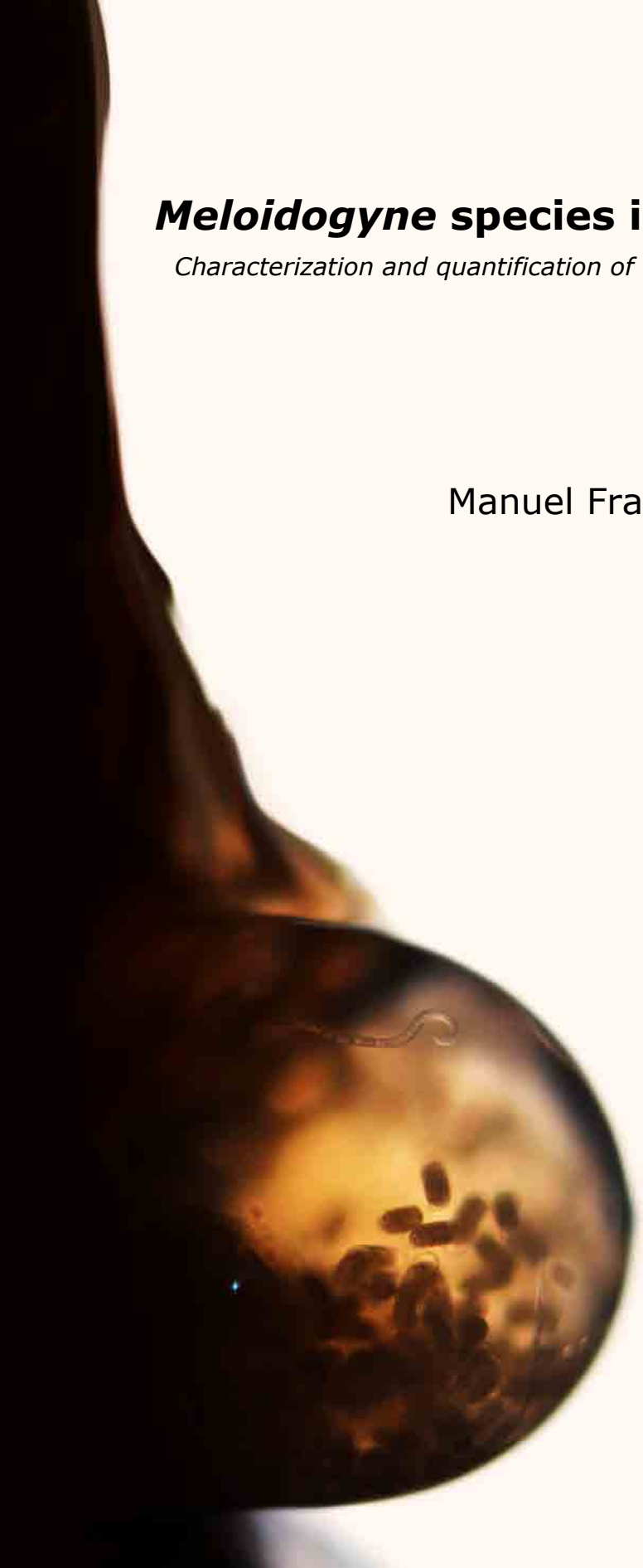
Meloidogyne species in cucurbit crops

Characterization and quantification of the host-parasite relationship



Manuel Francisco López Gómez

Front cover: Egg mass of *Meloigoyne javanica*. Eggs and juveniles in the second developmental stage are observed inside of egg mass.

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Doctoral Thesis

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Abstract

Cucurbits are often cultivated in rotation with Solanaceae in double cropping systems. Although most cultivated cucurbits are hosts of root-knot nematodes, comparative studies on their pathogenic effects on cucurbits are limited. The infection process of *Meloidogyne arenaria*, *M. incognita* and *M. javanica* was compared on zucchini squash cv. Amalthee, cucumber cv. Dasher II, melon cv. Pistolero, pumpkin cv. Totanera and watermelon cv. Sugar Baby in a growth chamber. All cucurbits were susceptible to the three isolates although *M. javanica* showed higher invasion rates, faster development and egg production than *M. arenaria*. Differences among cucurbits were primarily due to root invasion rates and formation of egg masses. Cucumber and melon were better hosts for nematode invasion and reproduction than zucchini followed by watermelon that was the poorest host. Large invasion rates followed by small reproduction traits were linked to *M. incognita* on zucchini. Reduced invasion rates, eggs mass numbers, and delayed early development were shown on watermelon.

Information on the host suitability of rotational crops to the nematode is useful because population densities rapidly build-up on susceptible crops under favorable conditions. The genetic background of the host plant and the parasitic variation in *Meloidogyne* affect the host-parasite relationship. In a series of experiments, the suitability of five zucchini and three cucumber genotypes to *M. incognita* (MiPM26) and *M. javanica* (Mj05) was determined in pot experiments in a greenhouse. The number of egg masses did not differ among the genotypes of zucchini or cucumber, but the eggs/plant and the reproduction factor (eggs per plant/nematode inoculum) did slightly. A marked differenced was observed between the nematode isolates; *M. incognita* MiPM26 showed lower reproduction traits than *M. javanica* Mj05. Examination of the zucchini galls for nematode post-infection development revealed unsuitable conditions for *M. incognita* since only 22% of the females produced egg masses compared to 95% of the *M. javanica* females. In cucumber, 86% of the *M. incognita* and 99% of the *M. javanica* females produced egg masses, respectively. In a second type of experiments, several populations of *M. arenaria*, *M. incognita* and *M. javanica* were tested on zucchini cv. Amalthee and cucumber cv. Dasher II to assess the parasitic variation amongst species and populations of

Meloidogyne. A greater parasitic variation was observed on zucchini than cucumber. Zucchini responded as a poor host for *M. incognita* MiPM26, MiAL09 and MiAL48, but as a good host for MiAL10 and MiAL15. Intra-specific variation was not observed amongst the *M. javanica* or *M. arenaria* populations. Cucumber was a good host for all the tested populations. Both cucurbits were suitable hosts for *Meloidogyne* but zucchini was a poorer host than the cucumber.

The host suitability of eight watermelon cultivars and seven cucurbit rootstocks to *M. incognita* and *M. javanica* was assessed in pot and field experiments. *Meloidogyne incognita* showed higher reproduction than *M. javanica* on the watermelon cultivars and cucurbit rootstocks. The watermelon cultivars did not differ in host status within each nematode isolate and they supported lower reproduction than the cucurbit rootstocks. Egg production increased with increasing initial inoculum level (*Pi*) on non-grafted Sugar Baby but the reproduction factor was similar at the two *Pi* levels. The total egg production on plant grafted onto the rootstocks RS841 and Titan (*C. máxima* x *C. mochata*) was higher at the higher *Pi,* but the reproduction factor was lower. The top development of field-grown non-grafted watermelon plants was significantly delayed in plots where the nematodes were detected at planting. However, no differences were observed in plots with grafted plants. In plots with nematodes, non-grafted and Titan-grafted plants had similar yield, which was higher than that of RS841-grafted plants. The Titan-Sugar Baby combination was tolerant *to M. javanica.* In the commercial plastic houses with grafted watermelon, the average reproduction factor was 42-fold which confirmed the high susceptibility of the squash hybrids as rootstocks for grafted watermelon.

Crop yield losses are influenced by preplanting population densities and the damage potential of the nematode to the crop has been described by mathematical models. Understanding the host-parasite relationship on cucurbit crops is necessary for predicting yield losses and modelling the nematode population dynamics. The relationship between the *Pi* and final (*Pf*) population densities of *M. javanica* in response to increasing initial inoculum levels and the effect on yield in zucchini cv. Amalthee were determined using a geometric series of 12 *Pi* from 0 to 51,200

eggs/100 cm^3 of soil in pot experiments in a greenhouse. The maximum multiplication rate of the nematode was 425, and the equilibrium density was 701,951 eggs/100 cm^3 soil. The relative yield, represented as dry top weight, fitted the Seinhorst damage function model and the minimum relative yield (*m*) was 0.82 and the tolerance limit (*T*) was 402 J_2/100 cm^3 soil. Regression analyses indicated a negative relationship between the *Pi* and the leaf chlorophyll content 40, 50, 60, and 70 days post-inoculation. The *Pi* and leaf chlorophyll content fitted the Seinhorst damage-function model. Zucchini cv. Dyamant was planted in a plastic greenhouse with a range of *M. javanica Pi* from 0 to 861 J_2/100 cm^3 soil. The maximum multiplication rate of *M. javanica* under field conditions was 3,093, and the equilibrium density was 1,485 J_2/100 cm^3 soil. The relationship between *Pi* and relative yield, represented as fruit weight, fitted the Seinhorst damage function model and *m* was 0.48, and *T* was 0.02 J_2/100 cm^3 soil.

The relationship between the *Pi* and *Pf* of *M. javanica* in response to increasing initial inoculum levels and the effect on yield in watermelon cv. Sugar Baby were determined in pot and field experiments. In the pots, the maximum reproduction rate of the nematode was 14, and the equilibrium density 49,400 eggs/100 cm^3 of soil. Yield data represented as fresh top weight fitted the Seinhorst damage function, and *m* was 0.65 and *T* was 74 eggs/100 cm^3 of soil. In the field experiments (2011 and 2012), the maximum reproduction rate was 73 and 70, and the equilibrium density 32 and 35 J_2/100 cm^3 soil. Yield data, represented as fruit weight, fitted the Seinhorst damage function in 2011 and the *m* and *T* values were 0.63 and 20 J_2/100 cm^3 of soil, respectively.

Resumen

Las cucurbitáceas se cultivan comúnmente en rotación con las solanáceas en muchas áreas de producción agrícola. Aunque la mayoría de los cultivos comerciales de cucurbitáceas son susceptibles a *Meloidogyne,* hay pocos estudios comparativos que aporten información sobre el efecto del patógeno en la planta. Por ello, se estudió el proceso infectivo y el desarrollo post-infeccional de *Meloidogyne arenaria, M. incognita* y *M. javanica* en calabacín cv. Amalthee, pepino cv. Dasher II, melón cv. Pistolero, calabaza cv. Totanera y sandía cv. Sugar Baby. Como resultado indicar que todas las cucurbitáceas ensayadas fueron susceptibles a los tres aislados, aunque *M. javanica* mostró mayor tasa de invasión, desarrollo más rápido y mayor producción de huevos que *M. arenaria*. Las diferencias observadas entre las cucurbitáceas se debieron principalmente a la tasa de invasión y a la formación de masas de huevos. El pepino y el melón presentaron mayor tasa de invasión y reproducción que el calabacín y la calabaza, seguidos de la sandía, que presentó los valores más bajos. El calabacín infectado por *M. incognita* mostró una elevada tasa de invasión, seguida de una baja tasa reproductiva. La sandía presentó menor tasa de invasión y formación de masas de huevos, y un retraso en el desarrollo del nematodo que las otras cucurbitáceas.

La información sobre la idoneidad de los cultivos frente al nematodo de los cultivos que componen las rotaciones es útil, ya que las densidades poblacionales del nematodo aumentan rápidamente en los cultivos susceptibles bajo condiciones favorables. El fondo genético de la planta huésped y la variación parasítica de *Meloidogyne* influyen en la interacción planta-nematodo. Se realizaron una serie de experimentos para estudiar la susceptibilidad de cinco genotipos de calabacín y tres genotipos de pepino a dos poblaciones de *Meloidogyne*: *M. incognita* (MiPM26) y *M. javanica* (Mj05). El número de masas de huevos no difirió entre los genotipos de calabacín o pepino, pero se observaron ligeras diferencias en la producción de huevos y la tasa de reproducción (huevos por planta/inóculo inicial). *Meloidogyne incognita* mostró menor número de masas de huevos y huevos/planta que *M. javanica*. El examen de las agallas formadas

en las raíces de calabacín reveló que el calabacín proporciona condiciones inadecuadas para el desarrollo de *M. incognita*, ya que sólo el 22% de las hembras produjo masas de huevos en comparación con el 95% de las hembras de *M. javanica*. En pepino, el 86% de las hembras de *M. incognita* y el 99% de *M. javanica* produjeron masas de huevos. En otra serie de experimentos, se inocularon varias poblaciones de *M. arenaria*, *M. incognita* y *M. javanica* en calabacín cv. Amalthee y pepino cv. Dasher II para determinar la variación parasítica entre las especies y poblaciones de *Meloidogyne*. En calabacín se observo una mayor variación parasitaria que en pepino, comportándose como huésped pobre para las poblaciones de *M. incognita* MiPM26, MiAL09 y MiAL48, mientras que respondía como buen huésped para las poblaciones MiAL10 y MiAL15. Las poblaciones de *M. javanica* o *M. arenaria* no mostraron variación intra-específica. El pepino fue un buen huésped para todas las poblaciones ensayadas. Ambas cucurbitáceas fueron huéspedes idóneos para las especies de *Meloidogyne,* pero el calabacín era peor huésped que el pepino.

Se estudió la idoneidad de ocho cultivares de sandía y siete patrones de cucurbitáceas frente a *M. incognita* y *M. javanica*, en condiciones de invernadero y en campo. *M. incognita* mostró mayor reproducción que *M. javanica* en los cultivares de sandía y patrones de cucurbitáceas. Los cultivares de sandía no difirieron en la idoneidad del huésped para cada aislado del nematodo y soportaron menos reproducción que los patrones de cucurbitáceas. Al estudiar el efecto de la *Pi* se observó que la producción de huevos aumentaba con el aumento de *Pi* en la sandía no injertada, pero no así la tasa de reproducción que no difería entre valores de *Pi*. La producción de huevos/planta en sandía injertada sobre los patrones híbridos de *C. máxima* x *C. mochata* RS841 y Titan fue mayor en la *Pi* superior, pero la tasa de reproducción disminuyó. En condiciones de campo, las sandías no injertadas mostraron un retrasó significativo en su desarrollo aéreo en las parcelas donde se detecto el nematodo al inicio del cultivo. Sin embargo, no se observo tal efecto en las sandías injertadas. En las parcelas donde se detectó en el nematodo al inicio del cultivo, las sandías no injertadas y las injertadas sobre Titan mostraron un producción similar, mayor que la de las plantas injertadas sobre RS841 en parcelas con presencia del nematodo al inicio del cultivo. El injerto

de la sandía sobre Titan proporciono tolerancia frente a *M. javanica*. En invernaderos comerciales donde se utilizaba las sandías injertadas, el valor medio de la tasa de reproducción fue 42 veces el nivel inicial, lo cual confirmó la alta susceptibilidad de los patrones híbridos que se utilizan como patrones de sandía.

Las densidades poblacionales de nematodos en el suelo antes de la siembra o trasplante influyen en las pérdidas de producción que puede experimentar un cultivo. Estas pérdidas pueden explicarse mediante modelos matemáticos que describen el daño potencial del nematodo en una especie vegetal determinada. Para poder predecir tales pérdidas y modelizar la dinámica poblacional del nematodo, es necesario estudiar la interacción huésped-parásito. Por ello, se determinó la relación entre *Pi* y *Pf* de *M. javanica* en respuesta a niveles crecientes de *Pi,* y el efecto sobre la producción de calabacín cv. Amalthee utilizando una serie geométrica de 12 *Pi* crecientes comprendidas entre 0 y 51.200 huevos/100 cm^3 de suelo en un invernadero. La tasa máxima de multiplicación del nematodo fue 425, y la densidad de equilibrio fue 701.951 huevos/100 cm^3 suelo. La producción, medida como peso seco de la biomasa aérea , se ajustó al modelo de daño de Seinhorst, dando como resultado una producción mínima (*m*) de 0,82 y un límite de tolerancia (*T*) de 402 J_2/100 cm^3 de suelo. Se realizó un análisis de regresión entre la *Pi* y el contenido de clorofila de las hojas a 40, 50, 60, y 70 días después de la inoculación, la cual indicó una relación negativa entre ambos parámetros, ajustándose al modelo de daño de Seinhorst. El calabacín cv. Dyamant se plantó en un invernadero de plástico infestado con *M. javanica* con *Pi* que oscilaba entre 0 y 861 J_2/100 cm^3 de suelo. La tasa máxima de multiplicación de *M. javanica* fue 3093, y la densidad de equilibrio fue de 1485 J_2/100 cm^3 de suelo. La relación entre la *Pi* y la producción, medida como peso de los frutos/parcela elemental, se ajustó al modelo de daño de Seinhorst; el valor de *m* fue 0,48, y el de *T* fue 0,02 J_2/100 cm^3 de suelo.

La relación entre la *Pi* y la *Pf* de *M. javanica* en respuesta inóculos iniciales crecientes y el efecto en producción de sandía cv. Sugar Baby se determinó en experimentos en maceta y en campo. En maceta, la tasa máxima de reproducción del nematodo fue de

14, y la densidad de equilibrio fue de 49.400 huevos/100 cm^3 de suelo. Los datos de producción, representados como peso fresco de la biomasa aérea, se ajustaron al modelo de daño de Seinhorst, siendo *m* igual a 0,65 y *T* igual a 74 huevos/100 cm^3 de suelo. En los experimentos de campo (años 2011 y 2012), la tasa máxima de reproducción fue de 73 y 70, y la densidad de equilibrio de 32 y 35 J_2/100 cm^3 de suelo. Los datos de producción, expresada en peso de los frutos/ parcela elemental, se ajustó a la función de daño de Seinhorst en 2011, siendo los valores *m* y *T* 0,63 y 20 J_2/100 cm^3 de suelo, respectivamente.

Introduction

Cucurbits

The *Cucurbitaceae* family includes 120 genera and over 800 species clustered in two subfamilies: *Cucurbitoideae* and *Zanonoideae*. The *Cucurbitoideae* subfamily integrates the most important genera from an agronomically point of view such as the *Cucumis*, *Citrullus* and *Cucurbita.* The genus *Cucumis* includes cucumber (*C. sativus* L.) and melon (*C. melo* L.), and *Cucurbita* includes pumpkins (*C. maxima* Dusch and *C. maxima* var *mochata* Dusch) and zucchini (*C. pepo* L.). The genus *Citrullus* includes watermelon (*C. lanatus* var. *lanatus* (Thunb.) Matsum. & Nakai).

The origin of the cucurbits occurred in different locations around the world. Cucumbers originated in Western Asia, where even now wild ancestors can be found in the foothills of the Himalaya Mountains. The domestication of these species was mostly done in Asia, and they were introduced from India to China, North Africa and Southern Europe. Later, they were carried to new world by Columbus in 1494 (Staub et al., 1997). The species of *Cucurbita* spp. originated in different locations of America. Pumpkins originated in Mexico, Peru and in the Eastern side of the United States, from 10000 BCE. However, the origins of zucchini occurred earlier than pumpkins. It was domesticated in Mexico as early as 7500 BCE and in the Mississippi valley between 3000 and 1000 BCE (Deyo and O´Malley, 2008). Both genera were introduced in Europe by Columbus. Watermelons originated in Africa, but they were domesticated in multiple areas of secondary diversity such as the Middle and the Near East and India. From there, they were spread across Mediterranean areas, and then, they were introduced in Europe and the Americas (McCreight et al., 2013).

Currently, these cucurbit crops are staple items in the diet. The worldwide cultivation area of the afore-mentioned species is 7.4 million hectares (ha), and the production is 195 x 10^6 tonnes (t) according to the United Nations Food and Agriculture Organization (FAO, 2012). The largest producer of cucurbits is China, distantly followed by Turkey. The European Union (EU) cultivation area is 193080 ha, and it produces 7 x 10^6 t. Spain is the highest producing country in the EU (30 %, 2 x 10^6 t) and the

second largest in cultivation area (22%, 42300 ha) after Romania (23%, 44879 ha). Among the cucurbit species cultivated in Spain, melon (39% of the cultivated surface of Spain) is the most extensively cultivated crop, followed by watermelon (26%). As far as the total production is concerned, melon and watermelon each represent 29%, of the total production of cucurbits, followed by cucumber (20 % of the surface area and 24% of production), squash and zucchini (both, 14% of the surface area and 17% of production).

The genus Meloidogyne Goeldi, 1892

In 1878, Jobert observed a new disease produced by little nematoid worms in coffee trees in Brazil (Jobert, 1878). However, it was ten years later when Göldi investigated the disease, and named the causing agent root-knot nematode (RKN) (Göldi, 1887). In his publication, the first RKN was described and named *Meloidogyne exigua.* It was included into the genus *Heterodera* (cyst nematodes) and was synonymized with *H. marioni* until 1949. In this year, Chitwood (1949) removed the RKN from *Heterodera* due to differences with cyst nematodes, and described a new genus named *Meloidogyne* that included *M. arernaria* (Neal) Chitwood, *M. exigua* Göldi, *M. incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood and *M. hapla* Chitwood.

The genus *Meloidogyne* falls into the Phylum Nemata (or Nematoda), Class Secernentea, Order Tylenchida, Superfamily Tylenchoidea and Family Heteroideride. Nowadays, the genus *Meloidogyne* is composed by more than ninety species (Hunt and Handoo, 2009). In Europe, twenty-three species have been found, and some of them have been added to the European and Mediterranean Plant Protection Organization Alert List (EPPO) (EPPO, 2008). In Spain, ten species of *Meloidogyne* have been reported (Andres et al., 1998, Castillo et al., 2003; 2009; Gómez Barcina et al., 1989; Peña-Santiago et al., 2004; Palomares-Ruis et al., 2007). The most common species of *Meloidogyne* worldwide are *M. arenaria*, *M. incognita* and *M. javanica*, and their distribution in Spain is detailed in Table 1.

Table 1[1]. Distribution of the most common species of *Meloidogyne* in Spain.

Region	*M. arenaria*	*M. incognita*	*M. javanica*
Andalousia	+	+	+
Aragon	+	+	-
Castilla La Mancha	+	+	-
Catalonnia	+	+	+
Madrid	-	+	-
Extremadura	+	+	+
Canary Islands	+	-	+
Murcia	+	+	+
The Basque Country	+	+	-

The (+) symbol indicates "present" and (-) nematode not detected.
[1]Adapted from Melgarejo et al. 2010.

The host range of *Meloidogyne* spp. is quite wide, including the majority of fruits, vegetables and ornamental plants. Only *M. arenaria*, *M. incognita* and *M. javanica* infect more than 2000 plant species (Hussey, 1985). In addition, they have been recognized as a menace for global food production due to the yield and economic losses they caused (Sasser, 1980). In Spain, the impact of *Meloidogyne* spp. in the current horticulture is increasing due to the intensification of the agriculture and monoculture mainly under protected cultivation. In these systems the nematode disposes of host plants and suitable temperatures all year long (Ornat et al., 1999). The estimated economic losses caused by *Meloidogyne* in cucurbit crops under protected cultivation in Spain were €918290 for cucumber, €649500 for zucchini and €451940 for watermelon (Talavera et al., 2012).

Biology and life cycle

Meloidogyne spp. is a poikilothermic organism, thus, temperature influences its life cycle, determining its length (Tyler, 1933). The nematode development occurs between 10ºC and 32ºC, and needs to accumulate an amount of degree-days (*K*) at a certain basal temperature (*Tb*) to complete its life cycle (Trudgill, 1995). Both constants are influenced by the host plant and the nematode population (Madulu and Trudgill, 1994).

Meloidogyne spp. is a sedentary endoparasit; this means that the nematode stays and feeds inside roots during the majority of its life cycle. Its life cycle comprises the egg stage, four juvenile stages, and the adult stage. Juveniles from the first (J1) and the second (J2) stage are vermiform. The third (J3) and fourth (J4) stages juveniles grow slightly in width, adopting a sausage-like shape. The nematodes from these four stages present a medium bulb and stylet. The adult stage presents sexual dimorphism; the female has a pear-shaped asymmetrical body, stylet, medium bulb, vulva and perineal pattern. It remains inside the root for the duration of its life cycle, and deposits the new progeny into a gelatinous matrix outside the root at the end of the cycle. On the contrary, the male presents a vermiform shape, large size and non-visible stylet (Karssen and Moens, 2006). Male migrates outside the roots, decreasing the intraspecific competition inside the galls. Thus, the reproduction strategy of RKN is mitotic parthenogenesis of the female, providing a conservation of the genome, which confers a wide host range.

The first moult occurs inside the egg, where the J1 moults to J2, which is the infective stage. J2 hatches from egg, and it migrates to soil. Once the J2 has hatched, it moves around the soil water phase, and is attracted by host plant exudates. J2 penetrates the root behind the root tip and migrates between cells through the interstitial space into the vascular cylinder. J2 punctures the stylet into previously selected cells, introducing secretions synthesized by the esophageal glands. A genetic transformation is induced in the cells, turning them into giant cells, and the feeding site is established. The giant cells are metabolically very active, with a dense cytoplasm formed by a high number of organelles such as

ribosomes and mitochondrias, which will supply the food to the nematode. At the same time, there is an intense cell multiplication (hyperplasia) around the giant cell, forming the gall. Once the feeding site is established, the nematode sex is differentiated (Taylor and Sasser, 1978), and J2 molts to J3, J4 and adult. Under favorable conditions, the nematode differentiates into female. On the contrary, under unfavorable conditions, the nematode differentiates into male, decreasing the competition between nematodes. At the completion of the life cycle, the female lays a gelatinous matrix and deposits the eggs of the new progeny inside it, forming the egg mass (*EM*).

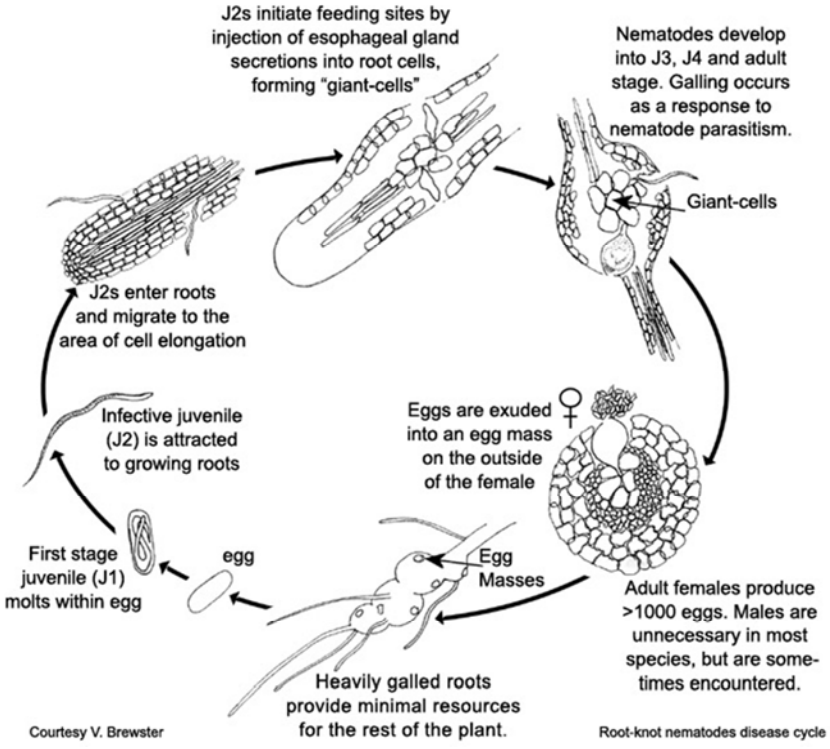

Fig. 1. Life cycle of *Meloidogyne* spp. Mitkowski and Abawi, 2003.

Epidemiology

In annual crops, the amount of nematode in the soil before transplantation is the initial population density (P_i). The relationship between the P_i with the final population density (population density at the end of the crop cycle; P_f) is studied by population dynamics, a mathematic model based on Nicholson´s competition model (1935). The population dynamics is defined as "the variation in the numbers of nematodes over time" (Greco and Di Vito, 2009), serving as an informative tool used to implement a correct integrate pest management. The relationship between *Pi* and *Pf* is measured by the reproduction factor (P_f/P_i, *Rf*), indicating how many times the P_i is multiplied at the end of the crop. In a theoretical situation, when the nematode has enough food and space, intraspecific competition is non-existing, the *Rf* adopts a constant value and the relationship between P_i and P_f is a straight line. However, in nature, the increase of the P_i raises the competition between nematodes as the available space and food decrease, thus, the *Rf* decreases with the increasing P_i. The relationship between P_f and P_i describes a logistic curve model (Fig. 2), where three parts can be differentiated:

i) *Lineal growth*. At low P_i, the P_f values increase constantly due to the absence of limiting factors, being lineal the relationship between P_f and P_i. The slope function in this linear growth ($P_f = a\ P_i$) is the maximum reproduction rate (*a*).

ii) *Maximum population density* (*M*). At medium P_i value, the P_f reaches its maximum value.

iii) *Equilibrium density* (*E*). It is a stabilization point, where there is enough food to maintain a P_f value similar to the P_i, being the $Rf = 1$.

