

RESEARCH NOTE/NOTA DE INVESTIGACIÓN

MOLECULAR IDENTIFICATION AND PATHOGENICITY OF *MELOIDOGYNE* SPP. IN *MUSA* AAB (PLANTAIN SUBGROUP) ‘DOMINICO HARTÓN’ SEEDLINGS

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ABSTRACT

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Meloidogyne (root-knot nematodes) are distributed worldwide and cause yield loss of economically important crops. The objectives of this research were to: (i) identify, with molecular techniques, the *Meloidogyne* species that parasitize *Musa* AAB ‘Dominico Hartón’ and (ii) evaluate the damage of *Meloidogyne* spp. to ‘Dominico Hartón’ seedlings. Second-stage juveniles (J2) of *Meloidogyne* spp. were collected from roots of ‘Dominico Hartón’ plants, at the Montelindo Research Farm of the Universidad de Caldas, Colombia, and molecular identification using PCR-RFLP and PCR-specific primers was performed at the Nematology and Molecular Techniques Laboratories, at Universidad de Costa Rica. A mixture of *Meloidogyne incognita* and *M. arenaria* was identified (1:7 ratio). Additionally, ‘Dominico Hartón’ seedlings were inoculated with mixtures of *M. incognita* + *M. arenaria* (0, 750, 1,500, 2,250, and 3,000 eggs + J2/seedling). Compared to the noninoculated control, from lowest to highest population density, it was observed that nematodes significantly ($P < 0.05$) reduced shoot dry weight from 25.4% to 52.6%, and root dry weight from 39.5% to 55.3%. Different nematode densities did not affect seedling height and number of leaves. In conclusion, the mixture of *M. incognita* + *M. arenaria* significantly affected growth of ‘Dominico Hartón’ seedlings.

Key words: *Musa* AAB, *Meloidogyne arenaria*, *M. incognita*, plantain ‘Dominico Hartón’

RESUMEN

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Los nematodos del género *Meloidogyne* (nematodos del nudo radical) están distribuidos alrededor del mundo y causan pérdidas en el rendimiento de los cultivos económicamente importantes. Los objetivos de esta investigación fueron: (i) identificar con técnicas moleculares las especies de *Meloidogyne* que parasitan

plantas de *Musa* AAB ‘Dominico Hartón’ y (ii) evaluar los daños de *Meloidogyne* spp. en plántulas de ‘Dominico Hartón’. Estados juveniles 2 (J2) de *Meloidogyne* fueron colectados de raíces de plantas de ‘Dominico Hartón’ en la granja de investigación Montelindo de la Universidad de Caldas, Colombia, y la identificación molecular con el PCR-RFLP y PCR-imprimadores específicos se realizó en los laboratorios de Nematología y Técnicas Moleculares de la Universidad de Costa Rica. Se identificó la mezcla de *Meloidogyne incognita* y *M. arenaria* (proporción de 1:7). Adicionalmente, la mezcla de *M. incognita* + *M. arenaria* (0, 750, 1.500, 2.250 y 3.000 huevos + estados J2 / plántula) fue inoculada en plántulas de ‘Dominico Hartón’. En comparación al control, se encontró que desde la menor hasta la mayor población, los nematodos significativamente ($P < 0,05$) redujeron el peso seco aéreo desde 25,36% a 52,58%, y el peso seco de raíces desde 39,47% a 55,26%. Las densidades de nematodos no afectaron la altura de las plántulas y el número de hojas. En conclusión, la mezcla de *M. incognita* + *M. arenaria* afectó el crecimiento de las plántulas de ‘Dominico Hartón’.

