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Plenary Address

Resistance genes for nematode management

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Molecular biology techniques are rapidly becoming essential tools in nematology, but their impact is felt the strongest in the search for resistance genes. Natural and synthetic resistance are the most environmentally-friendly and cost-effective means of reducing yield losses in agriculture, hence the emphasis on molecular-assisted breeding of natural resistance genes and engineering of synthetic resistance genes (Jung et al. 1998).

Molecular markers are now essential tools to map and clone natural resistance genes. Once cloned, these genes can be transferred to other crops using genetic engineering techniques. Synthetic resistance genes are genes involved in the biochemical signals that plants and nematodes exchange to establish or maintain their host–parasite relationship. As we unravel the genetics of the signalling process we find novel targets for manipulation (Williamson & Hussey 1996; Gheysen 1998). Plant genes activated by the nematode can be manipulated and transferred back into the plants. The expectation is that transgenic plants expressing disabled signal proteins will not sustain parasitic infection, i.e. the nematode will induce a hypersensitive reaction (apoptosis) instead of initiating a parasitic relationship. Nematode secretions (signals) can also be characterised and used to find the plant proteins they interact with. Nematode parasitism genes can be isolated by genetic analyses of virulent and avirulent populations. These genes could also indicate the plant proteins that nematodes interact with. Proteins toxic to nematodes or to plant cells can be expressed in transgenic plants. Delivery of the toxic protein to the relevant tissue is crucial to this strategy. This explains the emphasis on promoters that will procure expression only in giant cells or syncytia, or cells wounded by nematodes or other parasites.

Most plants have resistance genes

Most plants have defence processes that most nematodes are not able to breach, i.e. most plants are immune to most nematodes, and therefore non-hosts. These plants constitute a vast reservoir of potentially useful genes. Since these genes can now be cloned and transferred to other crops, we

have access to a virtually unlimited pool of genes to develop effective and durable resistance against nematodes. To transfer a natural resistance gene by genetic engineering, it must first be localised (mapped) on the plant chromosome and its sequence must be determined (Williamson 1998). DNA fragments (markers) that highlight the position of resistance genes can be found on the chromosome. The next step is to make a cDNA library (a collection of random DNA fragments where all genes are represented) and identify where the markers are on these fragments. The more markers that are identified, the closer some of them are to the gene and the shorter the fragment of chromosome that needs to be sequenced.

Two resistance genes against root-knot nematodes and the sugar beet cyst nematode have been mapped and transferred to sugar beet and tomato. The gene from sugar beet has been traced to a related wild species, *Beta procumbens.* These wild beets are not closely related to cultivated species and their chromosomes do not pair properly, so recombination mapping could not be used. Instead, a marker that always hybridised with the DNA of wild beets and the resistant hybrids of cultivated sugar beet was used to map the resistance gene. A collection of hybrid beets included plants carrying the resistance gene on small pieces of chromosomes from the wild beets. The DNA marker was used as a probe to identify the resistant plant with the smallest chromosome segment from the wild species. The cloned gene, *Hs1*$^{pro-1}$, was transferred to susceptible sugar beet and conferred resistance against the sugar beet cyst nematode (Cai et al. 1997). The gene *Mi* was isolated from tomato, *Lycopersicon esculentum* (Milligan et al. 1998). This gene has been used commercially in tomato since it was introgressed from a wild relative *L. peruvianum.* Large breeding experiments were necessary to identify the presence of markers close to the gene because of a lack of natural recombination in this area of the chromosome. Two genes, *Mi*-1.1 and *Mi*-1.2 were found, but only *Mi*-1.2 conferred resistance to a previously root-knot nematode-susceptible tomato cultivar (Kaloshian et al. 1998; Milligan et al. 1998; Williamson, 1998). There are a number of other candidate genes for resistance against both species of potato cyst nematode, the cereal cyst nematode and various races of the soybean cyst nematode. Several genes against root-knot nematodes exist in tobacco, tomato, soybean, sweet potato, potato, peanut and peach rootstocks, that are being localised. It is not clear at the time of this review whether some or all of them will be cloned and transferred to other crops.

Plant genes that can be turned into resistance genes

Plant genes that are specifically activated in nematode feeding cells can be isolated. When these nematode-activated genes are identified, synthetic resistance genes can be designed to disable their function (antisense strategies). Their promoters can be used to express toxic proteins to abort the formation of feeding cells (Fenoll et al. 1997). Genes expressed in nematode feeding cells, or the regulatory regions (promoters) that control these genes, have been isolated. Wilson et al. (1994) cloned cDNAs from 150 mature giant cells dissected from tomato roots infected with root-knot nematode. The analysis of this gene library is providing clues about the kind of plant genes that are expressed in giant cells (Bird 1996). Genes expressed in giant cells can be isolated through other means. The promoters of these genes are isolated by transforming plants with constructs to express a reporter gene but lacking a promoter. These experiments are yielding transgenic plants showing reporter gene expression in syncytia or giant cells. This occurs only when the construct has integrated behind the promoter of a gene that is active in these giant cells (Barthels et al. 1997; Fenoll et al. 1997). The promoter of a tobacco aquaporin gene (*tobRB7*) contains a sequence specifically triggered by root-knot nematodes. Opperman et al. (1994) made a deletion series at the 5' end of the tobacco RB7 promoter and they used the truncated promoters to drive a reporter gene (GUS) in tobacco. Transgenic tobacco plants transformed with the shortest promoter sequence (300 bp) and infected with root-knot nematodes showed GUS expression only in the giant cells and nowhere else in the roots. This nematode-specific promoter was used to direct the expression of barnase (a toxic RNase) to abort the initiation of giant cells in tobacco. Although the plants were resistant and nematodes did not develop, the truncated promoter was not exclusively giant cell-specific. Cotton plants transformed to express an attenuated barnase or independently transformed with a full-length cDNA antisense construct of the cotton homologue of *tobRB7* to interfere with the normal function of

giant cells, had a low level of resistance to *Meloidogyne incognita* but no resistance to *Rotylenchulus reniformis* (Robinson et al. 1998).

Since finding a promoter that will function in nematode giant cells is essential, other promoters that are highly active in roots have been tested. The 35S promoter – a strong constitutive promoter from the cauliflower mosaic virus – delivers effective levels of gene expression (Goverse et al. 1998; Bertioli et al. 1999). In tomato plants, the promoter of a defence-related gene (hydroxymethylglutaryl CoA reductase), triggered by fungal and bacterial pathogens, drives strong GUS expression in root-knot nematode giant cells (Cramer et al. 1993). The promoter of a haemoglobin gene from a nitrogen-fixing, non-leguminous plant can also be utilised (Ehsanpour & Jones 1996). A wound-inducible promoter, *wun1* from potato, is activated in cells penetrated by cyst nematodes, but not by root-knot nematodes (Hansen et al. 1996). Another wound-inducible promoter from asparagus can be used for expression in traumatised cells (Warner et al. 1993).

