SPATIAL DISTRIBUTION OF *MELOIDOGYNE* SPECIES AND RACES IN THE TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) PRODUCING REGION OF MORELOS, MEXICO

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ABSTRACT

Guzman-Plazola, R. A., J. Jaraba N., E. Caswell-Chen, E. Zavaleta-Mejía, and I. Cid del Prado V. 2006. Spatial distribution of *Meloidogyne* species and races in the tomato (*Lycopersicon esculentum* Mill.) producing region of Morelos, Mexico. Nematropica 36:215-229.

Regional spatial patterns of *Meloidogyne* species and races infecting tomato crops in Morelos state were analyzed from 50 tomato fields in the municipalities of Atlatlahucan, Tlayacapan, Totolapan and Yecapixtla. Rhizosphere soil was collected at each site and evaluated for soil texture, organic matter content, pH and electrical conductivity. Soil collected at each site was distributed in five 2-liter plastic pots. Four tomato seedlings cv. Rio Grande were planted in each pot. Ten to 20 egg masses per positive site were extracted and individual egg masses were placed in the root zone of an individual tomato plant of the same variety. Galling of each was assessed by the Taylor and Sasser Root Gall index. Fourteen out of fifty tomato fields contained *Meloidogyne* spp., but isolates from only eight fields were able to reproduce on tomato cv. Rio Grande. Thirty three isolates from all sites were identified to species using morphological and morphometric parameters. Species and races from twenty one of those isolates found in the region were: *Meloidogyne incognita* race 1, *M. arenaria* race 2 and *M. javanica* race 1 based on North Carolina Differential Host Test. At the regional level, *Meloidogyne* spp. were found distributed in three areas. Soils positive for *Meloidogyne* were moderately to slightly acid, and most of them had 38 to 71% sand and 13 to 29% clay content Soil organic matter content and pH were highly correlated with principal components I and II, respectively. This correlation allowed us to classify soils from all the sampled sites into four groups of edaphic similarity. Conduciveness of soils to *Meloidogyne* was assessed by means of a logistic regression model of *Meloidogyne* presence versus sand and clay content. Sand and clay content were useful to forecast the conduciveness of *Meloidogyne* spp. in the tomato producing soils of Morelos.

Key words: clay, *Meloidogyne arenaria* race 2, *M. incognita* race 1, *M. javanica*, regional spatial pattern, sand.

RESUMEN

Guzman-Plazola, R. A., J. Jaraba N., E. Caswell-Chen, E. Zavaleta-Mejía, y I. Cid del Prado V. 2006. Distribución espacial de especies y razas de *Meloidogyne* en la zona productora de tomate (*Lycopersicon esculentum* Mill.) en Morelos, Mexico. Nematropica 36:215-229.

Se analizó el patrón de distribución regional de las especies y razas de *Meloidogyne* asociadas al cultivo de tomate en el estado de Morelos, México. Se muestrearon cincuenta campos cultivados con tomate en los municipios de Atlatlahucan, Tlayacapan, Totolapan y Yecapixtla. De cada sitio se tomó una muestra de suelo rizosférico. En cada muestra se determinó la textura, el contenido de materia orgánica, el pH y la conductividad eléctrica. Estas variables se utilizaron para determinar, mediante regresión logística, cuáles características del suelo están asociadas a la ocurrencia de *Meloidogyne* en esa región. La muestra de suelo de cada sitio fue distribuida en cinco macetas de plástico de dos litros, donde se trasplantaron cuatro plántulas de tomate Río Grande. Se colectaron 10 a 20 masas de huevos por sitio positivo. Las masas fueron inoculadas por separado en tomate. Se evaluó el grado de aga-

llamiento mediante la escala de Taylor y Sasser. Las especies de *Meloidogyne* presentes fueron identificadas mediante variables morfológicas y morfométricas. Las razas se determinaron mediante la prueba de hospedantes diferenciales de Carolina del Norte. Se detectaron 14 sitios positivos para *Meloidogyne*, pero sólo de ocho se lograron incrementar las poblaciones de estos nematodos en tomate cv. Río Grande. Se obtuvieron 33 aislamientos que se identificaron hasta especie. En 21 de éstos se determinaron las razas. Las especies y razas identificadas fueron: *Meloidogyne incognita* raza 1, *M. arenaria* raza 2 y *M. javanica*. Se observó que a nivel regional *Meloidogyne* spp. se distribuye en tres áreas claramente identificadas. Los suelos positivos para *Meloidogyne* tuvieron un pH moderadamente a ligeramente ácido, porcentajes medios a altos de arena y bajos contenidos de limo y arcilla. El contenido de materia orgánica y el pH se encontraron altamente correlacionados con el componente principal I y II, respectivamente, lo que permitió agrupar los suelos en cuatro grupos de similaridad edafológica. Sin embargo, solamente los contenidos de arena y arcilla resultaron variables útiles para pronosticar la presencia o ausencia de poblaciones de *Meloidogyne* en la zona productora de tomate del estado de Morelos, México.

Palabras clave: arena, arcilla, *Meloidogyne arenaria* raza 2, *M. incognita* raza 1, *M. javanica*, patrón espacial regional.

