benefits of plant-nematode diagnostic and advisory services should closer cooperation between laboratories be attainable.

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Ad hoc but frequent observations on nematode-crop associations in the form of samples submitted for analysis to various laboratories provide invaluable information to scientists that would otherwise perhaps require substantial research inputs. Although not always repeatable and hence not publishable as such the information gained through the provision of these services frequently leads to new perspectives and adapted nematode-management approaches. This is based on the experience of the involved personnel of the laboratory of the North-West University in Potchefstroom. It could, therefore, be claimed with substantial certainty that this past summer season was fairly extraordinary in terms of the levels of plant-nematode infestation of various crops from various regions in the country. Prominence values, frequency of occurrence and population densities were determined for plant-parasitic nematode genera associated with each respective crop for which samples for diagnostic purposes were received. Root-knot nematodes were generally the predominant parasites found in root/tuber samples for the majority of grain, oilseed, vegetable and cover crops. Lesion, cyst, stubby root, ring, stunt and spiral nematodes were also identified in root/tuber and/or soil samples of various crops. In addition, based on previous experiences and records of some nematode species that sometimes are less abundant, increases in numbers were observed this season. It is, therefore, suggested that at least the respective public laboratories in the country endeavour to join forces in order to formalise data and publish them. Such an endeavour could benefit the individuals, their employer institutions, the growers who depend on our services, the relevant industries and the science of nematology at large.

The effect of South African entomopathogenic nematodes on Bt-resistant and non-resistant lifestages of *Busseola fusca*

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The maize stem borer (*Busseola fusca*) is indigenous to Africa and occurs throughout the maize production triangle. Maize stem borers are responsible for at least 10% yield loss but can also lead to total crop failure. The first field resistance of *B. fusca* to Bt maize was reported during 2007. The objective of this study was to determine the effect of two local entomopathogenic nematodes (EPNs), *Heterorhabditis zealandica* and *Steinernema yirgalemense* on Bt-resistant and non-resistant *B. fusca* under laboratory conditions. Different life stages of the insect were tested, which included non-resistant and Bt-resistant pupae, diapauses larvae and active larvae. Results indicated that both non-resistant and Bt-resistant pupae are less susceptible to EPNs infection compared to the high susceptibility of all larval stages tested. No significant differences existed in terms of susceptibility between non-resistant and Bt-resistant life stages. High numbers of *S. yirgalemense* penetrated and developed three days after inoculation of *B. fusca* larvae compared to the low numbers and poor development of *H. zealandica*. Larvae of less than 10 mm

were highly susceptible to penetration by both EPNs species, the latter of which had a high developmental rate.

Efficacy of *Steinernema* and *Heterorhabditis* spp. for control of fruit fly species (Diptera: tephridae) in laboratory studies in Tanzania

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Fruit flies (Diptera: Tephritidae) are among the most important insect pests in fruit production in Tanzania. *Bactrocera* spp., *Ceratitis* spp. and *Dacus* spp. can significantly reduce yield and fruit quality. Management of fruit flies is a challenge but include cultural control measures such as collecting and destroying infected host fruits, regulatory control (quarantine) and the use of insecticide baits. The best approach for control of fruit flies is to develop an IPM system that includes several strategies to reduce the build up of damaging pest populations. Biological control agents such as entomopathogenic nematodes (EPN) are potential candidates within such a system. Naturally occurring EPN isolates in the genera *Steinernema* and *Heterorhabditis* have been isolated from a fruit growing area in the Morogoro region in Tanzania. Selected EPN isolates have been tested against larval and pupal stages of three important fruit fly species, *Bactrocera invadens, B. cucurbitae* and *Dacus bivittatus*. Results are promising and show that several isolates caused 70% to 90% mortality of larvae for all three fruit fly species.

The biology of entomopathogenic nematodes in citrus mealybug, *Planococcus citri*

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Planococcus citri (Risso) (*Pseudococcidae*), commonly known as the citrus mealybug, is a serious pest of citrus in South Africa. Mealybugs display cryptic behaviour and are difficult to control chemically. Entomopathogenic nematodes represent biological control agents with the ability to actively seek their host, following them into cracks and crevices where chemicals cannot reach. The development of *Steinernema yirgalemens*e and *Heterorhabditus zealandica* in *P. citri*, was followed, as both of these species caused high mortality of mealybugs in bioassays where individual mealybugs were exposed to 200 IJ for 48 h in 50 l water, each in 24-well bioassay plates. In the biology study, adult female mealybugs were individually infected with 50 IJ in 50 l water each. After infection the number of nematodes which penetrated as well as their developmental stage was recorded every one to two days until a new consort of IJ emerged. Results obtained from the biological study between the two different nematode species in adult *P. citri* will be discussed.