Palabras clave: *Musa* AAB, *Meloidogyne arenaria*, *M. incognita*, plátano ‘Dominico Hartón’

Meloidogyne (root-knot nematodes) are distributed worldwide, attacking many economically important crops (Powers, 2004; Jones *et al.*, 2013; Sikora *et al.*, 2018). The most common species associated with banana and plantain are *Meloidogyne incognita*, *M. arenaria*, *M. javanica*, and *M. hapla* (De Waele and Davide, 1998; Sikora *et al.*, 2018). *Meloidogyne* spp. are sedentary endoparasites found in *Musa* roots. Along with other nematodes such as *Radopholus similis* and *Pratylenchus* spp., *Meloidogyne* spp. penetrates the root endodermis and migrates to the vascular cylinder where they induce multinucleated cell formations (De Waele and Davide, 1998; De Waele, 2000). Underground symptoms are galling on primary and secondary roots, sometimes causing them to bifurcate and distort (Sikora *et al.*, 2018). Above-ground symptoms include thinner pseudostems, narrower leaves, stunted growth, yellowing in the aerial organs, and a lower production yield (Razak, 1994; De Waele, 2000; Perry *et al.*, 2009). In a study done in the Ivory Coast, plant height, leaf number, and dry root weight were not affected in *Musa* AAB ‘Horse 1’ seedlings inoculated with *M. incognita* (Adiko, 1989). Nevertheless, in the same study, dry shoot weight was significantly reduced at an initial density of 20,000 *M. incognita* per plant. In Costa Rica, Moens *et al.* (2006) evaluated the reproduction and pathogenicity of *M. incognita* on *Musa* AAA ‘Grande Naine’ grown in pots and reported no effect on fresh root and shoot weights; in microplots, the same nematode species reduced bunch weight by 7.5 kg (32%) in *Musa* AAA ‘Grande Naine’. Additionally, in the Philippines, performance of giant Cavendish dessert bananas

under field conditions infected with *M. incognita* caused a 26.4% and 57.1% of yield loss with the lowest and highest inoculum densities, respectively (Davide and Marasigan, 1985).

Meloidogyne spp. parasitize banana and plantain roots, and it is common to find a mixture of *Meloidogyne* species where these crops are grown (De Waele and Davide, 1998). In Colombia, *Meloidogyne* spp. in *Musa* are mainly characterized by morphological and morphometric characteristics, for example, *M. arenaria*, *M. incognita*, and *M. javanica* were identified with these techniques in *Musa* AAB and *Musa* AAA roots (Jaraba *et al.*, 2008; Navarro *et al.*, 2010). Molecular diagnostics with mitochondrial DNA (mtDNA), however, are becoming more widely applied to provide accuracy, speed, reliability, and affordability in diagnostics, and even to enable characterization of new species (Powers, 2004; Hu and Glasser, 2006). Recently, applying molecular techniques, Riascos *et al.* (2019), reported *M. incognita*, *M. acrita*, *M. arenaria*, and *M. hispanica* associated with *Musa* spp. crops in Colombia. There is, however, little information in Colombia regarding losses and damage caused by plant-parasitic nematodes in *Musa* (Guzmán, 2011). Therefore, the objectives of this study were to: (i) identify, with molecular techniques, the *Meloidogyne* spp. that parasitize *Musa* AAB (plantain subgroup) ‘Dominico Hartón’ and (ii) evaluate the damage of *Meloidogyne* spp. to ‘Dominico Hartón’ seedlings.

Second-stage juveniles (J2) of *Meloidogyne* spp. were collected from roots of ‘Dominico Hartón’ plants, at the Montelindo Research Farm of the Universidad de Caldas (Palestina, Caldas,

Colombia). In the Laboratories of Nematology and Molecular Techniques-CIPROC, University of Costa Rica (San Jose, Costa Rica), DNA was extracted as described by Adam *et al.* (2007) with modifications. The region of the mitochondrial genome between the cytochrome oxidase subunit II (COII) and 16S rRNA mitochondrial DNA (mtDNA) genes was amplified using primers C2F3 and 1108 (Powers and Harris, 1993). The PCR products were digested with the restriction enzyme *Hinf*I (Thermo Fisher Scientific) according to the manufacturer's instructions. PCR amplification of the mtDNA resulted in a band of ~1100 bp (Fig. 1A) in 58 samples (each represents a single J2) and ~1600 bp (Fig. 1B) in 8 samples. No digestion was