INTRODUCTION

Morelos is the eighth largest tomato (*Lycopersicon esculentum* Mill.) producing state in Mexico. Tomatoes in this state are mainly produced under non-irrigated rainfall-dependent fields. Average tomato yield in Morelos is 20.4 ton/ha which is 17.2 and 27 ton/ha less than the average yield of Sinaloa and Baja California, respectively (SIAP-SIACON, 2004), the largest tomato producing states in Mexico. Low productivity of tomato crops in Morelos is caused by poor soil fertility, irregular rainfall, inappropriate crop management practices, and the incidence of pests and diseases. Virus diseases are the most important, but fungi and nematodes (*Meloidogyne* spp. and *Nacobbus aberrans* Thorne and Allen) may cause important yield losses (Flores, 1997). In 1985, 6187 ha were cultivated with tomatoes, but by 2001 this area was reduced to only 2830 ha (SIAP-SIACON, 2004). Moreover, only six out of 21 municipalities originally reported as tomato producers in 1980 continued to produce tomatoes in 1999 (Atlatlahucan, Tepoztlán, Tlalnepantla, Tlayacapan, Totolapan and Yecapixtla) (INEGI, 1999).

Nematodes cause an average of 21% yield loss in tomato crops worldwide (Sasser and Freckman, 1987). Ninety percent of yield losses due to nematodes are caused by root-knot nematodes (Agrios, 1997). Few reports are available on nematodes in tomato crops of Morelos State. In 1970, Palacios analyzed one tomato crop in each of the 22 municipalities reported as tomato producers at that time. He found *Meloidogyne incognita* and *M. arenaria* in all sampling sites. Pacheco (1986) and Cid del Prado *et al.* (2001) reported *M. incognita* race 1 in Tenextepango, municipality of Ayala. However, tomatoes are no longer produced in fields sampled in these three reports.

According to interviews with tomato growers from Morelos, nematicides are applied in a preventative manner. Growers routinely apply nematicides to the soil along with other chemicals to control soil pathogens. Generally, no technical criteria are followed. The identification of species and races of root-knot nematodes attacking tomato crops in the region, along with an analysis of their spatial distribution, and an assessment of nematode incidence and potential damage, would allow advisers

and growers to assess both the current and potential economic importance of nematodes. Such information would serve as decision support criteria for the application of management strategies.

The analysis of spatial distribution of nematodes has mostly been focused on single fields. At this scale, plant-parasitic nematodes are typically distributed in clusters (Barker, 1985). Aggregation of nematode populations is considered to be a consequence of their strategy of reproduction and their dispersal mechanisms. Few studies on regional distribution of nematodes have been carried out; however, this type of analysis may help explain the role of these organisms in agricultural systems. In this research, species and races of root-knot nematodes (*Meloidogyne* spp.) associated with tomato crops in Morelos and their regional distribution and relationships to soil factors were analyzed.

MATERIALS AND METHODS

Soil Sampling and Root-knot Nematode Detection

Fifty tomato fields in the tomato growing region of Morelos were sampled. Twenty seven tomato fields were sampled in the municipality of Totolapan, nine in Yecapixtla, eight in Tlayacapan and six in Atlatlahucan (Fig. 1). Fields to be sampled were selected arbitrarily. Tomato plants were close to senescence. In each field (0.5 to 1 ha), 10 tomato plants were selected randomly and rhizosphere soil was collected from each plant by extracting the root system and removing associated soil. Geographic coordinates were determined for each field by means of a Global Positioning Device (Trimble Navigator, model Ensign GPS, Trimble, Sunnyvale, CA). Soil samples from each field were mixed together and placed in five 2-L plastic pots. Four tomato seedlings cv. Rio Grande were planted into each pot. Three months later all plant roots were examined for the presence of galls and ten to twenty egg masses were collected from each positive plant.

Inoculum Production

Ten to 20 egg masses were collected from each field. Single egg masses of *Meloidogyne* spp. were placed close to the roots of individual tomato seedlings cv. Rio Grande. A mixture (1:1:1) of autoclave sterilized sand, organic soil, and perlite (Agrolite, Dicalite de Mexico, S.A.) was used as a growth substrate.

Isolate and Site Characterization

Ninety days after the last inoculation cycle roots from infected plants were collected and new egg masses were extracted. Females were stained using sodium hypochlorite-acid fuchsin technique (Daykin and Hussey, 1985). Second-stage juveniles (J_2) were collected by hatching eggs at room temperature. Males were extracted from rhizosphere soil by wet sieving and centrifugation (Hooper, 1986). Males and juveniles were fixed, dehydrated and mounted in glycerin. Seinhorst's Massive Dehydration Technique modified by Bongers (personal communication) was followed: Nematodes were killed by heating the suspensions to approximately 40°C before fixing them in formalin (4%). Nematode suspensions in Petri dishes were processed to glycerine by placing them in an alcohol chamber for 48 h then removing excess alcohol and placing the dishes in an oven at 40°C. Drops of glycerin were added over seven days until the nematodes were in pure glycerin.

