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Abstracts of Papers

Microbes as biological control agents for nematode pests

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An understanding of the population dynamics of pests and their natural enemies is essential for the successful deployment of biological control agents. Although there is much circumstantial evidence that nematophagous bacteria and fungi may regulate nematode infestations, a large proportion of the nematode population (>90 %) must be killed to prevent population increase. Few studies have demonstrated such control. In practice the use of biological control agents will depend on their integration with other control measures. There is no unifying model for biological control, so that predicting where it will succeed and the level of control that will be obtained, is difficult. There is usually a range of potential control agents associated with any given pest, which may be used in a number of ways. In addition, there are complex interactions between the nematode pest, its host plant and the natural enemy community as well as abiotic environmental factors that can determine success. However, when biological control is successful, the environmental and economic benefits can be quite considerable. Although studies on the biological control of plant-parasitic nematodes have moved from empirical to more manipulative and mechanistic approaches, few commercial products have been developed and none is in widespread use. The increasing number of reports of soils that are suppressive to specific nematode pests because of the activities of the natural enemy community suggests that, despite difficulties, biological control of nematode pests is a realistic objective. Such soils are useful sources of potential biological control agents but not all organisms that are effective in suppressive soils have the characteristics essential for commercial development. The selection of bacteria and fungi with potential as biological control agents for migratory and sedentary nematode pests will be reviewed and their production and application will be discussed.

Analysis of the pathogenic, morphological and genetic variability of *Scutellonema bradys* on yam in West Africa

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In West Africa the yam nematode, *Scutellonema bradys,* causes dry rot of yam (*Dioscorea* spp.). In order to identify resistance to *S. bradys* in yam, it is important that the conformity of the nematode across the yam-growing region is determined. Yam tubers with symptoms of dry rot and typical nematode damage produced in Nigeria, Togo, Benin, Burkina Faso, Mali, Ghana and the Ivory

Coast were collected and nematodes were extracted for measurement of genetic and morphological characteristics. They were also assessed for their pathogenicity on the yam species *Dioscorea rotundata.* Genetic assessment was undertaken following amplification of the internal transcribed spacer (ITS-1, 2) gene by PCR, using 10 individuals from each of 14 geographically separate isolates. In addition, PCR-RFLP, using Rsa1-digested samples was also performed. Species-specific primer sets were designed using the ITS-rDNA alignments of *S. bradys* isolates and other plant-parasitic nematodes, as well as amplified ITS-1,2 rDNA using genomic DNA as a template. Morphological characteristics (quantitative and qualitative) were measured using a compound microscope. Pathogenicity was assessed by inoculating 250 g seed sets with c. 1000 *S. bradys* before planting and measuring growth and damage parameters after six months. Nematodes from dry rot-infected tubers from all sites across the region were identified as *S. bradys.* Morphological characteristics differed little between sample sites (isolates). In Benin, however, one nematode isolate was different from others, particularly with relation to spear length. Furthermore, there was morphological variation among individuals belonging to the same isolate. Substantial genetic polymorphism was observed between different nematode individuals within an isolate and between isolates in Benin. Seven different genetic patterns were identified using Rsa1-digested PCR-RFLP patterns of the ITS gene. Some polymorphism was also observed between samples from sites across West Africa. All nematode isolates reduced yam tuber mass in comparison with uninoculated tubers, but not compared with each other.

A study of the host status of twelve common crops of the Cameroon Highlands to the nematode *Pratylenchus goodeyi*

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Knowledge of the host range of a given nematode species is important for recommending suitable rotation crops to farmers. *Pratylenchus goodeyi* is a well-known parasite of banana and plantain in African highland regions. The host range of this species has been documented as 'narrow', but crops identified as suitable hosts vary, depending on the location where the study was carried out. In order to identify host and non-host crops for *P. goodeyi* on the Cameroon Highlands a field trial was set up in an old banana plot. Twelve commonly cultivated crops were planted in a randomised complete block design with four replicates. Sampling was carried out four months after planting. A non-parametric analysis of variance revealed that there was a significant difference between treatments (crops) at $P = 0.0006$. Watermelon, maize and red bean were capable of hosting relatively high densities of the nematode where the *Musa* variety Grande Naine (AAA) was used as a reference crop. On watermelon and the maize cultivar CMS 8740, *P. goodeyi* was the major species extracted from the roots. On red bean and onion *P. goodeyi* was not the dominant species, even though densities were high enough to consider these crops as good hosts of this nematode. Other crops used in this study were red taro, coco, yam, sweet potato, okra, tomato and the maize variety Kasaï. Very low numbers to no *P. goodeyi* were recovered from these crops, indicating that they could be suitable rotation crops for reducing densities of this nematode.