Nematode genes essential to parasitism can be suppressed

As with other parasites and pathogens, biochemical signals are exchanged between nematodes and plants. Identifying nematode proteins required for the interaction provides other ways to interfere with the signals and abort the formation of feeding cells. If giant cells are formed when the juvenile nematodes release oesophageal gland secretions through their stylet into plant tissue, then the nematode genes that produce oesophageal proteins are potential targets to interfere with the nematode–plant interaction (Williamson & Hussey 1996; Gheysen 1998). Collaborative research between groups in Europe and the United States has identified oesophageal gland proteins from root-knot and cyst nematodes. The nematode's oesophageal secretions are collected and injected into mice to make monoclonal antibodies. These antibodies are then screened to identify those that bind to nematode secretory protein (Davis et al. 1994; Goverse et al. 1994; De Boer et al. 1996). One of the proteins injected by nematodes into plant cells is a cellulase (Ding et al. 1998; Smant et al. 1998; Rosso et al. 1999; Wang et al. 1999). The expression of at least one of these plantibodies in a transgenic plant should abort the formation of nematode feeding cells by binding to and altering the function of key proteins of the nematode oesophageal secretions. Several mouse antibody genes expressed in plants (plantibodies) as monoclonal antibodies or single-chain antibodies have not provided effective resistance (Baum et al. 1996; Rosso et al. 1996; Stiekema et al. 1997).

As with other pests and pathogens, specific gene products must be involved in the specificity parasitic nematodes display towards particular plants. When these genes are identified and cloned, new opportunities will arise to interfere with the host–parasite interactions. A genetic analysis of soybean cyst nematode parasitism on resistant and susceptible soybeans has been started with highly inbred (homozygous) lines of the cyst nematode (Dong & Opperman 1997). Several nematode genes control reproductive ability on resistant soybean cultivars and RAPD and AFLP markers linked to parasitism loci have been identified. The parasitism genes will be engineered into avirulent nematode lines to confirm their function, using transformation techniques adapted from *Caenorhabditis elegans* (Opperman & Bird 1998).

Proteins toxic to nematodes

Proteinase inhibitors

We expect that proteinase inhibitors in the diet of plant-parasitic nematodes bind to digestive proteinases in the gut and prevent protein hydrolysis and absorption of amino acids. The nematodes probably excrete undigested proteins along with their own digestive proteinases, resulting in a net loss of protein (Ryan 1990; Vrain 1999). The first proteinase inhibitor was engineered from cowpea to tobacco and it successfully controlled lepidopteran insect pests of tobacco (Hilder et al. 1987). Transgenic potato plants expressing this inhibitor did not support normal development of *M. incognita* or the reproduction of *Globodera pallida* (Hepher & Atkinson 1992). Michaud et al. (1996) found major cysteine proteinase activity in three species of root-knot nematodes. A cysteine proteinase inhibitor from rice, oryzacystatin I (OC-I), completely inhibited the proteolytic activity of all stages of *M. hapla.* The tighter the enzyme-inhibitor complex the more effective the inhibitor, but OC-I did not bind with high affinity to the proteinases of *M. incognita* and *M. javanica.* Potato and soybean cyst nematodes possess intestinal

cysteine proteinases that are, like those of root-knot nematodes, sensitive to OC-I (Koritsas & Atkinson 1994; Lilley et al. 1996). The proteinase inhibitor gene was modified by site-directed mutagenesis to make it bind with increased avidity to the proteinases of the potato cyst nematode (Urwin et al. 1995). Root-knot and sugar beet cyst nematodes did not develop normally in transgenic *Arabidopsis* roots expressing the wild rice inhibitor OC-I or the variant protein (Urwin et al. 1997).

Bacillus thuringiensis (Bt) *sporulation proteins*

When Bt proteins on plant parts are ingested by insects, they dissolve in the insects' mid-gut and are processed by digestive proteases into smaller polypeptides that bind to receptors and disrupt the insects' mid-gut membranes (Vadlamudi et al. 1995). Many strains of Bt that are not toxic to insects must rely on other hosts to multiply and to disseminate. Several Bt strains produce polypeptides that kill nematodes feeding on bacteria (Feitelson et al. 1992; Borgonie et al. 1996; Mena et al. 1997). However, the molecular structure and physiology of the intestine of plant-parasitic nematodes are not known and it is impossible to predict if their membranes contain receptors for these bacterial proteins.

Lectins

Lectins accumulate in large quantities in many seeds and in other storage organs of plants. We do not know what role lectin proteins have in the physiology of plants. However, since most lectins are toxic to animals, including insects and humans, we assume that lectins may act as defence proteins (Chrispeel & Raikhel 1991; Peumans & Van Damme 1995). A mannose-binding lectin engineered in various crops is toxic to aphids, plant hoppers and several nematodes, including root-knot, root lesion and potato cyst nematodes (Boulter et al. 1990; Anwar & McKenry 1998; Burrows et al. 1998).

Future prospects

Effective resistance against plant-parasitic nematodes is uncommon in many crops. Yet crop resistance is the cheapest and most environmentally-friendly way to reduce losses caused by nematodes. So the future of management strategies based on natural and synthetic resistance genes seems assured. This review outlined several natural and synthetic resistance genes that may soon be available to manage nematodes. Thanks to genomics and the elucidation of entire sequence of *C. elegans,* novel nematode and plant genes and their encoded protein products will be identified (Baillie & Bird 1999). They will present opportunities to interfere with the establishment and maintenance of host–parasite relationships. Can we think of a day when our crops will be immunised against plant-parasitic nematodes? The current reality is that we are still several years away from field-tested, effective, transgenic resistance against nematodes in any crop.

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Abstracts of Papers

PCR-based identification of root-knot nematodes

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Root-knot nematodes parasitise a large number of crops worldwide. They cause serious yield losses and/or external galls and internal spots that render products unmarketable. *Meloidogyne chitwoodi, M. fallax* and *M. hapla* cause numerous problems in European agricultural crops, whereas species such as *M. incognita, M. javanica* and *M. arenaria* are damaging crops in (sub)tropical areas or in greenhouses. In 1998 *M. chitwoodi* and *M. fallax* were declared quarantine organisms, emphasising the seriousness of the problems these nematodes cause. Methods that enable the detection and identification of these nematodes, as well as the determination of their species composition in mixtures are desirable and necessary. They form the basis for proper research into resistance and virulence, resistance management and reliable quarantine tests. Most methods of nematode diagnostics have some limitations. Species identification based on differences in morphological characteristics requires much skill and are often inconclusive for individuals. Isozyme analysis is a relatively quick way to identify species of the genus *Meloidogyne.* However, for reliable results, the isozyme analysis can only be carried out on females of a specific developmental stage. Therefore, DNA-based identification tests have been developed because they do not rely on the expressed products of the genome and are independent of environmental influence or developmental stage. The species *M. hapla, M. chitwoodi, M. fallax,* as well as the group of species *M. incognita, M. arenaria* and *M. javanica,* can be identified or distinguished by RAPD-PCR, ITS-RFLPs and by a multiplex PCR assay using four rDNA primers derived from ITS-sequences. The last two techniques can be applied to single juveniles and enable the estimation of species composition in mixtures. They can detect species present in mixtures in proportions as low as 5 %. More recently, sets of species-specific SCAR (sequence characterised amplified regions) primers have been developed for the identification of *M. chitwoodi, M. fallax* and *M. hapla* as well as

for *M. incognita, M. javanica* and *M. arenaria.* Additionally, a multiplex PCR assay was developed in which six primers are used, enabling the identification and differentiation of *M. chitwoodi, M. fallax* and *M. hapla* in mixtures. The sets of species-specific SCAR primers are very sensitive and enable the detection of as little as 0.001 juvenile in a PCR reaction.

