The threat of root-knot nematodes (*Meloidogyne* spp.) in Africa: a review

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Abstract

Meloidogyne species pose a significant threat to crop production in Africa due to the losses they cause on a wide range of agricultural crops. The direct and indirect damage caused by various *Meloidogyne* species results in delayed maturity, toppling, reduced yields and quality of crop produce, high costs of production and therefore loss of income. In addition, emergence of resistance-breaking *Meloidogyne* species has partly rendered various pest management programmes already in place ineffective therefore putting food security of the continent at risk. It is likely that more losses may be experienced in the future due to the on-going withdrawal of nematicides. To adequately address the threat of *Meloidogyne* species in Africa, an accurate assessment and understanding of the species present, genetic diversity, population structure, parasitism mechanisms and how each of these factors contribute to the overall threat posed by *Meloidogyne* species is important. Thus, the ability to accurately characterise and identify *Meloidogyne* species is crucial if we are to effectively tackle the threat of *Meloidogyne* species to crop production in Africa. In this review, we discuss the use of traditional versus molecular-based identification methods of *Meloidogyne* species and how accurate identification using a polyphasic approach can negate the eminent threat of root knot nematodes in crop production. The potential threat to Africa posed by highly damaging and resistance-breaking populations of 'emerging' *Meloidogyne* species is also examined.

Key words: *Meloidogyne*, identification, resistance-breaking, economic impact, pest management.

Introduction

In the recent past, the usage of nematicides has led to a significant reduction of *Meloidogyne* spp. populations in crop production. However, due to their toxicity and adverse effects on the environment, many nematicides have been or are currently being withdrawn from the market. This has now propelled *Meloidogyne* spp. to the forefront as important pathogens of many crops and other plants. In fact, in a 2013 Molecular Plant Pathology survey of the top ten plant parasitic nematodes, *Meloidogyne* spp. collectively were voted at the top of the list (Jones *et al*., 2013). In this article, we assess the current threat of *Meloidogyne* spp. to crop production with a specific focus on Africa. We also discuss the importance of an integrated approach towards accurate species identification and current as well as future *Meloidogyne* spp. management strategies.

Meloidogyne species present in Africa

There are currently nearly 100 recognised *Meloidogyne* spp., with 22 of these species reported to be present in Africa (Table 1). Historically, *Meloidogyne* spp. have been divided into major and emerging species. According to Moens *et al*. (2009), *M. arenaria*, *M. incognita*, *M. javanica* (occurring in tropical regions) and *M. hapla* (occurring in temperate regions) are considered to be the four major *Meloidogyne* spp. However, these authors consider a further five species as emerging species. These five emerging species are: *Meloidogyne chitwoodi*, *M. fallax*, *M. enterolobii*, *M. minor* and *M. paranaensis*. In Africa, *Meloidogyne arenaria, M. javanica* and *M. incognita* are regarded as the most dominant species reported in 26, 36 and 37 different cofig

untries across the continent, respectively (IITA, 1981; De Waele and Elsen, 2007). Collectively, these three species have been reported to cause damage in economically important crop plants such as sweet potato, banana, tomato, cabbage, potatoes, pineapple, cassava, maize, tobacco, cowpea as well as others such okra (*Abelmoschus esculentus*), papaya, buchu (*Agathosma betulina*), African spinach, (for a full list of hosts refer to Table 1). On the other hand, three of the five emerging species, namely *M. chitwoodi*, *M. enterolobii* and *M fallax*, are reported from Africa (Table 1). For example, the resistance-breaking apomictic species, *M. enterolobii* has been isolated from Burkina Faso, DRC, Malawi, Mozambique, Senegal, South Africa and Togo causing damage in potatoes and gauva (Marais, 2012; Onkendi and Moleleki, 2013b). On the other hand, M. chitwoodi and M. fallax have been reported to cause damage on Other *Meloidogyne* spp affecting economically important crop plants in Africa

