A comparative study of the development and reproduction of *Meloidogyne enterolobii* and other thermophilic South African *Meloidogyne* species

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DECLARATION

DECLARATION BY THE CANDIDATE

I, Raymond L Collett, declare that the work presented in this MSc thesis is my own work, that it is not been submitted for any degree or examination at any other University and that all the sources I have used or cited have been acknowledged by the complete reference.

Signature…… Date…27/05/2020……

DECLARATION AND APPROVAL BY SUPERVISORS

We declare that the work presented in this thesis was carried out by the candidate under our supervision and we approve this submission.

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ABSTRACT

Meloidogyne enterolobii, a highly pathogenic root-knot nematode species, infects fruit, grain, oilseed, ornamental and vegetable crops causing severe damage to agri-and horticultural crops worldwide. The species is infamous for its ability to render host plant resistance ineffective since it damages crops exhibiting resistance against other thermophilic species; *M. incognita* and *M. javanica.* This study commenced with an extensive desk-top study; a collective review (consulting 274 articles) about the global distribution, biology, pathogenicity and management of *M. enterolobii,* with special reference to sub-Saharan Africa. The research aim of the glasshouse study was to determine the life-stage development, life-cycle duration and reproduction potential of a South African *M. enterolobii* population compared to its counterpart species, *M. incognita* and *M. javanica*. Seedlings of three crops, maize ('P-2432-R'), soybean ('DM-5953-RSF') and tomato ('Moneymaker'), were inoculated with motile second-stage juveniles (J2) of each species. Ambient temperature regimes maintained in the glasshouse were 19-32 ºC, 15-32 ºC and 18-32 ºC for the maize, soybean and tomato experiments, respectively, over 25 days. Random isolation of 20 life stages of each species from root systems of crop seedlings followed at time intervals of 3, 5, 10, 15, 20, and 25 days after inoculation (DAI), including five replicates of each crop and species. Infected crop roots were removed, for each time interval, and the nematode life stages stained using the sodium-hypochlorite-acid-fuchsin method. Egg-masses were present on the crop's root surfaces 20 DAI and were first stained with eosin-Y before the life stages inside the roots were stained with the sodium-hypochlorite-acid-fuchsin method. Ten egg-masses were randomly removed from each crop seedling's root system, for each of the species, and the number of eggs per egg-mass counted. Data were subjected to Factorial Analyses of Variance and the degree days (DD) were calculated for each species. Morphological and molecular identification verified the identity of the three species used. Significant ($P \leq 0.05$) differences existed for the number of each of the life stages, of each species, among some of the time intervals. *Meloioigyne enterolobii* developed more rapidly from one life-stage to the other compared to the other two species. Although females were observed for all three species 15 DAI, single eggs were observed for *M. enterolobii* only. Egg masses were, however, produced by females of all three species 20 and 25 DAI. The presence of second J2 generations of *M. enterolobii* and *M. javanica* from 20 DAI compared to those of *M. incognita* (recorded from 25 DAI only) confirmed the quicker development of *M. enterolobii* as well as *M. javanica*. Ultimately, the shorter DD needed by *M. enterolobii* to complete its life cycle in roots of all three crop genotypes compared to those of *M. javanica*

and *M. incognita* represents novel information, both fundamentally and for its applicability. An improved advisory approach to farmers can now, for example, focus on rather using crop genotypes with shorter growing periods. Other management strategies can also be streamlined to focus on combatting *M. enterolobii* by, for example, interfering with its rapid life-cycle duration.

Keywords: Life cycle, maize, *Meloidogyne enterolobii, M. incongita, M. javanica,* reproduction, South Africa, soybean, sub-Saharan Africa, tomato

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CHAPTER 1: GENERAL INTRODUCTION

Root-knot nematodes, *Meloidogyne* species, are among the various plant-parasitic nematode genera that are highly pathogenic to a wide range of agricultural as well as ornamental crops and weeds. Second-stage juveniles (J2) of this genus, for example, infect the subterranean parts of various cultivated crops leading to severe economic losses globally (Jones *et al.,* 2013). Infection by root-knot nematodes reduces the ability of the plant to translocate water and nutrients effectively from the roots/other below-ground parts to the aerial parts due to the formation of giant cells. Hence such parasitism reduces the survival of the host plant and decreases crop yield/quantity (Abad *et al.,* 2009). Plant-parasitic nematodes are underestimated by commercial and subsistence producers due to the lack of information and misdiagnosis of crop symptoms. Nematode damage is usually diagnosed as or confused with nutrient deficiency, or damage inflicted by other diseases/pests (Coyne *et al*., 2018). Crop losses worldwide due to root-knot nematode infections have been estimated at 12.3%, representing a monetary loss of $157 billion US dollars (Singh *et al.,* 2015). An ever-increasing need for global food security and in the light of the adverse influence of climate change on pest management, the threat of increasing crop damage and lower yields as a result of parasitism by pests may accelerate (Chakraborty and Newton, 2011). Root-knot nematodes hence pose a severe threat to crop production in various climatic regions worldwide (Onkendi *et al.,* 2014) and concurrently also impact on the economy and the populace of various developing countries (Perry and Moens, 2013).

By February 2020, literature stated that 105 *Meloidogyne* species have been identified (Ghaderi and Karssen, 2020). However, this number is likely to increase annually with the continuous discovery of new species (Blok and Powers, 2009). The most abundant and commonly reported root-knot nematode species worldwide are: *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949*; Meloidogyne hapla* Chitwood, 1949, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949; and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Jones *et al.,* 2013; Sikora *et al.,* 2018). The following species have also been classified as highly damaging species: *Meloidogyne enterolobii* Yang and Eisenback, 1983*, Meloidogyne ethiopica* Whitehead, 1968, *Meloidogyne exigua* Göldi, 1892*,* and *Meliodogyne graminicola* Golden and Birchfield,

1965 (Jones *et al.,* 2013; Sikora *et al.,* 2018). Species that also threaten crop production, but are considered of lesser importance include: *Meloidogyne chitwoodi* Golden, O'Bannon, Santo and Finley, 1980, *Meloidogyne fallax* Karssen, 1996, *Meloidogyne hispanica* Hirschmann, 1986, and *Meloidogyne paranaensis* Carneiro, Carneiro, Abrantes, Santos and Almeida, 1996. However, identification of *Meloidogyne* species remains complicated due to intraspecies variations and morphological similarites among species. An example of initial misidentification is that of *M. enterolobii* that has in many cases been identified as *M. incognita* and is difficult to distinguish from this species (Yang and Eisenback, 1983; Blok *et al.,* 2002; Elling, 2013).

In South Africa, the most common root-knot nematode species identified as constraints to grain producers generally are *M. incognita* and *M. javanica* (Mc Donald *et al*., 2017). However, *M. arenaria* is also present in local grain-producing areas but to a lesser extent than *M. incognita* and *M. javanica* (Pretorius, 2018; Visagie *et al*., 2018). Furthermore, the recent discovery of *M. enterolobii* in a grain production area in the Highveld of the Mpumalanga Province (Pretorius, 2018; Visagie *et al*., 2018) qualifies it as a potential threat species to be added to the list of common root-knot nematode species that may damage grain crops, particularly maize (*Zea mays* L.) and soybean (*Glycine max* L. Merr). *Meloidogyne enterolobii* has also been reported from fruit crops, such as guava (*Psidium guajava* L.) (Willers, 1997), and vegetables, which includes pepper (*Capsicum annuum* L.) (Visagie *et al.,* 2018), potato (*Solanum tuberosum* L.) (Onkendi and Moleleki, 2013) and tomato (*Solanum lycopersicum* L.) (Rashidifard *et al.,* 2019).

1.1 Problem statement

The thermophilic root-knot nematode species *M. enterolobii* is referred to as 'an emerging threat' to cultivated crops, other higher developed plants and weeds (EPPO, 2014). *Meloidogyne enterolobii,* is currently listed as a quarantine pest in regions registered as members of the European and Mediterranean Plant Protection Organization (EPPO). For example, Portugal is a country where this species is listed as a quarantine pest (EPPO, 2014; Santos *et al.,* 2019). When compared to other *Meloidogyne* species, *M. enterolobii* is recorded to be highly pathogenic, due to its

severe infection of the subterranean parts of host plants, broad host range, and ability to render resistance that exist to other thermophilic species within crop cultivars ineffective (Castagnone-Sereno, 2012). A study by Westerich *et al.* (2011) has proven that *M. enterolobii* develops in roots of a tomato cultivar containing the *Mi*-gene; supporting the theory of its resistant breaking ability. The species has been reported from seven continents: Africa, Asia, Caribbean's, Central America, Europe, North America and South America (EPPO, 2019).

Reducing population densities of this root-knot nematode species to below damaging levels is crucial. To develop control strategies against *M. enterolobii* the basic biology and behaviour of the nematode must be understood, particularly the life-cycle duration, with such studies only being done in roots of guava (Ashokkumar *et al.,* 2019), passionfruit (*Passiflora* spp.) (Costa *et al.* (2017), and tomato (Westerich *et al.,* 2011). Limited information on the life cycle of *M. enterolobii* was found in literature. Hence to contribute towards our understanding and knowledge of why the species is so highly injurious, this facet of the biology of the species needs to be elucidated; especially for grain crops. Little is also known about the host status of major grain crops such as soybean and maize to *M. enterolobii*, with no information available for South Africa. The need for such knowledge is critical as *M. enterolobii* has been detected in the local grain production area of Bethal situated in the Highveld region of the Mpumalanga Province, South Africa, where soybean and maize are used in rotation (Pretorius, 2018). This species can hence have a severe impact on sustainable crop production.

1.2 Research aims and objectives

The aim of the study was to determine the life-cycle duration and life-stage development of *M. enterolobii* and evaluate its reproduction potential in roots of maize, soybean and tomato genotypes that are known to be susceptible to *M. incognita* and *M. javanica*.

The objectives of the study were:

- to determine the life-cycle duration and development of life stages of *M. enterolobii* compared to that of *M. incognita* and *M. javanica* in

greenhouse experiments in roots of root-knot nematode susceptible tomato, soybean and maize genotypes;

- to determine the reproduction potential of *M. enterolobii* compared to that of *M. incognita* and *M. javanica* in roots of susceptible soybean, maize and tomato genotypes.

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CHAPTER 2: A REVIEW ON *MELOIDOGYNE ENTEROLOBII*: A VIRULENT ROOT-KNOT NEMATODE SPECIES THREATENING CROP PRODUCTION WITH PARTICULAR REFERENCE TO SUB-SAHARAN AND SOUTH AFRICA

Since Castagnone-Sereno (2012) published an insightful review titled: "*Meloidogyne enterolobii* (=*M. mayaguensis*): profile of an emerging, highly pathogenic, root-knot nematode species", ample information has been generated worldwide about various aspects (e.g. advances in its identification, management, pathogenicity, virulence) of this pathogenic, thermophilic root-knot nematode species. Therefore, the need exists for a follow-up extensive, but condensed literature review on research that has been done for *Meloidogyne enterolobii* Yang and Eisenback, 1983; also considered a minor pathogen (Elling, 2013; Singh *et al.,* 2013), that has been increasingly detected across the world, especially since the start of the 21st century. The chapter addresses this issue by integrating results of 274 publications that have been published by the end of April 2020. Focus is particularly placed on research that has been done in terms of *M. enterolobii* in sub-Saharan Africa (SSA), which represents the part of the African continent lying south of the Sahara Desert (Merriam-Webster Inc., 2020). On this developing continent food security, defined by the United Nations as 'the physical and economic access to sufficient, safe, and nutritional food at all times that meets the dietary needs and food preferences for an active and healthy life for all people' (Smith and Gregory, 2013), is threatened by various diseases and pests of which root-knot nematodes is a major contributor (Coyne *et al*., 2009, 2018*)*. Furthermore, it is agreed with dos Santos *et al*. (2019) that the diversity, identity and distribution of root-knot nematode species in SSA, specifically for some countries, is generally lacking.

2.1 From origin to a global threat: insight into research done on *M. enterolobii*

To understand the impact that *M. enterolobii* has on a global scale, the extent and amount of research that has been conducted on the following topics will be considered in this article:

- occurrence and distribution across the globe, especially in SSA and particularly South Africa,

- identification: classical (morphology and morphometrics) and molecular, and/or genetic approaches,
- biology: focusing particularly on its genetics, life cycle and reproduction potential,
- hosts: crop genotypes identified and
- management strategies; referring to limited information being available regarding the chemical-, biological- and cultural control of the species opposed to ample information that has been generated on the host status (susceptible and resistant) of crops.

Meloidogyne enterolobii, (= *Meloidogyne mayaguensis* Rammah and Hirschmann, 1988) (Xu *et al*., 2004; Karssen *et al.,* 2012) is a thermophilic root-knot nematode species that poses a threat to the agri- and horticulture industries globally (EPPO, 2014) including Africa (Coyne *et al.,* 2018) and especially SSA. *Meloidogyne enterolobii* was described in 1983 from Hainan Island, China where it infected the roots of a pacara earpod tree [*Enterolobium contortisiliquum* (Vell.) Morong]. In 1988, *M. mayaguensis* was described from infected roots of eggplant (*Solanum melongena* L.) in Puerto Rico. The authors of this species state in the species description that *M. mayaguensis* superficially resembles *M. enterolobii* in terms of its general morphology, but listed distinct differences in terms of females regarding the stylet knobs as well as some perineal-pattern characteristic morphometrics for females. They also reiterated that the two species have different malate dehydrogenase patterns (Rammah and Hirschmann, 1988). In 2004, Xu and co-authors demonstrated sequence identity and the conspecificy between the two species and suggested that *M. mayaguensis* should be considered a junior of *M. enterolobii*. (Xu *et al.*, 2004). Karssen *et al.* (2012) compared the paratypes of the two species and confirmed the synonisation proposed by Xu *et al.* (2004). Hence the name *M. enterolobii* is further used in the article although some of the recent literature used still refers to it as *M. mayaguensis*.

The European and Mediterranean Plant Protection Organization (EPPO) placed *M. enterolobii* as an addition to the A-2 list in 2010; the species is not present in all EPPO regions but is of urgency and requires quarantine if possible (EPPO, 2010). This listing is based upon the high pathogenicity level of this species, its wide host range (infecting

cultivated crops: row and tree crops, ornamental plants and weeds) (CABI, 2020) and its particular ability to counteract host plant resistance that exists in various crops to other tropical species, *viz*. *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Karssen *et al*., 2013). The species is also classified as a high risk species, with a risk index of 0.636768, compared to that of *Heterodera zeae* Koshy, Swarup, and Sethi 1970 with the highest value of 0.642916 (Singh *al.* 2015a).

Major stumbling blocks in detecting *M. enterolobii* have been the intraspecies variation, and especially discriminating it from other thermophilic/tropical species that also belongs to the *M. incognita* group (MIG): *M. arenaria* and *M. incognita* in particular (Karssen *et al*., 2013). Identifying *M. enterolobii* was mainly done, until the early 2000s, using morphological and morphometrical approaches, which was really challenging. The inception of molecular technology in the early 2000s, however, provided the development of various improved molecular techniques that contributed towards identification of *M. enterolobii* from numerous countries and crops (Table 2.1; Table 1, Addendum 1) during the past two decades (Brito *et al*., 2004; Xu *et al.,* 2004).

Table 2.1. Reports of the occurrence of *Meloidogyne enterolobii* based upon information obtained from the European and Mediterranean Plant Protection Organisation (EPPO) and Centre for Agriculture and Bioscience International (CABI) databases as well as articles published in peer-reviewed, scientific journals.

Date	Date	Reference	Date	Continent and Region	Reference	Date	Continent and Region	Reference	Date	Continent and Region	Reference
1983	Asia, China	Yang and Eisenback (1983)	**2004 cont.**	S. America, Brazil	Torres *et al.* (2004)	**2010 cont,**	S. America, Brazil	Castro and Santana (2010)	**2016 cont.**	Africa, Kenya	Chitambo *et al.* (2016)
1984	Asia, China	Yang (1984)	**2005**	Asia, China	Liu *et al.* (2005)		S. America, Brazil	Silva and Oliveira (2010)		Africa, Nigeria	Kolombia *et al.* (2016)
1987	Africa, Burkina Faso	Fargette (1987)		N. America, Cuba	Molinari *et al.* (2005)		S. America, Brazil	Almeida *et al.* (2010)		Asia, China	Zhou *et al.* (2016)
	Africa, Côte d'Ivoire	Fargette (1987)		S. America, Brazil	Torres *et al.* (2005)		S. America, Venezuela	Perichi and Crozzoli (2010)		Asia, India	Poornima *et al.* (2016)
	Africa, Togo	Fargette (1987)		S. America, Brazil	Lima *et al.* (2005)	**2011**	N. America, Martinique	Quénéhervé *et al.* (2011)		N. America, Mexico	Ramírez-Suárez *et al* (2016)
	Asia, China	Zhang (1987)		S. America, Venezuela	Lugo *et al.* (2005)		S. America, Brazil	dos Reis *et al.* (2011)		N. America, Mexico	Villar-Luna *et al.* (2016)
1988	[1]N. America, Puerto Rico	Rammah and Hirschmann (1988)		S. America, Venezuela	Molinari *et al* (2005)		S. America, Brazil	Almeida and Santos (2011)	**2017**	Africa, Benin	Affokpon *et al.* (2017)
1989	N. America, Puerto Rico	Decker and Rodrigue (1989)	**2006**	N. America, United States of America	Kaur *et al.* (2006)		S. America, Brazil	Almeida *et al.* (2011a)		Africa, Kenya	Karuri *et al.* (2017)
1994	Africa, Côte d'Ivoire	Fargette *et al.* (1994)		S. America, Brazil	Carneiro, Mônaco *et al.* (2006)		S. America, Brazil	Almeida *et al.* (2011b)		Africa, Mozambique	Kisitu *et al.* (2017)
	Africa, Senegal	Diop (1994)		S. America, Brazil	Silva *et al.* (2006)	**2012**	Asia, China	Niu *et al.* (2012)		Africa, Niger	Assoumana *et al.* (2017)
1995	N. America, Cuba	Rodríguez *et al.* (1995)		S. America, Brazil	Carneiro, Almeida *et al.* (2006)		N. America, United States of America	Han *et al.* (2012)		Asia, India	Suresh *et al.* (2017)
1997	Africa, Senegal	Duponnois *et al.* (1997)		S. America, Venezuela	Perichi *et al.* (2006)		N. America, Costa Rica	Humphreys *et al.* (2012)		S. America, Brazil	da Costa *et al.* (2017)
	Africa, Senegal	Gueye *et al.* (1997)	**2007**	S. America, Brazil	Oliveira *et al.* (2007)		S. America, Brazil	Almeida *et al.* (2012a)		S. America, Brazil	Groth *et al.* (2017)
	Africa, South Africa	Willers (1997)		S. America, Brazil	Torres *et al.* (2007)		S. America, Brazil	Gomes *et al.* (2012)		Africa, South Africa	Van den Berg *et al.* (2017)
1999	N. America, Cuba	Cuadra *et al.* (1999)	**2008**	Europe, The Netherlands*	EPPO (2008)		S. America, Brazil	Paes *et al.* (2012)	**2018**	Africa, South Africa	Rashidifard *et al.* (2018)
	N. America, Cuba	Rodríguez *et al.* (1999)		Europe, Switzerland	Kiewnick *et al.* (2008)	**2012**	S. America, Brazil	de Sousa *et al.* (2012)		Africa, South Africa	Visagie *et al.* (2018)
2000	Africa, Burkina Faso	Trudgill *et al.* (2000)	**2008**	N. America, United States of America	Cetintas *et al.* (2008)		S. America, Brazil	Marques *et al.* 2012)		Asia, China	Xiao *et al.* (2018)

2000	Africa, Malawi	Trudigill *et al.* (2000)		S. America, Brazil	Silva *et al.* (2008)	**2013**	Africa, South Africa	Onkendi and Moleleki (2013a)		Asia, China	Lu *et al.* (2019)
	Africa, Senegal	Trudigill *et al.* (2000)		S. America, Brazil	Almeida *et al.* (2008)		Africa, South Africa	Onkendi and Moleleki (2013b)		N. America, United States of America	Overstreet *et al.* (2018)
	N. America, Martinique	Carneiro *et al.* (2000)		S. America, Brazil	Gomes *et al.* (2008a)		Asia, Thailand	Jindapunnapat *et al.* (2013)		S. America, Brazil	Soares, Lopes *et al. (*2018)
	N. America, Trinidad and Tobago	Trudgill *et al.* (2000)		S. America, Brazil	Gomes *et al.* (2008b)		N. America, United States of America	Ye *et al.* (2013)	**2019**	Asia, China	Long *et al.* (2019)
2001	[2]S. America, Brazil	Carneiro *et al.* (2001)	**2009**	Asia, Vietnam	Iwahori *et al.* (2009)	**2014**	Africa, Democratic Republic of the Congo	Onkendi *et al.* (2014)		Africa, South Africa	Rashidifard (2019)
2002	Africa, Burkina Faso	Blok *et al.* (2002)		Europe, Switzerland	Kiewnick *et al.* (2009)		Africa, South Africa	Marais *et al.* (2014a and b)		Africa, South Africa	Rashidifard *et al.* (2019a)
	Africa, Côte d'Ivoire	Blok *et al.* (2002)		S. America, Brazil	Siqueira *et al.* (2009)		Asia, China	Goa *et al.* (2014)		Africa, South Africa	Rashidifard *et al.* (2019b)
	Europe, France	Blok *et al.* (2002)		S. America, Brazil	Charchar *et al.* (2009)		Asia, China	Long *et al.* (2014)		Europe, Portugal	Santos *et al.* (2019)
	N. America, Puerto Rico	Blok *et al.* (2002)	**2010**	Africa, Republic of the Congo	Tigano *et al.* (2010)		Asia, China	Wang *et al.* (2014)		N. America, United States of America	Kirkpatrick *et al.* (2019)
2003	N. America, Cuba	Rodrigues *et al.* (2003)		Asia, China	Zhuo *et al.* (2010)		Europe, Belgium*	EPPO (2014)		N. America, United States of America	Rutter *et al.* (2019)
	S. America, Brazil	Guimarães *et al.* (2003)		Asia, Singapore*	Anonymous (2010)		N. Amerca, Mexico	Ramírez-Suárez *et al.* (2014)		S. America, Brazil	Luquini *et al.* (2019)
	S. America, Brazil	Maranhão *et al.* (2003)		N. America, Costa Rica	Tigano *et al.* (2010)	**2015**	S. America, Brazil	Rosa *et al.* (2014a)		Africa, Nigeria	Bello *et al.* (2020)
2004	N. America, Guatemala	Carneiro *et al.* (2004a)		N. America, Guadeloupe	Tigano *et al.* (2010)		Asia, China	Long *et al.* (2015)		Asia, China	Sun *et al.* (2019)
	N. America, Guatemala	Hernandez *et al.* (2004)		N. America, Guatemala	Tigano *et al.* (2010)		Asia, China	Wang *et al.* (2015)	**2020**	Asia, China	Zhang *et al.* (2020)
	N. America, United States of America	Brito *et al.* (2004)		N. America, Martinique	Tigano *et al.* (2010)		N. America, United States of America	Brito *et al.* (2015)		N. America, United States of America	Moore *et al.* (2020)
	S. America, Brazil	Carneiro *et al.* (2004a)		N. America, Puerto Rico	Tigano *et al.* (2010)	**2016**	S. America, Brazil	Peas-Takahashi *et al.* (2015)			

[1]North America, Central America and the Caribbean; [2]South America; *intercepted and/or absent

Figure 2.1 The occurrence of *Meloidogyne enterolobii* in South Africa as listed from 1997 to 2019 according to reports from Willers (1997); Onkendi and Moleleki (2013a, 2013b); Visagie *et al.* (2018); Rashidifard *et al.* (2018); Rashidifard *et al.* (2019)

Since its description in 1983, *M. enterolobii* has been reported 131 times, until the end of April 2020, from countries on all continents, except for Australia and Antarctica, where species has not been reported from to date (Table 2.1). The highest occurrence of this species was from the South American continent with 42 records, followed by North America, along with Central America and the Caribbean (32 records), Africa (28 records), Asia (23 records), while the least reports came from Europe (6 records, which includes absent and intercepted reports) (Table 2.1). The reported distribution of this thermophilic species generally is confined to warmer areas of the world, with its absence evident for the temperate regions of the America's, Asia, and Europe.

Considering the African continent, *M. enterolobii* was first reported from sub-Saharan Africa during 1987 from Côte d'Ivoire and Togo (Fargette, 1987), followed by South Africa (Willers, 1997) and Senegal 10 years later (Duponnois *et al.*,1997; Gueye *et al.,* 1997). Since 2000 it has been reported from Burkina Faso and Malawi (Trudgill *et al.,* 2000), the Democratic Republic of the Congo (Onkendi *et al.,* 2014), Kenya (Chitambo *et al,* 2016; Karuri *et al.,* 2017), Mozambique (Kisitu *et al.,* 2017), Nigeria (Kolombia *et al.,* 2016; Bello *et al*., 2020), Niger (Assoumana *et al.,* 2017), and South Africa (Marais, 2014a; Van den Berg *et al*., 2017; Visagie *et al*., 2018; Rashidifard, 2019; Rashidifard *et al.,* 2019a; Rashidifard *et al.,* 2019b). Interestingly, however, is that this species was unknowingly already present in South Africa in 1991 when rooted *Cactus* spp. that were exported were intercepted (Karssen *et al.,* 2008). *Meloidogyne enterolobii* was, however, only identified from genetic material harvested from this intercept in 1997 as part of a Pest Risk Assessment (PRA) using a molecular technique (Karssen *et al.,* 2008). The known distribution of *M. enterolobii* within South Africa has spread from the first report from the Mpumalanga Province in 1997 to the Gauteng, Limpopo, North West and Northern Cape provinces in 2019 (Figure 2.1).

The occurrence, and increased detection, of *M. enterolobii* in various SSA countries hence accentuates its potential to adversely affect crop production and ultimately food security in this developing part of the world.

2.1.1 Symptoms

Host plants infected by *M. enterolobii* are usually diagnosed due to the formation of galls on roots or other below-ground parts (Figure 2.2a), which is similar to the diagnosis of infection by other root-knot nematode species from which the genus receives its common name (Moens *et al.,* 2009). Although it is generally not possible and advisable to attempt distinguishing *Meloidogyne* spp. from each other using the form and/or size of galls, Cetintas *et al*. (2007) reported that galls formed by *M. enterolobii* on tomato roots (irrespective of the genotype used) were larger compared to those formed by *M. arenaria*, *Meloidogyne floridensis* Handoo, Nycsepir, Esmenjaud, Van der Beek, Castagneno-Sereno, Carta, Skantar and Higgins, 2004, *M. incognita* and *M. javanica* infection. The larger galls were, however, not recorded on roots of vetch (*Vicia sativa* L.) used in the same study since galls from all *Meloiodgyne* species were similar in size. Cetintas *et al*. (2007) furthermore reported that *M. enterolobii* galls represented a large and coalesced mass on primary roots, and large bead-like galls on the secondary roots of tomato. Severe root galling as a result of *M. enterolobii* infection was also found in roots of bell pepper (*Capsicum annuum* L.) and tomato from Brazil that are resistant to other species of the *M. incognita* group (Carneiro *et al*., 2006a). Scherer *et al.* (2012) has indicated the potential of *M. enterolobii* to cause complete failure of resistance in some guava

Figure 2.2 a and b. Below-ground symptoms of soybean roots (a) and above-ground symptoms showing yellowing and stunted soybean plants (b) infected by *Meloidogyne enterolobii* (Photo's: Raymond Collett, North-West University, Potchefstroom).

(*Psidium guajava* L.) genotypes. However, accessions of a similar crop, such as watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] genotypes, infected by *M. enterolobii* may react in differently ways (Filho *et al.* 2018a). The use of resistant genotypes against other *Meloidogyne* species, may hence be ineffective due to its susceptibility to *M. enterolobii* (Soares *et al.,* 2018b).

The formation of galls due to *M. enterolobii* infection of tomato roots follows after gaint cells were initiated 10-17 days after inoculation (DAI) (Westerich *et al.,* 2012). Multinucleate giant cells with thick cell walls, dence and granular cytoplasm, was evident on cellular level. Also compressed and disorganised vascular tissue, and hypertrophy of cortical paranchyma cells were evident due to *M. enterolobii* infection, with all histopathological changes appearing to be more pronounced when compared to those inflicted by *M. javanica* infection.

Above-ground symptoms result from feeding of root-knot nematode J2 and females in the giant-cells (acting as nutrient sinks) that minimize the uptake and translocation of water and nutrients to aerial plant parts. Symptoms of *M. enterolobii* infected plants hence become visible and are generally represented by chlorosis, stunting and/or wilting (Moens *et al.,* 2009); similar to those caused by parasitism of other root-knot nematode species (Figure 2.2b).

2.1.2 Damage potential/pathogenicity and crop losses

Useful tools to measure the pathogenicity of root-knot nematode species is root galling and egg and/or J2 numbers per root or g of root (Cetintas *et al.,* 2007; 2008; Martínez *et al.,* 2014). Interesting observations using gall indices were reported from a Brazilian study (Cetintas *et al*., 2007) showing that the extent of root galling induced by *M. enterolobii* in tomato differed significantly from its thermophilic counterpart species. In roots of genotype ‘Solar Set’, galling induced by *M. enterolobii* was 97%, compared to 79% for *M. arenaria*, 72% for both *M. incognita* and *M. javanica* and 13% for *M. floridensis*. However, no differences in this regard was recorded for genotype ‘Florida 47’, except that *M. floridensis* produced significant fewer galls than the other nematode species.

In terms of egg production, *M. enterolobii* and *M. arenaria* produced similar numbers of eggs per gram of root (tomato genotype 'Solar Set') regardless of the inoculum level, whereas *M. floridensis*, *M. incognita* and *M. javanica* produced significantly more eggs at a higher inoculum level (Cetintas *et al*., 2007).

Regarding the effect that *M. enterolobii* has on plant growth parameters, Cetintas *et al*. (2007) demonstrated that tomato plants infected with USA (Florida) populations of *M. enterolobii* and *M. incognita*, respectively, were significantly shorter than those infected by either *M. arenaria*, *M. floridensis* or *M. javanica*. In the same study, fresh shoot weight of tomato plants ('Florida 47') infected with *M. enterolobii* was also significantly reduced by 36% compared to that of a non-inoculated control. No further information could be found regarding the effect that *M. enterolobii* has on plant growth parameters, with none being published from Africa: particularly SSA.

In terms of yield, losses in guava exceeding 62 million US$, has been recorded from Brazil (Pereira *et al.,* 2009). This resulted from root infection and severe necrosis by the causal, fungal agent (*Fusarium solani*) that caused guava decline upon infection by *M. enterolobii* (Gomes *et al.,* 2010; 2012). This high yield loss figure has been reported as directly resulting from the synergistic interaction between *F. solani* and *M. enterolobii* infection, with the latter suggested to be a weak pathogen of the crop if it occurs on its own (Almeida *et al*., 2011a; Gomes *et al.,* 2014a). The *Meloidogyne*-based disease complexes (MDCs) may influence both plant and nematode systems aided by microbial metabolics and pathogenic genes (Lamelas *et al.* 2020). Other interactions of *M. enterolobii* on guava have been recorded; e.g. its interaction with *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961 *(= Helicotylenchus dihysterodes* Siddiqi, 1972) proved to have a synergistic effect on guava seedlings (Gomes *et al.,* 2014b). From the SSA region, the only crop yield loss data was reported by Willers (1997) indicating complete destruction of some guava orchards in South Africa due to *M. enterolobii* infection.

For crops other than guava, results from a microplot study in the USA (Florida) showed that parasitism by *M. enterolobii* reduced tomato ('Florida 47') fruit yield significantly by 65% compared to the non-inoculated control (Cetintas *et al*., 2007). The species has also been known to infect seedlings acquired from nurseries and can cause dying

of as much as 80% of the plants (da Silva and Santos, 2017). Crops that are dependent on above-ground/aerial parts as the main source of produce, e.g parsley (*Petroselinum sativum* L.) can experience severe losses depending on the *M. enterolobii* population density and exposure period; with yield losses of up to 57% experienced when exposed to high population densities and longer periods compared to 39% when exposed to lower population densities and shorter periods (Sangronis *et al.,* 2014). Even in susceptible and resistant tomato varieties an inoculation density of as low as 0.08 J2 per 500 cm^3 can reduce the aerial produce significantly (Zhang *et al.* 2015a).

A negative correlation also exists between *M. enterolobii* population densities and fruit number, with even low initial population densities of J2 (Pi = 100) being able to decrease fruit numbers to zero (Almeida *et al.,* 2011a). Not only does *M. enterolobii* parasitism affects the fruiting of crops, but it can also influence the level of micro- and macronutrients within the plant, for example it may decrease the foliar levels of nitrogen, phosphorus, and potassium (Gomes *et al.,* 2008b).

Ultimately, economic losses caused by *M. enterolobii* (and other *Meloidogyne* spp.) can impact negatively on human communities, particularly in the developing agricultural sector that exists in SSA and particularly a country such as South Africa. For example, it may lead to loss of employment (Pereira *et al.,* 2009), but more importantly threaten food security (Onkendi *et al.,* 2014). However, despite the steep increase in literature being published about the identification, occurrence and host plants of *M. enterolobii* since the beginning of the 21th century, very little research focused generally on determining the effect of *M. enterolobii* on plant growth parameters and yield of crops other than guava and tomato.

2.1.3 Identification

In order to develop and apply effective management strategies against nematode pests such as *M. enterolobii*, basic knowledge about its classification and identity is crucial and non-debatable (Brito *et al.,* 2004*;* Castagnone-Sereno, 2012). Except for classical techniques (Karssen, 2002), the North Carolina differential host test (Hartman and Sasser, 1985) has also (in limited instances only) been applied to

discriminate *M. enterolobii* from other species in the *M. incognita* group. Use of this method for this purpose is debatable (referred to later on). Other identification techniques include isozyme phenotyping (Esbenshade and Triantaphyllou, 1985), esterase phenotyping (dos Santos *et al*., 2019), molecular diagnostics (Hunt and Handoo, 2009; Moens *et al.,* 2009) and genetic approaches (Rashidifard *et al*., 2018; 2019; 2019a; 2019b). Tables 2.2a and 2.2b provide examples from literature of both morphological, molecular and genetic methods used to accurately identify *M. enterolobii*.

Table 2.2a. Morphological and morphometric characteristics used to identify *Meloidogyne enterolobii*.

Parameters	Reference
Morphometrics	Yang and Eisenback (1983), Rammah and Hirschmann (1988), Brito *et al.* (2004), Kaur *et al.* (2006), Perichi and Crozzoli (2010), Han *et al.* (2012), Wang *et al.* (2014), Long *et al.* (2015), Wang *et al.* (2015), Filho *et al.* (2016), Villar-Luna *et al.* (2016), da Cunha *et al.* (2018), Lu *et al.* (2019), Rashidifard *et al.* (2019c); Sun *et al.* (2019)
Lateral lines along the body	Yang (1984), Perichi and Crozzoli (2010)
Perineal pattern	Yang and Eisenback (1983), Yang (1984), Rammah and Hirschmann (1988), Brito *et al.* (2004), Torres *et al.* (2004), Lima *et al.* (2005), Carneiro *et al.* (2006b), Kaur *et al.* (2006), Silva *et al.* (2006), Charchar *et al.* (2009); Gomes *et al.* (2008b); Kiewnick *et al.* (2008), Silva *et al.* (2008), Iwahori *et al.* (2009), Perichi and Crozzoli (2010), Castro and Santana (2010), Zhuo *et al.* (2010), Almeida *et al.* (2011c), Almeida *et al.* (2011b), Humphreys *et al.* (2011), Quénéhervé *et al* (2011), de Sousa *et al.* (2012), Han *et al.* (2012), Paes *et al.* (2012), Rosa *et al.* (2013), Ye *et al* (2013); Rosa *et al.* (2014a); Wang *et al.* (2014), Correira *et al.* (2015), Long *et al.* (2015), Paes-Takahashi *et al.* (2015), Filho *et al.* (2016), Poornima *et al.,* (2016), Villar-Luna *et al.* (2016), da Cunha *et al.* (2018), Lu *et al.* (2019), Visagie *et al.* (2018), Xiao *et al.* (2018), Carrillo-Fasio *et al.* (2019); Chitambo *et al.* (2019), Rashidifard *et al.* (2019c); Sun *et al.* (2019)
Position of excretory pore	Yang and Eisenback (1983), Rammah and Hirschmann (1988), Perichi and Crozzoli (2010), Long *et al.* (2015), Filho *et al.* (2016), da Cunha *et al*. (2018), Xiao *et al.* (2018), Lu *et al.* (2019); Rashidifard *et al.* (2019c)
Dorsal-oesophageal gland opening	Yang and Eisenback (1983), Rammah and Hirschmann (1988), Perichi and Crozzoli (2010), Long *et al.* (2015), Filho *et al.* (2016), da Cunha *et al.* (2017), Xiao *et al.* (2018), Lu *et al.* (2019); Rashidifard *et al.* (2019c)
Male spicule and/or gubernaculum	Yang and Eisenback (1983), Rammah and Hirschmann (1988), Brito *et al.* (2004), Perichi and Crozzoli (2010), Filho *et al.* (2016), Lu *et al.* (2019); Rashidifard *et al.* (2019a); Rashidifard *et.* (2019b)
Head shape of male (shape of labial disc, lateral lips, medial lips)	Yang and Eisenback (1983), Yang (1984), Rammah and Hirschmann (1988), Brito *et al.* (2004), Silva *et al.* (2006), Perichi and Crozzoli (2010), Paes *et al.* (2012), Paes-Takahashi *et al.* (2015), da Cunha *et al.* (2017); Rashidifard *et al.* (2019c)
Stylet characteristics	Yang and Eisenback (1983), Yang (1984), Rammah and Hirschmann (1988), Brito *et al.* (2004), Perichi and Crozzoli (2010), Long *et al.* (2015), Filho *et al.* (2016), da Cunha *et al*. (2017), Visagie *et al.* (2018), Xiao *et al.* (2018); Rashidifard *et al.* (2019c)
Tail shape	Yang and Eisenback (1983), Rammah and Hirschmann (1988), Brito *et al.* (2004), Perichi and Crozzoli (2010), Wang *et al.* (2014), Long *et al.* (2015), Filho *et al.* (2016), Xiao *et al.* (2018), Lu *et al.* (2019); Rashidifard *et al.* (2019c); Sun *et al.* (2019)
Vulval slit length	Yang and Eisenback (1983), Rammah and Hirschmann (1988), Brito *et al.* (2004), Perichi and Crozzoli (2010), Lu *et al.* (2019); Rashidifard *et al.* (2019c)

Table 2.2b. Molecular and/or genetic techniques used to identify *Meloidogyne enterolobii*.

Technique	Reference
Protein-based analyses: isozymes	
Enzyme phenotyping using isozyme patterns of esterases (EST)	Yang (1984), Esbenshade and Triantaphyllou (1985), Fargette (1987), Fargette and Braaksma (1990), Brito *et al.* (2004), Carneiro *et al.* (2000); Carneiro *et al.* (2006b); Carneiro *et al.* (2006a); Carneiro, Tigano, Randig *et al.* (2004), Hernandez *et al.* (2004), Torres *et al.* (2004), Xu *et al.* (2004), Lima *et al.* (2005), Liu *et al.* (2005), Lugo *et al.* (2005), Molinari *et al.* (2005), Kaur *et al.* (2006); Brito, Stanley, Kaurm *et al.* (2007), Brito, Stanley, Mendes *et al.* (2007); Cetintas *et al.* (2007), Oliveira *et al.* (2007); Gomes *et al.* (2008a); Kiewnick *et al.* (2008), Silva *et al.* (2008; 2010; 2014), Charchar *et al.* (2009), Siqueira *et al.* (2009), Castro and Santana (2010), Zhuo *et al.* (2010), Almeida *et al.* (2011c), dos Reis *et al.* (2011), Quénéhervé *et al.* (2011), de Sousa *et al.* (2012), Paes *et al.* (2012), Rosa *et al.* (2013) Rosa, Oliveira *et al.* (2014); Pinheiro *et al.* (2013a; 2015), Villain *et al.* (2013), da Silva *et al.* (2014; 2017), Machado and Filho (2014), Correia *et al.* (2015), Paes-Takahashi *et al.* (2015), Wang *et al.* (2015), Janssen *et al.* (2016), da Silva *et al.* (2016), Kolombia *et al.* (2016, 2017), Freitas *et al.* (2017), Bellé *et al.* (2018), da Cunha *et al.* (2017), dos Santos *et al.* (2019), Lu *et al.* (2019), Santos *et al.* (2019)
Malate dehydrogenase (MDH)	Esbenshade and Triantaphyllou (1985), Brito *et al.* (2004), Hernandez *et al.* (2004), Carneiro *et al.* (2000), Xu *et al.* (2004), Lugo *et al.* (2005), Molinari *et al.* (2005), Kaur *et al.* (2006); Brito, Stanley, Kaurm *et al.* (2007); Brito, Stanley, Mendes *et al.* (2007);Cetintas *et al.* (2007), Oliveira *et al.* (2007), Kiewnick *et al.* (2008), Silva *et al.* (2010), Zhuo *et al.* (2010), Quénéhervé *et al.* (2011), da Silva *et al.* (2014), Wang *et al.* (2015), Kolombia *et al.* (2016; 2017), da Cunha *et al.* (2017)
Superoxide dismutase (SOD)	Esbenshade and Triantaphyllou (1985), Carneiro *et al.* (2000), Hernandez *et al.* (2004), Lugo *et al.* (2005), Molinari *et al.* (2005), Oliveira *et al.* (2007), da Cunha *et al*. (2017)
Glutamate-oxaloacetate transaminase (GOT);	Esbenshade and Triantaphyllou (1985), Carneiro *et al.* (2000), Hernandez *et al.* (2004), Oliveira *et al.* (2007), da Cunha *et al.* (2017)
DNA-based analyses	
Mitochondrial DNA (mtDNA)	
Cytochrome c oxidase subunit 1 (COI/CO1/COX 1)	Xu *et al.* (2004), Kiewnick *et al.* (2008), Kiewnick *et al.* (2015), da Cunha et al. (2017), Powers *et al.* (2018), Chitambo *et al.* (2019), Rashidifard *et al.* (2019a); Santos *et al.* (2019), Moore *et al.* (2020)
Cytochrome c oxidase subunit 2 (COII/CO2/COX 2)	Brito *et al.* (2004), Xu *et al.* (2004), Powers *et al.* (2005), Iwahori *et al.* (2009), Zhuo *et al.* (2010), Humphreys *et al.* (2011), Onkendi and Moleleki (2013a, 2013b), Ramírez-Suárez *et al.* (2014), Wang *et al.* (2014), Kolombia *et al.* (2016), Assoumana *et al.* (2017), da Cunha *et al*. (2017), Xiao *et al.* (2018), Rutter *et al.* (2019), Santos *et al.* (2019), Sun *et al.* (2019), Moore *et al.* (2020)

Cytochrome c oxidase subunit 3 (COIII/CO3/COX 3)	Janssen *et al.* (2016)
NADH dehydrogenase subunit 5 (NADH5)	Janssen *et al.* (2016), Kolombia *et al*. (2017), Pretorius (2018), Chitambo *et al*. (2019), Rashidifard *et al*. (2018); Rashidifard *et al.* (2019a)
COII/16S rRNA or COII/LRNA	Brito *et al*. (2004), Powers *et al*. (2005), Iwahori *et al*. (2009), Humphreys *et al*. (2011), Onkendi and Moleleki (2013a; 2013b), Ramírez-Suárez *et al*. (2014), Assoumana *et al*. (2017), Rutter *et al*. (2019), Rashidifard (2019); *et al.* (2019a); Santos *et al*. (2019), Sun *et al*. (2019), Blok *et al.* (2002), Zhuo *et al.* (2010)
Ribosomal DNA (rDNA)	
Large subunit (D2-D3 28S)	Ye *et al* (2013), Onkendi and Moleleki (2013a), Goa *et al.* (2014), Ramírez-Suárez *et al.* (2014), Xiao *et al.* (2018), Rashidifard, Marais, Daneel, Mienie *et al.* (2019); Rutter *et al.* (2019)
Small subunit (18S)	Blok *et al*. (1997), Brito *et al*. (2004), Powers *et al*. (2005), Lu *et al*. (2019),
Internal transcribed spacer (ITS)	Brito *et al*. (2004), Kiewnick *et al*. (2008), Ye *et al* (2013), Wang *et al*. (2014; 2015), Filho *et al*. (2016), Villar-Luna *et al*. (2017), Lu *et al*. (2019)
Intergenic spacer (IGS)	Blok *et al*. (1997), Tigano *et al*. (2010), Onkendi and Moleleki (2013a, 2013b), Ye *et al* (2013), Long *et al*. (2015), Assoumana *et al*. (2017), Sun *et al*. (2019)
External transcribed spacer (ETS)	Xu *et al*. (2004)
Other methods	
Restriction fragment length polymorphism (RFLP)	Xu *et al.* (2004), Powers *et al.* (2005), Gamel *et al.* (2014), da Cunha *et al*. (2017),
iRNA	Blok *et al*. (2002), Wang *et al*. (2014)
Sequence characterized amplified region – polymerase chain reaction (SCAR-PCR)	Adam *et al.* (2007), Tigano *et al.* (2010), Hu *et al.* (2011), Ye *et al* (2013), Villar-Luna *et al.* (2016), Freitas *et al.* (2017), da Cunha *et al.* (2017; 2018), Rashidifard *et al.* (2018) Rashidifard *et al.* (2019a); Visagie *et al.* (2018), Carrillo-Fasio *et al.* (2019), dos Santos *et al.* (2019), Koutsovoulos *et al.* (2019), Santos *et al.* (2019)
Random amplified polymorphic DNA (RAPD)	Carneiro *et al.* (2004a), Adam *et al.* (2007), Tigano *et al.* (2010), da Silva *et al.* (2014), dos Santos *et al.* (2019)
Satellite DNA (satDNA)	Randig *et al.* (2009)
Amplified fragment length polymorphism (AFLP)	Tigano *et al.* (2010)
Inter-simple sequence repeat (ISSR)	Tigano *et al.* (2010)

Multiplex-PCR	Hu *et al.* (2011)
Loop-mediated isothermal amplification (LAMP)	Niu *et al.* (2012), da Cunha *et al.* (2017), Zhou *et al.* (2017)
Lateral flow dipstick (LFD)	Niu *et al.* (2012)
Real-time PCR (qPCR)	da Cunha *et al*. (2017), Kiewnick and Braun-Kiewnick (2015), Braun-Kiewnick and Kiewnick (2018)
DNA microarrays	da Cunha *et al.* (2017)
Recombinase polymerase amplification (RPA)	Ju *et al.* (2019), Subbotin (2019)
Genotyping by Sequencing (GBS)	Rashidifard *et al*. (2018)

Similar to the identification of other root-knot nematode species (Moens *et al*., 2009), the classical approach applied to identify *M. enterolobii* has been the morphological and morphometrical characteristics of the i) perineal-pattern area (and its associated features), and ii) oesophageal area of adult females (Kleynhans, 1991; Rashidifard *et al.,* 2019c). In addition, characteristics of J2, males and females of *M. enterolobii* (amongst others body length, stylet length, tail shape and tail and hyaline terminus length) have been described (Table 2a).