The following morphological characteristics were assessed: stylet and stylet knobs shape, number and shape of labial rings in females, males and J_2. Perineal patterns from mature females were inspected for shape and type of dorsal arch, lateral

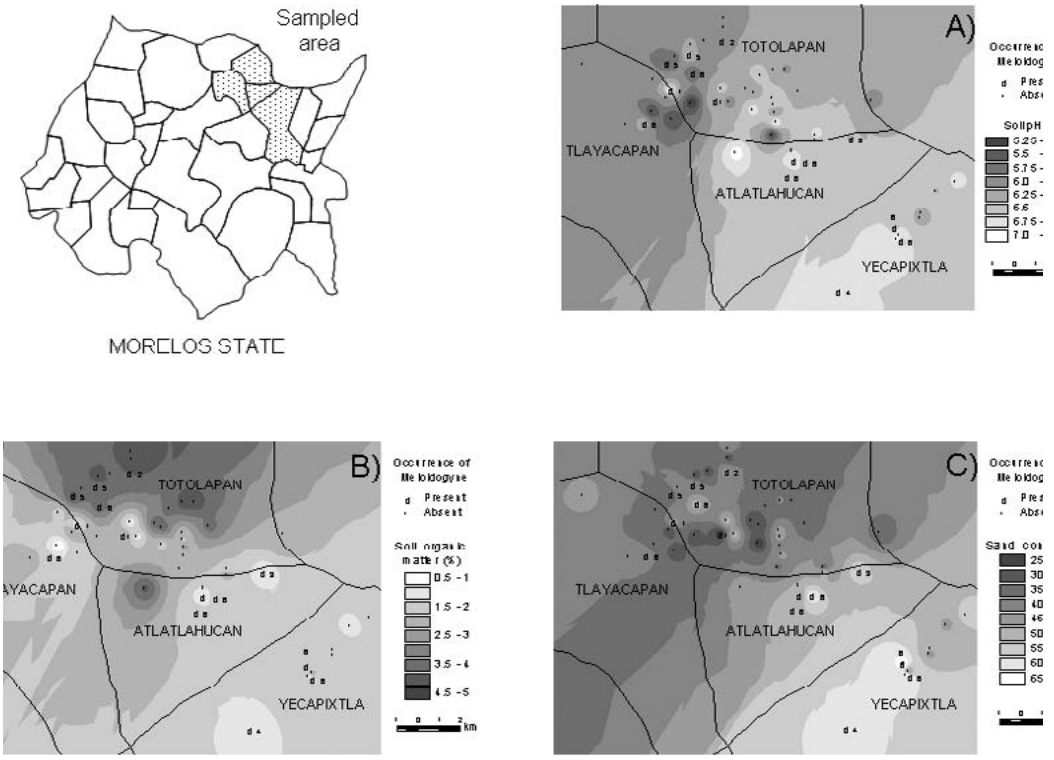

Fig. 1. (A) pH, (B) organic matter content and (C) percentage of sand in soils of the tomato producing region of Morelos. Numbers adjacent to sampling sites indicate presence of *Meloidogyne*. 1 = *Meloidogyne incognita*; 2 = *M. arenaria*; 3 = *M. incognita* + *M. arenaria* + *M. javanica*; 4 = *M. incognita* + *M. arenaria*; 5 = *M. incognita* + *M. javanica*; 6 = *Meloidogyne* spp. (not identified to species).

ridges and striae. Morphometric characteristics assessed in females, males and juveniles were stylet length and distance from the stylet knobs to the dorsal esophageal orifice. Body, tail and hyaline region length were also assessed in juveniles. All measurements and morphological assessments were made on 10 individuals of each type per isolate cultivated from single egg mass inoculations. Taylor and Sasser's (1978) galling scale was used in assessing the galling caused by each isolate at the end of the second cycle of population increase. In this cycle, plants were inoculated with infected roots from single egg mass inoculations to assess the capacity of each isolate to grow and multiply in tomato cv. Rio Grande, a commonly used cultivar in the tomato growing region of Morelos state.

The North Carolina differential host test (Sasser and Carter, 1985) was used to determine races of *Meloidogyne*. Depending upon inoculum availability, 1,000 to 5,000 eggs and juveniles were added to each of three replicates of each host plant. Plants were maintained in the greenhouse at an average temperature of 20°C. Sixty days after inoculation, roots were harvested to determine the presence or absence of galls.

A subsample was taken from each original compound soil sample. This subsample was analyzed in the Soil Fertility Laboratory in the Soil Science Department, Cole-

gio de Postgraduados. Texture, pH, electrical conductivity and organic matter content were measured for each sample. Soil texture was determined by Boyoucos hydrometer, soil pH was measured in soil:water mixture (1:2), electrical conductivity was measured in soil extract (1:5 soil: distilled and deionized water (Richards, 1990) and organic matter was estimated by the Walkley-Black method (Barreira, 1978). Soil pH classification was done according to Barreira (1978). Organic matter content of soils was classified according to Ortiz-Villanueva and Ortiz-Solorio (1980).

Statistical Analyses

To determine the pattern of spatial distribution of *Meloidogyne* at the regional level, a visual exploration of positive sites was carried out by means of Geographic Information System software (ArcView V.3.2, Environmental Systems Research Institute, Redlands California). Spatial interpolation of soil variables was done by distance-weighted average (Isaaks and Srivastava, 1989).

Edaphic similarity among sampling sites was analyzed by calculating principle components to the variance-covariance matrix of soil physical-chemical variables measured in this study. Similarity of soils among sites was analyzed by visual inspection of the scatter diagram made by the two main principal components. The meaning of each component was interpreted by its correlation with the original variables and the principle component loads (Afifi and Clark, 1990).

To determine which variables contributed best to discrimination between soils being favorable and non-favorable to *Meloidogyne* in the tomato producing region of Morelos, a backward variable selection procedure was followed and a logistic regression model (Ln (odds) = $aX_1 + bX_2 ...nX_n$) was fitted to the soil data. Model parameters were estimated by maximum likelihood. This model predicts the natural logarithm of the odds of a positive site for *Meloidogyne* as a linear combination of the selected variables (Afifi and Clark, 1990). Predicted values were compared with data on presence or absence of *Meloidogyne* for each sampled field. All statistical analyses were done with SAS software V. 9.1 (The SAS Institute, Cary, North Carolina). Princomp and Logistic procedures were used.

