RESEARCH/INVESTIGACIÓN

DISTRIBUTION OF *MELOIDOGYNE* SPECIES (TYLENCHIDA: MELOIDOGYNIDAE) IN TOMATO CROP IN SINALOA, MEXICO

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ABSTRACT

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Root-knot nematodes (RKN; *Meloidogyne* spp.) are one of the main constraints of tomato (*Solanum lycopersicum*) crops worldwide, and Sinaloa is one of the main producers of tomatoes in Mexico. There is little information on the distribution, prevalence, and incidence of RKN available in this crop and region. Identifying RKN species and estimating these epidemiology factors in Sinaloa are important for the design of specific strategies for RKN control. A total of 278 RKN samples from Los Mochis, Culiacan, La Cruz, and Escuinapa areas of Sinaloa were collected from tomato plants at production stage under greenhouse conditions to analyze the distribution, prevalence, and incidence of RKN. All samples were subsequently identified with morphological features of females and PCR amplification with species specific-primers. RKN were found in 100% of the surveyed greenhouses of tomato, confirming their widespread distribution and prevalence in this crop along the Sinaloa state. Based on molecular methods, *M. enterolobii* (*Me*) and *M. incognita* (*Mi*) were identified in the four regions. Incidence of *Me* was significantly greater than that of *Mi* and mixed infection (*Me*+*Mi*) occurred with 74.6% having *Me*, 16.8% having *Mi,* and 8.6% with both species of positive samples from all those analyzed. This information will enable tomato producers of this region to design and implement an appropriate control strategy for these RKN species.

Key words: Incidence, *Meloidogyne enterolobii*, *Meloidogyne incognita*, prevalence, *Solanum lycopersicum*

RESUMEN

Carrillo-Fasio, J. A., J. A. Martínez-Gallardo, R. Allende-Molar, S. Velarde-Félix, C. E. Romero-Higareda, y J. E. Retes-Manjarrez. 2019. Distribución de la especie *Meloidogyne* (Tylenchida: Meloidogynidae) en el cultivo de tomate en Sinaloa, México. Nematropica 49:71-82.

Los nematodos de los nudos de la raíz (RKN; *Meloidogyne* spp.) Son una de las principales limitaciones de los cultivos de tomate (*Solanum lycopersicum*) en todo el mundo y Sinaloa es uno de los principales productores de tomates en México. Hay poca información sobre la distribución, prevalencia e incidencia de RKN disponible en este cultivo y región. La identificación de especies de RKN y la estimación de estos factores epidemiológicos en Sinaloa son importantes para el diseño de estrategias específicas para el control de RKN. Un total de 278 muestras de RKN de las áreas de Los Mochis, Culiacán, La Cruz y Escuinapa de Sinaloa se recolectaron de plantas de tomate en la etapa de producción en condiciones de invernadero para analizar la distribución, prevalencia e incidencia de RKN. Todas las muestras se identificaron posteriormente con las características morfológicas de las hembras y la amplificación por PCR con cebadores específicos de especies. RKN se encontró en el 100% de los invernaderos de tomate encuestados, lo que confirma su amplia distribución y prevalencia en este cultivo a lo largo del estado de Sinaloa. Sobre la base de métodos moleculares, se identificaron *M. enterolobii* (*Me*) y *M. incognita* (*Mi*) en las cuatro regiones. La incidencia de *Me* fue significativamente mayor que la de *Mi* y la infección mixta (*Me* + *Mi*) ocurrió en 74,6% con *Me*, 16,8% con *Mi* y 8,6% con ambas especies de muestras positivas de todos los analizados. Esta información permitirá a los productores de tomate de esta región diseñar e implementar una estrategia de control adecuada para estas especies RKN.

Palabras clave: Incidencia, *Meloidogyne enterolobii*, *Meloidogyne incognita*, prevalencia, *Solanum lycopersicum*

INTRODUCTION

Mexico is one of the main producers of tomatoes (*Solanum lycopersicum* L.) worldwide, with 93.376 ha cultivated in 2016, representing about 21% of the total area cultivated in the Americas (FAO, 2018). The state of Sinaloa is one of the main producers of tomatoes in Mexico, where production reached 15,263 ha in 2016, (SAGARPA, 2018). Therefore, any limitation in productivity of these crops is of concern.