In terms of females, the use of perineal patterns as a diagnostic character to identify *Meloidogyne* species since the late 1940s (Chitwood, 1949) can be challenging due to intraspecies variation (da Cunha *et al*., 2018). These authors reiterated that the discovery of new *Meloidogyne* species particularly rendered the use of perineal-pattern morphology not to be accurate enough to distinguish among some species. Misidentification of, for example *M. enterolobii* and *Meloidogyne inornata* Lordello, 1956, as *M. incognita*, is a classical example since the perineal-pattern morphology of these species shows high similarity (Carneiro and Cofcewiez, 2008; Carneiro *et al*., 2016).

Brito *et al*. (2004), reported that the vulval-slit length of *M. enterolobii* may be useful as a diagnostic character to distinguish a Florida population of *M. enterolobii* from *M. incognita.* In addition Rashidifard *et al.* (2019c); identified three characteristics that can be used to characterise South African *M. enterolobii* populations. These include large phasmids surrounded by fine striae; fine striae on the lateral sides of the vulva; and the perineal pattern of mature females possessing a medium to high square-like dorsal arch. The authors emphasized that these characteristics have to be used in conjunction with all the others (including those of J2 and males) proposed to be used for *Meloidogyne* species identification. Although the morphological and morphometrical characteristics reported by Brito *et al*. (2004), Almeida *et al*. (2008) and Rashidifard *et al.* (2019c) may be useful in discriminating *M. enterolobii* from its tropical counterpart species, it remains a challenge due to the shortage of experience of both taxonomical and molecular skilled experts. Even with the use of an expert, chances exist that *M. enterolobii* can remain undetected using the classical approach. For example, *M. enterolobii*, as well as *Meloidogyne paranaensis* Carneiro, 1996 and *Meloidogyne izalcoensis* Carneiro, Almeida, Gomes and Hernandez, 2005 are

suggested to have remained undetected in coffee production areas of Brazil prior to the 2000s due to only morphological differentiation methods being available and/or used (Carneiro *et al.,* 2004a; Carneiro and Cofcewiez, 2008).

The North Carolina differential host test, was another technique utilised in the past to assist in differentiating among the four common root-knot nematode species, *viz*. *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica* (Hartman and Sasser, 1985). However, Rammah and Hirschmann (1988) also applied it to aid in verifying the classical and isozyme identification of *M. mayaguensis.* A disadvantage of this method in terms of identication purposes is that it has been developed to demonstrate the different physiological reactions of exclusively *M. arenaria, M. hapla, M. incognita* and *M.javanica* to only a few, specific genotypes of various crops: cotton (*Gossypium hirsutum* L.; 'Delta-pine 16'), pepper (*Capsicum annuum* L.; 'California Wonder'), resistant tobacco (*Nicotiana tabacum* L.; 'NC-70/93'), tomato ('Rutgers'), peanut (*Arachis hypogaea* L.; 'Florunner') and watermelon ('Charleston Grey') (Hartman and Sasser, 1985). More recently, Ye *et al.* (2013) however utilised the North Carolina differential host test to differentiate between *M. enterolobii* and its four commonly occurring counterpart species. These authors showed that *M. enterolobii* and *M. incognita* race 4 share similar host responses. Therefore, this test when used on its own, is inconclusive and not recommended for *M. enterolobii* identification.

Putting an end to challenging and doubtful identification of *M. enterolobii* using classical and host range test approaches was, however, seen with considerable developments in isozyme-, as well as molecular- and genetic-based techniques that enabled researchers to successfully distinguish it from others species belonging to the *M. incognita* group: *M. incognita* in particular (Table 2b). Using isozyme phenotyping, as well as molecular and genetic techniques to detect *M. enterolobii* and discriminate it from other species, with which it shares similar characteristics, have the following benefits: they are readily available; are nowadays routinely used in laboratories across the globe; are less expensive than when they were developed; and have already increased the number of reports on the occurrence and the host status of *M. enterolobii* during the last 20 years due to extensive surveys.

The first biochemical method used to identify a *M. enterolobii* population from China ('isolate E939') represented a technique that recorded the concurrent activity of a rare esterase phenotype (VS1-S1) and a malate dehydrogenase phenotype (Nla) from young female specimens (Esbenshade and Triantaphyllou, 1985). Thereafter various *M. enterolobii* populations were identified using this protein-based approached, with most studies being from Brazil (Esbenshade and Triantaphyllou, 1985; Fargette and Braaksma, 1990; Molinari *et al*., 2005; Lugo *et al*., 2005; Tigano *et al*., 2010; Paes-Takahashi *et al.,* 2015; da Cunha *et al*., 2018; dos Santos *et al*., 2019) (Table 2b).

The DNA-based techniques were developed since the early 2000s and included a wide range of methods used to identify *M. enterolobii* as reported in 96 publications (Table 2b). These mainly include amplification of various DNA fragment (e.g. ribosomal and mitochondrial DNA) using polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), sequence characterized amplified region – PCR (SCAR-PCR), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), multiplex-PCR, loop-mediated isothermal amplification (LAMP), real-time PCR, recombinase polymerase amplification (RPA) and genotyping by sequencing (GBS) (Table 2b).

Interestingly, most DNA-based studies showed that *M. enterolobii* populations grouped in separate clusters, but close to those containing the other tropical species, with high bootstrap values which are indicative of the homologous nature of the populations tested (Tigano *et al*., 2010; Onkendi and Moleleki 2013a; Visagie *et al*., 2018, Rashidifard *et al.* 2019a). For example, a study aimed at evaluating the genetic diversity of South American *M. enterolobii* populations (Brazil, Costa Rica, Guadeloupe, Guatemala, Martinique and Puerto Rico) and an African population (Republic of the Congo) using various DNA-based methods (IGS and mitochondrial DNA analysis, AFLPs, SCAR-PCR and ISSR) indicated a low diversity among the populations and suggested that it is a genetically homogeneous species (Tigano *et al*., 2010). From SSA, and specifically South Africa, this was also true for the homology of 23 *M. enterolobii* populations investigated by Onkendi and Moleleki (2013b) that had been isolated from infected potato tubers as well as for eight populations isolated from roots of green pepper (*Capsicum annuum* L.), guava and potato (Visagie *et al*. 2018). Furthermore, two *M. enterolobii* populations from Nigeria and Brazil (dos

Santos *et al*., 2019) shared a 100% sequence homology indicated by the use of 16S rRNA of mtDNA, D2-D3 28S rDNA, ETS, COII, SCAR-PCR, IGS-rDNA sequences amplification products.

To the contrary, various authors indicated that more variation exists among *M. enterolobii* populations. For example, Onkendi and Moleleki (2013a) showed that phylogenetic analysis of the D2-D3 gene (28S rDNA) suggested that *M. enterolobii* and *M. incognita* grouped in the same clade, while *M. arenaria* and *M. javanica* grouped in different clades. Janssen *et al.* (2016), grouped seven *M. enterolobii* populations from across the globe within the same clade as other *M. incognita*-group (MIG) species, but accentuated that virtually no mitochondrial variation was evident between the 23 *M. enterolobii* populations identified, except for a population, from Puerto Rico, that diverged from the others. Ultimately, a South African study using GBS showed that four South African *M. enterolobii* populations exhibited more variation within the species than that reported by other authors, suggesting that this species is more diverse than anticipated (Rashidifard *et al*., 2018).

The SCAR-PCR technique particularly is a popular tool used to identify *M. enterolobii* across the globe (Table 2a). During the first 10 years of the 21st century, various SCAR-PCR species-specific markers have been developed for the identification of *M. enterolobii* (Blok *et al*., 2002; Tigano *et al*., 2010). However, dos Santos *et al*. (2019) demonstrated that the use of SCAR-PCR markers alone can also result in erroneous results when identifying root-knot nematode species since it could not identify *M. ethiopica* and two atypic, unknown *Meloidogyne* spp. in their study. Nonetheless, this method has been the most commonly applied technique used to identify *M. enterolobii* populations with 15 reports being found to date (Table 2b).

Not only does molecular genetics aid in distinguishing *M. enterolobii* from other *Meloidogyne* species, but it may be used to identify unique genes to understand, and distinguish, connect or differentiate amongst species belonging to other genera. The actin protein gene sequence has shown 98.67-99.20% similarity to *Ditylenchus destructor* Thorne, 1945, and 97.33-98.67% to *Bursaphelenchus xylophilus* (Steiner and Buhrer, 1934) Nickle, 1970 (Lian *et al.,* 2014). Moreover, during a genetic study using GBS, five putatively virulent genes have been reported from *M. enterolobii*

populations whilst these could neither be found in the genome of *M. incognita* nor *M. javanica* (Rashidifard *et al*., 2018). This may contribute to understanding the overall biology and/or genetics of plant-parasitic nematodes and even aid in further development of control methods. Genomic studies of 12 mtDNA sequences have shown that the tropical-mitotic parthenogenetic species (*M. arenaria, M. enterolobii, M. incognita, and M. javanica*) together form Clade 1 [as indicated by Holterman *et al*. (2006)] based upon SSU rDNA, with *M. enterolobii* separated within this clade from the remaining *Meloidogyne* spp. (this can be due to the difference in numbers of non-coding regions when comparing *M. enterolobii* with others). This research also indicated that according to genome architecture, Clade 1 forms the closest common ancestor to another plant-parasitic nematode species, namely *Pratylenchus vulnus* Allen and Jensen, 1951 (Humphreys-Pereira and Elling, 2015). Isolates from the study of Santos *et al*. (2019) (which included populations of China, Portugal and Venezuela) have shown that the *M. enterolobii* populations grouped together when COI and COII sequences were used. Interestingly, *M. enterolobii* appears to be a sister-taxa of *Meloidogyne haplanaria* Eisenback, Bernard, Starr, Lee and Tomaszewski, 2004; with 29% (COI) and 92% (COII) bootstrap support.

The various and diverse DNA-based approaches assisted researchers in recording useful knowledge about *M. enterolobii*. It is agreed with dos Santos *et al*. (2019) that it is imperative to obtain knowledge that is based on the morphological, biochemical and molecular variability within a species (originating from different geographic localities) to enable scientists to recognise differential and stable characteristics for *Meloidogyne* species identification. Ultimately, combining morphology, isozyme and/or molecular/genetic techniques should be the preferred approach to accurately identify *Meloidogyne* species, in this case *M. enterolobii*.

2.1.4 Biology, reproduction and life cycle

Meloidogyne enterolobii reproduces by means of apomixis (obligatory mitotic parthenogenesis), with a chromosome number of 2n = 42-44 (Karssen *et al.,* 2013). This is similar to that of *M. incognita* and *Meloidogyne oryzae* Maans, Sanders and Dede, 1978 to name a few examples (Moens *et al*., 2009). While variability in the reproductive potential of *Meloidogyne* species is crucial to design suitable nematode

management strategies, little has been done in this regard for *M. enterolobii*. For example, Cetintas *et al.* (2007) compared the reproduction potential of Florida (USA) populations of *M. enterolobii* to that of other *Meloidogyne* species. According to this study, little difference existed regarding the reproduction potential of the species, but severe root galling was recorded for *M. enterolobii*.

Substantial variation was demonstrated for the reproductive potential of South African *M. enterolobii* single-species populations as well as mixed-species communities in roots of *M. incognita* susceptible tomato genotypes ('Rodade' and 'Florade', respectively) (Agenbag, 2016; Rashidifard *et al.,* 2019b). Agenbag (2016) reported a single-species population of *M. javanica* (initially isolated from potato roots) with a significantly higher reproduction potential (Rf = 203) than that reported for *M. enterolobii* populations (Rf values ranging between 8 and 14) which were initially isolated from guava roots. In both these South African studies single-species populations of *M. enterolobii* were identified with the lowest reproduction potential which is interesting and opposed to it being referred to as an 'aggressive' root-knot nematode species.

Limited literature regarding *M. enterolobii's* reproduction ability is, however, information about the duration of its life cycle compared to that of its tropical counterpart species. This is crucial information that has to be generated so that researchers can consider all factors that may contribute towards the high pathogenicity reported for this threat species. Such information, for example, whether *M. enterolobii* may have a shorter life cycle or produce more eggs per egg mass compared to other tropical species may shed light on its status as being a highly pathogenic species and is needed to complete the puzzle about this species' status as a threat. The life cycle of root-knot nematodes starts with the egg which is followed by three juvenile stages (the infective second/J2, third/J3 and fourth-stage juveniles/J4) that ultimately develop into sexually dimorphic mature males or females (Moens *et al.,* 2009).

Currently an extensive, comparative life-cycle study is conducted in roots of maize, soybean (*Glycine max* L. Merr) and tomato for South African *M. enterolobii*, *M. incognita* and *M. javanica* populations; following an initial study on tomato (Collett *et al*., 2019). Such information is crucial due to *M. enterolobii* infecting tomato coupled

with its discovery in a local maize production area (Pretorius, 2018). Since soybean is rotated commonly with maize in South Africa, the life-cycle duration of *M. enterolobii* in roots of these three crops may shed useful information on its reproduction potential compared to that of its counterpart species, that also occur in tomato and maize-soybean production areas. Maize is the predominant grain crop produced in South Africa, with 6.2 million metric tonnes (MT) recorded for 2019/20. It is a staple-food source for local inhabitants, particularly the poor, and an important fodder for cattle (GrainSA, 2017a). Soybean again is the most important oil-seed and oil-producing crop grown in South Africa, with 1.2 million MT being produced in 2019/20, and is also contributing substantially to human's diet while it also serve as feed for cattle (GrainSA, 2017b). Tomato on the other hand is the vegetable crop that has, except for potato, the highest production figure (537 thousand MT for 2017/18) for South Africa (FAO, 2019). This crop also forms part of the daily diet of South Africans and has numerous health and dietary benefits.

2.1.5 Host plants

Meloidogyne enterolobii is highly polyphagous and can infect a wide range of plant species: from ornamentals to agri- and horticultural crops (row and tree) as well as weeds that grow in tropical, subtropical and temperate areas of the world. Table 2.3 lists a wide range of host plants that is known to be parasitised by *M. enterolobii*.

Table 2.3. Host plants reported for *M. enterolobii*.

Host plant(s)	Reference
Enterolobium contortisiliquum (Vell.) Morong	Yang and Eisenback (1983)
Solanum melongena L.	Rammah and Hirschmann (1988)
Coffea arabica L.	Decker and Rodriguez (1989)
Bidens pilosa L., *Psidium guajava* L.	Willers (1997)
Impatiens balsamina L.	Rodríguez *et al.* (1999)
Crotalaria juncea L., *Lycopersicon esculentum* L. ('Santa Cruz','Viradoro'), *Phaseolus vulgaris* L. ('IPA-9'), *Psidium guajava, Vigna unguiculate* (L.) Walp. ('IPA-206')	Guimarães *et al.* (2003)
Ajuga reptans L., *Brugmansia spp.* Pers ('Sunray'), *Callistemon citrinus* (Curtis) Dum.Cours., *Capsicum annuum* var. *annuum* L., *Clerodendrum ugandense* (Hochst.) Steane and Mabb., *Lantana* spp. L., *Poinsettia cyathophora* Murry, *Psidium guajava, Myrica cerifera* L., *Ocimum* spp.L., *Solanum americanum* Mill., *Solanum melongena* L., *Tecomaria capensis* (Thunb.) Lindl., *Tibouchina elegans* Cogn., *Tibouchina* spp. Aubl. ('Compacta')	Brito *et al.* (2004)
Senefeldera multiflora Mart.	Lima *et al.* (2005)
Ananas comosus (L.) Merr., *Bidens pilosa, Cucurbita pepo* L., *Erechtites hieraciifolius* (L.) Raf. *ex* DC., *Oeceoclades maculate* (Lindl.) Lindl., *Synedrellapsis grisebachii* Hieron. & Kuntze ex O.Hoffm.	Carneiro *et al.* (2006b)
Angelonia angustifolia Benth.	Kaur *et al.* (2006)
Amaranthus hybridus L., *Carica papaya* L., *Cereus fernambucensis* Lem,, *Chamaesyce prostrata* Aiton., *Cnidoscolus urens,*(L.) Arthur, *Emilia sonchifolia* (L.) DC. ex Wight, *Euphorbia tirucalli* L., *Hidrocotyle bonariensis* Lam., *Malpighia punicifolia* DC., *Passiflora mucronate* Lam., *Senna alata* (L.) Roxb., *Senna occidentalis* (L.) Link., *Solanum americanum, Talinum triangulare* (L.) Juss.	Souza *et al.* (2006)
Brassica oleracea L. ('Florida Broad Leaf'), *Brassica oleracea var. botrytis* ('Waltham'), *Brassica oleracea var. esculenta*, *Canavalia ensiformis* (L.) DC., *Citrullus lanatus* (Thunb.) Matsum. & Nakai. ('Crimson Sweet'), *Curcurbito pepo* ('Yellow Crook Neck'), *Lycopersicon esculentum* ('Rutgers'), *Ocimum basilicum, Solanum melongena* ('Black Beauty'), *Vigna unguicilata* ('Iron Cay')	Brito *et al.* (2007b)
Abutilon theophrasti Medik., *Amaranthus spinosus* L., *Amaranthus retroflexus* L., *Brassica kaber* (DC.) L.C. Wheeler, *Cnidoscolus stimulosus* (Michx.) Engelm. and Gray, *Cucumis anguria, Ipomoea violacea* L., *Leonotis nepetifolia* (L.) R.Br., *Lycopersicon esculentum* 'Rutgers', *Phytolacca americana* L., *Senna obtusifolia*	Kaur *et al.* (2007)
Petunia hybrida Juss. ('Easy Wave Red', 'Easy Wave Rose Down', 'Easy Wave White', 'Madness Midnight 288', 'Milliflora Prostrate - Whisper Purple', 'Miniflora Prostrate - Whisper White', 'Petunia Mini Blue', 'Petunia Suncatcher - Lavender Imperator', 'Petunia Pink Vein – Florida', 'Suncatcher Dark Lavender', 'Suncatcher Saphire', 'Supertunia Blushing Princess', 'Supertunia Lavender Morn', 'Supertunia', 'Lavender Pink', 'Supertunia Lemon Plurne', 'Supertunia Mini - Blue	Mendes *et al.* (2007)

Veined', 'Supertunia Mini - Bright Pink', 'Supertunia Mini - Pastel Pink', 'Supertunia Mini Purple', 'Supertunia Mystic Pink', 'Supertunia Red', 'Surfinia Baby Compact - Amethyst Petunia', 'Surfinia Red Petunia', 'Surfinia Sugar Plum', 'Sweet Sunshine 5', 'Tidal Wave Silver'), *Solanum lycopersicon* 'Rutgers'	
Carica papaya, Capsicum annuum var. longum DC., *Eclipta prostrata, Fatoua villosa* (L.) L., *Panicum* spp. L., *Poinsettia cyathophora, Solanum americanum*, Acanthaceae Juss.	Brito *et al.* (2008)
Nicotiana tabacum L., *Psidium guajava* ('Paluma', 'Casca Dura')	Gomes *et al.* (2008b)
Ajuga reptans, Apium graveolens L., *Beta vulgaris* L., *Bidens alba* (L.) DC., *Bidens pilosa, Brassica oleracea* var. *italica* L., *Brugmansia* spp., *Capsicum annuum* L., *Citrullus lanatus, Coffea arabica, Cucurbita pepo, Euphorbia heterophylla* L., *Glycine max* (L.) Merr., *Ipomoea batatas* L., *Ipomoea* spp. L., *Lycopersicon esculentum, Nicotiana tabacum, Ocimum basilicum, Petroselinum crispum* (Mill.) Fuss, *Phaseolus vulgaris* L., *Psidium guajava, Solanum americanum, Solanum melongena, Solanum tuberosum* L., *Tibouchina* spp. Aubl., *Vica faba* L.	Hunt and Handoo (2009)
Canavalia ensiformis, Vigna unguiculate ('BR-17 Gurguéia'), *Lycopersicon esculentum*	Silva and Silva (2009)
Ajuga reptans, Brugmansia spp. Pers. ('Sunray'), *Buddleja davidii* Franch., *Callistemon* spp. R. Br., *Callistemon citrinus, Callistemon viminalis (*Sol. ex Gaertn.) Byrnes, *Clerodendrum ugandense, Gardenia* spp. J. Ellis, *Hibiscus grandifloras* Michx., *Lagerstroemia indica* (L.) Pers., *Lantana montevidensis* (Spreng.) Briq., *Ligustrum* spp. L., *Myrica cerifera*	Brito *et al.* (2010)
Maranta arundinacea L.	Zhuo et al (2010)
Apium graveolens, Capsicum chinense Jacq., *Capsicum frutescens* L., *Chamaesyce hypericifolia* (L.) Millsp., *Citrullus lanatus, Cucumis sativus* L,, *Leonotis nepetifolia, Lycopersicom esculentum, Petroselinum sativum, Psidium guajava, Urena lobate* L.	Quénéhervé *et al*. (2011)
Malpighia emarginata DC., *Psidium guajava* ('Tai-kua-bar')	Humphreys *et al*. (2011)
Eucalyptus grandis W. Hill. x *Eucalyptus urophylla* S.T. Blake	Almeida, Paes *et al.* (2012b)
Psidium spp. L., *Psidium acutangulum* DC. ('Araçazeiro-Pera 1', 'Araçazeiro-Pera 2') *Psidium cattleyanum* Sabine, *Psidium friedrichsthalianium* (O.Berg) Nied. ('Goiabeira da Cost Rica'), *Psidium guajava* ('Goiabeira Paluma')	Almeida, Wickert *et al.* (2012a)
Callistemon rigidus Schrad. and J.C.Wendl., *Capsicum baccatum* L., *Capsicum chinense, Cucurbita moschata* Duchesne ex Poir., *Physalis angulate* L., *Psidium guineensis* Sw., *Saccharum* spp. L. ('SP 801816', 'RB 867515', 'RB 92579', 'RB 956911'), *Solanum* spp. L. ('Gilo'), *Solanum melongena, Solanum lycopersycum* ('TRural l'),	Marques *et al*. (2012)
Byrsonima cydoniifoliai A. Jass.	Paes *et al*. (2012)
Psidium spp. L. ('ALU1', 'ALU2', 'ALU3', 'AROXO-C', 'AROXO-U')	Martins *et a*l. (2013)
Solanum tuberosum	Onkendi and Moleleki (2013b)

Capsicum spp. L. (‘CNPH 0780’, ‘CNPH 2825’, ‘CNPH 2850’, ‘CNPH 2851’, ‘CNPH 3040’, ‘CNPH 3188’, ‘CNPH 3234’, ‘CNPH 3269’, ‘CNPH 3272’, ‘CNPH 3278’,’CNPH 3375’, ‘CNPH 3471’, ‘CNPH 3480’, ‘CNPH 3522’, ‘CNPH 3573’, ‘CNPH 3627’, ‘CNPH 3864’, ‘CNPH 3871’, ‘CNPH 3937’, ‘CNPH 4137’, ‘CNPH 4159’, ‘CNPH 4160’ ‘CNPH 0060’, ‘CNPH 0436’, ‘CNPH 0578’, ‘CNPH 3260’, ‘CNPH 3283’, ‘CNPH 3429’, ‘CNPH 3449’, ‘CNPH 3454’, ‘CNPH 3458’, ‘CNPH 3459’, ‘CNPH 3466’, ‘CNPH 3467’, ‘CNPH 3485’, ‘CNPH 3511’, ‘CNPH 3521’, ‘CNPH 3523’, ‘CNPH 3536’, ‘CNPH 3543’, ‘CNPH 3553’, ‘CNPH 3572’, ‘CNPH 3633’, ‘CNPH 3713’, ‘CNPH 3714’, ‘CNPH 3717’, ‘CNPH 3883’, ‘BRS Garça’, ‘BRS Brasilândia’, ‘California Wonder’, ‘Silver’, ‘CNPH 148),	Pinheiro *et al.* (2013b)
Gossypium hirsutum L.(‘PHY 375 WR’, ‘PHY 565 WR’), *Glycine max* (‘7732’)	Ye *et al.* (2013)
Cucumis sativus (‘Menina Brasileira’, ‘Moranga Exposição’, ‘Shelper’, ‘Tetsukabuto Takaiama’, ‘B8-A Tetsukabuto’, ‘Excite Ikki’, ‘Yoshinari’, ‘Kouki’, ‘Taisho’, ‘Tsuyataro’)	Wilcken *et al*. (2013)
Ipomoea batatas	Goa *et al.* (2014)
Ziziphus jujuba Mill.	Long *et al.* (2014)
Ocimun basilicum L.	Martínez *et al*. (2014)
Citrullus lanatus	Ramírez-Suárez *et al.* (2014)
Manihot esculenta Crantz. (‘Inajá’)	Rosa *et al*. (2014a)
Daucus carota var. *sativus* Hoffm.	Wang *et al.* (2014)
Lactuca sativa L. (‘Lady’, ‘Winterset’, ‘Robinson’, ‘Sonoma’, ‘Raider’, ‘Lucy Brown’, ‘Bnondaga’, ’Summer Time’, ‘Tainá’, ‘Sundevil’, ‘L-109’)	Correia *et al*. (2015)
Ficus carica L. (‘Genoveso IAC’, ‘Roxo de Valinhos’, ‘White Adriatic’, ‘Caprifigo IAC’, ‘Celeste IAC’)	Costa *et al.* (2015)
Artocarpus heterophyllus Lam.	Long *et al.* (2015)
Beta vulgaris L. (‘Maravilha’, ‘Chata do Egito’, ‘Early Wonder’), *Coriandrum sativum* L. (‘Verdão’), *Raphanus sativus* L. (‘Comprido Vermelho’, ‘Redondo Vermelho’, ‘Comprido Branco’), *Solanum lycopersicum* L. (‘Block’)	Rosa *et al*. (2015)
Capsicum annuum	Wang *et al.* (2015)
Solanum spp., *Solanum scabrum* Mill.	Chitambo *et al.* (2016)
Solanum pseudocapsicum L.	Groth *et al*. (2016)
Dioscorea rotundata (Poir.) J.Miège	Kolombia *et al*. (2016)
Solanum tubersosum (‘BRS Ana’, ‘BRS Clara’, ‘SCS 365 Cota’, ‘BRS Eliza’, ‘Iapar Cris’, ‘Asterix’, ‘BRSIPR Bel’, ‘Agata’, ‘Epagri 361 – Catucha’)	Lima-Medina *et al.* (2016)
Stenocereus queretaroensis (F.A.C. Weber) Buxbaum	Ramírez-Suárez *et al.* (2016)
Capsicum annuum	Villar-Luna *et al*. (2016)
Cucumis melo L. (‘Orange lisa’, ‘Japonês’, ‘Caipira’, ‘Orange verde’, ‘Cantaloupe’), *Malpighia* spp. L. (‘P18A2B1’, ‘P24A2B1’, ‘P27A3B5’, ‘P29A3B5’, ‘P33A2’, ‘P34A3B5’), *Ficus carica* L. (‘Roxo de Valinhos’), *Musa* spp. L. (‘Thap Maeo’,	Freitas *et al*. (2017)

'Terra', 'Princesa', 'Tropical', 'Garantida', 'Galil 18', 'Prata anã', 'Japira', 'Preciosa', 'Grande Naine'), *Annona cherimola* Mill. x *A. squamosa* L. ('Orgulho Africano'), *Averrhoa carambola* L. ('Hart', 'Arkin', 'Tean-ma', 'Weller', 'Star King Sweet'), *Vitis* spp. L. ('Chardonnay', 'Solferino')	
Solanum lycopersiicon, Solanum melongena	SAPPNS[1] database (Marais *et al*. 2017)
Capsicum annuum	Assoumana *et al.* (2017)
Emilia fosbergii Nicolson.*, Psidium guajava, Solanum paniculatum* L.	da Silva and Santos (2017)
Ipomoea batatas ('K55', 'BS15')	Karuri *et al*. (2017)
Caladium x hortulanum Vent. ('Pink Beauty', 'White Christams', 'Freida Hemple', 'Red Flash', 'Carolyn Whorton', 'Postman Joyner')	Kokalis-Burelle *et al*. (2017)
Vigna inguiculata L. Welp,	Kisutu *et al*. (2017)
Bidens pilosa, Capsicum annum, Psidium spp., *Solanum tubersosum*	Van den Berg *et al.* (2017)
Cucurbitaceae ('Becada RZ F1', 'Bombo', HI 09 025 CUR FI'), *Cucurbita moschata* ('Bodygaurd F1', 'Security F1'), *Cucurbita maxima* Duchesne. *x C. moschata* ('Kazako'), Solananceae ('Beautfort', 'Optifort', 'Emperador'), *Solanum lycopersicum* ('Moneymaker', 'Oskar F1', 'Phantasia F1')	Hallmann and Kiewnick (2018)
Zea mays ('PHI-33H56')	Pretorius, 2018
Allium sativum L.*, Beta vulgaris var. cicla* L.*, Capsicum annuum* ('California Wonder'), *Solanum melongena* ('F1-100'), *Solanum tuberosum* ('Désirée), *Nicotiana tabacum* ('Virginia', 'Nat. H-92', 'Criollo', 'NC 95'), *Lycopersicon esculentum* ('Guardadjira', 'Nat. I-17', 'Campell-28'), *Canavalia ensiformis, Glycine max* ('Forrest'), *Phaseolus vulgaris* ('Icapijao'), *Psidium guajava* ('Cotorrera'), *Petroselinum crispum* ('Plain'), *Apium graveolens* ('Utah'), *Cucurbita* spp. ('Nat. I-F', 'Nat. I-M'), *Beta vulgaris* ('Detroit')	Rodriguez *et al*. (2018)
Capsicum annuum, Psidium guajava, Solanum tuberosum	Visagie *et al*. (2018)
Zingiber officinale Roscoe.	Xiao *et al*. (2018)
Acanthospermum australe (Loefl.) Kuntze.*, Amaranthus deflexus* L.*, Amaranthus hybridus, Amaranthus spinosus, Amaranthus viridis* L.*, Bidens pilosa, Bidens subalternans* (DC.) A. P. de Candolle.*, Cardiospermum halicacabum* L.*, Commelina benghalensis* L.*, Euphorbia heterophylla, Galinsoga parviflora* Cav.*, Ipomoea grandifolia, Ipomoea nil* (L.) Roth.*, Ipomoea purpurea* (L.) Roth.*, Leonurus sibiricus* L.*, Nicandra physaloides* (L.) Gaertn.*, Polygonum hidropiperoides* (Michx.) Small., *Portulaca oleracea* L.*, Rhynchelytrum repens* (Willd.) Zizka*, Sida rhombifolia* L.*, Solanum americanum, Solanum sisymbriifolium* Lam.*, Solanum pseudocapsicum, Talinum paniculatum* (Jacq.) Gaertn.*, Lycopersicum esculentum* Mill.	Bellé *et al*. (2019)

[1] Dr Mariette Marais of the Nematology Unit, Biosystematics, Agricultural Research Council–Plant Health and Protection is thanked for the use of data from the South African Plant-Parasitic Nematode Survey (SAPPNS) database; E-mail: maraism@arc.agric.za.

Solanum lycopersicum, Solanum tuberosum, Solanum scabrum, Solanum villosum,	Chitambo *et al.* (2019)
Morus alba L.	Long *et al.* (2019)
Gardenia jasminoides J. Ellis.	Lu *et al.* (2019)
Musa spp.	Luquini *et al.* (2019)
Phaseolus vulgaris, Solanum melongena, Arachis hypogaea L., *Lactuca sativa, Spinacia oleracea*	Rashidifard *et al.* (2019a)
Ipomoea batatas (‘Covington’)	Rutter *et al*. (2019)
Manihot esculenta Crantz.	Akinsanya *et al.* (2019)
Citrullus lanatus	Bello *et al*. (2020)
Artocarpus heterophyllus, Byrsonima cydoniifolia, Capsicum annuum, Citrullus lanatus, Coffea spp., *Cucumis sativus, Daucus carota* L., *Dioscorea rotundata, Enterolobium contortisiliquum, Euphorbia punicea* Sw., *Glycine max, Gossypium hirsutum, Ipomoea batatas, Malpighia* spp., *Manihot esculenta, Maranta arundinacea, Morus* spp. L., *Morus nigra* L., *Musa* spp., *Phaseolus* spp. L., *Psidium guajava, Solanum lycopersicum, Solanum pseudocapsicum, Zingiber officinale, Ziziphus jujuba*	CABI (2020)
Elaeocarpus decipiens L.	Moore *et al.* (2020)

The presence of *M. enterolobii* is of great concern to producers in sub-Saharan Africa regions where it has been found in roots/other below-ground parts of various crops as indicated in Table 2.3. These host include agri- and horticultural crops such as fruits, grains, legumes, abd vegetables, while weeds are also listed as hosts of *M. enterolobii.*

An interesting addition to the list of host plants for *M. enterolobii* is groundnut which was found for South Africa (Rashidifard *et al.,* 2019a). Guimarães *et al.* (2003) from Brazil also reported that groundnut roots contained mature *M. enterolobii* females, but without egg masses. This indicated that *M. enterolobii* infected the crop but were most probably not being able to reproduce in its roots. Brito *et al*. (2004), however, listed groundnut as a non-host to an USA population of *M. enterolobii*. Interestingly, a similar controversial scenario exists for its counterpart species *M. incognita* that has also been reported to infect groundnut in South Africa (Kleynhans*,* 1991; Kleynhans *et al*., 1996; Fourie *et al*., 2015; Rashidifard *et al.,* 2019a) although it has also been reported to be a non-host of the species in the USA (Dickson and De Waele, 2005). It is hence foreseen that more hosts of *M. enterolobii* will be listed, specifically in SSA since this species has been increasingly detected in areas of Eastern, Western, Central Africa and Southern Africa to date (Pagan *et al*., 2015; Janssen *et al*., 2016; Coyne *et al*., 2018; Pretorius*,* 2018; Visagie *et al.,* 2018; dos Santos *et al*., 2019; Rashidifard *et al.,* 2019a; Rashidifard *et al.,* 2019c).

2.2 Management strategies

Research on various management strategies to reduce *M. enterolobii* infections has shown partial success when the four major pillars of an integrated pest management (IPM) namely biological, chemical and cultural methods, as well as genetic host plant resistance approaches were applied (Table 2.4). Although most strategies included methods used to manage other *Meloidogyne* species, novel approaches have also been reported to reduce *M. enterolobii* densities in crop fields. These included the study of protease clones as biological toxins to target *M. enterolobii* parasitism (Zhang *et al.,* 2015b)*,* and the examination of 'knocking down' gene expression mechanisms that prevents *M. enterolobii* infection of host plants (Long *et al.,* 2017). Molecular studies that focus upon the mechanisms of infection by *M. enterolobii*, such as

MeTCTP (*M. enterolobii* Translationally Controlled Tumour Protein) that suppresses the host's immune response (Li *et al.,* 2016; Zhuo *et al.,* 2017) and cellulose binding proteins (CBP) (Menezes *et al.,* 2019) that aid penetration of J2 are novel and show potential to control this species.

2.2.1. Genetic host plant resistance

The use of genetic host plant resistance will probably be one of the major strategies investigated to reduce infections of *M. enterolobii*. Burla *et al*. (2010), however, accentuated the importance of using the optimum inoculation levels of *M. enterolobii* (<3500 eggs/seedling) and evaluation time (135 or 180 days after nematode inoculation) as well as reproduction factor (Rf) values to get accurate results for guava genotypes in terms of its hosts status. Although a host plant may present low gall and egg mass indices, the Rf may still remain supportive of the *M. enterolobii* populations (Costa *et al.,* 2015). Another factor to bear in mind is that host plants previously classified as resistant or non-hosts can be elevated to the level of moderately resistant or poor-host. An example includes the USA peach cultivar 'Flordagaurd', classified as a non-host in 2008 (Nyczepir *et al.,* 2008) which had the highest root-gall index value and Rf, but was reclassified as a host in 2013 (Souza *et al.,* 2013). Important too is that a crop plant may be infected, but still provide an abundant yield; indicating that the plant has a higher tolerance towards *M. enterolobii* (de Siqueira *et al.,* 2009).

Results from mainly Brazilian studies showed promise in terms of the poor host status of row and tree crops to this highly pathogenic species. Probably the most remarkable study in terms of the number and variety of crop genotypes identified as non- or poor hosts of an aggressive population of *M. enterolobii*, using esterase phenotyping and SCAR markers (Tigano *et al*., 2010), was reported on by Freitas *et al*. (2017) (Table 2.4). Resulting from this glasshouse study numerous tree crop genotypes were listed as non-hosts (Rf≤0.09) using various parameters (egg- and gall indices, number of eggs per root system and Rf, *viz*. assai (*Euterpe olereacea* Mart.), avocado (*Persea americana* Mill.), cashew (*Anacardium occidentale* L.), citrus (*Citrus sinensis* [L.] Obs. x *Poncirus trifoliata* [L.] Raf. 'Troyer'), coconut (*Cocos nucifera* L.), grapevine (*Vitis* spp.), mango (*Mangifera indica* L.), papaya (*Carica papaya* L.), passion fruit (*Passiflora* spp.), sapodilla (*Manilkara zapota* L.), star fruit (*Averrhoa carambola* L.)

and soursoup (*Annona muricate* L.) (Freitas *et al.,* 2017). Also from Brazil, a prune (*Prunus mume* Sieb and Zucc) rootstock was identified as highly resistant (using Rf values) to *M. enterolobii*, while four peach rootstocks showed resistance and one showed moderate resistance (Table 2.4.).

Research from various South American and Carribean countries indicated reproduction on perinneal crops such as atemoya (*Annona cherimolia* M. and *A. squamosa* L.) and bitter orange (*Citrus aurantium* L.) (Freitas *et al.* 2017), grapefruit (*Citrus paradisi* Macf.) (Rodriguez *et al.,* 2003) were low. Although accessions of cattley guava (*Psidium cattleyanum* Sabine) were also identified as resistant to *M. enterolobii* (Carneiro *et al*., 2007; Miranda *et al*., 2011), the low grafting success rate for guava with cattley guava showed no promise for developing resistant guava genotypes (Robaina *et al*., 2015). However, with new development of molecular methods, insight will be obtained in understanding characteristics of resistant genes (such as specific alleles from paternal plants, general combining ability, development of hybrid plants, resistance gene analogs and structures of resistant genes such as *Ma*-genes against *M. enterolobii*) which can be inherited by the next generation of crop plants (Damaceno *et al.,* 2016; Gomes *et al.,* 2016; Claverie *et al.,* 2017; da Costa *et al.,* 2017; Noia *et al.,* 2017). Accessions of *Psidium* species can however react differently and be either resistant or susceptible, according to the degree of inoculation densities (de Oliveira *et al.,* 2019). Although research for resistant hybrids of various crops has progressed, the search for resistant tomato hybrids remains elusive (Cantum *et al.,* 2009; Rosa *et al.,* 2014b); however a tolerant tomato genotype has shown potential in managing *M. enterolobii* (da Silva *et al.,* 2019). Effective breeding programs can be developed by estimating genetic parameters for interspecific resistance to *M. enterolobii* and for further identification of resistant rootstocks (Ribeiro *et al.,* 2018).

To successfully determine resistance within a cultivar it is of utmost importance to access and analyse the following factors: i) penetration of J2, ii) development of nematode life-stages, and iii) reproduction properties of the nematode. For example, the delayed life-stage development of *M. enterolobii* in roots of *P. cattleyanum* and *Psidium friedrichsthalianum* (O. Berg) Nied adversely impacted on the development of mature females, with subsequent low reproduction abilities recorded (de Sousa *et al.,* 2016).

In terms of annual row crops, strawberry (*Fragaria* x *ananassa* Duch), was identified as the only non-host of a *M. enterolobii* population used in a Brazilian study (Freitas *et al*., 2017). Other Brazilian studies also listed various genotypes of carrot (*Daucus carota* L.), lettuce, cauliflower (*Brassica oleracea* var. *botrytis* L.), cabbage (*Brassica oleracea* var. *capitate* L.), broccoli (*Brassica oleracea* var. *italica* L.) and cole (*Brassica* spp.) as non-hosts (Correira *et al.*, 2015; Rosa *et al.,* 2015). Furthermore, the USA soybean genotype 'Forrest' exhibits resistance to this species, while sweet potato (*Ipomoea batatas* L., clones 'UFLA07-49' and 'UFLA07-53') are also known as resistant (Hunt and Handoo, 2009, de Melo *et al.* 2011). From Cuba, genotypes of garlic (*Allium sativum* L.) and groundnut were identified as non-hosts (Rf = 0), while soybean, cabbage, broccoli, sugarbeet (*Beta vulgaris* var. *cicla* L.) and lettuce are listed as poor hosts (Rf>1) (Rodriguez *et al.,* 2003). Although the cucumber genotypes ('Shelper' and 'Exite Ikki KY') showed as susceptibility, due to lower Rf values of 1.01, they have been classified as resistant (Wilcken *et al.,* 2013). However, this brings into question, can these cucumber genotypes be classified as resistant or susceptible?

A potential contribution to such research and development of genetic resistance to *M. enterolobii* may include investigating the resistance of weed species in Southern America which includes *Sonchus oleraceus* L.*, Commelina benghalensis* L.*, Tagetes minuta* L. and *Manihot esculenta* (Carneiro *et al*., 2006b). Considering the African indigenous vegetable (AIV) species *Amaranthus cruentus* L. and *Amaranthus dubius* Mart. and Thell.*,* from Kenya, resistance has been demonstrated against *M. enterolobii* while they were susceptability to *M. incognita*. Interestingly these AIV species present also had resistance against the cyst nematodes *Globodera pallida* Stone 1973 and *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 (Chitambo *et al.,* 2019).

Few records exist to indicate resistance of ornamental crops to *M. enterolobii,* but the chinaberry tree (*Melia azedarach* L.) from Cuba have shown promising resistance to the species (Rodriguez *et al.,* 2003). However, 26 petunia (*Petunia x hybrida*) genotypes screened to an USA population of *M. enterolobii* (Mendes *et al*., 2007) were susceptible, while the same was found for seven caladium (*Caladium* x *horulanum*) genotypes (Kokalis-Burelle *et al*., 2017).

Despite the existence of resistant crop genotypes, *M. enterolobii* is known for its virulence [the ability to reproduce on resistant genotypes (Roberts, 2002)] against

crops that express resistance to its thermophilic counterpart species *M. arenaria, M. incognita* and *M. javanica* (Maranhão *et al.,* 2003; Brito *et al.* 2007a; Han *et al.,* 2011; Westerich *et al.,* 2011; Karuri *et al.,* 2017). For example, *M. enterolobii* was only recognized as a problem in Florida (USA) when galled tomato roots of root-knot nematode resistant tomato were noticed in response to what was thought to be 'persistent' populations of *M. incognita* (Brito *et al*., 2004). Furthermore, despite this species reacting similar to *M. incognita* race 4 in standard differential host plant tests, it compromises the *Mi-1* and *Tabasco* genes in tomato and pepper since it reproduces in roots of these crops (Brito *et al.,* 2007a). Virulence attributed to *M. enterolobii* hence limits the use of host plant resistance in managing this species in the majority of crops. It is thus crucial to exploit host plant resistance and integrate it along with other management strategies (Pinheiro *et al.,* 2014a). As with all management strategies, resistance also have shortcomings, namely that it is subjected to virulent nematode populations and it is dependent on environmental conditions, particularly high soil temperatures (beyond 28 °C) which may destabilise the trait (Greco and Vito, 2011).