Participatory identification of vegetable production constraints in the North Rift, Kenya

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Maize used to be a major crop grown by most farmers in Kenya. However, due to a high population growth rate and subdivision of land in most parts of the North Rift region, most farmers reverted to growing vegetables such as kales, cabbage and tomato, which take a shorter time to mature and provide a quicker source of income. Participatory Rural Appraisals (PRAs) and household interviews were conducted in four districts in this region. The main objectives were to characterise the vegetable-based production systems in terms of gender-desegregated data on major vegetable production constraints, control practices used by farmers and available pest control options. This information is needed to generate sustainable integrated pest management technologies, suitable for different farmer categories and needs. It was found that most households were resource-poor, and male-

headed (50–85 %). Farmers listed 21 limiting constraints to vegetable production. The major constraints faced by both males and females were pests, diseases and high cost of inputs. Major constraints for females were inadequate training and lack of technical know-how for identification of pests, diseases and the use of pesticides, while males regarded unavailability of good quality, certified seed and agrochemicals as major constraints. Researchers identified root-knot nematodes (*Meloidogyne javanica* and *M. incognita*) as one of the major factors responsible for reducing tomato yields. Policy implications of the findings and future research focus areas will be discussed.

On the distribution of plant-parasitic nematodes associated with cereal-based cropping systems in Uganda

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In a nematode survey of plant-parasitic nematodes associated with cereal-based cropping systems in Uganda, 293 sites were visited in five districts that represent the major cereal-growing areas in the country. Root samples were collected from maize, millet, sorghum, wheat and barley, grown singly or as intercrops. Twenty-two species, representing ten genera of plant-parasitic nematodes were identified from the various root samples collected. Fifteen species were associated with maize, 14 with finger millet, eight with sorghum, five with wheat and three with barley. Five genera, namely *Pratylenchus, Ditylenchus, Helicotylenchus* and *Scutellonema,* occurred more frequently than the others. This report provides baseline information on the distribution and abundance of plant-parasitic nematodes associated with cereal crops in Uganda. While a wide diversity of genera and species were associated with these crops, the pathogenicity of the plant-parasitic nematodes most commonly associated with cereals in Uganda has never been studied. However, many of them are potential pathogens and their influence on growth of cereals will have to be established. The occurrence of *Aphelenchoides arachidis,* a seed-borne endoparasitic nematode of groundnut and cereals, in particular needs further investigation. This nematode has previously been reported to have a restricted distribution in Nigeria, suggesting that it is a significant phytosanitary risk to all groundnut- and cereal-producing countries.

Global status of knowledge on nematode parasites of summer cereals

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Cereals are the most important food source for humans in the world. Maize, rice and wheat are the most important cereals produced, whereas others such as sorghum and millets are restricted by growing conditions and their production is therefore localised. Nematodes are, among other pests and diseases, responsible for a considerable gap between production potential and actual yield of these crops. Summer cereals are often incorrectly considered non-hosts to root-knot nematodes. The most important plant-parasitic nematode species in maize are root-knot, root-lesion and cyst nematodes, but more than 60 species have been associated with this crop. From a global perspective two of these groups, namely lesion and root-knot, and to a lesser extent stunt nematodes, are very important parasites of sorghum. Despite the importance of millets in several countries, little is known about nematode associations in these crops. Current trends in agriculture such as genetic manipulation, multi-cropping, no-till and biological farming significantly affect the existing knowledge base. These modern crop production trends put great pressure on simplistic nematode management practices. In future the ability to reduce yield losses caused by nematodes in cereals will require greater understanding of nematode biology and interactions with other organisms and factors. This requires innovation in terms of management strategies, particularly for crops with low profit margins or those used in subsistence farming situations. Therefore, the accumulation of basic information in terms of population dynamics, host ranges, climatic effects, control measures and stable production systems needs to be stimulated among crop protection specialists.

Nematode response to intercropping and organic amendments in sugarcane

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A trial was planted on the KwaZulu-Natal north coast on a small-scale farmer field to study the

effect of intercropping and organic amendments on sugarcane yield. Velvet bean and sweet potato were planted in the middle of the 1-m spaced cane rows. Bagasse, fly ash and cattle manure were applied under and over the cane setts. However, for the latter the amount of manure was not sufficient to entirely cover the setts. These practices were compared to untreated control and nematicide treatments. The best yield was obtained with the cattle manure treatment, but without a noticeable effect on nematode communities. The nutritional effect was obviously compensating for potential nematode damage. No biological control process occurred in these plots. Conversely, very good nematode control was obtained following embedding of the setts with a high amount of inert bagasse or fly ash. These results suggested that soil restructuring by organic fibre was the main factor in preventing nematodes from reaching the roots. As for intercrops, velvet bean had a stronger effect on the nematological components than sweet potato. Intercropping increased cane infestation by nematodes, but compensatory processes occurred later in the crop cycle. Nematicide treatment was highly profitable, but led to a pathogenic nematode community that will require further treatments. On the contrary, 'biological' treatments seemed to allow the build-up of a less pathogenic community, with a lower proportion of *Meloidogyne* spp., and a higher proportion of *Pratylenchus* spp. as well as *Helicotylenchus dihystera.* Finally, the results proved that adequate use of organic amendments can sufficiently replace nematicide treatments and that intercropping is neither detrimental nor beneficial to sugarcane.