Table 1 *Meloidogyne* species reported from various parts of Africa

Meloidogyne sp.	Country/region	Crop(s) affected	References
M. acronea	Kenya, South Africa, Malawi	Cotton, pigeon pea, sorghum, millet, grasses, pea, bulrush, okra, potato, tomato	Whitehead & Kariuki (1960); Hunt & Handoo (2009)
M. africana	Kenya, Sudan, Tanzania	Coffee	Whitehead (1959); Eisenback (1997)
M. arenaria	Algeria, Cote d'Ivoire, Egypt, Gambia, Ghana, Liberia, Libya, Madagascar, Malawi, Mauritius, Morocco, Mozambique, Nigeria, Sao Tome and Principé, Senegal, South Africa, Sudan, Tanzania, Uganda, Zimbabwe	Date palm, peach, potato, tobacco, tea, carrot, tomato, lettuce, cucumber, aubergine, cotton, soybean, pineapple, pyrethrum, banana, papaya, pepper, cowpea, okra, velvet bean	IITA (1981); CABI (2003)
M. chitwoodi	South Africa, Mozambique	Potato, cassava, groundnut, wheat soil	Kleynhans *et al.* (1996); Fourie *et al.* (2001b); Coyne *et al.* (2006b)
M. decalineata	Tanzania, Sao Tome	Coffee	Whitehead (1968); Lordello & Fazuoli (1980)
M. enterolobii	Malawi, Senegal, South Africa, Cote d'Ivoire, Burkina Faso, Democratic Republic of Congo	Potato, guava	M. Marais (unpublished data); Onkendi & Moleleki (2013a,b)
M. exigua	Mozambique	Cassava	Coyne *et al.* (2006b)
M. ethiopica	Ethiopia, Mozambique, Tanzania, Zimbabwe, South Africa	Tomato, bean, black wattle (*Acacia mearnsii*), cabbage, tobacco, pumpkin, pepper, macadamia, pineapple, carrot, home gardens, natural veld, potato	Whitehead (1968, 1969); CABI (2005)
M. fallax	South Africa	Groundnut	Fourie *et al.* (2001b)
M. graminicola	South Africa	*Paspalum* spp.	Kleynhans (1991)
M. hapla	Algeria, Cote d'Ivoire, Egypt, Kenya, Libya, Malawi, Morocco, Nigeria, South Africa, Tanzania, Uganda, Zimbabwe	Potato, date palm, groundnut, native plants and numerous crops	Fourie *et al.* (2001b); CABI (2002a)
M. hispanica	Burkina Faso, Malawi, South Africa	Granadilla, sugarcane, *Ficus* spp., ornamental crops, grapevine	Kleynhans (1991)
M. incognita	Algeria, Angola, Botswana, Burkina Faso, Cameroon, Congo, Democratic Republic of Congo, Cote d'Ivoire, Egypt, Ethiopia, Gambia, Ghana, Guinea, Kenya, Liberia, Libya, Madagascar, Malawi, Mauritania, Mauritius, Morocco, Mozambique, Namibia, Niger, Nigeria, Reunion, Senegal, Seychelles, Somalia, South Africa, Sudan, Tanzania, Tunisia, Uganda, Zambia, Zimbabwe	Potato, grapevine, maize, date palm, tomato, tobacco, cowpea, upland rice, soybean, papaya, pepper, aubergine, cauliflower, okra, cabbage, Chinese cabbage, onion, watermelon, African spinach, coconut, mango, citrus, guava, yam, cassava and numerous crops	IITA (1981); CABI (2002b); Kwerepe & Labuschagne (2004); SAPPNS database[a]
M. javanica	Aldabra, Algeria, Angola, Botswana, Burundi, Comoros, Democratic Republic of Congo, Cote d'Ivoire, Egypt, Eritrea, Gabon, Gambia, Ghana, Kenya, Liberia, Libya, Madagascar, Malawi, Mauritania, Mauritius, Morocco, Mozambique, Namibia, Nigeria, Reunion, Rwanda, Senegal, South Africa, Sudan, Tanzania, Tunisia, Uganda, Zambia, Zimbabwe	Potato, buchu (*Agathosma betulina*), sugarcane, banana, yam, sweet potato, date palm, tobacco, broad bean, celery, tomato, upland rice, aubergine, cabbage, Chinese cabbage, cassava and numerous crops	IITA (1981); CABI (2002b); SAPPNS database[a]
M. kikuyensis	Kenya, South Africa, Tanzania	Kikuyu grass, sugarcane	De Grisse (1960); Kleynhans (1991)
M. megadora	Angola, Sudan	Coffee, carrot, banana	Whitehead (1968); Eisenback (1997)
M. morocciensis	Morocco	Peach	Rammah & Hirschmann (1990)
M. naasi	Mozambique	Cassava	Coyne *et al.* (2006b)
M. oteifai	Democratic Republic of Congo	Coffee	Elmiligy (1968)
M. partityla	South Africa	Pecan, walnut	Kleynhans (1991)
M. propora	Aldabra Atoll	Black nightshade (*Solanum nigrum*), *Cyperus obtusiflorus*	Spaull (1977)
M. vandervegtei	South Africa	Unidentified woody plant from coastal forest	Kleynhans *et al.* (1996)

[a]The SAPPNS database was made available courtesy of M. Marais (ARC, South Africa).

include those that infect coffee (*M. africana*, *M. decalineata*, *M.decalieata* and *M. oteifai*); cassava (*M. exigua*, *M. chitwoodi*, *M. megadora*, and *M.*

naasi); sugarcane (*M. hispanica* and *M. kikuyensis*) and cotton (*M. acronea*). A number of *Meloidogyne spp* have also been reported on trees and woody shrubs such as *M. ethiopica* infecting black wattle (*Acacia mearnsii*); *M. morocciensis* infecting peach trees; *M. partityla* infecting pecan and walnut trees *M. propora* reported on black night shade (*Solanum nigrum*) as well as *M. vandervegtei* which has been reported on woody plants and coastal forests. Two *Meloidogyne* species, *M. graminicola* and *M. kikuyensis* have been reported to affect members of the *Poaceae* family, *Paspalum* spp and kikuyu (*Pennisetum clandestinum*) respectively.

