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Tomato (*Solanum lycopersicum*) and root-knot nematodes (*Meloidogyne* spp.) – a century-old battle

Awol SEID [1,2,*], Chemeda FININSA [1], Tesfamariam MEKETE [3], Wilfrida DECRAEMER [2,4] and Wim M.L. WESEMAEL [2,5,6]

[1] *Haramaya University, College of Agriculture and Environmental Sciences, School of Plant Sciences, Plant Protection Program, P.O. Box 138, Dire Dawa, Ethiopia*
[2] *Ghent University, Faculty of Sciences, Department of Biology, Nematology Unit, Ledeganckstraat 35, B-9000 Ghent, Belgium*
[3] *University of Florida, Entomology and Nematology Department, Bldg. 970, Natural Area Drive, Gainesville, FL 32611, USA*
[4] *Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium*
[5] *Institute for Agricultural and Fisheries Research (ILVO), Burg Van Gansberghelaan 96, B-9820 Merelbeke, Belgium*
[6] *Ghent University, Faculty of Bioscience Engineering, Laboratory for Agrozoology, Coupure links 653, B-9000 Ghent, Belgium*

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Summary – The encounter between *Meloidogyne* species and tomato is many centuries old. *Meloidogyne* species are known to cause high levels of economic loss worldwide in a multitude of agricultural crops, including tomato. This review was initiated to provide an overview of the damage potential of *Meloidogyne* spp. on cultivars of tomato (*Solanum lycopersicum*), and to compile the different studies done on the management of *Meloidogyne* spp. on tomato with particular emphasis on the *Mi* resistance gene. Numerous studies have been conducted to assess the damage potential of root-knot nematode on various tomato cultivars; its yield loss potential ranges from 25 to 100%. A range of management options from using synthetic nematicides to soilless cultures have been tried and are available for managing *Meloidogyne* spp. Resistant commercial cultivars and rootstocks carrying the *Mi* gene have been used successfully to manage *Meloidogyne incognita*, *M. javanica* and *M. arenaria*. However, virulent populations have been detected. Relying on a single root-knot nematode management strategy is an outdated concept and different management options should be used in an integrated management context by considering the whole system of disease management. In future management of *Meloidogyne* species, care must be taken in directly extrapolating the tolerance limit determined elsewhere, since it is affected by many factors such as the type of initial inoculum and physiological races of *Meloidogyne* spp., environmental conditions, types of cultivars and experimental approaches used.

Keywords – damage potential, durability, management, *Mi* gene, nematode control, pest management.

Tomato (*Solanum lycopersicum*) belongs to the Solanaceae family. It is native to South and Central America. Tomato is a popular vegetable crop worldwide and it is grown on more than 5×10^6 ha with a production of approximately 161×10^6 metric tons. Africa and Asia account for more than 80% of the global tomato area, with about 70% of world output (FAO, 2012). It is ranked first in the world for vegetables and accounts for 14% of world vegetable production (US$ 1.6 billion market value) (FAO, 2010). Tomato is a rich source of micronutrients such as minerals, vitamins and antioxidants for a well-balanced human diet. It also contains high levels of lycopene, an antioxidant that reduces the risks associated with several cancers and neurodegenerative diseases

* Corresponding author, e-mail: awolseid07@gmail.com

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(Giovannucci, 1999). Apart from being an important food crop, tomato is an acknowledged model species for evolutionary studies and research on fruit development and metabolite accumulation.

There are many pests and diseases damaging both the quality and quantity of tomato production. Plant-parasitic nematodes are one of them. They represent an important constraint on the delivery of global food security. Damage caused by plant-parasitic nematodes has been estimated at US$ 80 billion per year (Nicol *et al.*, 2011). This is likely to be a significant underestimate of the actual figure as many growers in developing nations are unaware of the existence of plant-parasitic nematodes (Jones *et al.*, 2013). One of the major obstacles to the production of adequate supplies of food in many developing nations is damage caused by *Meloidogyne* spp. (Sasser, 1980). It is generally admitted that four major species, *i.e.*, *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla*, as well as a few emerging species such as *M. enterolobii* and *M. chitwoodi*, cause the vast majority of crop damage (Moens *et al.*, 2009). *Meloidogyne* species cause high levels of economic loss in a multitude of agricultural crops worldwide with dramatic yield losses being reported on vegetables in tropical and sub-tropical agriculture (Sikora & Fernandez, 2005). They are impacting both the quality and quantity of marketable yields. Next to direct losses due to nematode attacks, many indirect losses through loss of irrigation water and fertilisers can occur since damaged roots do not utilise water and fertiliser as efficiently as healthy roots (Mai, 1985). In addition, root-knot nematodes interact with other plant pathogens, resulting in increased damage caused by other diseases, affecting world food supplies (Sasser, 1980).

Numerous studies have been conducted to determine the damage potential of *Meloidogyne* species on several vegetable crops including tomato, and different management strategies have been proposed. With the phase-out of methyl bromide, in particular, the problem of *Meloidogyne* spp. on tomato gained new interest. However, these studies were not compiled and presented in a way to help different stakeholders. Thus, the objectives of this review are: *i*) to provide an overview of the damage potential of *Meloidogyne* spp. on tomato cultivars; and *ii*) to compile different studies on the management of *Meloidogyne* spp. on tomato with particular emphasis on the *Mi*-resistant gene.