Meloidogyne spp. is the most important genus of plant-parasitic nematodes that affect vegetable crops worldwide (Sasser and Freckman, 1987; Cid del Prado *et al*., 2001). Even though approximately 100 nominal *Meloidogyne* species are described to date (Karsen, 2002; Perry *et al*., 2009; Wesemahel *et al*., 2011), the vast majority of research has focused on just four species, which have commonly been referred to as 'major' species, *M. arenaria*, *M. hapla*, *M. incognita*, and *M. javanica* (Taylor *et al*., 1982).

Among major species of root-knot nematodes (RKN), *M. incognita* has been the most studied worldwide (Trudgill *et al*., 2001; Abad *et al*., 2008). It was first described from carrots originating from Texas, USA in 1949 (Chitwood, 1949). In the last 20 years, *M. enterolobii*, first described from roots of Pacara Earpod trees (*Enterolobium contortisiliquum*) on Hainan Island in China (Yang and Eisenback, 1983) (syn. *M. mayaguensis*) (Karssen *et al*., 2012) has become more important worldwide for its high aggressiveness, increasing geographical distribution, wide host range, and pathogenicity on plants carrying resistance genes toward tropical RKN (Brito *et al*., 2007; Castagnone-Sereno, 2012). These genes confer resistance to *M. incognita*, *M. arenaria*, and *M. javanica* in tomato and pepper cultivars, but are ineffective against *M. enterolobii* in both crops.

In Mexico, six *Meloidogyne* species (*M. incognita*, *M. arenaria*, *M. javanica*, *M. hapla*, *M. chitwoodi*, and *M. enterolobii*) have been reported to attack Solanaceous crops in different states (Esbenchade and Triantaphyllou, 1985; Carrillo-Fasio *et al*., 2000; Cid del Prado *et al*., 2001; Martínez-Gallardo *et al*., 2015; Villar-Luna *et al*., 2016). In Sinaloa Mexico, only *M. incognita*, *M. arenaria*, *M. javanica*, and *M. enterolobii* have been reported to attack tomato plants (Carrillo-Fasio *et al*., 2000; Cid del Prado *et al*., 2001; Martínez-Gallardo *et al*., 2015). However, there have been no studies of incidence of these RKN species in Mexico to support investment decisions for research projects related to the development of control strategies.

The first documented occurrence and distribution of RKN in Sinaloa, Mexico was in 2000, when *M. incognita*, *M. javanica* and *M. arenaria* were reported based on morphological diagnosis through perineal pattern morphology

(Carrillo-Fasio *et al*., 2000). The results were confirmed by a survey undertaken in 2001 through the same methodology (Cid del Prado *et al*., 2001). Then in 2015 and 2016, *M. enterolobii* was first reported attacking tomato and pepper plants in this state by Martínez-Gallardo *et al.* (2015) and Villar-Luna *et al*. (2016), respectively. Given the fluid nature of RKN epidemics across Sinaloa, Mexico and other parts of the world, periodic monitoring and reevaluation of the status of RKN are necessary in order to document the species diversity of RKN in the Sinaloa region of Mexico.

In greenhouse-produced agriculture crops, the warm and humid environment, the high plant density, and monoculture are conducive to the establishment and spread of RKN. Most of these plant-parasitic nematodes can be controlled by solarization, irrigation control, nematicides, or the use of resistant varieties (Salazar-Antón and Guzmán-Hernández, 2013). Varieties of tomato carrying the Mi-1 gene against *M. incognita*, *M. javanica,* and *M. arenaria* have been extensively used in the Sinaloa region and worldwide since the 1990s (Williamson, 1998; Barbary *et al*., 2015). Nevertheless, when a new nematode emerges, it becomes very difficult to find a control solution if the pathogen is not properly identified. Adequate nematode control requires proper species identification, especially in the case of quarantine pests. However, the examples of *M. fallax*, which was thought to be *M. chitwoodi*, and *M. paranaensis* and *M. enterolobii*, both of which were thought to be *M. incognita* (Yang and Eisenback, 1983; van Meggelen *et al*., 1994; Carneiro *et al*., 1996) clearly show that much research needs to be done with identification in the genus. Classic techniques to confirm the identity of a *Meloidogyne* species are based on morphology and requires expertise that is often lacking.