The potential of five weed species to maintain *Heterodera schachtii* populations and to serve as a source of infection of vegetable crops

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The aim of this study was to determine the potential of five weed species to maintain *H. schachtii* populations and to serve as a source of infection for follow-up vegetable crops. The most obvious host response was shown by shepherd's purse (*Capsella bursa-pastoris*) and wild radish (*Raphanus raphanistrum*). The final population density of juveniles/gram of root at Pi 0.66 eggs and juveniles/gram soil for the latter weeds were significantly higher (P=0.05) than those for the other weed species. Shepherd's

purse and wild radish also had the highest number of eggs and juveniles/gram of soil for all initial Pi levels as well as the highest Pf/Pi ratios. When the Pf/Pi ratio was considered, it was evident that vegetable production should be free from weeds. Agricultural producers do not realise the potential danger of such weeds that are commonly observed in vegetable fields and usually tolerated. The ability of commonly occurring weeds to support multiplication of *H. schachtii* demonstrates the need of an awareness program for effective weed management to keep *H. schachtii* populations under economic threshold levels.

Research and training interventions to raise awareness about the impact of root-knot nematodes on vegetable production in subsistence farming

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Plant-parasitic nematodes are soil-borne pests that cause significant damage to agricultural and horticultural crops worldwide, including weeds. Sixteen leafy vegetables were screened for host suitability to *M. javanica* and *M. incognita* in independent greenhouse, micro-plot and field trials. Each seedling was inoculated with ±5 000 root-knot nematode eggs and second-stage larvae (J2) of the appropriate spp. and evaluations were done 56 days after inoculation (DAI) for greenhouse trials. For micro-plot and field trials evaluations were done 30 and 60 DAI, respectively. Significant variation existed among vegetable spp. with regard to egg and J2 counts/root system, Rf-values and eggs/gram of root. Three vegetables spp. maintained more *M. javanica* eggs and J2/root system than the susceptible standard, while six had Rf values >1 for both *M. javanica* and *M. incognita*, indicating susceptibility. However, 11 vegetable spp. had RF-values 1 (poor hosts) for *M. javanica* and eight for *M. incognita*. Egg and J2 numbers/root system ranged between 94 and 16 306 for *M. javanica* and from 228 to 14 007 for *M. incognita*. Results of both micro-plot and field trials correlated with those of the greenhouse screenings and indicated that indigenous vegetables as well as weeds could be fairly susceptible to both *M. javanica* and *M. incognita*.

Assessing the reproductive ability of a new *Globodera* species from the Sandveld region in potato

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The presence of a new *Globodera* sp. in the Sandveld region, morphologically very similar to *G. rostochiensis*, has complicated the detection of quarantine nematodes in regulatory samples from this region. Determining if this nematode is a pest of potato will have considerable impact on accessibility to foreign markets and certification of seed potatoes. Two separate experiments were conducted to determine the pathogenicity of the unknown *Globodera* sp. on potato. In experiment A, cysts of *G. rostochiensis* and the unknown *Globodera* sp. were incubated in potato root exudate for 4 weeks and inoculated onto germinating potato tubers (cv. BP1) which were incubated in the dark at 20°C for 14 weeks in a laboratory incubator. In experiment B, cysts of both species were incubated in potato root exudate for 7 days and inoculated onto potato plants (cv. VanderPlank) which were maintained for 12 weeks at 25°C in a glasshouse. Mean multiplication rates, percentage of viable eggs and spontaneous hatch were estimated. PCR-RFLP confirmed the identity of cysts used in this study. Cysts, containing viable eggs from the new *Globodera* sp. from the Sandveld region were unable to reproduce on potato plants (cv. BP1 and VanderPlank) under laboratory and glasshouse conditions.

Combined response of *Meloidogyne incognita* and *Busseola fusca* to genetically modified maize

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The root-knot nematode (*Meloidogyne incognita*) and maize stem borer (*Busseola fusca*) occur throughout the maize production triangle, often on the same site. Genetically modified maize became a useful management tool for the management of *B. fusca* (Bt-maize) and weeds (Roundup Ready maize). The objective of this study was to determine the response of *M. incognita* and *B. fusca*, separately and combined, on genetically modified maize. The isohybrids tested during this trial included DCK8010 (conventional), DKC8012B (Bt), DKC8030R (Roundup Ready) and DCK8040BR (both RR and Bt). All four cultivars were subjected to treatments that included i) a non-inoculated control, ii) inoculated with + 5 000 *M. incognita* per plant at planting, iii) inoculated with 10 *B. fusca* per plant six weeks after planting and iv) inoculated with both *M. incognita* and *B. fusca* at the same rates and time. Each treatment consisted of 15 plants and was replicated six times. Nine weeks after planting DKC8012B and DCK8040BR showed a significant increase in nematode numbers. In the presence of *B. fusc*a, however, the nematode numbers decreased significantly in DKC8012B but remained significantly higher than that in DCK8010. Although the nematode numbers in DCK8040BR remained significantly higher than in the other cultivars in the presence of *B. fusca*, there was no significant increase compared to that in the same cultivar inoculated only with nematodes. *B. fusca* showed a significantly higher survival rate in DKC8030R in the presence of *M. incognita*.