Root-knot nematodes on tomato

Tomato is often referred to as a universal host for *Meloidogyne* species. However, as of August 2015, there were 101 described species in the genus and tomato is a non-host for several of them. Most likely the term 'universal host' comes from the fact that the economically most important species reproduce well on tomato. This was also shown in the North Carolina Differential Host Range test. Reports on *Meloidogyne* spp. infecting tomato plants date back to the end of the 19th century. In the botanical garden of Pavia (Italy), tomato plants showed severe galling on the root system and after investigation this was ascribed to *Heterodera radicicola* (Cavara, 1895), a former name for root-knot nematode (most likely *M. javanica*). During the same period, similar symptoms were observed on tomatoes in a vegetable garden in the Sahara (Cavara, 1895). In 1889, 'exceptionally knotty' tomato roots were found near an agricultural experiment station in Auburn, Alabama (USA). Similar symptoms were observed on the roots of various plants. In a bulletin following these observations, *Heterodera radicicola* was identified as being the cause of the symptoms (Atkinson, 1889). Since then, many reports of root-knot nematodes on tomato have become available and at present we know that several species can cause severe damage to the crop.

Damage and yield losses of tomato due to *Meloidogyne* species

Root-knot nematodes can cause severe damage to the roots of tomato. Symptoms are more prevalent with tropical species compared to temperate root-knot nematodes (Fig. 1). Tomato cultivars have different degrees of susceptibility towards different *Meloidogyne* spp. Damage and yield loss studies conducted so far have shown a considerable difference in degree of susceptibility among tomato cultivars. Moreover, different populations of the same species of *Meloidogyne* even exhibit different degrees of pathogenicity on a specific tomato cultivar. A tomato cultivar that is absolutely susceptible to one population may be moderately resistant to another population of the same species. Several studies report the damage potential of different *Meloidogyne* spp. on different tomato cultivars under pot, microplot and field experiment conditions throughout the world. Experiments were done in different conditions and localities with different experimental approaches, making it difficult to extrapolate the results. Many factors affect the results. These include:

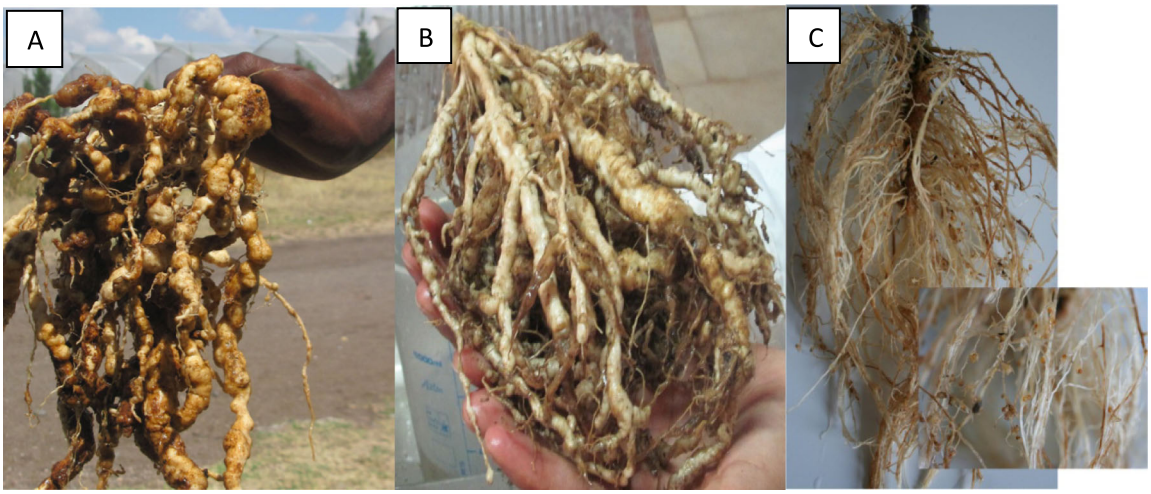

Fig. 1. Damage caused by tropical *Meloidogyne* spp. on roots of tomato (*Solanum lycopersicum*) from different parts of Ethiopia (A, B) and by *M. chitwoodi* on tomato from a pot test (C). This figure is published in colour in the online edition of this journal, which can be accessed *via* http://booksandjournals.brillonline.com/content/journals/15685411.

i) differences in laboratory extraction techniques and efficiency; *ii*) variations in soil type and environmental conditions that may affect nematode population development; *iii*) differing levels of resistance or tolerance among crops or crop varieties to be tested and cropping systems; *iv*) nematode species and population levels or inoculum densities; and *v*) inoculum types and inoculation techniques used (Greco & Di Vito, 2009; Nyczepir & Thomas, 2009). A frequently cited average yield loss due to *Meloidogyne* spp. is 10% (Koenning *et al.*, 1999). Nevertheless, much higher percentages have been documented (Table 1) in different regions, depending on population level, genus, frequency of infestations and crop species. Yield losses of 22-30% have been reported on tomato due to *M. incognita* (Sasser & Carter, 1985). In Western Anatolia (Turkey) *Meloidogyne* spp. caused up to 80% yield losses in processing tomato-growing areas (Kaşkavalci, 2007). In north-eastern Spain, an initial population density in soil of 4750 juveniles 250 cm^{-3} of *M. javanica* caused a 61% yield reduction in tomato cropped in summer plastic houses (Verdejo-Lucas *et al.*, 1994).