Susceptibility of the codling moth to South African isolates of entomopathogenic species of *Heterorhabditis* and *Steinernema*

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Codling moth, *Cydia pomonella,* is recognised as a serious pest of apple and pear worldwide. Alternatives to chemical control of codling moth are needed and the use of nematodes as an environmentally friendly biological control agent is being investigated. Infective juveniles of entomopathogenic species of *Heterorhabditis* and *Steinernema,* with their associated symbiotic bacteria occur naturally in soils where they infect and kill their insect hosts within a period of 24–48 hours. To obtain locally adapted isolates of entomopathogenic nematodes, a total of 150 soil samples was collected from natural habitats and orchards in the Cape Province. By means of laboratory trapping with the greater wax moth, *Galleria mellonella,* 12 of these samples were found to be infested with entomopathogenic nematodes. The nematodes were maintained in a laboratory on *G. mellonella* and infective juveniles were harvested for storage. Identified species were *H. bacteriophora, H. zealandica, H. marelatus* and a new species of *Steinernema.* Infective juveniles of these isolates were used in laboratory bioassays to test the susceptibility of fourth-instar larvae and pupae of codling moth to these nematode species in terms of moth mortality and penetration rate. An inoculum of 200 infective juveniles per insect was used and *G. mellonella* served as control. Preliminary results indicated high mortality rates of codling moth and *Galleria* larvae, but low mortality of codling moth pupae. Penetration rates were generally low, with the highest rate recorded for the new *Steinernema* isolate.

Effect of *Tephrosia vogelii* on the population dynamics of root-knot nematodes (*Meloidogyne* spp.)

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A total of eight researcher-designed and farmer-managed experiments were conducted near Blantyre, Machinga, Lilongwe and Kasungu from October 2001 to May 2002, to study the effect of *Tephrosia vogelii* on root-knot nematode populations. The experiments were all laid out in a complete randomised block design, with three replicates and two treatments, viz. maize monoculture and maize intercropped with *T. vogelii.* Soil samples were collected from the experiments four times during the 2001/2002 season and three times during 2002/2003. Results revealed higher population levels of root-knot nematodes in maize intercropped with *T. vogelii* than maize monoculture during both seasons. Significant differences were evident at all the sampling times.

Integrated management of *Radopholus similis* in banana

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Field experiments were conducted at Bvumbwe Research Station from 1994 to 1999 and fields at Satemwa in Thyolo and Mulanje between 2000 and 2004 to assess alternative control methods for *Radopholus similis* on banana. The experiments were of randomised complete block design, with six treatments and three replicates. Each mat occupied an area of 2.3 m^2 and each plot contained four mats. The six treatments included paring by removing all roots with a sharp knife and leaving the rhizome clean white, paring + hot water treatment (55 °C for 25 minutes) + chicken manure (600 g per mat), ground neem (*Azadirachta indica*) leaves, (40 g per mat), neem + hot water treatment + chicken manure, carbofuran 10G at 40 g per mat, and an untreated control where all infested roots were left undisturbed. There were significant differences in bunch mass, number of hands per bunch, number of fingers per hand, finger circumference and finger length at the Bvumbwe Research Station experiment in the 1997, 1998 and 1999 seasons. Significant differences were also recorded in bunch mass and number of fingers per hand in the on-farm experiments during the 2003/2004 season. *R. similis* numbers also differed significantly at Bvumbwe during 1997, 1998 and 1999 and in the on-farm field experiments at Thyolo and Mulanje during the 2002/2003 and 2003/2004 seasons. Neem + hot water + manure was the most effective method for controlling *R. similis* in banana, followed by neem only, paring + hot water + manure, and paring only.

Control of root-knot nematodes in potato, tobacco and vegetables with ethoprophos

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Ethoprophos has a water solubility of 750 to 843 mg l^{-1} at 21 °C and a half-life of up to 28 days in neutral soils, but longer in acidic soils. The pH stability of ethoprophos allows the product to be used over the range of pH 4 to 8. Many preliminary trials of applying the product in different ways produced variable results and crop tolerance was at times problematic. Considering that the major nematode pests on potato, tobacco and vegetables are *Meloidogyne* spp. and that soil conditions largely determine damage potential, a common approach was adopted in trials to evaluate ethoprophos activity from nematode population densities of 2000 and more. Ethoprophos was broadcast over the entire area and incorporated to a depth of 15 cm at three to 14 days before planting, using a 150 g kg^{-1} granular formulation at 5 to 10 kg a.i. ha^{-1}. The results of these trials indicated consistent and reliable control of root-knot nematodes, absence of crop-tolerance problems and favourable yield response, dependent on the severity of the *Meloidogyne* infestation. Further trials on additional crops such as citrus are in progress.