The effect of cover crops on root-knot nematode population levels in a potato-based cropping system

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A variety of cover crops, including *Brassica* and *Raphanus* spp., are used worldwide to reduce root-knot nematode population levels in agricultural and horticultural cropping systems. Locally, potato producers in the Western Free State Province experience substantial quality losses in potato due to root-knot nematode infection. Therefore, *Brassica* and *Raphanus* varieties were evaluated to establish their effect on a high root-knot nematode population that occurred in a producers' field in the Christiana area. A field trial where potato has been planted during the preceding season was subsequently conducted. The trial consisted of six treatments, viz. two *Brassica* varieties (Nemat and Caliente), two *Raphanus* varieties (Terranova and Doublet), an untreated control as well as a standard nematicide treatment (EDB@40 ha^{-1}). The trial layout represented a randomised complete block design with five replicates for every treatment. Root-knot nematode population levels/50g roots did not differ significantly (P0.05) among the untreated control, Nemat, Caliente, Terranova and Doublet treatments during tuber initiation of the potato crop. The EDB treatment, however, had a significantly lower number of root-knot nematodes/50g roots compared to the untreated control and other treatments.

Biological and physiological aspects in the host-parasite relationship of the nematophagous fungus *Pochonia chlamydosporia*

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The nematophagous fungus, *Pochonia chlamydosporia* (Clavicipitaceae), can parasitise eggs of cyst (*Globodera* spp., *Heterodera* spp.), root-knot (*Meloidogyne* spp.) and false root-knot (*Nacobbus* spp.) nematodes. Its potential as a biological control agent has been the subject of numerous studies to understand the micro-ecological conditions, including the tri-trophic (e.g. plant, fungus, nematode) and host-parasite relationships that allow the fungus to thrive in the soil and rhizosphere environment. *Pochonia* survives in soil in the absence of a nematode host and, although it behaves as a saprophyte, research evidence points

to a physiological 'switch' from saprophytic to parasitic stages triggered by nutrition. Fungal chlamydospore-based products for application to soil as an inoculum have been shown to be commercially viable. However, understanding the basic biology and physiology of the host-parasite interaction can provide new insights into commercial production methodology.

Paecilomyces lilacinus: an effective biological control agent?

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Two glasshouse trials on tomato (Rodade) were conducted during the 2010 season with *Paecilomyces lilacinus* and *Meloidogyne incognita*. The fungus originates from two different sources and was applied according to the manufacturer's recommendations. Treatments included different dosages and different timing of application of the biological product used. Results varied widely in terms of plant growth stimulation and nematode control. Product 1, when applied in seedling trays, showed significant growth stimulation of tomato plants. Application of the product on tomato roots planted in seedling trays followed with consecutive applications resulted in much better plant growth than those of the untreated control plants, irrespective of the dosage. Product 2 was applied to the soil at planting and had less of an effect on plant growth, although some of the treatments using different starters resulted in better growth of tomato plants than those of the untreated control. However, nematode control was not sufficient both for product 1 and 2. Laboratory tests indicated that *P. lilacinus* is effective in inhibiting hatching of nematode eggs, however development from spore to hyphen is estimated to be approximately five days. By the time the biological control agent is ready to inhibit egg hatching, nematodes already penetrated the roots of the host plant. As with many biological products, application methods and timing is crucial, much more so than for conventional nematicides. More research on these aspects is needed to ensure development of a more effective biological control agent.

Preliminary investigations into inducing resistance in sugarcane against *Meloidogyne incognita*

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Plants respond to nematode attack via a variety of mechanisms. One such mechanism is the production of jasmonic acid (JA) which is an elicitor of the plant's defence response. Indeed, systemic defence response induced by JA has been shown to protect susceptible tomato plants against infection by nematodes. Research at Rothamsted has shown that cis-jasmone, jasmonic acid and methyl-jasmonate can reduce infection of *Meloidogyne incognita* and *Globodera pallida*. Little has been published on using these chemicals to protect sugarcane against nematodes, apart from a few reports focussing exclusively on methyl-jasmonate. The aim of our work was to screen eight potential elicitor chemicals (BABA, Bion$^{(R)}$, cis-jasmone, ethrel, imidacloprid, methyl-jasmonate, salicylic acid, 2,6-dichloroisonicotinic acid) for their effects on reducing *Meloidogyne incognita* infection of sugarcane and sugarcane yield. A pot trial was conducted where the chemicals were tested on two sugarcane cultivars, N12 and N27. Results showed that three chemicals (BABA, Bion$^{(R)}$, salicylic acid) reduced *M. incognita* infection in both cultivars, although no effect on sugarcane yield was apparent. *Cis*-Jasmone and methyl-jasmonate had contrasting effects in the two cultivars. Based on these preliminary results, it appears that the salicylic acid pathway is the most dominant in sugarcane. Future work will involve testing these chemicals in naturally-infested soil and at different rates.