Management options

Control refers to specific acts designed to reduce the number of nematodes, while management has the objective of minimising economic losses and considers the whole system of care and treatment of crop pests (Hooper & Evans, 1993). Different management options that are commonly used for plant-parasitic nematodes are applicable for *Meloidogyne* spp. on tomato as presented below.

Prevention

Quarantine

Quarantine strategies are considered a preventive and not a curative approach in stopping the introduction and/or increased dissemination of economically important nematodes into a new site (Nyczepir & Thomas, 2009). With the aim of reducing the adverse impact of *Meloidogyne* spp. on agricultural crops, phytosanitary measures have major importance especially for resource poor farmers (Coyne *et al.*, 2009). To avoid introduction of *Meloidogyne* spp. into a field, awareness and regulation are required (Wesemael *et al.*, 2011). New nematode species to a country are often first intercepted by quarantine and inspection services, which assist in preventing the unintended spread of species to new areas (Coyne *et al.*, 2009). The four major root-knot nematode species, *M. arenaria*, *M. incognita*, *M. javanica* and *M. hapla*, are generally not regulated because these species of economic concern are distributed globally. The temperate root-knot nematodes, *M. chitwoodi* and *M. fallax*, are quarantine organisms in the EU (EC Directive 2000/29/EC) and the (sub)tropical *M. enterolobii* is placed on the EPPO A2 list as recommended for regulation as a quarantine pest (EPPO, 2014). The latter is known to be highly aggressive and able to

Table 1. Damage potential of the major root-knot nematodes (*Meloidogyne* spp.) on different tomato cultivars.

Species	Cultivar	Cultivar info	ET	YLP (%)	TL* (J2 cm^{-3} soil)	IP*	ST	Reference
M. hapla	Veebrite	NM	Microplots	40	2.6	0, 0.34, 2.39, 7.96, 36.35 J2 cm^{-3} soil	3	Potter & Olthof (1977)
M. hapla	Manapal	NM	Microplots	50	0.04	0, 0.4-1.6, 2-4.4, 8-16 eggs + J2 cm^{-3} soil	2	Barker *et al.* (1976)
M. hapla	Campbell 135	Susceptible	Field	NS	–	0, 0.29, 2.58, 22.94 J2 cm^{-3} soil	4	Sayre & Toyana (1964)
M. incognita	Manapal	NM	Microplots	85	0.04	0, 0.4-1.6, 2-4.4, 8-16 eggs + J2 cm^{-3} soil	2	Barker *et al.* (1976)
M. javanica	Campbell 135	Susceptible	Field	NS	–	0, 0.29, 2.58, 22.94 J2 cm^{-3} soil	4	Sayre & Toyana (1964)
M. incognita	Chico III	Susceptible	Field	100	4	0, 0.125, 0.25, 0.5, 1, 2, …, 256 eggs + J2 cm^{-3} soil	1	Di Vito *et al.* (1981)
M. incognita	U.C.105J	Susceptible	Pots	100	4	0, 0.25, 0.5, 1, 2, …, 512 eggs + J2 cm^{-3} soil	1	Di Vito & Ekanayake (1984)
M. incognita	IAS-1	Resistant	Pots	60	4	0, 0.25, 0.5, 1, 2, …, 512 eggs + J2 cm^{-3} soil	1	Di Vito & Ekanayake (1984)
M. incognita	Ventura	Susceptible	Field	100	0.55	0, 0.031, 0.062, 0.125, 0.25, …, 128 eggs + J2 cm^{-3} soil	1	Di Vito *et al.* (1991)
M. incognita	DISA N	Resistant	Field	30	0.55	0, 0.031, 0.062, 0.125, 0.25, …, 128 eggs + J2 cm^{-3} soil	1	Di Vito *et al.* (1991)
M. incognita	Shaft-Falat	NM	Pots	ND	0.33	0, 0.33, 0.65, 1.3, 2.6, 5.2 eggs + J2 cm^{-3} soil	2	Hamzehzarghani *et al.* (2012)
M. incognita	Pusa Ruby	Susceptible	Pots	25	–	1.3 J2 cm^{-3} soil	–	Ganaie & Khan (2011)
M. javanica	Ramon	Resistant	Microplots	29	–	10 000 eggs cm^{-3} soil	1	Ornat *et al.* (2001)
M. javanica	Cobra	Susceptible	Microplots	29	–	10 000 eggs cm^{-3} soil	1	Ornat *et al.* (2001)
M. javanica	Marglobe	Susceptible	Pots	60	0.28	0, 1, 2, 4, 8, 16 J2 cm^{-3} soil	1	Mekete *et al.* (2003)
M. javanica	Shaft-Falat	NM	Pots	ND	0.33	0, 0.33, 0.65, 1.3, 2.6, 5.2 eggs + J2 cm^{-3} soil	2	Hamzehzarghani *et al.* (2012)
Meloidogyne spp.	Asesewa	NM	Field	95.2	–	Natural infested field	2	Hemeng (1980)
Meloidogyne spp.	St. Peters	Resistant	Field	100	–	Natural infested field	2	Hemeng (1980)

NM: the information is not mentioned in the original article; ET: experiment type; YLP: yield loss potential; TL: tolerance limit; IP: initial population or inoculum density; ND: not determined; NS: not significant; ST: soil type: 1 = sandy soil, 2 = sandy loam, 3 = loam, 4 = clay soil.