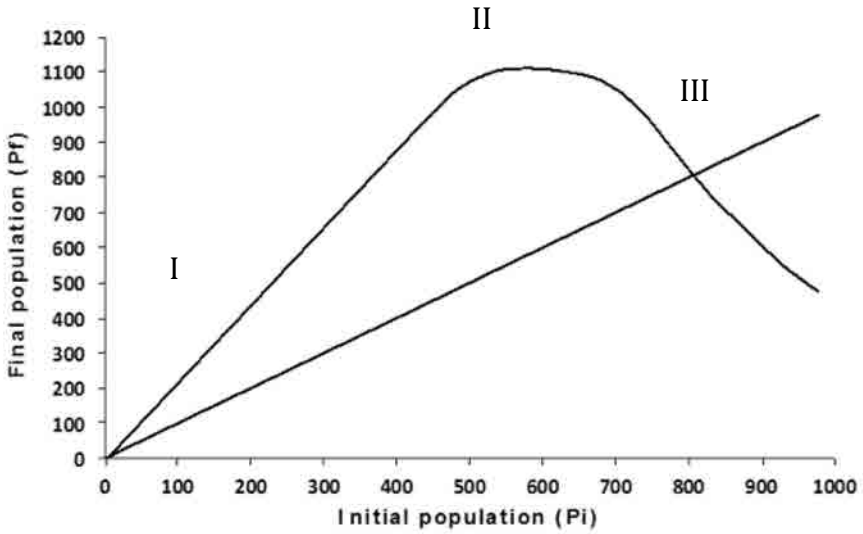

Fig. 2. Relationship between initial (P_i) and final (P_f) population densities of *Meloidogyne* spp. I) Linear growth ($P_f = a\ P_i$); II) maximum nematode population ($M = Ea/(a\text{-}1)$); III) equilibrium density ($Pf = Pi$).

From this model, two different constants can be calculated; the maximum reproduction rate (*a*) and the equilibrium density (*E*) (Seinhorst, 1966; 1976; 1970). The *a* value is defined as "the reproduction rate occurring at a very low nematode density on a given host plant" (Greco and Di Vito, 2009), and it is calculated as the slope of the strait line at very low P_i values (Fig. 1)

$$P_f = a\ P_i$$

The *E* value is defined as "the P_i at which *Rf* is equal to 1" (Greco and Di Vito, 2009), and it is calculated according to Schomaker and Been (2006) as:

$$M = E\ a/(a\text{-}1)$$

These two constants, *a* and *E,* are used to define the host status of a plant. On a good host, the values of the constants are larger than on poor hosts, and for non-hosts they are 0. Theoretical different host conditions are shown in Fig. 3.

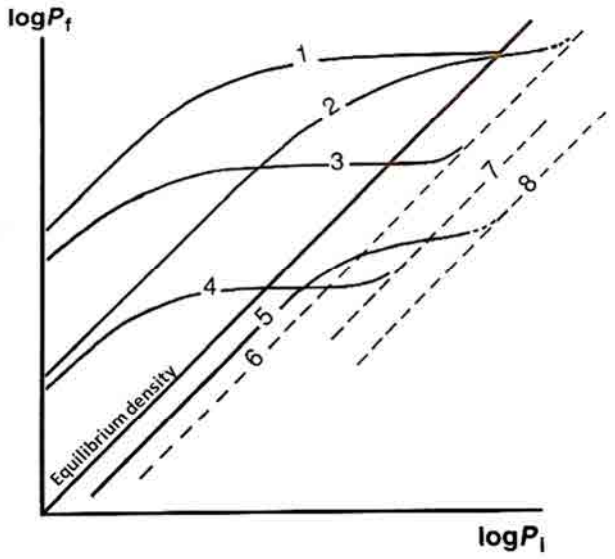

Fig.3. Host condition. Lines 1 and 2 are good hosts. Line 3 is an intermediate host. Line 4 and 5 are poor hosts. Lines 6, 7 and 8 are non-host plants. Seinhorst, 1965.

Plants infected by *Meloidogyne* spp. show several symptoms such as stunted growth, wilting and yellowing. These symptoms are the consequence of the damage occurred in the root system, which reduces the absorption of water and nutrients from the soil. Thus, the disease produces quantitative yield losses and decreases the quality of fruits. Yield losses are related to the P_i, which determines the nematode effect in plant production. The potential damage of the nematode population is described by a mathematical model (Seinhorst, 1965), providing information about the crop tolerance to the nematode infection and the crop damage. To model the damage function, it is necessary to determine the crop yield at different P_i levels. The formula of the model developed by Seinhorst (1965) is, when $P_i \geq T$, $y = 1$ when $P_i < T$:

$$y = m + (1-m)\, z^{(P_i-T)}$$

where y = yield, m = the minimum yield, z = a constant ≤ 1, P_i = initial population density, and T = tolerance limit. The tolerance limit *(T)* is the density of the nematode, below of which

yield loss does not occur, the minimum yield *(m)* is the yield that an infected crop can produce and the *z* value is the damage produced by a single nematode. These constants are indicators of crop tolerance, and they allow us to know the potential yield losses produced by the disease. The Fig. 4 presents an example of the Seinhorst model with *T* = 0.25 and m = 0.

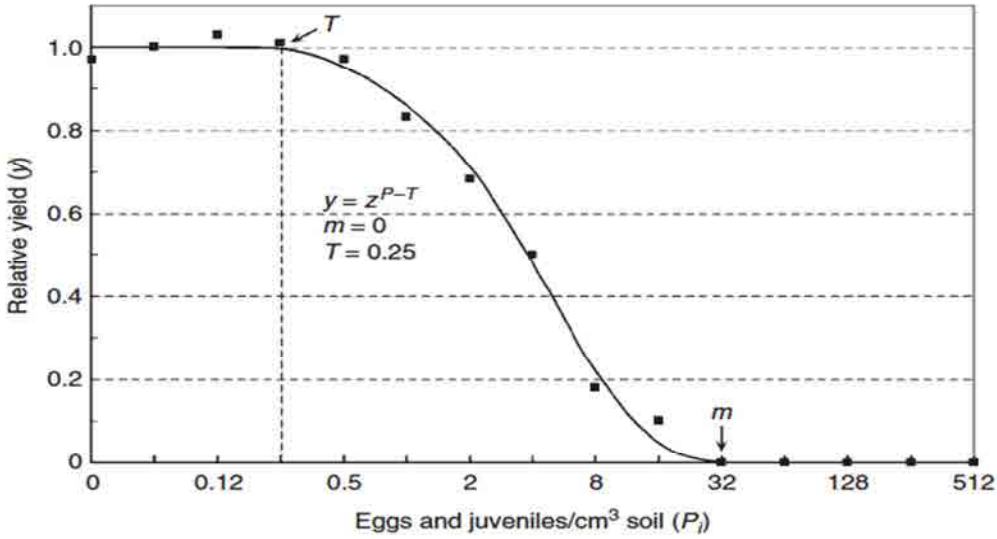

Fig. 4. Seinhorst damage function model, where y = relative yield, *m* = minimum yield, *z* = regression parameter, *P* = initial population level, *T* = tolerance limit. Greco and Di Vito (2009).

Management and control methods

The strategies for the management and control of RKN include chemical substances (fumigants and non-fumigant nematicides), physical, biological and cultural methods. The first use of chemicals to control the root-knot nematode disease was reported in 1881 (Rich et al., 2004). Since then, the use of chemicals has evolved over time. Traditionally, methyl bromide (*MeBr*) has been the most common chemical used in control method. However, it disappeared due to legislations such as the Montreal Protocol of 1987, where specific reduction measures were taken and subsequently led to the phase-out of production, import and consumption of ozone-depleting substances. Nowadays, there exist other fumigants and non-fumigants nematicides, which are widely used in the infected-fields of Spain such as 1,3-dichloropropene and metam-sodium (Talavera et al., 2012). On the other hand, the non-chemical control methods are gaining wide popularity. They can be combined with chemicals to increase the effectiveness against the disease.

Biological control employs natural enemies of nematodes to control the disease. Different organisms have been characterized as antagonists to nematodes including parasitic bacteria and fungi. Examples of these organisms are the endospore-forming bacteria *Pasteruria* spp., which infects the J2, and the *Pochonia chlamydosporia* var. *chlamydosporia*, which is an egg parasite. Both reduce nematode damage and nematode reproduction in the field.

Trap crops are plants used to decrease the nematode population in the soil. They can be antagonistic plants to *Meloidogyne* spp., as *Tagetes* spp., or *Asteraceae* spp. It can also be host plants to *Meloidogyne* spp. with short cycle like *Lactuca sativa* and *Raphanus sativus.*

*Cover crops***.** Non-host crops to *Meloidogyne* spp. are used to maintain low nematode populations densities. They are grown between cropping cycles or incorporated to the soils as green manure. Popular cover crops are cowpea (*Vigna unguiculata*), sorghum- (*Sorghum bicolor* × *S. sudanense*), sunn hemp (*Crotalaria juncea*), marigolds (*Tagetes* spp.), sesame (*Sesamum*

indicum), wheat (*Triticum aestivum*), oats (*Avena sativa*), crimson clover (*Trifolium incarnatum*), vetch (*Vicia* spp.), and lupine (*Lupinus angustifolius*).

*Biofumigation***.** The biodegradation of organic matter produces volatile substances that are used to control plant pathogens in infested fields. In addition, the biofumigation can contribute to the improvement of the soil characteristics.

Solarization is a technique that uses the radiant heat from the sun as a lethal agent against nematodes. It is practiced by disposing a clear polyethylene mulch to trap solar heat into the soil. The soil temperature increases over 45 ºC for a period of several weeks to a few months. Although this temperature is enough to kill the nematodes in the soil, is less effective against migratory juveniles than against eggs (Bello et al., 2001).

Steam heat is the sterilization of soil by water steam at high temperatures in order to sterilize a greenhouse soil. The recommended soil temperature increase is 45 ºC, enough to kill the parasitic nematodes. In recent years the use of this technique has been limited by the high costs of producing water steam (Nyczepir and Thomas, 2006).

Time of planting. Root-knot nematode is a poikilothermic organism, being medium temperatures their optimum temperature for development. Therefore, planting with low temperatures would provide a delay in the root invasion. Thus, the selection of a planting time that coincides with environmental conditions unfavorable for the nematode can reduce their impact in the crop (Bello, 1998).

Root destruction. Roots are removed from the ground at the end of the cropping cycle, being common to leave the roots into soil. In RKN-infested fields, nematode population can remain active in the roots. Therefore, removing plant roots from the precedent crop interrupt nematode life cycle, and thus, decreases the effective inoculum for the next crop (Ornat et al., 1999).

Resistant varieties. Resistance to parasitic nematodes is defined as "the ability of plants which have one or more resistance genes to inhibit or suppress nematode development and/or reproduction" (Roberts, 2002). When a plant is resistant to *Meloidogyne* spp., it means that it contains a specific gene or genes that react against the nematode. In tomatoes, a single dominant gen (*Mi*), discovered in *Solanum peruvianum*, interacts with a nematode protein, recognizing the nematode and switching on the plant defense (Medina-Filho and Stevens, 1980). This gen was introduced in commercial varieties of *S. lycopersicum*. In *Capsicum annuum*, the RKN resistance is controlled by several independent dominant genes (*Me*).

Grafting consists in the union of two tissues of living plants, which grow and develop as a single plant. In order for this process to be successful, the tissues next to the cambium layer of the scion and the rootstock must be compatible. Therefore, the species used as scions and rootstocks need to present a high botanical affinity. In horticulture, only cucurbits and Solanaceous crops are grafted onto compatible rootstocks. The rootstocks can bring new features to the grafted plants such as increase foliage, yield, and tolerance to abiotic stresses such as low temperatures and salinity (Davis et al., 2008). Aditionally, grafting can be used for integrate pest management. Thus, the susceptible scion is grafted onto tolerant or resistance rootstocks, counteracting the losses caused by the pathogen on susceptible crops. In cucurbits, the primary reason for grafting is to counteract damage caused by several forma species of *Fusarium oxysporum*.

Crop rotation is the successive cultivation of different crops following a specific order in the same field. This practice is widespread in Spain, such as rotational sequences of cucurbits (cucumber, watermelon or zucchini) and Solanaceous (tomato and pepper) in double cropping systems in protected cultivation (Meneses and Castilla, 2009; Talavera et al., 2012). Under the perspective of protected cultivation, the management actions related to soil diseases taken in a crop may affect the subsequent crop in the rotation (Westphal, 2011). The design of specific rotational sequences can be used as a preventive tool for integrated pest management against RKN diseases (Ferris et al.,

1994). These sequences may include less suitable or poor/resistant host plants to nematode, thus benefiting the following crop in the rotation. Poor/resistant hosts would reduce the effective inoculum for the following crop, with the subsequent reduction in yield losses (Ehwaeti et al., 1999; Ferris et al., 1994).

Current situation of root-knot nematode in cucurbits

Research related to RKN in cucurbit crops has focused largely on the search of resistance in the germplasm collections of wild *Citrullus* and *Cucumis* species (Thies and Levi, 2003, 2007; Cohen et al., 2014). Some accessions have shown potential to be exploited commercially because they proved to reduce the nematode reproduction and the disease severity. However, RKN-resistant cultivars in cucurbit crops are not currently available because of the difficulties encountered in the introgression of the desired traits into commercial cultivars. Assessment of RKN reproduction on cucurbits will be useful to determine variation within cultivars developed for growth under protected cultivation. Differences in the genetic background of the plants might provide tolerance to the nematode. Cucurbit crops are considered susceptible to *M. arenaria*, *M. incognita* and *M. javanica* but some differences amongst crops and within a crop have been reported due to the genetic background of the cultivars and the parasitic variation existing in the genus *Meloidogyne* (Edelstein et al., 2010; Mukhtar et al., 2013). The commercial cultivars currently used in the country were used to carry out the experimental work presented in this thesis.

Knowledge of the population dynamics of *Meloidogyne* and the potential yield losses are needed for the integrated management of the nematode. Damage and yield losses have been reported in cucumber and melon (Ornat et al., 1997; Ploeg and Phillips, 2001; Kim and Ferris, 2002; Webster et al., 2011) but few studies have focused on the development of damage-function models for zucchini and watermelon. Cultivation of these two crops has expanded under protected cultivation in Spain in recent years.

Objectives

The cucurbit crops are hosts of the major root-knot nematodes, *Meloidogyne arenaria*, *M. incognita* and *M. javanica*, but comparative studies are limited on their pathogenic effects on cucurbits. Since, cucurbits are often cultivated in rotation with solanaceae crops, information on their host suitability is useful to design strategies for integrated pest management. Understanding the host-parasite relationship on cucurbit crops is necessary for predicting yield losses and modelling the population dynamics of the nematode.

The general objective of this thesis was to characterize and quantified the host-parasite relationship on selected cucurbits in response to root-knot nematode infection. This objective was divided into specific objectives to determine:

1- The infection process and post-infectional development of Meloidogyne spp. on selected cucurbits crops.

The infection process of the host plant starts with the penetration of the roots by the second-stage juveniles. Once the nematode has established the feeding site, start their post-infectional development into third- and forth- stage juveniles until reaching the egg-laying-female adult stage. The life cycle is completed with the deposition of eggs into a gelatinous egg mass. The infection process and post-infectional development of *Meloidogyne arenaria*, *M. incognita* and *M. javanica* was investigated in selected cucurbit crops including zucchini, cucumber, melon, pumpkin, and watermelon (Chapter 1).

2- The suitability of zucchini, cucumber, watermelon and cucurbit rootstocks to Meloidogyne arenaria, M. incognita and M. javanica and the nematode parasitic variation on these crops.

Cultivation of less suitable or poor/resistant host crops could benefit the following crop in the rotation, because poor host crops reduce the nematode reproduction in the field. Assessment of nematode reproduction on cucurbits may discriminate tolerant genotypes, even if resistance genes are lacking, as the genetic

background of the plants can provide superior vigor, extensive root systems or tolerance to environmental stresses (Chapter 2 and 3).

3- Population dynamics of root-knot nematodes on zucchini and watermelon.

An estimation of the growth potential of the nematode population in a given crop provides information on the host status and, in turn, on the crop tolerance to the nematode. In susceptible crops, there is a negative relationship between the initial nematode population density and the reproduction rate. The potential growth of *Meloidogyne javanica* in response to increasing population densities was determined in zucchini and watermelon in greenhouse and field experiments. (Chapter 4 and 5, respectively).

4- Development of damage function models on zucchini and watermelon.

Damage caused by root-knot nematodes is determined by relating initial population densities to growth and yield. The minimal density that causes a measurable reduction in plant growth or yield varies with nematode species, host plants, cultivar and environment. The relationship between the initial population densities and yield losses, and their suitability to fit the Seinhorst damage function model was determined on zucchini and watermelon (Chapter 4 and 5, respectively).

Chapter 1

Penetration and reproduction of root-knot nematodes on cucurbit species

Penetration and reproduction of root-knot nematodes on cucurbit species

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Abstract

Cucurbits are often cultivated in rotation with Solanaceae in double-cropping systems. Most cucurbits have been described as susceptible to root-knot nematodes (RKN) but little is known on their relative levels of susceptibility. Because RKN species differ in rates of root invasion and reproductive traits, isolates of *M. arenaria*, *M. incognita* and *M. javanica* were compared on five cucurbit hosts in a climate growth chamber. They included zucchini squash cv. Amalthee, cucumber cv. Dasher II, melon cv. Pistolero, pumpkin cv. Totanera and watermelon cv. Sugar Baby. All cucurbits were susceptible to the three RKN isolates although *M. javanica* showed higher invasion rates, faster development and egg production than *M. arenaria* on the selected cucurbits. Apparent differences among cucurbits were primarily due to root invasion rates and formation of egg masses. Both *Cucumis* species (cucumber and melon) were better hosts for nematode invasion and reproduction than zucchini squash followed by watermelon. Large invasion rates followed by small reproduction factors were linked to *M. incognita* on zucchini squash. Reduced invasion rates and eggs mass formation along with delayed early development were shown on watermelon.

*Keywords***:** Cucurbitaceae, host suitability, *Meloidogyne arenaria*, *Meloidogyne incognita, Meloidogyne javanica*.

Introduction

Root-knot nematodes (RKN) are important pest for many vegetables worldwide (Karssen, 2002). The second-stage juveniles (J2) penetrate the roots and migrate through the intercellular space to the vascular cylinder to initiate and develop a permanent feeding site. Once established, J2 moult three times to become adults. Adult females lay eggs into a gelatinous matrix attached to the posterior end of the female.

Cucurbits are often cultivated in rotation with Solanaceae in double-cropping systems in several vegetable production areas. For instance, pepper is rotated with squash or cucumber (Thies et al., 2004) and tomato with melon or watermelon (Talavera et al., 2012). Most edible cucurbits are hosts of the most widespread root-knot nematodes *M. arenaria*, *M. incognita* and *M. javanica,* but comparative studies on their pathogenic effects on cucurbits are limited. *Meloidogyne* spp. differs in rates of root penetration (Arens et al., 1981; Khan and Khan, 1991a; Ehwaeti et al., 1999; Dutta et al., 2011), reproduction (Roberts and Thomason, 1989) on different hosts (Carneiro et al., 2000), and ability to withstand nematode damage (Ehwaeti et al., 1999). Non-host plants do not allow nematode attack often preventing root penetration and thereby nematode development and reproduction. Resistance is used to describe the ability of a plant to suppress development or reproduction of the nematode. Susceptible plants allow normal nematode development and the expression of any associated disease (Cook and Evans, 1987; Roberts, 2002). Susceptible plants building up great population densities are considered good host (Seinhort, 1967), and this ability is generally referred as the reproduction factor (*Rf*) that is measured as the final population density (*Pf*) divided by the initial population density (*Pi*). On the contrary, poor host plant often show low *Rf*. Host plants that multiply the nematode but suffer little damage are termed tolerant (Cook and Evans, 1987). Large soil infestations may result in high invasion rates that may cause great tissue injury of meristematic cells affecting initial plant growth. This situation can also be detrimental for nematode development as nematodes will compete for available feeding sites resulting in reduced multiplication rates (Arens et al., 1981). Conversely, high multiplication rates with no

plant damage might be achieved with slight soil infestations (Di Vito et al., 1985). Therefore, information on the host-parasite relationship in rotational crops like members of the cucurbit family will be valuable for a sustainable management of RKN in double cropping systems.

The objectives of this study were to compare penetration and reproduction of *Meloidogyne arenaria, M. incognita* and *M. javanica* isolates on a diversity of cucurbits including zucchini squash, cucumber, melon, pumpkin, and watermelon, and to select the most useful cultivar of cucurbits to include in double-cropping systems with Solanaceae.

Materials and Methods

Nematode root penetration

A time course experiment was conducted to compare root penetration by three RKN isolates on five cucurbit species. Each RKN isolate and cucurbit combination was replicated seven times. Tests were run separately and repeated two times. The cucurbits included zucchini squash (*Cucurbita pepo* L.) cv. Amalthee, cucumber (*Cucumis sativus* L.) cv. Dasher II, melon (*C. melo* L.) cv. Pistolero, pumkin (*Cucurbita maxima* Duschesne) cv. Totanera and watermelon [*Citrullus lanatus* (Thunb), Matsum & Nakai] cv. Sugar Baby.

Nematode isolates

The nematode isolates were *M. arenaria* (code Ma 68), *M. incognita* (code MiPM26) and *M. javanica* (code Mj05) from the nematode collection at IRTA, Centre of Cabrils. Nematode cultures were started from the progeny of one single female and they were maintained on susceptible tomato cv. Roma in spring-summer and on celery cv. D´Elne in autumn-winter in a greenhouse. Juveniles were obtained from infected tomato roots cv. Roma by the Baermann tray method (Whitehead and Hemming, 1965). Individual seedlings were inoculated with 200 freshly hatched J2

(less than 72h-old, *Pi*) of each isolate in approximately 0.5 ml of water.

Cucurbit seeds were soaked overnight and germinated in vermiculite trays for three days. Seedlings were transplanted at the cotyledon stage to 20-cm^3 capacity clay pots filled with sterilized river sand. Seedlings were allowed to growth 2 weeks for watermelon and 1 week for the others species before nematode inoculation. For each RKN isolate, seedlings were grouped into three sets, one set per harvest date 4, 7, and 11 days post-inoculation - dpi. Plants were maintained in a growth chamber at 26 ºC ± 1 with a photoperiod 16 h light, watered as needed and fertilized with a slow-release fertilizer (Osmocote ® Scotts Company, Netherlands, 15% N + 10% P_2O_5 + 12% K_2O + 2% MgO_2 + microelements) at the beginning of the test. At each harvesting time, plants were carefully removed from the pots, the root system washed free of soil. Roots were stained with acid fuchsin 0.05% (Bridge et al., 1982), and examined under a stereomicroscope to count the number of infection sites, and nematodes inside them. Infection sites were recognized because the root tissue was swollen and contained at least one nematode inside. Nematodes were categorized according to their post-embryonic developmental stages as J2 (vermiform), third-stage juveniles (J3, sausage-like) and fourth-stage juvenile (J4, sac-shape) (Taylor and Sasser, 1978).

Nematode reproduction

A comparison of RKN reproduction on cucurbit hosts was conducted using the same nematode isolates, cucurbit cultivars except pumpkin that was not included, and experimental conditions. Seedlings were obtained and inoculated as referred before to penetration test. The experimental design was a completely randomized block that included all possible combinations corresponding to 12 treatments with 12 replicates per treatment (4 cucurbit species x 3 RKN species). Five plants from each treatment were harvested 7 dpi to determine J2 penetration following the methodology previously described. The remaining seven plants/treatment were uprooted, the roots gently rinsed in water and transplanted into new pots filled with 500 cm^3 of sterilized river

sand to remove all J2 from root surface and prevent any further root penetration. The experiment was conducted twice.

At 45 dpi (728 degree-days, basal temperature of 10 ºC), the root systems were washed free of soil and weighed. Egg masses (EMs) were stained with a 0.1 g L^{-1} erioglaucine solution (Aldrich Chemical Company) for two hours (Omwega et al., 1988) and recorded. Eggs from the entire root system were extracted by maceration in a blender containing a 0.5% NaOCl solution for 10 min (Hussey and Barker, 1973) and counted to determine *Pf*. Both non-hatched eggs and empty eggs (egg shells) were recorded and the hatching rate was estimated. The fecundity of the females was estimated by dividing *Pf* by EMs and the *Rf* (*Rf*=*Pf*/*Pi*) was calculated.

Statistical analyses

The SAS system V8 (SAS Institute Inc., Cary, NC) was used for statistical analyses. Prior to the analyses, when needed, nematode data were log transformed [log10 (x+1)] to homogenize the variances. Data from the root penetration and nematode reproduction experiments were combined because there was no significant difference between the repeated experiments and they were analyzed using analysis of variance (ANOVA). Comparisons were conducted within cucurbit host and within nematode isolate (n=14; 7 replications x two experiments). In addition, data were grouped by RKN isolate (four cucurbits x 14 replications) and the new set of data subjected to ANOVA. Data from *Meloidogyne* were pooled (three RKN isolates x 14 replications) and subjected to ANOVA. When the analyses showed statistical differences (P=0.05), the means were separated according to Tukey HSD (Honestly Significant Difference) Test.

Results

Nematode root penetration

Significantly more (P<0.05) infection sites were found on zucchini squash infected by *M. incognita* (94 ± 12, mean ± standard deviation) at 11 dpi than *M. arenaria* (68 ± 7) followed by *M. javanica* (49 ± 16). The number of infection sites on watermelon roots (25 ± 7) at 11 dpi was significantly fewer (P<0.05) than on cucumber (44 ± 6), melon (44 ± 7) and pumpkin (59 ± 12) regardless of the RKN isolates.

Root penetration followed a similar pattern on both *Cucurbita* species (zucchini squash and pumpkin) although *M. incognita* J2 invaded zucchini squash roots more rapidly and in numbers significantly highest (P<0.05) than the other two isolates at all harvesting times (Fig. 1a). Final penetration rates on zucchini squash were 98% for *M. incognita*, 46% *M. arenaria* and 48% for *M. javanica* (Fig. 1a); on pumpkin were 99%, 70% and 68%, respectively (Fig. 1b). On cucumber, *M. incognita* and *M. javanica* showed significantly higher (P<0.05) penetration rates than *M. arenaria* at all harvesting times, and *M. javanica* was higher (P<0.05) than *M. incognita* at 11 dpi (Fig. 1c).The final penetration rates were 96%, 78%, and 59%, for *M. javanica, M. incognita* and *M. arenaria,* respectively (Fig. 1c). On melon, *M. javanica* invaded more rapidly and in numbers significantly highest (P<0.05) than *M. incognita* followed by *M. arenaria,* and final penetration rates were 100%, 84% and 54%, respectively (Fig. 1d). On watermelon, root penetration by *M. javanica* (51%) was significantly higher (P<0.05) than that of the other two RKN isolates (41% to 42%) (Fig. 1e).

At 4 dpi only vermiform J2 were found in roots of cucurbit species (data not shown). At 7 dpi, most penetrating J2 were at the J3-stage on zucchini squash, cucumber, and melon, with the exception of *M. javanica* on zucchini squash. RKN development was delayed on pumpkin and watermelon (Fig. 2a). At 11 dpi most nematodes were at J4-stage in zucchini squash, cucumber, and melon roots except for *M. arenaria* on melon (59%) (Fig. 2b). A mixture of J3 and J4 stages occurred on pumpkin, whereas, on

watermelon were found the three juveniles stages (J2, J3 and J4) with a dominance of the J3-stage (Fig. 2b).

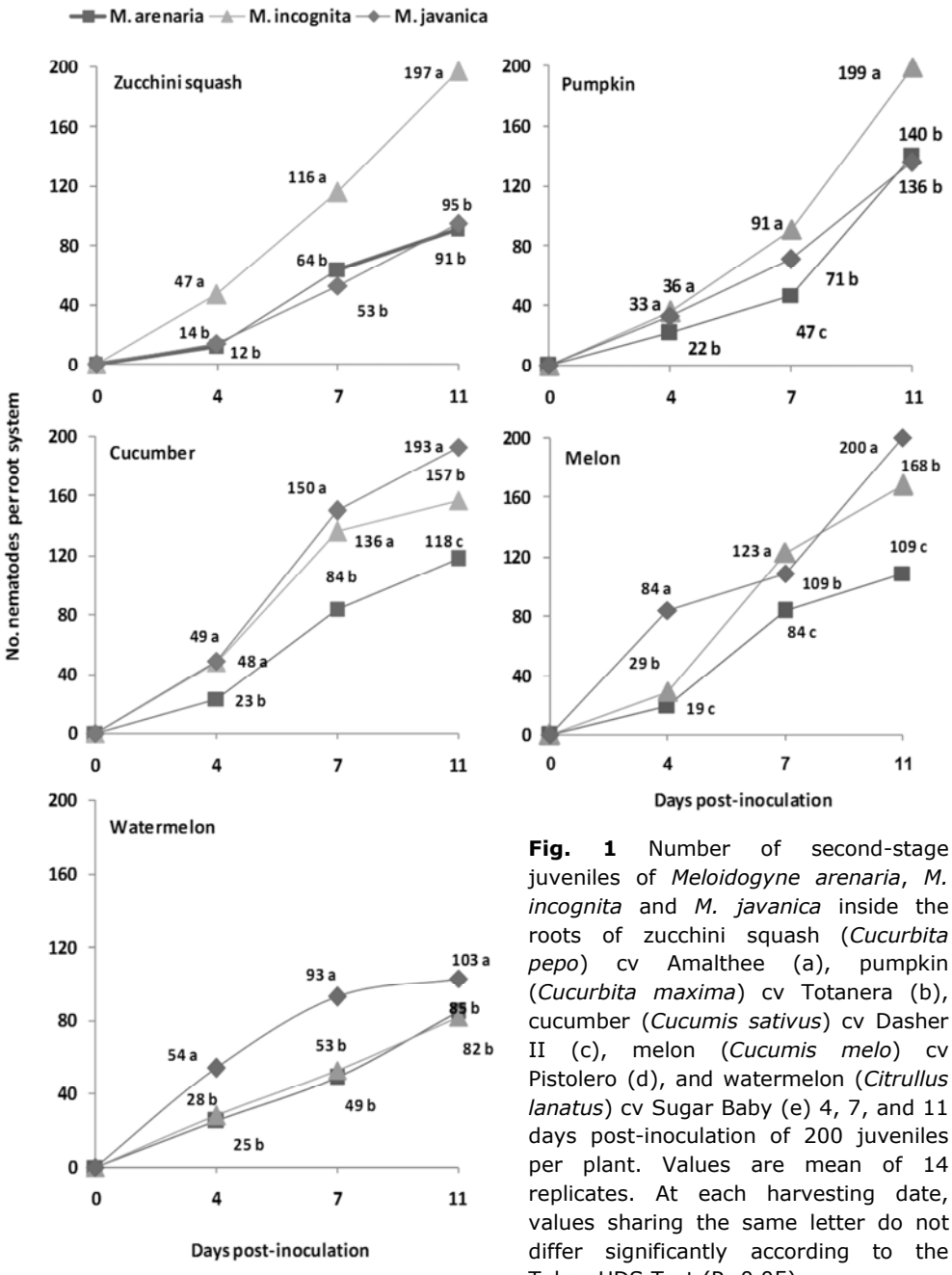

Fig. 1 Number of second-stage juveniles of *Meloidogyne arenaria*, *M. incognita* and *M. javanica* inside the roots of zucchini squash (*Cucurbita pepo*) cv Amalthee (a), pumpkin (*Cucurbita maxima*) cv Totanera (b), cucumber (*Cucumis sativus*) cv Dasher II (c), melon (*Cucumis melo*) cv Pistolero (d), and watermelon (*Citrullus lanatus*) cv Sugar Baby (e) 4, 7, and 11 days post-inoculation of 200 juveniles per plant. Values are mean of 14 replicates. At each harvesting date, values sharing the same letter do not differ significantly according to the Tukey HDS Test (P=0.05).