ITS-PCR sequence-based identification of *Meloidogyne chitwoodi* and screening of crops for host suitability to the Mooi River population

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Meloidogyne chitwoodi is an important nematode pest of potatoes. It has a wide host range and is pathogenic to many crops, including both mono- and dicotyledons. The identity of the species in South Africa was confirmed by means of a multiplex internal transcribed spacer-polymerase chain reaction (ITS-PCR)-based approach. DNA fragments containing parts of the ITS-regions and extracted from second stage juveniles were amplified by PCR using species-specific ITS sequence-derived primers. Amplified as well as digested fragments were loaded on an agarose gel, separated by electrophoresis and detected with ethidium bromide staining. Several crops were screened in the greenhouse (19 °C/26 °C night/day temperature regime and a 14 hour photoperiod) to a Mooi River population of *M. chitwoodi.* A randomised complete block design with six replicates was used and each seedling was artificially inoculated with 10 000 nematodes. Egg mass and egg counts were done 56 days after inoculation. *Brassica rapa, Eragrostis tef* and *Lolium multiflorum* proved to be good hosts, while *Eragrostis curvula* and *Zea mays* were poor hosts to this root-knot nematode species.

Effects of *Tylenchulus semipenetrans* on leaf osmotic potential, CO_2 assimilation and whole-plant-transpiration of *Citrus aurantium* seedlings under greenhouse conditions

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Tylenchulus semipenetrans infection produces characteristic changes in the partitioning of osmoticum ions in citrus. However, effects of *T. semipenetrans* on other physiological aspects of citrus remain undocumented. The objectives of this study were to evaluate the effects of *T. semipenetrans* on leaf osmotic potential (Lπ), CO_2 assimilation (CO_2A) and whole-plant-transpiration (WPT) of citrus seedlings over time. Fourteen six-month-old *Citrus aurantium* seedlings were inoculated with a total of 0 and 210 000 juveniles 12 weeks after potting in 20-cm-diameter clay pots. The experimental units were arranged in randomised complete block design, with each treatment replicated seven times, at ambient temperatures averaging 28 °C during the day and 24 °C at night. At harvest, 140 days after inoculation, females, juveniles and eggs per gram fresh roots averaged 518, 471 and 8117, respectively. The Lπ of infected seedlings was reduced by 37, 55, 89 and 82 % on day 56, 70, 98 and 140 after inoculation, respectively. CO_2A of infected seedlings was increased by 29, 41 and 28 % on day 56, 70 and 98, respectively. However, effects of infection on relative WPT were inconsistent. Infection decreased (18 %) and increased (16 %) WPT by days 56 and 98, respectively, whereas no effects were observed on days 70 and 140. The decrease in Lπ and increase in CO_2A were consistent with previous observations where infected plants consistently increased accumulation of osmoticum ions and carbohydrates.

SEM observations on the life cycle of the citrus nematode, *Tylenchulus semipenetrans* Cobb, 1913, on citrus roots

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The citrus nematode is an obligate plant parasite and apart from *Citrus* species it has only a few other host plants such as grapevine and olive. Young females feed on the outside of the rootlets for a while and then burrow into the feeder roots. The head region is deep inside the root cortex and with the rear end of the body protruding from the root surface. The posterior enlarges as the reproductive organs develop. Females normally occur in groups together on the root and are covered by a gelatinous layer into which the eggs are deposited. The juveniles hatch from the eggs and move freely in the soil. Nematode-trapping fungi were present

on all the citrus roots that were studied but especially in the vicinity of mature females with eggs. Two species of predacious fungi were found, namely *Arthrobotrys dactyloides* and *Dactylella ellipsospora. A. dactyloides* forms trapping rings and *D. ellipsospora* produces adhesion knobs borne on short stalks from the hyphae. A large number of rings and knobs with trapped juveniles and adults were observed. The presence of these fungi represent natural biological control of hatching nematode juveniles and adults on citrus roots.

Relative importance of nematodes, weevils and leaf diseases as *Musa* production constraints in central Uganda

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In 1996 a survey was conducted in central Uganda to establish nematode species profiles and related damage, banana weevil damage and leaf disease severity on East African highland banana (*Musa* AAA, Matooke group) and the more recently introduced cultivar Pisang Awak (*Musa* ABB). Stepwise multiple regression for this region revealed that the biotic production constraints of nematodes, weevils and leaf diseases contributed 22 % of the variation observed in bunch mass and 34 % for plant volume of highland banana, while 15 % variability in bunch mass and 19 % in plant volume for Pisang Awak. *Radopholus similis* and *Pratylenchus goodeyi* densities were the major biotic factors contributing to the variation in bunch mass and plant volume. Banana weevil (*Cosmopolites sordidus*) damage affected bunch mass but no significant effect of leaf diseases was evident. Banana streak virus symptoms were not observed, possibly due to the localised distribution. *Fusarium* wilt symptoms were also absent, possibly as a result of the sampling criterion of plants ready to harvest. *R. similis* may be associated with an overall bunch mass loss of 20 % for highland banana and 17 % for Pisang Awak country-wide. In addition, the associated reduction in plant cover may have severe implications. The greater area of exposed soil increases soil temperature, favouring the more aggressive nematode species such as *R. similis* and *P. coffeae,* and increases the risk of soil erosion and nutrient leaching following rainfall.

Role of the ring nematode (*Mesocriconema xenoplax*) in plum tree death

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High numbers of ring nematode (*Mesocriconema xenoplax*) were found in 93 % (n = 72) of plum tree orchards suffering from branch die-back and tree death. The main cause of these conditions is believed to be stem canker fungi and bacterial canker. Ring nematode is known to predispose fruit trees to these pathogens. In order to increase our knowledge of this nematode, the vertical and horizontal variation in its numbers was determined quarterly. There were significant differences in ring nematode numbers in samples from different seasons and between 0.8 and 2 m sampling distances from the trees. Numbers were never lower than 547 nematodes 250 cm^{-3} soil, which exceeds the threshold value of 300 250 cm^{-3}. Sampling at 20 cm depth intervals showed ring nematode numbers to decrease below 60 cm. However, even at 80–100 cm nematode numbers varied between 285 and 566 250 cm^{-3} soil, with the lowest numbers recorded during January (summer) and the highest during October (spring). These results indicate that ring nematodes can occur throughout an entire four-year-old plum tree orchard, to a depth of at least 1 m. This has serious implications for nematode control, as most available nematicides do not penetrate deeper than 30–40 cm into the soil.

The effect of ethylene dibromide on groundnut quality when applied at planting for control of *Ditylenchus africanus*

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Groundnut kernel quality may be affected by various abiotic and biotic factors, but *Ditylenchus africanus* is regarded as the most significant cause of groundnut downgrading in South Africa. A steady decline in national groundnut production suggests that control strategies presently recommended are inconsistent, ineffective or not cost-effective. The objective of this study was to evaluate reduced in-row applications of ethylene

dibromide (EDB) at planting for efficacy and economy on groundnut infected with *D. africanus.* Using three field trials and a microplot experiment, a range of reduced dosage rates, applied in the furrow at planting, was compared to conventional application before planting. In one field trial a range of EDB dosage rates was applied conventionally before planting. Regression analyses of nematode numbers, kernel yield, kernel quality and income parameters with EDB dosage rate as independent factor were carried out. Dosage rates of 10–15 l ha^{-1} proved to be effective and compared favourably with conventional application rates. Choice-grade kernels were obtained at relatively low input cost and no symptoms of phytotoxicity were observed. Adoption of this effective and economical control strategy for *D. africanus* may help restore groundnut production in South Africa to previous levels.

Evaluation of cadusafos G and EC formulations under drip and micro-sprinkler irrigation systems in South African citrus orchards

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As a result of a shortage of irrigation water in South Africa, many citrus growers have adopted drip irrigation in their orchards. Although both fenamiphos and ethoprophos are registered as EC formulations, the manufacturer of cadusafos requested that an EC formulation be compared with the existing granular formulation. This was conducted in four orchards, two of which were under micro-irrigation and two under drip-irrigation. The granular formulation of cadusafos performed slightly better than the EC formulation but the latter outperformed both the ethoprophos and aldicarb treatments with regard to nematode control.