obtained with the enzyme *Hinf*I of the ~1100 bp products, corresponding to the species *M. arenaria* (Fig. 1C). Similar results to this study were previously reported by Powers and Harris (1993) and Powers *et al.* (2005) where C2F3/1108 amplification products of 1.1 kb were designated as *M. arenaria*. In contrast, the PCR products of ~1600 bp had a restriction pattern of ~1150 and ~350 bp with the same enzyme, which is the pattern previously reported for *M. incognita* (Humphreys-Pereira *et al.* 2014; Humphreys-Pereira *et al.* 2017). The *M. arenaria* to *M. incognita* ratio was 7 to 1 in the population collected from plantain. The species-specific primers Far/Rar were used to confirm the identification of *M. arenaria* (Zijlstra

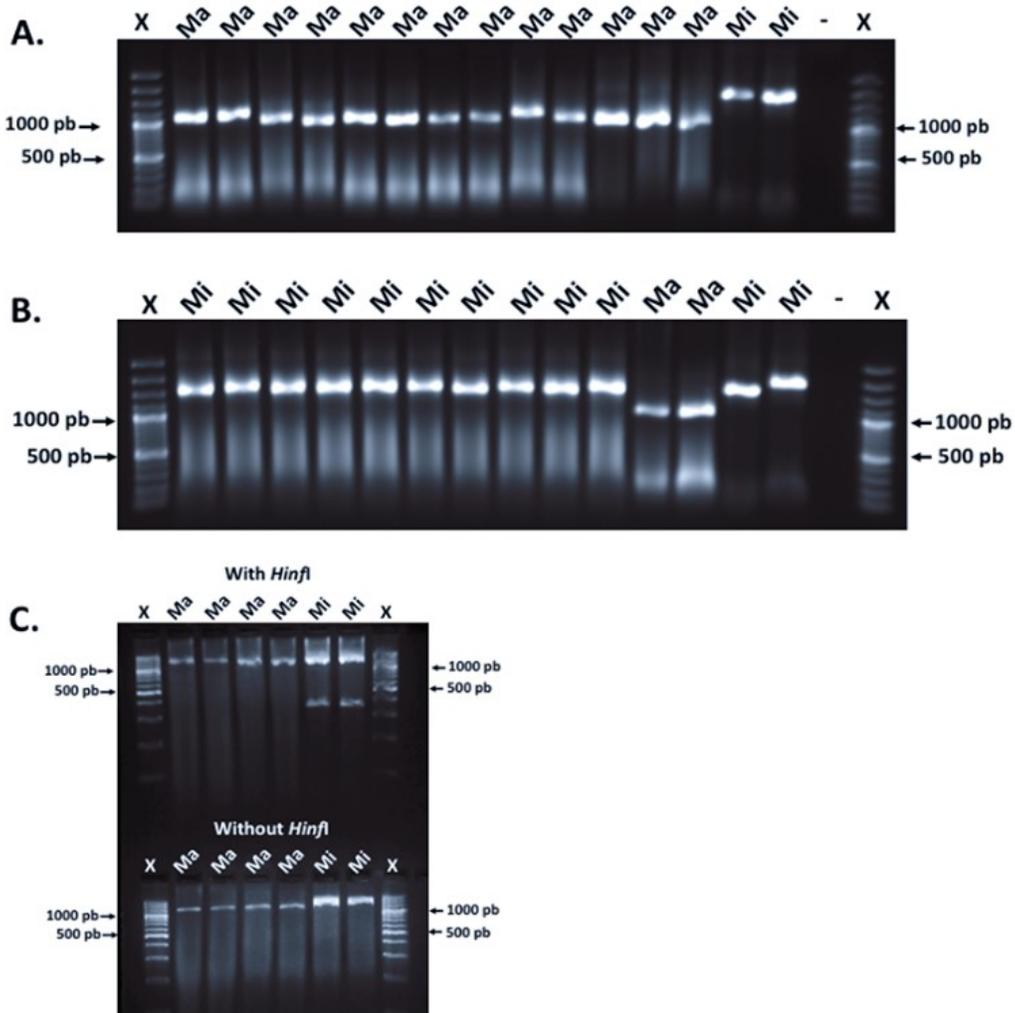


Figure 1. COII-16S rRNA PCR amplification products and PCR-RFLP patterns. A, B: Amplification products of DNA from *Meloidogyne* spp. using C2F3 and 1108 primers sets; C: Restriction patterns generated with *Hinf*I. Ma = *M. arenaria*, Mi = *M. incognita*; - = negative control; X = 100 bp DNA ladder.