The number of *Meloidogyne* species listed in table 1 is most certainly not exhaustive due to paucity of data in many regions. Because the three major species (*M. arenarea*, *M incognita* and *M. javanica*) are widespread and well-studied, this might have led to bias against accurate identification of the emerging species. Consequently, it is possible that many of the species listed as one of the major species could have been incorrectly diagnosed. Hence, it is conceivable that the potential impact of new and emerging species has been grossly understated. Similar problems of misidentified *Meloidogyne* species have been previously reported. For instance, for many years both *M. paranaensis* and *M. enterolobii* were misidentified as *M. incognita* (Yang *et al*., 1986; Carneiro *et al*., 1996). Thus we can anticipate that the wide adoption of molecular diagnostic tools in the future could lead to the number of species increasing as more cases of misidentification are made known or new species are recorded. It is important that the different *Meloidogyne* species are accurately identified in order to be able to evaluate their impact. It is also important to determine which quarantined *Meloidogyne* spp. are currently present in Africa and the extent of their distribution. If present, are they being accurately identified? Can we employ new diagnostic tools that are based on molecular technology together with classical methods to carry out accurate identification? For instance, Onkendi and Moleleki, 2013a&b have recently demonstrated the use of molecular approaches in accurately identifying various *Meloidogyne* spp. present in potatoes from South Africa. This information will enhance our knowledge of the current population densities and distribution of different *Meloidogyne* species and guide farmers in the implementation of integrated pest management strategies.

Economic impact

Meloidogyne spp. cause an estimated annual loss of $157 billion globally (Abad *et al*., 2008). However, in most cases, the impact of *Meloidogyne* spp. is grossly underestimated. This is more so in

Africa than anywhere else in the world. Hence it is likely that the overall annual losses due to these pathogens are much higher than estimated. In many crop producing regions in Africa, there has been no comprehensive assessment that focuses specifically on the economic impact of *Meloidogyne* spp. (Coyne *et al*., 2006a). There are several factors that have led to the scanty availability of information on the economic impact of *Meloidogyne* spp. across Africa. Firstly, there is a general lack of awareness of the effect of *Meloidogyne* spp. in crop production. As a result, these pathogens tend to be overlooked. Secondly, the long-term use of nematicides has led to an underestimated effect of *Meloidogyne* spp. however, with diminishing options for use of nematicides, *Meloidogyne* spp. problems are steadily beginning to resurface. Finally, the lack of information can be attributed to the acute lack of resources (both financial and human) to initiate large scale projects necessary to fully assess the *Meloidogyne* spp. situation in Africa (De Waele and Elsen, 2007). Even though in general there is limited information on the impact of *Meloidogyne* spp. in crop production in Africa, there is growing evidence that suggests that the problem of *Meloidogyne* spp. in most farms across the continent is a significant threat to crop production. Furthermore, through several projects, among others the International *Meloidogyne* Project (IMP), it is evident that *Meloidogyne* spp. cause considerable damage to various crops (Figure 1) (Jones, 2005; Coyne *et al*., 2006b).