Physical, chemical and biological properties of ethoprophos and their impact on product activity

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Ethoprophos is an organophosphate, acetyl cholinesterase-inhibiting nematicide, launched in southern Africa during 1995 on citrus as a 200 g l^{-1} EC formulation. This formulation was withdrawn during 2004 and replaced by a 150 g kg^{-1} granular product. Registrations include potato and some vegetables. Trials on the major crops damaged by nematodes are in progress. The physico-chemical and biological properties of ethoprophos suit the low organic matter and high sand content soils of southern Africa. The octanol water participation constant, log K_{ow} 2.15, precludes translocation from roots. High solubility in organic solvents results in nematicidal concentration in roots but precludes use in soils where the organic-matter content exceeds 7 %. The water solubility of the product (750 mg l^{-1} at 25 °C) permits full release in soils that have a moisture content of near field capacity but redistribution is limited and deep, even placement is therefore critical. Ethoprophos acts by contact in soil and roots and by feeding on treated roots. Inhibition of the neuro-transmitter enzyme is of a more permanent nature than with carbamates. Crop tolerance evaluation is critical as differential crop sensitivity exists. The half-life is dependent on many factors but is slightly shorter than comparable products, requiring an increased rate per hectare. Breakdown in soils is by microbial metabolism and chemical hydrolysis is rapid above pH 8.

Comparative cellular responses of resistant and susceptible soybean cultivars infected with *Meloidogyne incognita* race 2

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Meloidogyne incognita race 2 is abundant in existing soybean fields and areas of potential expansion of soybean production in South Africa. This presents a threat to the crop if the unique relationship between the nematode and the soybean plant is not understood and control measures are not adopted in time. As part of a comprehensive study, the local commercial soybean cultivars LS5995 (resistant) and Prima2000 (susceptible) were planted in pots in a greenhouse experiment to compare cytological changes in their roots caused by infection of J2 of *M. incognita* race 2. Root systems were artificially inoculated with approximately 3000 J2 and harvested at 2, 4, 10, 20, 30 and 45 days after inoculation (DAI), and subsequently prepared for transmission electron microscopic observations. J2 penetrated the roots of both soybean cultivars and migrated intercellularly through cortical to parenchyma cells at 2 DAI. Necrosis/degeneration of cells situated near the head region of J2 was evident at 2 DAI in the resistant cultivar LS5995, but early formation of giant cells was not always evident. No necrotic cells were, however, observed in the roots of the susceptible Prima2000. From 10 to 45 DAI, pronounced differences in giant cell development were observed in roots of both cultivars. Groups of five to 12 giant cells were associated with single developing *M. incognita* race 2 females in roots of Prima2000. These giant cells mostly replaced vascular tissues and were associated with distinct hyperplasia and hypertrophy of surrounding, uninfected cells. Giant cell formation in the roots of LS5995 differed substantially from that in Prima-2000 from 10 DAI onwards. Not only were giant cells in the roots of LS5995 smaller in size but generally also fewer in numbers than in Prima2000 at 10 DAI. Two additional types of giant cells, not reported before, were observed in the roots of LS5995, namely i) formation of abnormal cell wall ingrowths, and ii) giant cells with abnormally thick cell walls. Interference of the resistant LS5995 with the induction, development and maintenance of giant cells, as well as the reported restricted metabolic capacity of such giant cells, therefore adversely affected the development and fecundity of *M. incognita* race 2 in the present study.

Involvement of oxidative enzymes in the induction of defence-related proteins in cotton roots infected with root-knot nematodes

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Generation of active oxidative species (AOS) during the oxidative burst is an early key event during incompatible plant–pathogen interactions. AOS production in cotton roots during nematode infection was confirmed by blue staining observed in roots of the cotton cultivars DeltaOPAL, Tetra, Gamka and Acala OR3 infected with *Meloidogyne incognita* race 4. The staining varied from a dark blue staining in DeltaOPAL and Tetra to a faint reaction in Gamka and Acala OR3. Population development of *M. incognita* race 4 in the roots during growth and development of the above cotton cultivars was determined concurrently. The seeds of Tetra did not germinate. Growth response of *M. incognita* race 4 differed within the roots of the remaining three cotton cultivars. In DeltaOPAL where the dark staining was observed, the population of *M. incognita* race 4 reached a peak just after the first squares were formed, whereas in Acala OR3 and Gamka, where a faint reaction was observed, *M. incognita* race 4 populations only peaked at first flower and boll formation, respectively. It seems that AOS, which is generated during nematode attack, is recognised by the plant host as a signal for triggering defence responses.

Stimulation of egg hatching of *Tylenchulus semipenetrans*

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The citrus nematode's eggs can survive for up to nine years in soil and during favourable conditions are stimulated by host roots to hatch. All the eggs do not hatch simultaneously, however. The purpose of this study was to synchronise hatching of nematode eggs and subsequently eradicate nematode populations in soil by using only one or two

nematicide treatments. Initial results of trials in the laboratory and at Crocodile Valley Citrus Company were promising. There were clear indications that certain agents had a stimulating effect on the citrus nematode's eggs. Subsequently, an 11-year-old orchard with high nematode populations was identified in the Brits area. Three single-tree replicates per treatment, totalling 150 trees, were monitored. Three liquid formulation (EC) nematicides, viz. Rugby, Nemacur and Mocap were used as the chemical control component. A combination of different treatments and dosages, which included the liquid nematicides in combination with egg-hatch stimulants, egg-hatch stimulants on their own and the nematicides on their own, were monitored. Contrary to the initial trials the latest results showed no correlation and no conclusions could be drawn.