et al., 2000) and primers Inc-k14F/Inc-k14R (Randig *et al.*, 2002) for *M. incognita*. Each primer set was tested on at least twelve single J2 for each species. The PCR products using *M. arenaria*-specific primers had a single band of 420 bp whereas the primers for *M. incognita* yielded a single band of 399 bp (data not shown).

Phylogenetic relationships among *Meloidogyne* spp. were inferred using Bayesian analysis implemented in MrBayes 3.2 using the GTR + G substitution model (Ronquist *et al.*, 2012). Bayesian analysis of the COII-16S rRNA mtDNA region showed that *M. arenaria* was located within the same species group (PP = 100) and within a large monophyletic group (PP = 98) containing other tropical *Meloidogyne* spp. that

infect banana and plantain (Fig. 2). The results using molecular techniques revealed the presence of concomitant *Meloidogyne* species population (*M. arenaria* and *M. incognita*) parasitizing plantain. Both *Meloidogyne* species have been reported from roots of *Musa* plants (De Waele and Davide, 1998; De Waele, 2000, Araya, 2004; Moens *et al.*, 2006; Sikora *et al.*, 2018). In Colombia, *M. arenaria*, *M. incognita* and *M. javanica* have been detected in *Musa* and identified through morphological and morphometric characteristics (Grisales and Lescot, 1999; Jaraba *et al.*, 2008; Múnera, 2008; Navarro *et al.*, 2010). Recently, Riascos *et al.* (2019) amplified and sequenced the D2-D3 expansion segments of the 28S nuclear ribosomal RNA gene as well as partial