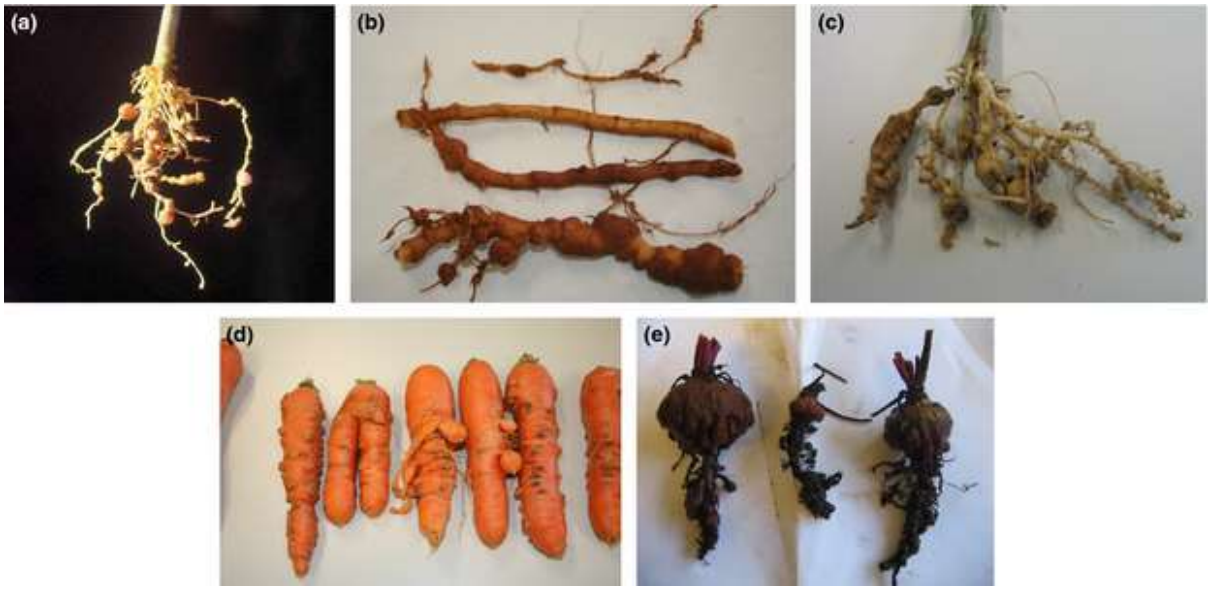

Figure 1. Galls and other symptoms caused by various Meloidogyne species on select crops. (a) Galls on tomato roots caused by Meloidogyne enterolobii. (b) Galls on grenadella roots caused Meloidogyne incognita. (c) Galls on cucumber roots caused by Meloidogyne javanica. (d) Galls and damage symptoms on carrot caused by Meloidogyne arenaria and M. incognita. (e) Galls on beetroot roots caused by M. javanica and M. incognita. Pictures (d) and (e) represent damage caused by Meloidogyne species during co-infection.

Based on the level of nematode populations, *Meloidogyne* spp. can cause high levels of crop loss during growth, increase the cost of production through increased fertilizer application and control programmes, and also significantly reduce post-harvest yields (Figure 2). Crop losses of 30% or more

Figure 2. Damage caused by Meloidogyne spp. in a tomato field in Mwea, Kenya.

in tobacco farms in some parts of Tanzania have been reported (Whitehead, 1969). In addition, crop losses of 50% in pyrethrum flower yields and a decrease in pyrethrin content in Kenya has also been attributed to *Meloidogyne* spp. infection (IITA, 1981). During surveys carried out by Fourie *et al*. (2001a) on soy bean in South Africa, *Meloidogyne* spp. that significantly hamper soybean production were observed in 16 of the 17 different localities sampled by the authors. In South Africa alone, potato production losses associated with plant parasitic nematode species in 1989 were estimated to be 16.7%, accounting for $7 Million annually (Keetch, 1989). Speijer and Kajumba (2000) identified *Meloidogyne* spp. and other plant parasitic nematodes as the phytoparasites that are responsible for 50% of banana loss in Uganda. Coyne *et al*. (2006a) also found out that 14.4% of galling on the yam tubers on sale in Mali were as a result of *Meloidogyne* spp. infection. Rejection of inferior quality crop produce both locally and internationally, increased scarcity of clean and healthy propagating materials and predisposing of growing crops to secondary infections by other organisms especially soil-borne pathogens are also some of the problems associated with *Meloidogyne* spp. infection (Powers *et al*., 2005). For example, interactions between *Meloidogyne* spp. and pathogens such as *Fusarium* spp. are well-documented while there may still be many other interactions which are less well-studied (Siddiqui *et al*., 2010; Mongae *et al*., 2013).

The presence of these *Meloidogyne* spp. populations puts agricultural production in Africa at a significant risk given the fact that most farmers do not have accurate information on the actual *Meloidogyne* spp. present in their farms (Onkendi and Moleleki, 2013a). This coupled with the ban and restrictions on some of the effective chemical compounds (such as methyl bromide) against a wide range of *Meloidogyne* spp. and lack of alternative strategies which are effective may greatly contribute to incidents of food crisis across the continent. *Meloidogyne fallax* and *M. chitwoodi* are listed as quarantine organisms in Europe as per the EC Directive of 2000/29/EC and EPPO region (Viaene *et al*., 2007; OEPP/EPPO bulletin, 2009; Wesemael *et al*., 2011). Furthermore, *M. enterolobii* is also listed as a quarantine organism across Europe (OEPP/EPPO bulletin, 2011). Therefore this scenario compounds the problems farmers in African countries face while exporting their farm produce especially to the European markets. The presence of these pathogens in their produce leads to rejection at the international markets (Powers *et al*., 2005). Resistance breaking species such as *M. enterolobii* may also contribute to a reduction in forest cover which may affect water catchment zones and also access to water in the longrun. This may happen in isolation or in conjunction with highly damaging forestry pathogens. Forest cover cushions various countries from adverse environmental conditions such as floods and drought.