Molecular biological methods to identify plant-parasitic nematodes of sugarcane

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Plant-parasitic nematodes are an important growth constraint to sugarcane. Current methods of identifying and enumerating nematodes from the soil and roots are based on the use of morphological characteristics. However, these methods are labour-intensive, require experienced personnel and a state-of-the-art microscope. With recent advances in molecular biology, powerful new methods such as the polymerase chain reaction (PCR) have been developed. These techniques have been widely used for the diagnosis of viral, bacterial and fungal pathogens. The aim of this work was to develop PCR-based methods for identifying plant-parasitic nematodes. Nematode genera commonly found associated with sugarcane, viz. *Pratylenchus, Helicotylenchus, Meloidogyne, Xiphinema* and *Paratrichodorus* were collected from trial sites in various regions of the South African sugar industry. Nucleic acids were extracted and amplifications were done using rDNA internal-transcribed spacer region (ITS1) for the identification of genera, as well as *Meloidogyne* and *Xiphinema* species-specific primers. Identification of the five genera was possible by size differentiation of the ITS1 amplification products. Sequencing of the ITS1 region was used to confirm the identity of the isolates by comparison with sequences in GenBank and to examine the phylogeny of the genera. Identification of certain species within *Meloidogyne* and *Xiphinema* was possible by the presence or absence of amplification products.

Identification of genetic markers associated with *Meloidogyne incognita* race 2 resistance in soybean

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Host plant resistance to *Meloidogyne incognita* race 2 is a useful and cost-effective tool for optimisation of soybean yield as well as profit margins. Locally, no nematicide is currently registered on soybean and most crops used in soybean rotations are also susceptible to *M. incognita.* The identification of molecular markers and subsequent application of marker-assisted selection is a quick and effective way to expedite a nematode resistance breeding process. The soybean cultivars LS5995 (resistant) and Prima2000 (susceptible) were used as parents in crosses to obtain a F_2 mapping population for the identification of genetic markers linked to the resistant trait. Inoculation with c. 10 000 *M. incognita* race 2 eggs and J2 was performed 16 days after plant emergence. The F_2 soybean population was screened with a number of SSR markers evenly distributed throughout the genome. A major QTL was identified on Linkage Group M between markers Satt201 and Satt590, accounting for 62.4 % of gall rating values and 80 % of eggs/root system variation in the segregating population. A minor QTL accounting for 31.7 % of the gall rating values was located between markers Satt487 and Satt358 on LG-O, which is in accordance with published reports. *M. incognita* race 2 marker-assisted selection could be used in a breeding programme using markers Satt201 and Satt358 that were identified in the present study.

A study of the virulence of root-knot nematode eggs after treatment with various concentrations of Crop Guard™ (furfural)

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Various concentrations of Crop Guard™ (furfural) were screened for the control of *Meloidogyne*

javanica (root-knot nematode) eggs over 1, 6, 12 and 24-hour periods of exposure, using controlled application methods. Concentrations of 1000, 5000, 10 000, 15 000, 20 000, 30 000, 40 000 and 50 000 ppm of the product were tested. Treatments, including an untreated control, were replicated five times. Each replicate consisted of 10 handpicked egg sacs that were transferred to 2 ml Eppendorf tubes filled with the various Crop Guard™ concentrations. Six-week-old tomato seedlings of the cultivar Rodade were used as host plants for gall and egg sac indexing, which was determined after six weeks. Root cuttings were stained with phloxine B and each cutting was examined individually for galls and egg sacs. Electron microscope photographs showed perforations on the outer surfaces of treated egg sacs, which became more pronounced in the 5000 ppm treatment after longer exposure. Gall development and egg sac production were low in the 5000 ppm treatment after 12–24 hours exposure. In treatments of 10 000– 20 000 ppm larger openings started to form in the outer surfaces of younger egg sacs that were exposed for 6, 12 and 24 hours. Eggs were damaged effectively as reflected by a decrease in the number of galls on the treated plants' roots. Gall index was nil at all exposure times. A prolonged exposure time is recommended for the lower Crop Guard™ concentrations (1000–5000 ppm).