* Original data were recalculated to cm^3 assuming an average soil density of 1.3 g cm^{-3}.

break the *Mi* resistance in tomato and *N* and *Tabasco* resistance in pepper (Fargette *et al.*, 1994; Brito *et al.*, 2007; Kiewnick *et al.*, 2009).

Sanitation

Sanitation is important to prevent new infestations (introduction into a production site) and to avoid secondary infestations (spreading within the production site). Human activities, such as the transport of infested planting material, soil, plant debris and irrigation water, can provide transfer channels between contaminated and healthy areas and easily spread *Meloidogyne* spp. (Collange *et al.*, 2011). For tomato production in protected cultures and in the open field, introduction with planting material poses a risk. Transplants are mostly provided in growth media that are free of pests and diseases, and should be obtained from reliable nurseries and, if possible, certified nematode-free plants should be used. At the farm level, cleaning all agricultural machinery and tools can avoid transporting plant-parasitic nematodes with the soil. Irrigation water can also be a source of nematode infection or a means to spread it within the field (Hugo & Malan, 2010). Due to environmental concerns and reduced water availability, closed systems (= recycling of irrigation water) are preferable. Proper sanitation of this water is of paramount importance to avoid the spread of pests and diseases. Moens & Hendrickx (1990) showed that *M. incognita* present in drainage water could re-infect tomato plants. Potential and available control measures for plant-parasitic nematodes in irrigation water are chlorination, electrical discharge, filtration, heat treatment, hydrogen peroxide, ozonation, sedimentation and flocculation and UV radiation. However, each treatment comes with advantages and disadvantages (Hugo & Malan, 2010).

Physical soil treatments

Steam heat and solarisation

The effectiveness of soil solarisation and steam heat in managing *Meloidogyne* spp. under field and glasshouse conditions is dependent on soil temperature (Nyczepir & Thomas, 2009). A soil temperature considered sufficient to control plant-parasitic nematodes is 45°C (Sikora & Fernandez, 2005). Lethal effects on eggs and second-stage juveniles of *M. incognita* have been observed below 45°C when nematodes were exposed to sub-lethal temperatures for a sufficient period of time (Wang & McSorley, 2008). In Florida, solarisation of a fine sandy soil for 3 months (July-September) suppressed *M. incognita* populations in tomato fields, resulting in increased yields (Overman & Jones, 1986). However, solarisation is more suited for annual crops, nurseries and raised beds (McGovern *et al.*, 2002). Disadvantages limiting the use of solarisation for the control of *Meloidogyne* spp. include the non-specificity (McSorley, 1998), the duration of time needed, decreasing efficacy with increasing soil depth below 5 cm and the size of the area to be treated (Nyczepir & Thomas, 2009).

The extensive use of steam heat in glasshouse conditions as a means to manage plant-parasitic nematodes has been limited in recent years, due to the high cost of heating fuel (Viaene *et al.*, 2013), non-specific effects on non-target (beneficial) microorganisms, possible emission of phytotoxic chemicals into treated soil and change in soil pH (Nyczepir & Thomas, 2009).

Flooding

Flooding and bare fallow treatments lowered soil populations of the four major *Meloidogyne* species. Rhoades (1982) reported that flooding reduced the density of *M. incognita* but the optimal duration of flooding depended on air temperature. Alternating drying cycles and flooding appeared to be more effective than prolonged flooding (Noling & Becker, 1994). However, a 3-week flooding period followed by a 5-week drying and a second 3-week flooding period in winter was not successful to control root-knot nematodes on tomato in Florida (Nelson *et al.*, 2002). Time duration and salinity problems limit the use of flooding in tomato production.

Rotation

In general, a rotation of a minimum 3 years is recommended for tomato to reduce pests and diseases. Due to the wide host range of several important root-knot nematode species, rotation options are limited. Rotation with corn and velvet bean reduced *M. incognita* population levels and increased tomato yield in a field in Puerto Rico (Acosta *et al.*, 1991). Rotation with *Mi* gene cultivars does not imply changes in farming systems or market supply (Ornat & Sorribas, 2008) and can be a solution for intensive tomato production.