Molecular biology techniques used in nematode diagnostics are usually based on nucleic acid studies. Most, particularly the DNA-based, are known to be robust, sensitive, specific and reliable in detecting and distinguishing various *Meloidogyne* spp. (Powers *et al*., 2005; Berry *et al.*, 2007). Molecular methods have been employed to accurately identify various *Meloidogyne* spp. (Hu *et al*., 2011). Polymerase chain reaction amplifications using species-specific primers derived from ribosomal DNA (rDNA) and regions (IGS) are useful to distinguish species (Zijlstra *et al*., 2000; Long *et al*., 2006; Hu *et al*., 2011).

Updated epidemiological information regarding *Meloidogyne* species in Sinaloa, Mexico, is lacking. The objectives of the present study were to analyze the distribution, prevalence, and incidence of the RKN species present in tomato crops in the four main tomato regions of Sinaloa, Mexico. Based on previous studies performed in tomato crop in Sinaloa, Mexico, we hypothesize that the prevalent species of RKN in this region of Mexico include *M. incognita*, *M. javanica*, and *M. arenaria*.

MATERIALS AND METHODS

Survey and sampling

The survey was conducted in tomato greenhouse production sites during the 2016/2017 growing season. A total of 278 samples from 45 tomato locations in the main four horticulture areas (Los Mochis, Culiacan, La Cruz, and Escuinapa) of Sinaloa, Mexico, were surveyed and sampled at fructification stage because these areas represent 90% of the tomato crop of this state (Fig. 1). Samples were stored at 4°C until nematode extraction.

Extraction and processing of nematodes

To identify the *Meloidogyne* species of the samples collected, females were dissected from galls of the roots with a needle using a stereo-microscope at 12× magnification. Females were rinsed with 1% NaOCl for 10 min and then with sterilized tap water and stored at 4°C before molecular and morphological identification. The frequency of incidence of the nematode species was calculated for each location.

Morphological identification

Perineal patterns of ten adult females per sample were used to discriminate among *Meloidogyne* species. Only this feature was determined because it is known to discriminate among these species (Sasser, 1954; Triantaphyllou and Sasser, 1960). Perineal patterns were prepared according to Riggs (1990). Observations and measurements of preparations were conducted