Abstracts of Posters

Occurrence of *Scutellonema bradys* on yam in Benin

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Scutellonema bradys is the major nematode species that affect yam (*Dioscorea* spp.) production and storage in Benin. A nematode survey was conducted during the storage periods of 2002 and 2003 in the yam-growing areas to study the distribution, population diversity and incidence of *S. bradys* on tubers marketed in Benin. The proportion of tubers infected with *S. bradys* varied between yam cultivars and species as well as the origin of tubers. Kokoro and *D. rotundata* were, respectively, the yam cultivar and species most frequently observed with symptoms of *S. bradys* damage. Higher nematode densities were found on *D. rotundata* than on *D. cayensis* and *D. alata.* Yoruba Dundu was most highly infected. Higher densities of *S. bradys* were recovered from tubers occurring in the southern Guinea Savannah agro-ecological zone compared to tubers from the northern Guinea Savannah. Molecular studies on nematodes extracted from yam tubers showed high levels of polymorphism. Three nematode isolates selected from geographically different localities showed different levels of pathogenicity on yam in the field and during storage.

Meloidogyne spp. and associated galling and damage on cassava in Kenya and Mozambique

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Using perineal patterns and female anterior measurements, five species of *Meloidogyne* were identified from cassava in Mozambique during a survey in 2003, namely *M. chitwoodi, M. exigua, M. incognita, M. javanica* and *M. naasi,* plus an unidentified species. In Kenya, *M. incognita* and *M. javanica* were identified from severely galled cassava plants from some of the 3710 clones in breeders' screening trials. Additional species were also observed but remained unidentified as they did not conform to the specifications of identified species or were too few for positive confirmation. *M. chitwoodi, M. exigua* and *M. naasi* are first records for Mozambique and for cassava, while *M. exigua* and *M. naasi* are first records for Africa. During the survey in Mozambique, observations on galling and other symptoms associated with

Meloidogyne spp. were also undertaken throughout the main cassava-growing provinces. Ten plants per field ($n = 202$) were evaluated for galling damage in terms of number of galls per 50 cm feeder roots per plant. *Meloidogyne* spp. were identified by collecting and 10 females per sample from galled root material were preserved in lactophenol. *M. incognita* was identified more often than other *Meloidogyne* spp., followed by *M. chitwoodi.* The severity of *Meloidogyne* spp. galling, expressed as number of galls per root, was generally slight but galling was observed in the majority of fields across the country. Some fields recorded moderate to severe galling. Severe damage to some of the breeding lines in Kenya provides evidence of resistance against *Meloidogyne* spp. in cassava and possible genotype × *Meloidogyne* spp. interaction. Observation of an unidentified *Meloidogyne* sp., as well as the newly recorded species, provides additional justification for a methodical examination and documentation of *Meloidogyne* spp. occurring on key crops in Africa, towards identification and development of resistant cultivars.

Effect of lectins on migratory and sedentary endoparasitic nematodes

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A complex of diseases and pests, including sedentary and migratory endoparasitic nematodes, threatens world crop production. Control of parasitic nematodes by transgenic plants could offer substantial benefits. Several classes of potential anti-nematode genes have been identified that can be used against nematodes as defence proteins such as plant lectins. In this study, *in vitro* assays were used to test two possible effects of plant lectins on *Radopholus similis, Pratylenchus coffeae* and *Meloidogyne incognita.* The effects of lectin ingestion were tested on transformed *Arabidopsis thaliana* plants expressing lectins from *Galanthus nivalis* (GNA) and *Epipactis helleborine* (EPA), as well as a ribosome-inactivating protein from an *Iris* sp. (IRIP). Experiments showed that IRIP-expressing *A. thaliana* could confer resistance to *R. similis.* Reproduction of *R. similis, P. coffeae* and *M. incognita* on GNA-expressing *A. thaliana* also appeared to be reduced. Binding experiments with lectins of *Calystegia sepium* (Calsepa), *Robinia pseudocacia* (RPA), *Triticum aestivum* (WGA) and *Arum maculatum* (AMA) showed that Calsepa, RPA, WGA and AMA might have a paralysing effect on *M. incognita,* but not on *R. similis* and *P. coffeae.* RPA, WGA and AMA also displayed some adverse effects on the hatching of *R. similis.* In chemotaxis assays, RPA and AMA appeared to affect the chemotactic behaviour of *P. coffeae.* The results need to be confirmed.

Differentiation of potato cyst nematode populations from South Africa by analysis of the rDNA Internal Transcribed Spacer

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A total of eight potato cyst nematode (PCN) populations from two different potato-producing regions in South Africa were analysed by means of rDNA-RFLP. The size of the PCR amplification products for all populations was typical for the genus *Globodera.* Restriction digestion of the amplified products with *MspI* and *HinfI* confirmed *G. rostochiensis* to be present in both the Sandveld and Ceres regions. However, several populations of the Sandveld region show no recognition for the *HinfI* restriction site and no digestion took place. Sequencing of the PCR products indicated the possibility of a new *Globodera* species.