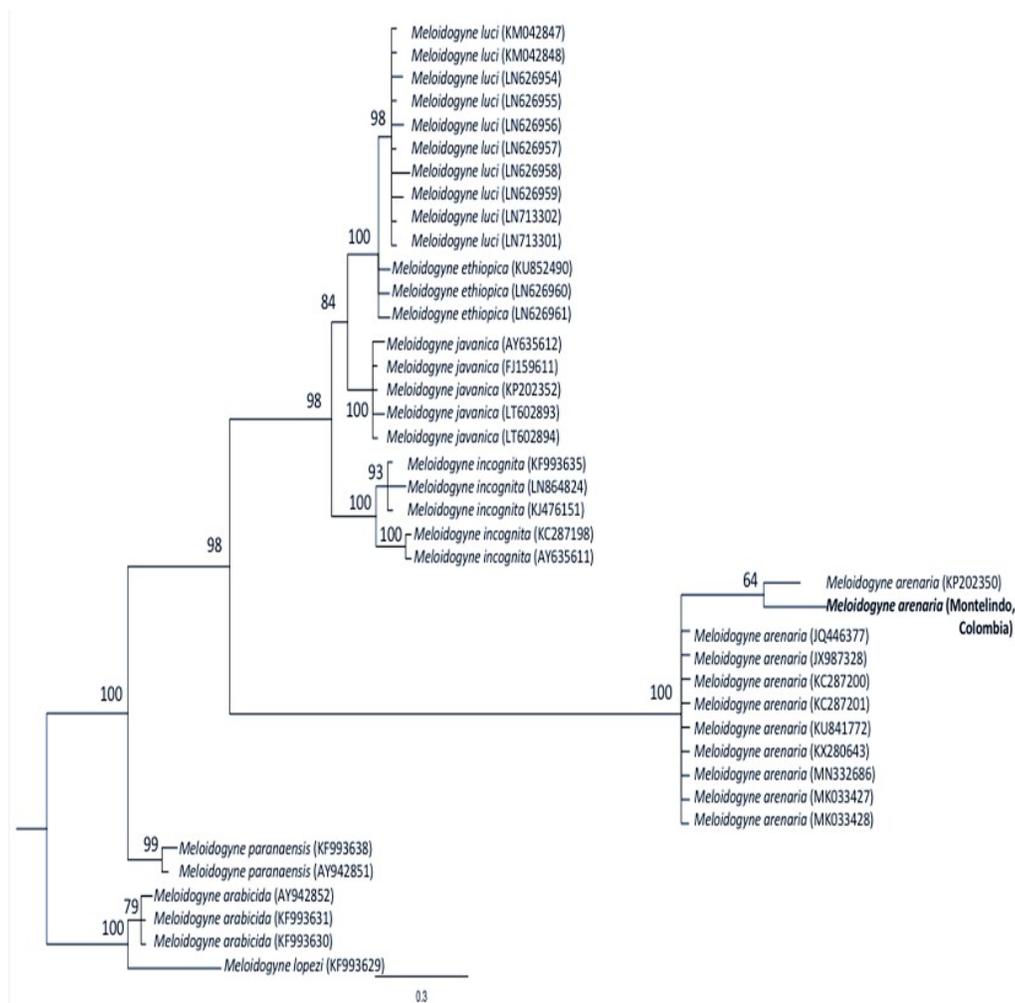


Figure 2. Bayesian analysis of *Meloidogyne* spp. based on mitochondrial DNA. Phylogenetic tree based on the region between the 3' region of COII gene and the 5' region of 16S rRNA gene. The consensus tree was generated using the GTR + G + I substitution model. Numbers indicate posterior probability values for nodes. Bold phrase indicates sequence generated in this study.

region of cytochrome oxidase subunit I (COI), and NADH dehydrogenase subunit 5 (Nad5) genes of mtDNA, reporting *M. incognita*, *M. acrita*, *M. arenaria*, and *M. hispanica* associated with *Musa* spp. in Colombia.

The *M. arenaria* + *M. incognita* mixture population was used in a nursery study to evaluate the effect (hereafter referred to as *Meloidogyne* spp.) on ‘Dominico Hartón’ seedlings. Two experiments were conducted in a nursery, under field conditions, at Montelindo Research Farm of the Universidad de Caldas. Samples were processed in the nematology laboratory at Universidad de Caldas (Manizales, Caldas, Colombia). The substrate used in the experiments consisted of a mixture of soil and sand in a 3:1 ratio, which was sterilized with 50 g/m² of Dazomet (Basamid®). Forty-five days after soil sterilization, black 5-kg plastic bags 20 W × 32 L cm were filled with the sterilized substrate. The substrate was a sandy loam texture (80% sand, 14% silt and 6% clay) with pH of 4.9, base content of Ca 3.87, Mg 1.53 and K 0.53 cmol(+)/kg, and P 17, Fe 165, Mn 26.74, Zn 10.75, Cu 4.18, S 41.48 and Bo 0.04 mg/kg. In order to obtain healthy plantain plants, 10 young sucker corms (approximately 1 kg each) were collected from plantain fields and determined to be free of plant-parasitic nematodes. Suckers were cleaned with a sanitary technique based on Loos and Loos (1960) and modified by Guzmán et al. (2012). Suckers were then subjected to the stem fragments technique developed by Moïse (2003) with modifications. Fifty days after applying the stem fragments technique, young seedlings with at least three leaves were carefully extracted with a knife. All roots were removed with a knife and shoots cut at 5 cm from the seedling base. Seedlings were weighed with a precision balance (Shimadzu) and planted in the black plastic bags containing sterilized soil. Forty days after planting, plantain seedlings were infested with 750, 1,500, 2,250 and 3,000 *Meloidogyne* spp. (eggs + J2) per plant by drenching the nematode suspension around the seedling roots. Non-inoculated plants were included as controls. A completely randomized design was used, with each plant as the experimental unit and three repetitions per treatment; the experiment was repeated. The first experiment was set up in October 2017 and was harvested in April 2018 (experiment 1) while the second experiment was conducted from January to July 2019 (experiment 2). At the end of each

experiment, shoot and root dry weight, plant height, and number of leaves were determined. Nematodes were extracted by macerating the tissue with a kitchen blender (Taylor and Loegering, 1953) and separated with the centrifugal flotation technique (Jenkins, 1964). *Meloidogyne* spp. reproduction factor (Rf = final population [*Meloidogyne* spp. (eggs + J2) in soil and roots]/initial population [treatment infestation levels]) and gall index (percent) of radical nodes (Bridge and Page, 1980) was also recorded. Data was subjected to analysis of variance (ANOVA) and mean separation was done with Dunnett’s test using R, version 3.5.1 (R Core Team, 2018).