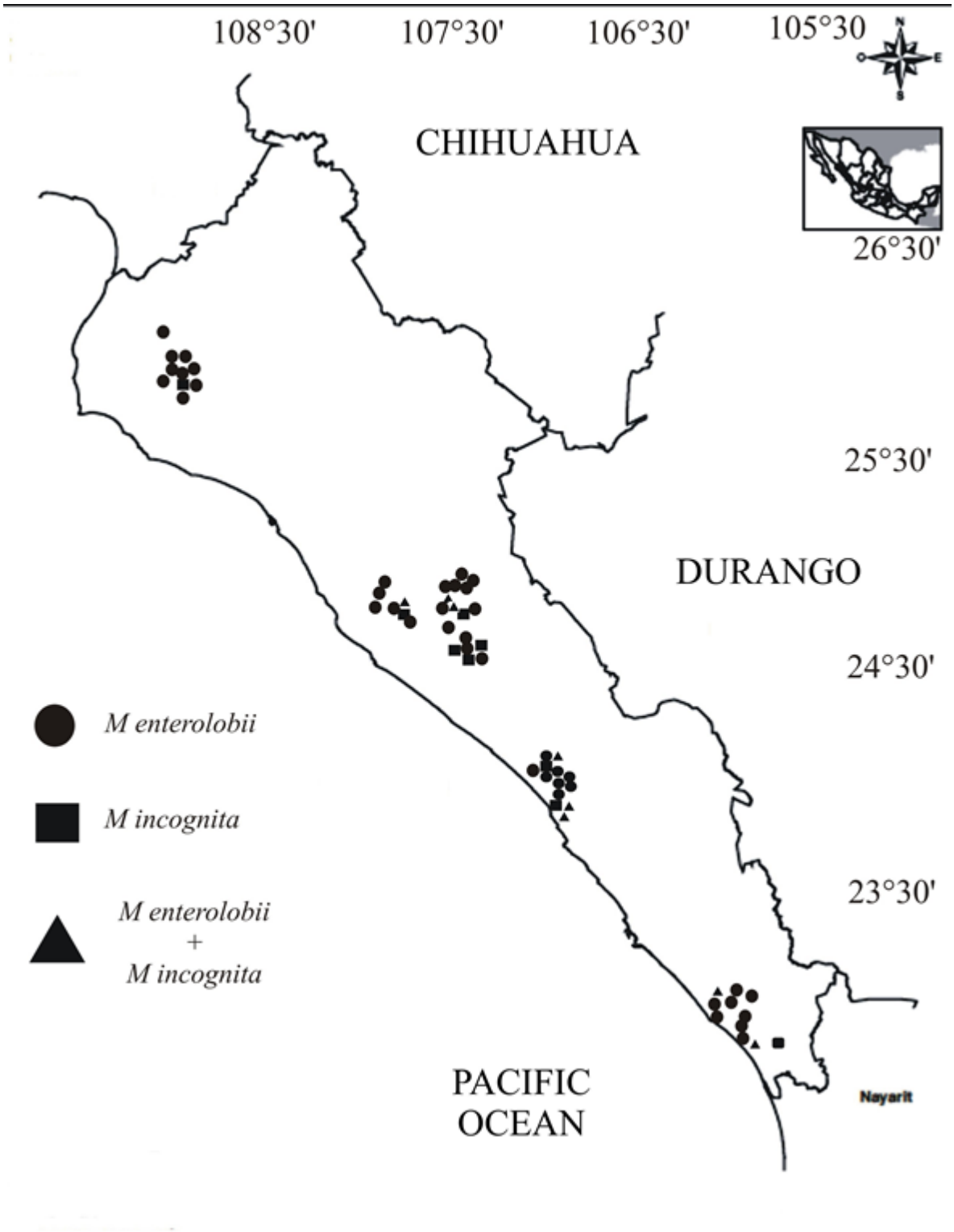

Figure 1. Distribution of *Meloidogyne* spp. identified from tomato production sites in Sinaloa, Mexico during the 2016/2017 growing season.

with a phase contrast microscope equipped with a digital camera. Morphological and morphometric identification was performed according to the original description of *M. enterolobii* (Yang and Eisenback, 1983; Rammah and Hirschmann, 1988) based on perineal pattern morphology and head region of adult females (EPPO, 2011).

Molecular identification

Roots of plants infected with *Meloidogyne* spp. were collected, flushed with water, and then washed with 0.52% NaOCl for 10 min. Ten individual galls with single egg mass of *Meloidogyne* were used for DNA extraction according to the methodology described by Hu *et al*. (2011).

To corroborate the identification of the *Meloidogyne* species, end point PCR was used according to the primers, reactions, and conditions described by Hu *et al.* (2011). *M. incognita*-specific primers (Mi-F 5′-GTGAGGATTCAGCTCCCCAG-3′ and Mi-R 5′-ACGAGGAACATACTTCTCCGTCC-3′) *M. javanica*-specific primers (Fjav 5′-GGTGCGCGATTGAACTGAGC-3′ and Rjav 5′-CAGGCCCTTCAGTGGAACTATAC-3′) and *M. arenaria* (F: Ma TCGAGGGCATCTAATAAAGG and R: Ma GGGCTGAATATTCAAAGGAA) that amplify SCAR markers as described by Zijlstra *et al*. (2000) were used. The rDNA-IGS2 internal primers (Me-F 5′-AACTTTTGTGAAAGTGCCGCTG-3′ and Me-R 5′-TCAGTTCAGGCAGGATCAACC-3′) described by Long *et al*. (2006) were used to amplify a specific sequence from *M. enterolobii*. *M. hapla* (F: Mha 5′-TCGAGGGCATCTAATAAAGG-3′ and R: 5′-Mha GGGCTGAATATTCAAAGGAA-3′) (Hu *et al*., 2011). *M. chitwoodi*-specific primers (JMV1 5′-GGATGGCGTGCTTTCAAC-3′ and JMV2 5′-TTTCCCCTTATGATGTTTACCC-3′) that amplify SCAR markers were designed by Wishart *et al*. (2002).