Table 2.4. Examples of crop genotypes that exhibit genetic host plant resistance to *Meloidogyne enterolobii*.

Crop and genotype(s)	References
Acca sellowiana (O.Berg) Burret	Freitas *et al.* (2014)
Allium ampeloprasum L. ('Poró Gigante'), *Allium schoenoprasum* L. ('Tokyo', 'Nebuka'), *Brassica* spp. L. ('Bruxelas', 'Tronchuda Portuguesa'), *Brassica oleracea var. botrytis* L. ('Teresópolis Gigante', 'Piracicaba Precoce'), *Brassica oleracea var. capitata* L. ('Chato de Quintal', 'Coração de Boi'), *Brassica oleracea var. italica* L. ('Piracicaba', 'Brasília', 'Santana', 'Cabeça'), *Daucus carota* L. ('Brasília', 'Brasília Irecê', 'Planeta'), *Lactuca sativa* L. ('Grand Rapids'), *Petroselinum crispum* (Mill.) Fuss ('Comum HT', 'Graúda Portuguesa')	Rosa *et al.* (2015)
Allium fistulosum L. ('Evergreen Nebuka'), *Hibiscus sabdariffa* L.	Bitencourtm & Silva (2010a)
Anacardium occidentale L., *Annona muricate* L., *Annona squamosa* L., *Annona squamosa x A. cherimola* Aiton., *Byrsonima crassifolia* (L.) Kunth, *Citrus aurantifolia* (Christm.) Swingle, *Citrus reticulate* Blanco., *Citrus sinensis* (L.) Osbeck., *Garcinia brasiliensis* (Mart.) Clusiaceae., *Hancornia speciose* Gomes., *Manilkara zapota* (L.) P.Royen, *Pouteria* spp. Aubl., *Theobroma grandiflorum* (Willd. ex Spreng.) K.Schum.	Silva and Krasuki (2012)
Arachis hypogaea L. ('Florunner'), *Gossypium hirsutum* L. ('Deltapine 61')	Filho *et al.* (2016)
Avena sativa L. ('IPR Afrodite', 'URS Corona', 'URS Tarimba', 'URS Taura', 'URS Guria', 'AL0509', 'URSFAPA Slava', 'URS Torena', 'IAPAR 61', 'URS Charrua', 'IRS 126')	Machado *et al.* (2015), Riede *et al.* (2015)
Avena sativa ('URS-21', 'IPR-126', 'URS-Gúria', 'URS-Tarimba', 'URS-Taura', 'IAC-7'), *Sorghum bicolor* (L.) Moench. ('BRS-332', 'BRS-310', 'BRS-330', 'BRS-308', '307.689', '307.671', '307.343', 'BRS-610', 'BRS-655', 'BRS-700'), *S. bicolor* × *S. sudanense* (Nees ex. Steud.) Millsp. & Chase ('Piper','BRS-800', 'BRS-802', 'BRS-801'), *Triticum aestivum* L. ('CD-118', 'CD-104', 'CD-108', 'CD-150', 'BRS-220', 'BRS-Pardela', 'BRS Tangará')	de Brida *et al.* (2018)
Brassica oleracea L. ('Acephala'), *Daucus carota* ('Royal Chanteny', 'Imperator')	Brito *et al.* (2007a)
Brassica oleracea var. *acephala* L, ('Manteiga'), *Coriandrum sativum* L., *Daucus carota* ('Brasília Irecê'), *Phaseolus lumatus* L.('UFPI-464', 'Raio de Sol', 'UFPI-463', 'MA-01', 'UFPI-469', 'UFPI-482', 'MA-03')	Bitencourtm & Silva (2010b)
Capsicum annuum L. ('CNPH 640', 'CNPH 677', 'CNPH 684', 'CNPH 690', 'CNPH 693'), *Capsicum frutescens*	Soares *et al.* (2017)
Capsicum annuum ('UTC81')	Carillo-Fasio *et al.* (2020)
Capsicum chinense Jacq. ('UENF 1730')	Conçalves *et al.* (2014)
Capsicum spp. L. ('BGH-433', 'BGH-4285', 'PIM-030', 'PIM-031', 'Charleston Belle', 'PIX-022I-31-07-01', 'PIX-022I-31-07-02', 'PIX-022I-31-13-01', 'PIX-0221-31-14-01', 'PI0221-31-20-01', 'PIX-022I-31-20-02'),	de Melo *et al.* (2011)

Ipomoea batatas (L.) Lam. (‘UFLA07-31’, ‘UFLA07-49’, ‘UFLA07-53’), *Lactuca sativa* (‘Elisa’, ‘Julia’, ‘Hortência’, ‘Luisa’, ‘Mirella’, ‘Vera’, ‘Verônica’, ‘Grand Rapids’, ‘Salinas 88’), *Phaseolus vulgaris* L. (‘Aporé’)	
Coffea arabica L. (‘Obatã IAC 1669-20’, ‘Apoatã IAC 2258’, ‘Catuaí Amarelo IAC 62’, ‘Catuaí Vermelho IAC 99’, ‘Catucaí Amarelo 17/02’, ‘Catucaí Vermelho 20/15’, ‘Mundo Novo IAC 379-19’)	Alves *et al.* (2009)
Cucumis melo L. (‘PI 414723’, ‘AC 29’, ‘PI 124112’)	Diniz *et al.* (2016)
Cucumis metuliferus E. Mey. (‘Kino’)	Pinheiro *et al.* (2019)
Eugenia stipitate McVaugh	Chiamolera *et al.* (2018)
Eugenia stipitata (‘Araçazeiro-bio’), *Psidium* spp. L. ‘Araçazeiro 1’, ‘Araçazeiro 2’, ‘Araçazeiro 6’	Almeida *et al.* (2009)
Glycine max L. Merr (‘PI 595099’, ‘PI 594427’)	Vieira *et al.* (2016)
Gossypium spp. L. (‘PRO 277’, ‘IAC 29-233’, ‘PR 136’, ‘IAC 24’, ‘IAC 03-979’), *Hibiscus sabdariffa* (‘Vinagreira’)	Martin *et al.* (2017)
Lactuca sativa (‘Ithaca’, ‘Raider Plus’, ‘RS-1397’, ‘L-104’, ‘Challenge’, ‘IP-11’, ‘Classic’, ‘Salinas 88’, ‘Vanguard 75’, ‘Calona’, ‘Desert Queen’)	Correira *et al.* (2015)
Lactuca sativa (‘L_2’, ‘L 1’, ‘L_4’, ‘L_3’, ‘L_7’)	Diniz *et al.* (2018)
Lactuca sativa (‘Verônica’, ‘Grand Rapids’, ‘Crespa para Verão’, ‘Black Seed Simpson’)	Sgorlon *et al.* (2018)
Malpighia emarginata DC. (‘002-SPE’. ‘015-CPA’, ‘026-CMF’, ‘027-CMF’, ‘028-CMF’, ‘029-CMF’, ‘031-CMF, ‘033-CMF’)	Moreira *et al.* (2016)
Malpighia glabra L. (‘Cm2’)	Castellano *et al.* (2011a)
Oryza sativa L.*, Zea mays* L, (‘AGN 2012’)	Silva and Silva (2009)
Oryza sativa (‘BRS Pepita’, ‘BRS Monarca’, ‘AN Cambará’, ‘ANA 9001’, ‘IAPAR 63’, ‘BRS Serra Dourada’, ‘Ricetec Ecco CL’, ‘BRS Talento’, ‘IAPAR 64’, ‘IAPAR 117’, ‘BRS Bonança’, ‘IAPAR 09’, ‘BRS Soberana’, ‘BRS Primavera’, ‘BRS Sertaneja’, ‘IAPAR 177’, ‘BRS Curinga’, ‘BRS Caravela’, ‘BRS Conai’)	Machado and Filho (2014)
Phaseolus vulgaris (‘Alabama #1’)	Crozzoli *et al.* (2011)
Passiflora edulis Sims. (‘Roxinho do Kênia’), *Passiflora edulis* f. *flavicarpa* (‘Afruvec’, ‘FB100’, ‘FB200’, ‘BRS Gigante Amarelo’, ‘BRS Ouro Vermelho’, ‘BRS Sol do Cerrado’)	Costa *et al.* (2017)
Prunus cerasifera Ehrh. (‘P.2175’, ‘P.1079’,’P.2646 x P.1079’ 3,’’P.2646 x P.1079’ 9)	Rubio-Cabetas *et al.* (1999)
Prunus mume Siebold & Zucc. (‘Mume’)	Souza *et al.* (2013)

Prunus persica (L.) Batsch (‘Nemaguard’, ‘Guardian®’, ‘Flordaguard’)	Nyczepir *et al.* (2008)
Psidium friedrichsthalianum (O. Berg) Nied (‘CL4’, ‘CL5’)	Castellano *et al.* (2011b)
Psidium cattleyanum Sabine., *Psidium friedrichsthalianum*	de Sousa *et al.* (2016)
Psidium cattleyanum, Psidium friedrichsthalianum	Chiamolera *et al.* (2018)
Psidium cattleyanum (nr. 117 ‘wild ‘araça’, 115 ‘wild araça’, and 116 ‘wild araça’), *Psidium guajava* L. (nr. 94 ‘Hitigio’, 95 ‘Tsumori’, 135 ‘Rica’, 87 ‘wild guava’, 101 ‘wild guava’, 102 ‘wild guava’, 35 ‘Século XXI’, 56 ‘wild guava’, 84 ‘wild guava’, 109 ‘wild guava’)	Miranda *et al.* (2010; 2011)
Psidium cattleyanum (‘Red Araçá’, ‘Leodoro’, ‘Ya-cy’)	Carneiro *et al.* (2007)
Psidium cattleyanum (‘U2’, ‘UI 1’, ‘UI 2’, ‘UI 4’, ‘CI 17’)	Biazatti *et al.* (2016)
Psidium guajava (‘Tailandesa’)	Pereira *et al.* (2016)
Psidium guineense Sw. (‘AR1’, ‘AR3’, ‘AR4’, ‘AR10’)	Filho *et al.* (2018b)
Psidium cattleyanum, Psidium friedrichsthalianum, Psidium rufum	Freitas *et al.* (2014)
Psidium spp. L. (‘A-Amar’, ‘A-30’, ‘A.S.V.’,’ A-23’, ‘A-30.4’, ‘A-R.S’, ‘A-30.3’, ‘A-R.E’, ‘A-Roxo-u’, ‘A-Roxo-c’, ‘A- lu1’, ‘A- lu2’, ‘A- lu3’, ‘A-Ufla’)	Souza *et al.* (2014)
Ricinus communis L. (’BRS Energia’, ‘AL Guarani’, ‘Sara’, ‘Nordestina’, ‘Lyra’, ‘CPACT 40’, ‘IAC 80’, ‘Nordestina’)	Santos & Gomes (2011)
Saccharum spp. L. (‘RB92579’, ‘RB863129’, ‘SP81-3250’, ‘RB867515’	da Silva *et al.* (2012)
Zea mays (‘Cati H – 2002’, ‘Pioneer 30F35’, ‘Dow 2B587’, ‘Pioneer 3862’, ‘Syngenta NB 8315’, ‘Cati AL – Piratininga’, ‘Dow PZ 240’, ‘Syngenta Somma’, ‘Dow 2B688’, ‘Dow PZ 242’, ‘Pioneer 30K64’, ‘Cati AL – Bandeirante’, ‘Dow 5K6086’, ‘Syngenta Maximus’, ‘Dow PZ 677’, ‘Cati AL 25’, ‘Dow 2B604’, ‘Cati H – 25’, ‘Dow 2B707’, ‘DKB 177’, ‘Dow 2B710’, ‘Syngenta Impacto’)	Rosa *et al.* (2012)

2.2.2 Biological control

Biological methods have proved to be effective in managing plant-parasitic nematodes, with research to identify potential biocontrol agents increasingly gaining popularity. The use of fungi *Trichoderma harzianum* (Jindapunnapat *et al.,* 2013) and *Athrobotrys oligospora* (Gueye *et al.,* 1997) indicated promising results in the management of *M. enterolobii*. Ferriera *et al.* (2011) have shown the indirect effects of toxic metabolites released by the symbiotic bacterium *Photorhabdus luminescens* of *Heterorhabditis baujardi* Phan, Subbotin, Nguyen and Moens, 2003, on J2 hatching and motility. However, studies using *Pasteuria penetrans* (Trudgill *et al.,* 2000; Carneiro *et al.,* 2004b) and rhizobacteria (Almeida *et al.,* 2011a) to control *M. enterolobii* were less successful.

2.2.3 Cultural control

Cultural practices mainly rely on knowledge about the host status of crop genotypes and non-essential plants, such as weeds, for their use in crop rotation. Equally important is detecting the target *Meloidogyne* species in a specific field and its accurate identification. Effective management of *M. enterolobii* is obtained by cultivating immune, non-hosts or resistant crops in crop rotation cycles, which can include crops such as oat, wheat, or sorghum (Table 2.4) (de Brida *et al.,* 2018). Although specific genotypes may exibit resistance responses towards *M. enterolobii,* an opposite response may occure for other *Meloidoggyne* species. Exampes of such potential responses include the utilization of the *M. enterolobii* resistant genotypes; oat 'IPR-126' (Machado *et al.,* 2015; Riede *et al.,* 2015; de Brida *et al.,* 2018) and lettuce 'black seed simpson' (Sgorlon *et al.,* 2018), which exibit susceptibility to *M. incognita* and *M. javanica*. Thus, it remains of great importance that producers contiuously are updated on the host status of crop genotypes that they can choose to cultivate. Nonetheless, also important is that continuous evaluation of crop genotypes be done to verify their resistance against *M. enterolobii* since although they can initially be classified as resistant, the trait can be rendered ineffective over time as demonstrated for peach cultivar 'Flordagaurd' (Filho *et al.,* 2016; Martin *et al.,* 2017). Other cultural

practices reported to be effective in reducing other *Meloidogyne* species densities in crop fields include utilising organic amendments, solarisation, heat treatment, and many others (Okendi *et al.* 2014; Jones, 2017). However, standard practices such as fallowing, ploughing, and weeding did not yield any reduction in *M. enterolobii* population densities in São João da Barra (Souza *et al*., 2006).

2.2.4 Chemical control

Chemical control is a popular management strategy due to its rapid reaction and effective results. Only a few classical, chemically-derived nematicides have been evaluated for their effects on *Meloidogyne* species population densities, namely those with active ingredients of 1,3 dichloropropene: 1,3-D (Coyne *et al.* 2009), dazomet (Nyczepir and Thomas, 2009), fenamiphos (Coyne *et al.* 2009), oxamyl (Nyczepir and Thomas, 2009), and metam-sodium (Nyczepir and Thomas, 2009) (Onkendi *et al.,* 2014), which may prove effective against *M. enterolobii*. The use of naturally occurring (not chemically synthesized) nematicides, e.g. those derived from secondary metabolites of biological control agents (e.g. bacteria, fungi or others), parts of animals and / or plants (leaves, roots/other below-ground parts, stems or fruits that contain aldehydes, essential oils, glucosinolate derivates and others) (see Table 2.5) have proven effective against *M. enterolobii*. Examples are the aqueous and ethanolic extracts of *Calotropis procera,* causing a mere 6.4% hatching of *M. enterolobii* J2 after 72 h (Vegas *et al.,* 2010), and 51.25 – 100% mortality of J2 within 24 – 48 h of exposure to *Chenopodium ambrosioides* (L.) (Quevedo *et al.,* 2010; Friere and Santos*,* 2018).

Table 2.5. Examples of biological, cultural, and chemical control strategies used to reduce population densities of *M. enterolobii* in crop rhizospheres

Biological control	
Bacteria	
Associated bacteria and the entomopathogenic nematodes containing them:	
Photorhabdus spp. *(Heterorhabditis bacteriophora*)	Molani *et al.* (2007)
Photorhabdus luminescens (Heterorhabditis baujardi)	Ferriera *et al. (*2011)
Xenorhabdus bovienii (*Steinernema feltiae*)	Molani *et al.* (2007)
Xenorhabdus spp. (*Steinernema carpocapsae*)	Molani *et al.* (2007)
Pasteuria penetrans	Trudgill *et al.* (2000)
Fungi	

Arbuscular mycorrhizal fungi *Acaulospora longula,* *Glomus etunicatum*	Pinheiro *et al.* (2014b) Campos *et al.* (2013) Campos *et al.* (2013)
Arthrobotrys oligospora	Gueye *et al.* (1997) Duponnois *et al*. (2001)
Lecanicillium psalliotae *Pochonia chlamydosporia var. catenulate* *Paecilomyces lilacinus*	Arévalo *et al*. (2009; 2012)
Paecilomyces lilacinus 'strain 251'	Kiewnick (2011)
Pochonia chlamydosporia *Paecilomyces lilacinus*	Carneiro *et al.* (2011)
Ponchonia chlamydosporia *Purpureocillium lilacinum (*formerly *Paecilomyces lilacinus)*	Silva *et al.* (2017)
Trichoderma harzianum	Jindapunnapat *et al.* (2013)
Cultural control	
Green manure used: *Crotalaria breviflora* DC., *C. juncea* L., *C. mucronate* Desv., *C. ochroleuca* G. Don., *C. spectabilis* Roth., *Dolichos lablab* (L.) Sweet., *Lollium multiflorum* L., *Mucuna deeringiana* (L.) DC., *M. cinereal* (L.) Pers., *M. aterrima* (Wall. ex Wight) Baker ex Burck., *Pennisetum glaucum* (L.) R. Br., and *Raphanus sativus* (L.) Domin.	Rosa *et al.* (2015)
Soil amendments used: *Acacia mangium* Willd., *A. holosericea* A. Cunn. ex G. Don., *Azadirachta indica* A. Juss., *Eucalyptus camaldulensis* Dehnh., *Casuarina equisetifolia* L. and *Sorghum vulgare* Pers.	Duponnois *et al.* (2001)
Soil amendments used: *Tagetes patula* L.	Moreira and Ferreira (2015)
Soil amendments used: *Azadirachta indica, Sorghum bicolor* (L.) Moench.	Moreira *et al.* (2015)
Soil amendments used: cow manure, *Azadirachta indica* oilcake	Souza *et al.* (2006)
Soil amendments used: *Azadirachta indica* filter cake, cow manure, chicken compost	Gomes *et al.* (2009)
Soil amendmensts used: meat and bone meal	Almeida *et al.* (2011c)
Crop rotation: refer to host plant resistance for crops used as well as *Avena sativa* L., *Triticum* spp. L., and *Sorghum bicolor*	de Brida *et al.* (2018)
Chemical control	
Aqueous extracts of: *Dieffenbachia amoena* L., *Ricinus communis* L., *Azadirachta indica, Morinda citrifolia* L., *Jatropha curcas* L., *Datura stramonium* L., *Spigelia anthelmia* Spig., *Plumbago scandens* L., *Chenopodium ambrosioides* L., *Solenostemon scutellarioides* (L.) R. Br.	Friere and Santos (2018)
Aqueous extracts of: *Anacardium occidentale* L., *Coffea arabica* L., *Chenopodium ambrosioides, Ruta graveolent* L. **Ethanolic extracts of:** *Chenopodium ambrosioides*	Quevedo *et al.* (2010)
Aqueous extracts of: *Chenopodium ambrosioides*, *Lycopersicon esculentum* L.	Vegas *et al.* (2010)

Ethanolic extracts of: *Calotropis procera* (Aiton) W.T.Aiton.*, Chenopodium ambrosioides*	
Essential oil: *Tephrosia toxicaria* Pers.	Moreira *et al.* (2018)
Synthetic nematicides: 1,3 dichloropropene, dazomet, fenamiphos, oxamyl, and metam-sodium	Coyne *et al.* (2009), Nyczepir and Thomas (2009), Onkendi *et al.* (2014)

2.2.5 An overall perspective of management

An IPM approach is advocated for managing *Meloidogyne* species and for *M. enterolobii* in particular the combined use of amendments, such as meat and bone-meal, along with nematophagous fungi, have proven effective in reducing its population densities in Senegal (Duponnois *et al.,* 2001) and Brazil (Almeida *et al.,* 2011c). Also, the incorporation of organic material into *M. enterolobii*-infested soil, such as manure (Souza *et al.,* 2006) and the phytomass of *Tagetes patula* L., studies in Brazil (Moreira *and* Ferrerira, 2015) reduced population densities of this species. The recommended and most effective, and most widely used strategy, however, is the use of resistant plants in a crop rotation system to manage *M. enterolobii* infections (de Brida *et al.,* 2018). Integration of resistant crop genotypes in combination with other strategies that proved to be effective have already been exploited for the potential management of *M. enterolobii* (Rosa *et al.,* 2015). Nonetheless, the potential for further research and development of novel and effective strategies are inevitable and highly sought after. Management strategies to mitigate the adverse effects on crop production due to parasitism by *M. enterolobii* will be of great value to producers with regard to ensure food security, especially in developing countries of SSA.

2.3. Focus on sub-Saharan Africa

Sub-Saharan Africa faces various challenges including an increasing population, the need for food security and the impact of climate changes (De Waele and Elsen, 2007; Coyne *et al.,* 2018; dos Santos *et al.,* 2019). The impact that a threat root-knot nematode species, such as *M. enterolobii*, can have on food production in SSA where developing farmers generally dominate, or in a country such as South Africa where this farming sector supports a substantial amount of daily food sources for underprivileged and poor people, must therefore not be underestimated. Staple food

sources such as maize, potato and tomato produced throughout SSA (and very common in South Africa), yam and cassava in Nigeria, banana in Côte d'Ivoire as well as other important crops (e.g. coffee, cowpea, sweet potato, other vegetable and fruit crops) are all susceptible to *M. enterolobii* and researchers have to plan investigations carefully to protect and optimise production of such crops. Determining the distribution of *M. enterolobii* in as many SSA countries as possible will be the first crucial step, followed by studies related to the biology (life cycle in particular), pathogenicity and most importantly the development of management strategies against it. Novel management tactics, focusing on the resources that are available to farmers, will have to be developed since the majority of farmers in SSA do not have applicable infrastructure and/or adequate financial means to use chemical control. Numerous chemical products are furthermore increasingly phased out due to their misuse, harmful effects on animals, humans and the environment (Castagnone-Sereno, 2012; Onkendi *et al.,* 2014; Jones, 2017). Hand in hand with this is training of nematologists, currently supported by various stakeholders in SSA (Fourie and Mc Donald, 2014; Cortada *et al.,* 2019) as well as informing producers of the deleterious effects that *M. enterolobii* and other *Meloidogyne* species (as well as other economically important nematodes) are having on their crops.

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CHAPTER 3: MATERIAL AND METHODS

This project focused on three target crops, namely maize (*Zea mays* L.), tomato (*Solanum lycopersicum* L.) and soybean (*Glycine max* L.) in which the life-stage development, life-cycle duration and reproduction potential of three root-knot nematodes (*Meloidogyne*) species, *viz*. *Meloidogyne enterolobii* Yang and Eisenback, 1983; *Meloidogyne incognita* (Kofoid andand White, 1919) Chitwood, 1949 and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, were studied comparatively.

Furthermore, several facets of nematological research formed part of this study including: i) the *in vivo* rearing of each of these three *Meloidogyne* spp., ii) extraction of eggs of each *Meloidogyne* sp. from infected roots of the three target crops, iii) hatching of second-stage juveniles (J2) from extracted eggs of each species, iv) inoculation of the roots of the target crops with motile J2 of each species, v) staining of crop roots to isolate *Meloidogyne* spp. individuals during dissection for the classification of life-stages, vi) staining of the root-knot nematode egg masses present on infected roots of the target crops to determine the reproduction potential of each species and vii) utilisation of pre-programmed statistical computerised tools to analyse the collected data using Analysis of Variance (Anova) and Factorial Anova's.

Under glasshouse conditions, these activities were conducted with the following objects that formed the basis of the project, namely:

i) to compare the life-stage development and life-cycle duration of each of the three *Meloidogyne* spp. (*M. enterolobii*, *M. incognita* and *M. javanica*) in roots of the three target crops maize, soybean, and tomato,
ii) assessing the reproduction potential of the three *Meloidogyne* spp. in roots of the three crops.

3.1. Comparison of the life-stage development and life-cycle duration of three *Meloidogyne* spp. in roots of maize, soybean, and tomato

The main aim of this study was to determine the life-stage development and life-cycle duration of a South African *M. enterolobii* population in comparison to that of South

African populations of its tropical/thermophilic counterpart species *M. incognita* and *M. javanica*.

3.1.1. Identification and *in vivo* rearing of the three *Meloidogyne* spp. used

Single-species populations of the three *Meloidogyne* spp. used, *M. enterolobii*, *M. incognita* and *M. javanica*, were obtained (see Table 3.1) and reared *in vivo* in roots of tomato (cv. 'Floradade') in a glasshouse at the EcoRehab premises of the North-West University (Potchefstroom, North West Province). The pots were filled with soil obtained from Witpoort Sand & Stone (Ventersdorp, North West Province) and contained 97.6% sand, 1.2% silt, 1.3% clay and 0.06% organic matter, and had a pH of 5.73 (H_2O). These analyses were done by personnel of the Eco Analytica, North-West University, Potchefstroom. To ensure all soil-borne pathogens were eliminated, the soil was fumigated with Telone® II (97,5% active ingredient 1,3-dichloropropene) at 150 $L.ha^{-1}$ one month before the onset of the root-knot nematode rearing process.

3.1.2 Morphological/classical identification

The identity of each species was determined using the morphological approach of Marais *et al*. (2017) using selected characteristics of the perineal-pattern area of 10, mature females from each of the three single-species populations (see Paragraph 3.1.1). The publication of Kleynhans (1991) was used for identification of *M. incognita* and *M. javanica* females, while those of Visagie *et al*. (2018), Brito *et al*. (2004) and Rashifidard *et al*. (2019) were used to identifying *M. enterolobii* females.

A modified method of isolation and dissection of the posterior part of each mature *Meloidogyne* spp. female's (cuticle that contains the perineal pattern) was utilized by replacing the use of a scalpel by a 23G (gauge) hypodermic syringe needle (Figure 3.1). The globular female's cuticle was first punctured at the base of the neck; and an incision was made horizontally at the midsection of the female's body. The anterior region was isolated and no content was removed from this region in order to study the stylet knobs, the lumen of the oesophagus and medium bulb of each specimen.

Table 3.1. Locality and hosts of the three South African *Meloidogyne* spp. populations used in the study

Species	Locality	Host
Meloidogyne enterolobii	Mpumalanga Province	Gauva (*Psidium guajava* L.)
Meloidogyne incognita	Western Cape Province	Potato (*Solanum tuberosum* L.)
Meloidogyne javanica	Western Cape Province	Potato (*Solanum tuberosum* L.)

The poster region was isolated and by applying slight pressure to the globular structure the content was removed. The cuticle was then cut with two vertical incisions and the small part of the cuticle containing the perineal pattern was situated facing upwards. Pressure was carefully applied again to remove any remaining internal tissue, then the cuticle with the perineal pattern was mounted in a drop of glycerol onto a glass microscope slide and covered with a glass cover slide. The same process was followed for the anterior part. Further investigation was done using a light microscope (100x magnification).

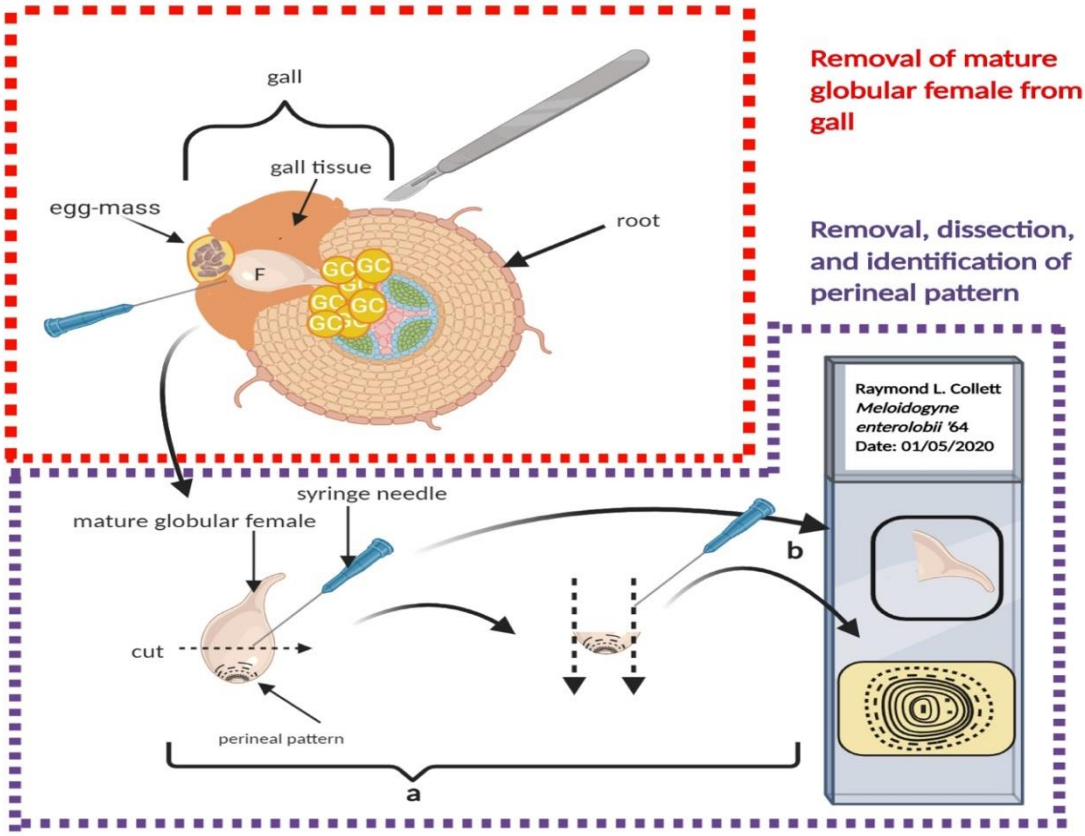

Figure 3.1. A step-by-step illustration of how mature females (containing egg masses) of *M. enterolobii*, *M. incognita* and *M. javanica*, respectively were removed from infected tomato roots (a); the posterior part of the body separated from the anterior part and the small cuticle area containing the perineal part cut (b); the perineal pattern and head of the female transferred and mounted next to each other on a microscopic slide that was labelled for identification purposes using a light microscope at 100x magnification (Created by Raymond L. Collett, NWU, Potchefstroom campus).

3.1.3 Molecular identification

The sequence characterised amplified region – polymerase chain reaction (SCAR-PCR) was used to obtain deoxyribonucleic acid (DNA) bands for each root-knot nematode species on an electrophoresis gel for identification. The procedure followed was adapted by Rashidifard (2019) from Musapa *et al.* (2013) and was as follows:

1. Isolation of females for DNA extraction
    a. Ten mature females were isolated from infected tomato roots in which the single-species populations of each species were mass-reared (see Paragraph 3.1.1) and transferred to individual Eppendorf tubes that were sterilised.
    b. Eppendorf tubes containing the females were stored in a fridge at 4 °C until DNA extraction was done.
2. DNA extraction
    a. The females in each tube for each of the species were homogenised using a pestle, which was sterilised with a 96% alcohol solution in between to prevent cross-contamination of the female's body tissue among the three species, and vortexed.
    b. 20 µl chelex (5% w/v) and 3 µl proteinase K (20 $mg.ml^{-1}$) were added to each sterilised Eppendorf-tube, containing the homogenate female tissue.
    c. The tubes were incubated for 2 h at 56 °C followed by 10 min incubation of 95 °C to enable the release and purification of the DNA extracts of each species' females.
3. Amplification of DNA (by Vacutec thermocycler)
    a. 25 µl PCR mix a 5.5 µL ddH_2O, 12.5 µl master mix (Promega Corporation, USA), 1 µl forward primer (10 µM), 1 µl reverse primer (10 µM), and 5 µl of extracted DNA from females of each of the three species were added to another set of individual Eppendorf tubes.
    b. The species-specific primers as indicated by Table 3.2.a. and b, was utilized in the amplification processes by adding 5 µl of each to the content of Eppendorf tubes as prepared in the step above (3a) for each of the three species.

c. The prepared Eppendorf tubes were transferred to PCR machines; one PCR machine was used for each of the three species.
d. The polymerase chain reaction (PCR) amplification profiles for each species were applied according to Table 3.2.b., from the process of denaturation temperature and time to final extension temperature and time to allow the production of the PCR products for each of the species.

4. Gel electrophoresis
    a. A 1% agarose gel solution containing TAE (Tris – Acetate – EDTA) was solidified into the characteristic rectangular form, used for gel analyses, containing individual wells at the top side in which the PCR products of the three species were loaded.
    b. Prior to loading these PCR products, a 1 bp ladder was loaded into the first well indicating the band location of each DNA fragment size that will be generated for the three species under investigation.
    c. Each well, after the first one containing the 1 bp ladder, was loaded with a 4 µl droplet of PCR product, drawn with a micropipette from each of the Eppendorf tubes of which the content was subjected to the PCR process (see 3a, b, c and d above), blended with 1 µl GelRed.
    d. After loading these PCR products into the wells of the gel, the same procedure was repeated with 4 µl droplets of the standards' DNA used for each of the three species.
    e. The process of separating the specific root-knot nematode DNA fragments utilizing an electrical current in the gel (lasting approximately 35 min) represented the next step after which the gel was exposed to an ultraviolet transilluminator for inspection of the gel to determine the size of the DNA fragments present.
    f. The species standards and the 1 bp ladder were inspected to enable determination of each of the three species' DNA bands that were visible on the gel (See Chapter 4, Figure 4.4.).

Information about the primers used for each root-knot nematode species is listed in Table 3.2a as well as the origin of the standard populations used for each species. Table 3.2b lists the primer pairs used for each of the three species and the PCR conditions to which the DNA samples were subjected.

Table 3.2.a. Identification of South African populations of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* using the sequence characterised amplified region – polymerase chain reaction (SCAR-PCR) (Zijlstra, 2000; Zijlstra, Donkers-Venne *et al.*, 2000).

Primer code for *Meloidogyne* sp.	**Primer sequence (5' →3')**	**Specificity**	**Reference**	**Origin of standard populations used[1]**
Me-F	AACTTTTGTGAAAGTGCCGCTG	*M. enterolobii*	Long *et al*. (2006)	Guava (*Psidium guajava*); Mpumalanga, Population M36[1]
Me-R	TCAGTTCAGGCAGGATCAACC			
F-inc	CTCTGCCCAATGAGCTGTCC	*M. incognita*	Zijlstra *et al*. (2000)	Maize (*Zea mays*), , Northern Cape, Population M5[1]
R-inc	CTCTGCCCTCACATTAGG			
Fjav	GGTGCGCGATTGAACTGAGC	*M. javanica*	Zijlstra *et al*. (2000)	Pumpkin (*Curcurbita pepo*), Northern Cape, Population M76[1]
Rjav	CAGGCCCTTCAGTGGAACTATAC			

Table 3.2.b. Polymerase chain reaction (PCR) amplification profiles used with the species-specific primers for identification of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica*. (Zijlstra, 2000; Zijlstra, Donkers-Venne *et al.*, 2000; Long *et al*., 2006).

Primer pair	**Denaturation temperature and time**	**Cycle**	**Denaturation temperature and time**	**Annealing temperature and time**	**Extension temperature and time**	**Final extension temperature and time**
Me-F/Me-R	94°C, 2 min	35	94°C, 30 s	64°C, 30 s	72°C, 1 min	72°C, 5 min
F-inc/R-inc	94°C, 2 min	45	94°C, 30 s	54°C, 30 s	72°C, 1 min	72°C, 5 min
F-jav/R-jav	94°C, 2 min	45	94°C, 30 s	64°C, 30 s	72°C, 1 min	72°C, 5 min

3.1.4 Preparation of *Meloidogyne* spp. inoculum

Tomato roots infected with the individual, *in vivo* reared populations of *M. enterolobii*, *M. incognita*, and *M. javanica* (Table 3.1) were separated from the aerial plant parts 30-40 days after the rearing processes commenced. The roots infected with the three individual species were washed under running tap water to remove excess soil and decomposed plant material. The roots were then subjected to the adapted NaOCl method of Riekert (1995) for the extraction of eggs as demonstrated in Figure 3.2. The entire root system was cut into 0.5-1 cm pieces and submerged in 250 ml of a 1% NaOCl solution in a 400-ml capacity container. The NaOCl solution dissolved the gelatinous matrix of the root-knot nematode egg-masses and released the eggs. The plastic container was placed onto an orbital shaker and shaken for 4 min to aid the release and suspension of the eggs in the solution.

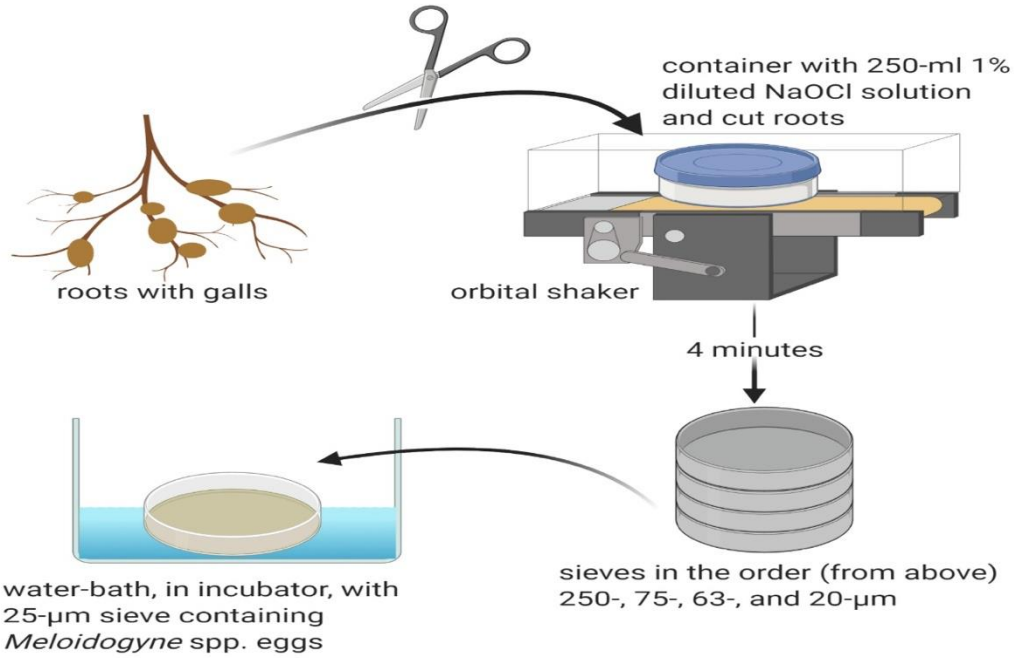

Figure 3.2 Extraction of *Meloidogyne* spp. eggs utilizing the adapted NaOCl method and placing extracted eggs into a water-bath that was transferred into an incubator at a controlled temperature (Created by Raymond L. Collett, NWU, Potchefstroom campus).

The content of the container (root pieces and *Meloidogyne* eggs suspended in the NaOCl solution) was decanted onto a stack of test sieves: from top to bottom in the order of 250-µm, 75-µm, 63-µm, and 20-µm, and thoroughly washed with a gentle stream of running tap water. The eggs obtained this way were counted in a de Grisse counting dish using a 10-µl droplet method (Figure 3.3) and a stereomicroscope (60× magnification). After obtaining the egg counts in 30 (5 droplets x 6 replicates) of these droplets for each of the three species, the total number of eggs obtained as a result of the extraction process were calculated.

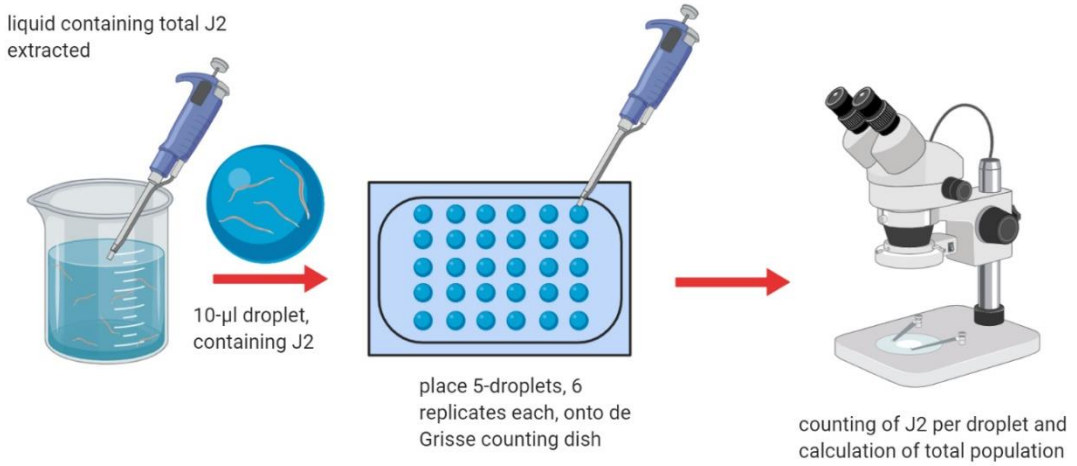

Figure 3.3 Counting of either extracted eggs or hatched second-stage juveniles (J2) per 10-µl droplet extracted from the base solution utilising a stereomicroscope; 60× magnification. (Created by Raymond L. Collett, North-West University, Potchefstroom).

The eggs of each species were next placed onto a 25-µm mesh aperture sieve that was submerged in a plastic container filled halfway with tap water. As the J2 started to hatch from the eggs on the sieve, they moved through the mesh of the sieve into the surrounding water. The hatching of J2 occurred in a temperature-regulated growth chamber at an ambient temperature of 28 °C. At each 2 h interval, the J2 that hatched were isolated by decanting them onto a 20-µm aperture mesh sieve and washing them into a glass beaker.

The J2 were then counted in the 30 droplets to determine their total population size and subsequently the number of J2 to be inoculated onto the roots of seedlings of each of the three target crops. Counting of J2 was done using a stereomicroscope (60× magnification).

3.1.5 Inoculation of crop seedlings with *Meloidogyne* sp. juveniles

Maize and soybean seeds were obtained from the respective suppliers, namely Monsanto South Africa (Pty) Ltd (now known as Bayer (Pty) Ltd) and Agricol (who has a trade agreement with DonMario Seeds, Argentina). The seeds were not treated with any fungi-, herbi- or pesticides since it was requested to be used in nematode experiments. Two-leaf stage tomato seedlings were obtained from Ezigro seedlings (White River, Mpumalanga Province, South Africa).

Maize, soybean and tomato seedlings were obtained by growing it from the respective seeds or seedlings (see previous paragraph) in 4-L capacity pots within a glasshouse under the same controlled conditions as indicated for the *in vivo* rearing of the *Meloidogyne* spp. (see Paragraph 3.1.1). During sowing of soybeans, each seed was inoculated with 5 mg of the nitrogen fixing bacteria (*Bradyrhizobium japonicum*), which was obtained from Soygro (Pty) Ltd (Potchefstroom, North West Province), to stimulate and promote germination and nitrogen fixation. After sowing of the seeds (maize and soybean) and getting seedlings (tomato), 250 ml tap water was added to each pot. The seedlings were watered every 2nd day for 10 days.

Table 3.3. Information about the three crops used for life-stage development, life-cycle duration and reproduction of three *Meloidogyne* spp. as well as information about the conditions under which the experiments were conducted.

Crop	Crop cultivar	Mean min. temp (C°)	Mean max temp (C°)	Number of J2 inoculated per seedling	Glasshouse
Maize					
Initial experiment	P-2432-R	21.7	32.2	2000	√
Repeat experiment	P-2432-R	16.0	32.0	950	√
Soybean					
Initial experiment	DM-5953-RSF	13.0	31.0	2000	√
Repeat experiment	DM-5953-RSF	16.0	32.0	950	√
Tomato					
Initial experiment	Moneymaker	18.5	32.0	1 200	√
Repeat experiment	Moneymaker	16.0	32.0	950	√

The following nutrient solution was applied to the pots of the three crops at transplanting: 100 ml of a 1 $g.L^{-1}$ solution of Nutrifeed (Stark Ayers) containing the following nutrient: Ca=7,0%; K=13,0%; Mg=2,2%; N=6,5%; P=2,7%; S=7,5%; <0,1% micro-elements (B, Cu, Fe, Mn, Mo, and Zn). At the second- and/or early third-leaf stage the seedlings were uprooted from the pots and transplanted into fumigated soil in 400-ml capacity white, plastic PVC tubes (Figure. 3.4).