Leaching of root-knot nematode eggs and larvae in sandy soils

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More than double the average rainfall in maize-producing areas during the 1996/97 planting season resulted in a decline in root-knot nematode counts from maize root samples. Since flooding is a well-known control method in water-abundant countries, excessive rainfall could result in leaching of root-knot nematode eggs and larvae. To test the hypothesis, a microplot rainfall-simulation trial with simulated rip-on-row cultivation to a depth of 70 cm (similar to maize field trials), was conducted. Eggs and larvae were inoculated at a depth of 10 cm prior to planting. Plots irrigated with 600 mm water (long-term annual rainfall) had an infestation level of 5906 eggs and larvae 50 g^{-1} roots at flowering. Plots irrigated with 1100 mm water, similar to that recorded during the 1996/97 season, resulted in average counts of 891 eggs and larvae 50 g^{-1} roots, indicating that eggs and larvae were reduced by leaching. In plots irrigated with 1600 mm water (1000 mm more than the average) the infestation 50 g^{-1} roots were 1274 eggs and larvae. This could be ascribed to saturation of the soil in microplots, thus limiting leaching.

Early root and nematode development in *Musa* as a function of time

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Three experiments were conducted to determine the effect of time on root development and nematode reproduction. In the first experiment in vitro propagated banana plantlets were planted in the greenhouse, inoculated with 0 or 1000 *Radopholus similis* after four weeks and harvested at weekly intervals. Root development started after a lag phase of four weeks and showed a large increase around week 7. Nematode reproduction increased with time but only low numbers of nematodes survived after inoculation. In the second experiment the plantlets were inoculated four or eight weeks after planting to determine the best period for inoculation. Nematode reproduction increased as a function of time, but there was no difference between the treatments. However, compared to the previous experiment, higher nematode populations were found. In the third experiment the effect of light and temperature on root and nematode development was studied for one year. During periods of low sunshine, root development decreased and, as a consequence, also nematode reproduction. High temperatures also reduced nematode reproduction. Both root development and nematode reproduction were a function of time and were affected by these environmental factors.

A new *Aphelenchoides* sp. causing 'black leaf streak' on *Ensete ventricosum* in Ethiopia

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During 1991 a foliar disease called 'black leaf streak', was observed on *Ensete ventricosum* in Ethiopia. A J Quimio identified an *Aphelenchoides* sp. as the cause of the disease. 'Black leaf streak' usually occurs on leaf margins and near the bases of newly-expanded leaves. On very young seedlings the black streaks may cover the entire leaf blade. Several streaks often fuse to form large necrotic spots in which the black streaks can still be seen. During 1998 the Nematology Section at PPRI received *Ensete ventricosum* leaves with 'black leaf streak' from Ethiopia for extraction and subsequent identification of the *Aphelenchoides* sp. A SEM study revealed the presence of nematodes and bacteria in close association. Nematodes and bacteria were observed in cavities in the leaf parenchyma tissue as well as on the leaf surface. The bacterium was provisionally identified as *Xanthomonas campestris* pv. *strelitzia* and the *Aphelenchoides* sp. is being described. The nature of the association between the nematode and the bacterium needs to be determined.

The effect of root-knot nematodes on plant growth and tuber formation of cassava at different stages of development

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Young cassava plants of cultivar SS4 were inoculated with 1000 juveniles of *Meloidogyne incognita* at 1, 14, 40, 70, 88 and 127 days after planting. At 150 days after planting plants were uprooted. The tuber number and total tuber mass of plants aged 14, 40, 70 and 88 days at inoculation were significantly ($P < 0.05$) reduced compared to the uninoculated plants. Plant height was not affected. Cassava plants initiate tubers when they are one to two months old. At this stage the plants are most vulnerable to root-knot nematode attack. Most of the damage caused by *M. incognita* is through a reduction in the number of tubers.

Identification of resistance to *Radopholus similis* in *Musa* in Cameroon

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The nematode *Radopholus similis* is one of the major constraints to banana and plantain production in Cameroon. Studies were carried out between 1994 and 1998 to search for sources of resistance to this nematode. One-hundred-and-forty-two accessions from different genomic groups were evaluated in pot experiments using monoxenic populations of *R. similis.* Planting material was suckers collected from the *Musa* germplasm in Njombé (Cameroon). Accessions were compared with French Sombre (Plantain, AAB) and Yangambi Km5 (Ibota, AAA) used as susceptible and resistant standards, respectively. All plantains (*Musa* AAB), Cavendish (*Musa* AAA) and Lugijura (*Musa* AAA) were susceptible to *R. similis.* Most clones of the Ibota (*Musa* AAA) subgroup (e.g. Yangambi Km5) were resistant to *R. similis.* Different levels of susceptibility to *R. similis* exist among diploids. All the *M. balbisiana* accessions were significantly less susceptible than the Cavendish and Plantain cultivars. Resistance of the diploid Pisang Jari Buaya was confirmed. Among the diploids with moderate levels of susceptibility were Calcutta 4, Truncata, Selangor, Thong Dok Mak and Sefet Velchy. FHIA hybrids were less susceptible to *R. similis* than most Plantains and Cavendish clones.

CORESTA working group for nematodes

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During 1995 an international project, the CORESTA Working Group for Nematodes, was initiated, with ARC-TCRI as coordinator. The main objectives of this project are to promote and assist in the development of tobacco cultivars with resistance to root-knot nematodes and to facilitate the exchange of suitable resistant germplasm. The establishment of a suitable tobacco cultivar

host range to distinguish among the various root-knot nematode species and races, is a long-term objective to assist tobacco growers in their local breeding programmes. The basic project procedures consist of replicated field trials planted in various tobacco-growing areas and countries. Greenhouse trials are conducted to obtain specific information regarding less common *Meloidogyne* spp. Six standard tobacco entries with varying levels of resistance or susceptibility to root-knot nematodes are provided to all participants. These standards are compared with promising local entries to determine their levels of resistance to *Meloidogyne* spp., using a root-gall index, supported by nematode population densities, yield and quality data. A total of 21 datasets from different localities, including Bulgaria, Colombia, Iran, Italy, Malawi, Mozambique, Portugal, Senegal, South Africa, Uruguay, USA (North Carolina) and Zimbabwe have been received. The susceptibility of tobacco to four *Meloidogyne* spp., viz. *M. incognita, M. javanica, M. arenaria* and *M. mayaguensis* was determined using the standards Coker 371, NC 95, TL 33, M/S STNCB 2-28 and NOD 8. Numerous promising breeding lines and cultivars (total of 34 additional entries) from various breeding programmes were compared to the standards. Preliminary results indicate that the highest resistance to *M. javanica* was obtained with the standard entries M/S STNCB 2-28 and NOD 8 and with other lines such as RL 2-1-1, T 14 (Zimbabwe) and KRK 3 (Colombia) with GI < 1.5 (scale 0–5). Different reactions of *M. incognita* on the standard NC 95, which is resistant to *M. incognita* races 1 and 3, indicate that STNCB 2-28, NOD 8 or related lines could be used as sources resistant to *M. incognita* races 2 and 4. Evaluations undertaken in Iran showed that two entries, CO 319 and N 2, had high resistance to *M. incognita* (possibly race 1 or 3). A host-range test with the inclusion of cotton will give a better indication of the specific race present in the field, until specific tobacco entries have been found to indicate the difference. In the USA, NC 95 proved to have the highest resistance to *M. arenaria* , but this needs to be confirmed. Reproduction of a South African population of *M. mayaguensis* on tobacco was higher than that of the population naturally occurring in Senegal. M/S STNCB 2-28, NC 95 and TL 33 had high gall indices. The lowest gall index was found in NOD 8 (RSA 1.6), but this is still too high for such an aggressive nematode. A more effective source of resistance may have to be sought for *M. mayaguensis.*