Data collected

The survey consisted of estimating the prevalence and incidence of the RKN problematic for each tomato area (Los Mochis, Culiacan, La Cruz, and Escuinapa), where samples were collected. The frequency of occurrence (prevalence = number of sites with RKN/total number of sites surveyed) and incidence of RKN species (number of samples positive to each species of RKN/total number of samples) were calculated for each site (Imren *et al*., 2016). Data from the distribution of RKN and symptom severity within each site were also gathered. Distribution of RKN within each location was assessed by monitoring RKN incidence in terms of percentage of the total production area affected. Disease severity was rated on a scale of 1 to 9, where: 1 = 0% of roots with galls; 3 = 25% of roots with small galls; 5 = 50% of roots with small galls; 7 = 75% of roots with large and extensive galling; and 9 = 100% of roots with large galls and rotten roots.

Data analysis

Data obtained were subjected to non-parametric variance analysis with the Kruskal-Wallis and Dunn median test to determine the significant difference among the variables ($P \leq 0.05$). All statistical analyses were performed with the SAS software (SAS, 1999).

RESULTS

Morphological identification of RKN

The morphological and morphometric characteristics of the mature females of RKN examined in this research were similar to those reported in the original descriptions of *M. enterolobii* and *M. incognita* (Chitwood, 1949; Eisenback *et al*., 1981; Yang and Eisenback, 1983; Rammah and Hirschmann, 1988). Females of *M. enterolobii* showed cuticular annulations, clear lateral fields, pyriform shape, and variable size. The distance of head to excretory pore ranges from 3.6-4.7 µm. The stylet was 13.2-17.8 µm long. The perineal pattern of *M. enterolobii* was variable, although some of the features were useful to discriminate among *Meloidogyn*e species (Fig. 2). Adult females showed a perineal pattern of oval shape, dorsal arch usually high and round, weak lateral lines sometimes present, large phasmids, typical characters of *M. enterolobii* (Fig. 2). On the other hand, *M. incognita* showed two annuls

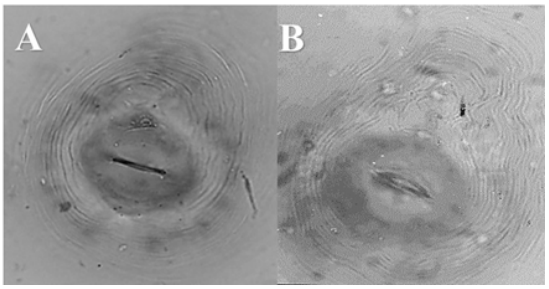

Figure 2. Perineal patterns of: A) *M. incognita* and B) *M. enterolobii*, obtained from females extracted from roots of tomato plants in Sinaloa Mexico.

behind the head cap and the anterior region of the stylet with rounded end, basal knobs rounded, and very short distance from the base of the knobs to the dorsal esophageal gland. The perineal patterns of *M. incognita* were variable in shape, although some of the features were useful to discriminate among *Meloidogyne* species (Fig. 2). Adult females showed a perineal pattern of oval shape, dorsal arch usually high formed by smooth and wavy striae, without lateral lines.

Molecular identification of RKN

PCR amplification was positive for all the individual egg mass samples analyzed for at least one set of the species specific primers, *Me*-F/*Me*-R and *Mi*-F/*Mi*-R, used in this study produced a 256 bp and a 1000 bp product for *M. enterolobii* and *M. incognita*, respectively. In both cases, a single fragment was observed (Figs. 3 and 4). On the other hand, the PCR amplification was negative in all the 278 samples analyzed for the specific primers for *M. arenaria*, *M. javanica*, *M. hapla*, and *M. chitwoodi*.