The initial experiment was conducted with tomato only, but subsequently the study was extended to include maize and soybean since the tomato plants were severely damaged by tomato leaf miner *Tuta absoluta* (Meyrick) and it was impossible to obtain leaf miner-free tomato seedlings.

Meloidogyne spp. used (Figure 3.4). Seven sampling intervals were used for the first tomato experiment, *viz*. 3, 5, 10, 15, 20, 25, and 30 days after inoculation (DAI) were used for each of the crops and for each of the three *Meloidogyne* spp.; with five replicates per sampling interval.

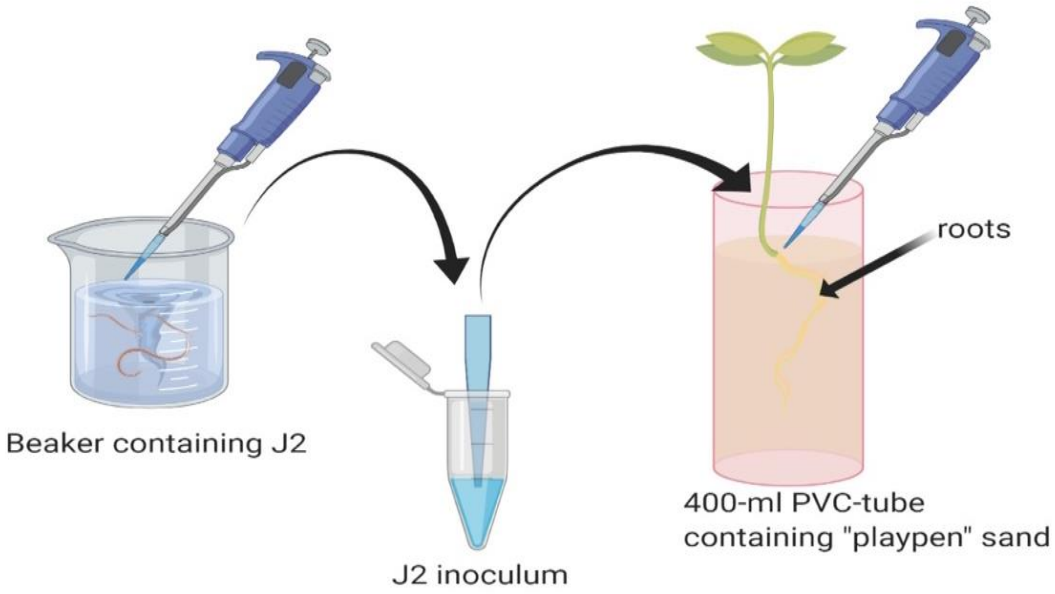

Figure 3.4 Inoculation of crop seedlings with second-stage juveniles (J2) of the respective root-knot nematode species used for life-stage development and life-cycle duration studies, and transplanting of seedlings three days after inoculation into 400-ml PVC-tubes filled with sterilized soil (Created by Raymond L. Collett, North-West University, Potchefstroom).

For each experiment the roots of each crop seedling were inoculated with the designated number of motile J2 (indicated in Table 3.3.) of each of the three species. Three DAI, the seedlings (of each crop) were removed from the soil, carefully rinsed with tap water and transplanted into another set of 400-ml capacity PVC tubes.

These plants were kept until the termination of each experiment. This was done to ensure that only the J2 that penetrated during the first three days were present in the roots and developed to the subsequent life stages

3.1.6. Staining of *Meloidogyne* spp. life stages for life-stage determination

The sodium-hypochlorite-acid-fuchsin method (Byrd *et al.,* 1983) was used to stain *Meloidogyne* spp. individuals in roots of the whole root system of each plant in order to facilitate their removal for further investigation (Figure 3.5).

Such staining was done by placing each root system, after it had been rinsed with tap water, into a 1:5 NaOCl:water (100-ml NaOCl to 500-ml tap water) solution for 6 min. The individual root systems were then rinsed under running tap water for 1 min and placed into 400 ml tap water for 15 min. After 15 min the root systems were rinsed for 1 min under running tap water.

Placed within a fume-hood, each rinsed root system was submerged for 30 s into a boiling solution consisting of 40 ml water and 1-ml acid-fuchsin solution (3.5 g acid-fuschin dissolved in 250 ml acetic acid). The root systems were left in this solution to cool down to room temperature for 30 min. After this period each root was washed for 1 min under running tap-water and placed into 10 ml glycerol. Up to this stage of the experimental procedure, the root systems were kept in the fume-hood

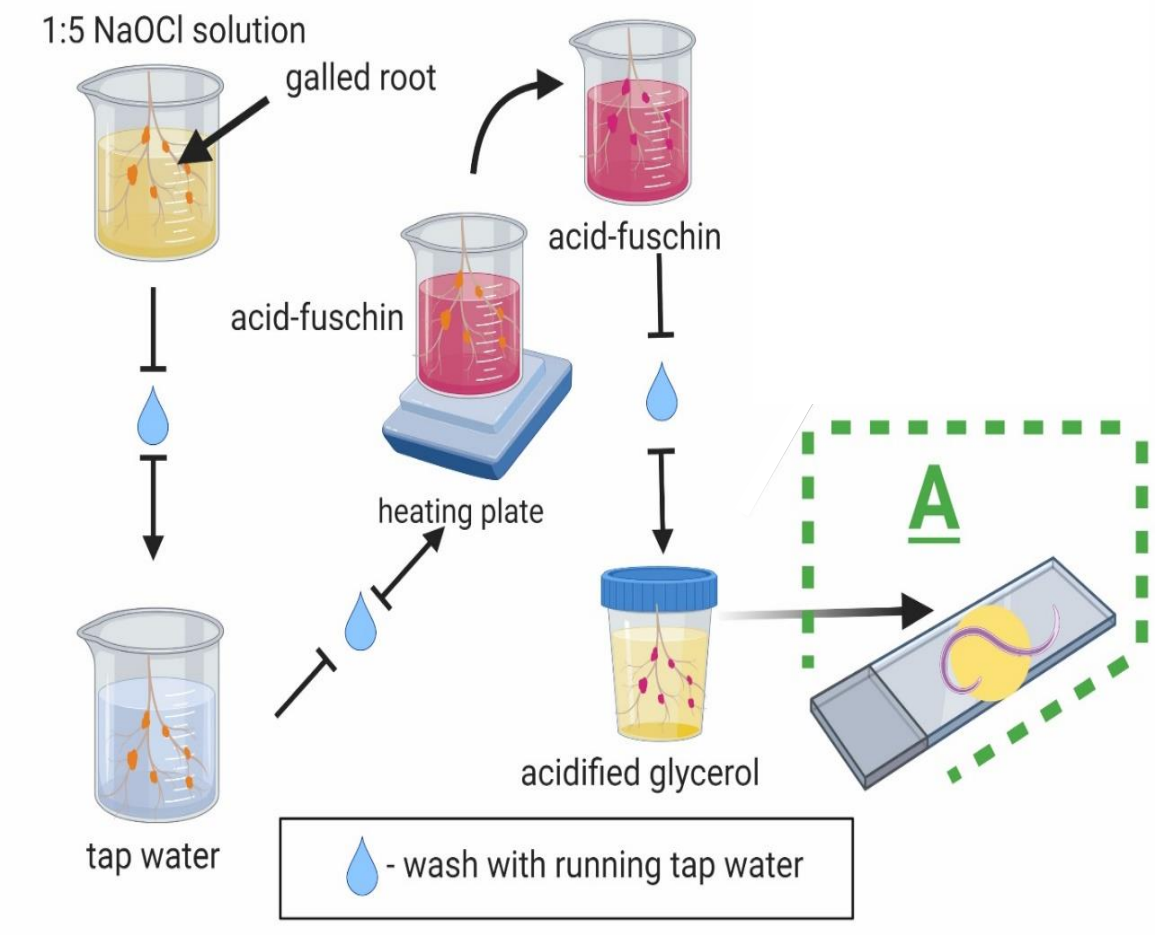

Figure 3.5 The sodium-hypochlorite-acid-fuchsin method used to stain roots and *Meloidogyne* spp. individuals to enable the isolation of 20 individuals per root system per species that were placed onto a glass-microscope slide for identification of the life stages using a light microscope (100× magnification) (Created by Raymond L. Collett, North-West University, Potchefstroom).

Important to note is that the root systems of all crop plants were stained only by using sodium-hypochlorite-acid-fuchsin method (Byrd *et al.,* 1983; Figure 3.5) until 15 DAI. The reason for this approach is that egg masses were found from 20 DAI onwards. Therefore the root systems obtained for the latter sampling intervals were first stained with eosin-Y (Figure 3.7) to remove egg-masses, prior to staining them with the sodium-hypochlorite-acid-fuchsin method. This procedure enabled the isolation of the 20 stained individuals per species per root system for determining their life stages as is explained below.

After staining, the roots were inspected using a stereomicroscope (40× magnification), and 20 nematode individuals of different life-stages were randomly removed for each root-knot nematode species and placed into a drop of glycerol on glass microscope slides (Figure 3.5).

The various *Meloidogyne* life stages were identified using the protocol of Triantaphyllou and Hirschmann (1960) (Figure 3.6). This was done using a light microscope (100× magnification).

The main criteria used for life-stage identifications were the presence of the number of cuticles/moults of each of the 20 nematodes that were isolated. For example, the presence of the 2nd, and 3rd moult indicated that the individual had reached the 4th juvenile stage.

3.1.7. Determining of reproduction potential of *Meloidogyne* spp.

The root systems collected, for the three species and the three crops, for the sampling intervals from 20 DAI onwards were stained using the eosin-Y method (Figure 3.7).

Each root system was placed into a 1:20 diluted eosin-Y solution; 20-ml eosin-Y stock solution (0.25-mg eosin-Y dissolved in 1-L water) diluted in 400 ml tap water for 1 h (Figure 3.7) (Cousins and Walker, 2000).

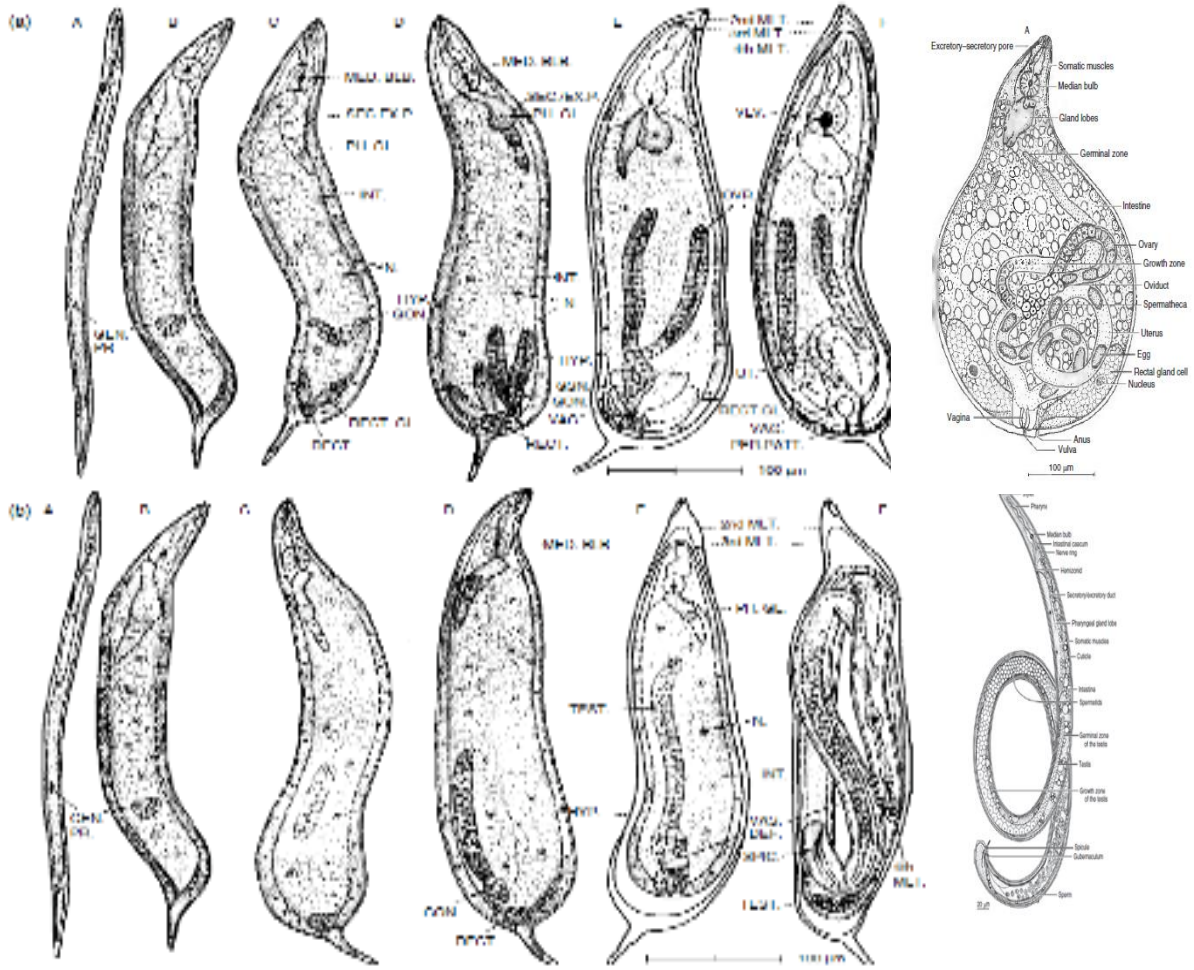

Figure 3.6. A representation of the various life stages that occur during the life cycle of *Meloidogyne* spp. The infective, motile second-juvenile (J2) stage (A), swollen, immotile J2 stage (B), immotile, third-juvenile stage (C-D) and the immotile, fourth-juvenile stage (E), an immotile, immature female (F), an immotile, mature female (G) and a motile, mature male (H) (Image obtained from an adapted version of Karssen *et al*., 2013).

Ten root-knot nematode egg-masses were then removed from each root system (for each crop) and each placed separately into 10 Eppendorf tubes (2-ml capacity) containing 1.5-ml of an 1% NaOCl solution for the extraction of eggs (Riekert, 1995). This was done by shaking each tube by hand for 2 min to enable the release of the eggs from each of the individual egg-masses. The eggs were then counted using a De Grisse dish and a stereomicroscope (60× magnification) (Figure 3.7).

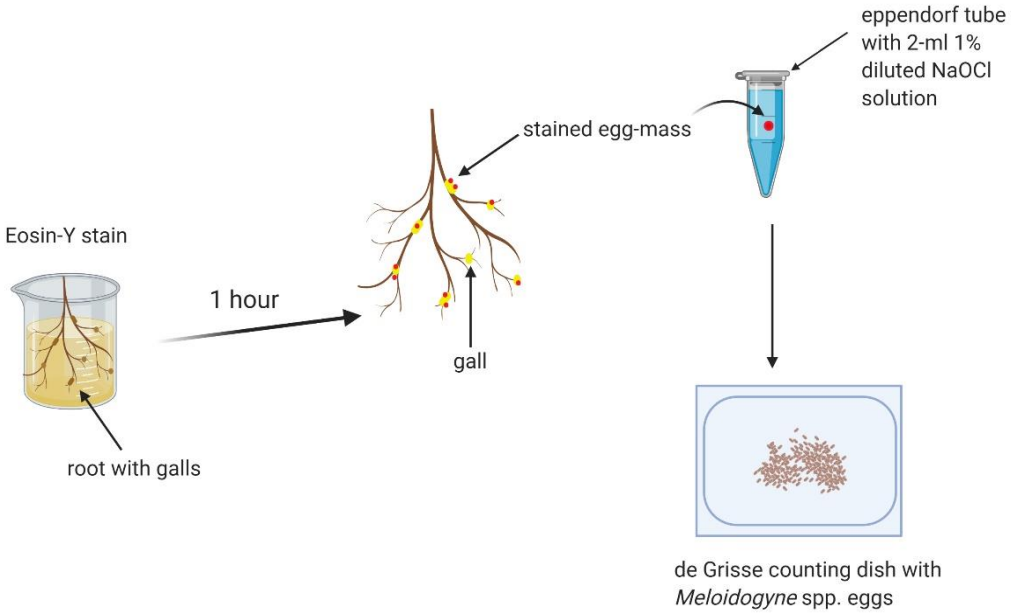

Figure 3.7. Eosin-Y staining of egg-masses present on galled roots to determine the reproduction potential of each *Meloidogyne* spp. and extraction of eggs from single egg masses to enable counting the eggs contained per egg mass using a stereo-microscope (60× magnification) (Created by Raymond L. Collett, NWU, Potchefstroom campus)

The rest of the root system, available after removal of the egg masses, of each plant for each root-knot nematode species, was subsequently stained using acid-fuschin and 20 individuals removed to determine their life stages as indicated in Paragraph 3.1.6.

3.1.8. Calculating degree-days for the *Meloidogyne* spp. life-cycle durations

The degree-days (DD) are described by Arnold (1960) as the thermal constant used to determine the measurement of the physiological time required for the completion of a process. In the context of this study, it includes the completion of the life cycle of the respective *Meloidogyne* spp. It is thus the heat units required for the development of a motile J2 to a mature life stage (egg-laying female or a male). A crucial element to the calculation of the DD is the base temperature (T_b) of each species which has been determined as the temperature at which the lowest rate of development occurred for such a species (Negron, 2006). The T_b was determined to be 10.0, 9.8 and 10.6 °C for

M. enterolobii (Jacobs *et al.,* 2011)*, M. incognita,* and *M. javanica* (Negron, 2006), respectively.

To calculate the DD for a specific *Meloidogyne* spp. the following equation was used: DD = $[(T_{max} + T_{min})/2 - T_b]$ x number of days required to complete; T_{max} = maximum temperature for the period of development; T_{min} = minimum temperature for the period of development; and T_b = base temperature of the *Meloidogyne* sp. in question.

3.2. Data analyses

Data obtained for the life stages identified per sampling interval and number of eggs per egg mass of the three *Meloidogyne* spp. studied for each of the three crops (and for the five replicates per crop per species) were recorded and subjected to Analysis of Variance (ANOVAs) first for each species. After such analyses, Factorial ANOVAs were done (Statistica, Version 13.3; www.statsoft.com) with *Meloidogyne* spp. as the main factor, Sampling Intervals as factor 1 and crops as factor 2.

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CHAPTER 4: RESULTS AND DISCUSSION - IDENTIFICATION OF THE THREE *MELOIDOGYNE* SPECIES USED DURING LIFE-STAGE DEVELOPMENT AND LIFE-CYCLE DURATION EXPERIMENTS

This study comprised 1st (initial) and a 2nd (repeated) experiments for each of three *Meloidogyne* species and three crops namely a maize, soybean, and tomato cultivar (See Chapter 3; Table 3.3.). The species *Meloidogyne enterolobii* Yang and Eisenback 1983, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 were reared *in vivo* as single-species populations in roots of a susceptible tomato cultivar (see Chapter 3, Paragraph 3.1.1. in order to elucidate their life-stage development and life-cycle duration. Individuals of each life-stage was stained (see Chapter 3; paragraph 3.1.6.) and identified according to the method of Triantaphyllou & Hirschmann (1960).

4.1 Results and discussion

4.1.1 Morphological/classical identification

A morphological approach, using oesophageal and perineal-pattern characteristics, of mature root-knot nematode females (Fig. 4.2) of *M. enterolobii*, *M. incognita* and *M. javanica* was followed as described by Marais *et al*. (2017).

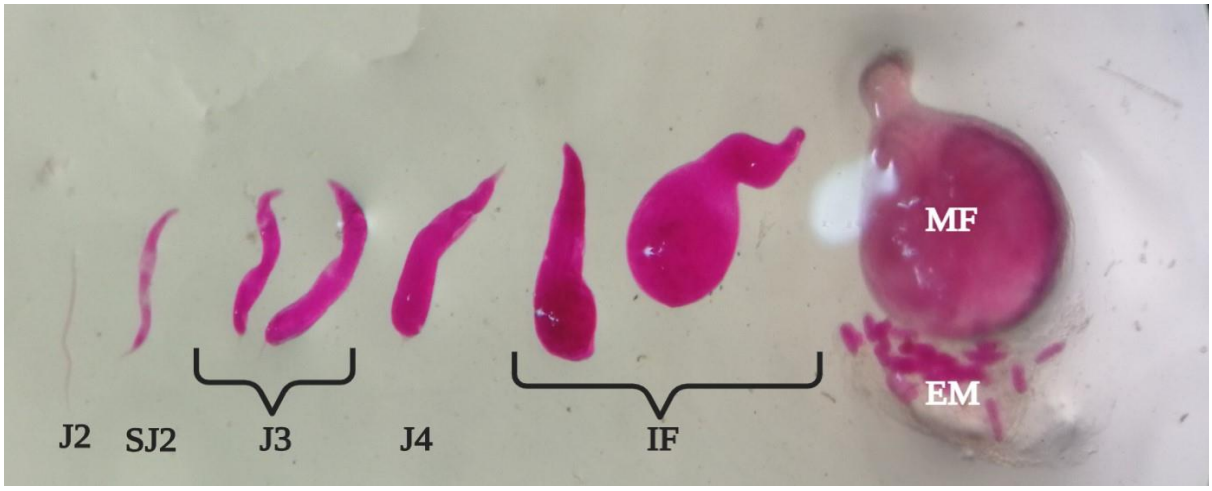

Figure 4.1. Life stages of *Meloidogyne enterolobii* indicating the motile second-stage juvenile (J2), swollen second-stage juvenile (SJ2), swollen third-stage juvenile (J3), swollen fourth-stage juvenile (J4), immature female (IF), and mature female (MF) with an egg-mass (Created by Raymond L. Collett, North-West University, Potchefstroom).

Thereafter, using the protocols of Kleynhans (1991), Brito *et al*. (2004) and Rashidifard *et al*. (2019) 10 mature females were isolated from infected roots in which the single-species populations of each species were mass-reared, for the identification of the three root-knot nematode species used in this study.

The characteristics used to attempt distinguishing among the females of the three species according to oesophageal morphology included mainly the shape of the lumen of the esophagi as well as the shape of the lumen of the metacorpus and procorpus linings (Table 4.1).

Perineal-pattern morphology was, however, the main focus to attempt elucidating the identity of the three species in terms of the classical approach. Figure 4.1a-c presents the typical appearance of the perineal patterns of *M. enterolobii* (a), *M. incognita* (b) and *M. javanica* (c), respectively. The identification of a species, according to the morphology of a perineal pattern, requires the successful recognition of each of the characteristics of the apex, dorsal arch, lateral lines, phasmids, striae, and the tail regions as indicated in Table 4.1. for each of the three species.

Table 4.1. The perineal pattern and the shape of the lumen of the oesopagi of the three species namely *M. enterolobii, M. incognita,* and *M. javanica;* according to [1]Yang and Eisenback (1983), [2]Eisenback (1985), [3]Kleynhans (1991); [4]Brito *et al.* (2004); [5]Karssen *et al.* (2013); [6]Agenbag (2016); [7]Rashidifard (2019).

		***Meloidogyne* species**		
	Characteristics	***M. enterolobii***	***M. incognita***	***M. javanica***
Perineal-pattern morphology[1, 2, 3, 4, 5, 6, 7]	**General shape**	Round to ovoid	Circular to oval	Circular to oval
	Dorsal arch	Moderately high to high	Medium high to high	Low to medium high
	Apex	Rounded, however square in some specimens	Broadly rounded or squared	Squarish to broadly rounded
	Phasmids	Distinct phasmids in tail-terminus	Distorted phasmids	Not clear, but visible
	Tail region	Tail tip terminus visible	Tail tip terminus visible	Tail tip terminus visible
	Lateral lines	Not distinct	Not visible	Clearly visible as double lines
	Striae	Finer and smoother pattern on ventral side; non present in peri-vulval area	Smooth, wavy, and even zigzag pattern	Smooth to wavy

	Miscellaneous	Perivulvar region free of striae	Whorl around tail terminus	Distinct rectal punctations
Shape of lumen of the oesophagus [3, 4, 6, 7]	**Lumen lining**	No information recorded	Expands then narrows towards spheroid	No information
	Metacorpus lining	No information recorded	Visible as an ovoid	Spheroid
	Procorpus lining	No information recorded	No information	Cylindrical, may expand/narrow immediately in front of usual ovoid

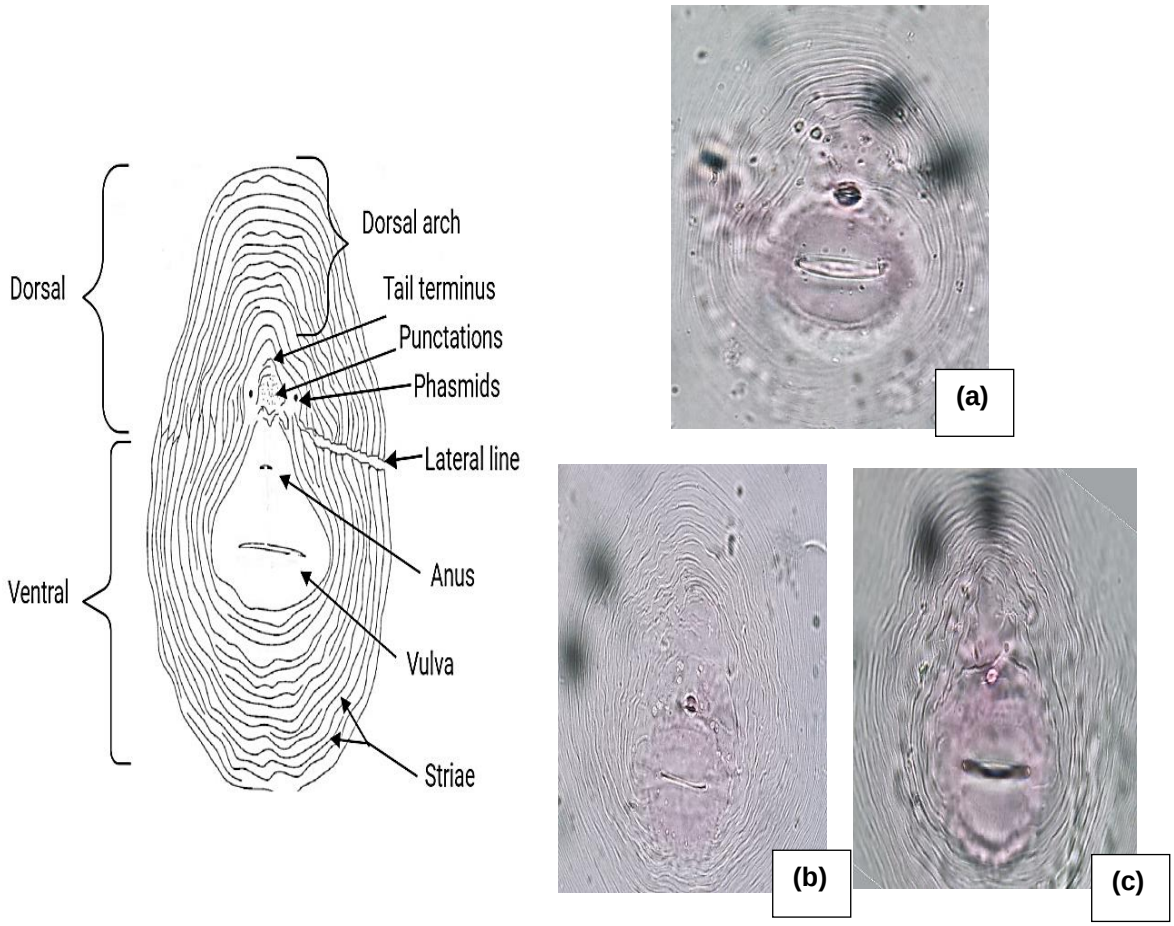

Figure 4.2. Perineal patterns of mature females of *Meloidogyne enterolobii* (a), *M. incognita* (b), and *M. javanica* (c). Images taken at 100x magnification, compared to perineal pattern diagram indicating characteristics of interest (Photo's: Raymond L. Collett, North-West University, Potchefstroom).

Although, according to Table 4.1., each species possesses a set of defined characteristics that are used to distinguish amongst them. It was, however, evident by observing the oesophageal morphology and perineal patterns of the females (Figure 4.3) that distinguishing between the three species was a challenge and remains problematic for the inexperienced and untrained eye. This was especially the case for *M. enterolobii* and *M. incognita* which were very difficult to distinguish between using these morphological characteristics. It agrees with reports that differenetiating between the latter species is challenging, even for experienced and expert taxonomists (Carneiro and Cofcewiez, 2008; Carneiro *et al*., 2016).

Thus, misinterpretation of the identity of the *Meloidogyne* species used in this study was avoided by the implementation of molecular methods such as the sequence characterized amplified region – polymerase chain reaction (SCAR-PCR) method (Zijlstra *et al*., 2000) as explained in Chapter 3, Paragraph 3.1.4, Tables 3.2a and b.

4.1.2 Molecular identification

The identity of the three species, verified using the SCAR-PCR technique (Zijlstra *et al*., 2000), are demonstrated in Figure 4.3. Three species-specific primers for the identification of the three *Meloidogyne* species were used to identify *M. enterolobii, M. incognita,* and *M. javanica* (See Chapter 3; Table 3.2.a). The species was identified from *in vivo*, mass-reared, single-species populations, that originated from a single egg mass of each of the species.

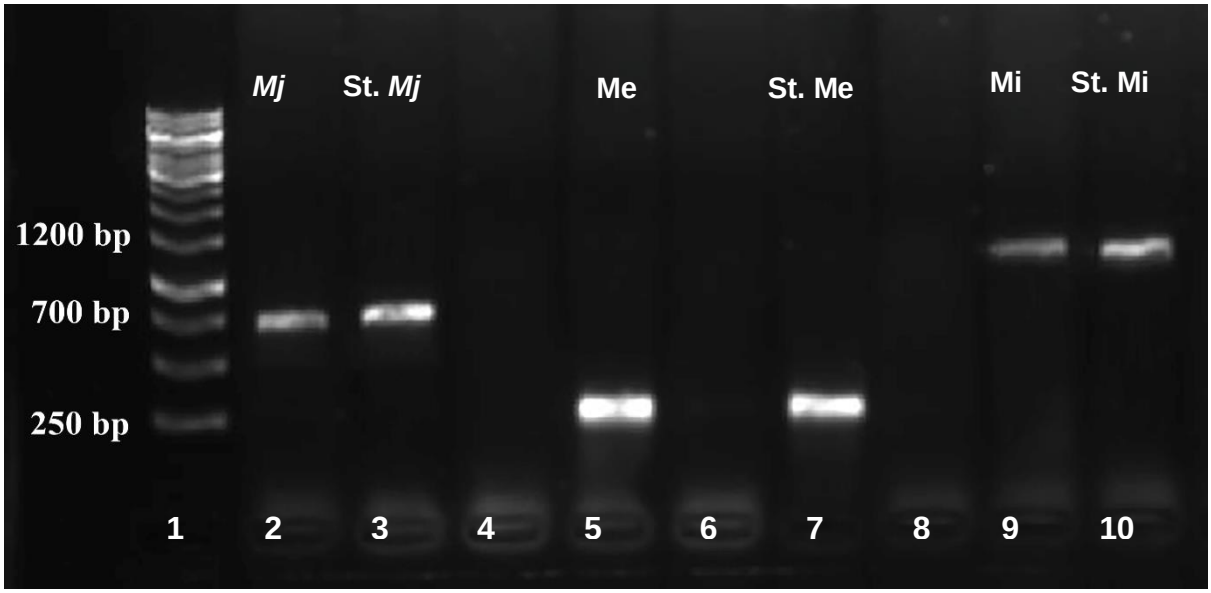

Figure 4.3. Gel electrophoresis results indicating DNA fragments of specific DNA sizes associated with specific *Meloidogyne* spp.; *viz*. (*Mj* – well 2) indicates the presence of *M. javanica*; (St. *Mj* – well 3) indicates the *M. javanica* standard; (*Me* – well 5) indicates presence of *M. enterolobii*; (St. *Me* – well 7) is the *M. enterolobii* standard*;* (*Mi* – well 9) indicates the presence of *M. incognita,* and (St. *Mi* – well 10) is the *M. incognita* standard. The 1 kb DNA ladder is indicated in well 1.

From the SCAR-PCR procetures the following *Meloidogyne* species was identified according to the PCR products, namely the DNA fragments, visualized by Figure 4.3. For *M. javanica* the DNA results indicate the presence of this species due to the presence of a 700 bp DNA SCAR fragment, in the the 2nd well, which is simular to that of Zijlsta *et al*. (2000). It also coincides with the 700 bp DNA fragment of the *M. javanica* standard in the 3rd well, thus supporting the positive results (Figure 4.3). Thus, results support the presence of this species in a single-species mass reared population since no fragments were amplified when the DNA of this species was subjected to the SCAR-PCR primers of *M. enterolobii* and *M. incognita* (data not showed).

For *M. enterolobii*, the DNA results from the SCAR-PCR method indicated a 250 bp fragment being amplified, present in the 5th well, which is simular in bp size of the fragment to that Long *et al.* (2006) reported for this species. This result is also in agreement with that of the *M. enterolobii* standard DNA fragment in the 7th well (Figure 4.3); hence the presence of an *in vivo*-reared, single-species *M. enterolobii* is verified since no fragments were amplified when the DNA of this species was subjected to the SCAR-PCR primers of *M. incognita* and *M. javanica* (data not showed).

For *M. incognita*, the DNA test indicates a possitve result for the presence of this species due to amplification of the 1 200 bp fragment in the 9th well, similar to the fragment reported by Zijlstra et al. (2000). This 1 200 bp fragment also mirrors that of the amplified standard (in the 10th well) supporting the result that the species was positively identified as a single-species population of *M. incognita* (Figure 4.3) since no fragments were amplified when the DNA of this species was subjected to the SCAR-PCR primers of *M. enterolobii* and *M. javanica* (data not showed).

4.1.3. Discussion

The *Meloidogyne* spp. was successfully identified, using both morphological and molecular approaches, to allow a comparative investigation of the life cycle, life stage and reproduction potential study of the three species: *M. enterolobii*, *M. incognita* and *M. javanica*.

Although results of the morphological perineal-pattern approach supported the molecular identification of the three species, it was difficult to distinguish the perineal patterns of especially *M. enterolobii* and *M. incognita* from each other; especially for an inexperienced MSc student. It his therefore agreed with Carneiro *et al.* (2017), Visagie *et al*. (2018) and

Rashidifard *et al*. (2019) that using morphology only, particularly the perineal-pattern approach, is challenging and may lead to misidentify *M. enterolobii*. Ultimately, combining the SCAR-PCR molecular results, that yielded species specifc DNA fragments of a particular bp length for each of the three species (250 bp or *M. enterolobii*; 1 200 bp for *M. incognita* and 700 bp for *M. javanica* and were each similar to the positive standards included for the respective species) and the morphological approach, yielded accurate identificaiton of the species.

Various authors advocated the combined use of morphological and molecular identification for *Meloidogyne* species and especially those belonging to the *M. incognita* group to prevent misidentification of species. For example this was done for South African *Meloidogyne* species, of which some was represented by *M. enterolobi* by Visagie *et al*. (2018) and Rashidifard *et al*. (2019); for Florida species Brito *et al* (2004); for North Carolina species (Ye *et al.*, (2013); and for Mexican species (Villar-Luna *et al.*, 2016).

It is crucial to correctly identify *Meloidogyne* species since *M. incognita* and *M. javanica* are known to cause problems in local maize (Mc Donald *et al*., 2017), soybean (Fourie *et al*., 2017) and tomato (Jones *et al*., 2017; Van den Berg *et al*., 2017; SAPPNS[1]) production areas. However, *M. enterolobii* was only recently identified from the second biggest soybean production area (Highveld of Mpumalanga) (Pretorius, 2018), in which the crop is commonly rotated with maize. Furthermore, the distribution of *M. enterolobii* was also recently reported from more crops grown in South Africa (Visagie *et al*., 2018; Rashidifard *et al*., 2019) and from other areas as those where it has been originally been identified (Willers, 1997; SAPPNS[1]).

The data obtained during this part of the study could hence be trusted and the comparative life-stage and life-cycle development, and reproduction potential study that followed was conducted with confidence since the identity of the three species was confirmed.

[1] Dr Mariette Marais of the Nematology Unit, Biosystematics, Agricultural Research Council–Plant Health and Protection is thanked for the use of data from the South African Plant-Parasitic Nematode Survey (SAPPNS) database; E-mail: maraism@arc.agric.za.

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CHAPTER 5: RESULTS AND DISCUSSION – COMPARISON OF THE LIFE-STAGE DEVELOPMENT, LIFE-CYCLE DURATION AND REPRODUCTION OF THREE *MELOIDOGYNE* SPECIES

Results are given in this chapter about the life-stage development, life-cycle duration and reproduction potential of the three species *Meloidogyne enterolobii* Yang and Eisenback, 1983; *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949*;* and *Meloidogyne javanica* (Treub, 1885) Chitwoodi, 1949, of which populations were obtained locally (see Chapter 3, Paragraph 3.1.5.; Table 3.3.). The species identification was verified using both morphological and molecular approaches (see Chapter 4, Paragraphs 4.1.1. and 4.1.2.)

The roots of target crops in which the life-stage development and life-cycle duration of the three selected species were conducted included maize (*Zea mays* L.), soybean *Glycine max* (L.) Merr. And tomato (*Solanum lycopersicum* L.). The purpose of this study a completed life-cycle (Figure 5.1) of a species represented the period from the initial infection of the motile second-stage juvenile (J2) to the stage in which a mature, egg-producing female was detected.

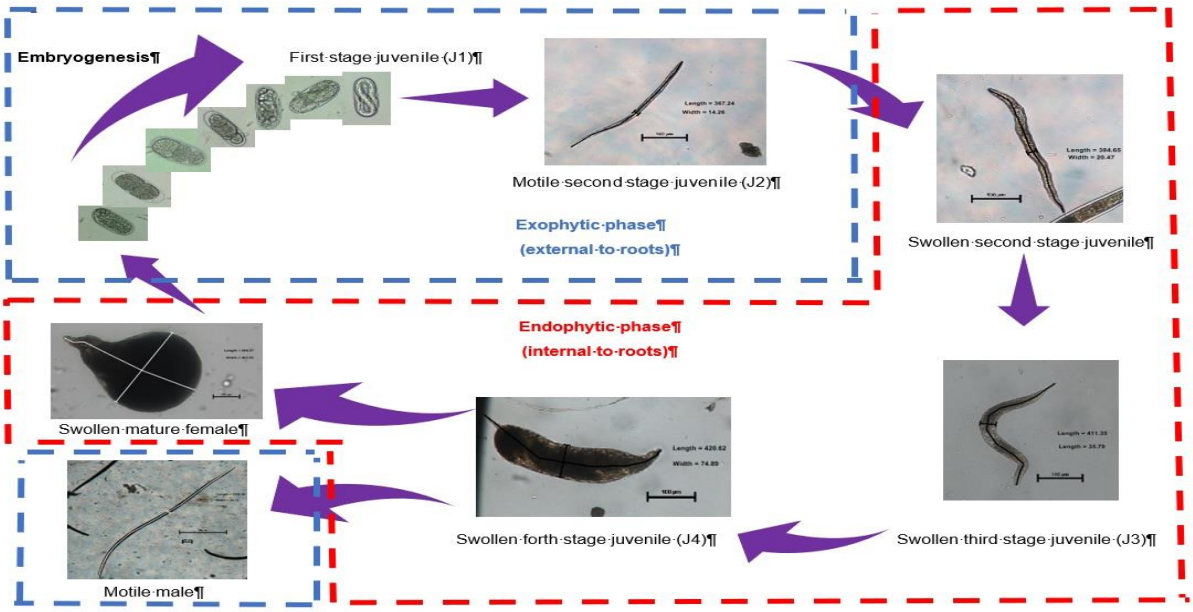

Figure 5.1. Representation of the life cycle of *Meloidogyne* species, starting with embryogenesis of the eggs, developing into motile second-stage juveniles (J2) (represented by blue) and development to swollen J2, swollen third-stage (J3), swollen fourth-stage (J4), and either a swollen mature female (which takes place in the endophytic phase, represented by red) or a motile male (represented by blue) (Created by R.L. Collett, NWU, Potchefstroom).

The order in which the results are given is maize first, followed by soybean and tomato.

5.1. Comparing the life-stage development, life-cycle duration and reproduction potential of *M. enterolobii, M. incognita,* and *M. javanica* in maize roots

Two experiments were performed during which seedlings of maize of genotype P-2432-R' (see Chapter 3, Table 3.3) were inoculated separately with 2 000 (1st experiment) and 950 (2nd experiment) second-stage juveniles (J2), respectively, of the following three species: *M. enterolobii, M. incognita,* and *M. javanica*.

5.1.1. Motile second-stage juvenile (J2)

Significant ($P \leq 0.05$) interactions existed for each of the three *Meloidogyne* species for Experiment x Species for the number of motile J2: *M. enterolobii* ($P=0.001$; F-ratio=23.0); *M. incognita* ($P=0.001$; F-ratio=18.0); and *M. javanica* ($P=0.001$; F-ratio=5.1). Similarly, significant ($P \leq 0.05$) interactions were evident for Species x Time Interval/DAI ($P=0.001$; F-ratio=14.0) and for Species x Experiment x Time interval ($P=0.001$; F-ratio=5.0) (Table 5.1.1).

Comparison of the two experiment's data for each of the three species showed that the motile J2 numbers differed significantly ($P \leq 0.05$) for some of the time intervals between the 1st and 2nd experiments (Table A1a in Addendum 1A; Figure 5.1.1a and b).

Comparatively, the three species' motile J2 numbers did not differ significantly from one another 3 DAI for both experiments (Figure 5.1.1a and b; Table A1a in Addendum 1A). However, 5 DAI the numbers of motile J2 of *M. incognita* and *M. javanica* were significantly ($P \leq 0.05$) higher compared to those of *M. enterolobii* for the 1st experiment while the three species have similar ($P > 0.05$) motile J2 numbers for the 2nd experiment.

For 10 and 15 DAI the motile J2 numbers for the three species did not differ significantly ($P \leq 0.05$) from each other for both experiments (Figure 5.5.1a and b; Table A1a, Addendum 1).

At 20 and 25 DAI, *M. enterolobii* had significantly ($P \leq 0.05$) higher motile J2 numbers than *M. incognita* and *M. javanica* for the 1st experiment, but it did not differ from the latter two species for the 2nd experiment (Figure 5.5.1a and b; Table A1a, Addendum 1).

Table 5.1.1. Number of motile second-stage juveniles (J2) of *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of maize genotype P-2432-R during a 25-day period under glasshouse conditions.

Time interval/DAI	*M. enterolobii*		*M. incognita*		*M. javanica*	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
3	19.6 d*D**	20.0 bD	20.0 cB	20.0 bB	20.0 cB	20.0 bB
5	16.2 cE	18.4 bDE	20.0 cB	20.0 bB	19.8 cB	18.0 bB
10	3.2 aBC	5.6 cC	4.0 bC	6.2 cD	5.8 bC	7.8 cC
15	2.8 aABC	0.02 aA	0.01 aA	0.01 aA	1.2 aA	0.8 aA
20	5.6 aC	1.6 aAB	0.8 aA	0.01 aA	1.0 aA	0.01 aA
25	9.6 bF	1.4 aAB	2.6 bC	0.6 aA	5.2 bC	0.6 aA
P	0.001	0.001	0.001	0.001	0.001	0.001
***F* ratio**	93.1	341.9	985.7	2465.0	111.5	293.3
Interaction data:						
Species x Experiment						
P	*0.001*		*0.001*		*0.001*	
F-ratio	*23.0*		*18.0*		*5.1*	
Species x Time interval (DAI)						
P	*0.001*					
F-ratio	*14.0*					
Species x Experiment x Time interval (DAI						
P	*0.001*					
F-ratio	*5.0*					

*Indicates the differences in means among the time intervals for each species and experiment in each column, with the same lower case letter showing no significant difference according to the Tukey HSD Test ($P \leq 0.05$); **Indicates the differences in means between the two experiments of each species across rows, with the same capital letter showing no significant difference for a particular species at a given time interval between the two experiments according to the Tukey HSD Test ($P \leq 0.05$).