Organic amendments

Organic amendments cover several sources and products, including green manures from cover crops or crop residues, industrial or town waste, animal manures, composted or not composted. They are incorporated into the soil or applied on top of the soil as mulches. In general,

soil amendments improve the nutrient and water holding capacity of the soil, improve soil fertility and structure, reduce erosion and release specific compounds that may be nematicidal and stimulate microbial activity in the soil (Akhtar & Malik, 2000; Oka, 2010; Thoden *et al.*, 2011). The results of studies on organic amendments to control root-knot nematodes are not straightforward. Biofumigation with *Brassica juncea* and *Eruca sativa* showed promising results both in increased yield of tomato and reduction of *M. incognita* population in Italy (Colombo *et al.*, 2008). By contrast, Noling & Gilreath (1999) found no reduced levels of *M. incognita* in amended plots compared to an untreated control, and lower tomato yields than in fumigated plots. There are studies that show increased root-knot nematode populations after application of the amendment (Thoden *et al.*, 2011). This gave rise to the hypothesis that interactions between several factors may contribute to the results, including the dosage of organic amendment and the number of application years, the chemical characteristics of different products, such as release of nematotoxic compounds, physiological stages of the incorporated plant tissues, compost maturity and decomposition stage of organic matter, C/N ratios of the organic amendment and soil infestation level, and nematode community structures.

Chemical control

Historically, chemical control has been the most important strategy to reduce *Meloidogyne* populations (Nyczepir & Thomas, 2009). According to Talavera *et al.* (2012), 78.3% of the farm advisors in south-eastern Spain mentioned chemical soil fumigation as the most commonly used management method for root-knot nematodes, followed by non-fumigant nematicides (59.8%). A combination of nematicides with soil solarisation and grafting on resistant rootstocks were considered to be the most effective methods of root-knot nematode management. Increasing environmental and health concerns resulted in the ban of methyl bromide, and chemical nematicides are being discouraged specifically as a sole management method. However, new generations of less harmful nematicides are becoming available as a result of renewed interest by the crop protection industry (Desaeger, 2014).

Biological control

Natural enemies are promising for root-knot nematode control. Several fungi and bacteria have been identified and classified based on their nematophagous and antagonistic characteristics, respectively. Nematophagous fungi include trappers, endoparasites, egg parasites and toxin producers. The egg-parasitising *Purpureocillium lilacinum* was reported to reduce *M. javanica* and *M. incognita* on tomato crops (Goswami *et al.*, 2006; Kumar *et al.*, 2009) but results have been difficult to reproduce (Hallmann *et al.*, 2009). A single pre-plant application of the fungus *P. lilacinum* strain 251 reduced root galling of *M. incognita* on tomato by 66% and egg mass formation by 74%, and also for *M. hapla* sufficient control was achieved on tomato (Kiewnick & Sikora, 2006). This strain of *P. lilacinum* has been commercialised in several countries. A one-off application of *Pochonia chlamydosporia* was able to slow down the build-up of *M. javanica* for at least 5-7 months in tomato and lettuce rotations in glasshouses (Van Damme *et al.*, 2005). However, Tzortzakakis & Petsas (2003) reported that *P. chlamydosporia* did not show any effect on *M. javanica* on tomato in glasshouse studies in Greece, and also in a double cropping system of lettuce and tomato in Spain *M. javanica* could not be controlled (Verdejo-Lucas *et al.*, 2003). *Aspergillus* spp. and *Trichoderma* spp. have shown potential to reduce populations of *M. incognita* on tomato (Goswami & Mittal, 2004; Goswami & Tiwara, 2007; Affokpon *et al.*, 2011a). When inoculation of arbuscular mycorrhizal fungi (AMF) was done 3 weeks before *M. incognita* inoculation, tomato plants were protected against *M. incognita* and its reproduction reduced (Talavera *et al.*, 2001). In a split-root experiment, Dababat & Sikora (2007) showed that *Fusarium oxysporum* Fo162 induced systemic resistance in tomato against *M. incognita*. In Benin (West Africa), a field application of AMF on a *Meloidogyne*-infested field increased tomato yields by 26% compared to the non-AMF control treatment (Affokpon *et al.*, 2011b). Important for control with AMF is successful root colonisation before nematode attack (Talavera *et al.*, 2001; Hallmann *et al.*, 2009).

The obligate endoparasitic bacteria *Pasteuria penetrans* effectively parasitised *M. incognita* in rotations that included tomato, eggplant and common beans or cabbage (Amer-Zareen *et al.*, 2004). In a *M. incognita*-infested microplot the application of 5×10^{10} spores m^{-2} increased tomato yield by 46% (Talavera *et al.*, 2002). The efficacy of *P. penetrans* depends on soil conditions, temperature and nematode age (Talavera & Mizukubo, 2003). Moreover, its host specificity requires mixtures to enable proper management of mixed *Meloidogyne* infestation (Hallmann *et al.*, 2009). *Streptomyces* spp. are important producers of antibiotics. Avermectins, which are produced by them, were found to have strong nematici-

dal effects (Hallmann *et al.*, 2009). Bélair *et al.* (2011) showed in a glasshouse bioassay that a combined soil treatment with *Streptomyces* and chitin reduced *M. hapla* populations and galls on tomato. However, the high cost of the soil treatments and variability in the results prevent the use as an alternative control method. Seed treatments proved to be successful to manage *M. incognita*, *M. arenaria* and *M. javanica* on tomato (Cabreira, *et al.*, 2009) and might be more promising compared to soil treatments. However, biological control agents alone rarely provide adequate management and should be integrated with other management methods such as resistant cultivars, crop rotations, trap crops or antagonistic plants, either to promote the establishment of biological control agents or to reduce nematode populations in the soil (Viaene *et al.*, 2013).