RESULTS

Sampling Results

Fourteen of the 50 fields sampled were positive for presence of *Meloidogyne* spp. Five positive fields were detected in the municipality of Totolapan, four in Atlatlahucan, three in Yecapixtla and two in Tlayacapan (Figs. 1 and 4). From these fourteen fields, nematodes from only eight fields were able to reproduce continually on tomato cv. Río Grande. Thirty three isolates from these fields were identified to species, but only 21 isolates were increased sufficiently to run differential host tests. Seventy four percent of total isolates were identified as *M. incognita*, 16% as *M. javanica* and 10% as *M. arenaria*.

Soil pH varied from 5.3 to 7.2. The organic matter content ranged from 0.5 to 4.5% and the electrical conductivity varied from 0.01 to 0.04 mmhos/cm. Soil texture included loam, sandy loam, sandy clay loam, clay loam, and clay.

Nematode Morphology

Five types of perineal patterns were detected among the 33 *Meloidogyne* isolates. Three of these types were very similar to the patterns described in the literature for *M. incognita*, *M. arenaria* and *M. javanica*. The other two types were similar to *M. incognita* patterns but with some varia-

tions (Table 1, Fig. 2). Isolates 2-3 (sampling site 2-isolate number 3), 2-5 and 47-33 had a high and squarish dorsal arch, with smooth to wavy striae, which is typical of *M. incognita* (Fig. 2A) (Eisenback, 1985). Isolates 11-7, 11-8, 11-9, 12-10, 12-11, 12-12, 36-16, 36-17, 36-18, 36-19, 36-20, 38-21, 38-22, 40-25, 40-26, 40-29, 40-30, 40-31 and 40-32 had a high and squared to rounded dorsal arch, with wavy striae (Fig. 2B). Isolates 40-24, 40-27 and 40-28 had a slightly low rounded to square dorsal arch, with smooth striae (Fig. 2C). Females in the previous three groups had a stylet cone

Table 1. Morphological characteristics observed among the isolates of root-knot nematode (*Meloidogyne* spp.) collected in the tomato producing region of Morelos State, Mexico.

Isolate[z]		Females			Juveniles	Males	
Site	Strain	Dorsal arch	Lateral ridges	Striae	Head region	Head region	Species
2	1	Low and rounded	Present, well defined	Smooth to slightly wavy	No annulations	No males	*M. javanica*
2	2						
36	14						
36	15						
38	22						
2	3	High and squarish	Absent	Smooth to wavy	1-3 annulations	2-3 annulations	*M. incognita*
2	5						
47	33						
2	4	Low and rounded	Absent	Smooth to wavy	No annulations	No males	*M. arenaria*
11	6						
31	13						
11	7	High, squarish and slightly rounded	Absent	Wavy	1-3 annulations	2-3 annulations	*M. incognita*
11	8						
11	9						
12	10						
12	11						
12	12						
36	16						
36	17						
36	18						
36	19						
36	20						
38	21						
38	23						
40	25						
40	26						
40	29						
40	30						
40	31						
40	32						
40	24	Slightly low, rounded or squarish	Absent	Wavy		No annulations	*M. incognita*
40	27						
40	28						

[z]Data from ten individuals per isolate.

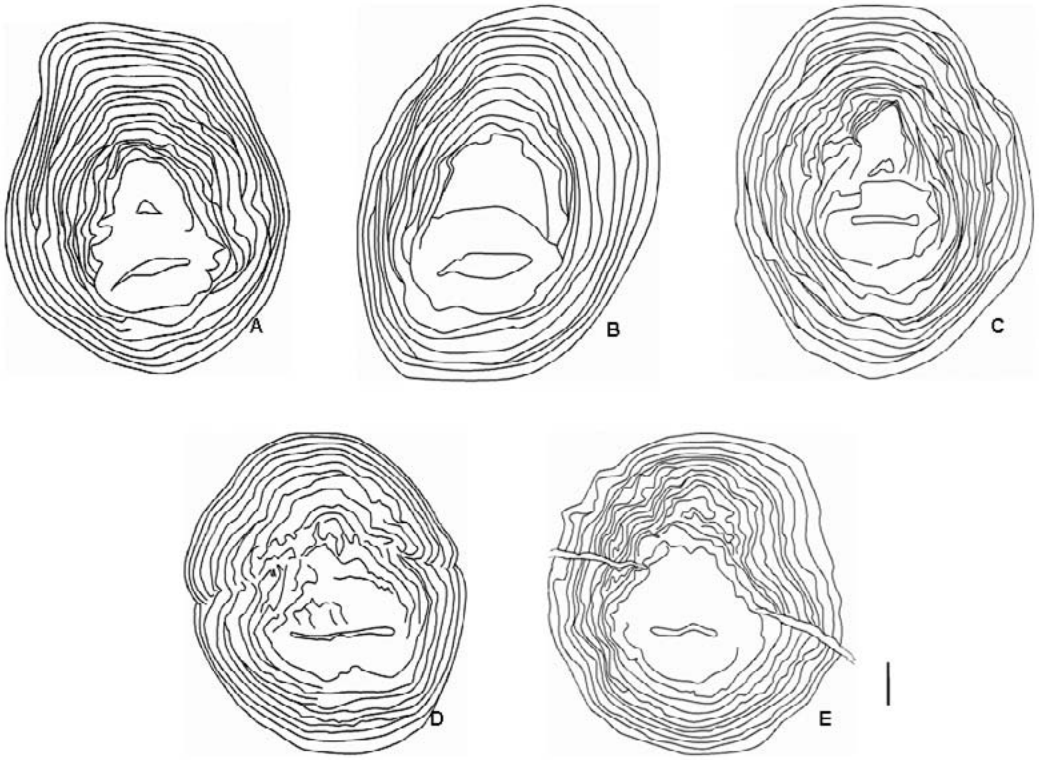

Fig. 2. Perineal patterns collected from the tomato producing region of Morelos: *Meloidogyne incognita*: (A) High and squarish, (B) High and rounded and (C) Rounded and slightly low, (D) *Meloidogyne arenaria* and (E) *Meloidogyne javanica*. Bar = 10 µm.