Screening of local cowpea cultivars and lines for resistance to *Meloidogyne javanica*

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Cowpea is an important rotation crop in commercial, subsistence and small-scale farming. Local cowpea cultivars are highly susceptible to root-knot nematode infestation and often induce nematode population increases both in cowpea and subsequent crops. A single inclusion of cowpea in a crop rotation sequence resulted in a twenty-fold increase in root-knot nematode infestation in a subsequent maize crop. Seven local cowpea cultivars and 14 lines were screened in a greenhouse for host suitability to *Meloidogyne javanica.* All cultivars and 12 of the lines had a reproduction factor (Rf) higher than one, indicating root-knot nematode population build-up. Two of the lines, T182b-889 and R6A, were poor hosts, with Rf values less than one. Screening results need to be confirmed in field trials.

Pathogenicity of two *Pratylenchus* spp. to flue-cured tobacco cultivars and breeding lines

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Pratylenchus zeae has a varied host range, while the host range of *P. delattrei,* which was first found on tobacco in the Groblersdal area, includes maize and grain sorghum. Large populations of these lesion nematode species could be present in the soil before tobacco is transplanted. Although the two species do not reproduce on tobacco, they could have a detrimental effect on young tobacco plants. The effect of these two nematode species on the various flue-cured tobacco cultivars and breeding lines can be divided into three categories, viz. slight or no effect, stimulative effect and negative effect.

The effect of *Bacillus chitinosporus* on egg walls of the nematode *Meloidogyne javanica*

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A suspension of the bacterium *Bacillus chitino-*

sporus was tested for its effect on the egg wall of the nematode *Meloidogyne javanica.* Nematode eggs were added to filtered, inoculated media with the microbe suspension and incubated for 18 hours at 34 °C. Under the SEM, walls of the untreated eggs appeared as thin, translucent, elastic, soft structures that covered a nematode juvenile. Morphological structures of larvae such as lateral fields and cuticle segments could be seen through the translucent egg wall. Eggs that were exposed to the bacterium had opaque, inelastic walls and no regular morphological features were observed. The number of bacteria attached to eggs differed, although all the contaminated egg walls were opaque. It is concluded that a bacterial secretion causes egg wall structures to transform and become opaque, hence prohibiting hatching of nematodes. The result is death of juveniles before hatching. Contaminated eggs disintegrate as a result of bacterial attack. After this, *Bacillus chitinosporus* breaks down chitin in the egg wall fragments. If the chitin in the walls has been degraded during earlier stages of exposure, egg walls become permeable and egg contents can be stained by the stain used for light microscope studies.

Nematode-induced production loss for the banana cultivars Nabusa (*Musa* AAA, 'Matooke' group), Pisang Awak (*Musa* ABB) and Sukali Ndizi (*Musa* AB) in Uganda

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The East African highland banana (Matooke and Mbidde groups) is a staple crop in Uganda. Over the last fifteen years there has been a decline in production as a result of increasing pest and disease pressure and a reduction in soil fertility. Farmers are to some extent replacing highland cultivars with exotic cultivars, such as Pisang Awak and Sukali Ndizi, which are considered to be less affected by pests and diseases. To confirm the farmers' observations, an experiment to compare the susceptibility of Nabusa, Pisang Awak and Sukali Ndizi to nematode attack was established at Sensusu, Uganda, during June 1994. The planting material was pared, selected for absence of weevil tunnels and hot-water treated for 20 minutes at 53 °C. The suckers were either inoculated with nematode-infested roots or not inoculated at planting in a field that had been under bush fallow for over eight years. The infested roots contained a species mixture of *Radopholus similis, Helicotylenchus multicinctus* and *Pratylenchus goodeyi,* which are the common species in central Uganda. In the second banana production cycle the nematode-inoculated Nabusa and Sukali Ndizi plants produced 29 % and less 17 % less fresh bunch mass, respectively, than the uninoculated plants, while the production of Pisang Awak was not affected by nematode inoculation. Unfortunately the cultivars Pisang Awak and Sukali Ndizi were severely affected by *Fusarium* wilt. At harvest of the second cycle, 78 % of the nematode-inoculated Pisang Awak plants versus 47 % of the uninoculated plants and 16 % nematode-inoculated Sukali Ndizi plants versus 6 % of the uninoculated plants, had died as a result of *Fusarium* wilt.

A more environmentally friendly approach to nematode control in bananas

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Nematodes are a serious problem in bananas and can cause yield losses amounting to millions of rands. It is therefore important to control nematodes. However, the focus has shifted from chemical control to integrated nematode and pest management in which both chemical and more environmentally-friendly nematode control measures are used. For the past few years, several environmentally-friendly nematicides have been evaluated against nematodes on bananas in greenhouse and field trials. PL Plus and Ditera were found to increase yields compared to the untreated control in the field. Agrimec controlled nematodes effectively in the greenhouse and is now being tested in the field. Tri-Mat induced vigorous plant and root growth, relative to untreated banana plants, in the greenhouse.

Nematode management in home gardens and small-scale farming systems: a preliminary study

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The global crop loss of about 10 % ascribed to nematodes would have more significance if all producers were equally affected. Most of these

losses are, however, experienced by those least able to afford it, namely resource-poor farmers. Some producers experience losses in the range of 25–50 %, but it can be closer to 100 % in cases of severe nematode infestations. Maize and tomatoes were included in two randomised split-plot design field trials at Dingleday near Bosbokrand (Mpumalanga). Treatments consisted of EDB (45 l ha^{-1}), soil solarisation, *Tagetes erecta,* kraal manure (20 t ha^{-1}) and an untreated control. The EDB and solarisation treatments suppressed a mixed *M. javanica* and *M. incognita* population significantly, whereas control provided by the kraal manure and *Tagetes* treatments was not significant. The maize cultivar SR52 was identified as an excellent host for this root-knot nematode population.

Evaluation of biological control agents against the citrus nematode *Tylenchulus semipenetrans*

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Nematicides have been used for several years to control citrus nematodes worldwide. The first nematicide of importance was DBCP. This was followed by the post-plant granular nematicides, e.g. aldicarb, fenamiphos and cadusafos. The efficacy of these products varied between orchards because of problems caused by several factors, e.g. poor movement through the soil profile and enhanced degradation rates because of high soil pH or accelerated microbial degradation (AMD). Health risks when handled by untrained labourers are a constant danger. Outspan's concern over the unnecessary use of nematicides was demonstrated more than a decade ago when the Outspan Diagnostic Centre was established to conduct nematode analyses for citrus growers to provide scientifically based advice on the need to apply a nematicide. Outspan has for many years also been involved in rootstock research to identify more tolerant rootstocks and has studied physical control of nematodes, e.g. soil solarisation and low-pressure steam (Agrelek). The only aspect not investigated was biological control. This work had to be conducted to develop an integrated pest management (IPM) approach for nematodes. The experiment was conducted for several private companies and the results as such are confidential. Both fungal and bacterial agents were involved and the trial was conducted over a period of two years. Neither the *Paecilomyces* formulations nor Biostart (*Bacillus* spp.) controlled the citrus nematode effectively. More research is necessary to explain why these biological control agents are effective against the root-knot nematode but not against *T. semipenetrans.*

Nematode control on bananas – an alternative approach

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Nematodes can cause severe problems on bananas and must be controlled. However, registered nematicides are expensive, toxic and unaffordable to some farmers. Therefore, a study was undertaken to evaluate the possible control of nematodes with common plants that are readily accessible. These plants were selected on the basis of containing compounds that have insecticidal and/or nematicidal effects. Extracts were obtained by pouring hot water on the plant material, mixing it and leaving it overnight. In a greenhouse trial these extracts were applied three times at fortnightly intervals to tissue culture banana plants infested with nematodes. Compared to the other extracts, tobacco, followed by garlic and radish resulted in a growth increase of the banana plants, as well as the lowest root necrosis index. An extract of *Tagetes* did not reduce the number of nematodes in the roots. In a separate trial the test plants were sown to pots containing soil infested with nematodes. After a few months these plants were mixed into soil in which tissue culture banana plants were planted. Plant growth was increased by many of the treatments. *Tagetes* resulted in the lowest number of nematodes of all the treatments.