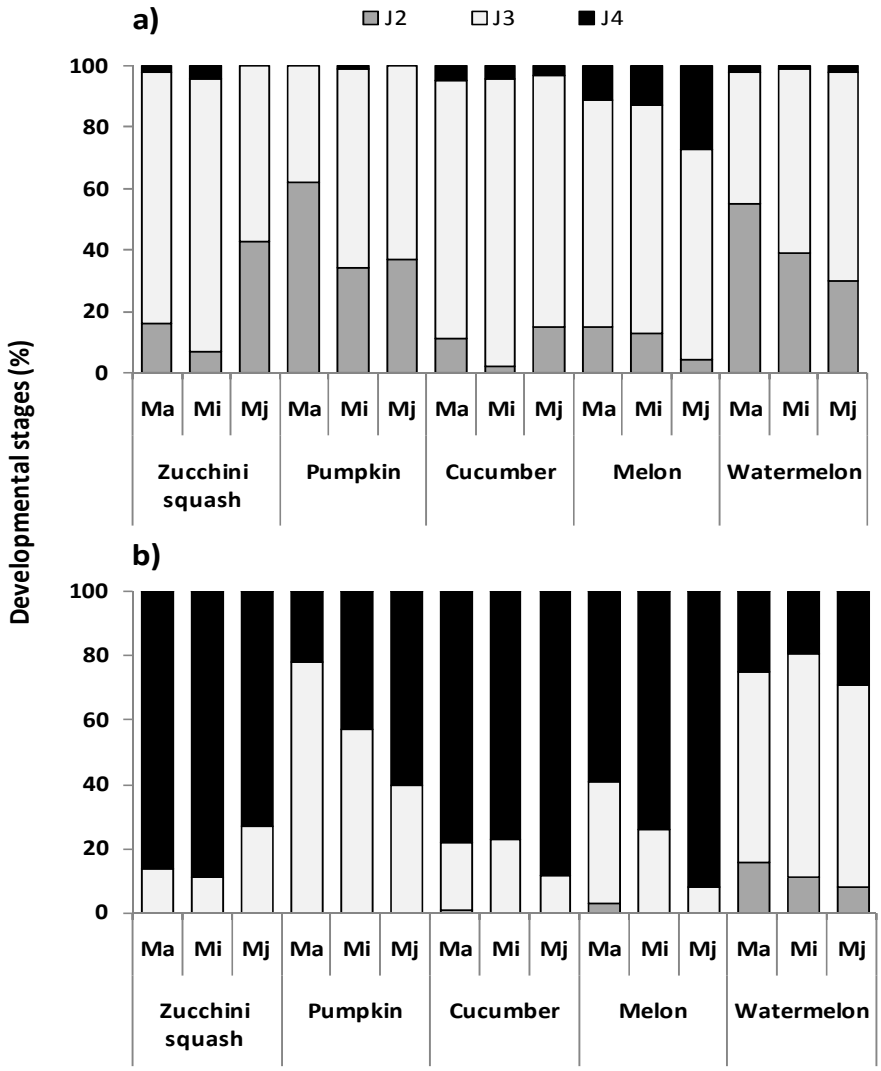

Fig. 2 Post-embryonic development of *Meloidogyne arenaria*, *M. incognita* and *M. javanica* in roots of zucchini squash (*Cucurbita* pepo) cv. Amalthee, pumpkin cv. Totanera (*Cucurbita maxima*), cucumber (*Cucumis sativus*) cv. Dasher II, melon (*Cucumis melo*) cv. Pistolero, and watermelon (*Citrullus lanatus*) cv. Sugar Baby at 7 (a) and 11 (b) days post-inoculation of 200 juveniles per plant. Values are mean of 14 replicates. J2 - second-stage juveniles; J3 - third-stage juveniles; J4 - fourth-stage juveniles.

On melon, the *Pi* values of *M. javanica* were significantly higher (P<0.05) than those of *M. incognita* followed by *M. arenaria*. Besides, *M. javanica* and *M. incognita* showed significantly higher (P<0.05) *Pf* than *M. arenaria*. On watermelon, *M. javanica* showed significantly higher (P<0.05) *Pi* values than the other two isolates, but only *M. incognita Pf* was higher (P<0.05) than *M. arenaria Pf* (Table 1).

Table 1. Reproductive traits of *Meloidogyne arenaria, M. incognita* and *M. javanica* on four cucurbit species after inoculation with 200 second-stage juveniles (J2) in a growth chamber.

Plant species Common name (Cultivar)	Nematode species	Iitial population density [a]	Egg masses	Final population density
Cucurbita pepo	*M. arenaria*	50 ± 6 b C	18 ± 9 a B	13857 ± 6542 a AB
Zucchini squash	*M. incognita*	104 ± 10 a C	24 ± 12 a B	5678 ± 4013 b C
(Amalthee)	*M. javanica*	47 ± 6 c D	25 ± 10 a B	15078 ± 7249 a B
Cucumis sativus	*M. arenaria*	101 ± 7 c A	46 ± 16 b A	24762 ± 14072 b A
Cucumber	*M. incognita*	136 ± 9 b A	73 ± 21 a A	32918 ± 10394 ab A
(Dasher II)	*M. javanica*	155 ± 4 a B	77 ± 21 a A	48863 ± 20384 a A
Cucumis melo	*M. arenaria*	87 ± 5 c B	16 ± 7 b B	7620 ± 3791b BC
Melon	*M. incognita*	134 ± 10 b B	30 ± 21 ab B	17481 ± 8929 a B
(Pistolero)	*M. javanica*	152 ± 7 a A	46 ± 22 a B	28355 ± 15089 a AB
Citrullus lanatus	*M. arenaria*	42 ± 10 c D	6 ± 5 b C	4982 ± 5077 b C
Watermelon	*M. incognita*	54 ± 7 b D	22 ± 11 a B	17926 ± 8769 a B
(Sugar Baby)	*M. javanica*	82 ± 13 a C	14 ± 11 ab C	9474 ± 11937 ab C

Values are mean ± standard deviation of ten replicates for initial population and 14 for reproductive traits. Values within cucurbit crop in the same column sharing the same lower-case letter are not significantly different. Values within nematode isolate in the same column sharing the same upper-case letter are not significantly different. Mean separation by Tukey HSD Test (P=0.05).[a] Number of J2 inside the roots 7 days post-inoculation.

Nematode reproduction

The *Pi* for this experiment was the number of nematodes that penetrated the roots at 7 dpi. Therefore, differences in *Pi* values were due to different penetration rates of the isolates on the cucurbit hosts (Table 1). Within cucurbit host, *M. incognita* showed higher *Pi* values on zucchini squash than *M. arenaria* followed by *M. javanica* but there was no difference in EMs among the isolates (Table 1). However, *M. incognita Pf* was significantly lower ($P<0.05$) than that of the other two isolates. On cucumber, *M. javanica* showed significantly higher ($P< 0.05$) *Pi* values than the other two isolates; but only *M. javanica Pf* significantly differed ($P<0.05$) from *M. arenaria Pf* (Table 1).

Table 2. Reproductive traits of three isolates of *Meloidogyne* spp. on four cucurbit species (*Cucurbita pepo* cv. Amalthee, *Cucumis sativus* cv. Dasher II, *C. melo* cv. Pistolero and *Citrullus lanatus* cv. Sugar Baby), grouped according to the species of the nematode, 45 days-post inoculation with 200 second-stage juveniles per plant.

Meloidogyne species	Root weight (g)	Egg masses	Final population density	Fecundity [b]	*Rf* [c]
M. arenaria	9.8 ± 0.6 b	22 ± 2 b	12997 ± 1635 c	731 ± 68 a	64 ± 8 c
M. incognita	11.9 ± 0.6 a	38 ± 2 a	18734 ± 1619 b	629 ± 67 a	93 ± 8 b
M. javanica	10.2 ± 0.6 ab	40 ± 2 a	25389 ± 1619 a	710 ± 67 a	127 ± 8 a

Values are mean ± standard deviation of 56 replicates. Values in the same column sharing the same letter do not differ according to Tukey HSD Test ($P=0.05$). [a] *Pf*/egg masses[b] *Rf* (Reproduction factor) = *Pf*/*Pi*.

The combined analysis indicated that root weight was higher in *M. incognita* than *M. arenaria* infected plants (Table 2). *Meloidogyne incognita* and *M. javanica* formed significantly more ($P<0.05$) EMs than *M. arenaria*, but *M. javanica Pf* was significantly higher ($P<0.05$) than *M. incognita Pf* (Table 2). However, the fecundity of the females did not differ among the three RKN

isolates (Table 2). The hatching rate of *M. arenaria* (20 ± 2) was lower (P<0.05) than that of *M. incognita* (32 ± 2) or *M. javanica* (30 ± 2).

Both *Cucumis* species (cucumber and melon) showed significantly higher (P=0.05) root weight was than zucchini squash, followed by watermelon (Table 3). Significantly higher (P=0.05) EMs were observed on cucumber than on melon or zucchini squash followed by watermelon (Table 3). Statistical differences in *Pf*, and *Rf* among the cucurbits consistently corresponded with those observed for EMs. There was no difference in the fecundity of the females among the cucurbit hosts (Table 3). Hatching rates on cucumber (34 ± 2), melon (30 ± 2) and watermelon (28+ 2) were comparable and significantly higher than on zucchini squash (22 ± 2).

Table 3. Reproductive traits of three isolates of *Meloidogyne*spp, grouped according to the cucurbit species.

Plant species Commonname (Cultivar)	Root weight (g)	Egg masses	Final population density	Fecundity [a]	*Rf*[b]
Cucurbita pepo Zucchini squash (Amalthee)	9 ± 0.7 b	22 ± 2.5 bc	11680 ± 1876 bc	708 ± 77 a	58 ± 9 bc
Cucumis sativus Cucumber (Dasher II)	15.3 ± 0.7 a	65 ± 2.4 a	35514 ± 1853 a	569 ± 77 a	178 ± 9 a
Cucumis melo Melon (Pistolero)	15.4 ± 0.7 a	31 ± 2.5 b	18071 ± 1923 b	716 ± 79 a	90 ± 10 b
Citrulluslanatus Watermelon (Sugar Baby)	3 ± 0.7 c	14 ± 2.4 c	10794 ± 1853 c	768 ± 77 a	53 ± 9 c

Values are mean ± standard deviation of 42 replicates. Values in the same column followed by the same letter are not significantly different according to Tukey HDS Test (P=0.05). [a]*Pf*/egg masses[b] *Rf* (Reproduction factor) = *Pf*/*Pi*.

Discussion

All cucurbits were susceptible to the three *Meloidogyne* isolates but significant differences in root penetration and nematode reproduction that persisted through the experimental period were detected. Cucurbits also differ in root galling severity (Edelstein et al., 2010). The present study confirms that the genetic background of the host as well as that of the nematode affect the host-nematode interaction in good hosts and poor/resistant hosts (Ehwaeti et al., 1999; Fournet et al., 2012; Verdejo-Lucas et al., 2013). Root invasion and formation of egg masses were the primary factors explaining differences among cucurbits in this study and the observed differences were thereafter consistently shown in final population densities and reproduction factors. Nevertheless, females that reached maturity laid similar numbers of eggs regardless the RKN isolate or the cucurbit host which corroborates that female fecundity is not a major factor in the response of the host to the nematode (Arens et al., 1981; Faske, 2013). The exception to this trend was *M. incognita* on zucchini squash that showed reduced fecundity (236 eggs/female) in comparison to the other isolates (603 eggs/female). In general, *M. javanica* showed greater all root penetration, faster development and reproduction than *M. arenaria* on the selected cucurbits. Seemingly, *M. javanica* invaded tobacco roots more rapidly and in greater numbers than *M. arenaria* or *M. incognita* (Arens et al., 1981). The lessen reproductive ability of *M. arenaria* in comparison to *M. javanica* on cucurbits is consistent with similar observations on tomato (Verdejo-Lucas et al., 2013) and could explain the restricted distribution and detection of *M. arenaria* in some vegetable areas (Giné et al., 2012; Talavera et al., 2012).

Both *Cucumis* species (cucumber and melon) were better host for nematode invasion and reproduction than zucchini squash followed by watermelon. *Meloidogyne incognita* and *M. javanica* showed similar root penetration pattern, infection and reproduction on cucumber and melon.

The *M. incognita* isolate showed a remarkable interaction with zucchini squash cv. Amalthee with significantly greater numbers of penetrating J2, similar egg mass production and lower *Pf* than the

other two RKN isolates. Accordingly, zucchini squash was a poorer host of *M. incognita* than *M. arenaria* or *M. javanica*. The specificity of this interaction for the crop cultivar or RKN isolate is not presently known but deserves further exploration since it resulted in increased root penetration and reduced *Pf*. This is an interesting combination of effects that might be of utility for the sustainable management of nematode infestations. Zucchini squash has been described as RKN susceptible (Thies et al., 2004) but whether squash cultivars differ in susceptibility levels is unknown. Species-specific and even race or population specific, non-host or resistance responses to *Meloidogyne* spp were found on cultivars of cauliflower, tomato rootstocks and several wild plants (Khan and Khan, 1991b; Ehwaeti et al., 1999; Cortada el al., 2009).

Apparently, the zucchini squash cv. Amalthee hindered the development of the nematode from the J4-stage to mature egg-laying-female with no effect on penetrating J2 since they progressively developed into immotile J3 and J4 stages. J4-stage juveniles may have died or stop developing as occurred on *Cucumis sativus* infected by *M. hapla* (Stephan and Trudgill, 1982). Faske (2013) found empty galls on *Cucumis melo* var. *texanus* and suggested that juveniles developed to males and had left the roots. An increase in the male/female ratio on *Cucumis myriocarpus*, a non-host for *M. incognita,* was reported by Pofu and Mashela (2011). Egression of juveniles after penetration of the roots occurred on resistant *Cucumis* genotypes (Faske, 2013) although it seems little likely to be the case here because large numbers of penetrating J2 developed into J4-stages. Sub-optimal development of feeding sites unable to supply sufficient nutrients for the nematode could possibly explain the *M. incognita*-zucchini squash interaction. Such resistance mechanism has been suggested on RKN resistant *Cucumis* (Walters et al., 2006).

The response of watermelon cv. Sugar Baby was differentiated by a great reduction in J2 penetration which suggested a pre-infectional mechanism that was not related to the RKN isolate. Allelochemicals released into the rizhosphere could affect nematode behavior, and thus modify the host recognition process (Dutta et al., 2012). Other mechanisms were retardation in juvenile development and low rates of penetrating J2 becoming

egg-laying females (24%). All these mechanisms together make watermelon cv. Sugar Baby the less suitable host to the three RKN isolates among the tested cucurbits. Montalvo and Esnard (1994) found that Sugar Baby supported the lowest *M. incognita* root galling and *Rf* among ten watermelon cultivars and all *Rf* values were significantly lower on watermelon than tomato. Other cucurbits also showed reduced *Rf* values in comparison to tomato or eggplants (Anwar and McKenry, 2010). These results support the worth of searching for less suitable RKN host since resistance to *M. arenaria, M. incognita* and *M. javanica* is not commercially available on *Cucumis, Cucurbita* or *Citrullus*. Resistance to *M. hapla* has been reported on some cultivars of squash and melon (Carneiro et al., 2000).

In summary, the main results from this comparative study were i) high root penetration and reproduction on cucumber and melon irrespective of the RKN isolate, ii) high penetration rates linked to low *Pf* values on *M. incognita*-infected zucchini squash, and iii) reduced invasion and delay in post-embryonic development on watermelon. These mechanisms operate on resistant germoplasm (Khan and Khan, 1991a) and poor host plants (Ehwaeti et al., 1999). Although the tested cucurbits were all susceptible to the three RKN isolates, differences in susceptibility levels were significant; cucumber cv. Dasher II followed by melon cv. Pistolero were the most susceptible hosts, and watermelon cv. Sugar Baby the least. The host status affects not only the damage a crop is likely to suffer but also the residual populations left in the soil which are the inoculum for the next crop in the rotation. Thus, watermelon cv. Sugar Baby could be used for the sustainable management of the disease in double-cropping systems with Solanaceae since *Rf* were a third of that on cucumber. Similarly, zucchini squash cv. Amalthee could be used in *M. incognita* infested fields.

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Chapter 2

Suitability of zucchini and cucumber genotypes to populations of Meloidogyne arenaria, M. incognita and M. javanica

Suitability of zucchini and cucumber genotypes to populations of *Meloidogyne arenaria, M. incognita* and *M. javanica*

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Abstract

The host suitability of five zucchini and three cucumber genotypes to *Meloidogyne incognita* (MiPM26) and *M. javanica* (Mj05) was determined in pot experiments in a greenhouse. The number of egg masses (EM) did not differ among the genotypes of zucchini or cucumber, but the eggs/plant and reproduction factor did slightly. *Meloidogyne incognita* MiPM26 showed lower ($P < 0.05$) EM, eggs/plant and reproduction factor than *M. javanica* Mj05. Examination of the zucchini galls for nematode post-infection development revealed unsuitable conditions for *M. incognita* MiPM26 since only 22% of the females produced EM compared to 95% of the *M. javanica* females. As far as cucumber was concerned, 86% of the *M. incognita* and 99% of the *M. javanica* females produced EM, respectively. Several populations of *M. arenaria*, *M. incognita* and *M. javanica* were tested on zucchini cv. Amalthee and cucumber cv. Dasher II to assess the parasitic variation amongst species and populations of *Meloidogyne*. A greater parasitic variation was observed on zucchini than cucumber. Zucchini responded as a poor host for *M. incognita* MiPM26, MiAL09 and MiAL48, but as a good host for MiAL10 and MiAL15. Intra-specific variation was not observed amongst the *M. javanica* or *M. arenaria* populations. Cucumber was a good host for all the tested populations. Overall, both cucurbits were suitable hosts for *Meloidogyne* but zucchini was a poorer host than the cucumber.

Key words: Cucumis sativus; *Cucurbita pepo*; infection; parasitic variation; reproduction; root-knot nematodes.

Introduction

Root-knot nematodes (RKN), *Meloidogyne* spp, are significant nematode pests for vegetable production in temperate, subtropical and tropical regions (Sikora and Fernández, 2005). Vegetables are grown under protected cultivation in many areas and nematode management in these systems is a major challenge due to crop intensity, short fallowing, and environmental conditions that favor pest and disease development. Cucurbits such as zucchini and cucumber are frequently rotated with tomatoes and peppers in double cropping systems in plastic houses in Spain (Meneses and Castilla, 2009), and thus, management actions taken in a crop may affect the subsequent crop in the rotation (Westphal, 2011). Information on the host suitability of rotational crops is useful because RKN population densities rapidly build-up on susceptible crops under the favorable conditions prevailing in plastic houses. Cultivation of less suitable or poor/resistant host crops would benefit the following crop in the rotation as poor hosts are less likely to be damaged than good hosts since invasion, root damage, and nematode reproduction are reduced (Ehwaeti et al., 1999). Lower RKN levels were recorded in cucurbits compared to solanaceous crops (Verdejo-Lucas et al., 2002; Talavera et al., 2012) pointing to different host suitability amongst these crops. Host range studies have shown that some crop cultivars differ in suitability to RKN (Fourie et al., 2012; Maleita et al., 2012) which also vary with the RKN species or populations. For example, bean and pea cultivars were good hosts for *M. hapla* and *M. chitwoodi* but the reproduction factor (*Rf*, final/initial population density) of *M. hapla* was greater than that of *M. chitwoodi* in some of the tested cultivars (Santo and Ponti, 1985). Cultivars of celery and carrot showed varying degrees of host suitability due to the parasitic variation in *M. hapla* (Melakeberhan and Wang, 2012, 2013). Extensive studies have been conducted on the suitability of watermelon germplasm (Thies and Levi, 2003, 2007) but those on current cultivars of other cucurbits are limited (Edelstein et al., 2010; Mukhtar et al., 2013).

Assessment of RKN reproduction on cucurbits may discriminate tolerant genotypes, even if resistance genes are lacking, as the genetic background of the plants can provide

superior vigor, extensive root systems or tolerance to environmental stresses (i.e. salinity) in the production area. It is generally accepted that cucurbit crops such as cucumber, melon, zucchini and watermelon, are susceptible to the most widespread RKN, *M. arenaria*, *M. incognita* and *M. javanica* (Sikora and Fernández, 2005), although resistance has been found in some *Cucumis* species (Fassuliotis, 1970; Walters et al., 2006, Faske, 2013). In the context of this study, host suitability refers to the ability of a host plant to reproduce the nematode and it is measured as the reproduction factor *Rf*. Therefore, good hosts show high *Rf* values whereas poor hosts often show low *Rf*.

Zucchini and cucumber are cultivated around the world in a variety of environmental conditions and are common ingredients in the daily diet in many countries. In Spain, around 8000 ha zucchini and cucumber are cropped annually of which 70% and 89%, respectively, are grown under protected cultivation (MAGRAMA, 2011). The economic losses due to RKN on zucchini and cucumber in southeastern Spain were estimated in €640504 and €918293, respectively, in 2010 (Talavera et al., 2012).

This study was conducted to determine i) the host suitability of zucchini and cucumber genotypes to *M. incognita* and *M. javanica,* and ii) the parasitic variation of populations of *M. arenaria*, *M. incognita* and *M. javanica* on these crops.

Materials and methods

Host plants

Five genotypes of zucchini (*Cucurbita pepo* L.) and three of cucumber (*Cucumis sativus* L.) were selected for the study. The zucchini genotypes represented fruits with different shapes and colors, and included cv. Amalthee (long light green), cv. Parador (long yellow), cv. Pixar (long green), cv. Floridor (round yellow) and cv. Satelite (round deep green). Cucumber genotypes represented fruits with different length, cv. Taray (long), cv. Dasher II (medium-long) and cv. Urano (short). Zucchini and cucumber seeds were soaked overnight and germinated in seed trays with vermiculite. When the first true leaf was fully expanded,

the seedlings were transplanted to Styrofoam pots filled with 500 cm^3 of sterilized river sand. Plants were arranged randomly on a greenhouse bench and allowed to grow for one week before nematode inoculation. They were watered daily as needed, and fertilized with a slow-release fertilizer (Osmocote® Scotts Company, Netherlands; 15% N + 10% P_2O_5 + 12% K_2O + 2% MgO_2 + microelements) at the beginning of the experiments.

Host suitability

The suitability of selected genotypes of zucchini and cucumber to *M. incognita* MiPM26 and *M. javanica* Mj05 was determined in two experiments conducted in a greenhouse. The RKN isolates, collected from infested tomato roots (Ornat et al., 2001), were established as single egg mass cultures and maintained on susceptible tomato cv. Roma in spring-summer and on celery cv. D´Elne in autumn-winter in a greenhouse. The nematode was multiplied on tomato cv. Roma to obtain the inoculum for the experiments. Eggs were extracted by blender maceration of infected roots in a 0.5% NaOCl solution for 5 min (Hussey and Barker, 1973). The egg suspension was passed through a 74-µm aperture sieve to remove root debris, and the dispersed eggs were collected on a 25-µm sieve, counted, and used as inoculum. Plants were inoculated with 4,000 eggs by adding aliquots of the respective RKN isolates into two holes made in the soil 3-cm apart from the base of the plant. Non-inoculated plants, included as control for reference, received the same volume of water. Each treatment (genotype-RKN isolate) was replicated seven times. The hatching rate of the egg inoculum was determined by placing aliquots of egg suspension on three replicated Baermann trays that were incubated at 26 ± 1ºC in darkness for 21 days. Emerged second-stage juveniles (J2) were collected once a week, stored at 4ºC until counted, and the sum of emerged J2 was used to calculate the hatching rate (%). The egg inoculum was converted to the number of emerged J2 and considered as the *Pi* for the experiments. Soil temperatures were recorded daily at 30-min intervals with temperature probes (Em50 Data Logger®, Decagon Devices Inc, USA, accuracy ± 1ºC, resolution 0.1ºC) inserted into the pots.

The experiment (Exp. 1) was terminated 65 days after nematode inoculation allowing the nematode to complete one generation. Tops were cut at ground level and their fresh weight determined. Root systems were washed free of soil, weighed, and stained in a 0.1 g/liter erioglaucine solution (Aldrich Chemical Company) for two hours (Omwega et al., 1988), then washed in tap water, and the number of egg masses per plant (EM) counted as an indication of nematode infectivity. The infection frequency was calculated by dividing the number of EM by the J2 inoculum x 100. Eggs were extracted from 3 g root subsamples for 10 min as previously described (Hussey and Barker, 1973), to determine the final population densities (*Pf*) that were expressed as eggs/plant. Host-suitability was based on the reproduction factor (*Rf*) calculated by dividing the number of eggs/plant (*Pf*) by the number of emerged J2 (*Pi*). To determine the fecundity of the females, five EM from each genotype of zucchini and cucumber × RKN combination were hand-picked and placed individually into Eppendorf tubes. The eggs were dispersed in a 0.5% NaOCl solution for 10 min, as described previously, and counted. Experiment two (Exp. 2) is a repetition of Exp. 1 with similar experimental conditions, plant maintenance and nematode assessments but was run for 74 days.

Post- infection development

To assess the RKN developmental stages inside the galls, a random sample of about 100 galls per treatment were dissected under a stereoscopic microscope. Root samples had been previously stained with acid fuchsine 0.05% (Bridge and Page, 1982), cleared in acidified glycerol, and stored until the developmental stages were categorized as females with and without EM, distorted females, males and juvenile stages (J3 and J4). The distorted females were small with an abnormal sausage-like shape with no EM as opposed to the globose pear shape of the females with EM.

Parasitic variation in Meloidogyne

Thirteen RKN populations were tested on zucchini cv. Amalthee, and five on cucumber cv. Dasher II. These populations had been previously characterized for their Mi-gene virulence status (Ornat

et al., 2001, Verdejo-Lucas et al., 2012), and were maintained as described previously. The identity of the populations was verified according to their enzymatic and molecular patterns (Esbenshade and Triantaphyllou, 1990; Zijlstra et al., 2000). They included the three most common species, *M. arenaria*, *M. incognita*, and M*. javanica,* in plastic greenhouses in southern Spain (Verdejo-Lucas et al., 2002; Talavera et al., 2012). Plants were inoculated with 500 freshly hatched J2 (less than 72 hours-old) and each treatment was replicated seven times. The experiment was conducted twice. Plants were harvested at 56 and 58 days after nematode inoculation. The root systems were washed free of soil, weighed and stained in a 0.1 g/liter erioglaucine solution. The number of galls per root system with and without EM was counted. The infection frequency was calculated as described previously.

Statistical analyses

The SAS system V8 (SAS Institute Inc., Cary, NC) was used for statistical analyses. Analysis of variance was carried out by the general lineal model (Proc GLM). Data were transformed to $\log_{10}$ (x + 1) when needed to homogenize the variances prior to the analyses. The experiments on host suitability were analyzed separately due to differences in egg hatching rate of the nematode inoculum. The response of the genotypes to each RKN isolate was compared and the Tukey Honestly Significant Difference (HSD) test used to separate means ($P < 0.05$). Comparisons between RKN isolates on individual genotypes and between cucurbit crops for individual populations were done by Student t Test ($P < 0.05$).The experiments on parasitic variation were analyzed together since no differences were found between the repeated experiments. The RKN populations were compared within each cucurbit crop and means separated by the Tukey HSD test ($P < 0.05$).