Pre-plant nematode control in pineapples in Northern KwaZulu Natal

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Ethylene dibromide (EDB) has been the most popular nematicide used for pre-plant control of plant-parasitic nematodes on Queen pineapple in South Africa to date. Lately a shortage of product is, however, often experienced. For this reason and because it is often very difficult to obtain optimal soil conditions for fumigation on heavy soils, three alternative nematicides as well as 1,3 dichloropropene (fumigant, applied at 204 l ha^{-1}), were evaluated for pre-plant nematode control on heavy soils (22% clay) on two pineapple cultivars (Queen and MD2) in Northern KwaZulu Natal. The three alternative nematicides included were: ethoprophos 10G (70 kg ha^{-1}), cadusafos 15G (50 kg ha^{-1}) and furfural (aldehyde) at 400l ha^{-1}. Nematodes were counted every three months after nematicide application was done. Plant vigour was also measured and yield data collected. Two nematode genera were recorded in root and soil samples, namely *Pratylenchus brachyurus* and *Meloidogyne* spp. Control of these nematodes was more effective in the Queen than in the MD2 planting. The lowest nematode infestation and highest yield were found for the furfural treatment in the Queen planting, while cadusafos gave the best nematode control in the MD2 planting. Furfural was consequently evaluated at different dosages and compared with EDB in a Queen planting on sandy soil.

Presto©, a new potential tool for control of the root-knot nematode *Meloidogyne hapla*

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Alternative methods of crop protection against plant-parasitic nematodes, preferring application of natural ingredients instead of synthetic pesticides, are applied in ecological production of agriculture commodities in as well as outside the European Union. Plant extracts and essential oils show antimicrobial, insecticidal as well as nematicidal activity. Traditionally, protection against root-knot nematodes in Central Europe was entirely focused on complete soil sterilisation using fumigant pesticides. Following restrictions on the use of methyl bromide, crop protection was limited to use the nematicide DAZOMET, which is not suitable for root vegetable production. Different plant extracts and essential oils have been tested for restriction of the negative effect of *Meloidogyne hapla* on the quality and yield of root vegetables. The aim of this work was to test three plant extract-based substances (Pongamia, Tagetes and Presto©). Trials were carried out under controlled conditions in the greenhouse. Nematode-infested soil originated from fields used for experiments. Effectiveness was evaluated using index of root galls, weight of roots and weight of leaves. Results revealed the nematicidal effect of Presto© that has a patent pending at this time. Results from field trials are expected in September 2011. The research was supported by MEYS; project number MSM 6046070901.

Nemalan®, a fermented plant extract with the potential for suppressing root-knot nematodes (*Meloidogyne* spp.) on tomatoes

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Management strategies for the control of root-knot nematodes (*Meloidogyne* spp.) on tomatoes are limited, with the registered chemical options being undesirable and costly. Plant extracts might offer an alternative and economically feasible option. The first aim of the study was to determine if an anaerobic fermented extract of *Lantana camara* supplemented with garlic, Nemalan®, could suppress *M. javanica* in a glasshouse trial. Nemalan was able to reduce the galling index, larvae, the number of eggs- and M. javanica females per /root system to levels that were comparable to the standard. Most of the Nemalan® application rates caused a reduction in root weight, but not plant weight or height. The other aims of the study were to develop a chemical fingerprint for quality control, and to investigate the presence of compounds with known activity against *Meloidogyne*. Although Nemalan® was shown to be complex, a chemical fingerprint consisting of 14 compounds, which mainly included the most abundant peaks on the GC-MS chromatogram, was developed using extracts manufactured over a six month period. Compounds, with known activity against *Meloidogyne* included -terpineol, borneol, camphor, diallyl disulphide, salicylic acid, vanil-

lic acid and butanoic acid (by-product from fermentation). The concentrations of these compounds were lower than the known effective concentrations, suggesting possible synergistic interactions between these compounds and/or unidentified compounds. These could include triterpenoids, which have activity against *Meloidogyne* and are known to occur in other *L. camara* extracts, but this requires further investigation.