Introduction and possible spread of *Aphelenchoides arachidis* in South Africa

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Aphelenchoides arachidis, the groundnut testa nematode, was described from northern Nigeria on groundnut. It is a facultative endoparasite and infests tissues of pods, testae, roots and hypocotyls, but not cotyledons, embryos and other parts of the groundnut plant. It was not known to be a pest of

groundnut outside of Nigeria until May 2003 when large numbers of *A. arachidis* were found in groundnut from the Vaalharts area, South Africa. D Hunt from CABI in England confirmed the identification. The present study involves a thorough morphological study of the South African specimens of *A. arachidis,* a comparison of local specimens with type-specimens from Nigeria, and listing of *A. arachidis* in South Africa as a quarantine nematode in terms of groundnut export. Unless appropriate precautions are taken, *A. arachidis* may become a serious pest worldwide, especially since it can be disseminated by infested seed. Of special interest is the possible existence of two biotypes of *A. arachidis,* one that occurs on cereals and the other on both cereals and groundnut. Therefore an ongoing survey of *A. arachidis* is envisaged, especially in areas of South Africa where groundnut and cereals are produced in close proximity.

Introgression of root-knot nematode resistance into local maize genotypes

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Local maize production is increasingly being hampered by infection by economically important root-knot nematodes (*Meloidogyne* spp.). Since chemical control of nematodes on this crop is seldom cost-effective and current crop rotation practices favour build-up of root-knot nematode populations, the demand for identification of resistant maize germplasm is increasing. Two foreign maize lines with resistance to *M. javanica* have been identified (RF-values < 1) in a greenhouse trial. The resistance of these two lines, together with the F_1 progeny resulting from crosses between the resistant and susceptible parents, was subsequently verified in a field trial where a mixed population of *M. javanica* and *M. incognita* had been artificially established. Subsequently, six F_2 populations resulting from these crosses were identified for resistance to this mixed root-knot nematode population. Root-knot nematode numbers were generally low in the majority of F_2 plants from population one and ranged between 0 and 113 17 per 50 g roots. Fifty-one per cent of these F_2 plants maintained between 0 and 100 root-knot nematodes individuals per 50 g roots. Although root-knot nematode numbers from plants from the other four F_2 populations were also generally low, the majority of these plants maintained more than 100 root-knot nematode individuals per 50 g roots. A search for molecular markers associated with the resistance trait will be conducted to facilitate marker-assisted selection and expedite the breeding process of root-knot nematode-resistant commercial maize hybrids and varieties.

Screening of groundnut accessions for resistance to the groundnut pod nematode

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The seedborne groundnut pod nematode, *Ditylenchus africanus,* is omnipresent in groundnut-producing areas of South Africa and is one of the economically most important pests of the crop. Resistant genotypes could play an important role in sufficiently reducing *D. africanus* populations. Various screenings have been done on local germplasm, selected elite breeding lines, as well as hybrids. Eighteen new accessions have been screened against this parasite in microplots since the 2001 growing season. The groundnut cultivars Sellie and Kwarts were used as susceptible and tolerant standards, respectively. Each seed was artificially inoculated with c. 3000 *D. africanus* individuals at planting. During the growing seasons of 2001/2002 and 2002/2003 none of these accessions maintained significantly lower *D. africanus* numbers in hulls and seeds than the susceptible cultivar Sellie. The tolerant cultivar Kwarts also did not differ significantly from the susceptible standard with regard to total number of nematodes in pods. During the 2003/2004 season, however, significantly lower *D. africanus* numbers were observed in seeds and hulls of PC 254 than in both standards. PC 287 also showed potential for maintaining significantly lower *D. africanus* numbers compared to the susceptible cultivar Sellie. PC 254, however, maintained the lowest numbers and has the additional benefit of containing high oleic acid levels, which is favourable for better shelf-life. These sources of resistance are currently being incorporated into the breeding programme, whereafter identification of molecular markers associated with the resistance trait will be undertaken. Marker-assisted selection will subsequently be used to expedite the breeding process.

Effect of organic amendments in integrated crop production of vegetables in rural farming

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A survey conducted in rural areas in the eastern and northern parts of South Africa revealed serious problems with nematodes, especially *Meloidogyne* spp. Nematodes were found in high numbers in vegetable gardens, e.g. up to 64 000 and 25 000 *Meloidogyne* larvae per 30 g roots of tomato and pepper, respectively. Several gardens have been abandoned because of this. As these communities do not have sufficient financial resources to purchase nematicides, other practices should be developed to alleviate to the problem and allow the people to produce acceptable yields. To determine the effect of organic amendments on production, a trial was conducted at Nelspruit with tomato, beetroot and spinach. Treatments included cattle manure, chicken manure, compost, permaculture, clear plastic cover, an untreated control and fenamiphos as a nematode-free control. Yield data showed that permaculture produced the best results for both spinach and beetroot. For beetroot this treatment provided the highest mass of leaves and tubers, as well as number of tubers recovered. Chicken manure gave the second-best results on beetroot. More tubers were harvested at the first harvesting date but as the trial continued the number of tubers harvested declined, contrary to the permaculture treatment where the number of tubers first increased and subsequently stabilised. The same tendency was observed for spinach. With tomato, chicken manure gave the best results, followed by cattle manure. For this crop the permaculture treatment did not perform well. Indications are that several organic amendments can be used successfully in an integrated crop production system, but different crops could require different treatments.