curved to slightly curved dorsally and a stylet shaft slightly wider near its base, with wide and flat knobs. These stylet and dorsal arch shapes are characteristics for *M. incognita* (Orton, 1973; Eisenback, 1985). For the purposes of this work, the second and third groups were referred to as *M. incognita* variation 1 and *M. incognita* variation 2, respectively.

Isolates 2-4, 11-6 and 31-13 had a perineal pattern and stylet morphology very similar to that reported by Eisenback (1985) for *M. arenaria* (Fig. 2D), whereas isolates 2-1, 2-2, 36-14, 36-15 and 38-22 had characteristics of *M. javanica* (Orton, 1972; Eisenback, 1985) (Fig. 2E). Males were recovered only from isolates 36-16, 36-18, 38-21, 38-23, 40-26 and 40-27. Morphological characteristics of these males were similar to the description made by Eisenback (1985) for *M. incognita*.

Juveniles from isolates 2-5, 11-7, 11-8, 11-9, 36-16, 36-17, 36-18, 38-21, 38-22, 40-24, 40-25, 40-26, 40-27, 40-28, 40-29, 40-30 and 40-32 had a head region, stylet and tail terminus similar to *M. incognita* (Orton, 1973; Eisenback, 1985). Juveniles from isolates 2-4, 11-6, and 31-13 had characteristics of *M. arenaria* (Orton, 1975; Eisenback, 1985). Second-stage juveniles of isolate 2-1 were similar to *M. javanica* (Orton, 1972; Eisenback, 1985).

Morphometry

Values of distances of the dorsal esophageal gland orifice (DEGO) to the base of the stylet for the isolates identified as *M. incognita*, *M. arenaria* and *M. javanica* varied from 2.1 to 4.0 µm, 3.3 to 6.3 µm and 2.2 to 5.0 µm, respectively. However, the most common values for *M. incognita* and *M. javanica* were 2.5 to 3.5 µm, whereas those for *M. arenaria* were 4.5 to 5.5 µm. Stylet lengths for *M. incognita*, *M. arenaria* and *M. javanica* were 14.2 to 18.2 µm, 13.25 to 17.3 µm and 14.5 to 18.2 µm, respectively. The most common values in *M. incognita* and *M. javanica* were 15.5 to 16 µm and 16.5 to 17 µm, respectively, whereas those for *M. arenaria* were 17.5 to 18 µm. A high degree of overlap was observed in the frequency distribution of the two variables in the three species.

Plant Response

Gall rating in tomato plants, at the end of the second cycle of inoculum increase (90 days after inoculation), showed contrasting differences depending on which of the 33 *Meloidogyne* isolates was used. Isolates 2-1, 2-2, 2-3, 12-10, 12-11, 12-12, 36-14, 36-15, 36-19 and 38-22 received a rating of 2 and produced small and few individual galls. Isolates 2-4, 2-5, 11-6, 11-7, 11-8, 11-9, 31-13, 36-17,36-18, 38-21 and 47-33 were rated as 3, with less than 30 galls per root. Isolates 36-19 and 38-23 received a rating of 4 because of the abundant and mostly individual galls. Limited gall coalescence was also observed in these isolates. Isolates from site 40 (strains 24 to 32) had a galling index of 5. In the early stages of gall development, only single galls were observed. By the end of the test, galls coalesced to produce large tumor-like galls.

Differential Host Test

All isolates of *M. incognita*, with the exception of 26-17 and 36-18, reproduced on pepper (*Capsicum annum*), watermelon (*Citrullus lanatus*) and tomato but not on tobacco (*Nicotiana tabaccum*), peanut (*Arachis hypogaea*) and cotton (*Gossypium herbaceum*), which is typical for race 1. Isolate 2-4 of *M. arenaria* reproduced on pepper, watermelon and tomato but not on cotton, tobacco and peanut. Isolate 31-13 of this species reproduced only on watermelon and tomato, and was tentatively classified as race 2. Isolate 11-6 did not infect any of the differential hosts. Isolate 2-1 of *M. javanica* infected watermelon and tomato and did not infect cotton, tobacco, peanuts and pepper. Lack of infection in the last two hosts are typical of race 1 (Rammah and Hirschman, 1990).

Analysis of the Distribution of Meloidogyne

Soils favorable to *Meloidogyne* in the tomato producing region of Morelos are distributed in three areas. The first area is located between the municipalities of Totolapan and Tlayacapan (seven fields), a second area is located in the municipality of Atlatlahucan (four fields), and a third area is located in the municipality of Yecapixtla (three fields) (Figs. 1 and 4).