Soilless culture systems

Soilless culture is a good alternative to soil-based culture particularly in glasshouse vegetable production. The use of soilless culture systems as a management strategy for plant-parasitic nematodes has long been tried. It is widely practised because it is more practical and cheaper than repeated soil fumigation (Hochmuth & Hochmuth, 2012). However, the development from growing plants in field soil to soilless culture systems has not resulted in the eradication of problems caused by plant-parasitic nematodes (Hallmann *et al.*, 2005; Ornat & Sorribas, 2008). *Meloidogyne incognita* and *M. arenaria* were found on roses grown in soilless culture in Sicily (D'Errico & Ingenito, 2003) and *M. hapla* was found in rock wool and coconut-peat cultures of roses in Germany (Hallmann *et al.*, 2004; Ornat & Sorribas, 2008). Almost all commonly used substrates are suitable for nematode infestation (Stapel & Amsing, 2004), and the most common sources of nematode infestation are infested planting material and irrigation water (Hallmann *et al.*, 2005). Control of plant-parasitic nematodes in soilless culture systems is extremely difficult. Nevertheless, heat treatment of circulation water (Evans, 1991; Runia & Amsing, 2001a, b), ultra violet radiation and filtration (Moens & Hendrickx, 1989; Amsing & Runia, 1995), resistance, plant growth management, avoidance of nematode infestation, routine monitoring of planting material and recirculation water, and the use of certified planting material can substantially reduce nematode problems (Hallmann *et al.*, 2005). In organic farming, hydroponics and the use of inorganic growing media are not allowed.

Resistant cultivars

Resistant cultivars are an economical and environmentally safe method for controlling *Meloidogyne* species. They are cultivated with a dual purpose; to reduce nematode population levels and to avoid crop damage by nematodes. Therefore, resistant cultivars also need to be tolerant to *Meloidogyne* species. It is particularly important for organic farming or integrated production since these systems do not allow, or they restrict, the use of chemical control (Ornat & Sorribas, 2008). Resistant cultivars do not require significant changes in farming operations or in market supply (Ornat & Sorribas, 2008).

Resistance against *Meloidogyne* spp. has been reported in many agricultural crops (Cook & Starr, 2006; Starr & Mercer, 2009) but is not often used (Cook, 2004; Wesemael *et al.*, 2011). Tomato is one of the few crops in which *Meloidogyne* resistance has been widely used, and commercial resistant cultivars and rootstocks are available for tomato (Ornat & Sorribas, 2008). Resistance against *M. incognita*, *M. javanica* and *M. arenaria* has been developed in widely used tomato cultivars bearing the *Mi* gene (Ornat *et al.*, 2001). Fruit yields of the susceptible tomato cv. Blitz were higher when grafted on cvs Beaufort and Hypeel45 tomato rootstocks carrying the *Mi* gene and inoculated with different populations of *M. incognita* (Lopez-Perez *et al.*, 2006). Nematode-resistant tomato rootstocks can be used for grafting desirable tomato scions. However, expression of resistance is affected by different factors such as soil temperature, species and populations of *Meloidogyne*, *Mi* dosage and tomato genetic background (Ornat & Sorribas, 2008). Thus, tomato cultivars should be carefully chosen, particularly when they are followed by a nematode-susceptible crop (Lopez-Perez *et al.*, 2006). The use of the *Mi* gene and its limitations are discussed below.

Integrated nematode management

The primary aims of integrated nematode management are to improve crop yield using a combination of management options, thereby targeting a key nematode species such as *Meloidogyne* species (Nyczepir & Thomas, 2009), and consideration of the ecosystem. The decision as to which management options will be part of the integrated nematode management strategy is governed by many factors such as *Meloidogyne* species present, perennial *vs* annual crops, economics, technology and societal considerations (Nyczepir & Thomas, 2009). To develop an effective integrated nematode management strategy, knowledge is

needed on plant damage or crop loss caused by resident *Meloidogyne* species on the crop(s) that will be produced, population densities and population dynamics of root-knot nematode populations with and without the use of control measures, and the economic consequences associated with different control methods (McSorley & Phillips, 1993). In integrated nematode management strategies there are interactions within a soil system, among management options, and among microorganisms. According to Collange *et al.* (2011), there are at least four main processes for controlling *Meloidogyne* species using an integrated approach: killing nematodes in the soil with thermal or chemical agents, breaking the nematode biological cycle to limit or delay reproduction sequences, enhancing the competitions from other microorganisms in the soil to reduce nematode populations by predation, trophic competition, or parasitism, and limiting dissemination from a contaminated to an uncontaminated area.