Using a Pest Risk Analysis model to regulate introduction and spread of nematodes of economic importance

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As a member of the World Trade Organization (WTO), South Africa follows certain principles, disciplines, procedures and guidelines as set out in the Agreement on the Application of Sanitary and Phytosanitary Measures and the International Standards on Phytosanitary Measures of the International Plant Protection Convention (IPPC). The IPPC was adopted by the Food and Agriculture Organization of the United Nations in 1951 and is acknowledged by the WTO as the body setting international standards for phytosanitary issues. South Africa, therefore, has the sovereign right to protect its export and import status and credibility by protecting human, animal and plant health through taking the necessary sanitary and phytosanitary measures. Personnel of the National Plant Protection Organisation of South Africa, Directorate Plant Health, have drafted a model for assessing the phytosanitary risks associated with importing plant material into South Africa. The model sets out procedures for scientific analysis of the potential dangers posed by organisms that may accompany imports of fresh plant commodities such as fruit, vegetables and grain, seed and other propagating material. We illustrate the use of this procedure when establishing the potential risk involved for nematode introduction and spread when importing host material into the country.

Plant-parasitic nematodes in Ethiopia

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Agriculture constitutes the livelihood of 85–90 % of the inhabitants of Ethiopia. Many different crops are grown but many problems are experienced with the cultivation of these crops, which include poor agricultural practices, the non-use of pesticides and the incidence of plant pests and diseases. Sporadic surveys over the past 20 years indicated that several plant-parasitic nematode genera and species are associated with many crops, but very little is known about nematode prevalence, spread or species diversity. A survey was done in 2002 during the June–September cropping season to reduce this knowledge gap. Two-hundred samples were collected from different parts throughout the southwestern regions of the country. The nematodes were extracted and fixed at the National

Plant Protection Centre in Ambo, Ethiopia, and sent to the ARC-Plant Protection Research Institute for species identification. Thirty-five different species were identified. Specimens that could belong to unknown species were also found. Taxonomic studies on the nematode fauna of Ethiopia are almost non-existent. Some species known to occur in neighbouring countries were recorded in Ethiopia for the first time. Two relatively scarce nematodes are illustrated in SEM photographs. *Paratylenchus pandatus* was found for the first time after its original description. Taxonomic studies are underway on some of the material and a new *Helicotylenchus* sp. is being described.

Radopholus similis associated with the giant swamp taro on the island of Yap

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A corm rot disease caused by nematodes gradually became a serious threat to the production of giant swamp taro (*Cyrtosperma merkusii*) on the island of Yap. Although the association of *Radopholus similis* with infected corms was known, it is only now that a detailed investigation is being carried out of biology of the nematode in such a unique, anaerobic swamp environment. Small pieces of infected corm were sent to the ARC-Plant Protection Research Institute in Pretoria for further processing and identification. Morphological studies confirmed the nematode species to be *R. similis.* Plants seldom show aboveground symptoms. *C. merkusi* is a perennial crop and the disease becomes more severe with age. The corm rot is wet, brown with a loose mass of dead tissue hosting thousands of nematodes. By the time corms are harvested from the third year onward there are cavities of dead tissue visible, with an unpleasant odour. Deep brown necrotic centres develop in the corms and the cavities advance towards the edible portion of the corm, leaving a perforated exterior that greatly reduces corm quality. Further investigations are underway to establish the life-cycle and spread of *R. similis* in this environment. Large actinolaimid nematodes were also found in the corm and their role will be investigated.

Inhibitory effects of culture filtrates of endophytic *Fusarium oxysporum* isolates on motile stages of the banana nematode, *Radopholus similis*

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The burrowing nematode, *Radopholus similis,* is one of the most damaging pests of *Mus*a spp. worldwide. Effective control is mainly achieved by the use of nematicides although costs for subsistence farmers and adverse environmental effects are matters of concern. A promising alternative to nematicides is the use of mutualistic endophytic fungi. Some endophytic strains have been demonstrated to possess potential as biological control agents, protecting their host plant against pests and diseases. Currently, research at the IITA focuses on the isolation and screening of endophytic fungi from banana plant tissue to identify potential isolates for nematode control. Culture filtrates (CF) of nine endophytic *Fusarium oxysporum* isolates from Uganda were tested for their effect on the mobility and mortality of *R. similis* females, males and juveniles. Nematodes were added to 100 % CF for 3, 6 and 24 hours and mobility assessed on each occasion. Mortality rates were determined after 24 hours. Two isolates were tested further at six CF concentrations (2.5, 5, 10, 25, 50 and 100 % v/v). Undiluted CFs of all isolates immobilised 39.4–100 % of the nematodes. The effect on nematode mobility increased with longer exposure periods and with higher CF concentrations. More males were immobilised than females. For all isolates tested, 76.4–100 % mortality rates were observed in the undiluted CFs. Nematode mortality increased with higher CF concentrations. Results demonstrated the production of metabolites by *F. oxysporum* endophytes that are toxic to *R. similis* and the potential of endophytic *F. oxysporum* isolates as commercial biological control agents.