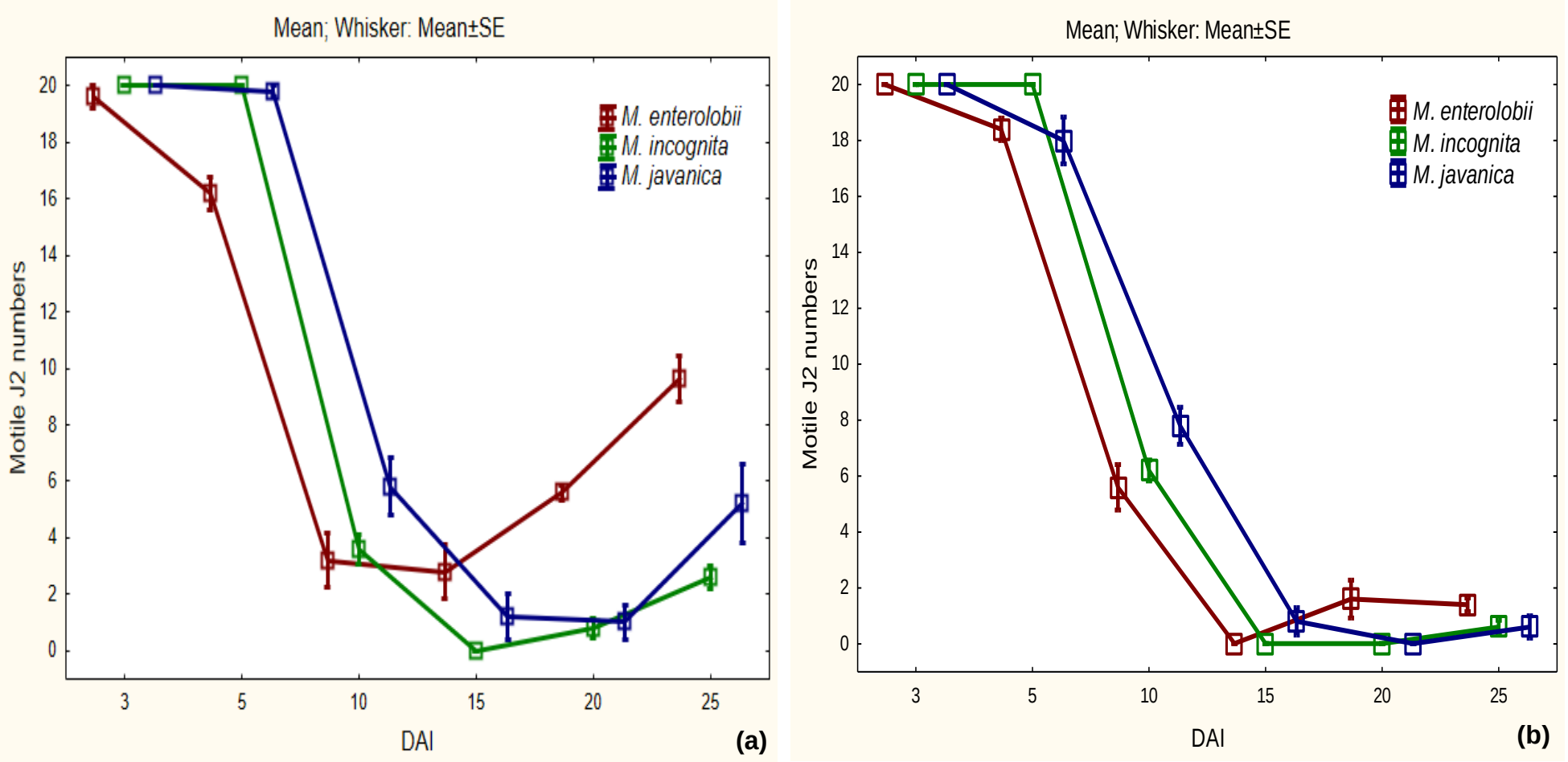

Figure 5.1.1. The number of motile second-stage juveniles (J2) of *M. enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) in roots of a maize genotype P-2432-R' from 3 to 25 days after inoculation (DAI) during a 1st (a) and 2nd (b) under glasshouse conditions.

5.1.2. Swollen second-stage juvenile (J2)

Significant (*P*≤0.05) interactions existed for each of the three *Meloidogyne* species for Experiment x Species for the number of motile J2: *M. enterolobii* (*P*=0.001; F-ratio=4.0); *M. incognita* (*P*=0.001; F-ratio=36.8); *M. javanica* (*P*=0.001; F-ratio=9.0). Similarly, significant (*P*≤0.05) interactions were evident for Species x Time Interval/DAI (*P*=0.001; F-ratio=7.7) and for Species x Experiment x Time interval (*P*=0.001; F-ratio=10.5) (Table 5.1.2).

Comparison of the two experiment's data for each of the three species showed that the swollen J2 numbers differed significantly (*P*≤0.05) between the 1st and 2nd experiments for some of the time intervals (Table 5.1.2; Figure 5.1.2a and b).

Comparatively the three species' swollen J2 numbers did not differ significantly from one another 3 DAI for both experiments (Figure 5.1.2a and b; Table A1b, Addendum 1A). However, 5 DAI the numbers of swollen J2 of *M. enterolobii* were significantly (*P*≤0.05) higher compared to those of *M. incognita* and *M. javanica* for the 1st experiment, while the three species have similar (*P*>0.05) swollen J2 numbers for the 2nd experiment.

For 10 DAI the swollen J2 numbers for *M. enterolobii* were significantly (*P*≤0.05) higher than those of the other two species for both experiments. However, 15 DAI the three species did not differ significantly for both experiments (Figure 5.1.2a and b; Table A1b, Addendum 1A).

At 20 DAI *M. enterolobii* had significantly (*P*≤0.05) higher swollen J2 numbers than *M. incognita* and *M. javanica* for the 1st experiment, while the three species had similar (*P*>0.05) numbers for the 2nd experiment (Table A1b, Addendum 1A).

At 25 DAI the swollen J2 numbers of *M. enterolobii* and *M. javanica* were significantly (*P*≤0.05) higher than those of *M. incognita* for the 1st and 2nd experiments (Figure 5.1.2a and b; Table A1b, Addendum 1A).

Table 5.1.2. Number of swollen second-stage juveniles (J2) of *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of maize genotype P-2432-R during a 25-day period under glasshouse conditions.

Time interval/DAI	*M. enterolobii*		*M. incognita*		*M. javanica*	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
3	0.4 b*AB**	0.02 aA	0.02 aA	0.01 aA	0.02 aA	0.01 aA
5	3.0 aDE	1.6 bABCD	0.02 aA	0.02 aA	0.2 aAB	2.0 bBC
10	8.8 cF	5.0 cE	6.0 bC	1.6 bB	5.8 cD	7.2 cD
15	0.8 abABC	0.01 aA	1.8 aA	0.6 abAB	0.8 aAB	0.6 abAB
20	2.2 abBCD	1.0 abABCD	0.02 aA	0.02 aA	0.01 aA	0.02 aA
25	2.8 bCD	2.2 bBCD	0.01 aA	0.02 aA	3.2 bC	0.2 abAB
P	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***
F--atio	***32.0***	***35.1***	***112.7***	***7.5***	***41.2***	***43.8***
Interaction data:						
Species x Experiment						
P	***0.004***		***0.001***		***0.001***	
F ratio	***4.0***		***36.8***		***9.0***	
Species x Time Interval (DAI)						
P	***0.001***					
F-ratio	***7.7***					
Species x Experiment x Time interval (DAI						
P	***0.001***					
F-ratio	***10.5***					

*Indicates the differences in means among the time intervals for each species and experiment in each column, with the same lower case letter showing no significant difference according to the Tukey HSD Test ($P \leq 0.05$); **Indicates the differences in means between the two experiments of each species across rows, with the same capital letter showing no significant difference for a particular species at a given time interval between the two experiments according to the Tukey HSD Test ($P \leq 0.05$).

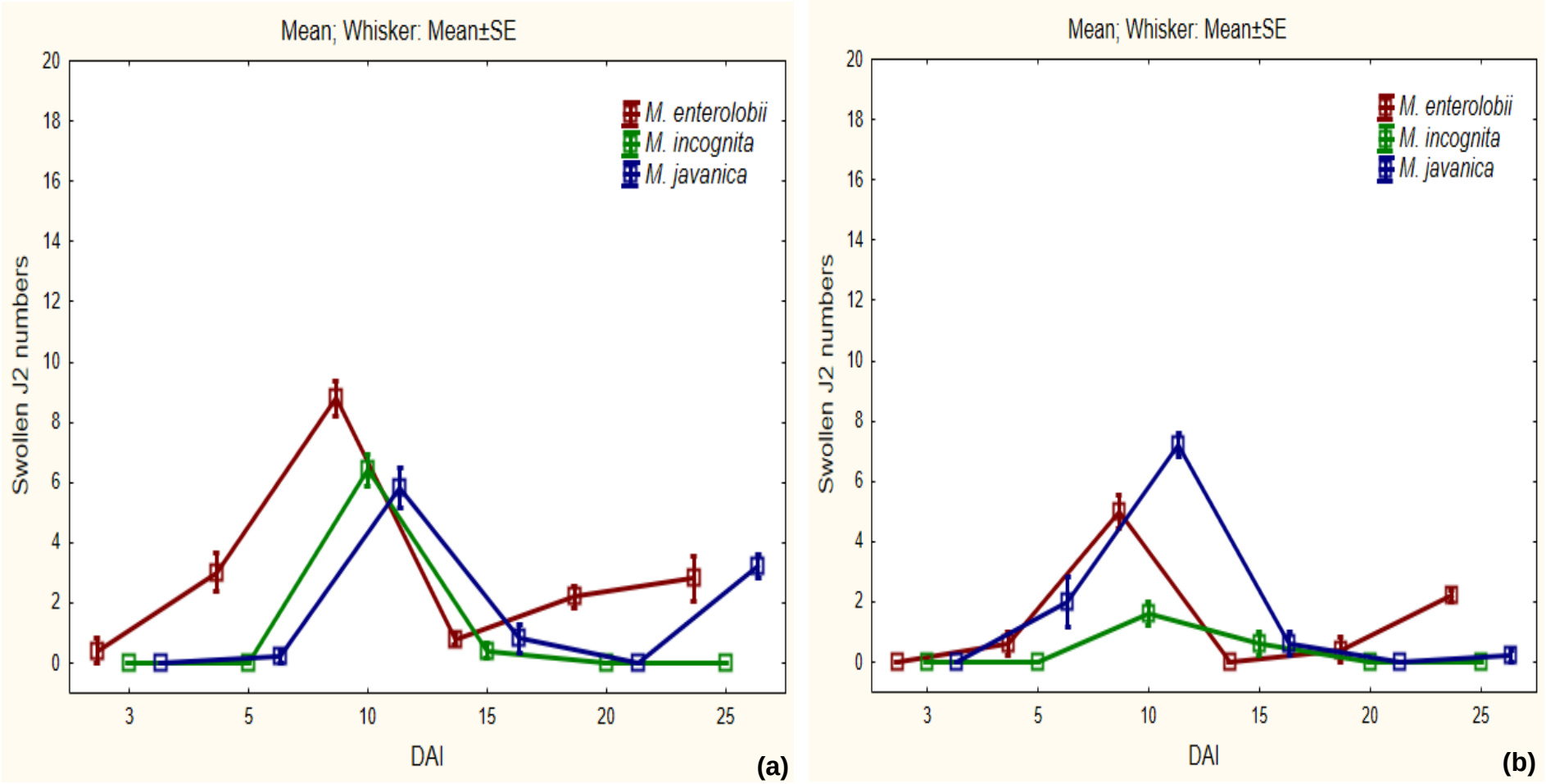

Figure 5.1.2. The number of swollen second-stage juveniles (J2) of *M. enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) roots of maize genotype P-2432-R from 3 to 25 days after inoculation (DAI) during a 1st (a) and 2nd (b) under glasshouse conditions.

5.1.3. Swollen third-stage juvenile (J3)

Significant (*P*≤0.05) interactions existed for Experiment x Species for the number of swollen J3 for *M. incognita* (*P*=0.001; F-ratio=27.7) and *M. javanica* (*P*=0.003; F-ratio=4.3), but not for *M. enterolobii* (*P*=0.055; F-ratio=2.4). While no significant interaction was evident for Species x Time Interval/DAI (*P*=0.054; F-ratio=1.9), a significant interaction did exist for Species x Experiment x Time interval (*P*=0.001; F-ratio=6.2) (Table 5.1.3).

Comparison of the two experiment's data for each of the three species showed that the swollen J3 numbers differed significantly (*P*≤0.05) for some of the time intervals between the 1st and 2nd experiments (Table 5.1.3; Figure 5.1.3a and b).

Comparatively the three species' swollen J3 numbers did not differ significantly from one another 3 and 5 DAI for both experiments (Table A1c, Addendum 1A; Figure 5.1.3).

For 10 DAI the swollen J3 numbers of *M. incognita* and *M. javanica* were similar and significantly (*P*≤0.05) higher than those of *M. enterolobii.* The three species, however, had similar J3 numbers for the 2nd experiment (Table A1c, Addendum 1A; Figure 5.1.3).

For the 15, 20 and 25 DAI the swollen J3 numbers for all three *Meloidogyne* species indicated no significant (*P*≤0.05) differences for both experiments.

Table 5.1.3. Number of swollen third-stage juveniles (J3) of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* in roots of maize genotype P-2432-R during a 25-day period under glasshouse conditions.

Time interval/DAI	*M. enterolobii*		*M. incognita*		*M. javanica*	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
3	0.02 a*A**	0.01 aA	0.03 aA	0.02 aA	0.02 aA	0.02 aA
5	0.61 aAB	0.02 aA	0.02 aA	0.02 aA	0.02 aA	0.02 aA
10	3.8 bCD	4.8 cD	7.2 cD	3.0 bC	5.4 bC	4.2 cBC
15	2.2 abABC	2.6 bBCD	1.41 bAB	1.8 bB	0.81 aA	3.0 bB
20	2.0 abABC	0.21 aA	0.02 aA	0.21 aA	0.02 aA	0.21 aA
25	1.01 aAB	0.02 aA	0.02 aA	0.01 aA	0.21 aA	0.01 aA
P	0.003	0.001	0.001	0.001	0.001	0.001
F-ratio	5.3	35.7	226.7	20.3	20.7	51.9
Interaction data:						
Species x Experiment						
P	***0.055***		***0.001***		***0.003***	
F-ratio	***2.4***		***27.7***		***4.3***	
Species x Time interval (DAI)						
P	***0.054***					
F-ratio	***1.9***					
Species x Experiment x Time interval (DAI)						
P	***0.01***					
F-ratio	***6.2***					

*Indicates the differences in means among the time intervals for each species and experiment in each column, with the same lower case letter showing no significant difference according to the Tukey HSD Test ($P \leq 0.05$); **Indicates the differences in means between the two experiments of each species across rows, with the same capital letter showing no significant difference for a particular species at a given time interval between the two experiments according to the Tukey HSD Test ($P \leq 0.05$).

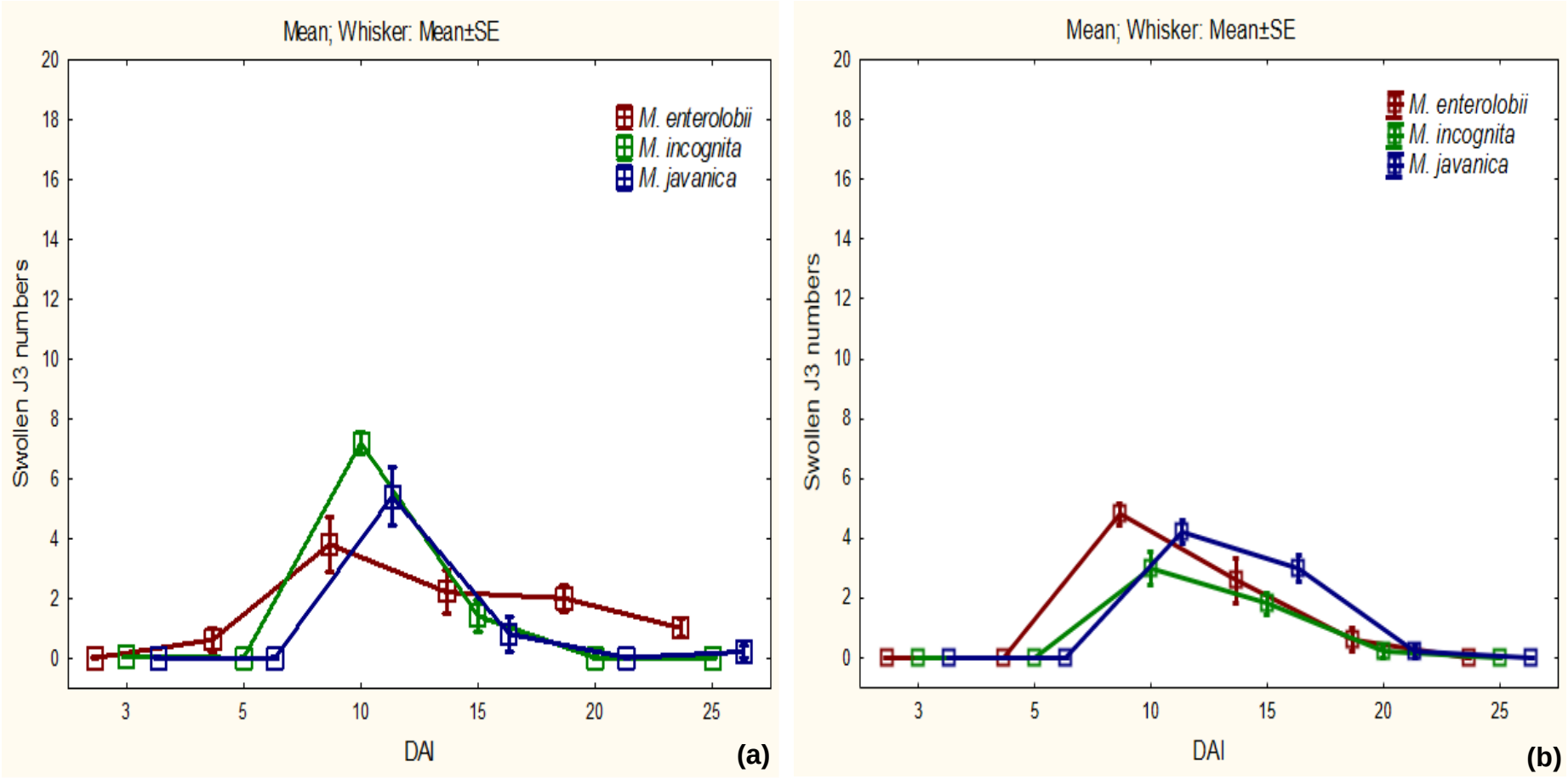

Figure 5.1.3. The number of swollen third-stage juveniles (J3) of *M. enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) roots of maize genotype P-2432-R from 3 to 25 days after inoculation (DAI) during a 1st (a) and 2nd (b) under glasshouse conditions.

5.1.4 Swollen fourth-stage juvenile (J4)

Significant ($P \leq 0.05$) interactions existed for Experiment x Species for the number of swollen J4: *M. enterolobii* ($P=0.001$; F-ratio=11.8); *M. incognita* ($P=0.001$; F-ratio=10.6); *M. javanica* ($P=0.001$; F-ratio=11.9). Similarly, significant ($P \leq 0.05$) interactions were evident for Species x Time Interval/DAI ($P=0.001$; F-ratio=8.7) and for Species x Experiment x Time interval ($P=0.001$; F-ratio=13.4) (Table 5.1.4).

Comparison of the two experiment's data for each of the three species showed that the swollen J4 numbers differed significantly ($P \leq 0.05$) for some sampling intervals for the 1st and 2nd experiments.

Comparatively the three species' swollen J4 numbers did not differ significantly from one another for 3 and 5 DAI for both experiments (Table A1d, Addendum 1A; Figure 5.1.4).

For 10 DAI the three species had similar ($P > 0.05$) swollen J4 numbers for the 1st experiment, while the numbers of this life stage for *M. incognita* were significantly ($P \leq 0.05$) higher numbers for the 2nd experiment compared to those of the other two species (which had similar J4 numbers) (Table A1d, Addendum 1A; Figure 5.1.4).

For 15 DAI the J4 numbers of the three species were similar ($P > 0.05$) for the 1st experiment, but for the 2nd experiment those of *M enterolobii* were significantly ($P \leq 0.05$) higher than those of the other two species.

At 20 and 25 DAI, all three species presented no significant ($P \leq 0.05$) differences for J4 numbers for both the 1st and 2nd experiment (Table A1d, Addendum 1A; Figure 5.1.4).

Table 5.1.4. Number of swollen fourth-stage juveniles (J4) of *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of maize genotype P-2432-R during a 25-day period under glasshouse conditions.

Time interval/DAI	*M. enterolobii*		*M. incognita*		*M. javanica*	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
3	0.02 a*A**	0.01 aA	0.02 aA	0.01 aA	0.02 aA	0.01 aA
5	0.02 aA	0.02 aA	0.02 aA	0.02 aA	0.02 aA	0.02 aA
10	3.4 bBD	4.6 bB	2.8 abABC	9.2 cD	3.0 bC	0.8 abAB
15	4.2 bB	11.4 cE	5.4 bC	3.4 bBC	2.8 bBC	3.4 cD
20	1.0 aACD	2.8 abBCD	1.21 aAB	0.8 aAB	0.21 aA	0.8 abAB
25	0.4 aAC	2.2 abABCD	0.81 aAB	1.8 abAB	0.02 aA	2.4 bBC
P	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***
F-ratio	***24.30***	***31.68***	***8.31***	***45.0***	***10.83***	***41.47***
Interaction data:						
Species x Experiment						
P	***0.001***		***0.001***		***0.001***	
F-ratio	***11.8***		***10.6***		***11.93***	
Species x Time interval (DAI)						
P	***0.001***					
F-ratio	***8.7***					
Species x Experiment x Time interval (DAI)						
P	***0.001***					
F-ratio	***13.4***					

*Indicates the differences in means among the time intervals for each species and experiment in each column, with the same lower case letter showing no significant difference according to the Tukey HSD Test ($P \leq 0.05$); **Indicates the differences in means between the two experiments of each species across rows, with the same capital letter showing no significant difference for a particular species at a given time interval between the two experiments according to the Tukey HSD Test ($P \leq 0.05$).

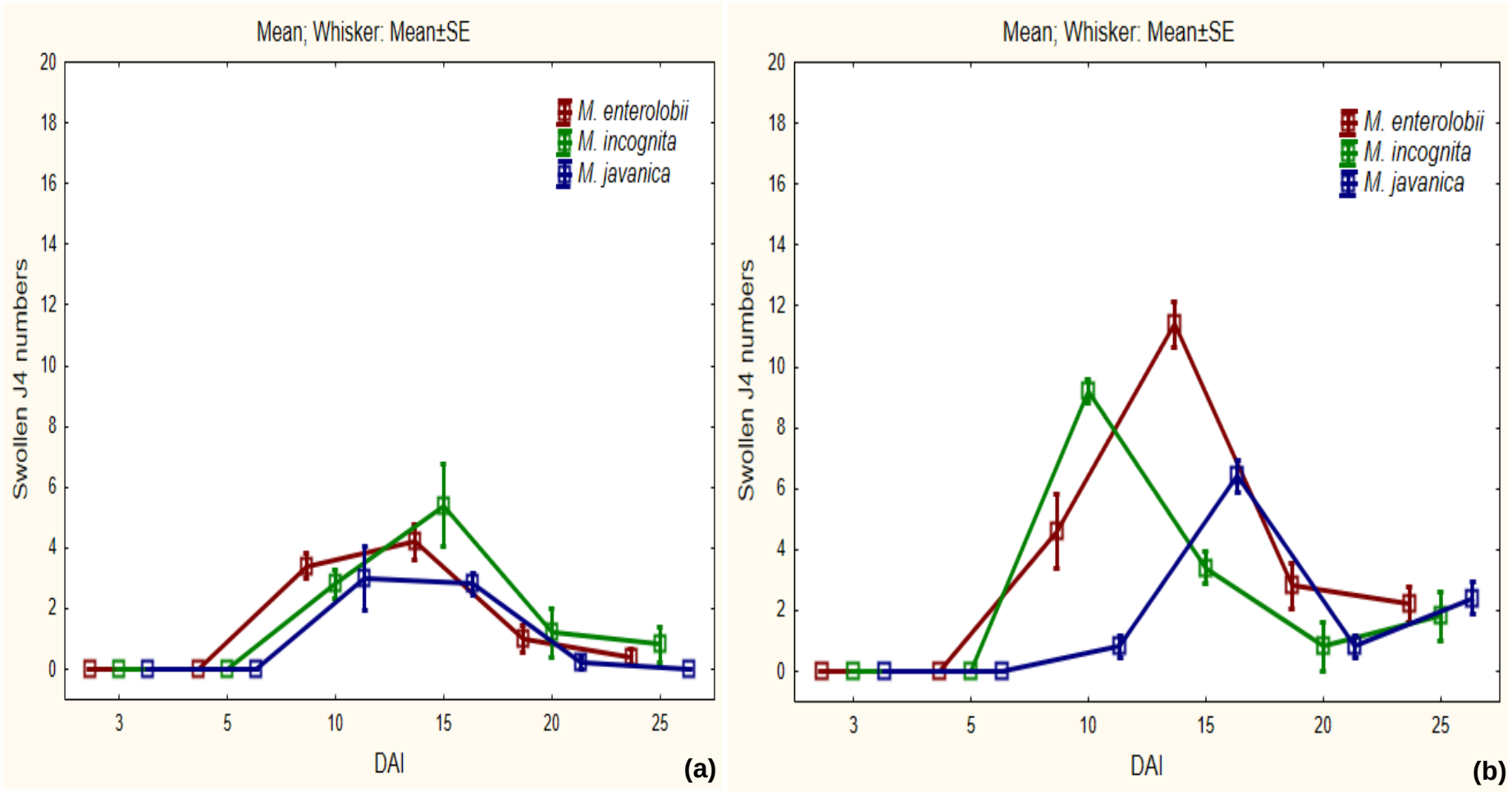

Figure 5.1.4. The number of swollen fourth-stage juveniles (J4) of *M. enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) in roots of maize genotype P-2432-R from 3 to 25 days after inoculation (DAI) during a 1st (a) and 2nd (b) under glasshouse conditions.

5.1.5 Females (immature and mature)

Significant (*P*≤0.05) interactions existed for Experiment x Species for the number of females of *M. enterolobii* (*P*=0.001; F-ratio=22.4) and *M. javanica* (*P*=0.001; F-ratio=19.6), but not for *M. incognita* (*P*=0.186; F-ratio=1.6). Significant (*P*≤0.05) interactions were evident for Species x Time Interval/DAI (*P*=0.001; F-ratio=19.1) for Species x Experiment x Time interval (*P*=0.001; F-ratio=6.7) (Table 5.1.5).

Comparison of the two experiment's data for each of the three species showed that the female numbers differed significantly (*P*≤0.05) for some of the time intervals between the 1st and 2nd experiments (Table A1e, Addendum 1A; Figure 5.1.5a and b).

Comparatively the three species' female numbers did not differ significantly (*P*≤0.05) from one another among the time intervals for 3 and 5 DAI for both experiments (Table A1e, Addendum 1A; Figure 5.1.4a and b).

For 10 DAI all three species had similar (*P*>0.05) female numbers for both experiments.

For the 1st experiment 15 DAI, *M. javanica* had significantly (*P*≤0.05) higher female numbers compared to *M. enterolobii*, while it was similar (*P*>0.05) to *M incognita* (Table A1e, Addendum 1A; Figure 5.1.4). For the 2nd experiment *M. incognita* had significantly (*P*≤0.05) higher female numbers compared to those of the other wo species, which were similar (*P*>0.05).

At 20 DAI, for the 1st experiments *M. javanica* female numbers, which were similar (*P*>0.05) to those of *M. incognita*, were significantly (*P*≤0.05) higher than those of *M. enterolobii* (Table A1e, Addendum 1A; Figure 5.1.4).

Table 5.1.5. Number of females (immature and mature) of *Meloidogyne enterolobii, M. incognita*, and *M. javanica* in roots of maize genotype P-2432-R during a 25-day period under glasshouse conditions.

Time interval/DAI	*M. enterolobii*		*M. incognita*		*M. javanica*	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
3	0.02 a*A**	0.02 aA	0.02 aA	0.02 aA	0.02 aA	0.02 aA
5	0.02 aA	0.02 aA	0.02 aA	0.02 aA	0.02 aA	0.02 aA
10	0.02 aA	0.01 aA	0.02 aA	0.02 aA	0.02 aA	0.02 aA
15	10.0 bC	6.0 cB	13.2 cC	14.2 cCD	14.4 bD	9.2 bC
20	9.4 bC	14.4 bD	18.0 bB	19.0 bB	18.8 bB	19.0 dB
25	6.2 cB	14.2 bD	16.6 bBCD	17.6 bBD	11.4 cC	16.8 cBD
P	0.001	0.001	0.001	0.001	0.001	0.001
F-ratio	61.5	113.1	168.7	176.5	147.2	805.9
Interaction data:						
Species x Experiment						
P	***0.001***		***0.186***		***0.001***	
F-ratio	***22.4***		***1.6***		***19.6***	
Species x Time interval (DAI)						
P	***0.001***					
F-ratio	***19.1***					
Species x Experiment x Time interval (DAI)						
P	***0.001***					
F-ratio	***6.7***					

*Indicates the differences in means among the time intervals for each species and experiment in each column, with the same lower case letter showing no significant difference according to the Tukey HSD Test ($P \leq 0.05$); **Indicates the differences in means between the two experiments of each species across rows, with the same capital letter showing no significant difference for a particular species at a given time interval between the two experiments according to the Tukey HSD Test ($P \leq 0.05$).

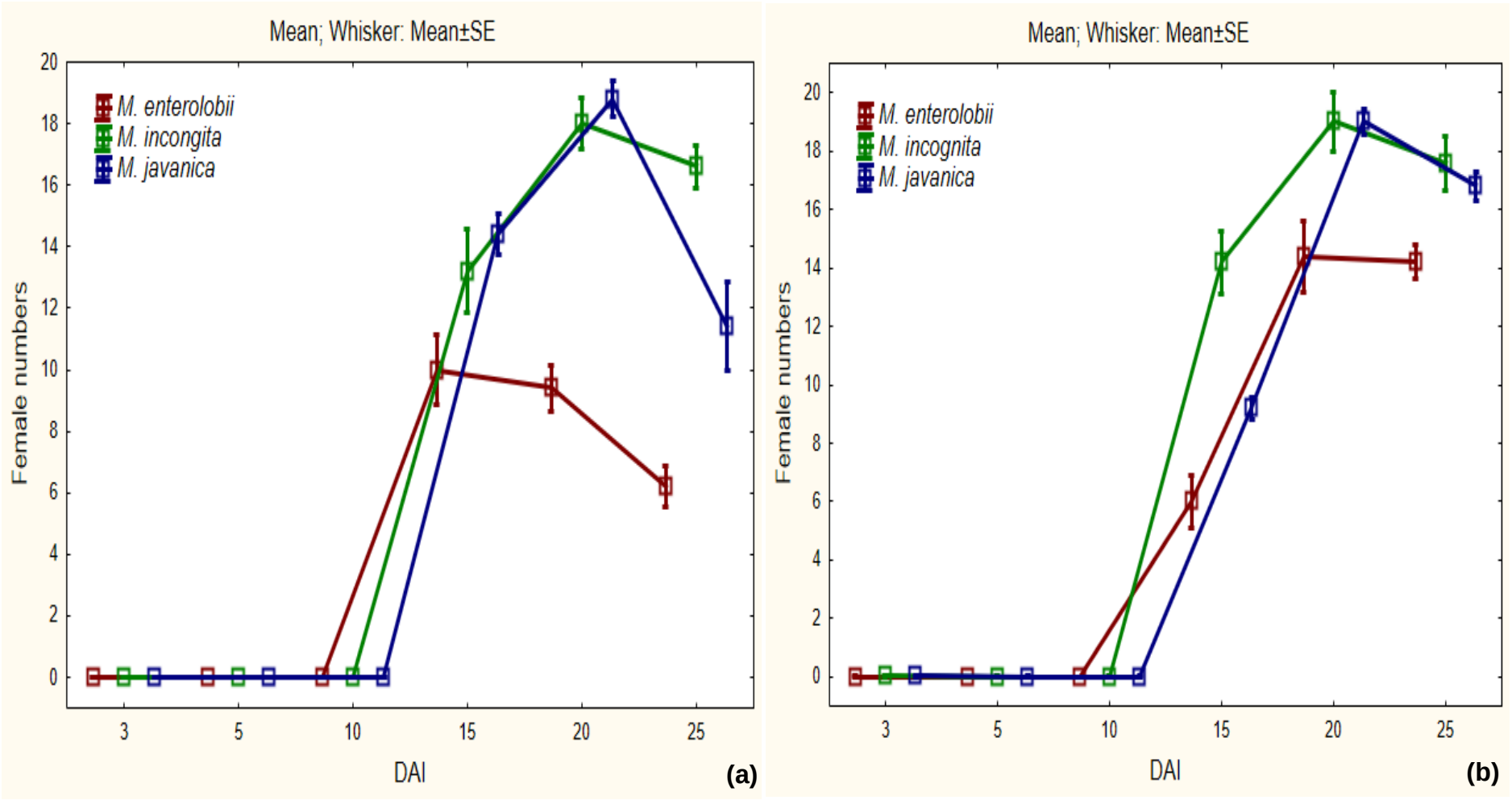

Figure 5.2.5 . The development of females (immature and mature) of *M. enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) in roots of maize genotype P-2432-R from 3 to 25 days after inoculation (DAI) during a 1st (a) and 2nd (b) under glasshouse conditions.

5.1.6 Reproduction (number of eggs produced per egg mass) of *M. enterolobii, M. incognita* and *M. javanica*

According to t-Test analyses, the number of eggs per egg mass 20 DAI differed significantly (*P*≤0.05) between the two experiments for *M. incognita* and *M. javanica*, but not for *M. enterolobii* (Table 5.1.6.; Figure 5.1.6a and b). When the species' egg numbers per egg mass were compared among each other per experiment, significant (*P*≤0.05) differences were evident for the following combinations for the 1st experiment only: *M. enterolobii* and *M. javanica,* and *M. javanica* and *M. incognita*.

Table 5.1.6. The average number of eggs produced per egg mass for *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of maize genotype P-2432-R 20 days after inoculation (DAI) under glasshouse conditions.

	M. enterolobii		***M. incognita***		***M. javanica***	
	Experiment 1	**Experiment 2**	**Experiment 1**	**Experiment 2**	**Experiment 1**	**Experiment 2**
Means	57 a*	36 a	70 a	49 b	91 a	49 a
P	*0.105*		***0.013***		***0.001***	
t-value	*1.8*		*3.2*		*11.4*	
Species compared among each other per experiment						
	Experiment 1			Experiment 2		
	Me vs. Mj	*Me vs. Mi*	*Mj vs. Mi*	*Me vs. Mj*	*Me vs. Mi*	*Mj vs. Mi*
P	***0.013***	*0.299*	***0.005***	*0.056*	*0.249*	*0.633*
t-value	*-3.2*	*-1.1*	*3.9*	*-2.2*	*-1.2*	*0.5*

*The same letter indicates no significant differences in egg numbers between the experiments for each species as well as for the species compared to one another using the using Student's T-test where (*P*≤0.05).

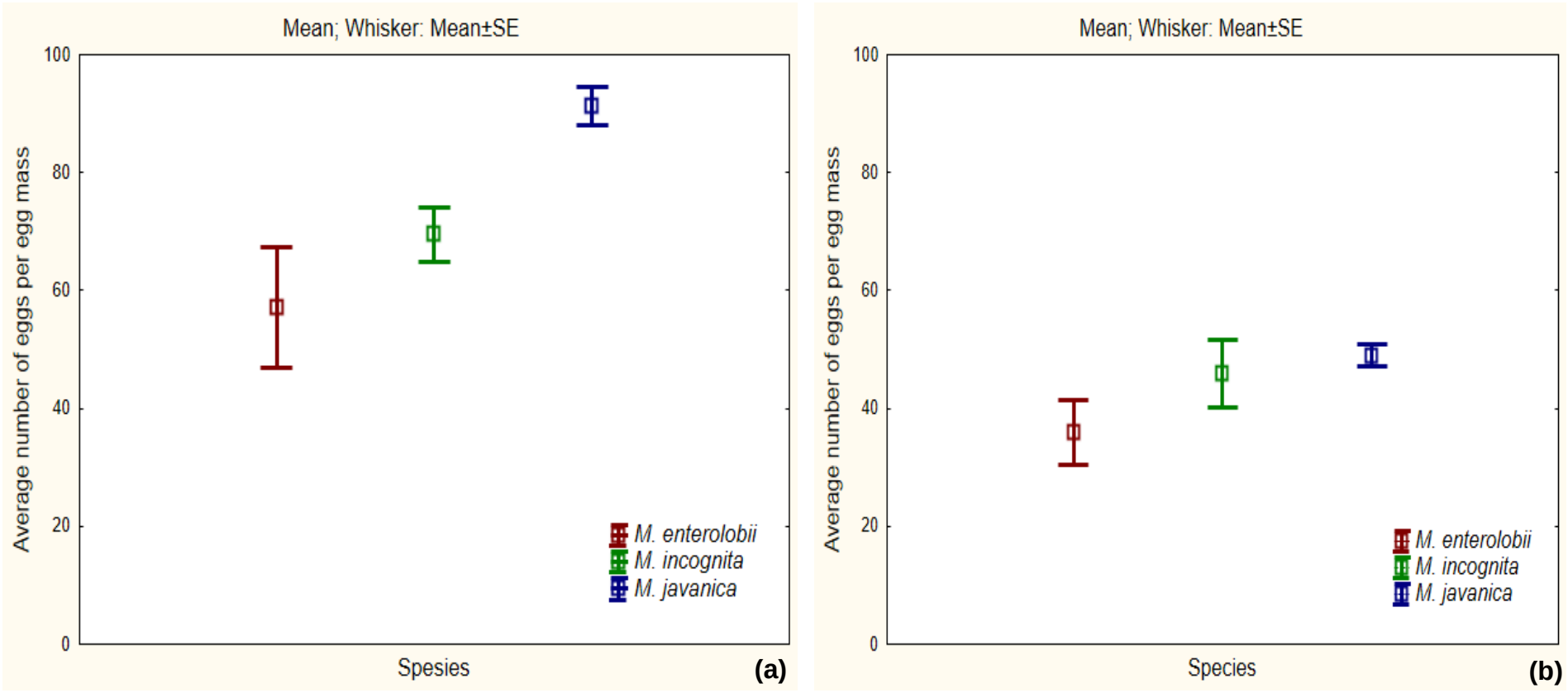

Figure 5.1.6. The number of eggs produced per egg mass for *M. enterolobii* (red), *M. incognita* (green) and *M. javanica* (blue) that were present on roots of maize genotype P-2432-R 20 days after inoculation (DAI) during a 1st (a) and 2nd experiment (b) under glasshouse conditions.

5.1.7. Degree days (DD) for *M. enterolobii*, *M. incognita* and *M. javanica*

The temperature analysis of the environment under glasshouse conditions yielded the temperature required for completion of a single life cycle (from motile J2 to mature egg laying female) as degree days (DD) or each species (Table 5.1.7).

Important to mention is that although single eggs were found for *M. enterolobii* 15 DAI when females and other life stages were removed from the maize roots for the life-stage development study. However, no egg masses were present at this time interval for this species. Therefore, the DD was calculated for *M. enterolobii* using 15 DAI. No single eggs were, however, found for *M. incognita* and /or *M. javanica* 15 DAI and egg masses for both species were only recorded from 20 DAI. Therefore, the DD for these two species were calculated using the latter time interval.

The required DD for *M. enterolobii* to complete a single life cycle was substantially lower in maize roots, for both experiments, compared to that of both *M. incognita* and *M. javanica.*

Table 5.1.7. Degree-day data for *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of maize genotype P-2432-R under glasshouse conditions.

Species	Min. Temperature (°C)	Max. Temperature (°C)	Base temperature (Tb)	Time interval (DAI)	Degree days (DD)
Experiment 1					
M. enterolobii	**21.7**	**32.2**	**10.0**	**15**	**254**
M. incognita			**9.8**	**20**	**343**
M. javanica			**10.6**	**20**	**327**
Experiment 2					
M. enterolobii	**16.0**	**32.0**	**10.0**	**15**	**210**
M. incognita			**9.8**	**20**	**284**
M. javanica			**10.6**	**20**	**268**

*****Negron, 2006; Jacobs *et al.,* 2011

5.2. Comparing the life-stage development, life-cycle duration and reproduction potential of *M. enterolobii, M. incognita,* and *M. javanica* in soybean roots

Two experiments were performed during which seedlings of soybean genotype DM-5953-RSF were used (see Chapter 3, Table 3.3) and inoculated separately with 2 000 (1st experiment) and 950 (2nd experiment) motile J2, respectively, of the following three species: *M. enterolobii, M. incognita,* and *M. javanica*.

5.2.1. Motile second-stage juveniles (J2)

Significant ($P \leq 0.05$) interactions existed for Experiment x Species for the number of motile J2 of all three species: *M. enterolobii* ($P=0.001$; F-ratio=9.2); *M. incognita* ($P=0.001$; F-ratio=14.9); and *M. javanica* ($P=0.007$; F-ratio=3.7). Similarly, significant ($P \leq 0.05$) interactions were evident for Species x Time Interval/DAI ($P=0.001$; F-ratio=27.0) and for Species x Experiment x Time interval ($P=0.001$; F-ratio=6.0) (Table 5.2.1).

Comparison of the two experiment's data for each of the three species showed that the motile J2 numbers differed significantly ($P \leq 0.05$) for some of the time intervals between the 1st and 2nd experiments (Table 5.2.1; Figure 5.2.1a and b).

Comparatively the three species' motile J2 numbers did not differ significantly ($P \leq 0.05$) for 3 DAI for both experiments (Table 1B1a, Addendum 1; Figure 5.2.1). This is similar to that of the 1st experiment for 5 DAI, while for the 2nd experiment the motile J2 numbers of *M. incognita*, which were similar ($P > 0.05$) to those of *M. javanica*, were significantly ($P \leq 0.05$) higher than those of *M. enterolobii*.

For 10 DAI the motile J2 numbers were significantly ($P \leq 0.05$) higher for *M. incognita* compared to those of both *M. javanica* and *M. enterolobii* for both experiments (Table B1a, Addendum 1; Figure 5.2.1). For the 2nd experiment, the trend mirrored that for the 1st experiment except that *M. incognita* had significantly ($P \leq 0.05$) higher motile J2 numbers than *M. javanica* and *M. enterolobii.*

For 15 DAI the swollen J2 numbers were significantly ($P \leq 0.05$) higher for *M. incognita* compared to those of both *M. javanica* and *M. enterolobii*

The number of motile J2 were similar ($P > 0.05$) for the three species 20 and 25 DAI for both experiments.

Table 5.2.1. Numbers of motile second-stage juveniles (J2) of *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of soybean genotype DM-5953-RSF during a 25-day period under glasshouse conditions.

Time interval/DAI	*M. enterolobii*		*M. incognita*		*M. javanica*	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
3	20.0 b*C**	19.2 dC	20.0 bB	20.0 bB	20.0 bB	20.0 bB
5	19.6 bC	13.2 cD	20.0 bB	17.2 bB	19.8 bB	16.8 bB
10	2.6 aAB	0.01 abAB	17.0 dB	1.2 cD	11.6 cD	1.6 cC
15	0.02 aA	0.01 aA	3.4 aC	0.01 aAC	1.2 aA	0.01 aA
20	0.02 aA	1.4 abAB	2.6 aAC	0.01 aA	0.02 aA	0.41 aA
25	2.0 aAB	3.2 bB	0.03 cA	1.4 aAC	0.22 aA	2.0 aA
P	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***
F-ratio	***259.1***	***142.8***	***413.9***	***190.7***	***129.2***	***218.7***
Interaction data:						
Species x Experiment						
P	***0.001***		***0.001***		***0.007***	
F-ratio	***9.2***		***14.9***		***3.7***	
Species x Time interval (DAI)						
P	***0.001***					
F ratio	***27.0***					
Species x Experiment x Time interval (DAI)						
P	***0.001***					
F ratio	***6.0***					

*Indicates the differences in means among the time intervals for each species and experiment in each column, with the same lower case letter showing no significant difference according to the Tukey HSD Test ($P \leq 0.05$); **Indicates the differences in means among the three species for each experiment (across rows), with the same capital letter showing no significant difference among species at a given time interval according to the Tukey HSD Test ($P \leq 0.05$).