Results

Host suitability

Inoculated and non-inoculated zucchini plants showed similar fresh top weight (data not shown), indicating that RKN infection and reproduction was not associated with plant damage after a single nematode generation. The hatching rate of the egg inoculum in Exp. 1 was 14% and 21% for *M. incognita* MiPM26 and *M. javanica* Mj05, respectively, which provided statistically different *Pi* values of 560 and840 J2 per plant. The hatching rate in Exp. 2, 26.4% and 27.5% for *M. incognita* and *M. javanica*, respectively, provided similar *Pi* values of 1055 and 1099 J2 per plant, respectively. Nevertheless, the numbers of *EM* were similar statistically, although numerically different, amongst zucchini genotypes within RKN isolate and experiment (Table 1). On average, *M. incognita* showed similar infection frequency (%) in both experiments (8.6 ± 0.5 and 8.7± 0.5 in Exp. 1 and 2, respectively) despite differences in J2 inoculum as also did *M. javanica* (65 ± 3 and 72 ± 4 in Exp.1 and 2, respectively). Zucchini genotypes differed in eggs/plant and *Rf* (Table 1), and the general trend in both experiments was that Amalthee supported less *M. incognita* eggs/plant than Satelite whereas Parador, Pixar and Floridor provided intermediated values although statistical differences were only shown in Exp. 2. The *Rf* of *M. incognita* on Amalthee and Pixar was lower ($P < 0.05$) than on Satelite. The *M. javanica* eggs/plant were lower ($P < 0.05$) on Amalthee, Parador and Pixar than on Floridor and Satelite in Exp. 2 (Table 1) and the *Rf* was lower ($P < 0.05$) on Amalthee than Parador, Pixar or Floridor in Exp. 1, but only Amalthee differed from Floridor in Exp. 2, (Table 1). In relation to the RKN isolates, all reproductive traits of *M. incognita* MiPM26 were lower ($P < 0.05$) than those of *M. javanica* Mj05 in both experiments (Table 1). Female fecundity did not differ amongst the zucchini genotypes but *M. incognita* (253 ± 6 eggs/*EM*, mean ± standard error) showed lower ($P < 0.05$) fecundity than the *M. javanica* (538 ± 23 eggs/*EM*).

Table 1. Root weight, number of egg masses, eggs per plant and reproduction factor (*Rf*) of *Meloidogyne incognita* (MiPM26) and *M. javanica* (Mj05) on genotypes of zucchini (*Cucurbita pepo*) and cucumber (*Cucumis sativus*) in pot

Cultivar	Exp.	Root weight		Egg masses		Eggs per plant		*Rf* [a]	
		MiPM26	Mj05	MiPM26	Mj05	MiPM26	Mj05	MiPM26	Mj05
Zucchini									
Amalthee	1	6.3 ± 0.4 a	6.8 ± 0.5 b	48 ± 3 a	466 ± 68 a	440 ± 80 a	22531 ± 2442 a	0.8 a	27 b
Parador		7.6 ± 0.6 a	7 ± 0.5 b	37 ± 7 a	643 ± 50 a	822 ± 262 a	59623 ± 12879 a	1.5 a	72 a
Pixar		6.9 ± 0.4 a	7.5 ± 0.4 ab	57 ± 5 a	652 ± 50 a	883 ± 288 a	59461 ± 26619 a	1.6 a	72 a
Floridor		na	9.5 ± 0.4 a	na	501 ± 74 a	na	56274 ± 12879 a	na	68 a
Satelite		6.2 ± 0.6 a	5.4 ± 0.2 b	54 ± 7 a	456 ± 24 a	1272 ± 244 a	28274 ± 4768 a	2.2 a	34 ab
Mean		6.7 ± 0.3	7.2 ± 0.3	50 ± 10*	541 ± 61	870 ± 127*	44815 ± 6357	1.5*	54
Amalthee	2	7 ± 0.6 b	4.8 ± 0.6 b	90 ± 17 a	787 ± 76 a	2707 ± 559 b	66530 ± 10999 b	2 b	42 b
Parador		6.9 ± 0.5 b	6.6 ± 0.5 ab	110 ± 9 a	721 ± 67 a	4175 ± 546 ab	52238 ± 9265 b	3.4ab	58 ab
Pixar		6.5 ± 0.7 b	5.6 ± 0.8 b	83 ± 8 a	905 ± 81 a	3864 ± 675 ab	55882 ± 10449 b	3 b	59 ab
Floridor		12 ± 0.6 a	8.8 ± 0.6 a	127 ± 14 a	1106 ± 88 a	4410 ± 918 ab	157027 ± 21258 a	3.6 ab	100 a
Satelite		6.5 ± 0.2 b	5 ± 0.6 b	127 ± 21 a	771 ± 124 a	8442 ± 1432 a	127957 ± 17492 a	7 a	71 ab
Mean		7.8 ± 0.5*	6.1 ± 0.4	108 ± 15*	858 ± 99	4719 ± 502*	91927 ± 9548	3.8*	78
Cucumber									
Taray	1	17 ± 0.5 a	13 ± 1 a	740 ± 51 a	752 ± 106 a	81583 ± 7705 a	77588 ± 10277 a	77 a	71 a
Dasher II		12 ± 0.5 b	8 ± 0.8 b	515 ± 15 a	603 ± 60 a	33503 ± 3723 b	65944 ± 6514 a	32 b	60 a
Urano		15 ± 0.4 ab	6 ± 1 b	499 ± 36 a	513 ± 64 a	55490 ± 10031 ab	68648 ± 7509 a	52 ab	62 a
Mean		15 ± 0.7 *	9.4 ± 0.8	584 ± 34	622 ± 76	60064 ± 6784	70945 ± 4793	54	65
Taray	2	14.4 ± 2.2 ab	13.4 ± 3 a	734 ± 87 a	1061 ± 115 a	81114 ± 16769 a	120700 ± 23363 ab	66 a	87 a
Dasher II		8.9 ± 0.8 b	10.7 ± 1.2 a	532 ± 34 a	915 ± 119 a	63994 ± 7668 a	102980 ± 11945 b	52 a	102 a
Urano		17 ± 2 a	14.3 ± 0.6 a	488 ± 69 a	1069 ± 114 a	45011 ± 6135 a	186966 ± 20469 a	37 a	158 a
Mean		13.1 ± 1.2	12.7 ± 1.2	585 ± 63*	1015 ± 116	63373 ± 6074*	135097 ± 14613	51*	114

experiments (exp.) conducted in a greenhouse for 65 and 74 days in Exp. 1 and 2, respectively.
Values are mean ± standard error of seven replicated plants/genotype. Values within each crop and experiment sharing the same letter are not significantly different according to Tukey test ($P < 0.05$). [a] Eggs/plant divided by emerged second-stage juveniles. *Indicates statistical differences ($P < 0.05$) between *Meloidogyne* isolates.

Table 2. Percentage of females with and without egg masses (*EM*), distorted females, males, juveniles stages (J3 + J4) and empty galls on genotypes of zucchini (*Cucurbita pepo*) and cucumber (*Cucumis sativus*) inoculated with 4000 eggs of *Meloidogyne incognita* MiPM26 (*Mi*) and *M. javanica* Mj05 (*Mj*) in pot experiments conducted in a greenhouse.

			Composition of the nematode population inside the galls (%)											
	No.galls dissected		Females with EM		Females without EM		Distorted females		Males		Juvenile stages		Empty galls	
Cultivar	*Mi*	*Mj*	*Mi*	*Mj*	*Mi*	*Mj*	*Mi*	*Mj*	*Mi*	*Mj*	*Mi*	*Mj*	*Mi*	*Mj*
Zucchini														
Amalthee	89	105	18	82	10	3	30	5	4	0	4	1	33	10
Parador	112	100	28	97	12	1	17	0	4	0	11	0	29	2
Pixar	109	100	22	98	19	2	18	0	0	0	6	0	34	0
Floridor	91	100	15	100	20	0	17	0	10	0	3	0	35	0
Satelite	101	101	26	97	17	1	19	1	2	0	8	0	29	1
Mean	96	101	22*	95	16*	1	20*	1	4*	0	4*	0	32*	3
Cucumber														
Taray	101	100	89	100	10	0	1	0	0	0	0	0	0	0
Dasher II	100	100	89	97	7	0	0	0	0	0	0	0	4	3
Urano	100	100	81	99	13	0	0	0	0	0	0	0	6	1
Mean	100	100	86	99	10*	0	0	0	0	0	0	0	3*	1

*indicates statistical differences between nematode species according to Student´s t test ($P < 0.05$).

Inoculated and non-inoculated cucumber plants showed similar fresh top weight independently of the genotype. The hatching rate of the egg inoculum was 30.8% and 29.6%, and 26.4% and 27.5%, for *M. incognita* and *M. javanica,* in Exp. 1 and 2, respectively, which provided similar *Pi* values: 1234 and 1184 J2/plant in Exp. 1, and 1055 and 1099 J2/plant of *M. incognita* and *M. javanica,* respectively, in Exp. 2. The number of *EM* was similar amongst cucumber genotypes within RKN isolate in both experiments (Table 1). *Meloidogyne incognita* produced less ($P<0.05$) eggs/plant on Dasher II than Taray in Exp. 1, whereas *M. javanica* did on Dasher II than Urano in Exp. 2 (Table 1).

The *Rf* of *M. incognita* on Dasher II was lower ($P < 0.05$) than on Taray but only in Exp. 1 (Table 1). Cucumber genotypes did not differ in *Rf* values when infected by *M. javanica*. The fecundity of the females in the cucumber plants was similar amongst genotypes irrespective of the RKN isolate: 568 ± 48 and 553 ± 58 eggs/*EM* for *M. incognita* and *M. javanica*, respectively.

Post-infection development

Zucchini galls induced by *M. incognita* MiPM26 showed both small and large galls. Small galls contained single pear-shape females with a large *EM* (22%) exposed on the root surface. Large galls showed hyperplasic and hypertrophic tissue and contained females without egg masses (16%), distorted females (20%), males (4%), and juvenile stages (4%) or they were empty (32%) (Table 2). *Meloidogyne javanica* galls were small, discrete and 82% to 100% contained single pear-shape females with large *EM*. Males or juveniles stages were not observed and only 3% of the galls were empty (Table 2). *M. incognita* females produced fewer ($P < 0.05$) *EM* than *M. javanica* (22% and 95%, respectively) on zucchini (Table 2). Examination of the cucumber galls showed that 86% of the *M. incognita* females had *EM*, 10% did not, and 3% of the galls were empty, whereas 99% of the *M. javanica* galls showed females with *EM* (Table 2).

Parasitic variation in Meloidogyne

Zucchini showed a large variation in the number of galls (Table 3). As a general trend, the *M. javanica* populations showed the highest numbers of *EM*, followed by *M. incognita* MiAL10 and MiAL15, and those of *M. arenaria.* However, the exceptions to this trend were *M. incognita* MiPM26, MiAL09, MiAL48 that showed high numbers of galls but few *EM* (Table 3). Cucumber showed a high correspondence between the numbers of galls and *EM* as more than 75% of the galls had *EM*. Only *M. arenaria* MaAL47 showed a lesser ($P < 0.05$) ability than the remaining populations to form galls and produce *EM* in the cucumber plants. When populations were grouped by *Meloidogyne* species (Table 4), *M. incognita* and *M. javanica* showed similar gall numbers on zucchini but higher ($P < 0.05$) than *M. arenaria*. The number of egg masses of *M. incognita* on zucchini was lower ($P < 0.05$) than that of *M. arenaria* followed by *M. javanica* (Table 4). On cucumber, *M. incognita* and *M. javanica* showed similar gall and egg mass numbers but *M. arenaria* produced less ($P < 0.05$) galls than *M. incognita,* and fewer ($P < 0.05$) *EM* than *M. incognita* and *M. javanica* (Table 4). The five individual populations tested on both cucurbit hosts showed lower ($P<0.05$) *EM* numbers on zucchini than cucumber (Fig. 1), which is in support of the differential host status of these cucurbits to *Meloidogyne*. The average number of *EM* was 33 ± 3.4 on zucchini, and nearly twice as many on cucumber (102 ± 12.8 *EM*).

Table 3. Total numbers of galls, with and without egg masses (*EM*) and infection frequency (%) on zucchini cv. Amalthee and cucumber cv. Dasher II inoculated with 500 second-stage juveniles of *Meloidogyne arenaria* (*Ma*), *M. incognita* (*Mi*) and *M. javanica* (*Mj*) in pot experiments conducted in a greenhouse.

Population code	Total galls	Galls with *EM*	Galls Without *EM*	Infection frequency[a]
		Zucchini		
Mj05	80 ± 5.9 abc	79 ± 6 a	1 ± 0.5 d	16 a
MjAL 39	78 ± 4.5 abc	78 ± 4.5 a	0 d	15.6 a
MjAL21	64 ± 6 abcd	64 ± 6 ab	0 d	12.8 ab
MjAL26	58 ± 6 cde	56 ± 6 ab	1 ± 0.8 d	11.2 bc
MiPM26	101 ± 4 a	12 ± 1 fg	89 ± 4 a	2.4 fg
MiAL09	81 ± 7 ab	3 ± 0.5 g	78 ± 7 a	0.6 g
MiAL48	38 ± 3.5 defg	5 ± 1 g	33 ± 2.4 b	1 fg
MiAL15	51 ± 3 def	42 ± 7 bcde	8 ± 1.2 c	8.3 cde
MiAL10	47 ± 4 defg	46 ± 3.5 abcd	1± 0.5 d	9.2bcd
Ma68	45 ± 3 defg	45 ± 3 abcd	0 d	9 b-e
MaAL47	37 ± 6 efg	36 ± 4 cde	1 ± 0.8 d	7.2 de
MaAL30	27 ± 4 fg	27 ± 4 de	0 d	5.4 def
MaGrau	27 ± 4 fg	25 ± 3 e	2 ± 1.6 d	5 ef
		Cucumber		
MjAL26	135 ± 15 ab	133 ± 15 a	2 ± 1 d	25 a
MiAL09	154 ± 14 a	116 ± 12 a	38 ± 8 a	24 ab
MiAL10	111 ± 14 ab	103 ± 14 a	8 ± 1 bc	26 a
MaGrau	117 ± 9 ab	102 ± 8 a	15 ± 2.4 ab	21 ab
MaAL47	61 ± 14 b	56 ± 15 b	5 ± 1.3 bcd	11.2 b

Values are mean ± standard error of 14 replicates per treatment (seven replicates plants /population x two experiments). Values in the same column within each cucurbit species followed by the same letter are not significantly different according to Tukey HSD (Honestly Significant Difference) ($P < 0.05$). [a] Egg masses per plant divided by J2 inoculum x 100.

Table 4. Number of galls and egg masses per plant of *Meloidogyne* populations grouped by nematode species on zucchini cv. Amalthee and cucumber cv. Dasher II inoculated with 500 second-stage juveniles in pot experiments conducted in a greenhouse.

Cucurbit	*Meloidogyne* species	Galls	Egg masses
Zucchini	*M. arenaria* (n= 4)	33 ± 2 b	32 ± 17 b
	M. incognita (n= 5)	63 ± 3 a	22 ± 24 c
	M. javanica (n= 4)	66 ± 3 a	67 ± 23 a
Cucumber	*M. arenaria* (n=2)	89 ± 11 b	78 ± 52 b
	M. incognita (n=2)	160 ± 27 a	102 ± 54 a
	M. javanica (n=1)	130 ± 13 ab	124 ± 54 a

Values are mean ± standard error of n x 7 replicated plants /experiment x 2 experiments. Values in the same column within each cucurbit crop followed by the same letter are not significantly different according to Tukey HSD (Honestly Significant Difference) ($P < 0.05$).

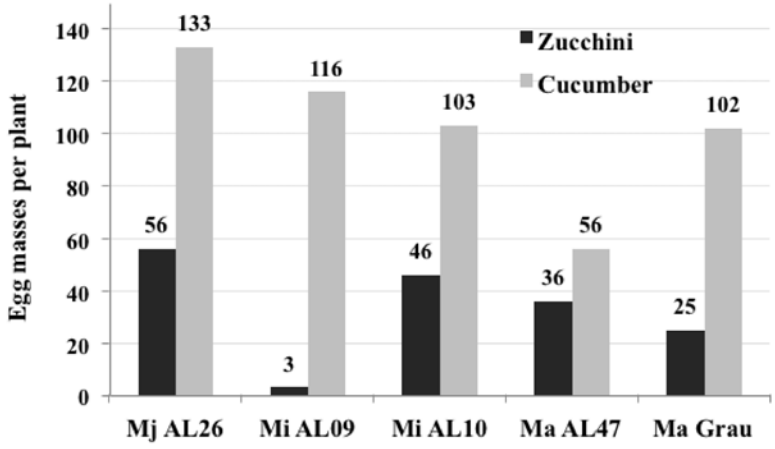

Fig. 1. Number of egg masses per plant, of *Meloidogyne* populations tested on both zucchini cv. Amalthee and cucumber cv. Dasher II inoculated with 500 second-stage juveniles in pot experiments conducted in a greenhouse. Bars represent the mean values plus standard error of 14 replicated plants per treatment. Cucurbit hosts differed in number of egg masses according to Student T-test ($P<0.05$).

Discussion

The interaction between *Meloidogyne* spp. and two cucurbit crops was investigated in relation to plant genotype and RKN parasitic variation. Differences in host suitability amongst zucchini genotypes occurred consistently in nematode reproduction (eggs/plant and *Rf*) but not infection (*EM* and infection frequency) despite differences in initial J2 inoculum levels. The lack of significant differences in Exp. 1 was probably due to the lower hatching rate of the egg inoculum in this experiment related to Exp. 2, which made any difference amongst genotypes more difficult to detect. In contrast, the RKN isolate had a striking effect on zucchini revealed by small infection frequency of 8.65% and *Rf* < 3.8 fold increase for *M. incognita* MiPM26 compared to a 65% to 72% infection frequency and *Rf* > 54 fold for *M. javanica* Mj05. The post-infection exam showed the inability of a high percentage of MiPM26 individuals to reach the egg-laying female stage on zucchini but such an effect was not observed for *M. javanica*. Pre-infection mechanisms were not involved because 98% of MiPM26 J2 inoculum penetrated and developed to J4 on zucchini (López-Gómez and Verdejo-Lucas, 2014). The slight genetic variability among these genotypes possibly reflects that fruit shape and size, phenotypic characters used for cucurbit domestication, have little effect on the host-nematode interaction.

The emptiness of a third of the galls could be due to crowding of large numbers of invading J2 leading to competition for available feeding sites, which may have stopped nematode development and eventually caused their death or derived them to males (Stephan and Trudgill, 1982; Faske, 2013). Insufficient nutrient supply by non-fully functional feeding sites may have produced distorted females unable to lay eggs (McClure et al., 1974). Overall, the zucchini genotypes we tested provided unsuitable conditions for MiPM26 development, as did Amalthee for MiAL09 and MiAL48 infection. All zucchini genotypes, however, were excellent hosts for Mj05 and all *M. javanica* populations showed high infectivity on Amalthee. Strawberry genotypes that were susceptible to *M. hapla* were non-hosts to *M. incognita* (Edwards et al., 1985). Similarly, soybean genotypes were less

suitable hosts to *M. incognita* than *M. arenaria* (Kirkpatrick and May, 1989).

The cucumber genotypes we tested showed similar susceptibility levels to both RKN isolates. However, cucumber cultivars grown in the Pothovar region of Pakistan differed in suitability levels to *M. incognita* with *Rf* from 0.33 to 10.52-fold increase in response to a 3000 J2 inoculum (Mukhtar et al., 2013) which point out the genetic variability within this crop.

Parasitic variation was greater on zucchini than cucumber. Thus, the suitability of zucchini varied from being a poor to good host depending on the *M. incognita* population which suggests that the severity of the disease would change from site to site and site-specific management would be necessary (Melakeberjan and Wang., 2012; Melakeberjan et al., 2012). Intra-specific variation was not observed amongst the *M. javanica* or *M. arenaria* populations on either crop. The reduced parasitic ability of *M. arenaria* was consistent on both zucchini and cucumber. On cucumber, little parasitic variation was observed, but the high infection and reproduction levels point to the need for nematode management strategies due to the low tolerance limit of cucumber to the nematode (Giné et al., 2014). Cucumber is also a suitable host to *M. hapla, M. floridensis* and *M. hispanica* (Stephan and Trudgill, 1982, Sikora and Fernandez, 2005; Maleita et al., 2012).

Root galling indicates successful establishment of the feeding sites that will allow further nematode development and life cycle completion. However, rating host suitability based on root galling may be misleading (Fassuliotis and Dukes, 1972; Edwards et al., 1985; Fourie et al., 2012; Maleita et al., 2012), as gall formation is not always followed by successful nematode development. This was exemplified by *M incognita* on zucchini that produced similar gall numbers but lower *EM* than *M. javanica*. Therefore, crop or cultivar recommendation cannot be made based only on the observation of root galling due to the RKN parasitic variation. In general, little host damage has been associated to low nematode reproduction (Ehwaeti et al., 1999), and differences among RKN populations have been observed on poor hosts or resistant genotypes such as pepper, asparagus, tomato and celery

(Khan and Khan, 1991; Dudash and Barker, 1992; Cortada et al., 2009; Melakeberhan and Wang, 2012).

Although the susceptibility of zucchini cv. Amalthee and cucumber Dasher II to RKN had been reported (Coyler et al., 1998, López-Gómez and Verdejo-Lucas, 2014, Giné et al., 2014, Vela et al., 2014), this study showed that zucchini was a poor host for MiPM26 and MiAL09 whereas cucumber was a good one. Also, the infectivity of the RKN populations was less on zucchini (4.8 times) and cucumber (2.5 times) than susceptible tomatoes (Verdejo-Lucas et al., 2012) which support the rotational value of cucurbit crops as contributors to moderate RKN build-up in double cropping systems. Lower remnant populations would remains in the soil after growing cucurbits than solanaceous crops, which concur with field observations (Talavera et al., 2012; Verdejo-Lucas et al., 2002). Additional tests should be done to corroborate the variation in zucchini and cucumber genotypes adapted to protected cultivation.

The size of the *M. incognita* galls and tissue disturbance on zucchini suggests that damage would be more severe in *M. incognita* than *M. javanica* infestations as reported on cucumber and melons (Edelstein et al., 2010) but *Pf* values will be smaller due to lower *EM* production. Conversely, higher *Pf* would be expected in *M. javanica*- than *M. incognita*-infested fields. Consequently, it could be argued that it would be more effective to grow zucchini instead of cucumber as a rotation crop in a RKN management program. This choice would be more successful in *M. incognita* and *M. arenaria* than *M. javanica*-infested soils. Populations of *M. hapla* showed different reproductive potential on celery, carrot, and potato (Melakeberhan and Wang, 2012, 2013; Melakeberhan et al., 2012).

In conclusion, the selected genotypes of both cucurbits were suitable hosts for *M. arenaria, M. incognita* and *M. javanica* but the zucchini was poorer host than the cucumber regardless of the genotype or RKN population. Post-infection mechanisms involved in the response of zucchini genotypes to *M. incognita* MiPM26 resulted in reduced egg production, *Rf* and female fecundity. The parasitic variation amongst RKN populations was strongly associated to the host suitability; larger on zucchini than cucumber.

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Chapter 3

Differential reproduction of Meloidogyne incognita and M. javanica in watermelon cultivars and cucurbit rootstocks

Differential reproduction of *Meloidogyne incognita* and *M. javanica* in watermelon cultivars and cucurbit rootstocks

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Abstract

The host suitability of watermelon cultivars and cucurbit rootstocks to *Meloidogyne incognita* and *M. javanica* was determined in pot and field experiments. *Meloidogyne incognita* showed higher reproduction than did *M. javanica* than watermelon and cucurbit rootstocks. The watermelon cultivars did not differ in host status when challenged with these two species and supported lower nematode reproduction than did the cucurbit rootstocks. Rootstocks *Lagenaria siceraria* cv. Pelops and *Cucurbita pepo* AK15 supported lower reproduction than did the squash hybrid rootstocks (*C. maxima* × *C. moschata*). Egg production increased ($P < 0.05$) with a rising initial inoculum level (*Pi*) in the non-grafted Sugar Baby but the reproduction factor *Rf* (eggs per plant / *Pi*) was similar at two *Pi* levels. The total egg production in the plants grafted onto RS841 and Titan squash hybrids were greater ($P < 0.05$) at the higher *Pi,* but the *Rf* values were lower. The development of field-grown non-grafted watermelon plants was significantly stunted in plots where the nematodes were detected at planting. However, no differences were observed in plots with grafted plants. In plots with nematodes, non-grafted and Titan-grafted plants had similar yield which was higher than that of RS841-grafted plants. In the commercial plastic houses with grafted watermelon, the average *Rf* value was 42-fold, confirming the high susceptibility of squash hybrid as rootstocks for grafted watermelon. The Titan-Sugar Baby combination was tolerant to *M. javanica.*

Key words: *Citrullus lanatus*, grafting, host suitability, root-knot nematodes, RS841, Titan.

Introduction

In protected cultivation, cucurbitaceous and solanaceous crops are commonly rotated to maximize land use and boost productivity and thus profits from a costly production system. Members of the Cucurbitaceae family, including watermelon, cucumber, squash, and melon, differ in susceptibility to nematodes of the genus *Meloidogyne* (RKN) and resistant cultivars are not commercially available (Thies & Levi, 2007; Levi *et al*., 2009; Thies & Levi, 2010; Pofu *et al*., 2011). Hence, alternating with less susceptible crops or cultivars would help to manage nematode population densities, because the host-plant status affects RKN egg production (Ehwaeti *et al.*, 1999), and thus the primary inoculum for the following crop.

Watermelon represents 46% of the cucurbit yield production worldwide. In Spain, watermelon cultivation has expanded to reach 37% in the last 10 years with approximately 18,600 ha, of which, 60% are under protected cultivation and 40% in open fields (MAGRAMA, 2013). Watermelon is susceptible to the most common RKN species, *M. arenaria, M. incognita,* and *M. javanica,* causing a reproduction factor (*Rf*) ranging from 1.2 to 14 (Montalvo & Esnard, 1994; Thies & Levi, 2007; Davis, 2007; Xing & Westphal, 2012; López-Gómez & Verdejo-Lucas, 2014; López-Gómez *et al*., 2014).

Grafting a susceptible cultivar onto resistant or tolerant rootstocks is a way to avoid losses caused by *Meloidogyne* in susceptible crops in continuous cropping systems. Grafting vegetables has been used in Far East Asian countries since the early 20th century but was not widely adopted on a commercial scale in the western world until the soil fumigant methyl bromide was banned. The primary reason for grafting is to counteract damage caused by soil-borne pests and pathogens. The rootstocks also bring new features to the grafted plants such as increased foliage and yield and tolerance to abiotic stress such as low temperature and salinity (Davis *et al*., 2008). Watermelon is highly susceptible to fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum,* and grafting is used to control this disease (Miguel *et al*., 2004). In Spain, approximately 50% of watermelon, representing 30 million plants, are grafted each year (Davis *et al*., 2008).

Watermelon is grafted mainly onto interspecific squash hybrids, (*Cucurbita maxima* × *Cucurbita moschata)*, although it is also grafted onto *Cucurbita pepo, Lagenaria siceraria*, *Momordica charantia*, and more recently, *Citrullus lanatus* (Davis *et al.*, 2008). However, these rootstocks have not been widely tested for their efficacy to suppress the RKN disease, although some reports on this are available (Levi *et al*., 2009; Thies *et al*., 2010; Kokalis-Burelle & Rosskoff, 2011). Furthermore, the efficacy of grafting for pathogen suppression may vary with the genetic background of the rootstocks and that of the nematode (Cortada *et al*., 2008; Mohamed *et al*., 2012). It is useful to know the host suitability to *Meloidogyne* spp. of current watermelon cultivars and cucurbit rootstocks in order to select poor hosts for the nematode.

Therefore, this study was conducted to determine i) the host suitability of watermelon cultivars and cucurbit rootstocks to *M. javanica* and *M. incognita,* and ii) the effect of initial inoculum level (*Pi*) on nematode reproduction in grafted and non-grafted watermelon under greenhouse and field conditions. RKN population densities were monitored on watermelon rotated with other vegetable crops in commercial plastic houses.

Materials and methods

Nematode

The RKN isolates were *M. incognita* (MiPM26) and *M. javanica* (Mj05) from the collection of IRTA, Centre of Cabrils, Barcelona, Spain. Nematode cultures were started from the progeny of a single female and maintained in tomato cv. Roma during spring-summer and in celery cv. D´Elne during autumn-winter in a greenhouse. The identity of the nematodes was verified according to their perineal and enzymatic patterns (Esbenshade & Triantaphyllou, 1990). The nematode was multiplied in tomato cv. Roma to obtain the inoculum for the experiments. Eggs were extracted by blender maceration of infected tomato roots in a 0.5% NaOCl solution for 5 min (McClure *et al,* 1973). The egg suspension was passed through a 74 µm aperture sieve to remove root debris, and the dispersed eggs were collected on a 25 µm sieve. The

second-stage juveniles (J2) were obtained by incubating egg suspensions in Whitehead trays at 26ºC ± 1 (Whitehead & Hemming, 1965). The J2 emerging within 72 h were used as the inoculum.