Populations of *Meloidogyne* were found associated with neutral to slightly acid soils. Two populations were found in moderately acid soils (5.5-6.0), six were found in slightly acid soils (6.0-6.5) and six in neutral soils (6.5-7.5). Root-knot nematodes were also found in soils of a wide range of organic matter content (OMC) (Fig. 1). Four populations were detected in soils having high (3 to 5%) OMC, one population was found in a medium soil (2 to 3% OMC), eight populations were found in poor soils (1 to 2% OMC) and one in a very poor soil (0.5 to1% OMC).

Meloidogyne populations were also found associated with soils having medium to high sand and low clay and silt content. Eight populations were found in soils hav-

ing more than 50% sand, four were found in soils with 40 to 50% sand and two were found in soils with a sand percentage of 20 to 40%. Two *Meloidogyne* populations were found in soils having 30 to 40% silt, nine were found in soils with 20 to 30% silt and three were found in soils having 10 to 20% silt. Clay percentage in positive soils varied from 10 to 30% (Fig. 1).

Principal components I and II accounted for 87.5 and 11.5 percent of total variance, respectively. All other components accounted for just the remaining 1% of total variance. The first component was highly correlated with organic matter content (r = 0.99) whereas the second component was highly correlated with soil pH (r = 0.95). The scatter diagram of points defined by principal components I and II shows that the tomato fields that were sampled can be classified into four similarity groups (Fig. 3). The distribution of these similarity groups is shown in Fig. 4. The first group consisted of fields 27, 35, 37 and 38. This group is characterized by acid to moderately acid soils of high to medium organic matter content. The second group consisted of fields 24, 25, 26, 28, 29, 30, 31, 32, 33, 34 and 36, and is characterized by slightly acid to neutral soils with medium to rich organic matter content. The third group consisted of fields 17, 45 and 50, and is characterized by moderate to slightly acid soils with poor to medium organic matter content. The fourth group consisted of fields 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 39, 40, 42, 43, 44, 46, 47, 48 and 49, which are characterized by moderately acidic to neutral soils with poor to

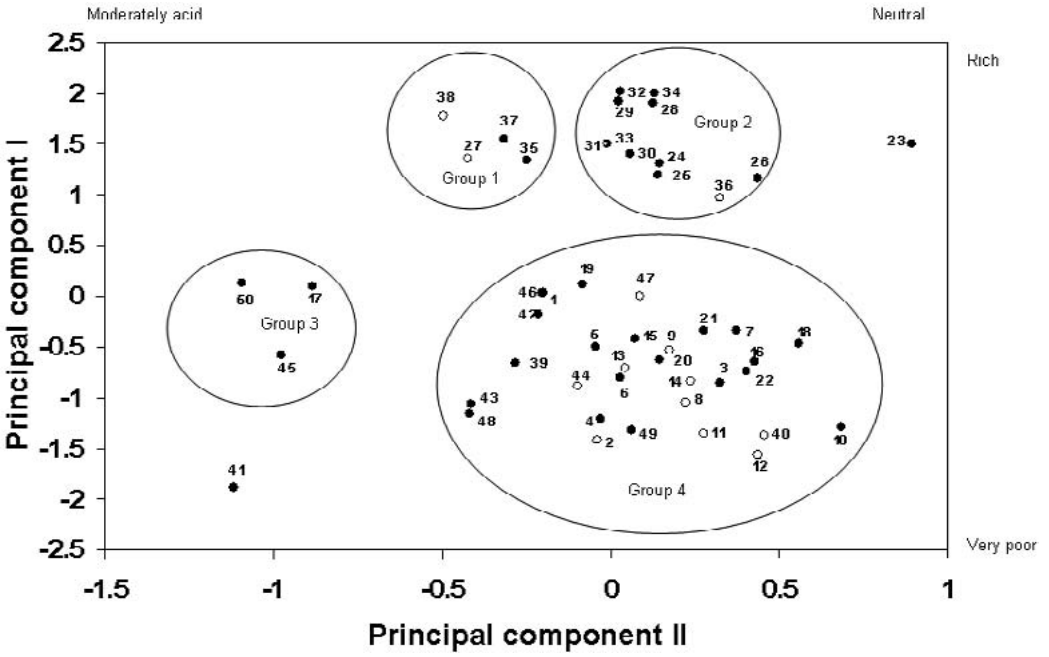

Fig. 3. Scatter diagram of tomato fields sampled in the tomato producing region of Morelos. Principal Component I is correlated with the organic matter content in the soil. Principal Component II is correlated with soil pH. Number at each point indicates the label for the tomato field. o = presence of *Meloidogyne* in that field, • = absence of *Meloidogyne*.