In both experiments, plants infected by *Meloidogyne* spp. had significantly ($P < 0.05$) reduced shoot and root dry weights compared to noninoculated plants (Fig. 3). Also, compared to the uninoculated control in experiment 1, from lowest to highest density, the inoculated mixture of *Meloidogyne* spp., on average, reduced shoot dry weight by 52.6% to 52.2%, respectively, and root dry weight by 49.0% to 40.4%, respectively (Fig. 3A). In experiment 2, results were similar with

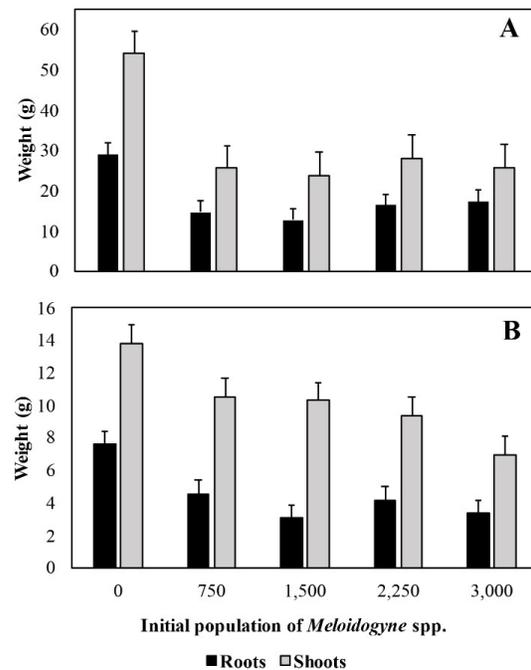


Figure 3. Effect of *Meloidogyne* spp. populations on root and shoot biomass (gram dry weight) of *Musa* AAB (plantain subgroup) ‘Dominico Hartón’ seedlings. A. Experiment 1 (October 2017 - April 2018). B. Experiment 2 (January - July 2019).

reductions in shoot dry weight of 25.4% to 49.3%, and root dry weight reduction of 39.5% to 55.3% (Fig. 3B). In general, most of the nematode densities did not affect plant height and leaf number (Table 1). Similarly, Moens *et al.* (2006) evaluated reproduction and pathogenicity of *M. incognita* on *Musa* AAA ‘Grande Naine’ grown in pots and reported no effect on fresh root and shoot weights. They found that mean root weight of inoculated plants was 6.7% higher compared with noninoculated plants, and fresh root weight in plants inoculated with *M. incognita* was reduced by 9%, compared to the control, in a microplot experiment. Van den Bergh *et al.* (2002) evaluated the effects of *Meloidogyne* spp. on selected Vietnamese *Musa* germplasm and found that inoculation with 4,000 eggs and J2 resulted in increased root weight and decreased number of standing leaves; there was no effect on plant height and shoot weight. These authors also reported that inoculation with 500 *Meloidogyne* spp. eggs and J2 resulted in increased root weight and decreased shoot weight and standing leaves number. In South Africa, Daneel *et al.* (2015), evaluated banana

tissue-culture plantlets ‘Chinese Cavendish’, ‘Williams’, ‘Grand Nain’ and ‘High Noon’ (AAAB) for their response to mixed populations of *M. incognita* and *M. javanica*. Initial densities of 0, 500, and 2,000 *Meloidogyne* per plant did not have a negative effect on fresh shoot and root weights of greenhouse-grown banana plants.