GENETICS-BASED MANAGEMENT

Brief history of Mi *gene from where to where?*

Resistance in tomato to root-knot nematodes was first observed in the wild species *Lycopersicon* (the genus *Lycopersicon* is now a synonym of *Solanum*) *peruvianum* Mill. P.I.128657 by Bailey (1941). It was later introgressed into the cultivated *S. lycopersicum* (Smith, 1944) and has proved useful in the management of *M. arenaria*, *M. incognita* and *M. javanica* (Roberts, 1992), the aphid (*Macrosiphum euphorbiae*) (Rossi *et al.*, 1998) and *Bemisia tabaci* biotypes Q (Nombela *et al.*, 2001) and B (Jiang *et al.*, 2001). Its resistance against *M. incognita* gave it its name – *Mi* gene (Williamson, 1998). *Mi* cultivars of tomato were introduced in the 1980s and have gained importance ever since. In California, USA, the majority of field-grown processing tomatoes have the *Mi* gene (Cook, 2004; Williamson and Roberts, 2009).

Structure and mechanism of action of the Mi *gene*

Although the exact numbers of responsible genes are unknown (Sidhu & Webster, 1975; Roberts *et al.*, 1990), the resistance in tomato cultivars against *Meloidogyne* species is thought to be controlled by a single dominant gene (Gilbert & McGuire, 1956; Roberts & Thomason, 1989; Messeguer *et al.*, 1991). The *Mi* gene was mapped to the short arm of tomato chromosome 6 near the centromere (Kaloshian *et al.*, 1998). It belongs to the NBS-LRR group of genes, which are characteristic of a family of plant proteins, including several that are required for resistance against bacteria, fungi and viruses (Milligan *et al.*, 1998). Two homologues of this gene, *Mi-1.1* and *Mi-1.2*, conferred resistance in an experimental assay (Milligan *et al.*, 1998). The functional *Mi-1.2* gene is referred to as '*Mi*'. *Mi*-mediated resistance triggers a hypersensitive reaction (Dropkin, 1969a) that involves cellular disorganisation, localised host-cell necrosis and restricted nematode development at the infection site near the vascular bundle. The tomato *Mi* resistance gene confers resistance, but not immunity, to *M. arenaria*, *M. incognita* and *M. javanica* (Roberts & Thomason, 1989), since a few juveniles are able to infect roots, but they develop slowly, resulting in a reproduction rate smaller than on susceptible cultivars (Talavera *et al.*, 2009). The same phenomenon was reported on alfalfa (Griffin & Elgin, 1977) and soybean (Pedrosa *et al.*, 1996). More detailed information about the structure and function of the *Mi* gene is given by Williamson (1998) and Williamson & Roberts (2009).

Effectiveness and profitability of the Mi *gene*

The *Mi* gene has been incorporated into many commercially available tomato cultivars (Devran *et al.*, 2010) and is used against root-knot nematodes in home gardens, tomatoes for the fresh market and processing tomato cultivars (Roberts & Thomason, 1989). For over 60 years it has been the only source of resistance in all commercial tomato cultivars and it has been effective for root-knot nematode management, especially when used in combination with other management techniques such as rotation and sanitation (Roberts, 1992). In successive field trials, the resistant cvs PSR 8991994 and Sanibel greatly suppressed root galling and *M. javanica* populations; fruit weight, number of fruit and weight per fruit as compared to the susceptible cv. Colonial increased significantly (Rich & Olson, 1999). Cultivars Monika (*Mi*-resistant) and Durinta (susceptible) tomatoes were cropped for three consecutive seasons in non-fumigated soil and soil fumigated with methyl bromide at 75 g m^{-2} and at a cost of € 2.44 m^{-2} to determine the effectiveness and profitability of the *Mi* gene. Growth of cv. Monika increased profits by € 30 000 ha^{-1} compared with cv. Durinta in non-fumigated soil (Sorribas *et al.*, 2005a). The resistant cv. Monika increased yield with 5.6, 4.4 and 4.7 kg m^{-2} after one, two and three consecutive crops, respectively, compared with the susceptible cv. Durinta in nematode-infested soil. The use of tomatoes with the *Mi* resistance gene was economically justified based on its cost efficacy (Sorribas *et al.*, 2005b). A cropping cycle with *Mi* tomato genotypes reduced initial population density for the next crop, and the effect was similar to the use of

nematicides on a susceptible crop (Tzortzakakis *et al.*, 2000; Talavera *et al.*, 2009). Maleita *et al.* (2011) reported that cv. Rapit can be used to control the three most common *Meloidogyne* spp. and inhibit the increase of *M. hispanica* populations. Four crop rotations including the *Mi*-resistant tomato cv. Monika and the susceptible cv. Durinta were assessed for three consecutive cropping seasons in three unheated plastic houses located in different parts of Spain. The *Mi*-resistant cv. Monika suppressed *M. javanica*, *M. arenaria* and *M. incognita* populations by more than 90% compared with the susceptible cv. Durinta. Substantial yield increase (+2.6 kg m^{-2} in the rotation including at least one resistant tomato cultivar and +6.1 kg m^{-2} when the resistant cultivar was cropped for 2 consecutive years) was only achieved when initial nematode populations were high and with suitable agroclimatic conditions for the resistant tomato cultivar (Talavera *et al.*, 2009). After growing a tomato cultivar with *Mi* in *M. javanica*-infested fields, yield losses of the succeeding cucurbit crop were significantly reduced (Ornat *et al.*, 1997) and yield was similar to two treatments with fenamiphos on susceptible tomato (Tzortzakakis *et al.*, 2000).