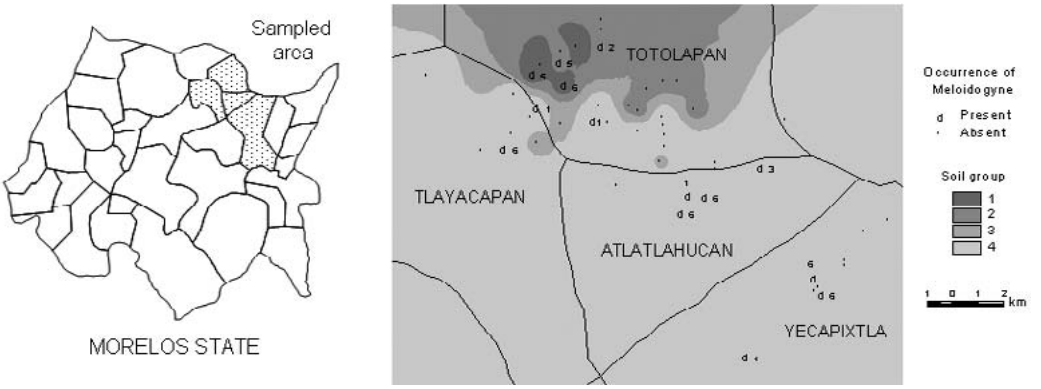

Fig. 4. Tomato soils classified by edaphological similarity. Group 1: Acid to moderately acid and high organic matter content (OMC). Group 2: Slightly acid to neutral and medium to rich OMC. Group 3: Moderately to slightly acid and poor to medium OMC. Group 4: moderately acid to neutral and poor to medium OMC. Numbers adjacent to sampling sites indicate presence of *Meloidogyne*. 1 = *Meloidogyne incognita*; 2 = *M. arenaria*; 3 = *M. incognita* + *M. arenaria* + *M. javanica*; 4 = *M. incognita* + *M. arenaria*; 5 = *M. incognita* + *M. javanica*; 6 = *Meloidogyne* spp. (not identified to species).

medium organic matter content. Fields 23 and 41 are outliers, having neutral pH and high organic matter content (field 23), or slightly acid pH and very poor organic matter content (field 41). Ten *Meloidogyne* populations were in group four, two of them were in group one, and two in group two. Groups one and two are similar in organic matter content and differ in soil pH, whereas groups two and four are similar in soil pH and differ in organic matter content. Group three has no similarity with the other soil groups.

Backward selection of soil variables to discriminate between positive and negative soils for *Meloidogyne* by means of logistic regression yielded only two selected variables. Sand ($Pr > X^2 = 0.0258$) and clay Sand ($Pr > X^2 = 0.0013$) content were positively and negatively associated with the presence of root-knot nematodes, respectively. Neither organic matter content, electrical conductivity nor pH contributed significantly to identification of soils conducive to *Meloidogyne*.

According to the logistic model the probability of a soil being favorable to *Meloidogyne*, in the tomato producing region of Morelos, is given by:

$$P = 1/[1 + e^{-(3.804\text{Sand} - 10.2229\text{Clay})}]$$

where: Sand = sand content on a scale from 0 to 1, Clay = clay content on a scale from 0 to 1. Standard error for sand and clay parameter estimates were 1.706 and 3.189, respectively. By running this model with data from all sampled fields, 78% of the cases were predicted accurately but in 21% of the cases the model failed; also, in 1% of the cases the prediction was equivocal.

Predictions of the model for a range of sand and clay combinations are shown in Fig. 5. According to the model, probability of root-knot occurrence increases monotonically with the increase of sand content, and drops below 0.5 when clay content increases above 30%.

DISCUSSION

Morphological and morphometric characteristics observed in the different *Meloidogyne* isolates from this study agree with the description of *Meloidogyne incog-*

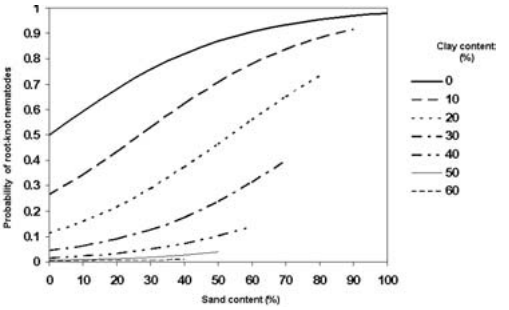

Fig. 5. Probability of a field being infested with root-knot nematodes, predicted by the model: $P = 1/[1 + e^{-(3.804\text{Sand} - 10.2229\text{Clay})}]$ for soils of the tomato producing region of Morelos. Sand = sand content on a scale from 0 to 1. Clay = clay content, on a scale from 0 to 1.

nita (Kofoid and White, 1919) Chitwood 1949; *Meloidogyne arenaria* (Neal 1889) Chitwood 1949, and *Meloidogyne javanica* (Trueb 1885) Chitwood 1949 (Sasser, 1979; Eisenback, 1985; Jepson, 1987). This is the first report of *M. javanica* in tomato crops of Morelos state. Our findings confirm the results of Palacios (1970) regarding the presence of *M. arenaria* and *M. incognita* in Morelos, but disagree regarding the presence of *M. javanica*. Species identifications of Palacios (1970) were preliminary and based on analysis of perineal patterns of a single field isolate for each municipality of the former tomato producing region in the state. However, distribution of tomato growing areas in Morelos has undergone considerable changes in the last 20 years. Other investigators (Pacheco, 1986; Cid Del Prado *et al.*, 2001) reported *Meloidogyne incognita* race 1 associated with tomatoes in the municipality of Ayala. However, this municipality is not currently producing tomatoes.

Variations in perineal patterns within isolates of *M. incognita* observed in this study agree with reports from other investigators, regarding isolates of *M. incognita* and *M. javanica*, propagated from single egg masses (Dalmaso and Bergé, 1979; Fargette, 1987; Fargette and Braaksma, 1990; Eisenback, 1993). These variations are considered to be a result of genetic heterogeneity.