Host suitability

Pot experiments were conducted to determine the host suitability for RKN of eight watermelon cultivars and seven cucurbit rootstocks, the characteristics of which are described in Table 1. Both groups of plants were tested simultaneously under the same environmental conditions in a climatic growth chamber. Seeds were soaked overnight and germinated at 26ºC ± 1 in vermiculite trays for 14 days for the watermelon cultivars and seven days for the rootstocks. Seedlings were transplanted at the cotyledon stage to pots filled with 500 cm^3 of sterilized river sand and allowed to grow for five days before inoculation with 500 RKN J2 per pot in approximately 2 ml of water. Each RKN isolate-cultivar combination was replicated seven times and the experiment was repeated once. Plants were maintained in the growth chamber at constant temperature of 26ºC ± 1 with 16 h light, and fertilized with a slow-release fertilizer Osmocote ® Scotts Company, Heerlen, Netherlands (15% N + 10% P_2O_5 + 12% K_2O + 2% MgO_2 + microelements) at the beginning of the experiment. The plants were watered daily as needed and soil temperatures in the pots at 7 cm in depth were registered at 30-min intervals (Em50 Data Logger®, Decagon Devices Inc, Pullman, WA, USA, accuracy ± 1ºC, resolution 0.1ºC). The number of generations completed by the nematode was calculated according to a base temperature *Tb* = 17.2°C and *K* 357 accumulated degree-days (*DD*) (López-Gómez *et al.*, 2014) being *Tb* the temperate below which the nematode development does not occurs, and the thermal constant *K,* the accumulated degree days (*DD*) above *Tb* necessary to complete one generation.

Table 1. Main characteristics of the watermelon cultivars and rootstocks mentioned in the study.

Species / type	Cultivar	Resistance / Feature	Company
		Watermelon, *Citrullus lanatus*	
Jubilee	Regus	2n, oblong, striped	Sakata
Sugar Baby	Sevilla	2n, round, black, microseed	Rijk Zwaan
	Sugar Baby	2n, round, black	Intersemillas
	Baronesa	2n, round, black	Rijk Zwaan
Crimson	Imperial	2n, oblong, striped	Akira
Yellow	Volga	3n, yellow Flesh	Ramiro Ar
Tiger	Akiless	3n, round, striped	Akira
Crimson	Paladin	2n, oblong, striped	Sakata
		Cucurbit rootstock	
Lagenaria siceraria	Pelops	HR: Fom; IR: Fon	Rijk Zwaan
Cucurbita pepo	AK 15	Unknown	Akira
Citrullus lanatus	Robusta	IR: *Mi*, *Ma*	Syngenta
C. maxima x C. mochata	RS841 (Klasico)	HR: Fom, Foc, Fon, Forc; IR: *Vd, Ma, Mi, Mj*	Akira
	Titan	HR Fol	Ramiro Ar
	Strongtosa	IR: *Fon*	Syngenta
	Routpower	IR: *Fon, Nematode*	Sakata
	Zadok	HR: Fom, Va; IR: Fon	Rijk Zwaan
	Shintoza	HR: Fon	Intersemillas
	Carnivor	HR: Fon	Syngenta

HR: high resistance, *IR*: intermediate resistence, *Foc*: *Fusarium oxysporum f*. sp. *cucumerinum*, *Fom*: *Fusarium oxysporum* f. sp. *melonis*, *Fon*: *Fusarium oxysporum* f. sp. *niveum*, *Forc*: *Fusarium oxysporum f.* sp. *radicis cucumerinum*, *Vd*: *Verticillium dahlie*, Nematode: *Meloidogyne* spp. / *Ma* / *Mi* / *Mj*: *M. arenaria, M. incognita, M. javanica*.

Plants were harvested 43 and 42 days after inoculation in experiment 1 and 2, respectively. Plants were carefully removed from the pots, the root system washed free of soil and weighed. Egg masses (*EM*) were stained with a 0.1 g L^{-1} erioglaucine solution (Aldrich Chemical Company, St Louis, Mo, USA) for two hours (Omwega *et al.*, 1988) and the number of *EM* counted. Eggs were extracted by macerating the entire root system in a blender with a 0.5% NaOCl solution for 10 min (McClure *et al*., 1973). The number of non-hatched eggs and egg shells were counted to record the total number of nematodes produced per plant and considered as the final population (*Pf*). The nematode reproduction factor *Rf* was calculated by dividing *Pf* by *Pi*. The percentage of emerged J2 from the newly formed eggs was calculated by dividing egg shells by *Pf* and multiplying by 100.

Effect of initial inoculum on nematode reproduction

The effect of *Pi* on reproduction of *M. javanica* and plant growth was determined in pot experiments. The treatments were i) non-grafted watermelon cv. Sugar Baby, ii) Sugar Baby grafted onto RS841, and iii) Sugar Baby grafted onto Titan. The *Pi* treatments were 0, 5,000 and 25,000 eggs / plant. All possible combinations were tested with nine replicates per treatment and the experiment was repeated once. Grafting was performed by a commercial nursery, and plants were transplanted to pots filled with 2.5 L of sterilized river sand after three weeks and inoculated one week after transplanting. Plants were placed at random on a bench in an unheated greenhouse with non-temperature controlled environment. Soil temperatures were recorded as described previously and the accumulated *DD* calculated as before. Plants were watered daily as needed and fertilized as described previously. Plants were harvested 100 days after nematode inoculation. Tops (stems, flowers and fruits) were cut at ground level and oven dried at 60^{o}C for 72 h to determine dry top weight. Roots were carefully removed from the pots, washed free of soil and weighed. Eggs were extracted from 10-g root subsample using a 0.5% NaOCl solution for 10 min. (McClure *et al*., 1973). The number of eggs including non-hatched eggs and egg shells were counted, and the *Pf* was expressed as eggs / plant. The *Rf* was

estimated by dividing *Pf* by the respective *Pi*. The percentage of J2 emerged was calculated by dividing egg shells by *Pf* × 100.

Field experiment

Thirty-six plots of 6.5 m^2 (2.6 × 2.5 m) were marked in an unheated plastic greenhouse infested with *M. javanica* located at the IRTA, (Cabrils, Barcelona, Spain). Individual plots consisted of a single row with four plants per plot spaced 60 cm apart. The treatments investigated were: i) non-grafted cv. watermelon Sugar Baby, ii) Sugar Baby grafted onto RS841 or iii) grafted onto Titan. The *Pi* treatment had two RKN levels: plots with *M. javanica* detected (*Pi* > 1) or not detected (*Pi* < 1) before planting. The treatments were arranged according to a stratified randomized-block design. All possible rootstock-*Pi* combinations were tested with six replicated plots per treatment. The soil of the plastic greenhouse was a sandy loam with 85.8% sand, 8.1% silt and 6.1% clay, pH 8.1, 0.9% organic matter w/w, and 0.40 dS m^{-1} electrical conductivity. The soil had been covered with black plastic polyethylene mulch before transplanting the seedlings to prevent weed growth following locally common practices for watermelon production. Plants were cultivated for 13 weeks. Plants were irrigated as needed through a drip irrigation system, and fertilized weekly with a solution consisting of NPK (15-5-30) 31 kg / ha, iron chelate, and micronutrients at a rate of 0.9 kg / ha.

Composite soil samples were collected at the beginning and end of the experiment to estimate the *Pi* and *Pf* of *M. javanica*. Individual samples consisted of five soil cores (c. 250 cm^3 each) taken to 25 cm deep with a sampling tube (2.5 cm diameter). Soil samples were mixed and nematodes extracted from two 500-cm^3 soil subsamples using Whitehead trays (Whitehead & Hemming, 1965). Nematodes were collected one week later, concentrated on a 25-µm-aperture sieve and counted. The *Rf* was calculated by dividing *Pf* by *Pi*. At harvest, roots were unearthed to assess disease incidence as the percentage of plants with galled roots and to estimate the disease severity (root galling) using a scale of 0 to 10, where 0 = no galls detected, and 10 = roots completely dead or dying (Zeck, 1971). Roots were then bulked, cut in 1-2 cm

sections and 10-g subsamples used to extract eggs as described in the previous section.

The above-ground plant growth was monitored by photographs taken weekly at each plot from the same perspective with a digital camera (Nikon D5100, 18-55 mm, f/3.5-566). The digital images were processed using ImageJ 1.48v software (http:\\rsb.info.nih.gov/ij/) to measure the surface area (m^2) of the plot covered by the plant foliage (Abrámoff *et al.,* 2004). The same scale was set for each image, and used as a reference for calculations.

Fruit yield (number and weight) was determined 10 and 13 weeks after transplanting. At the final harvest, tops were cut at ground level and oven-dried for two weeks to determine the dry weight. Soil temperatures were recorded as described above and the accumulated *DD* was calculated.

The Sugar Baby scion when grafted onto RS841, but not on Titan, grew adventitious roots that became RKN infected as they developed in the soil. Juvenile emergence from eggs produced in the same plant but different root system was compared to determine if the plant host affected egg hatching. Roots from the watermelon scion and cucurbit rootstock were manually separated at harvest, and 3-gram root subsamples from the respective root systems were incubated in Whitehead trays at constant temperature of 26ºC ± 1 in a climatic chamber. The hatched J2 were collected weekly for 12 weeks until hatching stopped.

Population densities in commercial fields

Soil population densities of *Meloidogyne* were monitored in six unheated commercial plastic houses, located in Southern Spain with a history of RKN problems. Cucurbits rotated with tomato were grown in three sites (10 crops), and grafted watermelon in the other three. The characteristics of the sites are presented in Table 6. At each site, a nematode-infested area of 81 m^2 (EP05 - EP09 sites) or 90 m^2 (Sanlu01) was marked and divided into nine plots of 9 m^2 (3 × 3 m) each in EP05 - EP09 sites, and 10 m^2 (2 × 5 m) in Sanlu01. Crops were managed by the growers according to

common agricultural practices in the area. Weeds were removed manually during and between cropping cycles. Composite soil samples were collected from each individual plot at the beginning and end of each cropping cycle to estimate *Pi* and *Pf* values, respectively. Individual samples consisted of six soil cores taken to a depth of 30 cm with a sampling tube (2.5 cm diameter). Soil was mixed thoroughly and nematodes were extracted from 250 cm^3 soil subsamples using the Whitehead tray method (Whitehead & Hemming 1965). Nematodes were collected and counted and the *Rf* calculated as described above.

Statistical analyses

The SAS V8 software (SAS Institute Inc., Cary, NC, USA) was used for the statistical analyses. Prior to the analyses, data were checked for normal distribution by the normal Shapiro-Wilk W test and the homogeneity of variances by the ANOM for variances. When needed, data were log transformed [$\log_{10}$ (x + 1)] to homogenize the variances. Data from the pot experiments on host suitability and grafting were combined because there were no differences ($P < 0.05$) between the repeated experiments. All data were analysed using analysis of variance (ANOVA), and when significant, the means were separated by Tukey Honestly Significant Difference (HSD) test ($P < 0.05$). In the host suitability experiment, comparisons were made within watermelon cultivars and cucurbit rootstocks for each RKN isolate. In addition, data were grouped by nematode isolate, and the new set of data was analysed using Student's t-test ($P < 0.05$). In the experiments on initial inoculum, comparisons were made within rootstock treatments and inoculum levels. Data from the field experiment were compared within plots with *M. javanica* detected (*Pi* > 1) or not detected (*Pi* < 1) before planting, and between rootstock treatments.

Results

Host suitability

Meloidogyne javanica and *M. incognita* had accumulated 405 and 412 *DD* in experiments 1 and 2, respectively, this corresponding to completion of one generation (*Tb*=17.2; 357 *DD)*. The watermelon cultivars did not differ in *EM*, eggs / plant or *Rf* (Table 2), although *M*. *incognita* had higher ($P < 0.05$) reproductive traits than did *M. javanica.* Juvenile emergence from the newly produced eggs was 10% and 7% for *M. incognita* and *M. javanica*, respectively, confirming the start of the second nematode generation. Regarding the cucurbit rootstocks, *Lagenaria siceraria* cv. Pelops was not as good a host (lower ($P < 0.05$) *EM*, eggs / plant and *Rf*) as the squash hybrids, and *M. incognita* reproduced more ($P < 0.05$) than did *M. javanica*. However, in *C. pepo* AK15, *M. javanica* reproduced more ($P < 0.05$) than did *M. incognita*. Both RKN isolates reproduced similarly on the squash hybrids (Table 2). The percentage of emerged J2 from the rootstocks was 41% and 26% for *M. incognita* and *M. javanica*, respectively. The combined analyses of both groups of plants indicated lower ($P < 0.05$) root weight (3 ± 0.4 and 9.9 ± 0.8 g / root, respectively, mean ± standard error) and reproductive traits in the watermelon cultivars than cucurbit rootstocks, irrespective of the RKN isolate.

Effect of initial inoculum on nematode reproduction

The mean daily soil temperature was 21.6ºC ± 5 and the minimum and maximum temperatures were 9.6ºC and 36.3ºC in experiment 1, and 10ºC and 36ºC in experiment 2, respectively. *Meloidogyne javanica* had accumulated 457 and 462 *DD* in experiments 1 and 2 respectively, indicating the completion of the first generation (*Tb* = 17.2 ºC, 357 *DD*) and the start of the second one. Total egg production augmented ($P < 0.05$) with increasing *Pi* in the non-grafted Sugar Baby from 51,500 to 286,500 eggs / plant and such an increase was proportional to the 5-fold increase in *Pi*. However, the *Rf* was similar at both *Pi* levels (Table 3). The root weight of non-grafted Sugar Baby plants was higher in RKN-inoculated than non-inoculated plants.

Table 2. Number of eggs masses, eggs per plant and reproduction factor (*Rf*) of *Meloidogyne javanica* (*Mj*) and *M. incognita* (*Mi*) in eight watermelon cultivars and seven cucurbit rootstocks

Botanical name and cultivar	Egg masses		Eggs / plant x 100		*Rf*[a]	
	Mj	*Mi*	*Mj*	*Mi*	*Mj*	*Mi*
Watermelon, *Citrulus lanatus*						
Regus	1.3 ± 0.3 a*	10 ± 2 a	4.8 ± 1.6 a*	22 ± 2 a	1 a*	5 a
Sevilla	1.2 ± 0.9 a*	7 ± 0.8 a	5.4 ± 3 a*	30 ± 10 a	1.1 a*	6 a
Sugar Baby	1.6 ± 0.7 a*	9 ± 1.5 a	3.6 ± 2 a*	19 ± 2 a	0.7 a*	4 a
Baronesa	1 ± 0.5 a	nt	1.6 ± 0.7 a	nt	0.3 a	nt
Imperial	0.5 ± 0.2 a	nt	2.1 ± 1.2 a	nt	0.4 a	nt
Volga	0.8 ± 0.3 a	nt	0.26 ± 0.1 a	nt	0.05 a	nt
Akiless	nt	8 ± 1 a	nt	16 ± 2 a	nt	3 a
Paladin	nt	12 ± 1 a	nt	20 ± 2 a	nt	4 a
Mean cultivars	1 ± 0.2*	9 ± 0.6	2.9 ± 1*	21 ± 2	0.6*	4
Rootstock						
Lagenaria siceraria						
Pelops	16 ± 2 b*	122 ± 11 b	148 ± 86 b*	365 ± 22 c	30 b*	73 c
Cucurbita pepo						
AK15	120 ± 9 a	117 ± 6 b	1073 ± 83 a*	468 ± 24 b	215 a*	94 b
C. maxima x C. moschata						
RS841 (Klasico)	139 ± 16 a	191 ± 8 a	1189 ± 161 a	1017 ± 57 a	238 a	203 a
Titan	100 ± 10 a	171 ± 12 a	940 ± 121 a	976 ± 47 a	188 a	195 a
Strongtosa	122 ± 15 a	nt	1177 ± 116 a	nt	235 a	nt
Routpower	127 ± 12 a	nt	1179 ± 87 a	nt	236 a	nt
Zadock	103 ± 6 a	nt	973 ± 51 a	nt	195 a	nt
Mean rootstocks	105 ± 6*	150 ± 6	969 ± 50*	706 ± 44	194*	141
Cultivar vs rootstock	S	S	S	S	S	S

nt: not tested. Values are mean ± standard error of 14 replicates per treatment (seven replicates per watermelon cultivar or rootstock x two experiments). Different letters within watermelon cultivar or rootstocks in the same column show differences by Tukey HSD (Honestly Significant Difference) test ($P < 0.05$). * indicate statistical differences between *M. incognita* and *M. javanica* according to Student´s t test ($P < 0.05$). [a] Eggs per plant / J2 inoculum.

Plants grafted onto RS841 and Titan produced more ($P < 0.05$) eggs at the higher than lower inoculum level, representing a 2.7- and 1.9-fold increase in rootstocks, respectively. The higher *Pi* resulted in a significant decrease ($P < 0.05$) in the *Rf* values and top dry weight of the grafted plants (Table 3). Plants grafted onto RS841 had ($P < 0.05$) heavier roots than did the non-inoculated plants at the lower but not at the higher inoculum level (Table 3). Overall egg production and *Rf* values were lower ($P < 0.05$) in non-grafted plants than in grafted ones (Table 3). Root weigh was higher ($P < 0.05$) in the non-grafted than grafted plants in the RKN-inoculated plants.

The percentage of J2 emerged at the end of the experiment was lower ($P < 0.05$) for the non-grafted than the grafted plants, as indicated by the number of empty egg shells, with no differences between *Pi* levels (Table 3).

Field experiment

The average mean daily soil temperature was 27.1ºC ± 4, and the minimum and maximum temperatures were 18ºC and 34.8ºC. *Meloidogyne javanica* accumulated 1086 *DD* by the end of the experiment, sufficient *DD* for completion of three generations (*Tb* = 17.2ºC, 357 *DD*). The *Pi* values in plots with *Pi* > 1 ranged from 17 to 172 J2 / 250 cm^3 soil and the average *Pi* did not differ amongst treatments (Table 4). The average *Pf* values and egg production were lower ($P < 0.05$) in plots with non-grafted than did the grafted plants. Disease incidence was 80% for the non-grafted plants and 96% for those grafted onto RS841 and Titan. Disease severity (gall rating) was lower ($P < 0.05$) in non-grafted plants (Table 4). Galled roots were found in all plots, including those with *Pf* < 1. The gall rating in these plots ranged from 0.25 to 1.75, and in plots with *Pf* > 1 ranged from 2 to 7.5. Dry top weight did not differ between the non-grafted and grafted plants (data not shown).

Table 3. Effect of initial inoculum on nematode reproduction, dry top weight, root weight and J2 emergence of *Meloidogyne javanica* in non-grafted watermelon cv. Sugar Baby and grafted onto rootstocks cv. RS841 and Titan inoculated with 0, 5,000 and 25,000 eggs per plant in pot experiments conducted in a greenhouse.

Cultivar / Rootstock	Inoculum	Eggs / plant x 100	*Rf*[a]	Dry top weight (g)	Root weight (g)	J2 emerg (%)[b]
Non-grafted Sugar Baby	0	0		22 ± 1.0 a B	15 ± 0.7 b A	
	5,000	515 ± 138 b B	10 a B	25 ± 2.0 a A	38 ± 3.0 a A	16 b B
	25,000	2865 ± 577 a B	11 a B	24 ± 1.0 a A	36 ± 1.2 a A	26 b A
Sugar Baby / RS841	0	0		28 ± 2.4 a A	15 ± 1.0 b A	
	5,000	9972 ± 1288 b A	199 a A	27 ± 2.5 ab A	23 ± 2.0 a B	60 a A
	25,000	26880 ± 3807 a A	108 b A	23 ± 2.0 b A	18 ± 1.6 b B	58 a A
Sugar Baby / Titan	0	0		25 ± 2.0 a A	18 ± 1.4 a A	
	5,000	7948 ± 9422 b A	159 a A	21 ± 2.4 ab A	19 ± 1.9 a B	51 a A
	25,000	15094 ± 2431 a A	60 b A	20 ± 2.0 b A	19 ± 1.7 a B	51 a A
Non-grafted versus grafted[c]		S	S	NS	S	S

Values are mean ± standard error of 18 replicates per treatment (nine replicates/ experiment x two experiments).Values within cultivar in the same column sharing the same lower-case letter are not significantly different. Values within inoculum level in the same column sharing the same upper-case letter are not significantly different. Mean separation by Tukey HSD Test ($P < 0.05$). [a] Eggs per plant / egg inoculum. [b] Egg shells /egg per plant x100. [c] S and NS indicate significant or non-significant differences between non-grafted and grafted plants, respectively, according to the Student's t- test ($P < 0.05$).

Table 4. Initial (*Pi*) and final (*Pf*) population densities of *Meloidogyne javanica*, reproduction factor (*Rf*), eggs per plant and gall rating of non-grafted watermelon cv. Sugar Baby and grafted onto rootstocks cv. RS841 and Titan in a plastic greenhouse infested with the nematode 13 weeks after transplanting.

Pi[a]	Treatment	*Pf*[a]	*Rf*[b]	Eggs / g root x 100	Gall rating[c]
<1	Non-grafted Sugar Baby	0		0.9 ± 0.4 b	0.8 ± 0.2 b
	Sugar Baby /RS841	8604 ± 3679 a (230-30500)		297 ± 133 a	3.4 ± 1 a
	Sugar Baby/ Titan	5362 ± 2988 a (0-18200)		250 ± 83 a	4.7 ± 2 a
64 ± 22 (17-144)	Non-grafted Sugar Baby	355 ± 211 b * (0-1250)	4 b	20 ± 6 b *	2.2 ± 0.2 b *
70 ± 20 (18-136)	Sugar Baby /RS841	13750 ± 4067 a (8700-22100)	368 a	233 ± 20 a	5.3 ± 1 a
69 ± 23 (23-172)	Sugar Baby/ Titan	13385 ± 3597 a (6600-30500)	381 a	400 ± 112 a	5.9 ± 0.4 a

Values are mean ± standard error of six replicated plots per treatment (four plants / plot). In parenthesis, range of *Pi* and *Pf* values. Values within *Pi* level with different letters in the same column show differences by Tukey HSD (Honestly Significant Difference) test ($P < 0.05$). * Statistical differences between plots with *Pi* <1 and *Pi* >1 according to Student´s t test ($P < 0.05$). [a] Juveniles/ 250 cm^3 soil. [b] *Pf* / *Pi.* [c] Scale from 0 to 10.

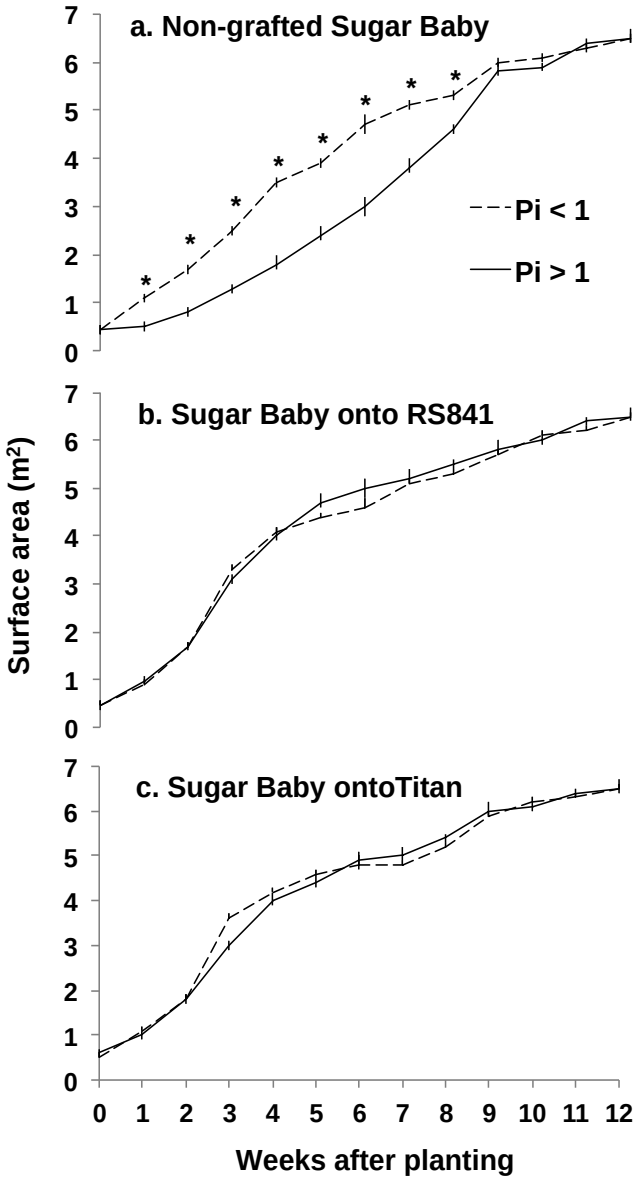

Fig. 1. Surface area (m^2) covered by non-grafted watermelon cv. Sugar Baby (a) and grafted onto squash hybrid rootstocks cv. RS841 (b) and Titan (c) in a plastic greenhouse infested with *Meloidogyne javanica.* Each treatment consisted of six replicated plots. Plots had detected (*Pi* > 1) or non-detected (*Pi* < 1) nematode levels at planting. Bars indicate the standard error of the mean. * indicate statistical differences between *Pi levels.*

The monitoring of the top development of the plants showed a significant reduction ($P < 0.05$) in the surface area covered by the non-grafted Sugar Baby plants in plots with $Pi > 1$ in comparison to those with $Pi < 1$ (Fig. 1a) from the first week after transplanting. Plant stunting lasted for eight weeks although plant growth caught up with that in plots with $Pi < 1$. By contrast, plant stunting was not detected in the plots with the RS841 and Titan grafted plants (Fig. 1b and 1c) despite being challenged to similar *Pi* than the non-grafted Sugar Baby plants. Symptoms of nematode damage such as chlorosis, wilting, and vine senescence were observed in the grafted but not in the non-grafted plants.

Fruit production of non-grafted Sugar Baby was delayed in plots with $Pi > 1$ in comparison with $Pi < 1$ (Table 5). The yield of the RS841 grafted plants was significantly ($P < 0.05$) reduced in plots with $Pi > 1$ in comparison to those with $Pi < 1$ producing fewer and smaller fruits (Table 5). In plots with $Pi < 1$, yield of RS841 grafted plants was 12% lower than of Sugar Baby. In plots with $Pi > 1$, the RS841 grafted plants yielded less ($P < 0.05$) than when grafted onto Titan. Relative to plots with $Pi < 1$, yield losses in plots with $Pi > 1$ were 27% for the non-grafted Sugar Baby, 45% for the RS841 grafted plants, and 3% for Titan grafted plants.

The total number of J2 emerged from adventitious roots of Sugar Baby grafted onto RS841 was 6,956 ± 2761 J2 / g root in comparison to 136,945 ± 31,861 J2 / g root from RS841 roots. Although J2 emergence peaked in the 2nd week (Fig. 2), the spread of emergence from the RS841 roots (11 weeks) was larger than from the adventitious Sugar Baby roots (8 weeks). About 80% of the J2 had already emerged from the rootstock roots after 2 weeks of incubation whereas only 54% did from the scion roots.

Table 5. Number of fruits and kilograms per plot, total yield and yield losses of non-grafted watermelon cv. Sugar Baby and grafted onto rootstocks cv. RS841 and Titan

Pi [a]	Treatment	10 weeks		13 weeks		Total yield	Yield losses (%)[b]
		No. fruit / plot	Kg/plot	No. fruit / plot	Kg / plot		
<1	Non-grafted Sugar Baby	1 ± 1* a	4 ± 2 a*	8 ± 1 a	29 ± 6 a	33 ± 5 a	0
	Sugar Baby/RS841	1 ± 1 a	5 ± 3 a	8 ± 1 a	24 ± 5 a	29 ± 4 a	12
	Sugar Baby/Titan	2 ± 1 a	9 ± 2 a	7 ± 1 a	24 ± 4 a	33 ± 3 a	0
64 ± 22 (17-144)	Non-grafted Sugar Baby	0 b	0 b	7 ± 1 a	24 ± 3 a	24 ± 3 a	27
70 ± 20 (18-136)	Sugar Baby/RS841	1 ± 1 ab	4 ± 1 ab	5 ± 1 a	13 ± 3 a	17 ± 3* b	45
69 ± 23 (23-172)	Sugar Baby/Titan	2 ± 1 a	7 ± 3 a	5 ± 1 a	25 ± 7 a	32 ± 5 a	3

Values are mean ± standard error of six replicated plots per treatment (four plants / plot). In parenthesis, range of *Pi* values. Values within *Pi* level with different letters in the same column show differences by Tukey HSD (Honestly Significant Difference) test ($P < 0.05$). * Statistical differences between plots with $Pi < 1$ and $Pi > 1$. [a] Juveniles/ 250 cm^3 soil [b] Referred to yield in plots with non-grafted Sugar Baby with $Pi \leq 0$.

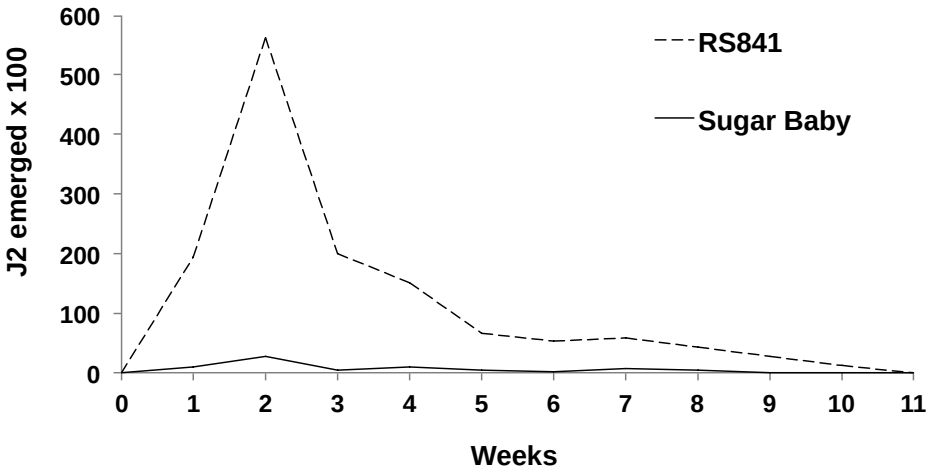

Fig. 2. Number of second-stage juveniles of *Meloidogyne javanica* emerged from adventitious roots of watermelon cv. Sugar Baby grafted onto RS841 and from the rootstock roots over 8 and 11 weeks respectively. Values are mean of three 3-gram replicated root samples.