On average, at all *Meloidogyne* spp. inoculum densities, a Rf value above one was observed (Table 1), confirming that plantain ‘Dominico Hartón’ is a good host for *Meloidogyne* spp. (Barriga and Cubillos, 1980; Tenente *et al.*, 2008; Van den Bergh *et al.*, 2002) (Fig. 4). Also, in both experiments, plants inoculated with *Meloidogyne* spp. at all densities had a root gall index from 15.5% to 41.7% (Table 1). Similar to this study, Adiko (1989) found a decrease in reproduction of *M. incognita* as the inoculum level increased. Additionally, Jonathan and Rajendran (2000) reported that the multiplication of *M. incognita*, in terms of Rf, was negatively related to the initial inoculum level. The highest Rf value of 491.1 was observed at an inoculation density of 10 *M. incognita* J2, whereas Rf was only 3.3 at the

Table 1. Plant growth of (height and number of leaves) and *Meloidogyne* spp. outcomes (reproduction factor and gall index) on *Musa* AAB ‘Dominico Hartón’ inoculated with varying initial densities of a mixed population of *M. incognita* and *M. arenaria*.

Experiment	Nematode density ^x	Plant height (cm) ^y	Number of leaves ^y	Rf (Pf/Pi) ^z	Gall index (%) ^z
1	0	47.5	6	0	0
	750	37.0*	4.7	14	15.5
	1,500	35.0*	5	24.3	33.3
	2,250	41.5	4.5	23.2	41.7
	3,000	41.7	5	2.4	41.7
2	0	26.7	6.3	0	0
	750	31	4.3	69.5	20
	1,500	30.3	4.3	22	41.7
	2,250	27	3.7*	11.1	33.3
	3,000	23	3.3*	17.9	33.3

^x Infestation densities of 0, 750, 1,500, 2,250, and 3,000 *Meloidogyne* spp. (eggs + J2) / plant.

^y Response variables used in analysis of variance (ANOVA); (*) indicates significant differences ($P < 0.05$) compared to the uninfested control, according to Dunnett’s test.

^z *Meloidogyne* spp. reproduction factor (Rf = Pf/Pi), where Pf: final population [*Meloidogyne* spp. (eggs + J2) in soil and roots] at the end of the experiment, and Pi: initial population [treatment infestation levels]; and gall index (percent) of radical nodes (Bridge and Page, 1980).



Figure 4. *Meloidogyne* spp. symptoms on *Musa* AAB (plantain subgroup) ‘Dominico Hartón’ seedlings: A. Plant with root knots; B. Healthy plant (left) and parasitized plant (right); C, D. Root knots caused by nematodes. E. Roots with *Meloidogyne* spp. nematode females on root knots; F. Roots proliferation in parasitized (left) and healthy roots (right). G. Excessive root proliferation with knots.

inoculum density of 10,000 *M. incognita* J2. This is probably due to fewer roots being available when inoculation density is high, thus creating crowded conditions around feeding sites which adversely affected the rate of nematode development (Gonçalves, 1998).

Cofcewicz *et al.* (2004) also obtained high Rf values that ranged between 3.0 and 14.6 when evaluating the effect of *M. incognita*, *M. arenaria* and *M. javanica*, alone and in combination, on five *Musaceae* genotypes in Brazil, with initial population densities of 6,000 eggs for each species. Sikora *et al.* (2018) reported *M. arenaria*, *M. incognita*, and *M. javanica* as widely distributed in different *Musa* spp.-producing regions in the world. In the Philippines, *M. incognita* and *M. arenaria* were detected in 82% of giant Cavendish dessert banana crops. In Western Malaysia, *M. incognita* and *M. javanica* are widely distributed in commercial banana plantations, with populations of up to 2,300 eggs and J2/g root, with knots along their roots (De Waele and Davide, 1998).

In conclusion, *M. arenaria* and *M. incognita* were molecularly identified and their damage to *Musa* AAB (plantain subgroup) ‘Dominico Hartón’ seedling growth evaluated. Accurate identification of *Meloidogyne* spp. affecting *Musa* AAB crops is essential in order to establish integrated pest management tactics and to reduce losses caused by these nematodes.

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