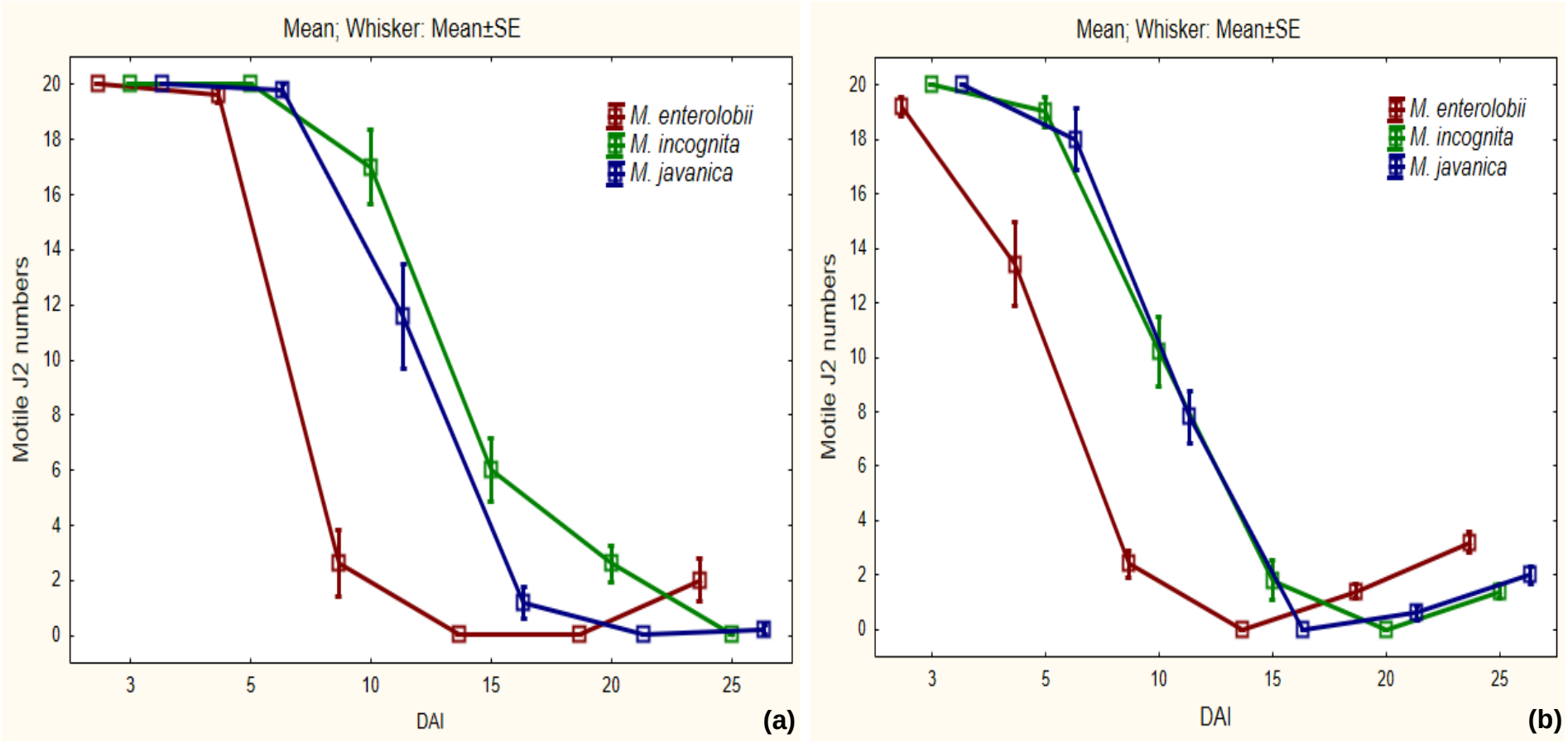

Figure 5.2.1. The number of motile second-stage juveniles (J2) of *M. enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) in roots of soybean genotype ('DM-5953-RSF') from 3 to 25 days after inoculation (DAI) for a 1st (a) and 2nd experiment (b) under glasshouse conditions.

5.2.2. Swollen second-stage juveniles (J2)

Significant (*P*≤0.05) interactions existed for Experiment x Species for the number of swollen J2: *M. enterolobii* (*P*=0.001; F-ratio=17.4); *M. incognita* (*P*=0.001; F-ratio=12.9); and *M. javanica* (*P*=0.001; F-ratio=3.9) (Table 5.2.2). Similarly, significant (*P*≤0.05) interactions were evident for Species x Time Interval/DAI (*P*=0.001; F-ratio=3.7) for Species x Experiment x Time interval (*P*=0.001; F-ratio=4.6) (Table 5.2.2).

Comparison of the two experiment's data showed that the swollen J2 numbers differed significantly (*P*≤0.05) for some time intervals between the 1st and 2nd experiments for all three species (Table 5.2.2).

Comparatively among the three species, the swollen J2 numbers did not differ significantly 3 DAI for both experiments (Table B1b, Addendum 1B; Figure 5.2.2a and b). However, while the swollen J2 numbers were similar (*P*≤0.05) for the three species 5 DAI for the 1st experiment those of *M. enterolobii* were significantly (*P*≤0.05) higher compared to those of *M. incognita* and *M. javanica* in the 2nd experiment.

For 10 DAI, the swollen J2 numbers of all three species differed significantly (*P*≤0.05) from one another for the 1ste experiment, with *M. enterolobii* being the highest; however, for the 2nd experiment the number of swollen J2 numbers were similar (*P*>0.05) for the three species (Table B1b, Addendum 1B; Figure 5.2.2a and b).

For 15 DAI the swollen J2 numbers for the three species did not differ significantly from one another for both experiments (Table B1b, Addendum 1B; Figure 5.2.2a and b).

At 20 DAI, the swollen J2 numbers were significantly (*P*≤0.05) higher for *M. enterolobii* for the 1st experiment compared to those of the other two species, while the three species did not differ from one another in this regard for the 2nd experiment (Table B1b, Addendum 1B; Figure 5.2.2a and b).

For 25 DAI, the swollen J2 numbers were similar (P>0.05) for the three species for both experiments.

Table 5.2.2. Numbers of swollen second-stage juveniles (J2) of *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of a soybean genotype ('DM-5953-RSF') during a 25-day period under glasshouse conditions.

Time interval/DAI	*M. enterolobii*		*M. incognita*		*M. javanica*	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
3	0.01 a*A**	0.8 aA	0.02 aA	0.01 aA	0.02 aA	0.01 aA
5	0.6 aA	6.4 bB	0.02 aA	2.8 aA	0.2 aA	3.2 abAB
10	10.4 cC	9.0 bBC	3.01 aAB	11.4 bC	6.2 cC	8.4 cC
15	6.0 bB	1.4 aA	5.2 bBC	1.61 aA	2.6 abcAB	2.4 bAB
20	8.2 bcBC	0.01 aA	5.6 bBC	0.02 aA	4.0 bcBC	0.02 aA
25	1.4 aA	1.8 aA	0.61 aA	0.02 aA	1.1 abAB	0.02 aA
P	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***
F-ratio	***21.5***	***25.8***	***15.0***	***10.0***	***8.7***	***23.2***
Interaction data:						
Species x Experiment						
P	***0.001***		***0.001***		***0.004***	
F-ratio	***17.4***		***12.9***		***3.9***	
Species x Time interval (DAI)						
P	***0.001***					
F-ratio	***3.7***					
Species x Experiment x Time interval (DAI)						
P	***0.001***					
F-ratio	***4.6***					

*Indicates the differences in means among the time intervals for each species and experiment in each column, with the same lower case letter showing no significant difference according to the Tukey HSD Test ($P \leq 0.05$); **Indicates the differences in means among the three species for each experiment (across rows), with the same capital letter showing no significant difference among species at a given time interval according to the Tukey HSD Test ($P \leq 0.05$).

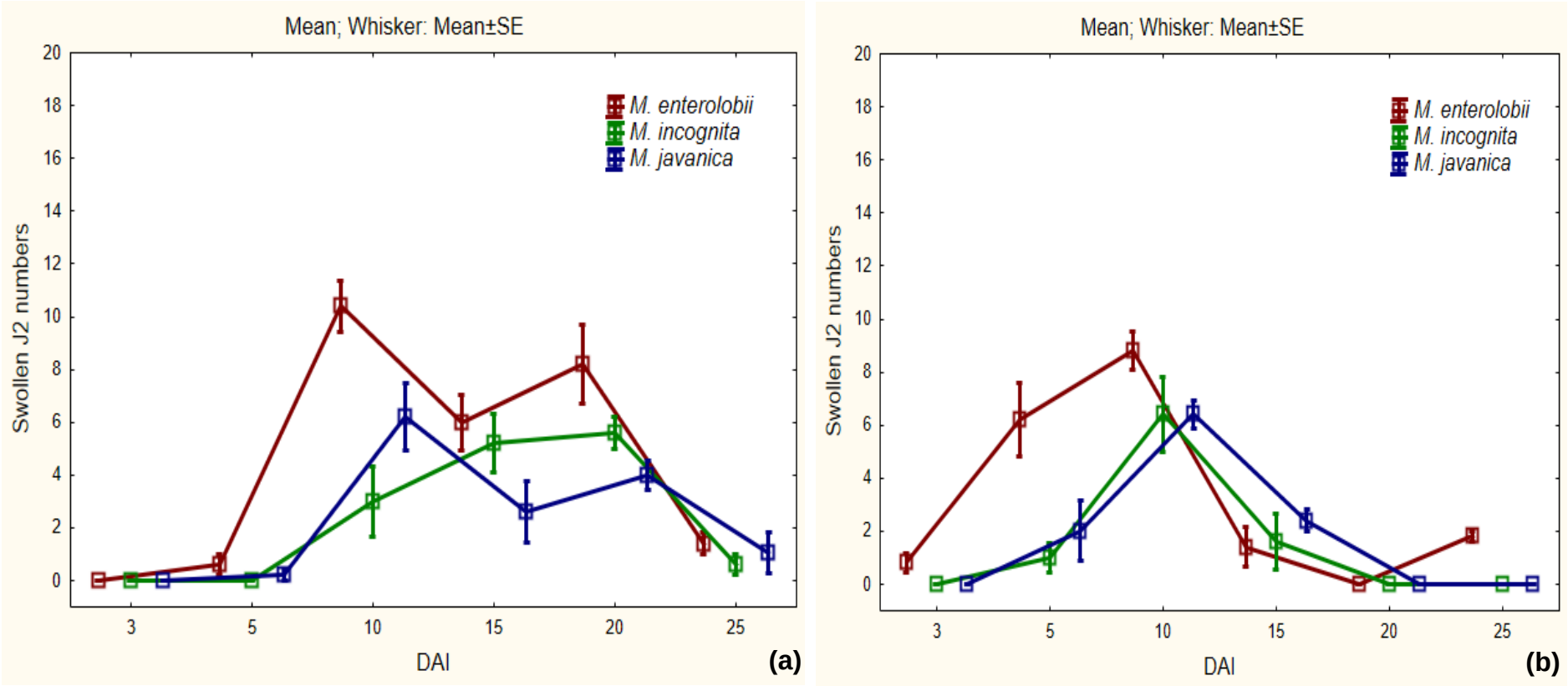

Figure 5.2.2. The number of swollen second-stage juveniles (J2) of *M. enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) in roots of soybean genotype DM-5953-RSF from 3 to 25 days after inoculation (DAI) during a 1st (a) and 2nd experiment (b) under glasshouse conditions.

5.2.3. Swollen third-stage juvenile (J3)

Significant ($P \leq 0.05$) interactions existed for Experiment x Species for the number of swollen J2: *M. enterolobii* ($P=0.025$; F-ratio=2.9); *M. incognita* ($P=0.001$; F-ratio=12.1); and *M. javanica* ($P=0.009$; F-ratio=3.5). Similarly, significant ($P \leq 0.05$) interactions were evident for Species x Time Interval/DAI ($P=0.001$; F-ratio=4.5) and for Species x Experiment x Time interval ($P=0.024$; F-ratio=2.2) (Table 5.2.3).

Comparison of the two experiment's data for each of the three species showed that the swollen J3 numbers differed significantly ($P \leq 0.05$) between the 1st and 2nd experiments for some of the time intervals (Table 5.2.3; Figure 5.2.3a and b).

Comparatively the three species' swollen J3 numbers did not differ significantly from one another 3 and 5 DAI for both experiments (Table 1Bc, Addendum 1B; Figure 5.2.3a and b).

For 10 DAI the 1ste experiment showed significant ($P \leq 0.05$) higher swollen J3 numbers of *M. enterolobii*, while the three species did not differ in this regard from one another for the 2nd experiment (Table 1Bc, Addendum 1B; Figure 5.2.3a and b).

For 15 DAI the swollen J3 numbers for the three species did not differ significantly from one another for both experiments (Table 1Bc, Addendum 1B; Figure 5.2.3a and b)..

At 20 and 25 DAI, no significance differences were evident among the three species for the swollen J3 numbers for both the 1st and 2nd experiment (Table 1Bc, Addendum 1B; Figure 5.2.3a and b).

Table 5.2.3. Numbers of swollen third-stage juveniles (J3) of *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of soybean genotype DM-5953-RSF during a 25-day period under glasshouse conditions.

Time interval/DAI	*M. enterolobii*		*M. incognita*		*M. javanica*	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
3	0.01 a*A**	0.02 aA	0.02 aA	0.02 aA	0.02 aA	0.02 aA
5	0.02 aA	0.4 aA	0.02 aA	0.02 aA	0.02 aA	0.02 aA
10	6.4 bD	6.2 bCD	0.02 aA	6.8 bBC	2.2 abAB	6.6 bAB
15	5.8 bCD	2.6 bABC	4.8 bE	3.4 bCD	4.4 bB	3.0 bAB
20	3.8 abBCD	0.4 aA	5.2 bDE	0.4 aAB	4.0 bB	0.4 aA
25	0.8 aAB	0.5 aAB	1.21 aABC	0.01 aA	3.2 abAB	0.01 aA
P	***0.001***	***0.001***	***0.001***	***0.001***	***0.005***	***0.001***
F-ratio	***9.9***	***23.8***	***23.2***	***11.9***	***4.7***	***11.6***
Interaction data:						
Species x Experiment						
P	***0.025***		***0.001***		***0.009***	
F-ratio	***2.9***		***12.1***		***3.5***	
Species x Time interval (DAI)						
P	***0.001***					
F-ratio	***4.5***					
Species x Experiment x Time interval (DAI)						
P	***0.024***					
F-ratio	***2.2***					

*Indicates the differences in means among the time intervals for each species and experiment in each column, with the same lower case letter showing no significant difference according to the Tukey HSD Test ($P \leq 0.05$); **Indicates the differences in means among the three species for each experiment (across rows), with the same capital letter showing no significant difference among species at a given time interval according to the Tukey HSD Test ($P \leq 0.05$).

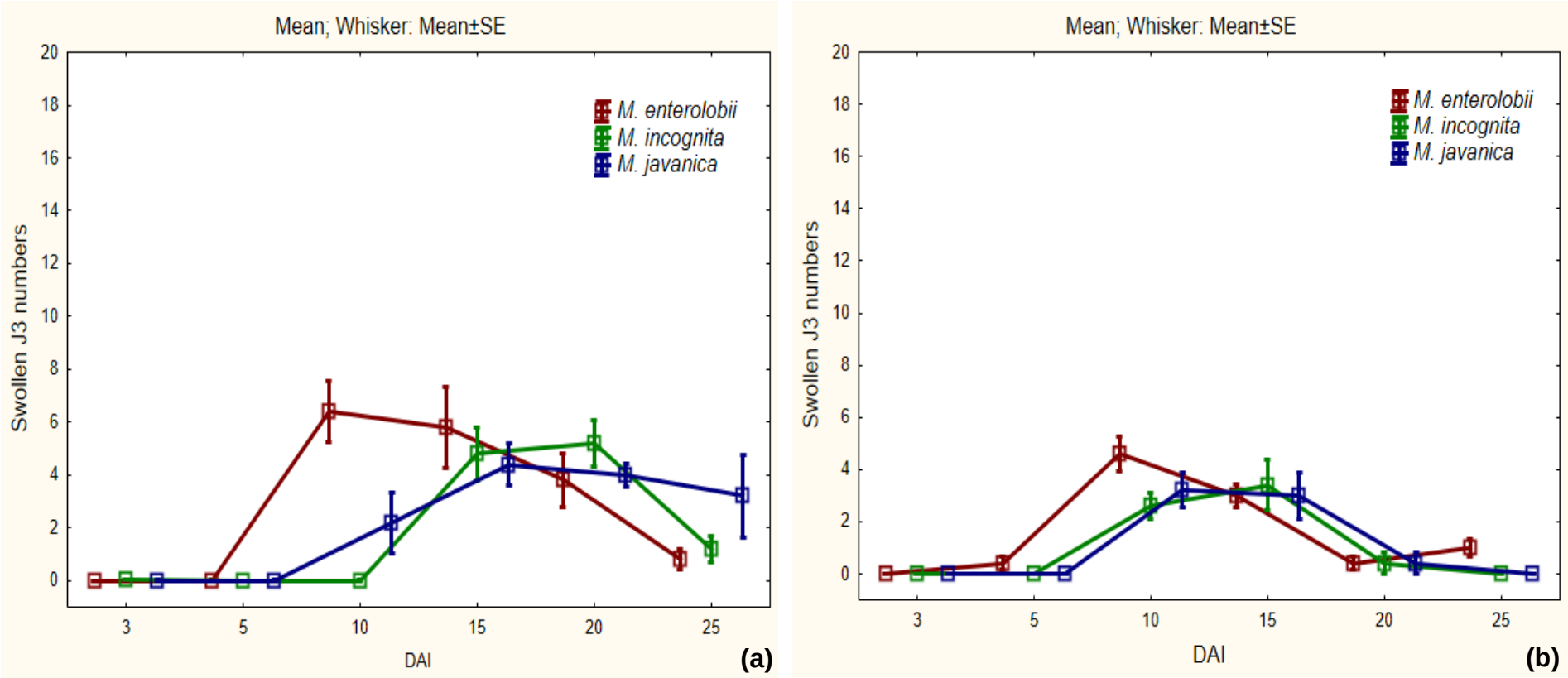

Figure 5.2.3. The number of swollen third-stage juveniles (J3) of *M. enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) in roots of soybean genotype DM-5953-RSF from 3 to 25 days after inoculation (DAI) during a 1st (a) and 2nd experiment (b) under glasshouse conditions.

5.2.4. Swollen fourth-stage juveniles (J4)

Significant (*P*≤0.05) interactions existed for Experiment x Species for the number of swollen J4: *M. enterolobii* (*P*=0.001; F-ratio=4.9); *M. incognita* (*P*=0.001; F-ratio=5.2); *M. javanica* (*P*=0.001; F-ratio=5.6). Similarly, significant (*P*≤0.05) interactions were evident for Species x Time Interval/DAI (*P*=0.001; F-ratio=6.6) and for Species x Experiment x Time interval (*P*=0.001; F-ratio=4.8) (Table 5.2.4).

Comparison of the two experiment's data for each of the three species showed that the swollen J4 numbers differed significantly (*P*≤0.05) for some of the time intervals between the 1st and 2nd experiments (Table 5.2.4; Figure 5.2.4a and b).

Comparatively the three species' swollen J4 numbers did not differ significantly from one another 3 and 5 DAI for both experiments (Table B1d, Addendum 1B; Figure 5.2.4a and b).

For 10 DAI no significant differences were evident for J4 numbers of the three species for both experiments (Table B1d, Addendum 1B; Figure 5.2.4a and b).

For 15 DAI the swollen J4 numbers for *M. javanica* were significantly (*P*≤0.05) higher than those of the other two species for the 1st experiment, while the species had similar (P>0.05) swollen J4 numbers for the 2nd experiment (Table B1d, Addendum 1B; Figure 5.2.4a and b).

At 20 DAI the swollen J4 numbers for *M. javanica* were significantly (*P*≤0.05) higher than those of the other two species for the 1st experiment; for the 2nd experiment *M. incognita* swollen J4 numbers were significantly (*P*≤0.05) higher than those of the other two species (Table B1d, Addendum 1B; Figure 5.2.4a and b).

For 25 DAI the three species had similar (P>0.05) swollen J4 numbers for both experiments (Table B1d, Addendum 1B; Figure 5.2.4a and b).

Table 5.2.4. Numbers of motile fourth-stage juveniles (J4) of *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of soybean genotype DM-5953-RSF during a 25-day period under glasshouse conditions.

Time interval/DAI	*M. enterolobii*		*M. incognita*		*M. javanica*	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
3	0.01 a*A**	0.02 aA	0.03 aA	0.01 aA	0.02 aA	0.01 aA
5	0.01 aA	0.02 aA	0.02 aA	0.0 aA	0.02 aA	0.02 aA
10	0.6 aA	4.8 bcB	0.02 aA	0.81 aAB	0.02 aA	2.0aA
15	4.2 bB	6.0 cB	2.6 bAB	7.6 bC	10.0 cB	9.6 bB
20	3.2 abAB	2.8 bAB	2.8 bAB	7.0 bC	7.6 bcB	1.41 aA
25	2.6 abAB	0.01 aA	3.6 bB	3.0 aAB	3.2 abA	1.2 aA
P	***0.002***	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***
F ratio	***5.8***	***19.0***	***8.7***	***18.2***	***17.2***	***38.4***
Interaction data:						
Species x Experiment						
P	***0.001***		***0.001***		***0.001***	
F-ratio	***4.9***		***5.2***		***5.6***	
Species x Time interval (DAI)						
P	***0.001***					
F-ratio	***6.6***					
Species x Experiment x Time interval (DAI)						
P	***0.001***					
F-ratio	***4.8***					

*Indicates the differences in means among the time intervals for each species and experiment in each column, with the same lower case letter showing no significant difference according to the Tukey HSD Test ($P \leq 0.05$); **Indicates the differences in means among the three species for each experiment (across rows), with the same capital letter showing no significant difference among species at a given time interval according to the Tukey HSD Test ($P \leq 0.05$).

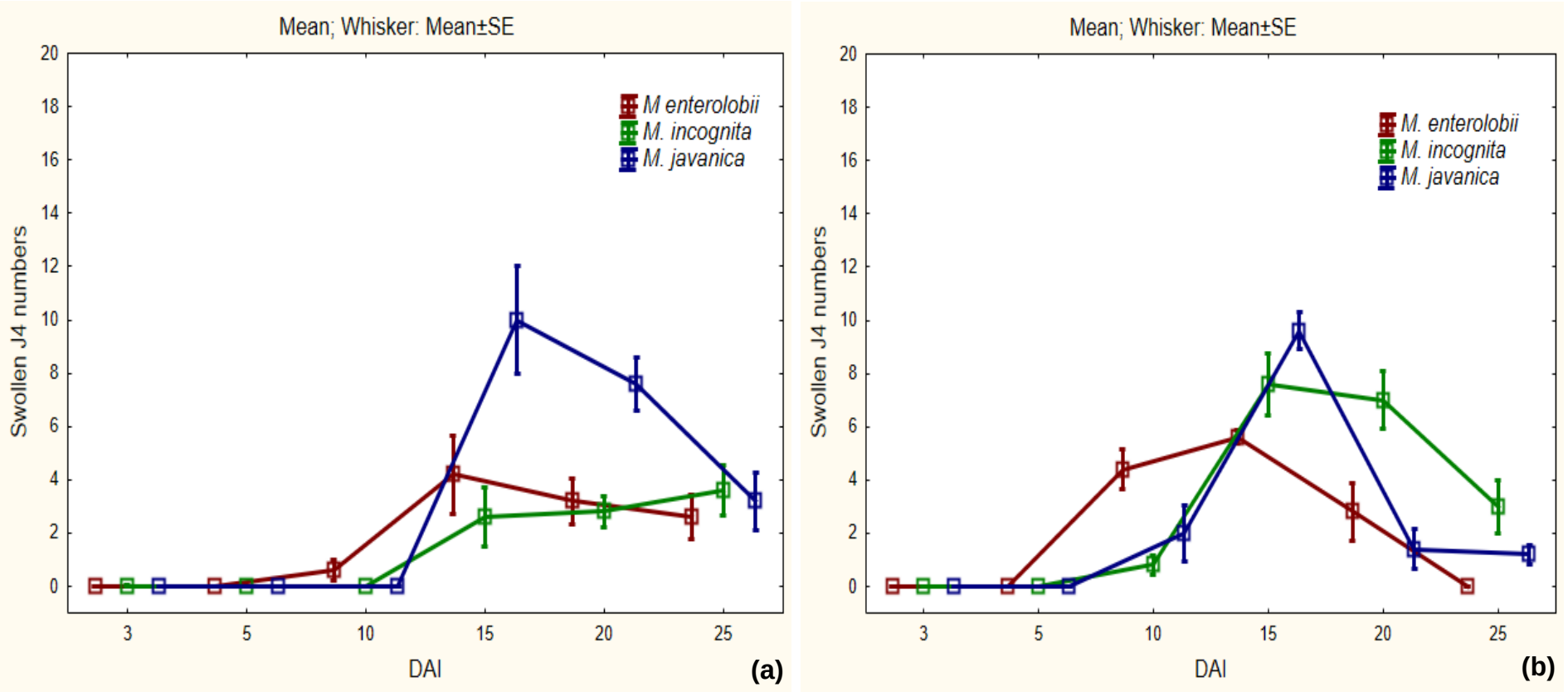

Figure 5.2.4. The number of motile fourth-stage juveniles (J4) of *M. enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) in roots of soybean genotype DM-5953-RSF from 3 to 25 days after inoculation (DAI) during a 1st (a) and 2nd experiment (b) under glasshouse conditions.

5.2.5. Females (immature and mature)

Significant (*P*≤0.05) interactions existed for Experiment x Species for the number of motile J2: *M. enterolobii* (*P*=0.001; F-ratio=17.9); *M. incognita* (*P*=0.001; F-ratio=14.1); and *M. javanica* (*P*=0.001; F-ratio=17.6). Similarly, significant (*P*≤0.05) interactions were evident for Species x Time Interval/DAI (*P*=0.001; F-ratio=3.4) and for Species x Experiment x Time interval (*P*=0.049; F-ratio=1.9) (Table 5.2.5).

Comparison of the two experiment's data for each of the three species showed that the female numbers differed significantly (*P*≤0.05) from one another for some of the time intervals between the 1st and 2nd experiments (Table 5.2.5; Figure 5.2.5a and b).

Comparatively the three species' female numbers did not differ significantly from one another 3 and 5 DAI for both experiments (Table B1b, Addendum 1; Figure 5.2.5a and b).

For 10 DAI the female numbers of the three species did not differ significantly from one another for both experiments, which mirrored the effect for the first experiment at 15 DAI (Table B1b, Addendum 1; Figure 5.2.5a and b). However, for the 2nd experiment 15 DAI the female numbers for *M. enterolobii* were significantly (*P*≤0.05) higher than those of the other two species (Table B1b, Addendum 1; Figure 5.2.5a and b).

At 20 DAI the female numbers of the three species were similar (*P*>0.05) for the 1st experiment; for the 2nd experiment those of *M. javanica* were significantly (*P*≤0.05) higher than those of *M. incognita* but similar (*P*≤0.05) to those of *M. enterolobii* (Table B1b, Addendum 1; Figure 5.2.5a and b).

For 25 DAI, the female numbers of the three species did not differ significantly from one another for both experiments (Table B1b, Addendum 1; Figure 5.2.5a and b).

Table 5.2.5. The number of females of *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of soybean genotype DM-5953-RSF during a 25-day period under glasshouse conditions.

Time interval/DAI	*M. enterolobii*		*M. incognita*		*M. javanica*	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
3	0.02 a*A**	0.02 aA	0.02 aA	0.03 aA	0.02 aA	0.03 aA
5	0.02 aA	0.02 aA	0.02 aA	0.02 aA	0.02 aA	0.02 aA
10	0.02 aA	0.02 aA	0.03 aA	0.02 aA	0.02 aA	0.02aA
15	4.0 bC	10.0 cD	2.0 abAC	5.4 cD	1.4 aAB	5.0 cB
20	5.0 bC	15.6 bB	3.8 bCD	12.6 bB	2.6 aAB	17.6 bD
25	13.2 cBD	14.0 bB	14.6 cB	15.6 bB	12.4 bC	16.8 bCD
P	*0.001*	*0.001*	*0.001*	*0.0301*	*0.001*	*0.001*
F ratio	*33.2*	*150.0*	*83.5*	*98.3*	*13.4*	*300.5*
Interaction data:						
Species x Experiment						
P	*0.001*		*0.001*		*0.001*	
F ratio	*17.9*		*14.1*		*17.6*	
Species x Time interval (DAI)						
P	*0.001*					
F ratio	*3.4*					
Species x Experiment x Time interval (DAI)						
P	*0.049*					
F ratio	*1.9*					

*Indicates the differences in means among the time intervals for each species and experiment in each column, with the same lower case letter showing no significant difference according to the Tukey HSD Test ($P \leq 0.05$); **Indicates the differences in means among the three species for each experiment (across rows), with the same capital letter showing no significant difference among species at a given time interval according to the Tukey HSD Test ($P \leq 0.05$).

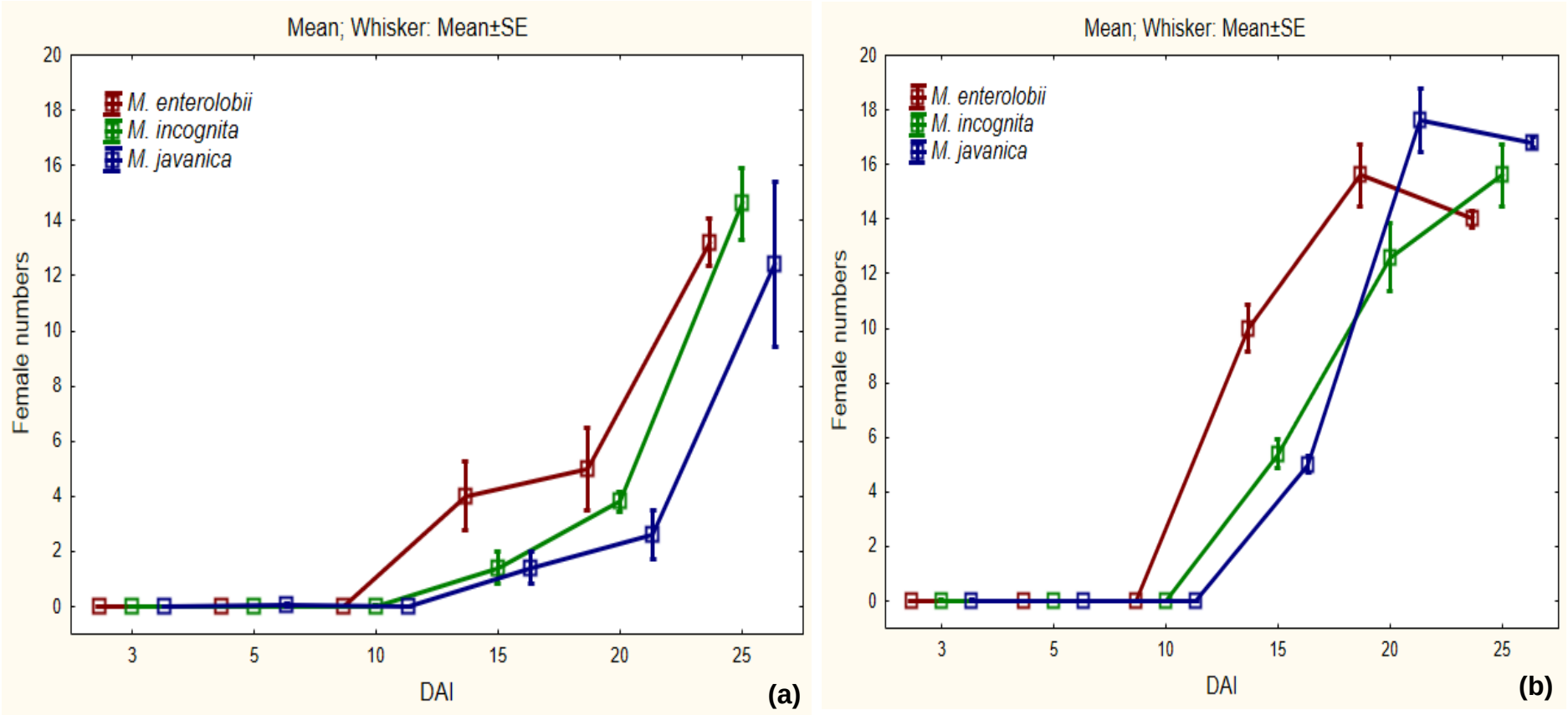

Figure 5.2.5. The number of swollen females of *M. enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) in roots of soybean genotype DM-5953-RSF from 3 to 25 days after inoculation (DAI) during a 1st (a) and 2nd experiment (b) under glasshouse conditions.

5.2.6. Reproduction (number of eggs produced per egg mass) of *M. enterolobii, M. incognita* and *M. Javanica*

According to t-Test analyses, the number of eggs per egg mass 20 DAI did not differ significantly ($P \leq 0.05$) between the two experiments for the three species (Table 5.2.6.; Figure 5.2.6a and b). When the species' egg numbers per egg mass were compared among each other per experiment, significant ($P \leq 0.05$) differences were evident for the 2nd experiment and only for *M. enterolobii vs*. *M. incognita*.

Table 5.2.6. The number of eggs produced per egg mass for *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of soybean genotype DM-5953-RSF 20 days after inoculation (DAI) under glasshouse conditions.

	M. enterolobii		***M. incognita***		***M. javanica***	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Means	98 a*	*88 a	184 a	70 a	74 b	77 a
P	*0.677*		*0.467*		*0.849*	
t-value	*0.4*		*0.8*		*-0.2*	
Species compared among each other per experiment						
	Experiment 1			Experiment 2		
	Me vs. Mj	*Me vs. Mi*	*Mj vs. Mi*	*Me vs. Mj*	*Me vs. Mi*	*Mj vs. Mi*
P	*0.399*	*0.582*	*0.482*	*0.126*	***0.015***	*0.366*
t-value	*0.9*	*-0.6*	*-0.7*	*1.7*	*3.1*	*1.0*

*The same letter indicates no significant differences in egg numbers between the experiments for each species as well as for the species compared to one another using the using Student's T-test where ($P \leq 0.05$).

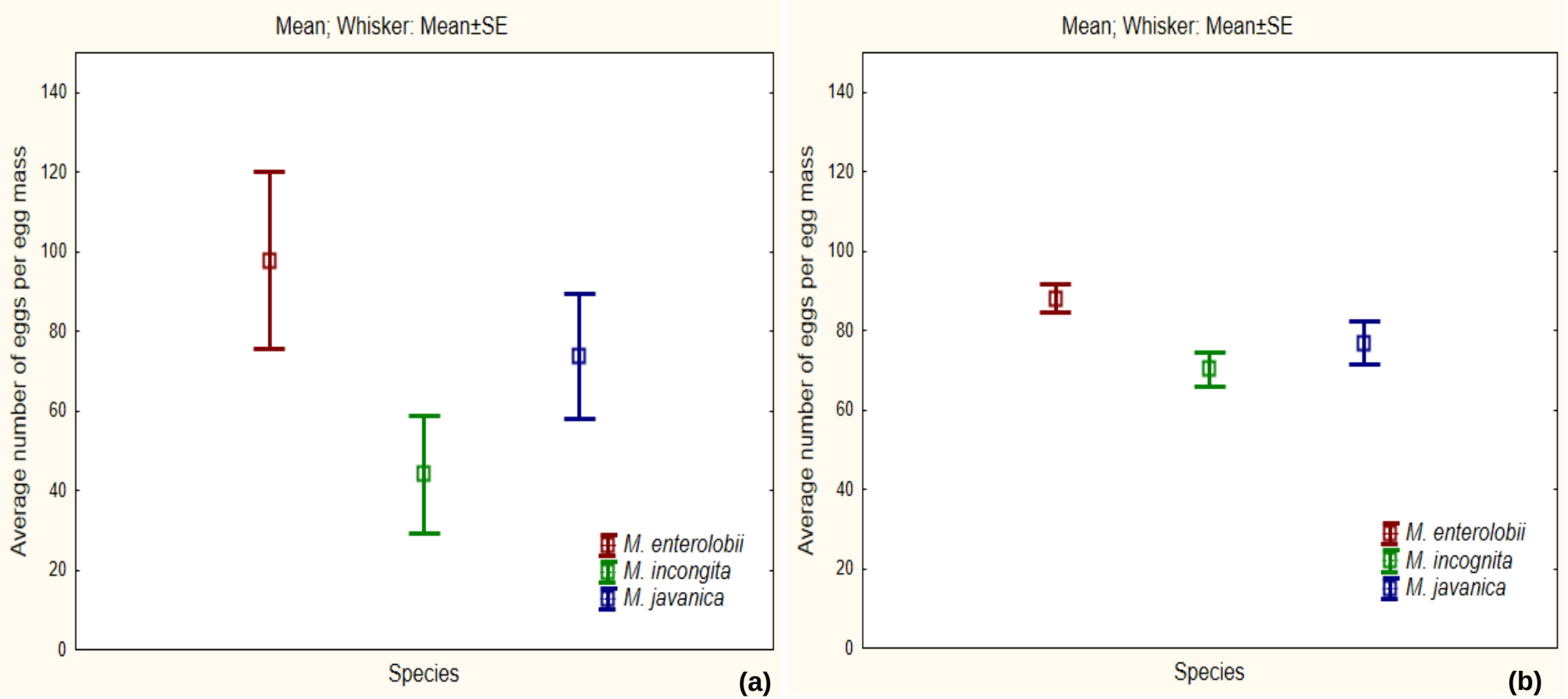

Figure 5.2.6. The number of eggs produced per egg mass for *M. enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) in roots of soybean genotype DM-5953-RSF 20 days after inoculation (DAI) during a 1st (a) and 2nd experiment (b) under glasshouse conditions.

5.2.7. Degree days (DD) for *M. enterolobii*, *M. incognita* and *M. javanica*

Similar to the maize experiment, see Paragraph 5.1.7, single eggs of *M. enterolobii* were recorded 15 DAI when females and other life stages were removed from the soybean roots for the life-stage development study. However, no egg masses were present. Therefore, the DD was calculated for *M. enterolobii* using 15 DAI. Since no single eggs were found for *M. incognita* and/or *M. javanica* 15 DAI and egg masses only recorded from 20 DAI the DD for these two species were calculated using the latter time interval.

The required DD for *M. enterolobii* to complete a single life cycle was substantially lower in roots of soybean, in both experiments, compared to that of both *M. incognita* and *M. javanica* (Table 5.2.7).

Table 5.2.7. Degree-day data for *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of soybean genotype DM-5953-RSF under glasshouse conditions.

Species	Min. Temperature	Max. Temperature	Base temperature (Tb)	Time interval (DAI)	Degree days (DD)
Experiment 1					
M. enterolobii	**13.0**	**31.0**	**10.0****	**15**	**180**
M. incognita			**9.8***	**20**	**299**
M. javanica			**10.6***	**20**	**244**
Experiment 2					
M. enterolobii	**16.0**	**32.0**	**10.0****	**15**	**210**
M. incognita			**9.8***	**20**	**284**
M. javanica			**10.6***	**20**	**268**

*****Negron (2006); **Jacobs *et al.* (2011)

5.3. Life-cycle comparison of *Meloidogyne enterolobii*, *M. incognita*, and *M. javanica* in tomato

Two experiments were performed during which seedlings of tomato genotype Moneymaker were used (see Chapter 3, Table 3.3) and inoculated separately with 1 200 (1st experiment) and 950 (2nd experiment) second-stage juveniles (J2), respectively, of the following three species: *M. enterolobii, M. incognita,* and *M. javanica*.

5.3.1. Motile second-stage juvenile (J2)

Significant ($P \leq 0.05$) interactions existed for Experiment x Species for the number of motile J2: *M. enterolobii* ($P=0.001$; F-ratio=5.0); *M. incognita* ($P=0.032$; F-ratio=2.7); but not for *M. javanica* ($P=0.430$; F-ratio=1.0). Similarly, a significant ($P \leq 0.05$) interaction was evident for Species x Time Interval/DAI ($P=0.001$; F-ratio=3.1) but not for Species x Experiment x Time interval ($P=0.150$; F-ratio=1.5) (Table 5.3.1).

Comparison of the two experiment's data for each of the three species showed that the motile J2 numbers differed significantly ($P \leq 0.05$) for some time intervals between the 1st and 2nd experiments (Table 5.3.1; Figure 5.3.1a and b).

Comparatively the three species' motile J2 numbers did not differ significantly from one another 3 and 5 DAI for both experiments (Table C1a, Addendum 1C; Figure 5.3.1a and b). The same trend was evident for 10, 15, 20 and 25 DAI, with the three species having similar ($P > 0.05$) motile J2 numbers.

Table 5.3.1. The number of motile second-stage juveniles (J2) of *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of tomato cultivar Moneymaker during a 25-day period under glasshouse conditions.

Time interval/DAI	*M. enterolobii*		*M. incognita*		*M. javanica*	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
3	18.6 c*D**	16.2 dD	20.0 cC	20.0 dA	20.0 bB	20.0 bB
5	9.0 bC	11.0 cC	11.4 bD	14.4 cE	15.4 bBC	16.6 bB
10	0.02 aA	0.01 aA	2.0 aA	2.8 bB	2.4 aA	1.8 aA
15	0.02 aA	0.02 aA	0.02 aA	0.8 abB	0.02 aA	0.02 aA
20	1.6 aAB	1.4 abAB	0.02 aA	0.01 aA	2.8 aA	0.81 aA
25	0.02 aA	3.2 bB	1.2 aB	1.6 abAB	2.6 aA	1.01 aA
P	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***
F ratio	***211.2***	***94.4***	***282.5***	***269.9***	***44.0***	***103.7***
Interaction data:						
Species x Experiment						
P	***0.001***		***0.032***		***0.430***	
F ratio	***5.0***		***2.7***		***1.0***	
Species x Time interval (DAI)						
P	***0.003***					
F ratio	***3.7***					
Species x Experiment x Time interval (DAI)						
P	***0.134***					
F ratio	***1.5***					

*Indicates the differences in means among the time intervals for each species and experiment in each column, with the same lower case letter showing no significant difference according to the Tukey HSD Test ($P \leq 0.05$); **Indicates the differences in means among the three species for each experiment (across rows), with the same capital letter showing no significant difference among species at a given time interval according to the Tukey HSD Test ($P \leq 0.05$).

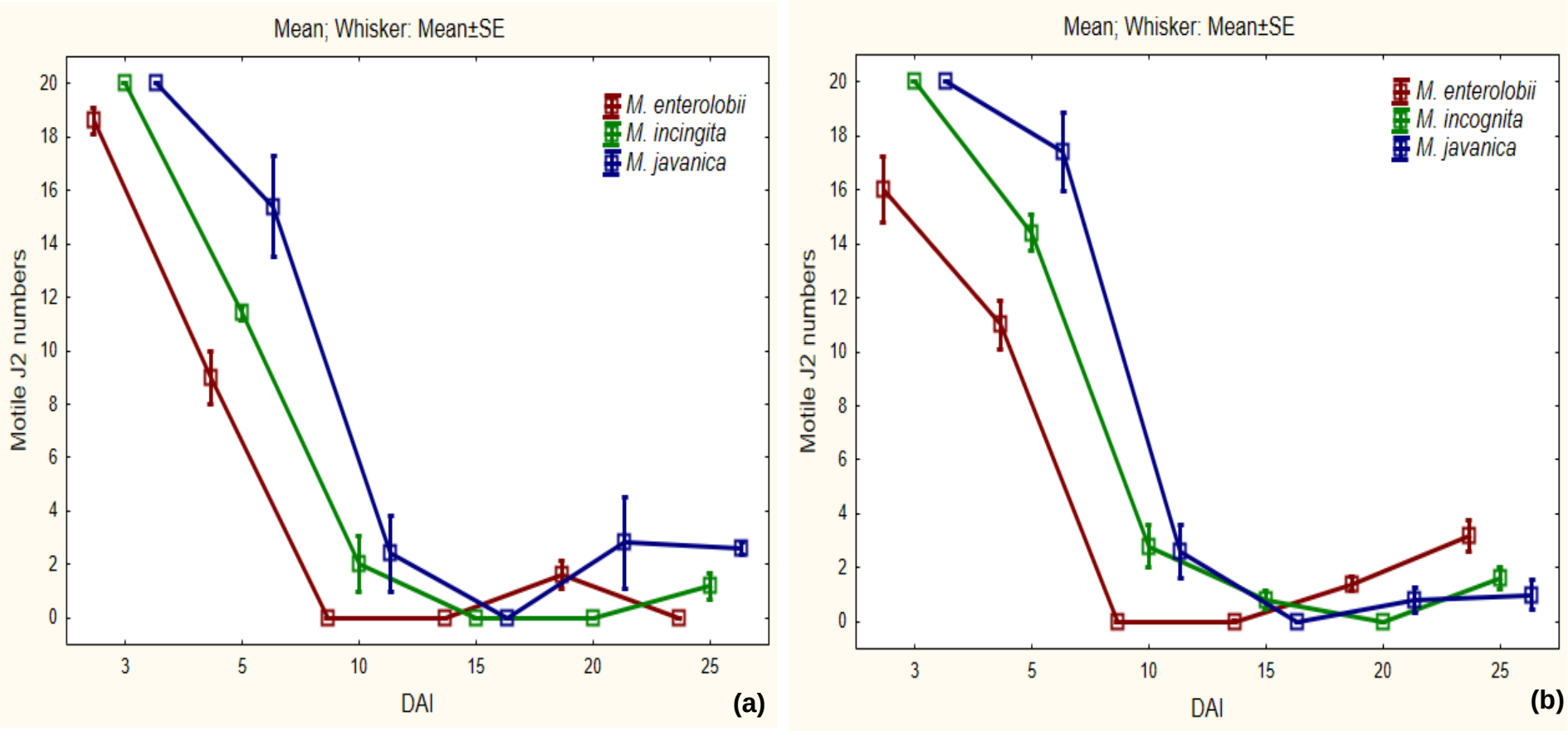

Figure 5.3.1. The development of motile second-stage juveniles (J2) of *M. enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) in roots of tomato genotype Moneymaker from 3 to 25 days after inoculation (DAI) during a 1st (a) and 2nd experiment (b) under glasshouse conditions.