Population densities in commercial fields

Meloidogyne javanica was the RKN species infesting all the commercial plastic greenhouses except for *M. incognita* in site Sanlu01 (Table 6). Watermelon was grafted onto squash hybrids in all the plastic greenhouses but at site EP05 the graft was onto watermelon cv. Robusta. The *Pi* values in grafted watermelon ranged from 2 to 9 J2 / 250 cm^3 soil, and the *Pf* from 5 to 720 J2 / 250 cm^3 soil. Population increases (*Rf* > 1) were recorded at the end of each cropping cycle irrespective of the crop. Cultivating grafted watermelon (n = 6) resulted in a 42-fold RKN population increase compared to a 133-fold population increase on susceptible tomato (n = 5) (Table 6).

Table 6. Initial (*Pi*) and final (*Pf*) population densities of *Meloidogyne javanica* and *M. incognita* in grafted watermelon rotated with other vegetable crops in commercial plastic greenhouses.

			Juveniles/ 250 cm^3 soil		
Site	Crop, cultivar/ rootstock	*Meloidogyne species*	*Pi*	*Pf*	*Rf*[a]
EP05 Nijar (Almería)	Watermelon cv. Dulce Maravilla/ Robusta F1	*M. javanica*	2 ± 4	5 ± 8	3
EP06 Nijar (Almería)	Watermelon cv. Dulce Maravilla / RS841	*M. javanica*	4 ± 5	163 ± 221	41
EP07 Nijar (Almería)	Watermelon cv. Fashion / RS841	*M. javanica*	3 ± 5	99 ± 152	33
EP08 Cañada (Almería)	Watermelon cv. Dulce Maravilla/ Shintoza F1	*M. javanica*	7 ± 8	380 ± 427	54
	Tomato cv. SD5017		71 ± 95	3852 ± 4538	54
	Tomato cv. Duraton		1 ± 1	311 ± 352	311
EP09 Cañada (Almería)	Watermelon cv. Dulce Maravilla/ Shintoza F1	*M. javanica*	9 ± 11	720 ± 829	80
	Tomato cv. Ikram		284 ± 327	3827 ± 4298	14
	Cucumber cv. Ciclon		1 ± 2	256 ± 414	256
	Tomato cv. Ikram		2 ± 3	550 ± 850	275
Sanlu01 Sanlucar Bda. (Cádiz)	Watermelon cv. Dulce Maravilla/ Carnivor	*M. incognita*	3 ± 5	135 ± 226	45
	Tomato cv. Matias (R)		2 ± 3	27 ± 37	14
	Squash cv. Amalthee		4 ± 5	122 ± 161	31

Values are mean ± standard error of nine replicated plots per site.
[a] Final population / initial population.

Discussion

The cultivars of watermelon were poorer hosts for both RKN species than were the cucurbit rootstocks in all our experiments. The watermelon cultivars were better hosts for *M. incognita* than for *M. javanica* but the squash hybrids were equally good hosts for these species. The *Rf* values we obtained (0.3 to 1.1 for *M. incognita* and 3 to 6 for *M. javanica*) in the watermelon cultivars were within the range reported for others watermelon cultivars. Thus, Montalvo & Esnard (1994) reported *Rf* values for *M. incognita* ranging from 2.9 in cv. Sugar Baby to 9.6 in cv. Charleston 76 compared to *Rf* of 24.8 in tomato cv. Rutgers. Thies & Levi (2003) reported *Rf* = 2.8, 1.8 and 5.4 for *M. arenaria* in watermelon cv. Crimson Sweet, Dixie Lee, and Charleston Gray. In another set of tests, the *Rf* values were 1.1, 0.6, and 5.4 for *M. incognita*, and 1.8, 3.3, 2.2 for *M. arenaria* in the same watermelon cultivars (Thies & Levi, 2007). Davis (2007) reported *Rf* = 6.6 for *M. incognita* on watermelon cv. Cooperstown whereas in cv. Royal Sweet, the *Rf* was 1.2 for *M. incognita* (Xing & Wesphal, 2012). However, *Pf* values lower than the *Pi* have been recorded in watermelon under several environmental conditions (David, 2007, Xing & Wesphal, 2012; López-Gómez *et al.,* 1014). The cucurbit rootstocks supported high RKN reproduction typical of highly susceptible hosts with no RKN resistance genes, as noted elsewhere on cv. Shintoza and Strongtosa (Kim & Ferris, 2002; Thies *et al*., 2010). Rootstocks RS841 and Titan supported a high RKN reproduction whether they had been grafted or not (Tables 2 and 3).

In these experiments, the *Pi* (1 J2 /cm^3 soil) was larger than the estimated tolerance limit (0.2 J2 /cm^3 soil) for watermelon (López-Gómez *et al*., 2014), the incubation temperature (26ºC ± 1) appropriated for crop and nematode development, and the experimental period sufficient for completion of one generation as indicated by J2 emergence from the newly formed eggs. Therefore, the differences in RKN reproduction between the watermelon cultivars and cucurbit rootstocks were attributed to their differential host suitability.

The grafted watermelon showed increased RKN susceptibility compared to non-grafted plants probably due to the poor host status of the scion Sugar Baby and good host status of rootstocks RS841 and Titan, respectively. A 5-fold *Pi* increase affected neither *Rf* values nor dry top weight of the non-grafted Sugar Baby as previously noted in the resistant tomato cultivar cv. VFNT-Cherr (Maleita *et al.,* 2012b). Similarly, a 10-fold *Pi* increase resulted in *Rf* < 1 in watermelon cv. Royal Sweet (Xing & Westphal, 2012). In the grafted plants, however, a 5-fold *Pi* increase decreased the *Rf*, and the dry top weight of the scion that could be explained by the negative relationship between *Pi* and *Rf,* and *Pi* and plant growth on susceptible host plants (Ploeg & Phillips, 2001; Giné *et al*., 2014).

The J2 emergence from the newly formed eggs was lower in the watermelon cultivars than the cucurbit rootstocks in all the experiments being the host status, and not the *Pi* levels, responsible for the reduced J2 emergence as reported in previous studies (Huang & Pereira, 1994). Thus, more than 51% of the eggs produced in RS841 and Titan had already hatched whereas less than 26% did in Sugar Baby roots at the end of the experiments. Also, in the RS841-Sugar Baby combination, the emerged J2 from comparable root weight samples proved 19-fold higher than from the scion adventitious roots. The host plant may also affect the length of life cycle being slower in poor and resistant host than in good hosts (Zhang & Schmitt, 1995; Maleita *et al.*, 2012a). Early J2 development was more slowly into watermelon roots than into other cucurbit crops considered better RKN hosts than watermelon (López-Gómez & Verdejo-Lucas 2014).

A delay of plant growth, typical of soil infested with low to medium *Pi* of the nematodes, was observed in non-grafted Sugar Baby but not in the plants grafted onto the cucurbit rootstocks. Plants remained stunted for two-thirds of the cropping cycle but restored growth 9 weeks after transplanting, a week before the first harvest. As a result, non-grafted Sugar Baby yielded 27% less in plots with Pi > 1 than in those with Pi < 1 because no fruits were collected at the first harvest. Recovery of watermelon plants from early stunting was observed in plots with average *Pi* of 3830 J2/250 cm^3 soil (López-Gómez *et al*., 2014). Scion development was not impaired by the nematode in the grafted plants despite the

greater disease severity of the grafted compared with non-grafted plants. Rootstock cv. Strongtosa showed greater disease severity than did non-grafted diploid cv. Fiesta and triploid cv. Tri-X313 watermelons (Thies *et al*., 2010). Therefore, delayed top development seemed to depend on the interaction between the nematode and the host plant and not only on the *Pi* levels.

The Titan-Sugar Baby combination provided tolerance to *M. javanica,* as evidenced by the yield similarity in plots with $Pi < 1$ and $Pi \geq 1$, and between grafted and non-grafted plants despite high *Rf* and root galling. By contrast, the RS841-Sugar Baby combination showed no tolerance and underwent yield losses in comparison to the non-grafted plants in plots both with $Pi < 1$(12%) and with $Pi \geq 1$ (45%). The poorer performance of RS841 could be explained by the production of suckers below the grafting union and adventitious roots by the scion. Melon plants grafted onto *C. moschata* showed tolerance to *M. incognita* (Sigüenza *et al*., 2005) whereas the squash hybrid cv. Shintoza did not prevent yield losses caused by *M. arenaria* to oriental melon (Kim & Ferris, 2002). Therefore, the importance of selecting the optimum combination for a specific pathogen, production system or environment is emphasized due to the differential responses of the rootstock-scion combinations. For instance, RS841 has been described as having intermediate resistance (Table 1) but was susceptible to the RKN isolates used in this study.

In the commercial plastic greenhouses, the *Rf* values in grafted watermelon grafted onto RS841, Shintoza, and Carnivor were within the range found in the oriental melon-Shintoza combination (Kim & Ferris, 2002). Only watermelon onto watermelon cv. Robusta registered low *Rf* values, suggesting that planting *C. lanatus var. lanatus* cultivars (i.e. Sugar Baby, Robusta) would be more effective for RKN disease management than would be the highly susceptible squash hybrids. However, the susceptibility of *C. lanatus* to *Fusarium* wilt might restrict this option to Fusarium-free areas. In fact, the introduction of squash hybrid rootstocks was a direct result of the susceptibility of watermelon to *F. oxysporum* f. sp *niveum* (Davis *et al*., 2008). It is necessary to find effective rootstocks to manage both diseases. Some accessions of watermelon,*C. lanatus* var. *citroides* and wild *Cucumis* spp. such as

C. africanus and *C. myriocarpus* have shown RKN resistance (Thies & Levi, 2007; Thies *et al.,* 2010; Pofu *et al.,* 2011; Cohen *et al*., 2014) whereas some *Lagenaria* accessions with resistance to fusarium wilt disease showed reduced RKN susceptibility (Levi *et al.*, 2009).

In conclusion, the watermelon cultivars supported low to moderate population increases compared to the enormous increases in the cucurbit rootstocks. Watermelon reduced J2 penetration rates (López-Gómez & Verdejo-Lucas 2014), *EM*, egg production, and J2 emergence. Although all cucurbit rootstocks currently used for grafting watermelon are susceptible to *Meloidogyne,* some rootstocks provide tolerance to the nematode. The choice of rootstock-scion combination is decisive for the success of an integrated disease management in sustainable agriculture.

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Chapter 4

Population dynamics of Meloidogyne javanica and its relationship with the leaf chlorophyll content in zucchini

Population dynamics of *Meloidogyne javanica* and its relationship with the leaf chlorophyll content in zucchini.

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Abstract

The relationship between the initial (*Pi*) and final (*Pf*) population densities of *Meloidogyne javanica* in response to increasing initial inoculum levels and the effect on yield in zucchini cv. Amalthee (*Cucurbita pepo* L.) was determined using a geometric series of 12 *Pi* from 0 to 51200 eggs/100 cm^3 of soil in pot experiments in a greenhouse. The maximum multiplication rate was 425, and the equilibrium density was 701951 eggs/100 cm^3 soil. The relative yield, represented as dry top weight, fit the Seinhorst damage function model and the minimum relative yield (*m*) was 0.82 and the tolerance limit (*T*) was 402 J2/100 cm^3 soil. Regression analyses indicated a negative relationship between the *Pi* and the leaf chlorophyll content (*LCC*) 40, 50, 60, and 70 days post-inoculation. The *Pi* and *LCC* fit the Seinhorst damage-function model. Zucchini cv. Dyamant was planted in a plastic greenhouse with a range of *M. javanica Pi* from 0 to 861 J2/100 cm^3 soil. The maximum multiplication rate of *M. javanica* under field conditions was 3093, and the equilibrium density was 1485 J2/100 cm^3 soil. The relationship between *Pi* and relative yield, represented as fruit weight, fit the Seinhorst damage function model ($P < 0.0001$, $R^2 = 0.78$); *m* was 0.48, and *T* was 0.02 J2/100 cm^3 soil.

Key words: *Cucurbita pepo*, root-knot nematode, damage functions.

Introduction

Root-knot nematodes (RKN), *Meloidogyne* spp., are major limiting factors for vegetable production worldwide (Sikora and Fernández, 2005). Management of nematode problems in RKN conductive systems, such as protected cultivation in plastic greenhouses, is a major challenge since crop intensity and environmental conditions under the plastic cover favor pest and disease development. Crops with long and short cycles are cultivated generally in rotation with solanaceous (tomato, pepper and eggplants) and cucurbitaceous (cucumber, melon, watermelon, and zucchini) crops with short fallowing periods (4-8 weeks) between successive crops (Sorribas and Verdejo-Lucas, 1994; Talavera et al., 2012).

The European Directive 2009/128/EC on the sustainable use of plant protection products promotes integrated production as a means for reducing pesticide use. To achieve this goal, it is necessary to understand the host-parasite relationship in the rotational crops of the production system for estimating nematode damage thresholds, predicting yield losses and modelling the population dynamics. The damage potential of the nematode to the crop has been described by mathematical models (Seinhorst, 1965). Estimation of the potential growth of the RKN population will provide information on the suitability of the crop and tolerance to the nematode. A negative relationship between the initial population density (*Pi*) and the reproduction rate (*Pf*/*Pi*), *Pf* being the nematode population density at harvest, has been described in several susceptible annual crops (Ferris et al., 1986). Damage caused by RKN is determined by relating *Pi* to growth and yield. The minimal density that causes a measurable reduction in plant growth or yield varies with nematode species, host plants, cultivar and environment (Barker and Olthof, 1976). It is generally accepted that cucurbitaceous crops are susceptible to RKN but differences in susceptibility levels have been observed (López-Gómez and Verdejo-Lucas, 2014). Yield losses have been reported in cucumber and melon (Ornat et al., 1997; Ploeg and Phillips, 2001; Kim and Ferris, 2002; Giné et al., 2014). However, few studies have focused on the development of damage-functions models for zucchini, *Cucurbita pepo* L. (Ferris et al., 1986; Vela et al., 2014).

As obligate sedentary endoparasites, RKN interfere with plant physiological processes involved in water uptake and nutrient translocation and create an imbalance of macro and micro-nutrients; in consequence, leaf chlorosis and stunted growth may appear in nematode-infested plants (Melakeberhan, 2003). Leaf chlorophyll content (*LCC*) was decreased on *M. incognita*-infected okra, tomato, and cucumber (Wani, 2006; Flor-Peregrín et al., 2014; Giné et al., 2014). The *LCC* or plant greenness is positively correlated with the foliar nitrogen concentration and plant productivity in several crops including zucchini, (Rharrabti et al., 2001; Gholizadeh et al., 2009; Pôrto et al, 2011). The *LCC* can be measured with a Soil Plant Analysis Development (SPAD) portable apparatus used to determine the nitrogen status of the plant and the need for nitrogen fertilization. Above-ground symptoms exhibited by RKN-infected plants are unspecific and can be confused with damage due to poor nutrition or injury caused by pathogens that attack the root system (bacteria, fungi and virus). The SPAD reader does not detect the nematode-induced damage but could be used for the indirect evaluation of the damage caused by nematodes when they are present. However, it should be used with care because other factors may influence the health of the plants.

Cultivation of zucchini in Spain is expanding with a 23% increase during the last decade. Most of the production is concentrated in the southern part of the country with 70% of the surface under protected cultivation in plastic greenhouses (MAGRAMA, 2011). The annual economic losses due to RKN in zucchini in Spain were estimated at €640504 (Talavera et al., 2012). This study was undertaken to determine the relationship between *Pi* and *Pf* densities of *M. javanica* in zucchini, the yield losses in response to increasing initial inoculum levels and to assess the relationship between *Pi* and *LCC*, as an indirect indicator of nematode-induced damage.

Materials and methods

Pot experiments

A geometric series of 12 *Pi* of *M. javanica* was used to determine the relationship between *Pi* and *Pf* and yield in zucchini cv. Amalthee in a greenhouse. The experiment was conducted twice. Zucchini seeds were soaked overnight and germinated in seed trays with vermiculite. When the first true leaf was fully expanded, the seedlings were transplanted to Styrofoam pots filled with 500 cm^3 of sterilized river sand. Plants were allowed to grow for one week before nematode inoculation. The *M. javanica* isolate (Mj05) had been established as a single egg mass and maintained on susceptible tomato cv. Roma in a greenhouse. The identification of the species was confirmed using SCAR-PCR markers (Zijlstra et al., 2000). To obtain the inoculum, eggs were extracted by blender maceration of infected roots using a 0.5% NaOCl solution for 5 min (Hussey and Barker, 1973). The egg suspension was passed through a 74 µm aperture sieve to remove root debris, and the dispersed eggs were collected on a 25 µm sieve. Seedlings were inoculated with 0, 50, 100, 200, 400, 800, 1600, 3200, 6400, 12800, 25600 and 51200 eggs/100 cm^3 soil. An additional level of 102400 eggs/100 cm^3 of soil was included in the replicated experiment. Each treatment was replicated seven times. Plants were maintained on a greenhouse bench, watered at field capacity before nematode inoculation, and after 5 days they were watered daily as needed, and fertilized with a slow-release fertilizer (Osmocote® Scotts Company, Netherlands; 15% N +10% P_2O_5 +12% K_2O + 2% MgO_2 + microelements) at the beginning of the experiments. Soil temperatures were recorded daily at 30-min intervals with temperature probes (Em50 Data Logger®, Decagon Devices Inc, USA, accuracy ± 1 ºC, resolution 0.1 ºC) inserted into the pots. The number of *M. javanica* generations was calculated according to *Tb* = 10.8 ^oC and 526 accumulated degree-days (*DD*) (Vela et al., 2014).

The hatching rate of the egg inoculum was determined by placing three aliquots of the egg suspension on Baermann trays that were maintained at 26 ± 1 ºC in darkness for 21 days. Emerged second-stage juveniles (J2) were collected once a week

and stored at 4 ºC until counted. The hatching rate (%) was then calculated and the egg inoculum converted to number of emerged J2 that represented the effective inoculum for root invasion.

The *LCC* was measured with a portable chlorophyll meter SPAD 502® (Minolta, Osaka, Japan) at 40, 50, 60, and 70 days post-inoculation (dpi). Three SPAD readings were taken per plant in the largest healthy fully expanded leaf at two-thirds the distance from the leaf tip towards the stem.

Plants were harvested 75 and 70 dpi in experiment 1 and 2, respectively. At harvest, tops were cut at ground level, oven-dried at 70 ºC for 72 h and weighed. Roots were washed free of soil and the fresh weight recorded. Eggs were extracted from 10 g root subsamples using 0.5% NaOCl solution for 10 min as described previously and quantified (eggs and egg shells). The *Pi* is expressed as the number of emerged J2/100 cm^3 soil and the *Pf* as the total number of eggs/100 cm^3 soil.

Field experiments

Zucchini cv. Dyamant was cultivated in an unheated plastic-covered greenhouse in Cabrils, Barcelona, Spain for 134 days (4 March to 16 July). Seeds of cv. Amalthee were not available at the time of conducting this experiment. The field was infested with a range of *M. javanica Pi* due to the history of the field that included methyl bromide fumigation and resistant and susceptible tomatoes several years before starting this study (Sorribas et al., 2005). The soil was a sandy loam with 85.8% sand, 8.1% silt and 6.1% clay, pH 8.1, 0.9% organic matter w/w, and 0.40 dS m^{-1} electrical conductivity. The field was divided into 24 plots of 2.5 × 2 m, each consisting of two rows with four plants per row spaced at 60 cm within and between rows, totaling 192 plants. Plants were irrigated through a drip irrigation system and fertilized once a week.

Composite soil samples were collected at the beginning and end of the cropping cycle to estimate the *Pi* and *Pf* of *M. javanica*. Five soil cores were taken to 25 cm depth, mixed thoroughly and the nematodes extracted from 500 cm^3 soil sub-samples in Whitehead trays (Whitehead and Hemming, 1965). One week later,

the juveniles were collected, counted and expressed as J2/100 cm^3 soil. At the end of the crop cycle, disease incidence was evaluated as the percentage of plants with galled roots, whereas disease severity was assessed using a root-gall index, according to a scale of 0 to 10, where 0 = a complete and healthy root system and 10 = dead plants and roots (Zeck, 1971). Roots from each plot were bulked, cut into 1 cm-long segments, and two 10 g subsamples were used to extract eggs by blender maceration in a 0.5 % NaOCl solution for 10 min as described above. The number of eggs was expressed per gram fresh root weight. Fruits were collected weekly for six weeks, weighed and the cumulated yield expressed as g/plant.

Statistical analyses

The SAS system V8 (SAS Institute Inc., Cary, NC) was used for statistical analyses. Data from pot experiments were presented separately due to differences between experiments in the hatching rate of the egg inoculum. Prior to the analyses, data were subjected to the normal Shapiro-Wilk W test to check for normal distribution of data and the ANOM for variances to check the homogeneity of variances. When needed, data were log transformed [log10 (x + 1)] to homogenize the variances. Data were subjected to ANOVA and means separated by the Tukey HSD (honest significant difference) test. Data from *LCC* were referred to that of uninoculated control plants to remove the effect of plant aging. The *Pi* values, ranging from 22 to 5687 J2/100 cm^3 soil did not differ between experiments, and hence data within these *Pi* ranges were pooled and used to determine the relationship between *Pi* and *Pf*, and the fit of the data to the Seinhorst damage function model (Seinhorst, 1965). To estimate the maximum nematode reproduction rate (*a*) in zucchini, the *Pi* value with the highest slope in the regression line between *Pi* and *Pf* was selected, and the equilibrium density (*E*) was the value of *Pi* = *Pf* (Seinhorst, 1967). The maximum population density (*M*) was estimated from the experimental data, and *E*, according to the expression $M = aE/(a\text{-}1)$ (Schomaker and Been, 2006). The nonlinear procedure (proc nlin) was used to fit data to the Seinhorst damage function model (Seinhorst, 1965); $y = m + (1\text{-}m)\, z^{(Pi\text{-}T)}$ when $Pi \geq T$, and $y = 1$ when $Pi < T$, where *y* is the relative yield, *m* is the minimum

relative yield, *z* is a constant ≤ 1, and *T* is the tolerance limit (the nematode density below which there is no yield loss). Similarly, nonlinear regression analyses were used to determine the relationship between *Pi* and *LCC* at each post-inoculation time, and if the relationship fitted the Seinhorst damage function model. The relative values of dry top weight, fruit weight and *LCC* were calculated for a given *Pi* at *Pi* = 0. Linear regression analysis was used to determine the relationship between gall rating and yield in the field experiment.

Results

Pot experiment

The minimum and maximum temperatures were 12ºC and 30.9ºC and 11.6ºC and 30.7ºC in experiment 1 and 2, respectively. The nematode had accumulated 738 and 734 *DD* when experiments 1 and 2 were harvested which indicated that *M. javanica* had completed one generation (526 *DD*) and started a second one. The hatching rate of the egg inoculum in the pot experiments was 11% and 43% which provided an effective J2 inoculum ranging from 6 to 5687 J2/100 cm^3 soil and 22 to 44032 J2/100 cm^3 soil in experiments 1 and 2, respectively (Table 1). *M. javanica* reproduced on zucchini cv. Amalthee at all *Pi* levels, and the maximum *Pf*/*Pi* of 532 (*Pi* = 11/100 cm^3 soil) and 319 (*Pi* = 22 J2/100 cm^3 soil) decreased to 97 (*Pi* = 5687/J2 100 cm^3 soil) and 19 (*Pi* = 44032 J2/100 cm^3 soil) in experiments 1 and 2, respectively (Table 1). The maximum reproduction rate (*a*) and equilibrium density (*E*) of *M. javanica* was 425, and 701951 eggs/100 cm^3 soil, respectively (average of the two experiments, Fig. 1). Yield losses, represented as dry top weight, fit the Seinhorst damage function ($P < 0.005$, $R^2 = 0.49$), showing that *m* was 0.82 and *T* was 402 J2/100 cm^3 soil (Fig. 2).

Table 1. Initial (*Pi*) and final population densities (*Pf*) and reproduction rate (*Pf/Pi*) of *Meloidogyne javanica*, and dry top weight of zucchini, *Cucurbita pepo*, cv. Amalthee in response to increasing *Pi* in repeated experiments (1 and 2) in a greenhouse.

Pi (J2/100 cm^3 soil)[a]	*Pf* x 10^3 /100 cm^3 soil		*Pf/Pi*		Dry top weight (g)	
	1	2	1	2	1	2
0	0	0	0	0	5.5 ± 0.8 a	4 ± 0.5 ab
6	2 ± 4 e		428 a		5.1 ± 0.3 a	
11	6 ± 1.7 e		532 a		5.4 ± 0.4 a	
22	10 ± 2 e	7 ± 0.9 c	460 a	319 a	5.8 ± 0.4 a	3.9 ± 0.3 ab
45	16 ± 3 e	10 ± 1.7 c	366 ab	238 ab	6.1 ± 0.8 a	4.3 ± 0.3 ab
90	36 ± 6 e	19 ± 2 c	413 a	223 ab	5.5 ± 0.4 a	4.4 ± 0.3 a
181	67 ± 12 de	28 ± 1 c	379 ab	163 bc	5.3 ± 0.4 a	4.1 ± 0.3 ab
361	35 ± 6 e	61 ± 9 c	99 bc	177 bc	6.1 ± 0.9 a	4.3 ± 0.3 ab
723	266 ± 18 cd	112 ± 24 c	378 ab	162 bc	4.1 ± 0.3 ab	3.4 ± 0.2 ab
1446	359 ± 51 bc	184 ± 26 c	255 abc	134 bcd	5.1 ± 0.3 a	4.2 ± 0.2 ab
2893	755 ± 11 a	646 ± 42 ab	268 abc	235 ab	3.8 ± 0.3 b	3.3 ± 0.3 ab
5687	547 ± 97 ab	505 ± 54 b	97 bc	97 cde	4.3 ± 0.3 ab	3.5 ± 0.3 ab
11008		562 ± 68 b		51 de		2.8 ± 0.4 ab
22016		684 ± 107 ab		31 de		3.4 ± 0.4 ab
44032		813 ± 11 a		19 e		2.5 ± 0.3 b

Values are mean ± standard error of seven replicates. [a] Egg inoculum converted to emerged juveniles according to a hatching rate of 11% and 43% in experiment 1 and 2, respectively.

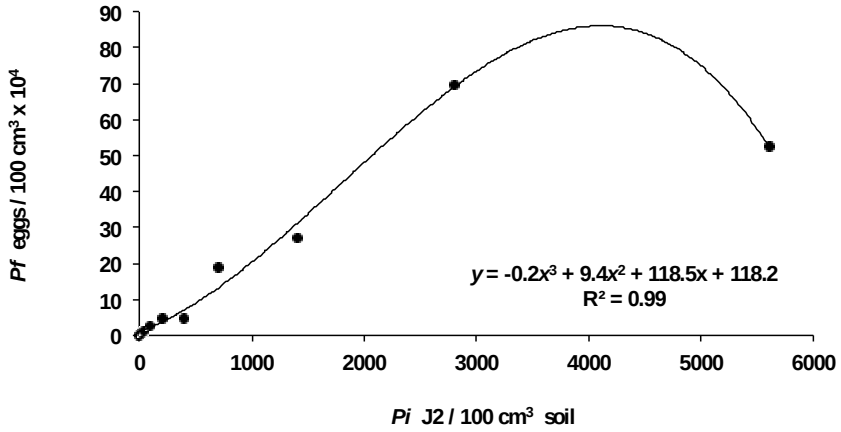

Fig. 1. Relationship between initial (*Pi*) and final (*Pf*) population densities (J2/100 cm^3 of soil) of *Meloidogyne javanica* in zucchini, *Cucurbita pepo*, cv. Amalthee inoculated with increasing inoculum levels of the nematode in pot experiments in a greenhouse. Values are means of 14 replicates/treatment.

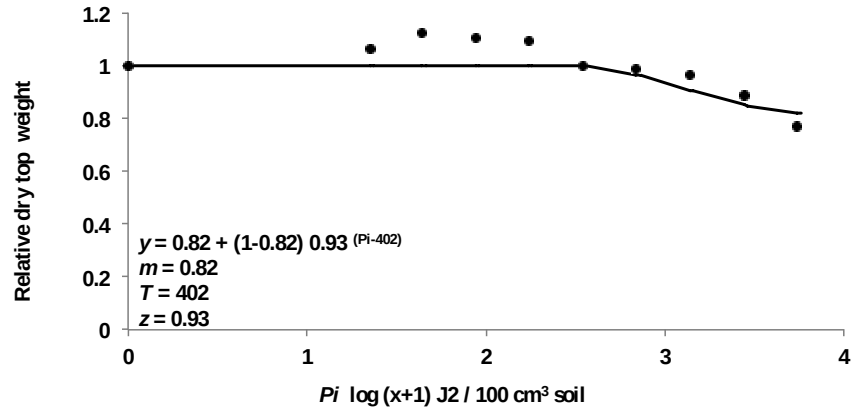

Fig. 2. Relationship between *Meloidogyne javanica* initial population densities (*Pi*) and relative dry top weight of zucchini, *Cucurbita pepo*, cv. Amalthee in pot experiments in a greenhouse. Values are means of 14 replicates/treatment.