Species identification

Many African countries have inadequate diagnostic capabilities to carry out reliable pathogen diagnostic services (Ogundiran, 2005). This has led to fragmented data on the presence and distribution of *Meloidogyne* spp. which has serious implications on various aspects of agriculture. There is the threat of introduction of new and possibly aggressive *Meloidogyne* spp. to an area, higher cost implications through management strategies that target the wrong organism and loss of revenue due to produce being denied entry into other countries based on the presence of a quarantine organism. Historically, nematologists have relied solely on morphological and morphometrical characters to identify *Meloidogyne* spp. The earliest use of isozymes as biochemical methods to identify *Meloidogyne* spp. was published by Esbenshade and Triantaphyllou (1985) and according to Blok and Powers (2009), isozymes are a convenient first stage approach in determining *Meloidogyne* spp. biodiversity. Antibodies, both polyclonal and monoclonal have been produced for *Meloidogyne* spp. diagnostics but the use of antibodies is limited to few examples as it has, according to Blok and Powers (2009) been superseded by DNA-based diagnostics (Davies *et al*, 1996; Tastet *et al*., 2001). In recent years, identification methods which are DNA-based have gained popularity and this has led to a combination

of both morphological, biochemical and molecular methods to describe and identify nematodes (Karssen *et al*., 2004: Castillo *et al*., 2009).

Morphological and morphometric characteristics

The use of morphological and morphometric characters was traditionally the most common method used by diagnosticians as the preliminary and routine method of identifying *Meloidogyne* spp. These methods rely heavily on the shape of body, labial region, stylet length, shape of stylet cone, basal knobs and nature of perineal pattern in female labial region and characteristics of size, stylet length, distance of the dorsal gland orifice (DGO) from the stylet base for males and J2 juveniles (Kleynhans, 1991; Hunt and Handoo, 2009). According to Hunt and Handoo (2009), clear interspecific boundaries that all *Meloidogyne* spp. diagnostician yearn for are becoming increasingly obscure due to factors such as existence of obscure species and an increasing occurrence of new or emerging species. These authors also cite variable morphometrics, conserved morphology host effects, intraspecific variation, and parthenogenetic mode of reproduction as obscuring factors. This problem is best illustrated by the fact that the existence of what is known as the *incognita* type perineal pattern is now acknowledged to occur in a number of species (Hunt and Handoo, 2009). Hence, this underscores the importance of an integrated diagnostic approach in identification of *Meloidogyne* spp. (Landa *et al*., 2008; Blok and Powers, 2009).

Isozyme phenotypes

Isozymes are variants of a particular enzyme. Isozyme phenotypes have been used for routine identification of various *Meloidogyne* spp. in several parts of Africa despite the fact that they are restricted to the adult female stage of development (Esbenshade and Triantaphyllou, 1990; Muturi*et al*., 2003). The adult female stage is the suitable stage since it is associated with the expression of a given gene product. The procedure is easy and quick to perform and given the fact that reference standards for certain *Meloidogyne* spp. (usually *M. javanica*) are used, it is easy to identify various *Meloidogyne* spp. which are usually common.

The limitations of diagnostic tests based entirely on isozyme phenotypes include lack of capacity to utilize other stages of development (second stage juveniles and eggs) and lack of a wide array of standards to compare results with (Molinari *et al*., 2005; Wesemael *et al*., 2011). In some cases, it is difficult to determine and differentiate band sizes between different species during identification. This has necessitated the use of more than one enzyme to resolve this problem. Malate

dehydrogenase (mdh) is known to separate *M. hapla* from *M. incognita*, *M. arenaria* and *M. javanica*, whereas glutamate dehydrogenase can separate *M. incognita* from *M. javanica*, *M. arenaria* and *M. hapla* (Esbenshade and Triantaphyllou, 1990; Muturi *et al*., 2003). Due to these limitations, in surveys aimed at the identification of *Meloidogyne* spp., the use of isozymes can be applied as an initial step of identification.

Molecular-based identification methods

Molecular-based methods used in nematode diagnostics are usually based on nucleic acid studies. Most of these methods, particularly the DNA-based ones, are known to be robust, sensitive, specific and reliable in detecting and distinguishing various *Meloidogyne* spp. compared to morphological or biochemical methods (Powers *et al*., 2005; Berry *et al*., 2007). Based on this, several molecular methods have been employed to accurately identify various *Meloidogyne* spp. For the purpose of this review, these will be grouped into gel or sequence-based methods as well other methods that do not strictly fall in either of these categories.

Gel-based molecular diagnostic methods include random amplified polymorphism DNA (RAPD), sequence characterized amplified region markers (SCAR-PCR), restriction fragment length polymorphisms (RFLPs) and amplified fragment length polymorphisms (AFLP). The RAPD method employs short sequence RAPD primers to distinguish several *Meloidogyne* spp. on the basis of species characteristic patterns. These characteristic patterns can be harnessed to design species-specific markers and/or primers (SCAR-PCR) (Zijlstra, 2000; Fourie *et al*., 2001b). Using RFLPs, genomic (g) DNA is digested with restriction endonucleases followed by probe hybridisation to generate polymorphisms. AFLPs generate unique fingerprints of gDNA through selective PCR amplification of restriction digested gDNA fragments that have been ligated to specific adaptors. Microsatellites are high tandem repeats of short sequences which are often located in the heterochromatin, centromeric and telomeric regions of the chromosomes. Polymorphisms generated using microsatellites are due to differences in copy numbers or sequence lengths (Mestrovic *et al.*, 2006). The use of microsatellites (satDNA) to discriminate various *Meloidogyne* spp. has been explored in a number of studies (Castagnone-Sereno *et al*., 1993; Piotte *et al*., 1994; Mestrovic *et al*., 2006). Other gel-based methods previously used for identification of *Meloidogyne* spp. include RFLP and AFLP (Curran *et al*., 1986; Williamson *et al*., 1997; Fargette *et al*., 2005).