Holism, catch-22 and nematode damage: introducing resource-limited farmers to nematodes

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Working with resource-limited farming communities that have low literacy levels challenged our traditional technology transfer methods. Participatory training methods that can be used by researchers and trainers to illustrate complex

concepts to field workers and resource-limited farmers are demonstrated. The importance of using farming systems as the basis is illustrated. In resource-poor communities the success of the farming system becomes the main objective, not researchers' fields of specialisation. It is very important to take note of communities' way of doing and assist them solving their problems. Problems should not be identified for them and then solved without their consent.

Movement of nematicides through the soil profile

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The nematicides aldicarb (Temik), fenamiphos (Nemacur), cadusafos (Rugby), terbufos (Counter) and isazofos (Mocap) are used in southern Africa to control the citrus nematode *Tylenchulus semipenetrans* in citrus orchards. Nematicides are applied when nematode populations in roots exceed 1000 females 10 g^{-1} roots. Unfortunately it has been proved that in many cases where nematicides were applied the citrus nematode was not effectively controlled. Factors contributing to this include poor application (distribution) of the product over the root area, adsorption of nematicides in soils, movement of the product through the soil profile and rapid degradation of the product, both chemically (pH) and as a result of microorganisms that utilise the products as a source of energy (accelerated microbial degradation). In South Africa the organic matter content of most soils is extremely low and the restriction that it has on the movement of nematicides is not as important as in humic and peat soils. The clay content and the amount of irrigation water applied could, however, play an important role in leaching products through the root zone. In this study, the role that these two factors play in the movement of certain nematicides was investigated, using the bioassay techniques described by Stirling to determine the presence of the chemicals in the soil at different depths over time.

On-farm testing and large scale distribution of nematode-free yam planting material in southern Nigeria

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Over 90 % of the world's yam (*Dioscorea* spp.) are produced in West and Central Africa. Soil fertility in many production areas is declining and a combination of pests and diseases further affects yam during crop growth and storage. Especially during storage, the yam nematode *Scutellonema bradys* and root-knot nematodes, *Meloidogyne* spp., can severely affect yam tubers. *S. bradys* causes dry rot in stored tubers, whereas *Meloidogyne* spp. reduce yield and marketability of harvested tubers. As a result of the high storage losses, yam planting material is generally costly. A strategy for control of nematode-related storage losses is heat treatment of yam tubers. Submersion of tubers before planting for a period of 25 minutes in water at 53 °C can reduce production and subsequent storage losses by 30–60 % and also reduces loss of planting material for the next season. Pilot trials at five farms in Western Nigeria suggested good potential for large-scale introduction of the yam heat-treatment method to yam producers. Therefore, trials in three major yam-growing areas in Nigeria were set up. Farmers and extension officers are actively involved in the evaluation of the technique, as well as in assessing prospects of incorporating the treatment in local yam-production systems. A preliminary economic assessment of thermotherapy costs in relation to farmers' gain has been done and will be used to determine the initiation of similar projects during following years.

Effects of *Tylenchulus semipenetrans* on the partitioning of osmoticum ions in citrus replants

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Various studies have demonstrated that *Tylenchulus semipenetrans* infection of citrus seedlings under greenhouse conditions alters the partitioning of osmoticum ions (K, Na and Cl). The effect of *T. semipenetrans* infection on the partitioning of osmoticum ions on citrus replants has not been studied before. A five-year study was conducted at Mokgalakwena Citrus Project to evaluate the effect of *T. semipenetrans* on the partitioning of these ions. The experiment consisted of 40 plots of 40 × 4.6 m each, half of them treated and the other half left untreated. Population densities of *T. semipenetrans* were reduced by two 53 kg ha^{-1} applications of methyl bromide during August and December 1994, respectively. Young Valencia trees on rough lemon rootstocks were planted

during February 1995 and all plots were mulched, using sisal residues. Leaf, root and nematode samples were collected during February of the next four years. *T. semipenetrans* infections in the untreated plots increased during the first year, but decreased during subsequent years. During the whole period replants in the untreated plots had significantly higher leaf Na and Cl and significantly lower leaf K and root Na, Cl and K contents. It is therefore concluded that *T. semipenetrans* infections alter the partitioning of osmoticum ions in citrus replants.

Abstracts of Posters

Nematodes limiting expansion of soybean production in South Africa

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Soybean production is currently expanding to areas of South Africa where maize is the major crop. These areas have lighter soils, and soybeans planted there are likely to be exposed to plant-parasitic nematodes that were not a limiting factor in production previously. Consequently, a nematode survey, based on soil and root samples, was conducted at 17 representative localities. Twelve plant-parasitic genera were identified and several genera and species are first reports on soybean in South Africa. *Meloidogyne* and *Pratylenchus* spp. were the predominant endoparasites and *Helicotylenchus* and *Scutellonema* spp. the predominant semi-ectoparasites. *Meloidogyne incognita* and *M. javanica,* which are predominant in maize-producing areas, were also the predominant root-knot nematode species in the survey. Soybean production will be at risk of yield losses due to plant- parasitic nematodes when grown in these areas.

Rotation crops for the cotton root-knot nematode in South Africa

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The root-knot nematode is considered to be one of the most important nematode pests of cotton in South Africa. As part of an integrated nematode control programme for cotton, wheat and oat cultivars, which are poor hosts for a South African population of *Meloidogyne incognita* race 4, were selected in greenhouse trials. A long-term crop rotation trial was initiated at Jan Kempdorp to determine whether the selected crops and cultivars can be used effectively to reduce damage caused by nematodes. Cotton (cv. Tetra) was planted on the same plots for three consecutive summer seasons. Two winter plantings of the wheat cultivars SST 825, Kariega, Marico, Palmiet, the oat cultivars Perdeberg and Maluti, or fallow plots followed the cotton. Fluctuation of plant-parasitic nematodes over seasons was monitored regularly. The density of nematode populations, which included *Meloidogyne, Pratylenchus, Paratrichodorus* and *Xiphinema* spp, increased on the cotton during all three seasons. Most of the oat and wheat cultivars suppressed the *Meloidogyne* spp. population. *Meloidogyne* juveniles could not be detected in the fallow plots at the end of the first cycle. *Meloidogyne* spp. numbers were slightly reduced during the 1997/98 season, compared to the same sampling period during the 1996/97 season. Towards the end of the third cotton season the *Meloidogyne* spp. population increased again. The average yield of the second cotton season was low due to very late planting of the crop, but the highest seed cotton yield was obtained from plots that followed the oat cultivar Maluti. On cotton, the lowest *Meloidogyne* spp. population density was found in plots following the wheat cultivar Marico. Continued research is needed to present more definite recommendations.