5.3.2. Swollen second-stage juveniles (J2)

Significant ($P \leq 0.05$) interactions existed for Experiment x Species for the number of swollen J2: *M. enterolobii* ($P=0.001$; F-ratio=12.1); *M. incognita* ($P=0.001$; F-ratio=23.7); but not for *M. javanica* ($P=0.638$; F-ratio=0.7). Similarly, significant ($P \leq 0.05$) interactions were evident for Species x Time Interval/DAI ($P=0.001$; F-ratio=8.2) and for Species x Experiment x Time interval ($P=0.001$; F-ratio=4.8) (Table 5.3.2).

Comparison of the two experiment's data for each of the species showed significant ($P \leq 0.05$) differences for swollen J2 numbers between the 1st and 2nd experiments (Table 5.3.2; Figure 5.3.2a and b).

Comparatively the three species' swollen J2 numbers did not differ significantly from each other 3 and 5 DAI for both experiments (Figure 5.3.2a and b; Table C1b in Addendum 1C).

For 10 DAI, the swollen J2 numbers of *M. javanica* for the 1st experiment were significantly ($P \leq 0.05$) higher than those of the other two species; for the 2nd experiment the swollen J2 numbers of the three species were similar ($P > 0.05$) (Table C1b in Addendum 1C; Figure 5.3.2a and b).

For 15, 20 and 25 DAI the swollen J2 numbers of the three species were also similar ($P > 0.05$); for both experiments.

Table 5.3.2. The number of swollen second-stage juveniles (J2) of *Meloidogyne enterolobii, M. incognita*, and *M. javanica* in roots of tomato cultivar Moneymaker during a 25-day period under glasshouse conditions.

Time interval/DAI	*M. enterolobii*		*M. incognita*		*M. javanica*	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
3	1.4 a*A**	2.8 bAB	0.02 aA	0.01 aA	0.02 aA	0.02 aA
5	10.2 bD	5.2 cBC	8.6 cC	4.8 bB	4.6 aAB	3.4 aA
10	2.4 aAB	6.0 cC	5.2 bB	11.8 cD	13.0 bC	8.8 bBC
15	0.02 aA	0.02 aA	1.4 aA	0.8 aA	1.0 aA	0.02 aA
20	0.8 aA	0.02 aA	0.2 aA	0.02 aA	0.2 aA	0.02 aA
25	0.02 aA	0.6 abA	0.4 aA	0.02 aA	1.2 aA	1.6 abA
P	0.001	0.001	0.001	*0.001*	*0.001*	*0.001*
F ratio	49.2	25.2	38.0	*136.0*	*20.2*	*11.6*
Interaction data:						
Species x Experiment						
P	*0.001*		*0.001*		*0.221*	
F ratio	*12.1*		*23.7*		*1.5*	
Species x Time interval (DAI)						
P	*0.001*					
F ratio	*8.2*					
Species x Experiment x Time Interval						
P	*0.001*					
F ratio	*4.8*					

*Indicates the differences in means among the time intervals for each species and experiment in each column, with the same lower case letter showing no significant difference according to the Tukey HSD Test ($P \leq 0.05$); **Indicates the differences in means among the three species for each experiment (across rows), with the same capital letter showing no significant difference among species at a given time interval according to the Tukey HSD Test ($P \leq 0.05$).

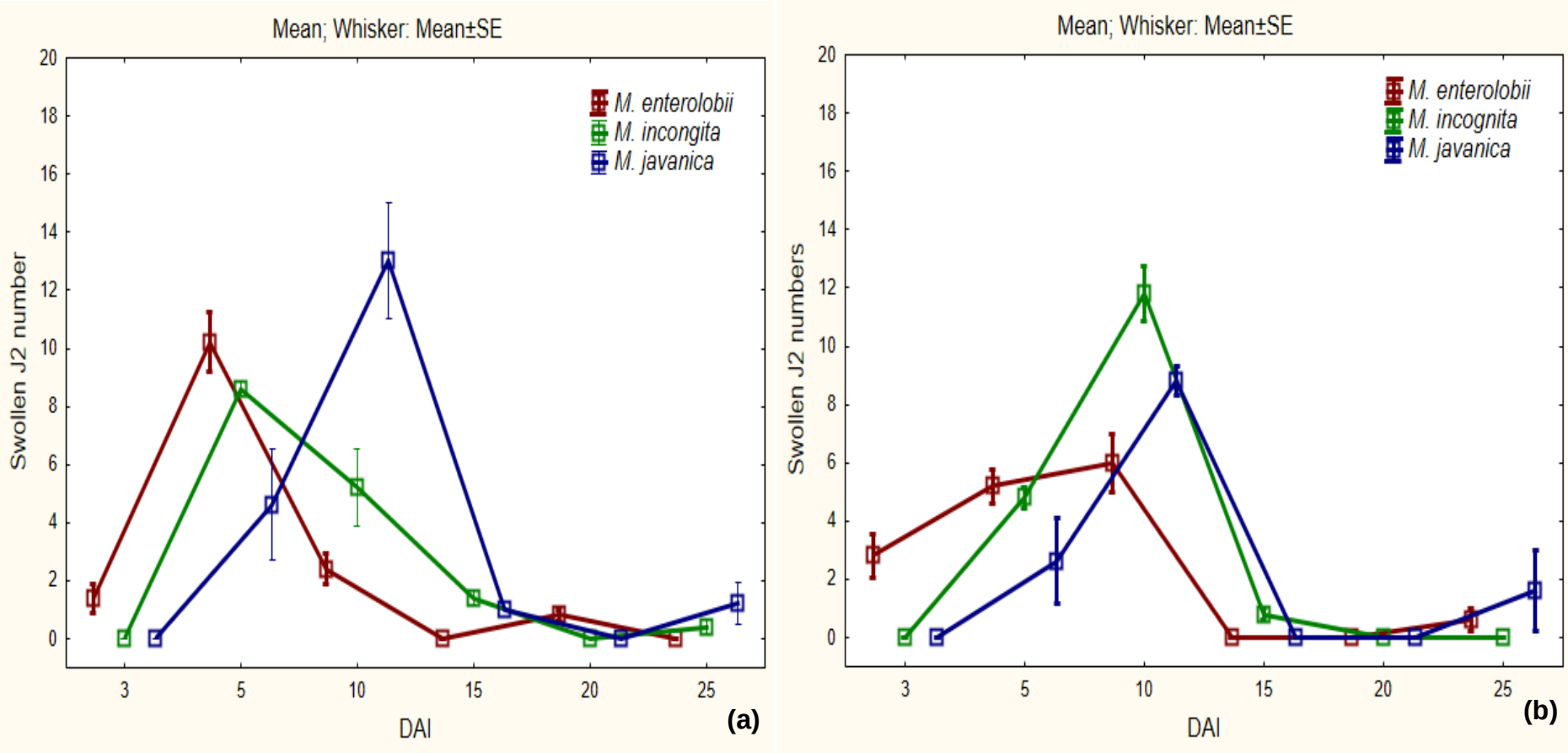

Figure 5.3.2. The number of swollen second-stage juveniles (J2) of *M. enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) in roots of tomato genotype Moneymaker from 3 to 25 days after inoculation (DAI) during a 1st (a) and 2nd experiment (b) under glasshouse conditions.

5.3.3. Swollen third-stage juvenile (J3)

Significant (*P*≤0.05) interactions existed for Experiment x Species for the number of swollen J3 for all three species: *M. enterolobii* (*P*=0.001; F-ratio=26.8) *M. incognita* (*P*=0.001; F-ratio=6.4); and *M. javanica* (*P*=0.021; F-ratio=3.0). Similarly, significant (*P*≤0.05) interactions were evident for Species x Time Interval/DAI (*P*=0.001; F-ratio=3.7) for Species x Experiment x Time interval (*P*=0.001; F-ratio=7.6) (Table 5.3.3).

Comparison of the two experiment's data for each of the three species showed that the swollen J3 numbers differed significantly (*P*≤0.05), for some of the intervals, between the 1st and 2nd experiments (Table 5.3.3; Figure 5.3.3a and b).

Comparatively the three species' swollen J3 numbers did not differ significantly from one another 3 and 5 DAI for both experiments (Table C1c in Addendum 1C; Figure 5.3.3a and b).

For 10 DAI and for the 1st experiment, the swollen J3 numbers of *M. enterolobii* and *M. incognita* were similar (*P*>0.05) and significantly (*P*≤0.05) higher than those of *M. javanica.* However, the three species' swollen J3 numbers did not differ significantly from one another for the 2nd experiment (Table C1c in Addendum 1C; Figure 5.3.3a and b).

For 15, 20 and 25 DAI, the three species were also similar (*P*>0.05) with respect to their swollen J3 numbers; for both experiments (Table C1c in Addendum 1C; Figure 5.3.3a and b).

Table 5.3.3. The number of swollen third-stage juveniles (J3) of *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of tomato genotype Moneymaker during a 25-day period under glasshouse conditions.

Time interval/DAI	*M. enterolobii*		*M. incognita*		*M. javanica*	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
3	0.02 a*A**	1.2 aA	0.02 aA	0.02 aA	0.02 aA	0.02 aA
5	0.8 aA	3.4 bcBC	0.02 aA	0.8 aAB	0.02 aA	0.02 aA
10	11.2 bD	4.8 cC	11.4 bC	4.2 bcAB	3.4 aA	8.0 cB
15	1.2 aA	1.6 abAB	3.0 aAB	5.2 cB	1.8 aA	1.8 bA
20	1.4 aAB	0.01 aA	1.0 aAB	1.4 abAB	1.6 aA	0.2 aA
25	0.02 aA	0.2 aA	0.02 aA	0.01 aA	1.2 aA	0.02 aA
P	***0.001***	***0.001***	***0.003***	***0.001***	***0.179***	***0.001***
F ratio	***97.4***	***14.8***	***14.8***	***9.1***	***1.7***	***192.5***
Interaction data:						
Species x Experiment						
P	***0.025***		***0.001***		***0.016***	
F ratio	***26.8***		***6.4***		***3.1***	
Species x Time interval (DAI)						
P	***0.001***					
F ratio	***3.7***					
Species x Experiment x Time interval (DAI)						
P	***0.001***					
F ratio	***7.6***					

*Indicates the differences in means among the time intervals for each species and experiment in each column, with the same lower case letter showing no significant difference according to the Tukey HSD Test ($P \leq 0.05$); **Indicates the differences in means among the three species for each experiment (across rows), with the same capital letter showing no significant difference among species at a given time interval according to the Tukey HSD Test ($P \leq 0.05$).

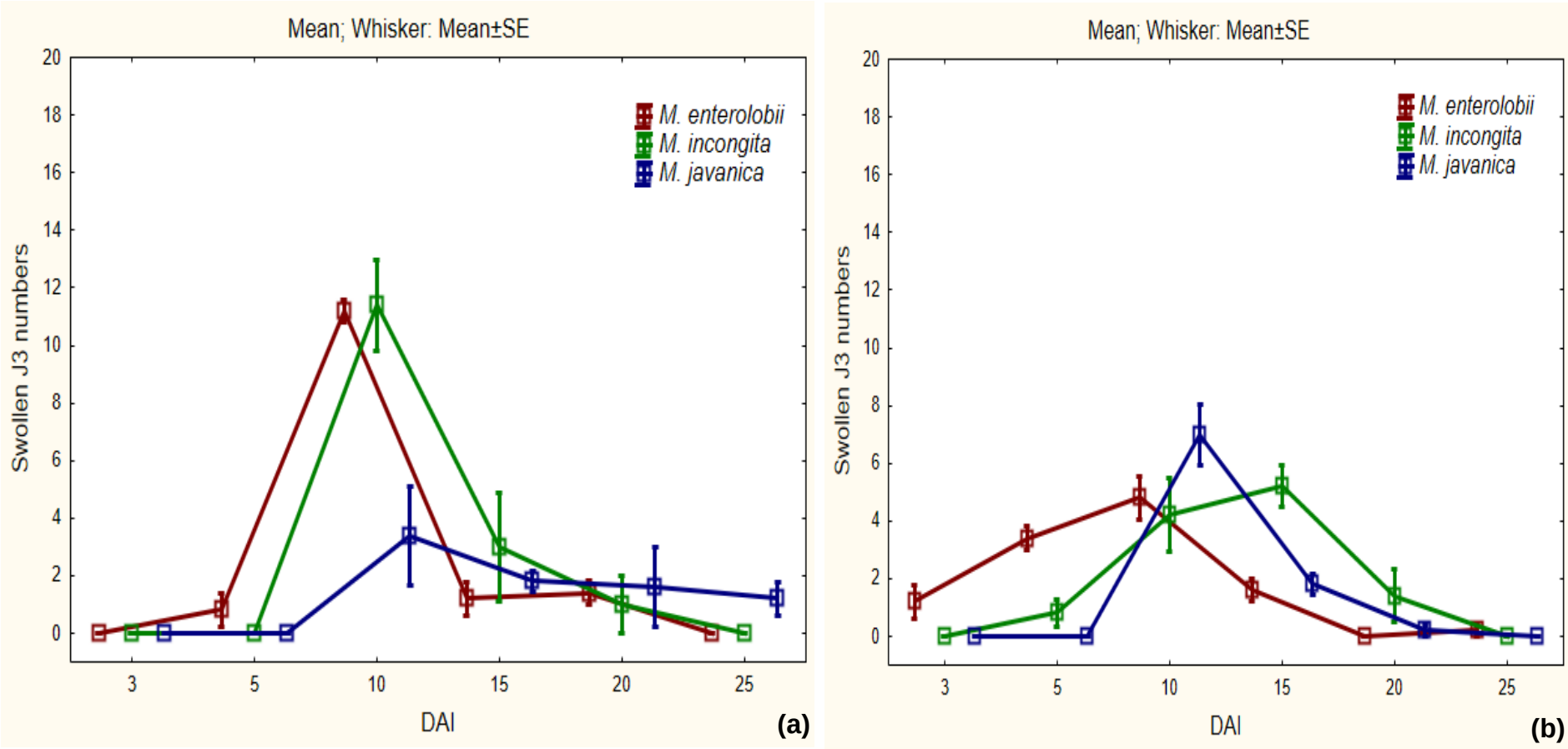

Figure 5.3.3. The number of swollen third-stage juveniles (J3) of *M. enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) in roots of tomato genotype Moneymaker from 3 to 25 days after inoculation (DAI) during a 1st (a) and 2nd experiment (b) under glasshouse conditions.

5.3.4. Swollen forth-stage juvenile (J4)

A significant (*P*≤0.05) interaction existed for Experiment x Species for the number of swollen J4 for *M. enterolobii* (*P*=0.001; F-ratio=6.8), but not for *M. incognita* (*P*=0.849; F-ratio=5.7) neither for *M. javanica* (*P*=0.917; F-ratio=0.3). Significant (*P*≤0.05) interactions were, however, evident for Species x Time Interval/DAI (*P*=0.001; F-ratio=16.6) and for Species x Experiment x Time interval (*P*=0.032; F-ratio=2.1) (Table 5.3.4).

Comparison of the two experiment's data showed that the swollen J4 numbers differed significantly (*P*≤0.05), for some of the time intervals, between the 1st and 2nd experiments (Table 5.3.4; Figure 5.3.4a and b).

Comparatively the three species' swollen J4 numbers did not differ significantly from one another 3 and 5 DAI for both experiments (Table C1d, Addendum 1C; Figure 5.3.4a and b).

The swollen J4 numbers of *M. enterolobii* were significantly (*P*≤0.05) higher 10 DAI, for both experiments, than those of the other two species (Table C1d, Addendum 1C; Figure 5.3.4a and b).

For 15 DAI for both the 1st and 2nd experiments, the swollen J4 numbers for *M. incognita* were significantly (*P*≤0.05) higher than those of the other two.

At both 20 and 25 DAI, the swollen J4 numbers of all three species were similar (P>0.05); for both experiments (Table C1d, Addendum 1C; Figure 5.3.4a and b).

Table 5.3.4. The number of swollen fourth-stage juveniles (J4) of *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of tomato genotype Moneymaker during a 25-day period under glasshouse conditions.

Time interval/DAI	*M. enterolobii*		*M. incognita*		*M. javanica*	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
3	0.02 a*A**	0.01 aA	0.02 aA	0.01 aA	0.02 aA	0.02 aA
5	0.02 aA	0.41 aA	0.02 aA	0.02 aA	0.02 aA	0.02 aA
10	6.4 cDEF	9.2 bF	1.41 aAB	1.2 aAB	1.2 aA	1.8 abA
15	3.8 bcBCD	8.6 bEF	12.0 bC	11.4 cC	5.4 bBC	5.6 bC
20	2.6 abABC	5.6 cCDE	4.4 aB	5.0 bB	1.8 aAB	1.2 aA
25	2.0 abAB	0.41 aA	1.61 aAB	0.02 aA	0.4 aA	1.6 abA
P	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***	***0.007***
F ratio	***9.1***	***80.1***	***18.54***	***63.8***	***20.54***	***4.5***
Interaction data:						
Species x Experiment						
P	***0.001***		***0.849***		***0.914***	
F ratio	***6.8***		***5.7***		***0.3***	
Species x Time interval (DAI)						
P	***0.001***					
F ratio	***16.6***					
Species x Experiment x Time Interval (DAI)						
P	***0.031***					
F ratio	***2.1***					

*Indicates the differences in means among the time intervals for each species and experiment in each column, with the same lower case letter showing no significant difference according to the Tukey HSD Test ($P \leq 0.05$); **Indicates the differences in means among the three species for each experiment (across rows), with the same capital letter showing no significant difference among species at a given time interval according to the Tukey HSD Test ($P \leq 0.05$).

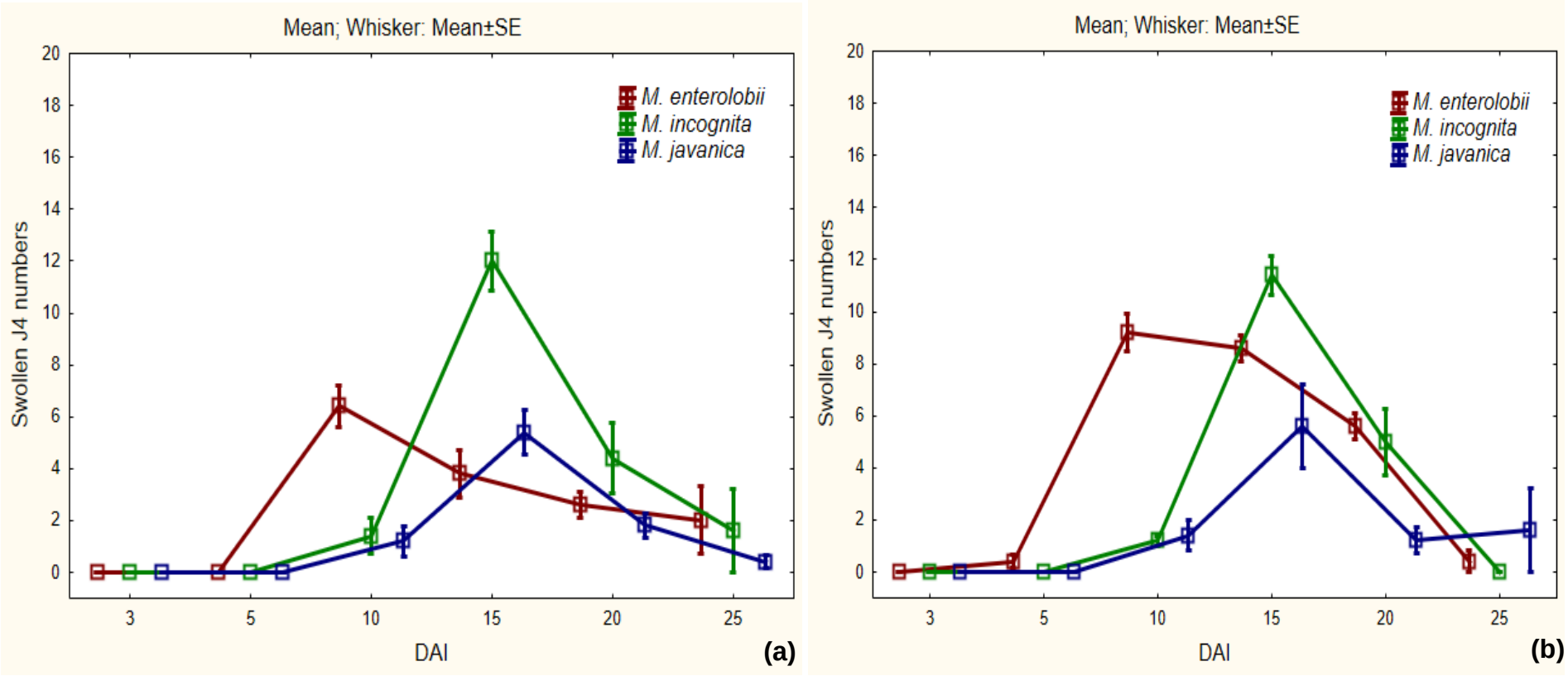

Figure 5.3.4. The number of swollen fourth-stage juveniles (J4) of *M. enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) in roots of tomato genotype Moneymaker from 3 to 25 days after inoculation (DAI) during a 1st (a) and 2nd experiment (b) under glasshouse conditions.

5.3.5. Females (mature and immature)

A significant ($P \leq 0.05$) interaction existed for Experiment x Species for the number of females for *M. enterolobii* ($P=0.001$; F-ratio=6.1), but not for *M. incognita* ($P=0.559$; F-ratio=0.8) and *M. javanica* ($P=0.447$; F-ratio=1.0). A significant ($P \leq 0.05$) interaction were evident for Species x Time Interval/DAI ($P=0.001$; F-ratio=13.6), but not for Species x Experiment x Time interval ($P=0.128$; F-ratio=1.5) (Table 5.3.5).

Comparison of the two experiment's data for each of the three species showed that the female numbers differed significantly ($P \leq 0.05$), for some of the time intervals, between the 1st and 2nd experiments (Table 5.3.5; Figure 5.3.5a and b).

Comparatively the three species' female numbers did not differ significantly from one another 3 and 5 DAI for both experiments (Table C1e, Addendum 1C; Figure 5.3.5a and b).

For 10 DAI the female numbers of the three species of both experiments were also similar ($P > 0.05$) (Table C1e, Addendum 1C; Figure 5.3.5a and b).

For the 1st and 2nd experiments 15 DAI the female numbers of *M. enterolobii* and *M. javanica* were significantly ($P \leq 0.05$) higher than those of *M. incognita* (Table C1e, Addendum 1C; Figure 5.3.5a and b).

Female numbers of the three species did not differ significantly from each other for both the 20 and 25 DAI time intervals (Table C1e, Addendum 1C; Figure 5.3.5a and b)

Table 5.3.5. The numbers of females of *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of tomato genotype Moneymaker during a 25-day period under glasshouse conditions.

Number of females						
Time interval/DAI	***M. enterolobii***		***M. incognita***		***M. javanica***	
	Experiment 1	**Experiment 2**	**Experiment 1**	**Experiment 2**	**Experiment 1**	**Experiment 2**
3	**0.02 a*A****	**0.02 aA**	**0.02 aA**	**0.03 aA**	**0.02 aA**	**0.02 aA**
5	**0.02 aA**	**0.02 aA**	**0.02 aA**	**0.02 aA**	**0.02 aA**	**0.02 aA**
10	**0.02 aA**	**0.02 aA**	**0.02 aA**	**0.02 aA**	**0.02 aA**	**0.02 aA**
15	**15.0 bcB**	**9.8 bB**	**4.0 aA**	**1.8 aA**	**11.8 bB**	**12.6 bBC**
20	**13.6 bB**	**13.0 cD**	**14.6 bB**	**13.6 bB**	**13.8 bBC**	**17.8 bC**
25	**18.0 cC**	**15.6 dBC**	**16.8 bB**	**18.4 cB**	**14.6 bBC**	**15.0 bBC**
P	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***	***0.001***
F ratio	***143.7***	***289.2***	***41.1***	***101.2***	***61.36***	***43.2***
Interaction data:						
Species x Experiment						
P	***0.001***		***0.559***		***0.447***	
F ratio	***7.1***		***0.8***		***1.0***	
Species x Time interval (DAI)						
P	***0.001***					
F ratio	***13.6***					
Species x Experiment x Time interval (DAI)						
P	***0.128***					
F ratio	***1.5***					

*Indicates the differences in means among the time intervals for each species and experiment in each column, with the same lower case letter showing no significant difference according to the Tukey HSD Test ($P \leq 0.05$); **Indicates the differences in means among the three species for each experiment (across rows), with the same capital letter showing no significant difference among species at a given time interval according to the Tukey HSD Test ($P \leq 0.05$).

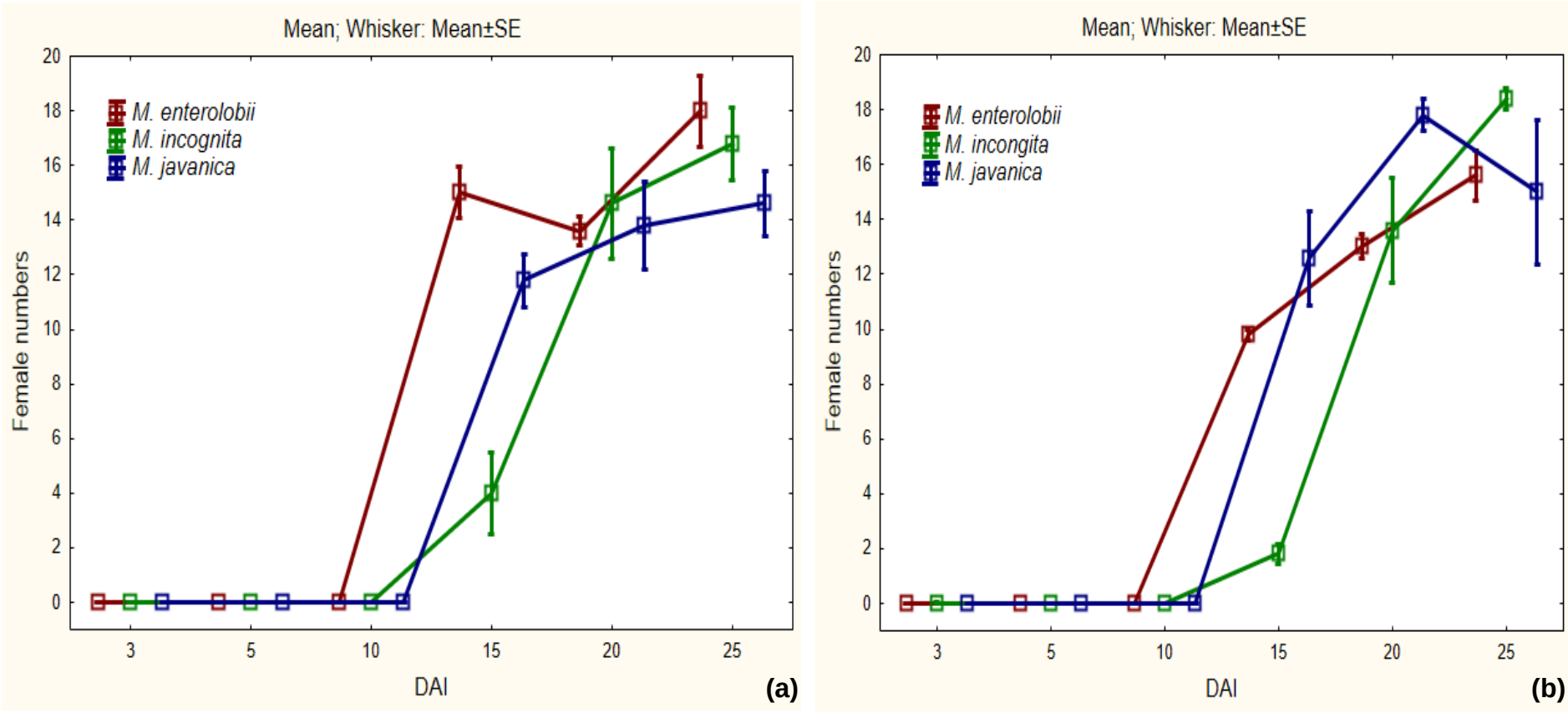

Figure 5.3.5. The number of females of *M. enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) in roots of tomato genotype Moneymaker from 3 to 25 days after inoculation (DAI) during a 1st (a) and 2nd experiment (b) under glasshouse conditions.

5.3.6. Reproduction (number of eggs produced per egg mass) of *M. enterolobii, M. incognita* and *M. javanica*

According to t-Test analyses, the number of eggs per egg mass 20 DAI did not differ significantly ($P \leq 0.05$) between the two experiments for the three species (Table 5.2.6.; Figure 5.2.6a and b). When the species' egg numbers per egg mass were compared among each other per experiment, significant ($P \leq 0.05$) differences were evident for the 2nd experiment and only for *M. javanica vs*. *M. incognita*.

Table 5.3.6. Average number of eggs per egg-mass of *Meloidogyne enterolobii, M. incognita*, and *M. javanica* in roots of a susceptible tomato genotype ('Moneymaker) at 25-days after inoculation under controlled conditions.

	M. enterolobii		***M. incognita***		***M. javanica***	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Means	77 a*	80 a	55 a	72 a	80 a	77 a
P	*0.905*		*0.390*		*0.914*	
t-value	*-0.1*		*-0.9*		*0.1*	
Species compared among each other per experiment						
	Experiment 1			*Experiment 2*		
	Me vs. Mj	*Me vs. Mi*	*Mj vs. Mi*	*Me vs. Mj*	*Me vs. Mi*	*Mj vs. Mi*
P	*0.935*	*0.477*	*0.492*	*0.611*	*0.209*	***0.040***
t-value	*-0.1*	*0.8*	*0.7*	*0.5*	*1.4*	*2.5*

*The same letter indicates no significant differences in egg numbers between the experiments for each species as well as for the species compared to one another using the using Student's T-test where ($P \leq 0.05$).

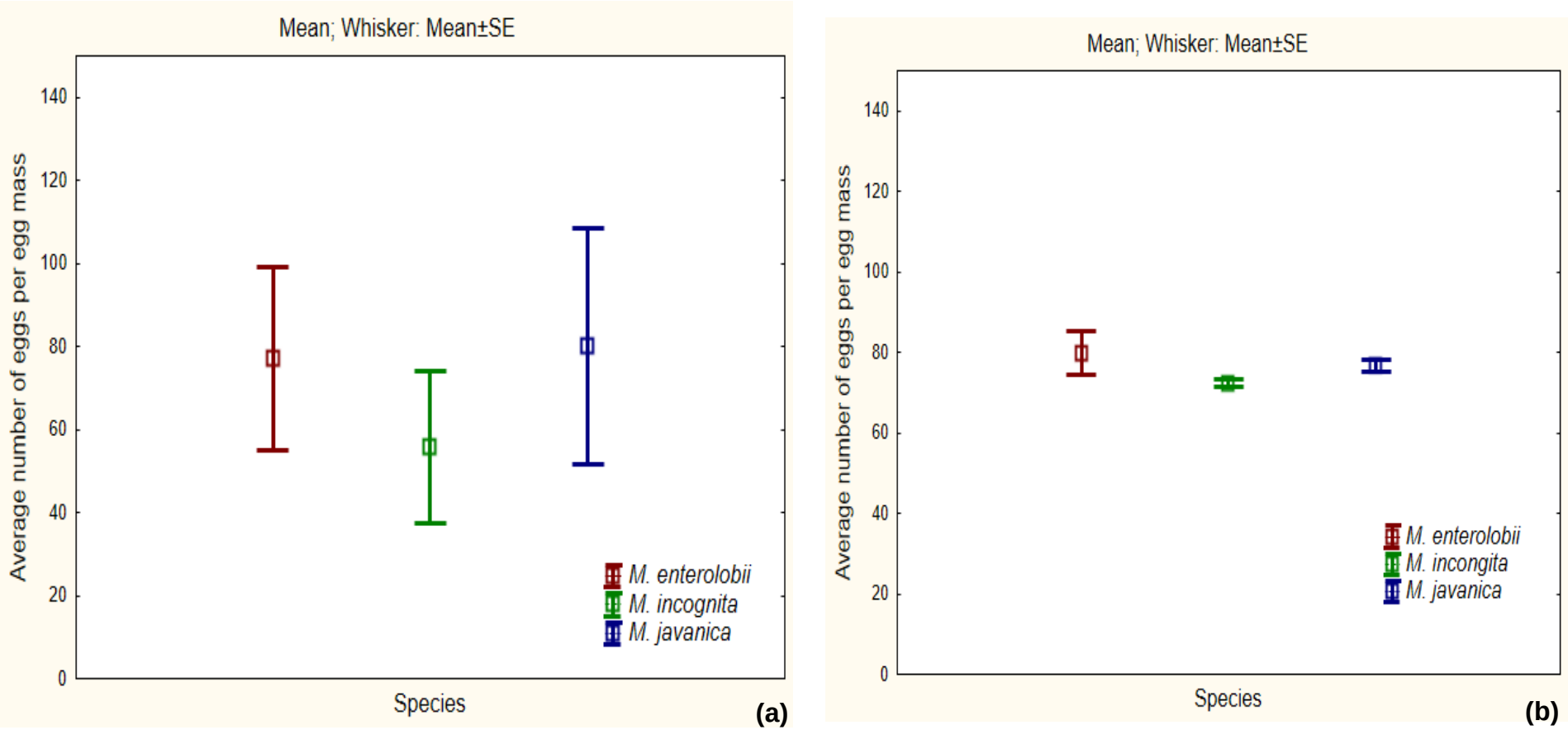

Figure 5.3.6. The number of eggs produced per egg mass for M. *enterolobii* (red line), *M. incognita* (green line) and *M. javanica* (blue line) in roots of tomato genotype Moneymaker 25 days after inoculation (DAI) during a 1st (a) and 2nd experiment (b) under glasshouse conditions.

5.3.7. Degree days (DD) for *M. enterolobii*, *M. incognita* and *M. javanica*

Similar to the maize and soybean experiments (see Paragraphs 5.1.7 and 7.2.7, respectively) single eggs of *M. enterolobii* were found 15 DAI from the tomato roots when females and other life stages were removed for the life-stage development study. However, no egg masses were present. Therefore, the DD was calculated for *M. enterolobii* using 15 DAI. Since no single eggs were found for *M. incognita* and /or *M. javanica* 15 DAI and egg masses only recorded from 20 DAI, the DD for these two species were calculated using the latter time interval.

The required thermal time for *M. enterolobii* to complete a single life cycle was substantially lower, for both experiments, in comparison to *M. incognita* and *M. javanica* (Table 5.3.7).

Table 5.3.7. Degree-day data for *Meloidogyne enterolobii, M. incognita* and *M. javanica* in roots of tomato genotype Moneymaker under glasshouse conditions.

Species	Min. Temperature (°C)	Max. Temperature (°C)	Base temperature (Tb)	Time interval (DAI)	Degree days (DD)
Experiment 1					
M. enterolobii	**18.5**	**31.0**	**10.0****	**15**	**221**
M. incognita			**9.8***	**20**	**299**
M. javanica			**10.6***	**20**	**283**
Experiment 2					
M. enterolobii	**16.0**	**32.0**	**10.0****	**15**	**210**
M. incognita			**9.8***	**20**	**284**
M. javanica			**10.6***	**20**	**268**

*****Negron (2006); **Jacobs *et al.* (2011)

5.4. Discussion

Novel information regarding the substantially shorter DD needed by *M. enterolobii* to complete its life cycle in roots of three crops (maize, soybean and tomato) compared to that of both *M. incognita* and *M. javanica* has been generated as a result of this greenhouse study. The only other information available regarding the life-cycle duration of *M. enterolobii* is that by Negron (2006) for an USA population (24 DAI in guava roots) and Ashokkimar *et al.* (2019) for an Indian population (28-30 DAI in tomato roots).

This is a first report of the DD being reported for *M. enterolobii* to complete its life cycle; particularly in roots of three crops that are economically important in South Africa (also sub-Saharan Africa and other parts of the world) regarding their uses as sources of food, protein and/or oil (DAFF, 2017; GrainSA, 2017a and b). The recorded DD values for *M. enterolobii* for these crops are in a fairly narrow range if the means of the two experiments for each crop was calculated, namely for maize (232 DD), soybean (195 DD) and tomato (216 DD). These values were, however, substantially lower than that of *M. javanica* (that followed *M. enterolobii* in this regard) being 298 for maize, 236 for soybean and 284 for tomato; and *M. incognita* that had the highest DD values (313 DD for maize, 272 DD for soybean and 292 DD for tomato). The DD information generated is valuable and has a practical application since it suggests that *M. enterolobii* will be able to produce eggs earlier than its counterpart species after infecting roots of these crops, with soybean being superior in terms of the shorter life-cycle duration of this species in its roots. Results of this study are however, different from DD values reported by Negron (2006) for *M. incognita* in roots of okra [*Abelmoschus esculentus* (L.) Moench] as being 303 DD and 344 DD for *M. javanica* at an ambient temperature range of 24-27 ºC. This difference could partly be explained by the difference in temperature ranges recorded during this South African study, being 19-32 ºC for maize, 15-32 ºC for soybean and 18-32 ºC for tomato for all three species. Nonetheless, the substantially lower minimum temperature values recorded for this South Africa study indicates that *M. enterolobii* and its two counterpart species are able to reproduce and complete their life cycles under cooler conditions. According to Yang and Eisenback (1983) the preferred temperature range for *M. enterolobii* is 17-28 ºC; the minimum value being well in range with that of the South African study, while the upper limit is 4 ºC lower than that of the South African study. For *M. javanica* (Madulu and Trudgill, 1994) and *M. incognita* (Tsai, 2008) the preferred temperature ranges are 18-30 ºC and 25-35 °C, respectively; also relatively well in range with those of the South African.

Pronounced differences in especially the generally quicker development of swollen J2 of *M. enterolobii* in roots of all three crops from 5 DAI compared to those of *M. javanica* and *M. incognita* (from 10 DAI) supports the shorter DD and earlier production of single eggs by *M. enterolobii* 15 DAI compared to the absence thereof for *M. javanica* and *M. incognita*. Furthermore, earlier peaks recorded for swollen *M. enterolobii* J2 numbers from 5-10 DAI for *M. enterolobii* and from 10-15 DAI for the other two species in roots of the three crops substantiate the earlier egg production by this species. This is in agreement with results by Ashokkimar *et al*. (2019) who studied an Indian population of *M. enterolobii* in guava roots at an ambient temperature range of 26-30 ºC.

Similarly to the quicker J2 life-stage development of *M. enterolobii*, was that for J3 and J4 development of the South African population. However, J4 of the South African *M. enterolobii* population peaked from 15 DAI compared to that of the Indian population that peaked from 19-21 DAI (Ashokkimar *et al*., 2019). For *M. javanica*, Negron (2006) recorded J3 of an USA population in roots of okra from 10 DAI, which differed from the South African study (recorded from 15 DAI). For *M. incognita,* J3 of the same USA population were recorded from 15 DAI which is similar to that of the South African study. A similar trend was also evident for J4 development in the study by Negron (2006) compared to that of the South African populations of *M. javanica* and *M. incognita*.

Regarding females, the three South African species all developed females from 15 DAI, which is in contrast to results by Negron (2006) who recorded females of USA populations of *M. incognita* and *M. javanica* only from 20 DAI. Westerich *et al*. (2011) recorded, for a Brazilian population, that egg-producing *M. enterolobii* females were found 24 DAI in tomato roots at a temperature of 26 ºC. Ashokkimar *et al.* (2019) recorded non-egg producing *M. enterolobii* females in guava roots in India from 21-24 DAI, while egg-producing females were found 28-30 DAI. Results of the Brazilian and Indian studies are different from results for the South African population of *M. enterolobii* for which egg-producing females were recorded much quicker (15 DAI) in roots of maize, soybean and tomato.

Interestingly *M. enterolobii* did not necessarily have the highest female numbers in roots of the three crops, which for the 20 and 25 DAI could be explained by the presence of substantially high numbers of 2nd generation J2, J3 and J4. By contrast for *M. incognita*, for example, high female numbers at 20 and 25 DAI coincided with low J2 numbers

representing a 2nd generation. The female numbers of *M. javanica* beyond 15 DAI appears to be very similar, but to a lesser degree, to that of *M. enterolobii*.

Due to the quicker development of single eggs by the South African *M. enterolobii* population, the onset of a second generation of J2 20 DAI further supports the quicker life-stage development and life-cycle duration of this species. However, a 2nd generation of J2 was also recorded for *M. javanica* from 20 DAI indicating the closer resemblance of its life-stage development and life-cycle duration with regard to *M. enterolobii*, opposed to that of *M. incognita* 2nd generation of J2 for the latter species were only recorded 25 DAI for all three crops. The presence of a 2nd generation of swollen J2 of *M. enterolobii* 25 DAI was recorded as well as for *M. javanica* but to a lesser degree for the latter species in terms of numbers of this life-stage being recorded.

Interestingly the egg production per egg mass of the three species 20 DAI were similar. Hence due to limited research regarding the number of eggs per egg mass for *M. enterolobii* in particular cannot be noted as a measure of pathogenicity.

The fundamental information generated on the life-stage development and life-cycle duration of the three South African root-knot nematode species are not only of scientific value, but of utmost practical importance. The data generated as a result of this study suggest that during a crop growth period of 60 days, for example *M. enterolobii* can go through four life cycles apposed to three bt *M. incongita* and *M. javanica.* This may offer partial explanation of the higher pathogenicity of *M. enterolobii. Meloidogyne incognita* and *M. javanica* are predominant nematode pests in local grain (Fourie *et al*., 2017; Mc Donald *et al*., 2017) and tomato (Jones *et al*., 2017) production areas. Of importance too is that, except for the presence of *M. enterolobii* in local guava (*Psidium guajava* L.) orchards (Willers, 1997) and various vegetable production sites (Onkendi and Moleleki, 2013; Van den Berg *et al*., 2017; Visagie *et al*., 2018; Rashifidard *et al.,* 2019; SAPPNS[1]), this highly pathogenic species (EPPO, 2010) has also been found in fields and protective structures (nethouses) where tomato is produced in South Africa (Van den Berg *et al*., 2017;

[1] Dr Mariette Marais of the Nematology Unit, Biosystematics, Agricultural Research Council–Plant Health and Protection is thanked for the use of data from the South African Plant-Parasitic Nematode Survey (SAPPNS) database; E-mail: maraism@arc.agric.za.

SAPPNS[1]). Particularly important to note is that this species has also been detected in roots of maize obtained from a field in the Highveld grain production area (Pretorius, 2018). The occurrence and adverse impact of these species on such crops in terms of yield losses experienced (Jones *et al*., 2013; Fourie *et al*., 2017; Mc Donald *et al*., 2017) in areas where these crops are produced thus put the root-knot nematode problem in perspective and accentuates why fundamental knowledge, such as obtained in this study should be acquired.

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CHAPTER 6: CONCLUSION

During this study the life-stage development, life-cycle, duration and reproduction potential of *Meloidogyne enterolobii* Yang and Eisenback 1983 was determined in comparison to its thermophilic counterparts *M. incognita* (Kofoid and White, 1919) Chitwood, 1949 and *M. javanica* (Treub, 1885) Chitwood, 1949*;* under glasshouse conditions. Maize (*Zea mays* L) P-2432-R, soybean (*Glycine max* L) DM-5953-RSF, and tomato (*Solanum lycopsericum* L.) Moneymaker were used as the target crops since they are economically important food and/or protein and oil sources for South Africans (DAFF, 2017; GrainSA, 2017a, 2017b) and inhabitants in other sub-Saharan countries (SSA) (IITS, n.d.; Fufa *et al.*, 2001; Coyne *et al.,* 2018,). *Meloidogyne enterolobii,* just as its counterparts, has a detrimental impact on food security globally (Singh *et al.,* 2015) especially in developing countries. The species has been classified as a highly pathogenic and virulent *Meloidogyne* species (Castagnone-Sereno, 2012) and understanding the integral development of this species in terms of the time it takes for its life stages to complete a life cycle may contribute to advancing in the management of this species.

Since information on the duration of the life-cycle of *M. enterolobii* falls short in comparison to that of its counterpart species (*M. incognita* and *M. javanica*), which are the predominant nematode pests in agricultural production areas in SSA (dos Santos *et al.* 2019) and South Africa (Fourie *et al*., 2017; SAPPNS[1]) in particular, this study aimed to address this limitation.

6.1 *Meloidogyne enterolobii*, a virulent root-knot nematode species threatening crop production with particular reference to SSA

The first part of this study represented a desk-top study in which 280 accessible, scientific literature resources (see Chapter 2) were consulted to summarize the following about *M. enterolobii*: global distribution; limitations reported and successes achieved regarding the identification and discrimination of the species from other similar species of the *M. incognita* group (MIG) others (based upon morphology, molecular studies, host responses,); and ultimately management strategies reported to combat the species.