The advantages of gel-based methods are that they are simple to perform and they are not limited to a certain stage of development (Blok and Powers, 2009). Despite being simple, there are

some challenges, associated with these methods and these include; low sensitivity, poor band visibility in some cases, lack of reproducibility between different laboratories as well as the need to use high amounts of DNA to achieve desired results(Adam *et al*., 2007; Blok and Powers, 2009). A further challenge, especially in the case of RFLP and AFLP is the need to, at times, use radioactive materials. These challenges are by far not limited to Africa. This is a global problem that would require global co-operation between researchers for cross-validation of samples if these methods are to be used effectively. However, given the stated lack of financial and other resources, this is a particularly bigger challenge for African researchers as validation would incur huge costs of shipping DNA samples across the globe.

Sequenced-based diagnostics rely, not exclusively, on obtaining sequences of specific gene regions and comparing them with reference sequences deposited in public databases. Sequence-based methods include the use of mitochondrial DNA (mtDNA), ribosomal DNA (rDNA) (Blok and Powers, 2009).The mitochondrial DNA (mtDNA) is one of the regions usually targeted for identifying various *Meloidogyne* spp. (Hyman, 1990; Hugall *et al*., 1994; Hyman and Whipple, 1996). Multiple copies present in the mitochondrial DNA in each cell offer a ready template for PCR assays and other molecular studies (Brown *et al.*, 1979). The low level of recombination that is associated with the mitochondrial DNA coupled with high rates of evolution provides a unique region that has also been utilized for phylogenetic studies and in studying different *Meloidogyne* spp. (Blouin, 2002; Blok and Powers, 2009). The cytochrome oxidase subunit I (*COI*) within the mitochondrial DNA is currently being viewed as a potential gene that can be used in bar coding all *Meloidogyne* spp. and also in studying evolutionary trends and intra-specific variations within *Meloidogyne* populations (Powers, 2004; Blok, 2005). Based on this barcoding concept, other studies have suggested the clustering of nematodes into molecular operational taxonomic units (MOTUs) which have individuals with highly similar sequence homology. These sequences are based on a specific gene which may not necessarily be the *COI* of the mtDNA (Floyd *et al*., 2002).

Molecular sequence-based identification can also be based on the ribosomal DNA (rDNA) to identify various *Meloidogyne* spp. The 18S, 28S (26S), 5.8S coding genes, the internal transcribed spacer (ITS), the external transcribed spacer (ETS) and the intergenic spacer (IGS) regions are usually employed in diagnostics and phylogenetic studies (Blok *et al*., 1997). The repetitive nature of rDNA provides a better template for PCR work due to more variations among *Meloidogyne* spp. than other regions such as the D2-D3 (Palomares-Rius *et al.*, 2007). Variations in sequences occur between

regions of the rDNA that codes for 18S, 28S (26S) and 5.8S. Therefore these repetitions and sequence variations can be exploited for identification of *Meloidogyne* spp.

The use of sequence based methods coupled with depositing of these sequences into publicly available databases presents huge opportunities for many African researchers for the identification of *Meloidogyne* spp. The advantage being that comparisons do not require obtaining and shipping voucher specimens or reference DNA from all over the world since the analysis can be done using platforms linked to international databases through the internet. Therefore, this is also a huge benefit to other researchers who may identify new *Meloidogyne* spp. and may need to compare their unique sequences with those deposited in databases for similar *Meloidogyne* spp. identified in other countries.

Microarrays, although not yet widely adopted for identification of *Meloidogyne* spp., are also useful in *Meloidogyne* spp. diagnostics due to their potential to target various regions simultaneously. The major challenge with microarrays is that they are costly for non-established resource poor laboratories. There are also other global challenges associated with microarrays such as sensitivity and specificity which will need to be properly optimised before they can be widely used (Blok and Powers, 2009).

As molecular technology continue to advance, the entry of real time PCR (qPCR) has significantly improved identification of *Meloidogyne* spp. This method has increased sensitivity, specificity and simultaneous detection of more than one *Meloidogyne* spp. can be done within one qPCR assay. Besides this, the method can be performed within a short period due to the fact that there are no post PCR procedures. Apart from detection, qPCR can be used to quantify the amount of nucleic acid present and in genotyping through the generation of high resolution melt curves (HRMC) that are only specific to certain species (Bates *et al*., 2002; Holterman *et al*., 2012). To this end, some progress involving different *Meloidogyne* spp. has been made in trying to use this approach (Zijlstra and Van Hoof, 2006; Berry *et al*., 2008; De Weerdt *et al*., 2011; Holterman *et al*., 2012). All these studies have demonstrated that using qPCR in *Meloidogyne* spp. studies is specific and efficient.