Prevalence of RKN population

Absolute presence of RKN was found in all the tomato sites surveyed in the four main regions of Sinaloa. *M. enterolobii* species was the most prevalent in all the four regions surveyed followed by *M. incognita* and mixed infestations of both *Me* and *Mi* having infestation percentages of 92.9, 57.1, and 46.4%, respectively (Table 1 and Fig. 1). There were significant differences among the distribution of the *Meloidogyne* species found in the sampled fields (P = 0.0001) (Table 2). *M. enterolobii* was the most prevalent species among the 45 locations followed by *M. incognita* and the mixed infection with both species that averaged 71.4, 56.0, and 46.2% (Table 2).

Incidence of RKN population

M. enterolobii was the most abundant species in all tomato areas sampled with incidence in

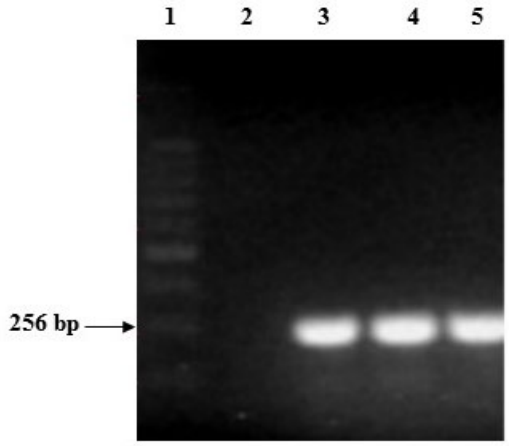

Figure 3. PCR products obtained from genomic DNA of isolates of *Meloidogyne enterolobii* with the specific primers pair *Me*-F and Me-R, viewed on a 1.0% agarose gel. Lane 1 = 1kb Plus; lane 2 = negative control; lane 3, 4 and 5 = isolates from galled tomato plants.

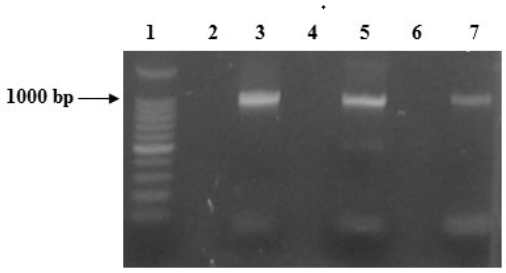

Figure 4. PCR products obtained from genomic DNA of isolates of *Meloidogyne incognita* with the specific primers pair *Mi*-F and *Mi*-R, viewed on a 1.0% agarose gel. Lane1 = 1kb Plus; lane 2, 4 and 6 = negative control; lane 3, 5 and 7 = isolates from galled tomato plants.

Table 1. Prevalence and incidence of single and combined populations of *Meloidogyne enterolobii* (*Me*) and *M. incognita* (*Mi*) in 278 samples taken from 45 greenhouse tomato production sites in four regions of Sinaloa, Mexico.

Area[z]	No. fields surveyed	No. samples collected	Prevalance[x] *Me*	Prevalance *Mi*	Prevalence *Me+Mi*	Incidence[y] *Me*	Incidence *Mi*	Incidence *Me+Mi*
CU	12	90	94.7	36.8	42.1	79.4	10.3	10.3
ES	7	13	85.7	42.9	62.5	69.2	30.8	7.5
LC	13	90	85.7	78.6	57.1	60.5	27.7	11.8
LM	13	91	100.0	68.8	0.0	84.3	8.2	0.0
Total	45	284	92.9	57.1	46.4	74.6	16.8	8.6

[x] Prevalence is the number of sites with RKN/total number of sites surveyed.
[y] Incidence is the number of samples positive to each species of RKN/total number of samples.
[z] CU = Culiacan, ES = Escuinapa, LC = La Cruz, LM = Los Mochis.