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Highlights from this review study are that: the distribution of the species has been substantially expanded on a global basis (including SSA, specifically South Africa) since its discovery in 1983; major successes were made regarding molecular methods being developed and used in combination with morphological approaches to accurately identify the species; management of *M. enterolobii* remains an ongoing struggle, as the virulent species has rendered various strategies ineffective due to its ability to overcome host plant resistance (Castagnone-Sereno, 2012). Despite global limitations and challenges existing in terms of research being done on *M. enterolobii*, information is particularly lacking in this regard for various developing countries of sub-Sharan Africa countries, with limited developing nematological resources (Fourie and Mc Donald, 2014; Cortada *et al.,* 2019), and irregular food security.

The research part of this study is the first in its kind in a sub-Saharan Africa country, South Africa, and includes three susceptible host crop genotypes, namely maize 'P-2432-R', soybean 'DM-5953-RSF', and tomato 'Moneymaker'.

6.2 Identification of the three *Meloidogyne* species used during life-stage development and life-cycle duration experiments

Morphological and molecular identification was done before the experiments began to verify the identity of the three South African species. Perineal-pattern and oesophageal identification of females verified the identity of the three species. This was evident for *M. incognita* and *M. javanica* using protocols of Kleynhans (1991). For *M. enterolobii* morphological studies by Visagie *et al*. (2018) and Rashidifard (2019) were consulted utilizing unique ridge characteristics of the perineal patterns (such as apex design, dorsal arch height, lateral lines, overall shape of the patterns, phasmids, striae, and tail region visibility) and the shape of the lumen, meso- and procorpus lining. Similarly to the classical identification approach, the sequence characterized amplified region – polymerase chain reaction (SCAR-PCR) method identified the three species with respect to the size of their deoxyribonuclease (DNA) fragments which coincided with those of the standards used for each species; 250 bp for *M. enterolobii* (Long *et al*., 2006); 1 200 bp for *M. incognita* (Zijlstra *et al*., 2000); and 700 bp for *M. javanica* (Zijlstra *et al*., 2000). These results hence enabled that the study could proceed with confidence regarding the accurate identity of the three species.

6.3 Comparing the life-stage development, life-cycle duration and reproduction potential of *M. enterolobii, M. incognita,* and *M. javanica* in maize roots

The experiments, initial and repeat, for each *Meloidogyne* species and crop were done under glasshouse conditions that was maintained at temperature ranges of 19-32 ºC, 15-32 ºC and 18-32 ºC for maize, soybean and tomato, respectively. Seedling of each crop genotypes was inoculated with second-stage juveniles (J2) of each species (see Chapter 3, paragraph 3.1.5., Table 3.3). Twenty specimens of various life stages of each *Meloidogyne* species were isolated (see Chapter 4, Figure 4.1.) per time-interval [3, 5, 10, 15, 20 and 25 days after inoculation (DAI)]. The identification of the life stages were successfully done with the aid of Triantaphyllou and Hirschmann (1960) and represented motile second-stage juveniles (J2), swollen J2, swollen third-stage juveniles (J3), swollen fourth stage juveniles (J4), swollen females (immature and mature) and motile males.

The ultimate and most important outcome of the experiments was that *M. enterolobii* needed substantially lower degree days (DD) in roots of all three crops (232 DD for maize; 195 DD for soybean; 216 DD for tomato), than its counterpart species, to complete its life cycle; from J2 infection until the production of eggs by females. *Meloidogyne javanica* generally followed (298 DD for maize; 236 DD for soybean; 284 DD for tomato), with *M. incognita* (313 DD for maize; 272 DD for soybean; 292 DD for tomato) needing the longest time to complete its life cycle in roots of the three crops.

The shorter DD for *M. enterolobii* is ascribed to its general quicker development through life stages compared to those of the other two species. Although all three species had similar trends for the motile J2 development until 15 DAI, *M. enterolobii* developed (in both experiments of the three crops) a 2nd generation of motile J2 at 20 DAI; which was also the case for *M. javanica* but not for *M. incognita*.

Meloidogyne enterolobii and *M. javanica* developed swollen J2 earlier than *M. incognita*, from 20 DAI.

Although *M. enterolobii* developed quicker than the other two species through the life stages up to the swollen J4, all three species had female stages 15 DAI. However, *M. enterolobii* did produce single eggs at the latter time interval while this was not evident for either *M.*

incognita or *M. javanica*. For the latter species egg masses was evident 20 DAI, which was also the case for *M. enterolobii.*

The reproduction potential of the three species with respect to the number of eggs produced per egg mass were similar, indicating that no one was superior in terms of the other. This specific parameter was used to determine the species' reproduction potential, instead of the final population density per root system, since although roots of the three crops were inoculated with the same number of motile J2 of the respective species when the experiments commenced, it is not known how many of these life stages of each species infected the roots when the seedlings were uprooted 3 DAI and transplanted to address the aim of the study.

6.4. Summary

The life stages of the South African population of *M. enterolobii* generally developed quicker to females that produced single eggs (not egg masses) 15 DAI than its counterpart species *M. incognita* and *M. javanica.* Resultantly the DD *M. enterolobii* needed to complete its life cycle was substantially lower than that of *M. javanica* and *M. incognita* (in descending order).

6.5. Recommendation

Since the life-cycle of *M. enterolobii* is shorter than those of *M. incognita* and *M. javanica* it is important that producers know the identity of the root-knot nematode species causing problems in their fields. Newly developed maize, soybean and tomato genotypes should be screened for their host status so that producers can choose those in which roots *M. enterolobii* has the lowest reproduction potential in order to enable maximum reduction of its population densities. Focus in this regard should particularly on genotypes with a shorter growing period too since this will also minimize an increase in *M. enterolobii* numbers during a growing season. Similarly, genotype screening should be done for *M. incognita* and *M. javanica* and other thermophiles, e.g. *M. arenaria* (also occurring in grain and tomato production areas but to a lesser degree). Also, other management strategies (than the use of host plant resistance or poor hosts) should be exploited to enable producers to combat *M. enterolobii* and other economically important root-knot nematode species that damage local crops.

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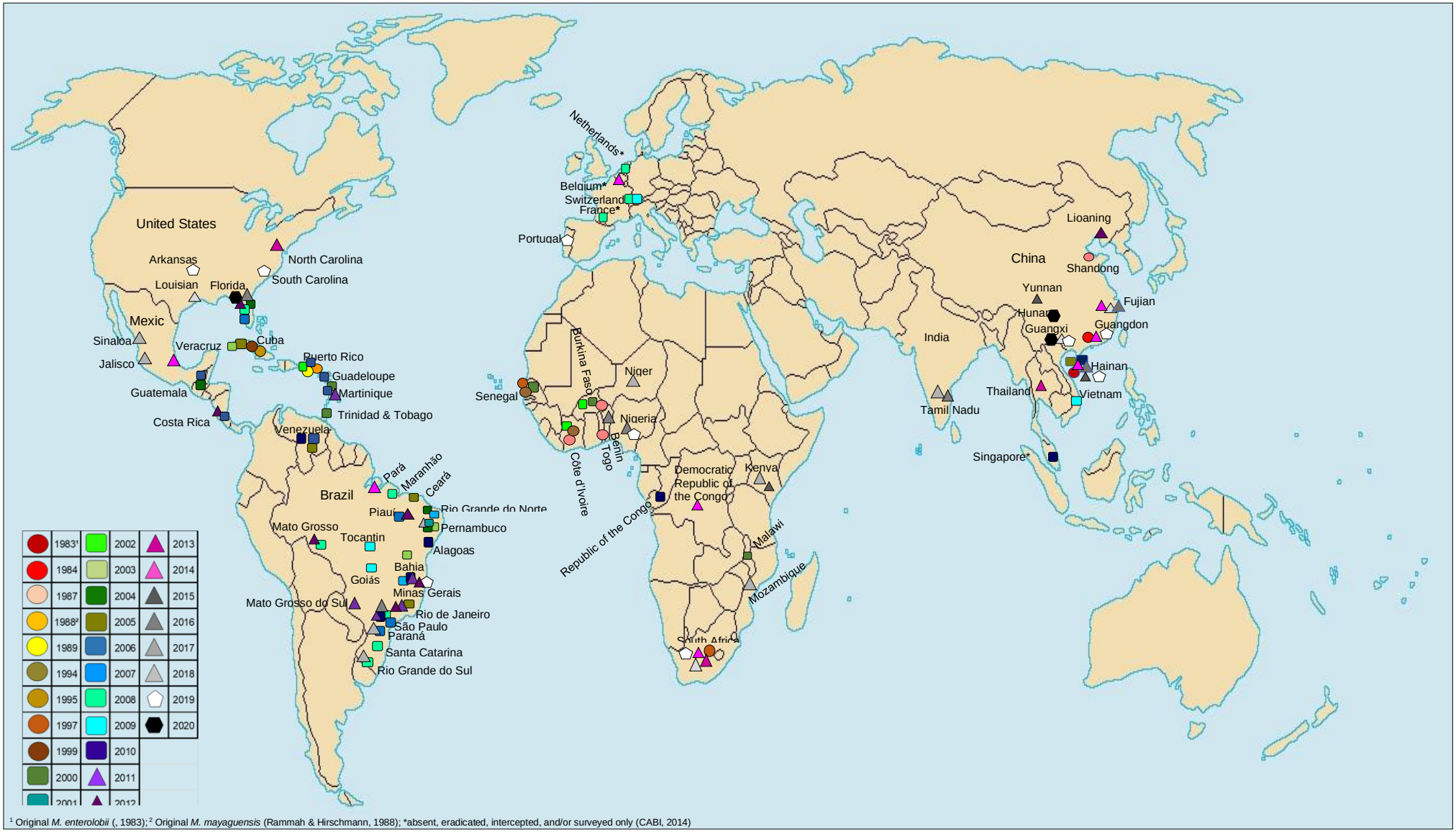
ADDENDUM

Addendum 1: The occurence of *Meloidogyne enterolobii* on a global scale according to Table 2.1



¹ Original *M. enterolobii* (, 1983); ² Original *M. mayaguensis* (Rammah & Hirschmann, 1988); *absent, eradicated, intercepted, and/or surveyed only (CABI, 2014)

Figure 1 The occurrence of *M. enterolobii* (=*M. mayaguensis*) since its discovery in 1983 until the end of 2019

Addendum 1A: Factorial tables for the different life stages of three *Meloidogyne* species isolated from maize roots for various time intervals (days after inoculation: DAI)

Table A1a. The number of motile second-stage juveniles (J2) of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* at different time intervals (days after inoculation: DAI) in roots of maize genotype P-2432-R according to a three-way Factorial Analysis of Variance (Statistica 13.3) with means separated at $P \leq 0.05$ according to the Tukey HSD Test.

Tukey HSD test; variable Motile J2 (Maize Exp 1 & 2) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between MS = 1,4976, df = 144,00

Interaction:
Species x Experiment x Time interval (DAI), *P*=0.001, F ratio=5.0

DAI	Species	Exp	Motile J2 Mean	A	B	C	D	E	F	G	H	I	J	K
20	Mi	2	0,01000	****										
15	Mi	2	0,01000	****										
20	Mj	2	0,01000	****										
15	Mi	1	0,01400	****										
15	Me	2	0,01400	****										
25	Mi	2	0,60400	****	****									
25	Mj	2	0,60600	****	****									
20	Mi	1	0,80600	****	****	****								
15	Mj	2	0,80600	****	****	****								
20	Mj	1	1,00600	****	****	****								
15	Mj	1	1,20600	****	****	****								
25	Me	2	1,40000	****	****	****								
20	Me	2	1,60400	****	****	****								
25	Mi	1	2,60000	****	****	****	****							
15	Me	1	2,80000	****	****	****	****	****						
10	Me	1	3,20000		****	****	****	****	****					
10	Mi	1	3,60000			****	****	****	****	****				
25	Mj	1	5,20000				****	****	****	****	****			
20	Me	1	5,60000					****	****	****	****			
10	Me	2	5,60000					****	****	****	****			

10	Mj	1	5,80000						****	****	****			
10	Mi	2	6,20000							****	****			
10	Mj	2	7,80000								****	****		
25	Me	1	9,60000									****		
5	Me	1	16,20000										****	
5	Mj	2	18,00000										****	****
5	Me	2	18,40000										****	****
3	Me	1	19,60000											****
5	Mj	1	19,80000											****
5	Mi	2	20,00000											****
3	Mi	2	20,00000											****
3	Mi	1	20,00000											****
3	Me	2	20,00000											****
3	Mj	1	20,00000											****
5	Mi	1	20,00000											****
3	Mj	2	20,00000											****

Table A1b. The number of swollen second-stage juveniles (J2) of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* at different time intervals (days after inoculation: DAI) in roots of maize genotype ('P-2432-R') according to a Factorial Analysis of Variance (Statistica 13.3) with means separated at $P \leq 0.05$ according to the Tukey HSD Test.

Tukey HSD test; variable Swollen J2 (mE mI mJ in Maize Exp 1 & 2) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between MS = ,67247, df = 144,00

Interaction:
Species x Experiment x Time interval (DAI), *P*=0.001, F ratio=10.5

Species	DAI	Exp	Swollen J2 Mean	A	B	C	D	E	F	G	H	I
Mj	3	2	0,010000	****								
Mi	3	2	0,010000	****								
Me	15	2	0,010000	****								
Mj	20	1	0,010000	****								
Mi	25	1	0,010000	****								
Mj	3	1	0,012000	****								
Mi	5	2	0,012000	****								

Mj	20	2	0,012000	****									
Mi	20	2	0,012000	****									
Mi	25	2	0,014000	****	****								
Mi	3	1	0,014000	****	****								
Mi	20	1	0,016000	****	****								
Me	3	2	0,016000	****	****								
Mi	5	1	0,018000	****	****								
Mj	5	1	0,208000	****	****								
Mj	25	2	0,212000	****	****	****							
Mi	15	1	0,406000	****	****	****							
Me	3	1	0,412000	****	****	****							
Mj	15	2	0,606000	****	****	****							
Mi	15	2	0,606000	****	****	****							
Me	15	1	0,802000	****	****	****							
Mj	15	1	0,806000	****	****	****							
Me	20	2	1,004000	****	****	****	****						
Mi	10	2	1,600000	****	****	****	****	****					
Me	5	2	1,600000	****	****	****	****	****					
Mj	5	2	2,004000		****	****	****	****					
Me	20	1	2,200000			****	****	****					
Me	25	2	2,200000			****	****	****					
Me	25	1	2,800000				****	****					
Me	5	1	3,000000					****					
Mj	25	1	3,200000					****	****				
Me	10	2	5,000000						****	****			
Mj	10	1	5,800000							****	****		
Mi	10	1	6,400000							****	****		
Mj	10	2	7,200000								****	****	
Me	10	1	8,800000									****	

Table A1c. The number of swollen third-stage juveniles (J3) of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* at different time intervals (days after inoculation: DAI) in roots of maize genotype ('P-2432-R') according to a Factorial Analysis of Variance (Statistica 13.3) with means separated at $P \leq 0.05$ according to the Tukey HSD Test.

Tukey HSD test; variable J3 (mE ml mJ in Maize Exp 1 & 2) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between MS = ,69566, df = 144,00

Interaction:
Species x Experiment x Time interval (DAI), *P*=0.001, F ratio=6.2

Species	DAI	Exp	J3 Mean	A	2	3	4	5	6	7	8	9
Mj	25	2	0,010000	****								
Me	3	2	0,010000	****								
Mi	25	2	0,010000	****								
Me	5	2	0,012000	****								
Mj	5	1	0,012000	****								
Mj	3	2	0,012000	****								
Mj	3	1	0,012000	****								
Me	25	2	0,012000	****								
Mi	25	1	0,012000	****								
Mi	3	2	0,012000	****								
Mj	20	1	0,012000	****								
Mi	5	1	0,012000	****								
Me	3	1	0,012000	****								
Mi	20	1	0,012000	****								
Mi	5	2	0,014000	****								
Mj	5	2	0,014000	****								
Mi	3	1	0,024000	****								
Mj	25	1	0,208000	****	****							
Mi	20	2	0,210000	****	****							
Me	20	2	0,210000	****	****							
Mj	20	2	0,210000	****	****							
Me	5	1	0,608000	****	****	****						
Mj	15	1	0,808000	****	****	****						
Me	25	1	1,002000	****	****	****	****					
Mi	15	1	1,002000	****	****	****	****					
Mi	15	2	1,800000	****	****	****	****	****				
Me	20	1	2,000000	****	****	****	****	****				
Me	15	1	2,200000		****	****	****	****	****			

Me	15	2	2,600000			****	****	****	****			
Mj	15	2	3,000000				****	****	****	****		
Mi	10	2	3,000000				****	****	****	****		
Me	10	1	3,800000					****	****	****	****	
Mj	10	2	4,200000						****	****	****	
Me	10	2	4,800000							****	****	
Mj	10	1	5,400000								****	****
Mi	10	1	7,200000									****

Table A1d. The number of swollen fourth-stage juveniles (J4) of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* at different time intervals (days after inoculation: DAI) in roots of maize genotype ('P-2432-R') according to a Factorial Analysis of Variance (Statistica 13.3) with means separated at $P \leq 0.05$ according to the Tukey HSD Test.

Tukey HSD test; variable J4 (mE mI mJ in Maize Exp 1 & 2) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between MS = 1,4546, df = 144,00

Interaction:
Species x Experiment x Time interval (DAI), *P*=0.001, F ratio=13.4

Species	DAI	Exp	J4 Mean	1	2	3	4	5	6	7	8
Mj	3	2	0,01000	****							
Me	3	2	0,01000	****							
Mi	3	2	0,01000	****							
Mj	25	1	0,01200	****							
Mi	5	2	0,01200	****							
Mj	5	2	0,01200	****							
Me	5	2	0,01200	****							
Mi	5	1	0,01200	****							
Mj	5	1	0,01400	****							
Mj	3	1	0,01400	****							
Me	3	1	0,01400	****							
Me	5	1	0,01400	****							
Mi	3	1	0,01600	****							
Mj	20	1	0,20800	****	****						
Me	25	1	0,40600	****	****						

Mj	10	2	0,80400	****	****	****						
Mj	20	2	0,80400	****	****	****						
Mi	25	1	0,80600	****	****	****						
Mi	20	2	0,81200	****	****	****						
Me	20	1	1,00600	****	****	****						
Mi	20	1	1,20600	****	****	****						
Mi	25	2	1,80400	****	****	****	****					
Me	25	2	2,20000	****	****	****	****					
Mj	25	2	2,40000	****	****	****	****					
Me	20	2	2,80000	****	****	****	****	****				
Mi	10	1	2,80000	****	****	****	****	****				
Mj	15	1	2,80000	****	****	****	****	****				
Mj	10	1	3,00000		****	****	****	****				
Me	10	1	3,40000			****	****	****				
Mi	15	2	3,40000			****	****	****				
Me	15	1	4,20000				****	****	****			
Me	10	2	4,60200				****	****	****			
Mi	15	1	5,40000					****	****			
Mj	15	2	6,40000						****	****		
Mi	10	2	9,20000							****	****	
Me	15	2	11,40000									****

Table A1e. The number of females of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* at different time intervals (days after inoculation: DAI) in roots of maize genotype ('P-2432-R') according to a Factorial Analysis of Variance (Statistica 13.3) with means separated at $P \leq 0.05$ according to the Tukey HSD Test.

Tukey HSD test; variable Female (mE mI mJ in Maize Exp 1 & 2) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between MS = 1,9917, df = 144,00

Interaction:
Species x Experiment x Time interval (DAI), *P*=0.001, F ratio=6.7

Species	DAI	Exp	Female Mean	1	2	3	4	5	6	7	8
Me	10	2	0,01000	****							
Mj	3	1	0,01200	****							
Mi	10	1	0,01200	****							

Me	5	1	0,01200	****							
Mi	5	1	0,01400	****							
Mj	10	1	0,01400	****							
Me	10	1	0,01400	****							
Me	5	2	0,01400	****							
Me	3	1	0,01400	****							
Mi	10	2	0,01600	****							
Mj	5	1	0,01600	****							
Mj	5	2	0,01600	****							
Me	3	2	0,01600	****							
Mj	10	2	0,01600	****							
Mi	3	1	0,01600	****							
Mi	5	2	0,01600	****							
Mj	3	2	0,02200	****							
Mi	3	2	0,02200	****							
Me	15	2	6,00000		****						
Me	25	1	6,20000		****						
Mj	15	2	9,20000		****	****					
Me	20	1	9,40000		****	****					
Me	15	1	10,00000			****	****				
Mj	25	1	11,40000			****	****	****			
Mi	15	1	13,20000				****	****	****		
Mi	15	2	14,20000					****	****	****	
Me	25	2	14,20000					****	****	****	
Me	20	2	14,40000					****	****	****	
Mj	15	1	14,40000					****	****	****	
Mi	25	1	16,60000						****	****	****
Mj	25	2	16,80000							****	****
Mi	25	2	17,60000							****	****
Mi	20	1	18,00000								****
Mj	20	1	18,80000								****
Mi	20	2	19,00000								****
Mj	20	2	19,00000								****

Addendum 1B: Factorial tables for the different life stages of three *Meloidogyne* species isolated from soybean roots for various time intervals (days after inoculation: DAI)

Table B1a. The number of motile second-stage juveniles (J2) of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* at different time intervals (days after inoculation: DAI) in roots of soybean genotype DM-5953-RSF according to a three-way Factorial Analysis of Variance (Statistica 13.3) with means separated at $P \leq 0.05$ according to the Tukey HSD Test.

Tukey HSD test; variable Motile J2 (Soybean experiment 1 & 2) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between MS = 2,1315, df = 144,00

Interaction:
Species x Experiment x Time interval (DAI), *P*=0.001, F ratio=6.0

Exp	Species	DAI	Motile J2 Mean	1	2	3	4
2	Me	15	0,01000	****			
2	Mi	20	0,01000	****			
2	Mj	15	0,01000	****			
1	Mj	20	0,01200	****			
1	Me	20	0,01200	****			
1	Mi	25	0,01200	****			
1	Me	15	0,01400	****			
1	Mj	25	0,21200	****			
2	Mj	20	0,60400	****			
1	Mj	15	1,20600	****			
2	Me	20	1,40000	****			
2	Mi	25	1,40000	****			
2	Mi	15	1,80200	****			
1	Me	25	2,00000	****			
2	Mj	25	2,00000	****			
2	Me	10	2,40000	****			

1	Mi	20	2,60000	****			
1	Me	10	2,60400	****			
2	Me	25	3,20000	****			
1	Mi	15	3,40000	****			
2	Mj	10	7,80000		****		
2	Mi	10	10,20000		****	****	
1	Mj	10	11,60000			****	
2	Me	5	13,40000			****	
1	Mi	10	17,80000				****
2	Mj	5	18,00000				****
2	Mi	5	19,00000				****
2	Me	3	19,20000				****
1	Me	5	19,60000				****
1	Mj	5	19,80000				****
1	Mj	3	20,00000				****
1	Mi	5	20,00000				****
1	Mi	3	20,00000				****
2	Mj	3	20,00000				****
1	Me	3	20,00000				****
2	Mi	3	20,00000				****

Table B1b. The number of swollen second-stage juveniles (J2) of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* at different time intervals (days after inoculation: DAI) in roots of a soybean genotype ('DM-5953-RSF') according to a Factorial Analysis of Variance (Statistica 13.3) with means separated at $P \leq 0.05$ according to the Tukey HSD Test.

Tukey HSD test; variable Swollen J2 (Me Mi Mj in Soybean experiment 1 & 2) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between MS = 2,7068, df = 144,00

Interaction:
Species x Experiment x Time interval (DAI), *P*=0.001, F ratio=4.57

Exp	Species	DAI	Swollen J2 Mean	A	B	C	D	E	F	G	H
2	Mi	3	0,01000	****							
2	Mj	3	0,01000	****							
2	Me	20	0,01000	****							

1	Me	3	0,01000	****								
2	Mi	20	0,01200	****								
1	Mi	3	0,01200	****								
2	Mj	20	0,01200	****								
2	Mi	25	0,01400	****								
2	Mj	25	0,01400	****								
1	Mj	3	0,01600	****								
1	Mi	5	0,01600	****								
1	Mj	5	0,21400	****								
1	Me	5	0,60600	****								
1	Mi	25	0,61000	****								
2	Me	3	0,80600	****								
2	Mi	5	1,00400	****								
1	Mj	25	1,04200	****								
1	Me	25	1,40200	****								
2	Me	15	1,40400	****								
2	Mi	15	1,60600	****	****							
2	Me	25	1,80000	****	****	****						
2	Mj	5	2,00400	****	****	****						
1	Mi	10	2,20200	****	****	****	****					
2	Mj	15	2,40000	****	****	****	****	****				
1	Mj	15	2,60400	****	****	****	****	****	****			
1	Mj	20	4,00000	****	****	****	****	****	****			
1	Mi	15	5,40000		****	****	****	****	****	****		
1	Mi	20	5,60000			****	****	****	****	****		
1	Me	15	6,00000				****	****	****	****		
2	Me	5	6,20000					****	****	****		
1	Mj	10	6,20000					****	****	****		
2	Mj	10	6,40000						****	****		
2	Mi	10	6,40000						****	****		
1	Me	20	8,20000							****	****	
2	Me	10	8,80000							****	****	
1	Me	10	10,40000								****	

Table B1c. The number of swollen third-stage juveniles (J3) of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* at different time intervals (days after inoculation: DAI) in roots of a soybean genotype ('DM-5953-RSF') according to a Factorial Analysis of Variance (Statistica 13.3) with means separated at $P \leq 0.05$ according to the Tukey HSD Test.

Tukey HSD test; variable J3 (Me Mi Mj in Soybean experiment 1 & 2) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between MS = 2,0737, df = 144,00

Interaction:
Species x Experiment x Time interval (DAI), *P*=0.001, F ratio=2.15

Exp	Species	DAI	J3 Mean	A	B	C	D	E	F	G	H
1	Me	3	0,010000	****							
2	Mi	25	0,010000	****							
2	Mj	25	0,010000	****							
1	Mi	5	0,012000	****							
2	Mi	3	0,012000	****							
2	Mj	3	0,012000	****							
2	Me	3	0,012000	****							
1	Me	5	0,014000	****							
1	Mj	3	0,014000	****							
2	Mi	5	0,014000	****							
1	Mi	10	0,014000	****							
2	Mj	5	0,014000	****							
1	Mj	5	0,014000	****							
1	Mi	3	0,020000	****							
2	Me	20	0,406000	****	****						
2	Me	5	0,406000	****	****						
2	Mi	20	0,410000	****	****						
2	Mj	20	0,410000	****	****						
1	Me	25	0,806000	****	****	****					
2	Me	25	1,002000	****	****	****	****				
1	Mi	25	1,204000	****	****	****	****	****			
1	Mj	10	2,204000	****	****	****	****	****	****		
2	Mi	10	2,600000	****	****	****	****	****	****	****	
2	Me	15	3,000000	****	****	****	****	****	****	****	****
2	Mj	15	3,002000	****	****	****	****	****	****	****	****

2	Mj	10	3,200000	****	****	****	****	****	****	****	****	
1	Mj	25	3,204000	****	****	****	****	****	****	****	****	
2	Mi	15	3,400000	****	****	****	****	****	****	****	****	
1	Me	20	3,802000		****	****	****	****	****	****	****	
1	Mj	20	4,000000			****	****	****	****	****	****	
1	Mj	15	4,400000				****	****	****	****	****	
2	Me	10	4,600000					****	****	****	****	
1	Mi	20	5,200000						****	****	****	
1	Me	15	5,800000							****	****	
1	Mi	15	6,000000							****	****	
1	Me	10	6,400000								****	

Table B1d. The number of swollen fourth-stage juveniles (J4) of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* at different time intervals (days after inoculation: DAI) in roots of a soybean genotype ('DM-5953-RSF') according to a Factorial Analysis of Variance (Statistica 13.3) with means separated at $P \leq 0.05$ according to the Tukey HSD Test.

Tukey HSD test; variable J4 (Me Mi Mj in Soybean experiment 1 & 2) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between MS = 2,7280, df = 144,00

Interaction:
Species x Experiment x Time interval (DAI), *P*=0.001, F ratio=4.84

Exp	Species	DAI	J4 Mean	1	2	3	4	5	6	7
1	Me	3	0,01000	****						
1	Me	5	0,01000	****						
2	Me	25	0,01000	****						
2	Mj	3	0,01000	****						
2	Mi	3	0,01000	****						
2	Mj	5	0,01200	****						

2	Mi	5	0,01200	****						
1	Mj	10	0,01200	****						
1	Mj	3	0,01400	****						
2	Me	3	0,01400	****						
2	Me	5	0,01400	****						
1	Mi	5	0,01400	****						
1	Mj	5	0,01600	****						
1	Mi	10	0,02000	****						
1	Mi	3	0,02200	****						
1	Me	10	0,61000	****	****					
2	Mi	10	0,80400	****	****					
2	Mj	25	1,20200	****	****					
2	Mj	20	1,40400	****	****					
2	Mj	10	2,00400	****	****	****				
1	Me	25	2,60000	****	****	****				
1	Mi	20	2,80000	****	****	****				
2	Me	20	2,80200	****	****	****				
2	Mi	25	3,00200	****	****	****	****			
1	Me	20	3,20000	****	****	****	****			
1	Mi	15	3,20000	****	****	****	****			
1	Mj	25	3,20000	****	****	****	****			
1	Mi	25	3,60000	****	****	****	****	****		
1	Me	15	4,20000		****	****	****	****		
2	Me	10	4,40000		****	****	****	****		
2	Me	15	5,60000			****	****	****	****	
2	Mi	20	7,00000				****	****	****	****
2	Mi	15	7,60000					****	****	****
1	Mj	20	7,60000					****	****	****
2	Mj	15	9,60000						****	****
1	Mj	15	10,00000							****

Table B1e The number of females of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* at different time intervals (days after inoculation: DAI) in roots of a soybean genotype ('DM-5953-RSF') according to a Factorial Analysis of Variance (Statistica 13.3) with means separated at $P \leq 0.05$ according to the Tukey HSD Test.

Cell No.	Tukey HSD test; variable Female (Me Mi Mj in Soybean experiment 1 & 2) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between MS = 3,2424, df = 144,00 Interaction: Species x Experiment x Time interval (DAI), *P*=0.049, F ratio=1.9									
	Exp	Species	DAI	Female Mean	A	B	C	D	E	F
8	1	Mi	5	0,01200	****					
15	1	Mj	10	0,01200	****					
20	2	Me	5	0,01200	****					
13	1	Mj	3	0,01400	****					
3	1	Me	10	0,01400	****					
21	2	Me	10	0,01400	****					
19	2	Me	3	0,01400	****					
2	1	Me	5	0,01400	****					
27	2	Mi	10	0,01600	****					
32	2	Mj	5	0,01600	****					
26	2	Mi	5	0,01600	****					
33	2	Mj	10	0,01600	****					
7	1	Mi	3	0,01600	****					
1	1	Me	3	0,01800	****					
31	2	Mj	3	0,02200	****					
25	2	Mi	3	0,02200	****					
9	1	Mi	10	0,02600	****					
14	1	Mj	5	0,04800	****					
16	1	Mj	15	1,40600	****	****				
10	1	Mi	15	2,00200	****	****				
17	1	Mj	20	2,60200	****	****				
11	1	Mi	20	3,80000	****	****				
4	1	Me	15	4,00400	****	****				
34	2	Mj	15	5,00000		****				
5	1	Me	20	5,00000		****				

28	2	Mi	15	5,40000		****				
22	2	Me	15	10,00000			****			
18	1	Mj	25	12,40000			****	****		
29	2	Mi	20	12,60000			****	****	****	
6	1	Me	25	13,20000			****	****	****	
24	2	Me	25	14,00000			****	****	****	****
12	1	Mi	25	14,60000				****	****	****
23	2	Me	20	15,60000				****	****	****
30	2	Mi	25	15,60000				****	****	****
36	2	Mj	25	16,80000					****	****
35	2	Mj	20	17,60000						****

Addendum 1C: Factorial tables for the different life stages of three *Meloidogyne* species isolated from tomato roots for various time intervals (days after inoculation: DAI)

Table C1a. The number of motile second-stage juveniles (J2) of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* at different time intervals (days after inoculation: DAI) in roots of tomato genotype Moneymaker according to a three-way Factorial Analysis of Variance (Statistica 13.3) with means separated at $P \leq 0.05$ according to the Tukey HSD Test.

Tukey HSD test; variable Motile J2 (1 and 2 in Tomato experiment 1 & exp 2 together) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between MS = 3,8512, df = 144,00

Interaction:
Species x Experiment x Time interval (DAI), *P*=0.134, F ratio=1.5

Exp	Species	DAI	Motile J2 Mean	A	B	C	D	E	F
2	Mi	20	0,01000	****					
2	Me	10	0,01000	****					
1	Me	10	0,01200	****					
1	Me	15	0,01200	****					
1	Mi	20	0,01200	****					
1	Me	25	0,01200	****					
2	Mj	15	0,01200	****					

1	Mi	15	0,01200	****					
1	Mj	15	0,01200	****					
2	Me	15	0,01400	****					
2	Mi	15	0,80400	****					
2	Mj	20	0,80600	****					
2	Mj	25	1,00400	****					
1	Mi	25	1,20200	****					
2	Mj	10	1,20600	****					
2	Me	20	1,40000	****					
2	Mi	25	1,60000	****					
1	Me	20	1,60400	****					
1	Mi	10	2,00400	****					
1	Mj	10	2,40400	****					
1	Mj	25	2,60000	****					
2	Mi	10	2,80000	****					
1	Mj	20	2,80600	****					
2	Me	25	3,20000	****					
1	Me	5	9,00000		****				
2	Me	5	11,00000		****	****			
1	Mi	5	11,40000		****	****	****		
2	Mj	5	13,20000		****	****	****		
2	Mi	5	14,40000			****	****	****	
1	Mj	5	15,40000			****	****	****	****
2	Me	3	16,00000				****	****	****
1	Me	3	18,60000					****	****
1	Mj	3	20,00000						****
1	Mi	3	20,00000						****
2	Mi	3	20,00000						****
2	Mj	3	20,00000						****

Table C1b. The number of swollen second-stage juveniles (J2) of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* at different time intervals (days after inoculation: DAI) in roots of tomato genotype Moneymaker according to a three-way Factorial Analysis of Variance (Statistica 13.3) with means separated at $P \leq 0.05$ according to the Tukey HSD Test.

Tukey HSD test; variable Swollen J2 (1 and 2 in Tomato experiment 1 & exp 2 together) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between MS = 3,9969, df = 144,00

Interaction:
Species x Experiment x Time interval (DAI), *P*=0.001, F ratio=4.8

Exp	Species	DAI	Swollen J2 Mean	A	B	C	D	E	F	G
2	Mi	3	0,01000	****						
2	Mj	20	0,01200	****						
2	Mj	15	0,01200	****						
1	Me	15	0,01200	****						
1	Mj	3	0,01200	****						
1	Me	25	0,01200	****						
1	Mi	3	0,01200	****						
1	Mj	20	0,01200	****						
2	Mj	3	0,01200	****						
1	Mi	20	0,01200	****						
2	Me	20	0,01200	****						
2	Me	15	0,01200	****						
2	Mi	20	0,01200	****						
2	Mi	25	0,01400	****						
1	Mi	25	0,40820	****	****					
2	Me	25	0,60800	****	****					
2	Mi	15	0,80200	****	****					
1	Me	20	0,80400	****	****					
1	Mj	15	1,00000	****	****					
1	Mj	25	1,20600	****	****	****				
1	Mi	15	1,40000	****	****	****				
1	Me	3	1,40200	****	****	****				
2	Mj	25	1,60820	****	****	****				
1	Me	10	2,40000	****	****	****	****			
2	Me	3	2,80000	****	****	****	****			
1	Mj	5	4,60800	****	****	****	****	****		
2	Mi	5	4,80000	****	****	****	****	****		
1	Mi	10	5,20000		****	****	****	****		

2	Me	5	5,20000		****	****	****	****		
2	Me	10	6,00000			****	****	****	****	
2	Mj	5	6,60800				****	****	****	
1	Mi	5	8,60000					****	****	****
1	Me	5	10,20000						****	****
2	Mj	10	10,20000						****	****
2	Mi	10	11,80000							****
1	Mj	10	13,00000							****

Table C1c. The number of swollen third-stage juveniles (J3) of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* at different time intervals (days after inoculation: DAI) in roots of tomato genotype Moneymaker according to a three-way Factorial Analysis of Variance (Statistica 13.3) with means separated at $P \leq 0.05$ according to the Tukey HSD Test.

Tukey HSD test; variable J3 (1 and 2 in Tomato experiment 1 & exp 2 together) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between MS = 2,6510, df = 144,00

Interaction:
Species x Experiment x Time interval (DAI), *P*=0.001, F ratio=7.6

Exp	Species	DAI	J3 Mean	A	B	C	D	E	F
2	Mi	25	0,01000	****					
2	Me	20	0,01000	****					
2	Mj	3	0,01200	****					
2	Mj	5	0,01200	****					
2	Mi	3	0,01200	****					
1	Me	25	0,01200	****					
1	Mi	3	0,01200	****					
1	Mi	5	0,01200	****					
1	Me	3	0,01200	****					
2	Mj	25	0,01200	****					
1	Mj	5	0,01200	****					
1	Mi	25	0,01200	****					
1	Mj	3	0,01200	****					
2	Mj	20	0,21000	****					
2	Me	25	0,21000	****					

1	Me	5	0,80800	****	****				
2	Mi	5	0,81000	****	****				
1	Mi	20	1,01000	****	****	****			
2	Me	3	1,20400	****	****	****			
1	Me	15	1,20600	****	****	****			
1	Mj	25	1,20600	****	****	****			
1	Me	20	1,40000	****	****	****	****		
2	Mi	20	1,40600	****	****	****	****		
2	Me	15	1,60000	****	****	****	****		
1	Mj	20	1,60800	****	****	****	****		
2	Mj	15	1,80000	****	****	****	****		
1	Mj	15	1,80000	****	****	****	****		
1	Mi	15	3,00800	****	****	****	****		
2	Me	5	3,40000	****	****	****	****	****	
1	Mj	10	3,40400	****	****	****	****	****	
2	Mi	10	4,20000		****	****	****	****	
2	Me	10	4,80000			****	****	****	
2	Mi	15	5,20000				****	****	
2	Mj	10	7,00000					****	
1	Me	10	11,20000						****
1	Mi	10	11,40000						****

Table C1d. The number of swollen fourth-stage juveniles (J4) of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* at different time intervals (days after inoculation: DAI) in roots of tomato genotype Moneymaker according to a three-way Factorial Analysis of Variance (Statistica 13.3) with means separated at $P \leq 0.05$ according to the Tukey HSD Test.

Tukey HSD test; variable J4 (1 and 2 in Tomato experiment 1 & exp 2 together) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between MS = 2,8062, df = 144,00

Interaction:
Species x Experiment x Time interval (DAI), *P*=0.031, F ratio=2.1

Exp	Species	DAI	J4 Mean	A	B	C	D	E	F	G	G	I
2	Mi	3	0,01000	****								
2	Me	3	0,01000	****								

2	Mj	3	0,01200	****								
2	Mi	5	0,01200	****								
2	Mj	5	0,01200	****								
1	Me	5	0,01200	****								
1	Mi	3	0,01200	****								
1	Mi	5	0,01200	****								
1	Me	3	0,01200	****								
1	Mj	5	0,01200	****								
1	Mj	3	0,01200	****								
2	Mi	25	0,01400	****								
1	Mj	25	0,40800	****	****							
2	Me	25	0,40800	****	****							
2	Me	5	0,40800	****	****							
2	Mi	10	1,20000	****	****	****						
2	Mj	20	1,20200	****	****	****						
1	Mj	10	1,20800	****	****	****						
1	Mi	10	1,40400	****	****	****	****					
2	Mj	10	1,40400	****	****	****	****					
1	Mi	25	1,60800	****	****	****	****	****				
2	Mj	25	1,60800	****	****	****	****	****				
1	Mj	20	1,80000	****	****	****	****	****				
1	Me	25	2,00600	****	****	****	****	****				
1	Me	20	2,60000	****	****	****	****	****	****			
1	Me	15	3,80000	****	****	****	****	****	****			
1	Mi	20	4,40000		****	****	****	****	****			
2	Mi	20	5,00000			****	****	****	****	****		
1	Mj	15	5,40000				****	****	****	****	****	
2	Mj	15	5,60000					****	****	****	****	
2	Me	20	5,60000					****	****	****	****	
1	Me	10	6,40000						****	****	****	
2	Me	15	8,60000							****	****	****
2	Me	10	9,20000								****	****
2	Mi	15	11,40000									****
1	Mi	15	12,00000									****

Table C1e. The number of females of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* at different time intervals (days after inoculation: DAI) in roots of tomato genotype Moneymaker according to a three-way Factorial Analysis of Variance (Statistica 13.3) with means separated at $P \leq 0.05$ according to the Tukey HSD Test.

Tukey HSD test; variable Female (1 and 2 in Tomato experiment 1 & exp 2 together) Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search) Error: Between MS = 4,3417, df = 144,00

Interaction:
Species x Experiment x Time interval (DAI), *P*=0.128, F ratio=1.5

Exp	Species	DAI	Female Mean	A	B	C	D	E
2	Me	10	0,01000	****				
1	Me	5	0,01200	****				
1	Me	10	0,01200	****				
2	Mj	10	0,01200	****				
2	Mj	5	0,01200	****				
2	Mj	3	0,01200	****				
1	Mi	3	0,01200	****				
1	Mi	5	0,01200	****				
1	Mi	10	0,01200	****				
1	Mj	10	0,01200	****				
1	Mj	5	0,01200	****				
1	Me	3	0,01200	****				
1	Mj	3	0,01200	****				
2	Me	5	0,01400	****				
2	Mi	10	0,01600	****				
2	Mi	5	0,01600	****				
2	Me	3	0,01600	****				
2	Mi	3	0,02200	****				
2	Mi	15	1,80000	****				
1	Mi	15	4,00000	****				
2	Me	15	9,80000		****			
1	Mj	15	11,80000		****	****		
2	Mj	15	12,60000		****	****		
2	Me	20	13,00000		****	****	****	

1	Me	20	13,60000		****	****	****	****
2	Mi	20	13,60000		****	****	****	****
1	Mj	20	13,80000		****	****	****	****
1	Mj	25	14,60000		****	****	****	****
1	Mi	20	14,60000		****	****	****	****
1	Me	15	15,00000			****	****	****
2	Mj	25	15,00000			****	****	****
2	Me	25	15,60000			****	****	****
1	Mi	25	16,80000			****	****	****
2	Mj	20	17,80000				****	****
1	Me	25	18,00000				****	****
2	Mi	25	18,40000					****

ADDENDUM B

DECLARATION OF LANGUAGE EDITING

Language editing statement

TO WHOM THIS MAY CONCERN

I, Prof. Hendrika (Driekie) Fourie, hereby declare that the thesis titled: **"A comparison of the development and reproduction potential of *Meloidogyne enterolobii* and other thermophilic South African *Meloidogyne* species"** by R L Collett has been edited for language correctness and spelling by the supervisors. No changes were made to the academic content or structure of this work.

21 May 2020

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ADDENDUM C

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A comparative study of the development and reproduction of *Meloidogyne enterolobii* and other thermophilic South African *Meloidogyne* species

RL Collett

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Dissertation submitted in fulfilment of the requirements for the degree *Master of Science in Environmental Sciences with Integrated Pest Management* at the North-West University

Supervisor: Prof H Fourie
Co-supervisor: Dr M Marais
Co-supervisor: Dr MB Daneel

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ETHICS APPROVAL LETTER OF STUDY

Based on the review by the **Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC)**, the Committee hereby clears your study as no ethical risk. This implies that the FNASREC grants permission that, provided the general conditions specified below are met, the study may be initiated, using the ethics number below.

Study title: A comparative study of the development and reproduction of Meloidogyne enterolobii and other thermophilic South African Meloidogyne species
Study Leader/Supervisor: Prof D Fourie
Student: RL Collett

Ethics number: NWU-01341-20-A9
(Institution | Study Number | Year | Status)

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application type: Single **Risk Category:** No Risk

Commencement date: 01/02/2020
Expiry date: 01/04/2021

General conditions:
The following general terms and conditions apply:

- *The commencement date indicates the date when the study may be started.*
- *In the interest of ethical responsibility, the NWU-SCRE and FNASREC reserves the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;*
  - *withdraw or postpone approval if:*
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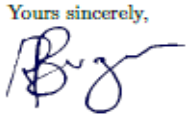
    - *it becomes apparent that any relevant information was withheld from the FNASREC or that information has been false or misrepresented;*
    - *submission of the annual (or otherwise stipulated) monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and / or*
    - *new institutional rules, national legislation or international conventions deem it necessary.*
- *FNASREC can be contacted for further information or any report templates via Roelof.Burger@nwu.ac.za 018 299 4269*

The FNASREC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the FNASREC or the NWU-SCRE for any further enquiries or requests for assistance.

Yours sincerely,



Prof Roelof Burger
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File reference: 9.1.5.4.2

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