Overall, gel-based methods for example SCAR-PCR provide a cost friendly and effective method of *Meloidogyne* spp. delineation. However, since there is a number of emerging *Meloidogyne* spp., it is difficult to say with certainty that a given method is capable of delineating all the *Meloidogyne* spp. alone. Several methods which are affordable, accurate, reproducible and widely adopted by other research laboratories should be considered too.

Management strategies

The ultimate goal of controlling various *Meloidogyne* spp. in the soil is to protect the crop from attack, cushion it from being predisposed to secondary infections and achieve maximum crop yield at the end of the growing season at a low cost (Coyne *et al*., 2006a; Norshie *et al*., 2011). Pest management strategies which have been adopted in most parts of Africa can be categorized broadly as cultural, biological or chemical. These are either practised singly or in combination to achieve desired results.

Chemical control methods

Chemical methods of control involve the application of different inorganic formulations to kill or interfere with the reproduction of *Meloidogyne* spp. in infested soils. In *Meloidogyne* spp. control programmes, nematicides are usually the most effective method of controlling high levels of *Meloidogyne* spp. in various farms. Nematicides containing active ingredients of methyl bromide and other harmful compounds have been banned in various parts of the world. Other nematicides which are known to control various *Meloidogyne* spp. include Fenamiphos, Oxamyl, 1, 3 dichloropropene (1, 3-D), Aldicarb, Dazomet and metam-sodium. Nematicides reduce high populations of various *Meloidogyne* spp. in the soil though they do not completely eliminate them particularly once the symptoms have started to be noticed (Sirias, 2011). They can be applied either as pre-plant nematicides, fumigants or as contact nematicides (Strajnar and Širca, 2011). Breaking of large lumps of soil, good soil humidity and removing crop remains of the previous season from the soil is very essential for these nematicides to work well.

The disadvantages of using chemical methods to control these *Meloidogyne* spp. is that, some of them are toxic to humans due to residues in the food chain, they contribute to environmental pollution through the pollution of the ozone layer (such as methyl bromide), they are expensive to small scale farmers and their continued use can lead to some level of resistance to the target nematode species. This resistance can be mainly as a result of mutation given the fact that the phylum Nematoda is associated with high evolution rates (Blouin *et al*., 1995).

Biological control methods

Biological methods entail the use of living organisms either in pure cultures or in mixtures to control *Meloidogyne* spp. Some biological products such as those developed by Pasteuria Inc., and Koppert Biological Systems against certain *Meloidogyne* spp. have demonstrated significant effect in

the control of these plant parasitic nematodes. These products are usually developed from micro-organisms such as *Pasteuria penetrans*, *Pasteuria hartismeri*, *Pochonia chlamydosporia*, *Bacillus firmus*, *Paecillomyces lilacinus* and *Trichoderma* spp., which attach to the nematode cuticle or parasitize the female eggs therefore killing the nematodes eventually (Kariuki and Dickson, 2007; Bishop *et al*., 2007). In addition, some studies have also shown another biological strategy where endophytes such as *Fusarium oxysporum* (FO162) can induce systemic resistance against *Meloidogyne* spp. in some crops such as tomato (Walters, 2009). Colonization of roots by *Fusarium oxysporum* (FO162) leads to the accumulation of root exudates in tomato roots which have a repelling effect on *M. incognita* (Mohamed, 2010).

Soil amendment procedures involving the application of organic materials (such as farm manure and extracts from Marigold (*Tagetes* species) to release toxic compounds that can kill plant parasitic nematodes have also been explored as a form of biological control (Mcsorley and Duncan, 1995). Antagonistic bacteria such as *Pseudomonas aeruginosa* in these decomposing organic materials either act as competitors or release metabolic toxins which may change the nature of root exudates (aimed at reducing the population of *Meloidogyne* spp. colonizing the roots) or kill various *Meloidogyne* spp. To achieve better results from soil amendments, organic materials should be applied at high rates to have a significant effect on nematode populations (Putten *et al*., 2006). In general, the use of organic material is not only cheap but also improves the efficiency of these antagonistic bacteria by offering them ready nutrients which are essential for their growth and survival.

Cultural control methods

Cultural practices include the development and use of resistant crop cultivars, planting clean planting materials, intercropping, crop rotation and cleaning of farm implements (Brown *et al*., 2006). Many of these practices have been used successfully in various parts of Africa to reduce the spread of *Meloidogyne* spp. in different crop fields for many years. However, the cost and availability of clean planting material can at times be a hindrance to many small scale growers. Furthermore, the limitation of employing crop rotation as a control strategy in commercial farms is that it is not economically feasible due to economic losses which may be incurred during the fallow periods and also in trying to establish a new crop in large scale as the previous one. Growth challenges associated with human population also make crop rotation virtually impractical in certain parts of the continent. Prior to use of methods such as crop rotation, the identity of *Meloidogyne* spp. should be understood, its host range and also the cropping history of the field evaluated. This is critical in decision making to avoid indiscriminate use of nematicides and also scale down management costs.