Damage threshold population densities of *Tylenchulus semipenetrans* as measured through the accumulation of foliar osmoticum ions on mature citrus

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Several studies have demonstrated that *Tylenchulus semipenetrans* Cobb induces slow decline of citrus through increased accumulation of foliar Na and Cl ions and/or alteration of the partition-

ing of nutrient ions. However, damage threshold population densities of the citrus nematode in terms of accumulation of Na and Cl have not been determined. The objective of this study was to determine the damage threshold densities of *T. semipenetrans* on mature citrus using foliar Na and Cl ions. The study was conducted at Mokgalakwena Citrus Project with 29-year-old Washington navels on rough lemon rootstocks. Eight rows were randomly selected from a total of 19 rows, each row subdivided into seven blocks of six trees each in the autumn of 1995. Within each block, root samples were collected at 25-cm depth using a 3-cm-diameter soil auger on the cardinal sides of two randomly selected trees at a distance of 0.5 m from the trunk. Also, leaf samples were collected from the nematode-sampled trees. Prior to regression analysis, ion and nematode data were transformed into $\log_e(x)$ and $\log_e(x + 1)$, respectively, to homogenise the variances. The constant terms in various linear models were small, thus demonstrating that in terms of the accumulation of Na and Cl to physiological and visual damage, high densities of *T. semipenetrans* were required.

In vitro mass culturing of nematode species

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Many plant-parasitic nematodes have a wide host range and limit commercial and small-scale agriculture globally. Large numbers of particular nematode species and races are often required as pure and sterile populations for studies on host plant ranges, pathogenicity, chemical control and host plant resistance. Various plant-parasitic and one free-living nematode species are well established and maintained in vitro at the ARC-GCI in Potchefstroom. *Ditylenchus africanus* is maintained on groundnut callus tissue (cv. Sellie) on a modified Murashige and Skoog medium at 25 °C, *Pratylenchus zeae* on maize roots (PAN6479) on Skoog, Tsui and White medium at 27 °C, *P. brachyurus* on carrot discs (cv. Nantes) at 27 °C and *Meloidogyne javanica* on *Agrobacterium rhizogenes*-transformed tomato roots (cv. Moneymaker) at 27 °C. The free-living species, *Caenorhabditis elegans,* is maintained in a haemoglobin-based solution at 25 °C. Availability of in vitro-cultured nematodes has the advantage of a constant supply of inoculum and experimental material. It is cost-effective, time-saving and species-specific.

Evaluation of host suitability for *Paratrichodorus meyeri* of rotational crops used in tobacco cultivation

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The stubby root nematode, *Paratrichodorus meyeri,* is a major limiting factor on flue-cured tobacco in the Vaalwater production area. This nematode can cause a reduction of 25 % and more in income, resulting in a loss of R6250 per hectare for flue-cured tobacco. Rotation with crops resistant to this nematode species is a possible solution to this problem. In a preliminary greenhouse trial, maize cv. SNK 2340, wheat cv. SST 86 and the standard South African babala were evaluated with tobacco cv. TL 33 and cotton cv. ACALA 1517/70 as standards. In a second trial, the host status of the maize cultivars SNK 2888, PAN 6479, SNK 2776, PAN 6364 and SNK 2147 was evaluated in the greenhouse. Cultivar SNK 2340 was included as a standard treatment. Soil infested with *P. meyeri* was used in the trials. All the crops evaluated to date are considered excellent, good or moderate hosts.

The reproduction and life cycle of a South African population of *Xiphinema index*

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The seasonal fluctuation of a South African *Xiphinema index* population, effect of four hosts on the length of the life cycle and the reproduction rate with fig as host at three different temperatures were studied. Reproduction was highest during early summer (November and December) when the maximum air temperature was between 25 and 30 °C. Under controlled conditions at 25 ± 2 °C the duration of the life cycle of *X. index* was between 75 and 85 days with the vine rootstock Fairy as host, between 55 and 65 days with rootstock Jacquez and between 45 and 55 days with fig and rootstock 775 Paulsen. Using fig as host the highest reproduction rate of the nematode

was at 28 °C, with a 216-fold increase in numbers and the lowest at 18 °C, with no adults after 85 days.

Discrimination of South African *Xiphinema* spp. by PCR-RFLP analysis

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PCR-RFLP analysis of the internal transcribed spacer region of ribosomal DNA was performed on individual nematodes to distinguish between the species *Xiphinema index, X. italiae, X. elongatum* and *X. americanum.* A single nematode of each species was ruptured in a drop of buffer and heat-treated. The PCR mixture was then prepared directly in the same tube. This extraction method was applicable to juveniles and adults. After PCR, the amplified products were digested with endonucleases, *Msp*I and *Cfo*I. Differences in restriction fragment lengths separated these species from one another.

Microplot screening of local lupin cultivars for resistance to *Meloidogyne javanica*

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Lupin is an important rotation crop in commercial farming owing to its high and balanced nutrient content. This is particularly true for farms with an animal production component. Local lupin cultivars are highly susceptible to root-knot nematode infestation and often result in a nematode population build-up in crop rotation trials. Local knowledge of the host status of lupin cultivars to root-knot nematodes is lacking. A microplot screening trial with 29 local cultivars was conducted. Infestation levels of *Meloidogyne javanica* in roots were determined to indicate host suitability. Significant differences were observed. The cultivar SAL 34, with an average of 7.6 eggs and larvae per gram roots had the lowest infestation, followed by SAL 101 with 13.2 and Lutop with 14.1. The highest infestation level was 667 eggs and larvae per gram roots for cultivar Esta. This information is valuable for cultivar recommendations when lupin is planted in *M. javanica*-infested soil.

A pilot survey of plant-parasitic nematodes on golf courses in South Africa

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Grasses planted on putting greens and fairways of some golf courses in South Africa were observed with symptoms of nutrient deficiency. Foliar symptoms included chlorotic patches and streaks, wilting and thinning of the grass cover. The roots sometimes showed brown lesions and frequently appeared stunted. As these symptoms are similar to the damage caused by plant-parasitic nematodes on grass in other parts of the world, we conducted a pilot nematode survey of selected golf courses in Gauteng, Eastern Cape, Western Cape and North-West Provinces. The following grasses were sampled: bent grass (*Agrostis palustris*), coach grass (*Cynodon* sp.), fescue grass (*Festuca pratensis*), winter grass (*Poa annua)* and kikuyu grass (*Pennisetum clandestinum*). The following nematode genera have been found on golf courses in South Africa and are also known pathogens of grass covers in other parts of the world: *Helicotylenchus, Heterodera, Meloidogyne, Mesocriconema, Paratrichodorus, Tylenchorhynchus* and *Xiphinema.* The genera *Paratrichodorus, Hemicycliophora* and *Xiphinema* were most abundant in the rhizosphere of bent grass, while *Scutellonema* and *Helicotylenchus* were predominant around the roots of kikuyu grass. The results of this survey suggest that plant-parasitic nematodes, especially *Paratrichodorus, Helicotylenchus, Hemicycliophora* and *Xiphinema* are, alone or in complexes with other pathogenic soil organisms, responsible for damage to grass cover of golf courses in South Africa.

Resistance of banana cultivars to burrowing nematodes (*Radopholus similis*) and root-knot nematodes (*Meloidogyne* spp.)