The decline in *LCC* was associated with *Pi* and time (dpi) since the *Pi* x dpi interaction was significant (P <0.0001). The analysis of the factor *Pi* on *LCC* indicated that *Pi* from 6 to 90 J2/100 cm^3 soil had no significant effect on *LCC* in comparison to the uninoculated plants. Significant reductions of *LCC* were observed for *Pi* higher than 181 J2/100 cm^3 soil (Table 2). As far as the effect of dpi was concerned, the *LCC* values progressively decreased over time with significant reductions between reading dates, except for 50 and 60 dpi (data not shown). The analysis of the *Pi* x dpi interaction showed significant declines at *Pi* ≥ 5687 J2/100 cm^3 soil at 40 dpi, ≥ 1446 J2/100 cm^3 soil at 50 and 60 dpi and ≥ 361 J2/100 cm^3 soil at 70 dpi. The relationship between the *Pi* and *LCC* fit the Seinhorst damage function at each measured data (Fig. 3). A positive relationship between *LCC* and dry top weight was observed at 40 (R^2 = 0.54, P >0.0002), 50 (R^2=0.51, P <0.0001), 60 (R^2 = 0.49, P <0.0001), and 70 dpi (R^2 = 0.66, P <0.0001).

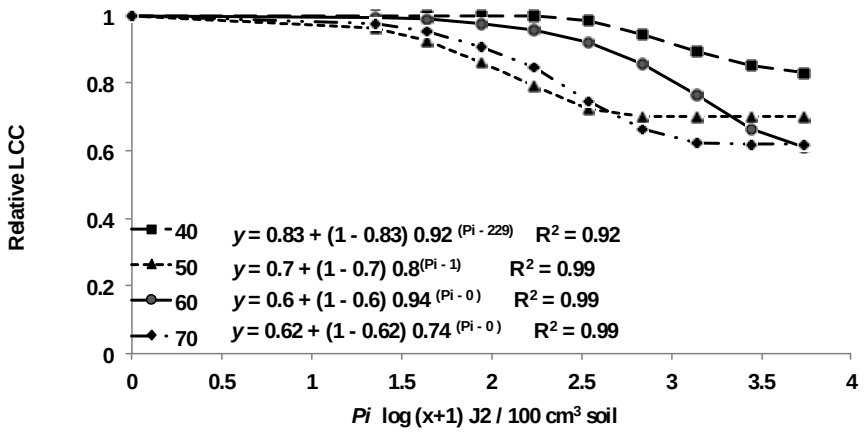

Fig. 3. Relationship between *Meloidogyne javanica* initial population densities (*Pi*) and relative leaf chlorophyll content (*LCC*) measured at 40, 50, 60, and 70 days post inoculation in pot experiments in a greenhouse. Values are means of 14 replicates/treatment

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Table 2. Leaf chlorophyll content (*LCC*) of zucchini, *Cucurbita pepo*, cv. Amalthee in response to increasing initial population densities (*Pi*) of *Meloidogyne javanica* 40, 50, 60, and 70 days post-inoculation in pot experiments conducted in a greenhouse. Values are referred to nematode-free plants.

Pi (J2/100 cm^3 soil)	Days-post-inoculation			
	40	50	60	70
0	1 abc	1 ± 0.02 a	1 ± 0.04 a	1 ± 0.04 a
6*	1 ± 0.04 a	0.96 ± 0.03 a	1 ± 0.03 a	1 ± 0.05 a
11*	1 ± 0.02 a	0.96 ± 0.03 a	0.94 ± 0.04 ab	0.94 ± 0.06 abc
22	1 ± 0.05ab	0.93 ± 0.03 ab	0.99 ± 0.05 ab	0.91 ± 0.04 abc
45	1 ± 0.04 ab	0.86 ± 0.03 a-e	0.92 ± 0.05 ab	0.96 ± 0.05 ab
90	1 ± 0.09 ab	0.87 ± 0.03 a-d	0.89 ± 0.05 ab	0.87 ± 0.04 abc
181	0.98 ± 0.03 abc	0.75 ± 0.03 c-g	0.83 ± 0.04 a-e	0.83 ± 0.05 a-d
361	0.92 ± 0.04 bcd	0.80 ± 0.02 b-f	0.86 ± 0.05 abc	0.73 ± 0.04 c-f
723	0.94 ± 0.04 a-d	0.80 ± 0.04 a-f	0.83 ± 0.03 a-d	0.64 ± 0.05 def
1446	0.90 ± 0.04 b-e	0.73 ± 0.05 d-h	0.74 ± 0.06 b-e	0.76 ± 0.05 b-e
2893	0.84 ± 0.03 c-f	0.58 ± 0.06 gh	0.60 ± 0.05 e	0.53 ± 0.05 efg
5687	0.81 ± 0.03 def	0.63 ± 0.03 fgh	0.62 ± 0.05 de	0.55 ± 0.04 fg
11008*	0.71 ± 0.03 ef	0.61 ± 0.04 fgh	0.62 ± 0.06 cde	0.33 ± 0.04 gh
22016*	0.65 ± 0.04 f	0.65 ± 0.08 e-h	0.66 ± 0.07 b-e	0.47 ± 0.08 fgh
44032*	0.68 ± 0.03 f	0.52 ± 0.04 h	0.66 ± 0.04 b-e	0.24 ± 0.04 h

Values are mean ± standard error of 14 replicates (7 replications x 2 experiments), except for *Pi* with * which are mean of 7 replicates. Values in the same column followed by the same lower-case letter are not significantly different according to Tukey HSD (Honestly Significant Difference).

Field experiment

Pre-planting *M. javanica* population densities ranged from 0 to 861 J2/100 cm^3 soil (477 ± 97 J2/100 cm^3 soil, mean ± standard error), and the *Pf* from 0 to 7402 (2581 ± 944 J2/100 cm^3 soil). The nematode was not detected in eight out of 24 plots at the beginning of the experiment and in three at the end of the cropping cycle. Eggs production ranged from 0 to 64240 eggs/g root (18630 ± 3585 eggs /g root). Disease incidence was 88% and disease severity was 4.9 with a range from 1 to 8.

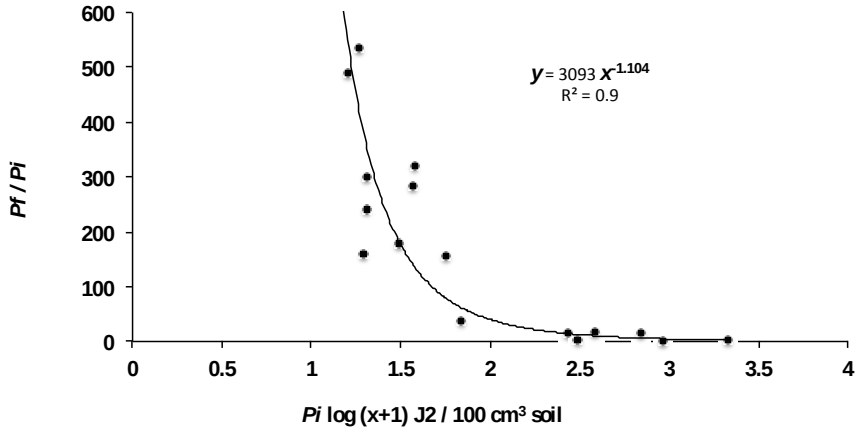

Fig. 4. Relationship between the initial population densities (*Pi*) and the reproduction factor (*Pf/Pi*) of *Meloidogyne javanica* in zucchini, *Cucurbita pepo*, cv. Dyamant under field conditions.

The relationship between *Pi* and *Pf*/*Pi* was described by a potential function (Fig. 4), and the *a* and *E* values were 3093, and 1485 J2/100 cm^3 soil, respectively. The relationship between *Pi* and the relative yield of zucchini cv. Dyamant, represented as fruit weight, fit the Seinhorst damage function model ($P < 0.0001$, $R^2 = 0.78$) showing that *m* was 0.48, *T* was 0.1 J2/100 cm^3 soil, and the *z* value was 0.94 (Fig. 5). The relationship between root galling and yield was fitted to a linear function ($R^2 = 0.85$, $P = 0.0004$) with a negative slope where yield decreased with increasing gall rating (data not shown). Non-infected plants (*GI* = 0) produced an average yield of 3854 g/plant, similar to that of slightly infected

plants (*GI*<4), whereas plants heavily infected (*GI* ≥ 5) yielded significantly less (P <0.001) (Table 3). Yield of plants with gall ratings between 1-2 and 3-4 were 7 % and 20 % less than those of non-infected plants, respectively. The maximum yield losses were observed in plants with *GI* ≥ 7.

Fig. 5. Relationship between *Meloidogyne javanica* initial population densities (*Pi*) and relative fruit weight of zucchini, *Cucurbita pepo*, cv. Dyamant under field conditions.

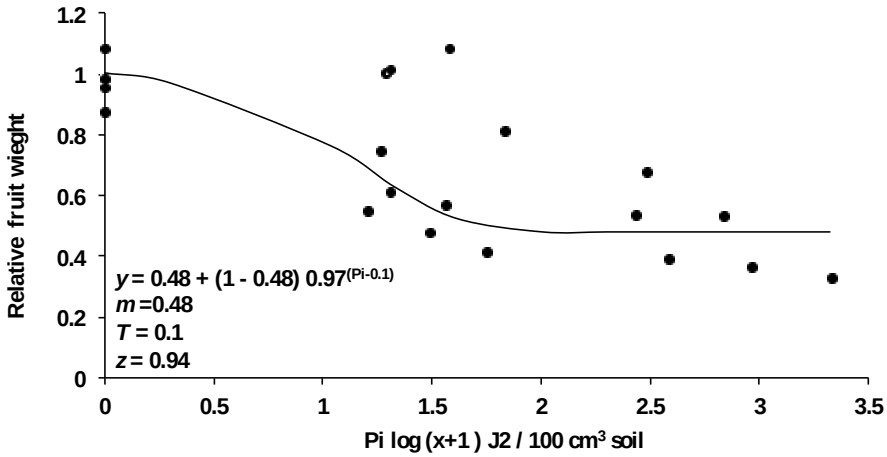

Table 3. *Meloidogyne javanica* root gall index, yield and percent yield losses on zucchini, *Cucurbita pepo*, cv. Dyamant under field conditions.

No. of plants	Gall index [a]	Yield (g/ plant)	Yield losses (%)
22	0	3854 ± 358 a	0
16	1 - 2	3584 ± 594 a	7
33	3 - 4	3096 ± 441 a	20
101	5 - 6	1971 ± 205 b	49
13	7 - 8	1255 ± 135 b	68

Values of yield are mean ± standard error of n plants. [a] Based on a scale of 1 to 10 (Zeck, 1971).

Discussion

The population dynamic of *M. javanica* in zucchini was used to estimate host suitability by measuring the maximum reproduction rate and equilibrium density (Seinhorst, 1970); both parameters showed high values (a= 425 and E = 701951 eggs/100 cm^3 soil) in the pot experiments. However, they were not as high as those observed in good hosts such as cucumber (a =833 and E = 1203400 eggs/100 cm^3 soil) under plastic greenhouse conditions (Giné et al., 2014). In contrast, poor hosts such as watermelon showed comparatively low a and E values (a =14 and E = 49400 eggs/100 cm^3 soil) (López-Gómez et al., 2014). The relationship between *Pi* levels and dry top weight resulted in a T value of 402 J2/100 cm^3 soil, which suggests that zucchini is more tolerant to the nematode than other crops such as cantaloupe (T = 19 eggs and J2/100 cm^3), tobacco (T = 200 eggs and J2/100 cm^3) or cucumber (T < 1 J2/100 cm^3) (Di Vito et al., 1983; Giné et al., 2014). Differences in m and T values observed between cv. Dyamant (0.48 and 0.02 J2/100 cm^3 soil, respectively) and cv. Amalthee (0.82 and 402 J2/100 cm^3 soil, respectively) probably reflect different experimental conditions, cultivar susceptibility or duration of the experiments (70-75 and 134 days in the pots and field, respectively), which suggests that yield losses would occur in zucchini if conditions for nematode development were suitable. The damage threshold in annual crops has been related to the planting date and the duration of the crop cycle (Ehwaeti et al., 1999; Talavera et al., 2009; Vela et al., 2014). The rotational crop preceding zucchini will also affect RKN reproduction and yield losses. For instance, squash produced less when preceded by RKN susceptible rather than resistant pepper (Thies et al., 2004). Similarly, cultivation of non-host crops before lemondrop squash reduced yield losses in squash (McSorley et al., 1994). Another consideration is the nematode survival rate during the short fallow periods between successive crops estimated in 50% of the *Pf* of the preceding crop (Ornat et al., 1999).

The negative relationship between gall rating and zucchini yield indicated that the root damage caused by RKN must be critical for the plant since it resulted in significant yield losses; 53% of the zucchini cv. Dyamant plants had $GI \geq 5$ which indicated that

25% of the root system was not functional according to Zeck (1971). A similar relationship has been observed in other RKN susceptible crops such as carrots and peanuts (Bélair and Boivin, 1988; Korayem and Bondok, 2013). Although gall rating is not a quantitative measure, the correlation between root galling and zucchini yield supports the fact that rating root galling is a practical tool due to its relationship with yield losses. Root galling also provided detailed information on the spatial distribution of the nematode in the field and the identification of areas of severe disease symptoms that could allow the construction of a reference map to document the crop history of the site.

Declines in chlorophyll with increasing *Pi* were also observed in tomato, French bean and cucumber (Loveys and Bird, 1973; Melakeberhan et al., 1985; Giné et al., 2014) and support the utility of the SPAD reading as an indirect way to evaluate nematode damage. Due to the strong correlation between *LCC* and the nitrogen status of the plant (Gholizadeh et al., 2009; Pôrto et al., 2011), SPAD readings can be considered a measure of RKN-induced nutrient deficiencies in infected plants. This non-destructive measure can be used to monitor in real time the health status of the plant and changes in *LCC* may be detected before the appearance of disease symptoms (Wagner et al., 2006). Declines in chlorophyll occurred at an earlier stage than those in growth parameters in *M. incognita*-infected beans (Melakeberhan et al., 1985). Zucchini is cultivated in a growth period of 3 to 5 months, thereby, SPAD readings in the course of the cropping cycle would allow sufficient time to adjust nitrogen fertilization or implement control measures to compensate nematode damage. However, the sensitivity of the SPAD 502 reader to detect differences in *LCC* at the early stages of nematode infection is unknown, and additional studies are needed. For instance, the appropriate time for SPAD readings needs to be established; the reading at 50 dpi concurred approximately with the completion of one nematode generation, a critical time, as roots would be invaded by the second nematode generation.

Conclusions

Zucchini was a susceptible host to *M. javanica* with *Pf*/*Pi* of 425 on cv. Amalthee and 3093 on cv. Dyamant, but it is more tolerant than other rotational crops of economic importance in protected cultivation. Damage function models were developed for the relationships between *Pi* and *Pf*, *LCC* and yield losses whose magnitude depended on the size of the nematode population at planting, the RKN species, the zucchini cultivar and the planting date (temperature). The *LCC* can be a practical tool to assess nematode damage and can have predictive significance. Measurement of this parameter can be done directly saving the time for taking and transporting soil samples to specialized laboratories, and it can be done by non-skilled personnel with brief elementary training.

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Chapter 5

Damage functions and thermal requirements of Meloidogyne javanica and M. incognita on watermelon


D-1-2

Damage functions and thermal requirements of *Meloidogyne javanica* and *M. incognita* on watermelon

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Abstract

The relationship between the initial (P_i) and final (P_f) population densities of *Meloidogyne javanica* and yield of watermelon, *Citrullus lanatus,* Thunb, Matsum & Nakai, cv. Sugar Baby were determined in pot and field experiments. In the pots, the maximum reproduction rate of the nematode was 14, and the equilibrium density 49400 eggs/100 cm^3 of soil. Yield data represented as fresh top weight fitted the Seinhorst damage function ($P<0.001$, R^2 = 0.7), and the minimum relative yield (*m*) was 0.65 at $P_i \geq 3200$ eggs/100 cm^3 of soil and the tolerance limit (*T*) 74 eggs/100 cm^3. In the field experiments (2011 and 2012), the maximum reproduction rate was 73 and 70, and the equilibrium density 32 and 35 J2/100 cm^3 soil. Yield data represented as fruit weight fitted the Seinhorst damage function in 2011 ($P<0.001$, R^2 = 0.92) and the *m* and *T* values were 0.63 and 20 J2/100 cm^3 of soil, respectively. *Meloidogyne incognita* and *M. javanica* needed similar length of time for development to egg-laying females and life cycle completion at 24.4ºC.

Key words: *Citrullus lanatus*, root-knot nematode, equilibrium density, reproduction rate, tolerance limit.

Introduction

Root-knot nematodes (RKN), *Meloidogyne* spp., are major limiting factors for growing vegetables worldwide (Sikora and Fernández, 2005). Several crops within the family Cucurbitaceae are severely damaged by RKN, including watermelon, *Citrullus lanatus,* Thunb, Matsum & Nakai (Thies and Levi, 2003; Pofu *et al.*, 2011). In Spain, watermelon is cultivated in about 18600 ha with an annual production of 782000 t, of which 48% are produced under protected cultivation (MARM, 2010). The estimated economic losses in south-eastern Spain due to RKN on watermelon were €451940 under protected cultivation (Talavera *et al.*, 2012). In this area, chemicals are frequently used for controlling soil-borne pathogens and nematodes. Currently, no commercial watermelon cultivars are resistant to *Meloidogyne* spp. (Thies and Levi, 2007). Therefore, understanding the host-parasite relationship on watermelon is necessary for predicting yield losses and modelling the population dynamics of the nematode. Heavy root galling was observed after inoculation of watermelon with *M. arenaria, M. incognita* or *M. javanica* (Winstead and Riggs, 1959). By contrast, Edelstein *et al.* (2010) found no galling or low gall indexes in plants inoculated with *M. javanica* and *M. incognita*, respectively. An estimation of the growth potential of the RKN population in a given crop will provide information on the host suitability and, in turn, on the crop tolerance to the nematode. In susceptible crops, there is a negative relationship between the initial nematode population density and the reproduction rate (Ferris, 1986). Crop yield losses are influenced primarily by preplanting population densities, and the damage potential of the nematode to the crop has been described by mathematical models (Seinhorst, 1965). Yield losses and damage-function models have been reported for several cucurbit crops (Ornat *et al*., 1997; Ploeg and Phillips, 2001; Webster *et al*., 2001; Kim and Ferris, 2002). However, data on the relationship between RKN preplanting populations and watermelon yield are scarce. As far as we are aware, only two reports refer to yield losses on watermelon caused by *M. incognita* (Davis, 2007; Xing and Westphal, 2012).

Root-knot nematodes are poikilothermic organisms and therefore temperature not only influences their survival in the soil

between crops but also their migration, root penetration, post-infection development, egg production, and embryogenesis (Tyler, 1933). In addition, development rates and life cycle duration vary among *Meloidogyne* species (Maleita *et al.*, 2012). In the scenario of climatic change, information on the thermal time requirements of *Meloidogyne* spp. are useful to estimate the generation time and to schedule planting dates or final harvests. This could help to delay root infection or reduce the number of nematode generations, and thereby reduce plant damage and the remnant inoculum for the subsequent crop. Yield losses in tomato were associated with the completion of three nematode generations in spring plantings (Sorribas *et al.*, 2005) but losses were not observed in autumn plantings when the nematode completed a single generation (Talavera *et al.*, 2009). The thermal requirements for RKN development on watermelon have not been investigated.

This study was undertaken to determine: i) the potential growth of *M. javanica* on watermelon in response to increasing initial population densities; ii) the relationship between the initial population densities and yield losses, and their suitability to fit the Seinhorst damage function; and iii) the thermal requirements of *M. javanica* and *M. incognita* on watermelon.

Materials and Methods

Plant material and nematode inoculum

In all the experiments, the watermelon cultivar used was Sugar Baby (Intersemillas S.A.). Seeds soaked overnight were placed in seed germination trays filled with vermiculite. When the first true leaf was fully expanded, the seedlings were transplanted to 500-cm^3 Styrofoam cups with sterilized river sand (A. Sanchez Abellan, Premia de Dalt, Barcelona, Spain). Plants were allowed to grow for one week before nematode inoculation. Watermelon plants, maintained in a greenhouse, were watered at field capacity immediately after transplanting and before nematode inoculation. Plants were fertilized at the beginning of the assays with a slow-release fertilizer Osmocote® Scotts Company, Netherlands (15% N +10% P_2O_5 +12% K_2O + 2% MgO_2 + microelements) and watered

as necessary to maintain the moisture level, although the moisture content of the growing medium was not quantified.

The *M. javanica* and *M. incognita* isolates cultures, started with a single egg mass, were maintained on susceptible tomato cv. Roma in a greenhouse. The species identification was confirmed using SCAR-PCR markers (Zijlstra *et al*., 2000). The nematode inoculum was obtained by extraction of the eggs by blender maceration of infected roots in a 0.5% NaOCl solution for 5 min (Hussey and Barker, 1973). The egg suspension was passed through a 74 µm aperture sieve to remove root debris, and the dispersed eggs collected on a 25 µm sieve, counted, and used in pot experiments. For the thermal time requirements, the egg suspension was placed on Whitehead trays (Whitehead and Hemming, 1965) to obtain second-stage juveniles (J2), collected daily for 5 days and stored at 9ºC until use.

Pot experiments

A geometric series of 12 initial population densities (P_i) of *M. javanica* were used to assess the relationship between the P_i and final (P_f) population densities in pots in a greenhouse. Aliquots of the egg suspension were added as to give 0, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 x 100 eggs/100 cm^3 soil. Each treatment (inoculum level) was replicated seven times and the experiment repeated twice. The first experiment was run from 24 May to 16 August 2011 (74 days), and the second from 17 May to 17 July 2012 (61 days). At the end of each experiment, the plants were removed from the pots, the roots carefully washed free of soil and the plant fresh root and top weight (leaves, stems, flowers and fruits) recorded. Eggs were extracted from 5 g root subsamples as described previously and quantified (eggs and egg shells) to determine the P_f and the reproduction rate (P_f/P_i). Soil temperatures at 7 cm deep were recorded daily at 30 min intervals with temperature probes (Em50 Data Logger, Decagon Devices Inc, USA, accuracy ± 1ºC, resolution 0.1ºC).

Field experiments

Field experiments were conducted in 2011 and 2012 in an unheated plastic covered greenhouse fully enclosed over field soil located at Cabrils, Barcelona, Spain. The soil, infested with *M. javanica*, had a history of resistant and susceptible tomato cultivars grown in rotation, which provided a spread of the population (Talavera *et al*., 2009; Verdejo-Lucas *et al*., 2009). The soil was a sandy loam with 85.8% sand, 8.1% silt, and 6.1% clay, pH 8.1, with 0.9% organic matter w/w, and 0.40 dS m^{-1} electrical conductivity. Thirty-two plots of 2.5 x 2 m consisted of a single row with three plants spaced 80 cm in 2011, or four plants spaced 60 cm in 2012. Soil was prepared by hand hoeing plots individually. Seedlings were transplanted when the first true leaf was fully expanded on 20 June and harvested on 29 August 2011 (97 days), and from 16 April to 31 July 2012 (138 days). Plants were irrigated through a drip irrigation system once a week for a month, and thereafter twice a week until harvest. They were fertilized weekly with a solution of NPK (15-5-30) at 31 kg/ha, iron chelate, and micronutrients at a rate of 0.9 kg/ha. Plants were pollinated by a colony of *Bombus* bees (Biobest, Belgium) placed inside the plastic house at first blossom. At harvest, plant tops and roots were removed from the plastic house. Soil temperatures were recorded daily at 30 min intervals, as referred before, at 15 cm deep.

To increase the nematode population density, a susceptible tomato cv. Roma was cultivated between the two watermelon experiments from September 2011 to February 2012.

Composite soil samples were collected at the beginning and at the end of each experiment to estimate *M. javanica* P_i and P_f. Individual samples, consisted of five soil cores taken from 25 cm deep with a sampling tube (2.5 cm diameter). Samples were mixed thoroughly and nematodes extracted from two 500 cm^3 soil sub-sample/ plot using Whitehead trays (Whitehead and Hemming, 1965). One week later, J2 were collected, counted, and expressed as J2/100 cm^3 soil. Disease incidence was measured as the percentage of plants with galled roots, whereas disease severity was assessed using the root-gall index. Plants were uprooted, and rated on a scale of 0 to 10, where 0 = complete and healthy root

system and 10 = plants and roots dead (Zeck, 1971). Roots from each plot were bulked, cut into 1 cm-long segments, and eggs extracted from two 10 g-subsamples by blender maceration in a 0.5% NaOCl solution for 10 min (Hussey and Barker, 1973). The number of eggs was expressed per gram fresh root weight. Fruits were harvested, counted and weighed at the end of the experiment, when they had reached 4-5 kg according to common practices for this watermelon cultivar.

Thermal time requirements

The thermal requirements of *M. incognita* and *M. javanica* on watermelon were determined on a range of constant temperature of 17, 21, 25, and 28 ºC in growth-chambers with a 16-h light photoperiod. The length of time from J2 inoculation until the first egg deposition into the gelatinous matrix by mature female (egg-laying females, *ELF*), and life cycle completion (*LCC*) were estimated. Watermelon seedlings (60/treatment) were transplanted to 200 cm^3 Styrofoam cups containing steam-sterilized sand, and one week later inoculated with 200 J2/ pot. Plants were irrigated at field capacity with water, and thereafter when necessary, to maintain the moisture level and fertilized as previously referred. Soil temperatures were recorded daily at 30-min intervals at 4 cm deep.

Assessment periodicity varied according to temperature and expected occurrence of *ELF* or *LCC*. Three plants per treatment were removed daily until infection (e.g. first swelling J2 observed). Roots were washed free of soil, stained with 0.05% acid fuchsin (Bridge and Page, 1982), and observed. From then on, plants were removed at 5 or 15 day-intervals, and again daily when the *ELF* and *LCC* processes were expected to occur according to the literature (Tyler, 1933; Trudgill, 1995). The entire root system were stained with a 0.1 g/L erioglaucine (Aldrich Chemical Company) solution for 2 h (Omwega *et al.*, 1988) to facilitate egg mass observation. All egg masses were handpicked, and the eggs were dispersed in a 1% NaOCl solution to determine the number of days from egg deposition to J2 emergence (empty eggs). The base temperature (*Tb*) was calculated according the regression equation, 1/D = a T-b when 1/D = 0; Tb = b/a, where 1/D is the reciprocal

of the number of days (*D*) needed for completion of a given process (days^{-1}) and '*a*' is the slope of the function. The thermal constant (*K*) is the accumulated temperature or degree-days (*DD*) over the *Tb* calculated as the reciprocal of the slope (1/*a*) of the function (Trudgill, 1995). The regressions lines of *ELF* development and *LCC* were compared between RKN species.

Statistical analyses

The data were analysed using the SAS system V9 (SAS Institute Inc., Cary, NC). The data from the pot experiments were subjected to ANOVA and pooled because there were no statistical differences between the repeated experiments. The relationship between P_i and P_f was used to estimate the maximum nematode reproduction rate (*a*) and the equilibrium density (*E*, $P_i = P_f$) (Seinhorst, 1970). The data on nematode reproduction in the field experiments were compared by Student's t-test ($P < 0.05$). The maximum reproduction rate (*a*) was estimated by selecting the P_i values with the highest slope in the regression line between P_i and P_f. The maximum population density (*M*) was estimated from the experimental data, and *E*, according to the expression $M=aE/(a-1)$ (Schomaker and Been, 2006). The relative fresh top weight (*RFTW*) was calculated as fresh top weight for a given P_i/fresh top weight for $P_i = 0$. The nonlinear procedure (proc nlin) was used to fit the data to the Seinhorst damage-function model; $y = m + (1-m)\, z^{(Pi-T)}$ when $P_i \geq T$, and y = 1 when $P_i < T$, where *y* is the relative yield, *m* is the minimum relative yield, *z* is a constant ≤ 1, and *T* is the tolerance limit (the nematode density below which there is no yield loss) (Seinhorst, 1965). The relative yield (fresh top weight, *RFTW*, for the pot, and fruit weight, *RFW*, for the field experiments) was calculated as yield for a given P_i/yield for $P_i = 0$.

Results

Pot experiments

The sum of Celsius degrees were 1878ºC and 1461ºC (*Tb*= 0) in the first and second experiment, respectively, and temperatures varied from 18.5 to 35.8ºC and 15.7 to 38.6ºC. *Meloidogyne javanica* reproduced on watermelon at all initial levels and the maximum P_f/P_i of 14-fold (P_i =50/100 cm^3 soil) decreased to two-fold at P_i of 6400 to 25600 eggs/100 cm^3 soil. Only a P_i of 51200 eggs/100 cm^3 soil resulted in a P_f/P_i value less than one (Table 1). The maximum reproduction rate was at the lowest P_i and the *E* was 49400 egg/100 cm^3 soil (Fig. 1). Yield losses, represented as *RFTW* fit the Seinhorst damage-function model (P<0.001, R^2 = 0.70), showing that *m* was 0.65, *T* 74 eggs/100 cm^3 soil, and the value of *z* = 0.76.