Meloidogyne incognita was the species most frequently found (76%) in the region, followed by *M. javanica* (15%) and *M. arenaria* (9%). Palacios (1970) found that *M. incognita* and *M. arenaria* had a similar frequency whereas Cid del Prado *et al.* (2001) found only *M. incognita*. These differences may be a result of variations in sampling methodology. In our work, each sample was a mixture of ten subsamples of rhizosphere soil, and we sampled a larger number of tomato fields. We detected mixed populations of *Meloidogyne* species in some tomato fields. Sites 2, 11, 36 and 38 had mixtures of *M. incognita*, *M. arenaria* and *M. javanica*; *M. incognita* and *M. arenaria*; *M. incognita* and *M. javanica*, respectively. Field 2 is the only one that had a mixture of the three species. Other researchers have also found mixed populations in other crops of Mexico (Sosa-Moss, 1985; Cid-Del Prado *et al.*, 1996). Existence of species mixtures in tomato fields of Morelos could hinder the control of root-knot nematode by means of changing the pattern of crop rotation as these nematode species have different and very wide host ranges.

The results of the North Carolina differential host test were inconsistent for the three isolates of *M. arenaria* tested, particularly 2-4 and 11-6. These isolates did not reproduce on tobacco, cotton or peanut and isolate 31-13 only failed to infect tobacco; therefore they were assigned tentatively to race 2 because the reaction on peanut is the key to differentiate between race 1 (+) and race 2 (-) of this species (Sasser and Carter, 1985). Any doubt about species identity was ruled out since perineal patterns, stylet and distance to DEGO were typical of that reported for *M. arenaria* (Eisenback, 1985). Isolates that did not reproduce on any host (e.g., 11-6) may have not had enough inoculum or the

inoculum may have lost viability. Lack of infection of this species on tobacco could also be due to a similar situation and to the fact that this test was conducted under colder conditions than those of the region where the isolates were collected. Some isolates may be difficult to assign to races by means of the differential host test of Sasser and Carter (1985). Fargette (1987) reported that *M. arenaria* race 2 and *M. javanica* cannot be distinguished by this differential host test since both groups have the same host range. Some populations of *M. javanica* can reproduce on pepper and peanut, whereas most populations do not (Eisenback *et al.*, 1981; Cetintas *et al.*, 2003). Host specificity has also been reported for geographic isolates of root-knot nematodes. Araya and Caswell-Chen (1995) reported that a California isolate of *M. javanica* was unable to infect *Coffea arabica* cvs. Caturra and Catuai even though tropical isolates of this nematode did cause infection.

Even though it was impossible to statistically assess the spatial pattern of soils positive for *Meloidogyne* in the region due to the lack of complete randomness of the samples, visual analysis of the distribution of positive tomato fields in the region indicated that *Meloidogyne* populations were distributed in three main patches. Clumping is a typical spatial distribution of nematode species in small patches of natural communities (Pen-Mouratov and Steinberger, 2005) and at the crop field level (Barker, 1985); however, in this study it was evident that even at the regional level it is possible to distinguish clumps of field crops infected with *Meloidogyne*. This result indicates that *Meloidogyne* populations are not randomly distributed in the tomato producing region of Morelos; therefore nematode control measures should be focused on specific areas.

The association of *Meloidogyne* populations with moderately acid to slightly alkaline soils and very poor to very rich organic matter content in the soil corroborates previous reports that species in this genus may adapt to a wide range of soil conditions (Ferris and Van Gundy, 1979). Most positive fields in the region have soils with a pH near neutral and very low organic matter content, which is consistent with the range (4-8) and optimum (6.4-7) pH reported by Ferris and Van Gundy (1979) for survival, hatching and reproduction of the different species of *Meloidogyne*.

Calculation of the principal components from the covariance matrix of physical and chemical soil variables gave evidence that soil organic matter content and soil pH are properties that can be used to group soils according to their degree of similarity. Soil texture and electrical conductivity did not contribute significantly to eigenvalue of each of these principle components due to the relatively high homogeneity in the values of these variables among sampled sites. A scatter diagram (Fig. 3) of sampled sites showed that *Meloidogyne* populations are located in groups of tomato fields having some degree of edaphic similarity. Most positive fields have poor organic matter content and moderate to slightly acid soil pH.

The association of *Meloidogyne* populations with soils having medium to high sand content found in this study has also been reported in other investigations. Starr *et al.* (1993) and Van Gundy (1985) found that most *Meloidogyne* populations are associated with soils having a medium to high sand content, and that they are rarely found in clay soils. Sand content may be the main explanation of the regional pattern of distribution in groups of field crops positive for *Meloidogyne* in Morelos. Results of logistic regression show that sand and clay content are the main factors, among those evaluated, contributing to forecast the condusiveness to root-knot nematodes

in the region. Soil texture is a very stable soil characteristic that has very important effects on the capacity of nematodes to move in the soil interphase; it also affects root penetration, reproduction, and population levels of the nematode in the roots (Windham and Barker, 1986).

The fact that soil organic matter content, soil pH and electrical conductivity were not found useful to identify conduciveness to *Meloidogyne* can be explained because pH and electrical conductivity values vary with the moisture content of soils (Van Gundy, 1985). It is to be expected that soil organic matter in the region changes rapidly due to a high rate of mineralization, which is favored by the high temperatures that are commonly observed in the region (Barreira, 1978). The increase in organic matter content due to soil amendments in the region can be considered a transient factor that may suffer considerable changes in the short term.

The identification of species and races of *Meloidogyne* and their distribution delineated in this study may be a useful tool for the design and implementation of control strategies with a rational utilization of chemicals and crop rotations. The relatively high number of sampled fields gives a greater degree of confidence about the diagnosis of current distribution of root-knot nematodes in the region than in previous works. Geographic coordinates of each sampled field are available and will be useful for future research.

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