Limitations of the Mi *gene*

Despite its effectiveness and profitability, the resistance conferred by the *Mi* gene has some critical limitations. Planting a resistant crop for several consecutive years can increase the risk of selection of virulent nematode populations. This has been reported for the *Mi* gene in Morocco after 3-8 years (Eddaoudi *et al.*, 1997), in Florida, USA, after five consecutive resistant tomato crops (Noling, 2000), and in Spain after three cropping cycles of resistant tomato rootstocks (Verdejo-Lucas *et al.*, 2009). Meher *et al.* (2009) showed that continuous growing of 13 resistant tomato cultivars during 10 years resulted in a 6.6% higher infection by *M. incognita* compared with a susceptible control. The presence of naturally occurring resistance-breaking populations has also been reported (Roberts, 1992; Ornat *et al.*, 2001; Maleita *et al.*, 2011). In Spain, 48% of 29 field populations of *Meloidogyne* spp. were found virulent against the *Mi* gene (Verdejo-Lucas *et al.*, 2012). It was unclear if the presence of virulent populations was due to selection pressure by repeated cultivation of resistant tomato cultivars. Virulent *Meloidogyne* spp. have been found in most tomato-growing areas (Castagnone-Sereno, 2006). Resistance mediated by *Mi* is broad with its effect on the tropical species *M. arenaria*, *M. incognita* and *M. javanica*. However, it is not effective against the aggressive *M. enterolobii* (Kiewnick *et al.*, 2009) and the temperate *M. hapla* and *M. chitwoodi* (Brown *et al.*, 1997; Liu and Williamson, 2006), all species that are known to infect tomato. Another constraint for the *Mi* gene is the irreversible breakage of resistance at high soil temperatures (>28°C) (Dropkin, 1969b; Haroon *et al.*, 1993; Talavera *et al.*, 2009). Mutation(s) in the *Mi* gene or a gene required in the *Mi*-mediated resistance pathway (Lopez-Perez *et al.*, 2006) and failed transcription due to DNA methylation (Liharska, 1998) can hamper the efficacy. The expression of the *Mi* gene is also affected by gene dosage, depending on whether the resistant cultivars are heterozygous (*Mimi*) or homozygous (*MiMi*) as shown by Tzortzakakis *et al.* (1998). These authors found much greater reproduction of partially virulent populations of *M. javanica* on heterozygous compared to homozygous tomato genotypes. Despite these constraints *Mi*-resistant cultivars remain important for management of *Meloidogyne* spp. on tomato.

Future considerations

Given the withdrawal of effective nematicides, alternative management strategies for *Meloidogyne* spp. in tomato production are needed. Prevention, physical management methods, organic amendments, biological control, resistant cultivars and an integrated nematode management have proved to be effective but have their limitations. Innovations are limited and take time to be accepted and implemented. The most promising results have been achieved with the successful implementation of the *Mi* gene in commercial cultivars. In total, nine resistance genes (*Mi 1-9*) are now known in tomato. In six of them heat stability was reported (Ammiraju *et al.*, 2003; Jablonska *et al.*, 2007; Wu *et al.*, 2009; Wang *et al.*, 2013) but these genes are not yet cloned or available in commercial cultivars. Pyramiding genes might be the key to overcome the problem of heat stability successfully. Techniques to cool soil temperature to below the critical 28°C through daily watering and the use of plastic mulch until the canopy covers the soil proved to be successful (Rich & Olson, 1999) but seem impractical.

The development of rootstocks containing a heat-stable gene should be a priority in order to control *Meloidogyne* spp. in tomatoes grown at high soil temperatures. The *Mi* resistance gene should also be used in an integrated management context to preserve its durability and prevent the selection of virulent populations of *Meloidogyne* due to variability in isolate reproduction, resistant genotypes, and environmental conditions. The use of tomato geno-

types with the *Mi* gene can be optimised in a rotation sequence of a cropping system.

It is advisable to evaluate the pathogenicity of local *Meloidogyne* populations associated with different environmental characteristics before growing the *Mi*-resistant tomato. Farmers can grow a few tomato plants in soil collected from their field to assess the presence of *Meloidogyne* and its aggressiveness under local circumstances. Response to temperature regimes or other abiotic factors, and system compatibility, including undesirable associations with other pests, diseases or agronomic traits, should also be assessed. The damage potential of root-knot nematodes on tomato crops depends on many factors, such as initial population density, aggressiveness, environmental conditions, cultivar and experimental approach. Thus, the tolerance limit should be determined locally and care must be taken in extrapolating the tolerance limit determined elsewhere. An estimate of the tolerance level can be made by diluting infested soil with sterilised soil and growing tomato plants in a series of nematode densities. However, more precise evaluation will require the aid of a specialist and specialised equipment. All methods of control likely to be used in developing countries should be adaptable to the small-scale farmer with minimum financial resources. Awareness and support are necessary to allow sustainable tomato production both in intensive as subsistence agriculture.

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