74.6% of positive samples (Table 1). *M. incognita* incidence was lower in all the areas sampled with detection in 16.8% of positive samples (Table 1). Incidence of mixed infection with *M. enterolobii* plus *M. incognita* occurred in 8.6% of the total samples (Table 1).

Distribution of RKN

All 45 locations from the four main horticulture areas (Los Mochis, Culiacan, La Cruz, and Escuinapa) of Sinaloa, Mexico, surveyed had different levels of RKN damage in their tomato crops. Growers surveyed in the 2016 season reported an average 67.1% damage from RKN (Table 2). We found significant differences in the distribution of RKN-problematic areas among the 45 production sites in the four horticultural areas mentioned above (>1% level) (Table 2). Los Mochis was the most problematic area followed by Culiacan, Escuinapa, and La Cruz with 88.6, 75.1, 61.8, and 42.8% incidence, respectively (Table 2). Tomato growers reported a wide distribution of RKN problematic areas within their growing region with an average distribution of 63.5% (Table 2).

Symptoms of RKN

All the 278 tomato root samples taken from the 45 location areas showed galls, although the severity and quantity varied significantly among the roots from the four areas sampled (>1% level) (Table 2). The Culiacan area had the most severe damage by RKN followed by Los Mochis, Escuinapa, and La Cruz with 8.2, 8.0, 7.8 and 6.2, respectively (Table 2).

Regarding the severity of symptoms of RKN species, we found significant differences among the gall symptoms caused by *M. enterolobii* and *M. incognita* single and mixed infections (H = 50.85; df = 2; P = 0.0001) (Table 2). *M. enterolobii* generated the greatest quantity and size of galls per root system, followed by *M. incognita* and the mixed infection with both species causing galling symptoms that averaged 7.8, 6.5, and 6.1, respectively (Table 2).

DISCUSSION

Meloidogyne spp. has a significant impact on tomato production worldwide, including Mexico. Most of the time, these RKN outbreaks result in losses through a reduction in growth, yield, and fruit quality (Sasser and Freckman, 1987; Cid del Prado *et al*., 2001; Karssen, 2002; Perry *et al*., 2009; Wesemael *et al*., 2011). Historically, *M. enterolobii* has been referred to as a minor species. During the last 20 years, *Me* has become more important worldwide because of its aggressiveness, increasing geographical distribution, wide host range and pathogenicity on plants carrying resistance genes (Brito *et al*., 2007; Castagnone-Sereno, 2012; Elling, 2013).

From the six *Meloidogyne* species (*M. incognita*, *M. arenaria*, *M. javanica*, *M. hapla*, *M. chitwoodi*, and *M. enterolobii*) that have been reported in Mexico, only *M. incognita*, *M. arenaria*, *M. javanica*, and *M. enterolobii* have been reported parasitizing tomato (Carrillo-Fasio

Table 2. Distribution and severity of *Meloidogyne enterolobii* (*Me*) and *M. incognita* (*Mi*) from 45 tomato production sites in the main four production regions of Sinaloa, Mexico.

Area or *Meloidogyne* spp.	Distribution[x]	Severity[y]
Culiacan	75.1 b [z]	8.2 a
Los Mochis	88.6 a	8.0 a
Escuinapa	61.8 c	7.8 a
La Cruz	42.8 d	6.2 b
M. enterolobii	71.4 a	7.8 a
M. incognita	56.0 b	6.5 b
Me + *Mi*	46.2 b	6.1 b

[x]Distribution is the RKN-problem in terms of percentage of the total production area affected by these nematodes inside each site studied.
[y]Severity was rated on a scale of symptoms of 1 to 9 as described above from the roots analyzed inside each site studied.
[z]Within columns, means with common letters are not significantly different (Dunn, $P \leq 0.05$).

et al., 2000; Cid del Prado *et al*., 2001; Martínez-Gallardo *et al.*, 2015). In the field, RKN outbreaks are difficult to manage. Effective nematode control requires proper species identification, especially in the case of a new nematode that can be confused with a similar species (van Meggelen *et al*., 1980; Yang and Eisenback, 1983; Carneiro *et al*., 1996). The lack of reliable and quantitative data regarding the importance of plant diseases is the factor that retards progress of agriculture and plant pathology. Hence, periodic monitoring of the status of RKN in this important horticulture region is necessary. No studies of the prevalence and incidence of these RKN species in Sinaloa, Mexico, have been conducted since 2001.