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Burrowing nematodes and root-knot nematodes were tested on Grand Nain, High Noon, Chinese Cavendish and Williams banana plants in two separate trials in a greenhouse. Three nematode

densities, namely 0, 500 and 2000 nematodes per plant were evaluated. Each treatment was replicated 10 times. All plants were removed after eight weeks and their stem, leaf and root masses determined. Roots were evaluated for burrowing and root-knot nematodes. The number of nematodes was also determined. High Noon seems to be more tolerant to both burrowing and root-knot nematodes. This is possibly due to vigorous root growth and abundance of hairy roots of High Noon banana plants. Although Chinese Cavendish had large numbers of root-knot nematodes in the roots, it was still growing vigorously and had the highest plant mass as well as the largest leaf area.

Control of *Meloidogyne incognita* on Bambara groundnut (*Vigna subterranea*) by means of biofumigation

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Cabbage (*Brassica oleracea capitata)* varieties Drumhead and Glory of Enkhuizen and forage rape (*Brassica rapa*) variety Forage star were tested for their biofumigation effect on *Meloidogyne incognita* race 2 on bambara groundnut. Chopped leaves and stems of each variety were applied at rates of 20, 40 and 60 kg ha^{-1} to soil inoculated with 100 egg masses of *M. incognita* per pot and incubated for six weeks to allow for decomposition. Bambara groundnut was planted as a test crop immediately after decomposition and allowed to grow for eight weeks before evaluation. All three *Brassica* crops reduced the *M. incognita* population significantly. The highest reduction was recorded at 60 kg ha^{-1} although this was accompanied by phytotoxicity. Drumhead applied at 40 kg ha^{-1} gave more satisfactory control, considering both nematode reduction and phytotoxicity.

Abstracts of Workshop Papers

Development of furfural as a nematicide on groundnuts

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Furfural is a natural, biodegradable plant-by-product that shows potential as an environmentally friendly nematicide. Field trials were conducted on three irrigated farm lands, representative of the groundnut-production area, exhibiting symptoms caused by either the peanut pod nematode (*Ditylenchus africanus*) or *Tylenchorhynchus brevilineatus,* during the summer prior to the trial. Treatments were furfural, aldicarb or oxamyl and an untreated control. Furfural was sprayed evenly over the surface of the soil with knapsack sprayers at dosage rates of 50, 100 and 200 l ha^{-1}, and then tilled into the top 10 to 15 cm. Groundnut (cultivar Kwarts) was planted within 24 hours of furfural application. In terms of plant dry mass early in the season and seed yield at harvest, none of the furfural treatments exhibited phytotoxicity symptoms. Nematode numbers and average seed yield of the different treatments varied. With regard to net profit, the furfural treatments were equivalent or sometimes better than the standard aldicarb or oxamyl treatments. Furfural could be applied prior to planting through a centre pivot irrigation system at 50 l ha^{-1}. Casual observations indicated that symptoms of *T. brevilineatus* appeared only four months after application on groundnut. A second application at that stage of crop growth again reduced nematode numbers without inducing phytotoxicity symptoms.

Reflections on nematology teaching in southern Africa in the coming five years

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During a visit to South Africa in 1991, the idea was born to initiate a Nematology course at the Agricultural Faculty of the University of Natal, Pietermaritzburg. The course became compulsory to advanced students in the Department of Microbiology and Plant Pathology and was open to students or participants of other departments, universities and institutions. The course lasts two weeks, with lectures in the morning and practicals in the afternoon. Lectures include morphology and biology of nematodes, ecology, soil life and soil physics, dynamic and genetic aspects of nematode populations; genetic variation, yield/density

relations, dynamic simulation and statistical approaches, factors affecting damage, ecophysiology, integrated nematode management and damage control. Nematode problems of major crops of southern Africa are discussed. Information about these crops in the South Africa was obtained from researchers at the various institutions during recent visits by the author. Such information provides the participants of the course with up-to-date information relevant to the area. Field trips are conducted and the participants are made familiar with symptoms of nematode infestations in various crops. They collect their own soil and plant samples, learn how to extract nematodes, to identify them (at least to genus level) and to determine nematode densities in the soil. The courses have been held since 1994 and the present capacity is 24 participants per course. The course is sufficient to obtain a sound knowledge about the role of nematodes in water, soil and crops. It may form the basis for further studies, e.g. in taxonomy of nematodes, molecular aspects, control (e.g. biological, chemical, breeding for resistance, tolerance). It is particularly suited to those who wish to understand farming systems, the role of the soil fauna and the particular position of nematodes in these systems. Extension officers with this kind of training, preferably interrelating through a network covering the country, would be able to recognise nematode problems, extract the nematodes from samples, fix them properly and submit them, when necessary, for identification. Regular soil sampling might also improve crop production and lower the risks of mismanagement simply by ensuring that nematodes are considered. Full-time nematologists with the larger institutions and universities may serve as referees for those in the field and as partners in accumulating insight in the role of nematodes. Even those who seek a career in administration can benefit from this course, as they will be better prepared when topics on nematology pass their desks.

Nematology training in South Africa: what can we learn from the past?

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A brief historical review of nematology training over the past sixty years is given. The founder of nematology in South Africa, the late Dr W J van der Linde, was trained at Cornell University in the USA, as was the acknowledged doyen of nematology in this country, Prof. Juan Heyns, who studied under Gerald Thorne in Wisconsin. Other colleagues were trained in Europe (England, the Netherlands, Germany and France) before sufficient expertise existed for initiating training courses locally. The first formal undergraduate training in nematology was offered at the University of Stellenbosch as part of a BSc (Agric.), majoring in entomology, nematology being a semester module. Gradually, more South African universities started to offer nematological training, mainly as specialised short courses or as integral parts of their plant pathology curricula. The question being asked is: is there sufficient need or potential for training nematologists in southern Africa or shall we return to our past practices by sending prospective candidates for training overseas?

Management of accelerated nematicide degradation in soils

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In tropical, subtropical and temperate zone soils, accelerated microbial degradation has caused failure of all currently available, soil-applied, biodegradable nematicides. This has affected a wide range of crops. Using the organophosphate nematicide fenamiphos as an example, we discuss the microbiological aspects of accelerated nematicide degradation in soils and present a management option to address the problem. In the 1970s, in commercial banana plantations in tropical Australia, West Africa, Central and South America, intensive treatment of soils with fenamiphos at 4 mg kg^{-1} soil repeated three times per year against *Radopholus simulus* resulted in a step-by-step build-up of communities of soil microorganisms that rapidly degraded the chemical. When treatments with fenamiphos were interrupted, communities that caused the accelerated degradation died back to near natural levels and degradation rates were again similar (but slightly higher) than those in previously untreated control soils. In acidic to slightly acidic soils from Africa, die-back of fenamiphos-degrading microbial communities took 12–16 months; in dry alkaline soils of Australia, population die-back or ‘soil recovery’

took 24 months or more. When 'recovered' soils were re-treated with fenamiphos, full efficacy of the product could be demonstrated. The possibility of 'cross-degradation' of other nematicides in soils where fenamiphos was degraded was tested using most currently available non-volatile nematicides. Laboratory and long-term field experiments showed that soils that degraded fenamiphos at accelerated rates degraded other organophosphate nematicides (e.g. cadusafos and terbufos) at control soil rates. Similarly, soils that degraded other organophosphate and/or carbamate (e.g aldicarb and carbofuran) pesticides at accelerated rates degraded fenamiphos at control soil rates. Based on this information, nematicide alternation experiments were conducted on commercial farms, plantations and bowling greens in the four regions. These showed that accelerated degradation of fenamiphos could be effectively controlled by 1:1 alternations with several different organophosphate nematicides, the choice of which depends on the pH of the soil.

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