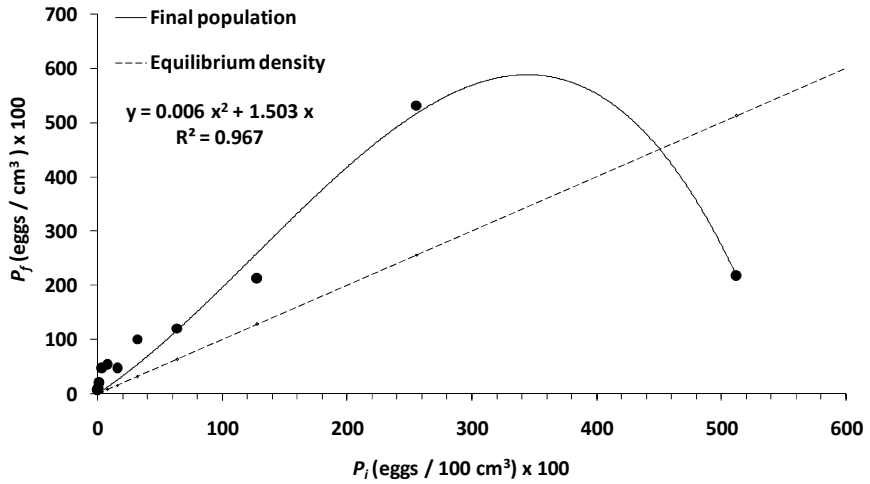

Fig. 1 Relationship between initial (P_i) and final (P_f) population densities (eggs/100 cm^{3}soil) of *Meloidogyne javanica* on watermelon cv. Sugar Baby. Values are mean of 14 replicates/ treatment.

Table 1 Final population densities (P_f) and reproduction rate (P_f /initial population density, P_i) of *Meloidogyne javanica*, and fresh top weight of watermelon cv. Sugar Baby in response to increasing P_i of the nematode in pot experiments conducted in a greenhouse (combined data from two experiments).

P_i/100 cm^3 of soil	P_f/100 cm^3 of soil[a]	P_f/P_i[b]	Fresh top weight (g)
0	0	0	37 ± 4.8
50	700 ± 130	14	36 ± 4.5
100	1000 ± 160	10	40 ± 5
200	2000 ± 690	10	28 ± 3.2
400	4600 ± 2480	12	29 ± 5.6
800	5400 ± 2350	7	27 ± 3.7
1600	4700 ± 1090	3	33 ± 5.3
3200	10100 ± 2490	3	22 ± 4.3
6400	12100 ± 2080	2	20 ± 3.7
12800	21200 ± 3770	2	22 ± 3.5
25600	53200 ± 9000	2	20 ± 2.9
51200	21700 ± 7100	0.4	28 ± 4.3

Values are mean ± standard error of 14 replicates (seven replicates/ experiment x two experiments). [a] Eggs and egg shells

Field experiments

In 2011, the average soil temperature was 28.5ºC (22-32ºC), and in 2012, 26.5ºC (18.4- 33.3ºC), and the sum of Celsius degrees being 1859 and 2813ºC, respectively. The nematode was not detected in 12 out of 32 plots in 2011, or in six in 2012. Pre-plant *M. javanica* population densities ranged from 0 to 1800 J2/100 cm^3 soil in 2011 and 0 to 3600 J2/100 cm^3 soil in 2012 (Table 2). The P_i values were 5.5 fold higher ($P<0.05$) and more widely spread in 2012 than 2011, although P_f densities were not statistically different with P_f/P_i <1 (Table 2). However, egg production was higher ($P<0.05$) in 2012 than in 2011 (Table 2). The disease incidence was 44% in 2011, and 63% in 2012 (data not shown), and disease severity was higher ($P<0.05$) in 2012 than 2011. Galled roots were observed in 25% of the plots with $P_f \leq 0$ in 2012.

Table 2 Initial (P_i) and final (P_f) population densities, reproduction rate (P_f/P_i), eggs per gram of root and disease severity (gall rating) of *Meloidogyne javanica* on watermelon cv. Sugar Baby in field experiments conducted in a nematode-infested plastic house in 2011 and 2012.

Year	P_i/100 cm^3	P_f/100 cm^3	P_f/P_i	Egg/g root	Gall rating [a]
2011	125 ± 62 (0–1800) b	23 ± 20 (0-370) a	0.18 (0-12) a	94 ± 35 (0-750) b	0.98 (0–4) b
2012	690 ± 180 (0–3600) a	26 ± 10 (0–230) a	0.037 (0-0.5) a	454 ± 128 (0-2984) a	2.42 (0–7) a

Values are mean ± standard error (range) of 32 plots for each field experiment. Values in each column followed by the same letter are not significantly different according to Student's t-test (P< 0.05). [a] Based on a scale from 0 (none, healthy plant) to 10 (dead plants) (Zeck, 1971).

The relationship between P_i and P_f/P_i was described by a potential function; this was used to calculate the theoretical maximum reproduction rate, which was 73 and 70 in 2011 and 2012, respectively, and the *E*, 32 and 35 J2/100 cm^3 soil, respectively. Yield data represented as fruit weight fit the Seinhorst damage-function model (P<0.0001, R^2 =0.92) in 2011; m was 0.63, T 20 J2/100 cm^3 soil, and the *z* value was 0.95 (Fig. 2). Nevertheless, data from 2012 did not fit the damage-function model. In this experiment, 13 out of 32 plots showed stunted top growth at average *Pi* of 1532 ± 306/100 cm^3 soil and *GI*=4 (scale 0 to 10) in comparison to RKN symptomless plots showing an average *Pi* of 303 ± 119/100 cm^3 soil and *GI*=2. Average P_f densities were 20 and 29/100 cm^{3}soil, respectively.

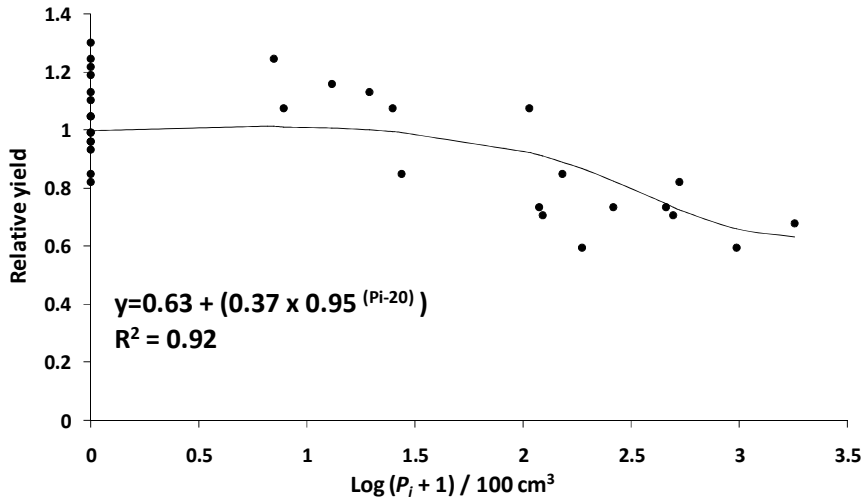

Fig. 2 Relationship between *Meloidogyne javanica* initial population densities (P_i)/100 cm^3 soil and relative fruit yield of watermelon cv. Sugar Baby, in a field experiment (2011).

The *M. javanica* population in the soil increased 64-fold from 12 ± 20 to 770 ± 130 J2/100 cm^3 soil (data not shown) after the tomato crop planted between the two watermelon crops.

Thermal time requirements

The length of time for completion of *ELF* and life cycle decreased as the temperature increased from 16.8 to 27.8°C (Table 3), and was similar between *M. incognita* and *M. javanica* for *LCC* at the two highest temperatures (24.3 and 24.4ºC and 27.6 and 27.8ºC, respectively). The thermal requirements for *ELF* were similar between the two RKN species (Table 3). The *Tb* for *M. incognita LCC* was 14.0°C and *K*=500 *DD*. The thermal requirements of *M. javanica LCC* were estimated from the three highest temperatures. At 17.2°C hatching did not occur 180 days after inoculation when the experiment was concluded. The estimated values were *Tb*=17.2°C and *K*=357.1 *DD* (Table 3), and the relationship between soil temperature and the reciprocal of time for *ELF* and *LCC* is presented in Fig 3.

Table 3 Number of days required to *Meloidogyne incognita* (*Mi*) and *M. javanica* (*Mj*) complete egg-laying females (*ELF*) development and life cycle (*LCC*) on watermelon cv. Sugar Baby after inoculation with 200 second-stage juveniles at four soil temperatures, base temperature (*Tb*) and accumulated degree days above *Tb* (*K*).

Soil temperature (° C)		*ELF*		*LCC*	
Mi	*Mj*	*Mi*	*Mj*	*Mi*	*Mj*
16.8	17	70	114	107	no
21.6	21.4	52	46	80	73
24.3	24.4	30	29	52	52
27.8	27.6	17	22	31	32
Tb (° C)		14.7	14.8	14	17.2
K (*DD*)		250	277.8	500	357.1

no: not observed until 114 days after inoculation.

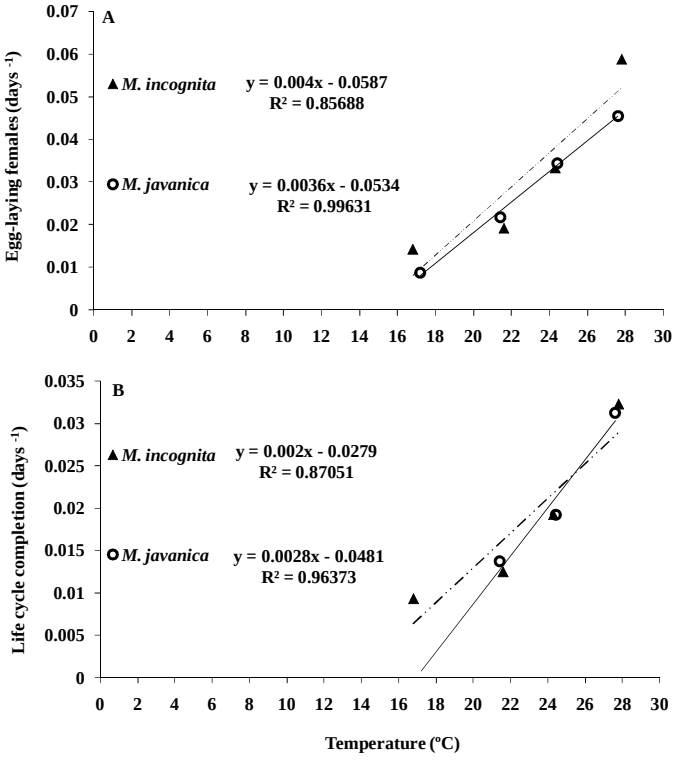

Fig. 3 Rate of development of *Meloidogyne incognita* and *M. javanica* for A) egg-laying females (*ELF*) and B) life cycle completion (*LCC*, from J2 to J2).

Discussion

Meloidogyne javanica reproduced on watermelon cv. Sugar Baby in pot confirming the results already reported of an increase of the RKN populations (P_f/P_i >1) on this crop (Montalvo and Esnard 1994; Thies and Levi, 2003, 2007). According to Di Vito *et al.,* (1991, 1992, 2004) on tomato, pepper, and spinach, the highest P_f/P_i occurred at the lowest P_i, in contrast to melon and cucumber that did at intermediate inoculum levels among the tested P_i (Giné *et al*., 2014; Ploeg and Phillips, 2001). The low magnitudes of the maximum reproduction rate and equilibrium density support the poor-host status of watermelon for *M. javanica* (Seinhorst, 1967). By contrast, a good-host such as susceptible tomato cv. Durinta showed a maximum reproduction rate of 3500 and equilibrium density of 1140 J2/100 cm^3 under similar environmental conditions whereas these values on the resistant tomato cv. Monika were 158 and 20 J2/100 cm^3, respectively (Talavera *et al.,* 2009). The low reproduction rate of *M. javanica* could be explained by a great reduction in J2 penetration, and delayed development of RKN into watermelon roots (López-Gómez and Verdejo-Lucas, 2014), a mechanism associated with poor-host condition (Ehwaeti *et al*., 1999).

Watermelon cultivation in the *M. javanica*-infested field decreased the P_f densities, as already noted in *M. incognita* (Davis, 2007; Xing and Westphal, 2012). Watermelon was planted during periods suitable for crop development and conducive to nematode infection, since the minimum soil temperatures (18.4ºC) exceeded the *Tb* for this RKN life-cycle completion. Therefore, the decline of *M. javanica* populations on watermelon cannot be attributed to soil temperature but to other factors. Overall, the P_f oscilated around the equilibrium density irrespective of low, medium, or high P_i. Reduced P_f could be also due to root damage which is related to the number of nematodes that attack the host. Watermelon is severely damaged by RKN that causes profuse root galling, and population increases ranging from 1.1 to 9-fold or decreases with P_f/P_i <1 (Montalvo and Esnard, 1994; Thies and Levi, 2003, 2007; Davis, 2007; Xing and Westphal, 2012). As damage increases, the competition for feeding sites increases which can impair growth of the nematode and host. Nematode development decreased as

result of nematode crowding within the roots and consequent reduction of available food or competition for suitable sites for giant cell formation (McClure and Viglierchio 1966). Younger plants supported smaller RKN populations than older plants probably due to lower number of available feeding sites (Ploeg and Phillips, 2001). Low P_f on melon was attributed to severely damaged root systems unable to support large RKN populations (Di Vito *et al*., 1983; Ploeg and Phillips, 2001). On the other hand, the host has the capacity to compensate nematode damage and repair injury by regenerating new tissue. Recovery of watermelon plants from early stunting was observed in plots with average P_i of 1532 J2/100 cm^3 soil and $GI \geq 4$ in the 2012 field experiment with wide spread P_i. Wallace (1971) considered that differences in the responses of plants to infection by *M. javanica* were the result of the interaction between inhibitory and stimulatory processes in the plant. Consequently, top growth in some plant species was stimulated by low numbers of J2, and root weight increased as numbers of nematodes increased. In a second group of plants, top growth was not influenced by P_i and in a third one, growth decreased linearly with increasing P_i.

Watermelon cv. Sugar Baby was considered tolerant to *M. javanica* because the T and m were higher than those reported for RKN susceptible crops, such as melon (T = 0.48 J2/100 g soil and m = 0.35), cucumber (T=1 J2/100 cm^3 soil and m=0.36), and pepper (T = 36 J2/100 cm^3 soil and m = 0.4) (Di Vito *et al.,* 1991; Ploeg and Phillips, 2001; Giné *et al*., 2014). The pot and field experiments gave similar minimum relative yield (m=0.65-0.63, respectively), which was comparable to that of resistant tomato cv. DINA S (m = 0.7) (Di Vito *et al.*, 1991). In addition, the estimated maximum yield loss (37%) on watermelon was consistent with the yield increases in response to methyl bromide fumigation (Davis, 2007). Xing and Westphal (2012) reported a T for watermelon top dry weight of 4 J2/100 cm^3 soil of *M. incognita*. Our results could reflect that watermelon has a higher tolerance to *M. javanica* than to *M. incognita* or the threshold level to reduce fruit yield is higher than that affecting plant growth.

The rate of RKN development is linearly related to the reciprocal of time between *Tb* and optimum temperature; although

less frequently, nonlinear functions have also been found (Tyler, 1933; Trudgill, 1995). In this study, the estimation of *Tb* was done by projecting the straight segment of the curve according to Wilson and Barnett (1983). This may result in an overestimation of *Tb*, although the authors considered it of minor concern because for most crops temperatures are above *Tb* during the cropping season. We tested a range of temperatures adequate for nematode development but did not include suboptimal temperatures. If they had been tested, the estimated *Tb* value of *M. javanica* would have been smaller and closer to the published values (Madulu and Trudgill, 1994; Trudgill, 1995).

Most studies on thermal requirements of RKN used tomato as the host plant although the number of studies using cucurbits is increasing. Both *M. incognita* and *M. javanica* showed similar thermal requirements for *ELF* on watermelon cv. Sugar Baby, as they did for *LCC* on squash (*Cucurbita moschata*) and cucumber cv. Dasher II (Davila *et al.,* 2005; Giné *et al.,* 2014). The negative relationship between the required thermal sum and the base temperature (Trudgill and Perry, 1994; Ploeg and Maris, 1999; Maleita *et al.*, 2012) may be affected by host status, population size, and root damage because any factor delaying nematode development will increase thermal constants, since there is a trade-off between the estimates of *Tb* and *K* (Madulu and Trudgill, 1994; Trudgill, 1995). Early J2 development was delayed on watermelon in comparison to other cucurbits which are better hosts than watermelon (López-Gómez and Verdejo-Lucas, 2014). Development of *M. konaensis* on coffee was slower than on tomato (Zhang and Schmitt, 1995), or that of *M. hispanica* on resistant than susceptible tomato (Maleita *et al.*, 2012). *Meloidogyne incognita* completed its life cycle in *Tagetes* at 30^{o}C but not at lower temperatures (Ploeg and Maris, 1999).

In protected cultivation, cucurbitaceous and solanaceous crops are frequently grown in rotation with little time between successive crops, which does not favour the natural decline of the nematode by starvation in the absence of plant hosts. Therefore, nematode management should be considered from a holistic point of view rather than a single crop approach as the management actions, in a given crop, would affect the subsequent crop

(Westphal, 2011). In a rotation strategy, the number of nematodes in the soil may cause damage to the crop depending upon the host (Ehwaeti *et al*., 1999). Two factors were at least involved in the decline of *M. javanica* population densities after cultivation of watermelon cv. Sugar Baby: the poor-host status of the crop and the root damage caused by the nematode. This is consistent with field observations of lower P_f after cucurbitaceous than after solanaceous crops (Talavera *et al*., 2012).

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General Discusion

The family Cucurbitaceae contains numerous genera, including economical revelant crops in current agriculture such as *Citrullus* spp., *Cucumis* spp. and *Cucurbita* spp. All of them are considered hosts to *Meloidogyne* spp. (Fassuliotis, 1971; Montalvo and Esnard, 1994; Edelstein et al., 2010; Wesemael et al., 2011; Talavera et al., 2012). However, differences such as nematode reproduction and crop tolerance have been reported amongst cucumber cultivars (Mukhtar et al., 2013), which could be interesting for rotation purposes due to cucurbit crops being frequently grown in rotation with species of solanaceae (Meneses and Castilla, 2009). Knowledge about host suitability of the main cucurbits crops and cultivars to *Meloidogyne* spp. populations is important in order for the best option to be included in the rotation to maintain the population densities under the damage threshold (Westphal, 2011).

The selected cucurbit crops were host to *Meloidogyne* spp., although they have shown differences in the number of juveniles that invaded the roots, developed until mature females and the number of egg masses produced. The species of *Cucumis* spp. (cucumber and melon) were the most suitable host to nematode invasion and reproduction, followed by zucchini and watermelon. Cucumber and melon provided favorable conditions for the nematode that presented high invasion rates, a progressive development and high egg masses production. However, cucumber provided higher reproduction traits than melon (chapter 1). The majority of the juveniles that infected the cucumber roots developed into egg-laying females, producing high reproduction rates (chapter 2).

Zucchini showed high penetration rates and egg masses production but lower than *Cucumis* spp. (cucumber and melon) (chapter 1). The infection process of *M. incognita* in zucchini was characterized by small reproduction traits with no effect on the number of penetrating J2 since they progressively developed into immotile J3 and J4 stages (chapter 1). Thus, it was assumed that no defense mechanism preventing the root penetration and the feeding site establishment was involved. However, examination of the zucchini galls revealed the inability of *M. incognita* individuals to reach the egg-laying female stage (chapter 2), showing reduced fecundity in comparison with *M. javanica* females. The sub-optimal

development of feeding sites unable to supply sufficient nutrients for the nematode ot their early degradation could possibly explain the *M. incognita*-zucchini squash interaction. In resistant plants, a high percentage of non-fertile females have been observed (Mc Clure et al., 1974). However, the infection process of *M. javanica* in zucchini was characterized by reduced penetration rates compared to the rates observed in *M. incognita*; yet the post-infection development was successful post-infection development with 95% of the penetrating juveniles reaching the stage of egg laying female (chapter 2) and high reproduction rates were achieved.

In watermelon, the infection process was characterized by reduced juvenile invasion, a delay in post-infectional development and a low number of egg-laying females irrespective of the *Meloidogyne* isolate (chapter 1 and 3). Reduction of the invasion rate has been reported as a pre-infectional defense mechanism, which involves morphological structures or root exudates that prevents the attraction or repel the nematodes (Huang, 1985; Anwar et al., 1994, Anwar and Mckenry, 2000). Besides, the delay in post-infection development prevented the life cycle completion of a high percent of the penetrating nematodes. The combination of reduced penetration rates and delay in nematode development resulted in the production a low number of egg-laying females.

The suitability of current commercial genotypes of zucchini, cucumber, watermelon and cucurbit rootstocks to *Meloidogyne* was assessed to investigate if the genetic background of the host plant and the parasitic variation associated to the genus *Meloidogyne* influenced the plant-nematode interaction. Such factors have been reported to affect the host-parasite interaction in beans, peas, asparagus and tomatoes (Jaquet et al., 2005; Cortada et al., 2009; Dudash and Barker, 1992; Santo and Ponti, 1985). The genotypes of zucchini slightly differed in egg production and in the reproduction factor but not in the ability of the nematode to infect the roots and produce egg masses. A large parasitic variation was observed in the number of galls amongst the population of *M. incognita* in zucchini that responded as a good or poor host depending on the tested population. However, intra-specific variation was not observed amongst the *M. javanica* or *M. arenaria* populations. The genotypes of cucumber showed similar suitability

to the nematode with some minor differences in egg production and reproductive factor when infected by *M. incognita*. These genotypes did not differ in values of reproductive factor when infected by *M. javanica.* Although zucchini and cucumber were suitable hosts for *Meloidogyne,* zucchini was a poorer host than cucumber as indicated by the number of egg masses (three times less than in zucchini than cucumber). In addition, the fecundity of the females in zucchini was lower than in cucumber (chapters 1 and 2). The genotypes of watermelon did not differ in host status within each nematode isolate and they supported lower reproduction in comparison with the cucurbit rootstocks. *Meloidogyne incognita* showed higher reproduction than *M. javanica* on the watermelon cultivars and cucurbit rootstocks (chapter 3).

The root-knot nematode population dynamics and damage functions were investigated in zucchini and watermelon as there was little information on these crops. In greenhouse experiments involving one nematode generation, zucchini was better host than watermelon to *M. javanica*. Zucchini showed high values for the maximum reproduction rate and equilibrium density, which is in support of its good host condition to *M. javanica* in comparison with watermelon (chapters 4 and 5). This coincides with the results of the studies presented in chapters 1 and 2. In watermelon, the low magnitudes of the maximum reproduction rate and equilibrium density support the poor host status of this crop for *M. javanica* (chapter 5).

In the field experiments involving several nematode generations, the zucchini cv. Dyamant showed high reproduction rates (chapter 4), similar to those of a good host as cucumber cv. Dasher II or tomato cv. Durinta (Talavera et al., 2009; Gine et al, 2014). Watermelon consistently proved to be a poor host to *M. javanica* (chapter 5); the nematode population decreased at harvest in relation to the initial population and the values oscillated around the equilibrium density. The reproduction traits were low, similar to poor hosts such as resistant tomato cv. Monika (Talavera et al., 2009), and lower than good hosts such as cucumber and susceptible tomato cv. Durinta (Talavera et al., 2009; Gine et al., 2014). Watermelon is a potential crop for managing the disease

produced by root-knot nematode, and a suitable candidate for rotation of Solanaceae with Cucurbitaceae crops.

The amount of yield losses that a crop can suffer is independent of the plant host status. Thus, the Seinhorst damage function model (Seinhorst, 1965) was used to estimate the tolerance and the yield losses caused by the nematode in zucchini and watermelon. In experiments involving one nematode generation, zucchini was more tolerant than watermelon (chapters 4 and 5). Both cucurbits were more tolerant and experimented less yield losses than other cucurbits of economic importance in protected cultivation such as melon and cucumber under similar conditions (Giné et al., 2014).

As obligate sedentary endoparasites, *Meloidogyne* interferes with plant physiological processes involved in water uptake and nutrient translocation and create an imbalance of macro and micro-nutrients; in consequence, leaf chlorosis and stunted growth may appear in nematode-infested plants. Leaf chlorophyll content is positively correlated with the foliar nitrogen concentration and plant productivity in several crops including zucchini. Measurements of leaf chlorophyll content at weekly intervals showed that the content was reduced by *Meloidogyne* infection in zucchini as occurred in cucumber (Giné et al., 2014), and there was a negative relationship between the initial population densities and the leaf chlorophyll content. Thus, this non-destructive measure can be used to monitor the health status of the plant in real time and adjust the nitrogen fertilization or to implement control measures to compensate the damage caused by the nematode.

In field experiments involving several nematode generations, zucchini showed a low tolerance to the nematode, and high maximum yield losses. Vela et al. (2014) reported that crop tolerance of zucchini infected by *M. incognita* depends on the cropping season, even so, the zucchini tolerance to *M. javanica* (Chapter 4) is lower than *M. incognita* in spring sesion (Vela et al., 2014), and the crops suffers more yield losses with *M. javanica* than *M. incognita*. Watermelon showed high tolerance and reduced yield losses compared to susceptible cultivars such as cucumber or tomato (Giné et al, 2014; Talavera et al, 2009) (chapters 4 and 5).

However, the nematode damage in watermelon was observed during the cultivation period as a delay in the plant growth. A significant reduction was observed in the surface area covered by the non-grafted Sugar Baby plants in plots where the nematode was detected at begining of the experiment in comparison to plots where the nematode was not detected. Plants remained stunted for two-thirds of the cropping cycle but restored growth 9 weeks after transplantation (chapter 3 and 5). As a result, non-grafted Sugar Baby yielded 27% less in infested plots than in those non-infested, due to the fact that no fruits were collected during the first harvest (chapter 3).

Watermelon is commonly grafted onto cucurbit rootstocks due to their susceptibility to *Fusarium* wilt (Davis et al., 2008). The effect of initial inoculum level on nematode reproduction and yield losses was determined in watermelon grafted onto two intraspecific rootstocks (*Cucurbita maxima* x *C. mochata)*, RS841 and Titan, and non-grafted watermelon under greenhouse and field conditions. It was observed that in both experimental conditions high population increases in the grafted compared to the non-grafted plants irrespective of the initial inoculum level. Furthermore, rootstock RS841 suffered a greater yield loss than the non-grafted watermelon or the Titan-grafted plants. The rootstock Titan conferred tolerance to the nematode in the *M. javanica*-infected plots. Thus, the grafting technique applied to watermelons is not viable as a integrate pest management strategies due to the increase in the nematode population, although, the selection of a suitable rootstock is important because Titan shows tolerance to the nematode.

The differences observed in the suitability of commercial cucurbits crops to root-knot nematode disease in this thesis have indicated that the ability of the watermelon to interfere with the nematode development makes it an ideal crop to be included in crop rotations to for maintain nematode density levels below those that do not cause economic damage for the following crops. On the other hand, the differential response of zucchini against nematode species makes it particularly suitable for interfering with the development of *M. incognita*, being useful to integrate pest management in field infested by this *Meloidogyne* specie. The

defense mechanisms that operate against to the nematode in each cucurbit are still unknown, opening new lines of research for the future. Finally, we have demonstrated that the commercial interspecific hybrids rootstocks do not inhibit the nematode reproduction, so it is necessary to look for rootstocks that are effective against root-knot nematode infestion.

Conclusions

The root invasion, post-infection development and reproduction of *Meloidogyne* spp. differed amongst the selected cucurbits. Watermelon showed lower reproduction traits than zucchini followed by pumpkin, melon and cucumber.

The host suitability tests indicated small differences in nematode reproduction amongst the selected genotypes within a nematode population in zucchini, cucumber, watermelon and cucurbit rootstocks. According to their susceptibility levels, cucumber and the cucurbit rootstocks were categorized as good hosts highly susceptible to the nematode. In contrast, zucchini and watermelon showed an intermediate and poor host status, respectively.

The parasitic ability of *Meloidogyne javanica* was higher than that of *M. incognita* in all cucurbits, except for zucchini. The nematode populations showed a great parasitic variation in zucchini, and the greatest variations were observed in the populations of *M. incognita*.

The studies on the population dynamics of *Meloidogyne* in cucurbits showed that watermelon is a poorer host than zucchini for *Meloidogyne javanica*. Watermelon consistently showed low values for maximum reproduction rate and equilibrium density in greenhouse and field conditions.

The yield loses caused by *Meloidogyne javanica* in zucchini cv. Dyamant reached up to 52 % under field conditions and the tolerance limit of the crop was close to the nematode detection level. However, the yield losses in watermelon were 27 % and a high tolerance limit of 20 nematodes/100 cm^3 of soil.

The cucurbit rootstocks were susceptible to *Meloidogyne* spp. and supported high reproduction levels. The selection of the scion–rootstock combination would be critical because only some rootstocks provide tolerance to the nematode

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Chapter 4: *Damage functions and termal requirements of Meloidogyne javanica and Meloidogyne incognita on watermelon*

Chapter 5: *Differential reproduction of Meloidogyne incognita and M. javanica on watermelon cultivars and cucurbit rootstocks.*

Anex A: List of publications related to this doctoral thesis

Publications included in SCR

Giné, A., **López-Gómez**, M., Vela, M.D., Ornat, C., Talavera, M., Verdejo-Lucas, S., Sorribas, F.J. 2014. Thermal requirements and population dynamics of root-knot nematodes on cucumber and yield losses under protected cultivation. *Plant Pathology* 63, 1446-1453. DOI: 10.1111/ppa.12217

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All the photographs showed in this thesis have been done by Manuel López-Gómez.