The presence of *M. enterolobii* and *M. incognita* parasitizing tomato plants in single and mixed infections was confirmed by this research. Morphometric characters of females usually were within the range reported in the original descriptions (Chitwood, 1949; Eisenback *et al*., 1980; Eisenback *et al*., 1981; Yang and Eisenback, 1983; Ramma and Hirschmann, 1988). Perineal patterns showed a dorsal arch rounded, which is typical of *M. enterolobii*, and usually sets it apart from *M. incognita*, *M. arenaria*, *M. javanica*, and *M. hapla* (EPPO, 2011). Perineal patterns of *M. incognita* were variable in shape, although the oval shape, dorsal arch, usually high formed by smooth and wavy striae without lateral lines, were useful to discriminate among *Meloidogyne* species. Some adult females showed variable perineal pattern morphology. Such situations do not allow accurate identification among these species, so the use of more than one identification technique is necessary.

Molecular analysis of the DNA amplified fragments of 256 and ~1000 bp obtained by using the species-specific primers revealed that they correspond to those reported by Hu *et al*. (2011). The results indicate that the RKN present in Los Mochis, Culiacan, La Cruz, and Escuinapa, Sinaloa, corresponds to *M. enterolobii* and *M. incognita*. These results coincide with those by Carrillo-Fasio *et al*. (2000) and Cid del Prado Vera *et al*. (2001), who reported the presence of *M. incognita* in tomato plants in this region of Mexico and with Martínez-Gallardo *et al*. (2015) and Villar-Luna *et al*. (2016) who reported the presence of *M. enterolobii* in tomato and pepper crops in this same region of Mexico. The absence of *M. javanica*, *M. arenaria*, *M. hapla*, and *M. chitwoodi* from all samples analyzed by PCR and morphological traits, could be attributed to the use of cultivars with Mi-1 resistance gene. The lack of species diversity may be also related to the cropping pattern or monoculture. It is well known that competition among species may result in the

dominance of one species after several generations (Manzanilla-Lopez and Starr, 2009). The dominance of one species over others can be favored by numerous factors, such as environmental conditions, inoculum level, and host suitability (Kolombia *et al*., 2017).

We found that *M. enterolobii* is the most predominant species of RKN followed by *M. incognita* and mixed infection with both nematodes. These results indicate that *M. enterolobii* has a greater distribution than that of *M. incognita*. Global warming could be a factor since temperature can modify the microorganism populations of the soil because of the different capacities for survival. It is known that *M. enterolobii* has a high reproduction rate, virulence, wide host range, and is also more resistant to some biocontrol agents, like *Pasteuria penetrans* compared with *M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla* (Brito *et al*., 2004, 2007; Castagnone-Sereno, 2012).

The presence of species interaction within *Meloidogyne* is well known in other parts of the world (Johnson and Nusbam, 1970; Kinloch and Allen, 1972; Khan and Haider, 1991; de Araujo Filho *et al*., 2016; Kolombia *et al*., 2017). The observation of the dominance of one *Meloidogyne* species in comparison to others also is a phenomenon that has been documented (de Araujo Filho *et al*., 2016; Kolombia *et al*., 2017), and according to Nobre-Maleita *et al.* (2012), it could be due to the fact that different populations of the same species of *Meloidogyne* also can react differently on the same plant species/cultivar.

This study is, to the best of our knowledge, the first on the prevalence and incidence of RKN species in tomato crop in the state of Sinaloa, Mexico. The RKN species affecting this crop were unequivocally identified using the perineal pattern morphology of adult females,and corroborated through PCR amplification with species specific-primers. This is also the first report of *M. enterolobii* and *M. incognita* mixed infections in tomato. Knowledge generated in this study regarding the distribution, prevalence, and incidence of RKN in this part of Mexico aids in predicting the potential spread in the future and provides a better understanding of the epidemiology of these important nematodes.

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