Physical methods such as heat treatment and solarization of the soil before planting can be combined with cultural methods for effective control of various *Meloidogyne* spp. (Ioannou, 2000). Solarization of nursery soil up to 40 cm for a period of three weeks has been found effective in reducing egg infectivity (Nico *et al*., 2003).

Resistant cultivars

The basis of using resistant cultivars to control *Meloidogyne* spp. relies on knowing exactly which species is being targeted. Several studies are underway to develop crops with resistance genes against various *Meloidogyne* spp. (Norshie *et al*., 2011). There are certain cases of known resistant crops as in the case of tomatoes (due to the *Mi-1* gene) and wild potato (*Solanum bulbocastunum*). Initially, the resistance gene (*Rmc-1*) located on chromosome 11 of wild potatoes was found to confer resistance against *M. chitwoodi* and other *Meloidogyne* spp. such as *M. fallax* and *M. hapla* (Gebhardt and Valkonen, 2001; Brown *et al*., 2006). But with the entry of resistance breaking *Meloidogyne* spp., some of the crops have been rendered susceptible (Janssen *et al*., 1998; Brown *et al*., 2009; Kiewnick *et al*., 2009). Future prospects of using resistant cultivars to manage *Meloidogyne* spp. require more research. Norshie *et al*. (2011), recently showed that certain potato lines are capable of resisting *M. chitwoodi* partially during infection. With the enormous amount of information being generated from the expressed sequence tags (ESTs), genome, transcriptome and proteomic sequences and attempts to introduce genes into plants to code for protein inhibitors such as chitinases, collagenases, cytotoxins, lectins and monoclonal antibodies against plant parasitic nematodes, it is hoped that an increase in transgenic crops with resistance to *Meloidogyne* spp. can be anticipated in the future (Fuller *et al*., 2008).

Resistant cultivars will not only reduce the cost of production but also safeguard the environment against pollution from chemical residues associated with nematicides. Resistance of various crops to *Meloidogyne* spp. infection is important since a resistant crop can allow little or no *Meloidogyne* spp. reproduction thus providing a better way of controlling nematodes in a crop field (Norshie *et al*., 2011). In order to achieve promising results with the use of resistant cultivars, there is need to constantly carry out accurate species identification and surveillance. It is also important to educate growers to try and contain resistance breaking *Meloidogyne* spp. such as *M. enterolobii* where they have been detected. Ultimately the cost and availability of resistant genotypes will be a huge influencing factor on whether these benefits will trickle down to small scale growers in Africa.

Concluding remarks

The recent identification of 'emerging' highly damaging and resistant *Meloidogyne* spp. in certain parts of Africa poses a considerable challenge to formulation of effective management strategies. Lack of accurate and current data on various *Meloidogyne* spp. present in each part of the continent and the polyphagous nature of these pathogens also poses a greater risk on the future of food production in Africa.

To adequately address these emerging and other *Meloidogyne* spp., it is imperative that resources are harnessed to drive more research aimed at assessing and understanding the species identity, genetic diversity, population structure, parasitism mechanisms and the overall threat posed by them (Fargette *et al*., 2010). Therefore there is need to embrace modern technology in conjunction with classical methods while carrying out *Meloidogyne* spp. identification.

It is imperative to have platforms that will allow those involved in research projects focussing on *Meloidogyne* spp. identification to share information with other projects targeting other phytoparasitic nematodes of economic importance. This is vital particularly in understanding the parasitism mode of these pathogens since they share some traits (Curtis, 2007). The training of more scientists in the field of plant parasitic nematodes and molecular biology should also be a key priority to various agricultural stakeholders if at all food sustainability and income generation is to be achieved (Barker *et al*., 1994; Barker, 2003).

To effectively manage these highly damaging pathogens and other *Meloidogyne* spp., application of biological, cultural and chemical methods should be done in line with integrated pest management (IPM) practices. This should be preceded with a thorough survey of farms in context and an accurate diagnosis of *Meloidogyne* spp. present. Molecular based methods of diagnosis should be used together with classical methods for accurate identification (Oliveira *et al*., 2011). This will lead to gradual management of *Meloidogyne* spp. and finally reduction in the high damage that they cause on various crops. This strategy will eventually benefit growers and avoid high costs of production.

With the phasing out of various effective nematicides such as methyl bromide, the search for effective and environmental friendly alternative methods should be pursued. At the same time more robust diagnostic techniques should be adopted to correctly identify and avoid further spread of the highly damaging, resistance breaking and emerging *Meloidogyne* spp. Growers should also be

educated on proper phytosanitary procedures to avert the introduction of *Meloidogyne* spp. into their farms.

Acknowledgement

Authors will wish to thank the Nematology Unit, ARC-PPRI for the use of data from the South African Plant-Parasitic Nematode Survey (SAPPNS) database.

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